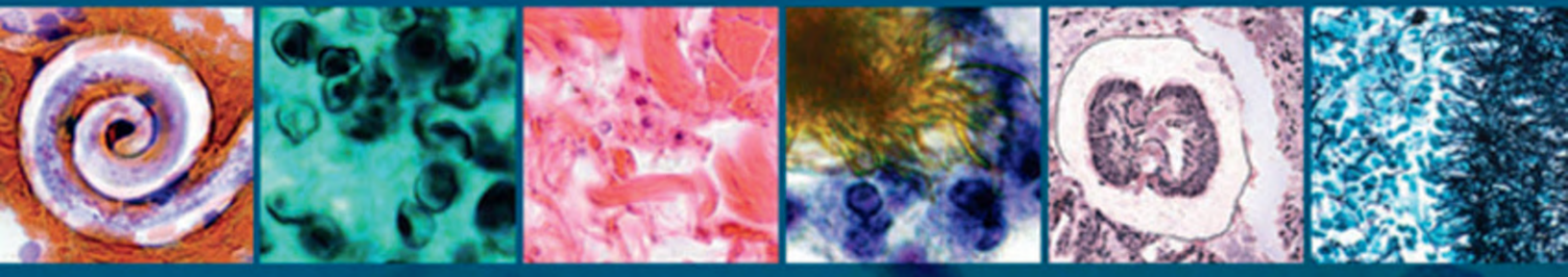
2nd Edition

DIAGNOSTIC PATHOLOGY *of* INFECTIOUS DISEASE



Richard L. Kradin

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Diagnostic Pathology of Infectious Disease

Diagnostic Pathology of Infectious Disease

Second Edition

Richard L. Kradin, MD, DTM&H

Associate Pathologist and Associate Physician
Department of Pathology and Pulmonary/Critical Care Unit
Massachusetts General Hospital
Associate Professor in Pathology and Associate Professor in Medicine
Harvard Medical School
Boston, Massachusetts

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1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

DIAGNOSTIC PATHOLOGY OF INFECTIOUS DISEASE, SECOND EDITION

ISBN: 978-0-323-44585-6

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Previous edition copyrighted 2010.

Library of Congress Cataloging-in-Publication Data

Names: Kradin, Richard L., editor.
Title: Diagnostic pathology of infectious disease / [edited by] Richard L. Kradin.
Description: Second edition. | Philadelphia, PA : Elsevier, [2018] | Includes bibliographical references and index.
Identifiers: LCCN 2017007825 | ISBN 9780323445856
Subjects: | MESH: Communicable Diseases–diagnosis | Communicable Diseases–pathology | Clinical Laboratory Techniques
Classification: LCC RA643 | NLM WC 100 | DDC 616.9–dc23 LC record available at https://lccn.loc.gov/2017007825

Content Strategist: Kayla Wolfe
Senior Content Development Manager: Katie DeFrancesco
Publishing Services Manager: Patricia Tannian
Senior Project Manager: Amanda Mincher
Design Direction: Teresa McBryan

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1

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I dedicate the second edition of this textbook to my colleagues in the departments of Pathology and Medicine at the Massachusetts General Hospital.

Contributors

H. Thomas Aretz, MD
Associate Professor of Pathology
Harvard Medical School
Vice President, Global Programs
Partners HealthCare International
Affiliate Pathologist
Massachusetts General Hospital
Boston, Massachusetts

Sandra Camelo-Piragua, MD
Clinical Assistant Professor of Pathology
University of Michigan
Ann Arbor, Michigan

Norman C. Charles, MD
Clinical Professor of Ophthalmology and Pathology
Director, Ophthalmic Pathology, Bellevue Hospital Center
New York University Langone Medical Center
Director, Ophthalmic Pathology, Bellevue Hospital Center
New York, New York

Elizabeth G. Demicco, MD, PhD
Assistant Professor of Pathology
Mount Sinai Hospital
New York, New York

Vikram Deshpande, MBBS
Associate Pathologist
Massachusetts General Hospital
Associate Professor of Pathology
Harvard Medical School
Boston, Massachusetts

Ralph C. Eagle Jr., MD
Director, Department of Pathology
Wills Eye Hospital
Philadelphia, Pennsylvania

Alton B. Farris III, MD
Associate Professor and Director
Laboratory of Nephropathology and Electron Microscopy
Department of Pathology
Emory University
Atlanta, Georgia

Judith A. Ferry, MD
Professor of Pathology
Harvard Medical School
Director of Hematopathology
Department of Pathology
Massachusetts General Hospital
Boston, Massachusetts

Jay A. Fishman, MD
Professor of Medicine
Harvard Medical School
Director
Transplantation Infectious Disease and Compromised Host Service
Massachusetts General Hospital
Boston, Massachusetts

Robert P. Hasserjian, MD
Associate Professor of Pathology
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts

E. Tessa Hedley-Whyte, MD
Professor of Pathology
CS Kubik Laboratory for Neuropathology
Massachusetts General Hospital
Harvard University
Boston, Massachusetts

A. John Iafrate, MD, PhD
Pathologist
Massachusetts General Hospital
Professor of Pathology
Harvard Medical School
Boston, Massachusetts

Frederick A. Jakobiec, MD, DSc
Director, David G. Cogan Ophthalmic Pathology Laboratory
Department of Ophthalmology
Massachusetts Eye and Ear Infirmary
Boston, Massachusetts

Matthew M. Johnson, MD
Pathologist
Carolinas Pathology Group
Charlotte, North Carolina

Susan V. Kattapuram, MD
Associate Professor of Radiology
Massachusetts General Hospital
Boston, Massachusetts

Richard L. Kradin, MD, DTM&H
Associate Pathologist and Associate Physician
Department of Pathology and Pulmonary/Critical Care Unit
Massachusetts General Hospital
Associate Professor in Pathology and Associate Professor in Medicine
Harvard Medical School
Boston, Massachusetts

Gregory Y. Lauwers, MD
Senior Member
Department of Anatomic Pathology
H. Lee Moffitt Cancer Center & Research Institute
Professor of Oncologic Sciences
University of South Florida
Tampa, Florida

Alice Z.C. Lobo, MD
Dermatologist
Clinica Alice Lobo
Presidente Prudente, SP
Brazil

Eugene J. Mark, MD
Pathologist
Massachusetts General Hospital
Subspecialty Head, Pulmonary Pathology
Professor of Pathology
Harvard Medical School
Boston, Massachusetts

Ricard Masia, MD, PhD
Instructor in Pathology
Harvard Medical School
Assistant in Pathology
Massachusetts General Hospital
Boston, Massachusetts

Martin C. Mihm Jr., MD
Director, Melanoma Program
Dermatology
Director, Mihm Cutaneous Pathology Consultative Service
Brigham and Women's Hospital
Professor of Pathology and Dermatology
Harvard Medical School
Boston, Massachusetts

Danny A. Milner Jr., MD, MSc (Epi), FASCP
Chief Medical Officer
Director, Center for Global Health
American Society for Clinical Pathology
Chicago, Illinois
Adjunct Professor of Immunology and Infectious Diseases
Harvard T.H. Chan School of Public Health
Boston, Massachusetts

Mari Mino-Kenudson, MD
Associate Professor of Pathology
Harvard Medical School
Associate Pathologist
Massachusetts General Hospital
Boston, Massachusetts

Joseph Misdraji, MD
Associate Professor of Pathology
Massachusetts General Hospital
Boston, Massachusetts

G. Petur Nielsen, MD
Pathologist, Department of Pathology
Director, Bone and Soft Tissue Pathology
Director, Electron Microscopy
Massachusetts General Hospital
Professor of Pathology
Harvard Medical School
Boston, Massachusetts

Nicole Pecora, MD, PhD
Assistant Professor of Pathology and Laboratory Medicine
University of Rochester Medical Center
Rochester, New York

Carlos N. Prieto-Granada, MD
Assistant Professor
Department of Pathology
The University of Alabama at Birmingham
Birmingham, Alabama

Drucilla J. Roberts, MA, MD
Associate Pathologist
Massachusetts General Hospital
Boston, Massachusetts

Andrew E. Rosenberg, MD
Professor of Pathology
Vice Chair; Director of Bone and Soft Tissue Pathology
Department of Pathology
Miller School of Medicine, University of Miami
Miami, Florida

Vicki J. Schnadig, MD
Professor Emerita
Department of Pathology
University of Texas Medical Branch
Galveston, Texas

Martin K. Selig, BA
Senior Medical Technologist
Massachusetts General Hospital
Boston, Massachusetts

Anna M. Stagner, MD
Fellow, David G. Cogan Ophthalmic Pathology Laboratory
Department of Ophthalmology
Massachusetts Eye and Ear Infirmary
Boston, Massachusetts

Rosemary Tambouret, MD
Department of Pathology
Massachusetts General Hospital
Boston, Massachusetts

Lawrence Zukerberg, MD
Associate Pathologist
Massachusetts General Hospital
Associate Professor of Pathology
Harvard Medical School
Boston, Massachusetts

Preface

I am pleased to have the opportunity to publish this second edition of *Diagnostic Pathology of Infectious Disease*. For some years now, I have served as the Director of Infectious Disease Pathology at Massachusetts General Hospital. In that capacity, I have the opportunity to see a vast array of infectious diseases as they present in the practice of surgical pathology. Infectious diseases are a staple of surgical pathology, and their accurate diagnosis is critical for patient care but may be diagnostically challenging.

The nuances of the pathology of infection require the surgical pathologist to have an understanding of clinical medicine, knowledge of infectious disease demographics, experience recognizing the varied morphological features of pathogens, the ability to recognize how host responses vary with infection and immunosuppression, and the facility to know when one is not dealing with a noninfectious diagnosis. While most surgical pathologists manage to do a very reasonable job in diagnosing infection, many would likely admit that their level of sophistication in this area is limited.

Most textbooks of infectious disease pathology emphasize details of microbial identification. However, what the practicing surgical pathologist needs to know is how to recognize the morphology of organisms and the spectrum of histological responses that they may cause. Few busy pathologists have the time or inclination to specialize in infection, so they need an accessible resource on this topic. That was the rationale for the first edition of *Diagnostic Pathology of Infectious Disease,* and that aim has not changed. The present text provides new information that has accrued in this field since the last edition and has new chapters devoted to infections of the orbit and to novel techniques developed in microbiology that will assist the surgical pathologist in confirming and speciating infections.

Like the previous edition, the present text is not designed to be an in-depth compendium of infection; there are other excellent textbooks available to serve that purpose. What it *does* provide is an up-to-date, practical, and well-illustrated presentation of the pathology of infection that will be both an informational and a visual resource for the practicing surgical pathologist.

Acknowledgments

It is difficult to know where to begin with respect to acknowledgments. Perhaps the best place is with my fellow authors who, with two exceptions, are all present or former colleagues in the Department of Pathology at the Massachusetts General Hospital (MGH).

The MGH Infectious Disease Unit is outstanding. I attend their weekly case conferences and am always impressed by the insight they bring to the diagnosis and management of challenging cases. They have been consistently supportive of the importance of pathology in the diagnosis of infection, and I trust that I have added to their knowledge of how infection manifests in tissues.

To the staff of the London School of Hygiene and Tropical Medicine, who gave me the opportunity to hone my skills with respect to infection as a Diplomate in Tropical Infectious Disease.

To the editorial staff at Elsevier, including Bill Schmitt, who recognized the merit in this project, and Katie DeFrancesco, who has patiently shepherded it along.

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1 Introduction

RICHARD L. KRADIN

Infection continues to account for the majority of human diseases. Substantial advances have occurred since the last edition of this textbook in the field of infectious disease. Technological advances have improved diagnostic yields for a variety of diseases. New diseases such a Middle East respiratory syndrome and the Zika virus epidemic have gained worldwide attention. Global travel and the migration of large populations from Africa and the Middle East have brought populations that were previously isolated into contact. The spread of Ebola virus from 2013–2016 from areas of Africa to travelers returning to the United States highlights this issue.

Increasing numbers of therapeutic agents that modify the immune system have led to major breakthroughs in the treatment of hepatitis C, but new antiinflammatory drugs have also increased the numbers of patients who are functionally immunosuppressed. Furthermore, the phenomenon of global warming threatens to foster conditions for new and reemergent diseases.

In response to the challenge of infection, surgical pathologists must continue to render diagnoses from both cytology specimens and biopsies. Both noninvasive and minimally invasive approaches have been developed that call upon the practicing pathologist to opine with respect to smaller samples. Furthermore, in addition to establishing the cause of infection, pathologists must consider a range of disorders in their differential diagnosis with respect to underlying disorders that can mimic the histology of infection.

Whereas the microbiology laboratory and infectious disease specialists have become increasingly skilled in the diagnosis of infectious diseases, the same unfortunately has not been uniformly true of surgical pathologists, who may continue to defer to their clinical colleagues in this area. This choice is fostered by the frustration of trying to identify small numbers of small pathogens and difficulties in diagnosing organisms with accuracy. Taken together, the time, effort, and expense of diagnosing infection can at times seem nongratifying for a busy surgical pathologist.

Nevertheless, the ubiquity of infectious diseases makes it highly unlikely that surgical pathologists can avoid being confronted with the diagnosis of infection in practice, so that it behooves them to be aware of the intricacies of how infection manifests in situ. Fortunately there have been advances in this regard that will assist the surgical pathologist in establishing an accurate diagnosis. Indeed the primary aim of this new edition of the *Diagnostic Pathology of Infectious Disease* continues to be to rekindle the interest that most surgical pathologists once held for the pathologic diagnosis of infection.

The present text continues to be organized unlike most other textbooks of infectious disease pathology. The contributors continue to be subspecialists in surgical pathology who have established expertise in diagnosing the infections that primarily affect their organ system, and for this reason the text is still organized based on organ system rather than as a litany of specific infectious organisms. As a consequence, the reader will be exposed to these disorders as they are encountered in a subspecialty practice of pathology. The nuances of infectious diagnoses are presented together with their differential diagnosis, so that the reader can better glean from the text how to narrow the differential diagnosis in practice.

The text includes a preliminary discussion of the types of inflammatory responses that can be elicited by various microorganisms and how host defenses modify these responses. There is a detailed explanation of how to apply histochemical stains differentially to narrow the differential diagnosis with respect to morphology of microbes. Advances in the application of immunohistochemical stains, in situ hybridization, and polymerase chain reaction are discussed prior to a discussion of the infections encountered in the major organ systems. New chapters that specifically focus on the role of the microbiology laboratory, as well as specific infections of the orbital region, have been added to this edition.

Because many microorganisms can affect a variety of human tissues, there is necessarily redundancy within the text with respect to their description. However, on balance, the superimposed constraints of tissue microanatomy lead to diversity with respect to the morphologic appearances of infection at different sites, so that repetitiveness in this regard has a didactic purpose. In addition, for the busy practitioner, it is possible to use this text as a single resource with respect to infection in an organ system of specific interest, in a case-dependent fashion, without having to consult a series of subspecialty texts.

As conceived, the present text is still designed to be functionally complete but not encyclopedic, and there is much information with respect to the clinical, epidemiologic, or mechanistic bases of infection that will not be found here. In addition, some exotic parasitic disorders have not been included. Other texts that include these data are available, and at times a pathologist may need to refer to them. However, for the most part, all that is required to diagnose the vast majority of infections can be found within the present revised text.

The diagnosis of infection, comparable to that of neoplasia, requires experience. The morphologic appearances of infection are

as diverse as those of malignancy. Because the variations encountered are virtually inexhaustible, no textbook can expect to illustrate all of what is encountered in practice. Indeed, at times textbooks tend to focus on one aspect of an infection and the inexperienced pathologist in this area may be misled in expecting to encounter examples that are comparable to those within selected illustrations. This text cannot replace experience. However, after the diverse appearances of infection are appreciated and accepted, the surgical pathologist may derive substantial pleasure from pondering their fine distinctions and take pride in the growing sense of competence that develops from his or her experience in this area.

2

General Principles in the Diagnosis of Infection

RICHARD L. KRADIN, VIKRAM DESHPANDE, A. JOHN IAFRATE

Sampling

Diagnosing Infection In Situ

Potential Limits of Biopsy Interpretation

Classification of Patterns of Infection

Histochemical Stains
- Hematoxylin and Eosin
- Gram Stain
- Silver Impregnation
- Fungal Stains
- Acid-Fast Bacteria Stains
- Connective Tissue Stains
- Giemsa Stains
- Mucicarmine
- Melanin Stains
- Viral Inclusion Body Stains

Immunohistochemical Methods

Molecular Diagnostics

In Situ Hybridization
- Polymerase Chain Reaction
- Branch Chain In Situ Hybridization

The identification of infection in biopsied tissues is the primary responsibility of the surgical pathologist. In an age when both noninvasive and minimally invasive approaches and techniques have increased, it is important to revisit the role of the biopsy in the diagnosis of infection (Box 2.1). Isolating microorganisms in the microbiology laboratory is a sensitive and accurate approach to their identification, but it has several important limitations. First, it cannot distinguish infection from colonization, nor can it ascertain the significance of the isolated organism. Only the presence of an organism in situ, together with an expected inflammatory response by the host, constitutes acceptable evidence of its role in infection.

For example, consider how to interpret the clinical significance of a fungus isolated from the airways of a patient with bronchiectasis who also has a new pulmonary infiltrate in the setting of immunosuppression. Is the fungal isolate the likely cause of the opportunistic infection, or might it be a benign commensal? Studies have attempted to address this question[1] with guidelines formulated for practice, but these are indeed merely "guidelines" because only identification of a potential pathogen within a site of infection can provide substantive evidence that the fungus is an invasive pathogen. For this and other reasons to be addressed in this text, the pathologic diagnosis of infection is a critical element in formulating optimal therapy.

Sampling

Tissue sampling is fundamentally important in the diagnosis of infection. All excised tissues should be considered as potentially infective. This approach fosters due diligence with respect to the possibility of contagion, as well as thoughtful concern as to how the tissues will be handled to optimize the chances of establishing an accurate diagnosis (Box 2.2). Samples of excised tissues should be harvested by sterile technique and sent to the microbiology laboratory with information concerning the types of organism that are being considered diagnostically. Directions to consider anaerobic and fastidious species should be clearly stated.

The surgical pathologist must ascertain that all diagnostic possibilities have been considered. Consultation with an infectious disease specialist can be invaluable in ensuring that specimens are properly handled ab initio. What must be avoided is thoughtlessly placing a biopsy specimen directly into formalin fixative without first considering a diagnosis of infection.

Touch imprints should be routinely prepared and can be stained in the frozen-section suite or in the microbiology laboratory. In general, 5 to 10 touch imprints will suffice, with sampling from the most suspicious portions of the biopsy specimen (e.g., areas of necrosis or suppuration).

Harvesting a portion of the biopsy specimen for ultrastructural analysis can foster the accurate diagnosis of many organisms (e.g., viruses, *Tropheryma whippeli,* microsporidia).[2] Specimens may be harvested for polymerase chain reaction (PCR) testing to establish the diagnosis of others (e.g., *Coxiella*, *Mycobacterium*, *Rickettsia*).[3]

The rapid diagnosis of a frozen section can help to focus on the diagnostic workup. All of the pertinent histochemical and ancillary studies can ideally be ordered before the permanent sections are processed, to avoid undue delay in diagnosis.

• BOX 2.1 Role of the Surgical Pathologist in the Diagnosis of Infection

Establish morphologic diagnosis of infection
Assess immunocompetence of the host
Narrow the differential diagnosis of possible pathogens
Confirm results of microbiologic cultures
Refute the relevance of microbiologic cultures
Establish diagnosis unrelated to infection
Identify concomitant infection in a primary inflammatory or neoplastic disorder
Identify new pathogens

• BOX 2.2 Optimal Handling of Tissue Biopsies: Always Consider Infection!

Make touch imprints for histochemical staining
Handle samples for microbiologic culture with sterile technique
Harvest samples for ultrastructural examination in glutaraldehyde fixative
Harvest fresh samples for appropriate polymerase chain reaction assays
Freeze portion of biopsy specimen for research
After all of this is done, place biopsy specimen in formalin

Diagnosing Infection In Situ

Because host immune mechanisms can greatly amplify the host response, the actual number of pathogens present in tissues is frequently surprisingly small. This means that many sections may need to be examined before a pathogen is identified. Although few surgical pathologists would balk at the idea of ordering additional sections to exclude malignancy in a biopsy they deemed suspicious, it is not uncommon for a pathologist to examine only a single histochemically stained tissue section in the diagnostic process of infection.[4] More egregious is the fantasy that the causative infectious agent will eventually be diagnosed by the microbiology laboratory, so there is no need for the surgical pathologist to belabor the process.

This approach is wrong minded for several reasons. First, the microbiology laboratory may fail to identify a causative organism.[5] Second, the organism isolated by the laboratory may not represent the actual infective agent in vivo. The analogy is the role for Gram staining of secretions in chronically intubated patients to determine whether there is a neutrophilic exudate consistent with infection and whether there is a predominating organism—steps that can promote the choice of appropriate antibiotic therapy.[6] In this setting, undue emphasis on culture results can lead to a seemingly endless process of adding or eliminating antibiotics in patients who are merely colonized by bacteria and not actually infected. Treatment decisions that do not take into account the host response and dominating organisms will tend to favor the production of increasingly antibiotic-resistant isolates and may potentially compromise public health. This is only one of several compelling reasons to consider diagnostic biopsies in patients with infections in situations that do not readily yield to noninvasive approaches.

Potential Limits of Biopsy Interpretation

Despite the merits of examining biopsy specimens in the diagnosis of infection, one must be aware of those situations in which the sensitivity and specificity of histochemically stained sections are limited. An example is tuberculosis, in which biopsies can fail to demonstrate mycobacteria in almost half of cases.[7] However, even in this setting, the appearance of the inflammatory response in situ should foster a working diagnosis that is often sufficiently reliable to institute empirical treatment.

TABLE 2.1 Tissue Responses to Infection

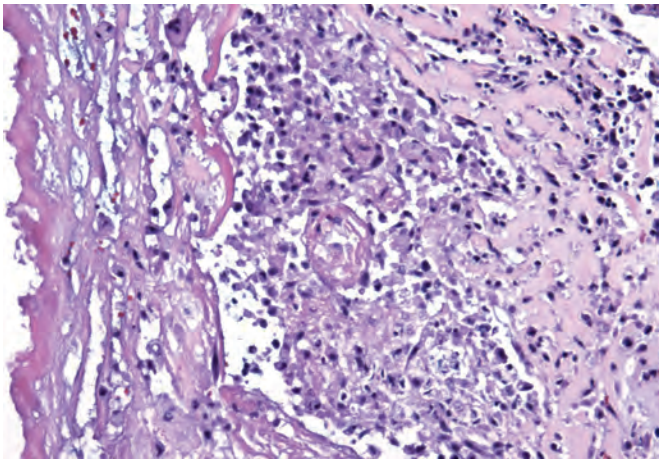
Type of Inflammation	Example
Exudative inflammation	Pyogenic bacteria
Necrotizing inflammation	Gram-negative bacteria, amebiasis
Granulomatous inflammation	Mycobacteria, fungi
Histiocytic inflammation	*Rhodococcus, Legionella,* Whipple disease
Eosinophilic inflammation	Fungi, parasites
Cytopathic changes	Viruses
No response	Host energy

Classification of Patterns of Infection

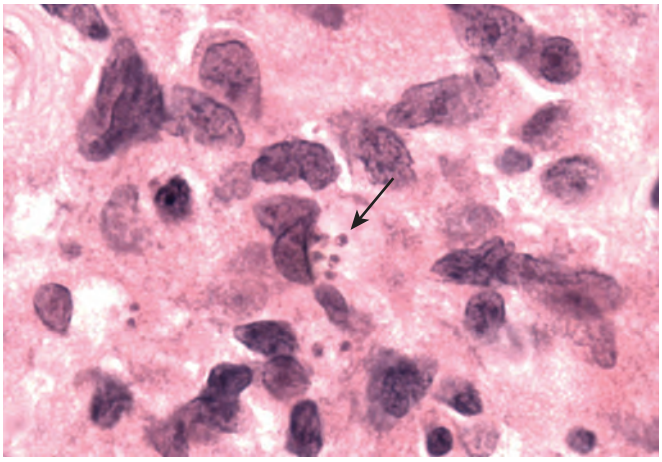
There is currently no uniformly accepted classification schema for the histologic patterns of response yielded by microorganisms. The inflammatory response in infection is a function of the host response, which is in turn a function of (1) the anatomy of the affected organ, (2) the virulence factors produced by the infective agent, and (3) host immunocompetence. The surgical pathologist must be aware that a single species of microorganism may be capable of evoking a variety of different patterns of inflammation. An example is the broad spectrum of disorders produced in response to infection with *Aspergillus* spp., which ranges from benign colonization, to hypersensitivity responses, to malignant angioinvasive infection.[8]

The characteristic types of inflammation elicited by infection (Table 2.1) can be broadly categorized as follows.

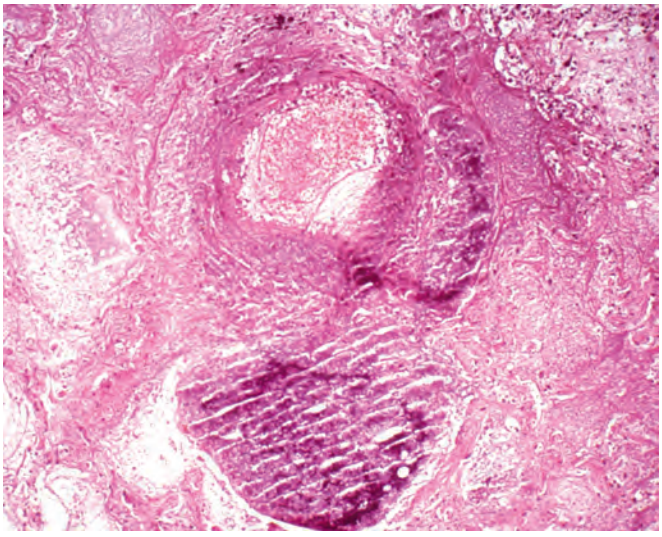
1. **Pyogenic responses.** In these responses, neutrophils predominate, leading to pus formation. They are evoked primarily by bacteria, although viruses and fungi can also elicit them (Fig. 2.1).
2. **Necrotizing inflammation.** Tissue necrosis can occur in several forms. In certain infections, such as those caused by amoebas or gram-negative bacteria, liquefactive necrosis is frequently seen (Fig. 2.2). Other forms, such as ischemic, mummefactive, and caseous necrosis, are often seen in mycobacterial and fungal infections.
3. **Granulomatous inflammation.** This response is characterized by the presence of epithelioid macrophages with multikaryon (giant cell) formation. It appears to reflect cell-mediated immunity to poorly catabolized antigens and is evoked by mycobacteria, fungi, and parasites (Fig. 2.3).
4. **Histiocytic inflammation.** These responses are characterized primarily by the presence of foamy macrophages and are a prominent component of infections caused by *Legionella, Rhodococcus, Calymmatobacterium, Leishmania,* and *T. whippeli* (Fig. 2.4). In patients who are severely immunocompromised,



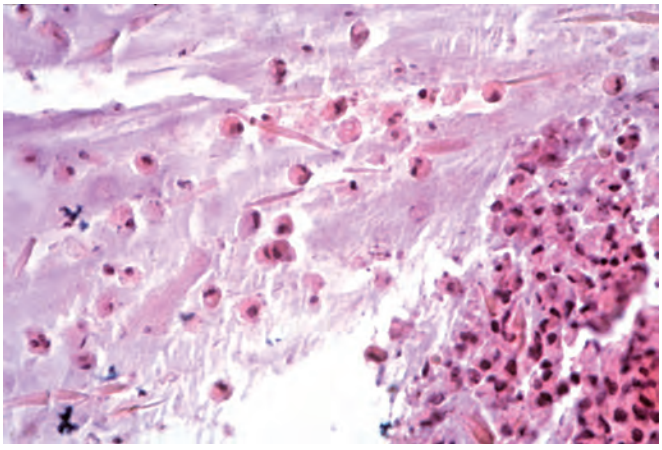
• **Figure 2.1** Pyogenic response in acute infective endocarditis due to *Streptococcus* spp. with neutrophilic exudate (×400).



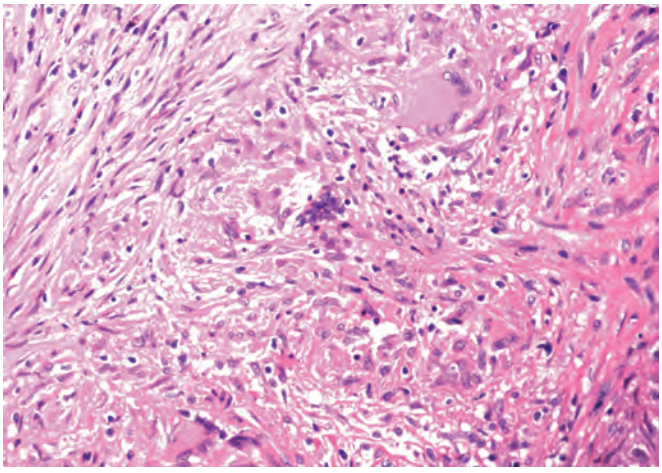
• **Figure 2.4** Histiocytic response shows "foamy" macrophages containing *Leishmania donovani (arrow)* (×600).



• **Figure 2.2** Necrotizing response to *Pseudomonas aeruginosa,* showing liquefactive destruction of lung tissue (×250).



• **Figure 2.5** Eosinophilic response to *Aspergillus fumigatus* (×400).

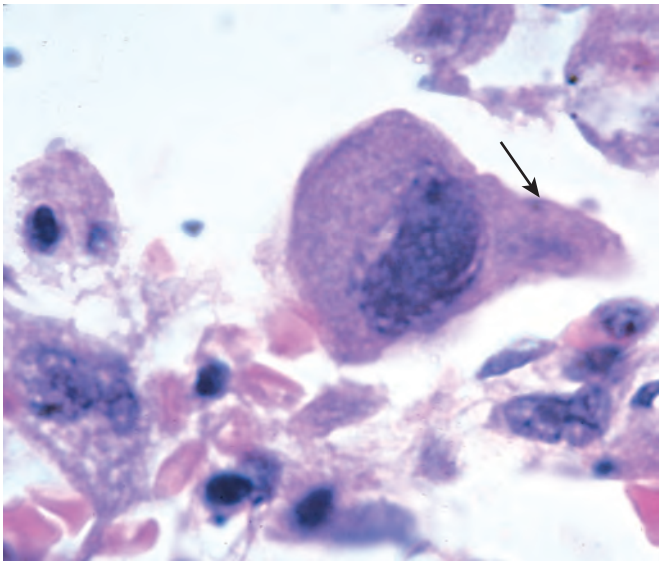


• **Figure 2.3** Granulomatous response to *Mycobacterium tuberculosis* (×25).

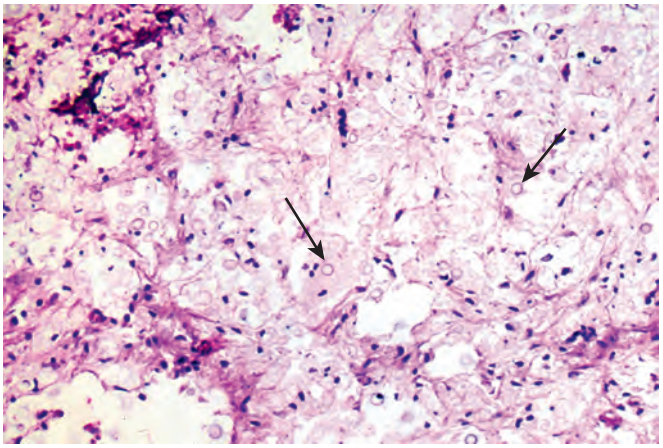
organisms that normally elicit granulomatous inflammation may instead evoke histiocytic infiltrates.

5. **Eosinophilic inflammation.** This is seen in response to multicellular parasites and certain fungi (Fig. 2.5).
6. **Cytopathic changes.** Although this is not properly a type of inflammation, cytopathic changes do reflect a response to viral infection. Nuclear inclusions are part of the response to DNA viruses, whereas cytologic inclusions are seen with some RNA and DNA viral infections, such as cytomegalovirus (Fig. 2.6).
7. **Null responses.** In the setting of profound immunosuppression, one may not see inflammation; only the uninhibited growth of microorganisms is apparent (Fig. 2.7).

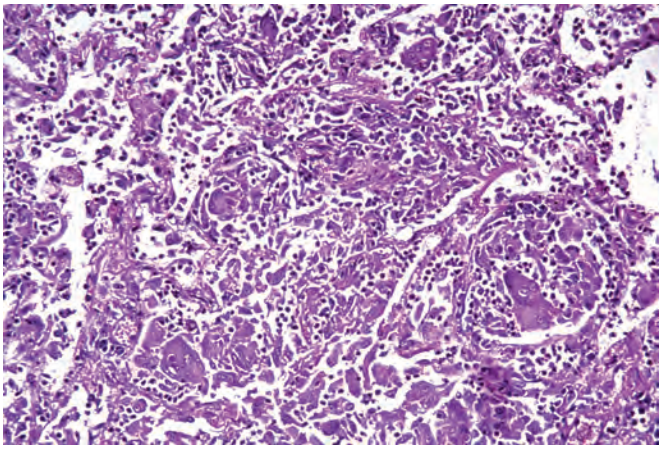
This classification schema is only a crude approximation because overlap patterns of inflammation are common, as with necrotizing granulomatous inflammation, granulohistiocytic inflammation (Fig. 2.8), and granulomatous inflammation with tissue eosinophilia (Fig. 2.9). The primary didactic element is that careful consideration of the histologic response in situ can help to narrow what would otherwise be a very broad differential diagnosis and can also provide invaluable information concerning host immunocompetence. For this reason, surgical pathologists must develop expertise concerning the inflammatory patterns that can accompany reduced immunocompetence resulting from genetic



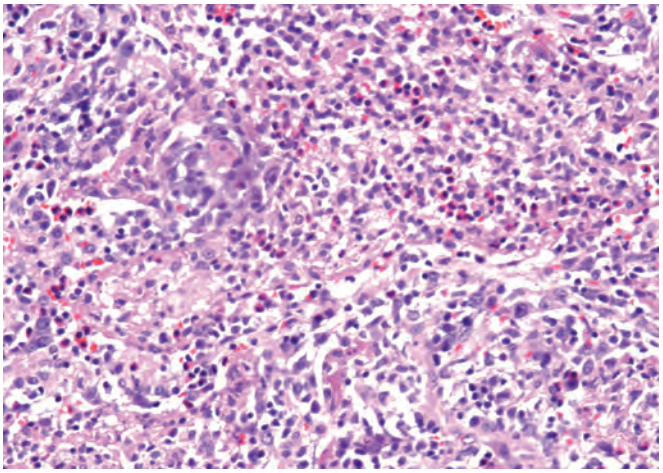
• **Figure 2.6** Cytopathic response to *Cytomegalovirus* with both nuclear and cytoplasmic *(arrow)* inclusions (×600).



• **Figure 2.7** Null response to *Cryptococcus neoformans (arrows)* (×400).



• **Figure 2.8** Granulohistiocytic response to *Blastomyces dermatitidis* (×250).



• **Figure 2.9** Granulomatous response with tissue eosinophilia due to *Coccidioides immitis* (×250).

factors, age, toxins, and drugs, because they can skew the expected pattern of inflammation and at times confound the diagnosis.

Histochemical Stains

The identification of microorganisms in biopsy samples is enhanced by the selective application of widely available histochemical stains (Table 2.2). Pathologists should be aware of the spectrum of histochemical staining by microorganisms and knowledgeable with respect to how to formulate combinations of stains to enhance diagnostic specificity.[9]

Hematoxylin and Eosin

The majority of pathogens can be identified with the standard hematoxylin and eosin (H&E) stain. These include cytopathic viruses, some bacteria, most fungi, and virtually all parasites (Box 2.3).

Gram Stain

The tissue Gram stain is a congener of the Gram stain used routinely to identify organisms in body secretions and fluids. The Brown-Hopps stain is currently the preparation of choice because it enhances gram-negative bacteria and rickettsia to a greater degree than the Brown-Brenn. In addition, the latter can be hazardous to technical personnel and has largely fallen into disfavor. The tissue Gram stain colors the cell walls of gram-positive bacteria a deep violaceous blue (Fig. 2.10A) and gram-negative bacteria a pale salmon pink (see Fig. 2.10B). Consequently, it is far easier to detect gram-positive species, and one must be careful not to overlook the presence of faintly stained gram-negative species. Gram variability is a potential pitfall in interpretation because it can raise the specter of polymicrobial infection. Attention to the uniform morphologic characteristics of stained organisms is the best way to avoid being misled by this phenomenon.

Nonbacterial pathogens can also be identified with the Gram stain. The blastoconidia (yeast) of *Candida* spp. (Fig. 2.11A) and the microconidia of *Aspergillus* spp. (see Fig. 2.11B) are gram positive, and this feature can help in distinguishing these species from other fungi. Microsporidia can be well demonstrated as gram-positive intracellular inclusions within cells (Fig. 2.12).

TABLE 2.2 Histochemical Staining Characteristics of Microbes

Organism	Staining Characteristics
Viruses	
Influenza	No cytopathic change
Coronavirus (SARS)	No cytopathic change
Adenovirus	H&E (smudge cells); IHC
Cytomegalovirus	H&E (intranuclear and cytoplasmic inclusions); IHC; PAS and GMS (intracytoplasmic inclusions)
Herpes virus	H&E (intranuclear inclusions); IHC
Measles	H&E (intranuclear inclusions, polykaryons)
Respiratory syncytial virus	H&E (polykaryons); IHC
Parainfluenza	H&E (intracytoplasmic inclusions)
Bacteria	
Gram positive	Tissue Gram, GMS (all)
Gram negative	Tissue Gram, GMS (some)
Legionella	Silver impregnation
Nocardia	Tissue Gram, GMS, modified ZN
Actinomyces	Tissue Gram, GMS
Mycobacteria tuberculosis	ZN and modified ZN; PCR
Atypical mycobacteria	Mo dified ZN, ± ZN, PCR
Fungi	
Histoplasma	GMS, PAS
Cryptococcus	H&E, GMS, PAS, mucicarmine; Fontana, IHC
Blastomyces	H&E, GMS, PAS, mucicarmine (weak)
Coccidiomyces	H&E, GMS, PAS
Candida	H&E, GMS, PAS, Gram stain; IHC
Aspergillus	H&E, GMS, PAS, IHC
Zygomyces	H&E, GMS, PAS
Pseudeallescheria	H&E, GMS, PAS
Alternaria and dematiaceous fungi	H&E, GMS, PAS, Fontana
Parasites	
Protozoa	H&E, PAS, Gram stain (microsporidia); IHC *(Toxoplasma)*
Metazoans	H&E, trichrome stain
Echinococcus	GMS in chitinous wall, modified ZN (hooklets)
Paragonimiasis	Ova birefringent
Schistosomiasis	Lateral and terminal spines stain with modified ZN

GMS, Gomori methenamine silver stain; *H&E*, hematoxylin and eosin stain; *IHC*, immunohistochemical methods; *PAS*, periodic acid–Schiff stain; *PCR*, polymerase chain reaction; *SARS*, severe acute respiratory syndrome; *ZN*, Ziehl-Neelsen stain.

• BOX 2.3 Microbes That Can Be Identified With Hematoxylin and Eosin Stain

Cytopathic viruses
Bacteria in colonies or in "granules"
Most fungi
Parasites

Silver Impregnation

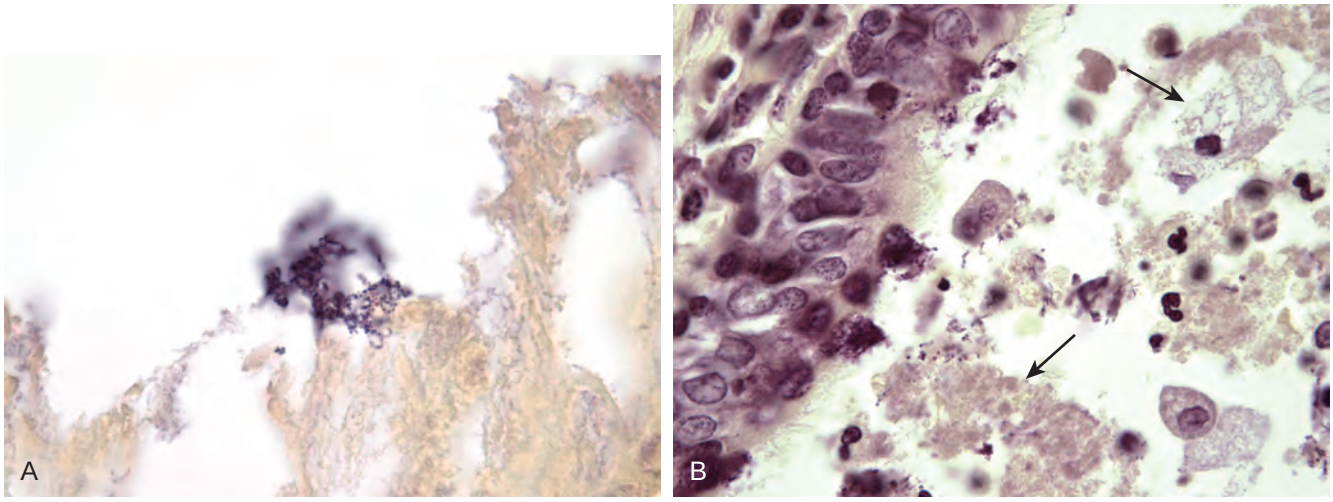
The impregnation of tissue sections with silver constitutes the basis of the Warthin-Starry, Dieterle, and Steiner stains. There is some controversy among experts as to whether these stains are equally efficacious in the identification of certain organisms, such as *Bartonella* spp., but they generally yield comparable results. In theory, all eubacteria, including mycobacteria, will stain positively with silver impregnation. However, in our experience, they do not do so reliably, and this approach cannot be recommended as a screening tool. In general, bacteria are enhanced both colorimetrically and in size by the deposition of silver salts on their cell walls, making them easier to identify but at times causing confusion in interpretation. Background staining presents a problem in interpretation, but the morphologic regularity of eubacteria usually allows for accurate identification after experience has been established with the technique.

Certain weakly gram-reactive or non–gram-reactive bacteria cannot be demonstrated reliably by any other histochemical method. These include *Treponema* spp. (Fig. 2.13), *Borrelia* spp., *Bartonella* spp., *Leptospira* spp., and *Calymmatobacterium*. Weakly staining gram-negative bacteria, including *Legionella* spp., *Burkholderia* spp., *Francisella* spp., and *Helicobacter*, are also best demonstrated by silver impregnation.

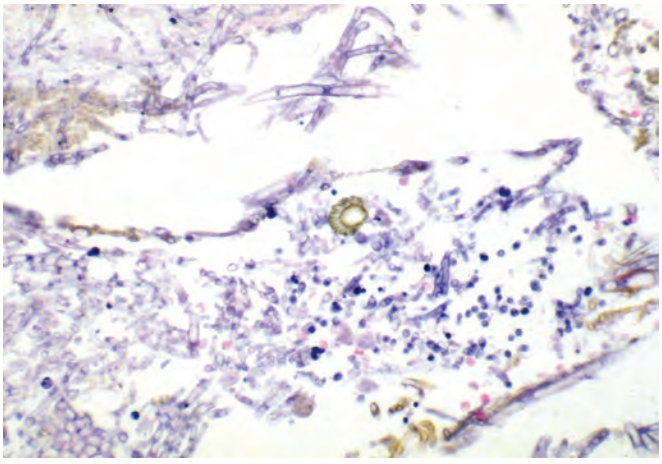
Fungal Stains

The Gomori methenamine silver (GMS) and Gridley stains are the preferred methods for demonstrating fungi (Table 2.3). Because certain fungi demonstrated by GMS do not consistently stain well with periodic acid–Schiff (PAS), the latter should be reserved as a secondary approach, but it can at times enhance morphologic detail. Although the GMS is often counterstained with methyl green for contrast, other counterstains can be applied. For example, it is possible to counterstain with H&E; this allows for a detailed assessment of the cellular immune response and promotes accurate identification of intravascular and perineural invasion by organisms.

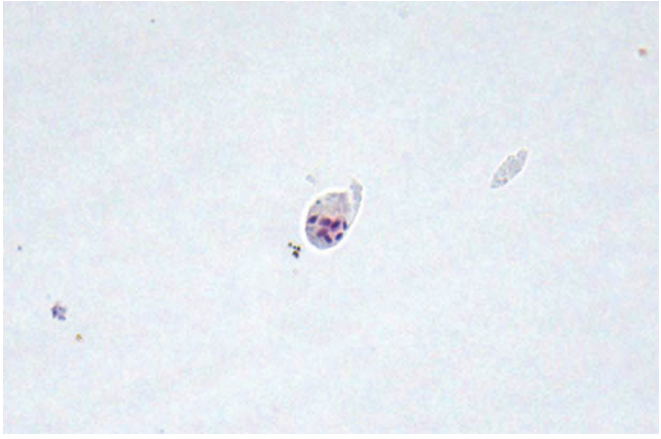
All gram-positive bacteria, including the actinomycetes, stain with GMS (Fig. 2.14), as do some encapsulated gram-negative bacteria, such as *Klebsiella* spp. Bacteria that have been treated before tissue sampling (e.g., infective endocarditis) may not be well decorated by the Gram stain, but they often retain their GMS positivity. For this reason, both stains should be examined before excluding a gram-positive bacterial infection. The actinomycetes, including mycobacteria, are gram-positive eubacteria and consequently also stain with GMS. The GMS is the stain of choice for demonstrating *Pneumocystis jiroveci* (Fig. 2.15), and it highlights the trophozoites of *Entamoeba histolytica*, encysted amoebas, the intracytoplasmic inclusions of cytomegalovirus-infected cells, the polar bodies of microsporidia, and the cyst wall of *Echinococcus* spp. (Fig. 2.16).



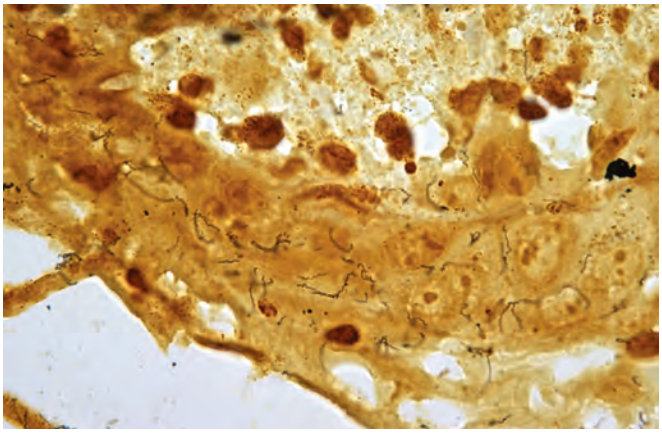
• **Figure 2.10** **A,** *Streptococcus* spp. stain deep blue-magenta (×600). **B,** Gram-negative bacteria are pale salmon-pink *(arrows)* (×600).



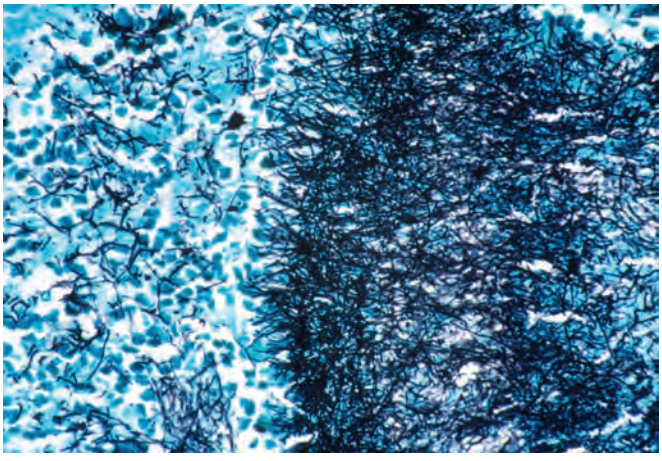
• **Figure 2.11** The microconidia of *Aspergillus fumigatus* stain intensely gram positive (×250).



• **Figure 2.12** Gram-positive intracytoplasmic microsporidia (×400).



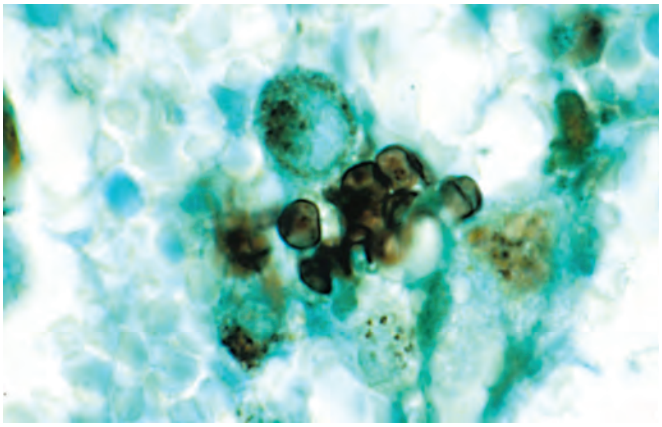
• **Figure 2.13** Spirochetes of *Treponema pallidum* stain with Warthin-Starry silver impregnation (×400).



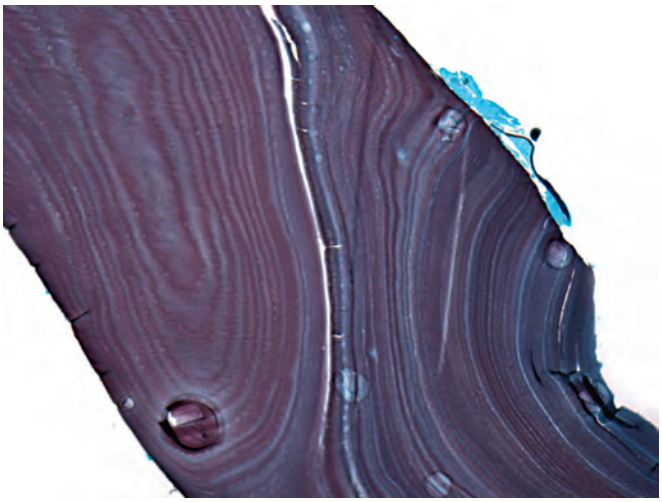
• **Figure 2.14** *Actinomyces israelii* stains with Gomori methenamine silver (×250).

TABLE 2.3 Fungal Identification in Tissue

Organism	Size (Width in µm)	Defining Morphology
Histoplasma capsulatum	2-5	Narrow-neck bud
Cryptococcus neoformans	5-20	Narrow-neck bud
Blastomyces dermatitidis	15-30	Broad-based bud
Candida glabrata	3-5	Budding, no pseudohyphae
Candida spp.	2-3	Yeast, pseudohyphae, hyphae
Aspergillus spp.	3-5	Acute-angle branching, septate, conidial head
Zygomyces spp.	5-8	Right-angle branching, ribbons, pauciseptate
Pseudallescheria spp.	3-4	Acute-angle branch, septate, terminal chlamydospore, pigmented conidia
Fusarium spp.	4-5	Acute and right-angle branch, septate, narrowed branch points
Coccidioides immitis	20-200	Endosporulation



• **Figure 2.15** Gomori methenamine silver-positive cysts of *Pneumocystis jirovecii* (×600).



• **Figure 2.16** Gomori methenamine silver-positive wall of cyst produced by *Echinococcus granulosus* (×200).

Acid-Fast Bacteria Stains

The Ziehl-Neelsen (ZN) stain and its modifications historically have been essential tools in the identification of mycobacteria.[3] *Mycobacterium* spp. (Fig. 2.17A) are also GMS positive (see Fig. 2.17B), and some atypical mycobacteria, such as *Mycobacterium avium-intracellulare* complex (MAC), also stain with PAS. The modified stain for acid-fast bacteria (Fite-Faraco or Putt) detects mycobacterial antigens that are sensitive to strong acid, a step in the decolorization of the ZN stain. For this reason, it can be used to screen for all mycobacteria and may be required to detect certain atypical mycobacteria, such as *Mycobacterium leprae*, as well as *Nocardia*, *Rhodococcus*, and *Legionella micdadei*. The cortical spines of *Schistosoma* spp. (Fig. 2.18), the hooklets of *Echinococcus*, and the spores of *Cryptosporidium* also stain well, but variably, with modified acid-fast bacillus stains.

Connective Tissue Stains

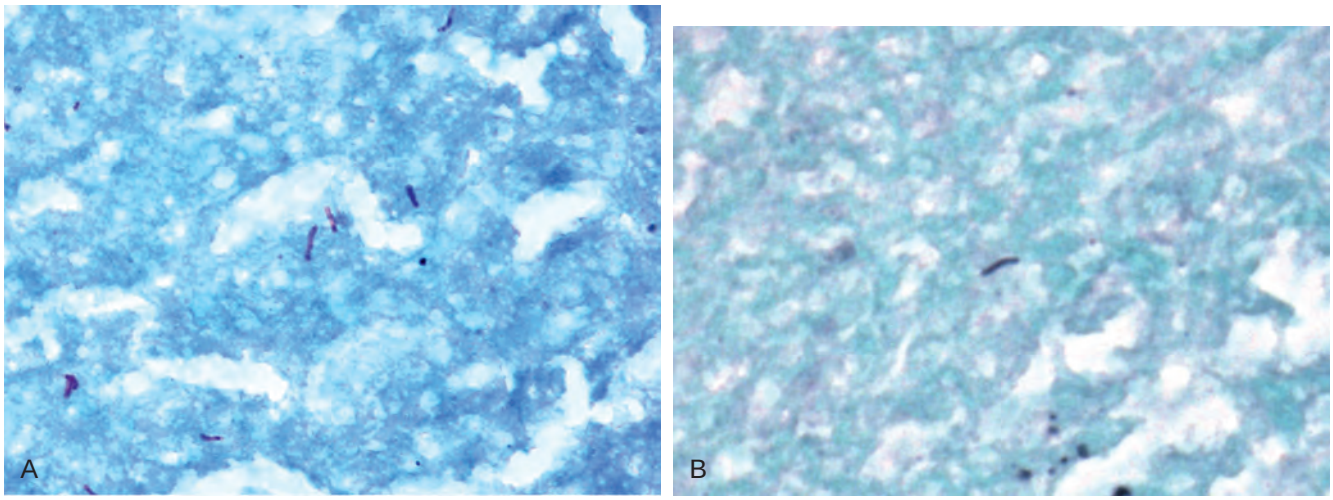
Masson trichrome, Movat pentachrome, and Wilder reticulin stains can be useful ancillary methods for classifying helminthic infections (Fig. 2.19). The inclusions of cytomegalovirus are demonstrated well by trichrome stains. The reticulin stain demonstrates the details of most helminths, the amastigotes of trypanosomes, and the rod-shaped kinetoplast of *Leishmania* spp.

Giemsa Stains

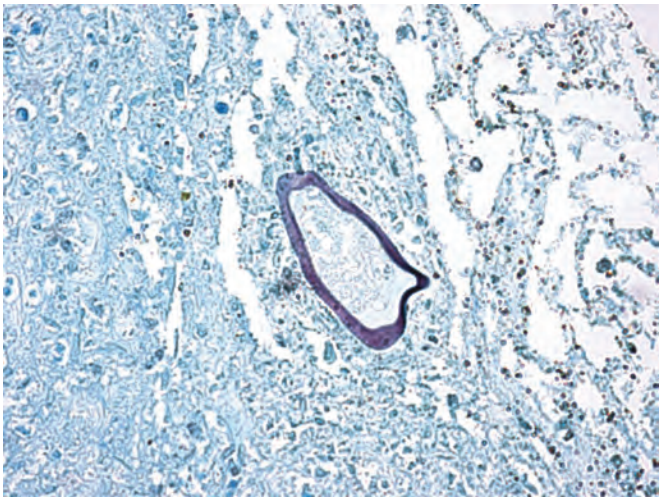
Giemsa stains and their variants can help in identifying a wide spectrum of pathogens, including protozoa, bacteria, chlamydia, and rickettsia. However, the small size of some of these organisms (e.g., rickettsiae) limits the degree of confidence in establishing an accurate diagnosis, and prior experience in diagnosing these infections is essential.[10]

Mucicarmine

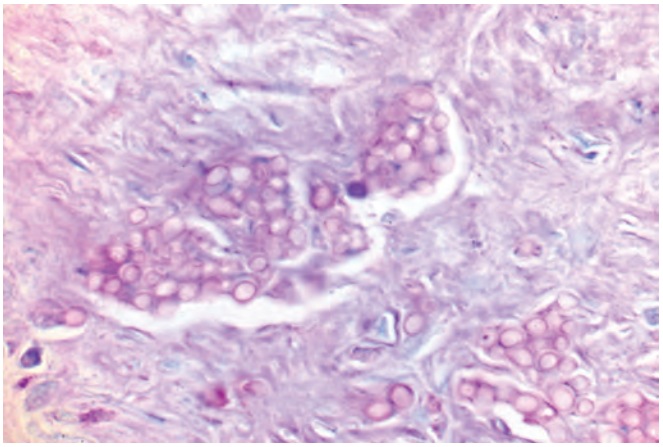
Several fungi, most notably *Cryptococcus*, *Blastomyces*, and *Rhinosporidium,* exhibit mucicarminophilia, either in their secreted capsules *(Cryptococcus)* or in their cell walls. Mucicarminophilia



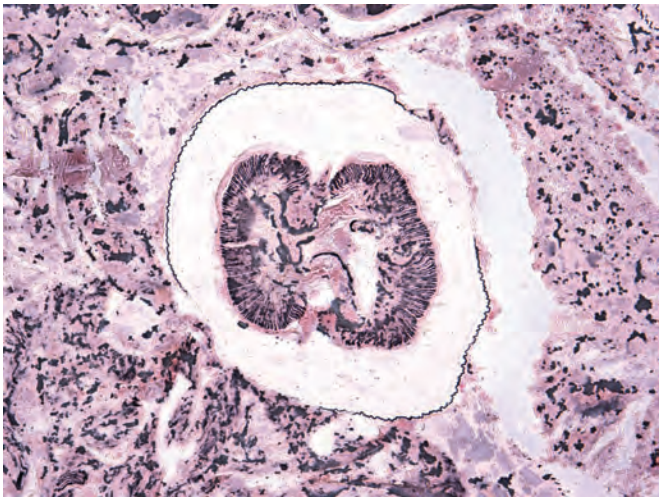
• **Figure 2.17** *Mycobacterium tuberculosis* stains with Ziehl-Neelsen stain (×600) **(A)** and Gomori methenamine silver stain (×600) **(B)**.



• **Figure 2.18** Cortical spine of ovum of *Schistosoma mansoni* stains positive with Fite-Faraco stain (×600).



• **Figure 2.20** Mucicarmine stain decorates the capsule of *Cryptococcus neoformans* (×400).



• **Figure 2.19** Wilder reticulin stain highlights *Dirofilaria immitis* (×150).

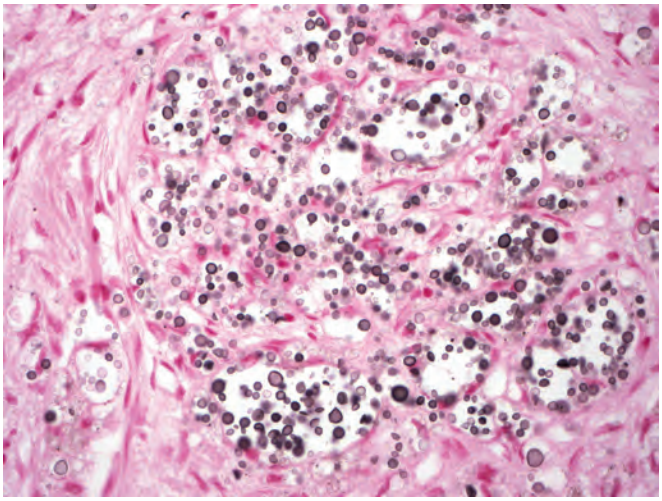
is an essential feature in the diagnosis of *Cryptococcus*; however, this staining, although invariably present, may be difficult to detect in capsule-deficient variants (Fig. 2.20).[11]

Melanin Stains

The Fontana-Masson stain demonstrates premelanin precursors within the cell wall of *Cryptococcus neoformans* and is an essential confirmatory approach to the in situ identification of capsular-deficient forms (Fig. 2.21).[12] All dematiaceous fungi are positive with Fontana-Masson, and this stain can help to confirm the impression of pigmentation seen in H&E sections.

Viral Inclusion Body Stains

A number of stains (e.g., Feulgen) can detect viral inclusions with cells. However, none add considerably to the H&E stain in this regard, and they are rarely adopted in practice, especially since confirmatory immunostains have become more widely available.



• **Figure 2.21** Fontana-Masson stain assists in the identification of an "acapsular" variant of *Cryptococcus neoformans* (×400).

TABLE 2.4 Immunohistochemical Stains Commercially Available for Microbe Identification in Paraffin-Embedded Tissues

Fungi	Viruses and Bacteria
Aspergillus (genus only)	Herpes virus 1 (cross-reacts herpes virus 2)
Cryptococcus	Varicella zoster
Histoplasma	Cytomegalovirus
Candida spp.	Respiratory syncytial virus
Coccidioides immitis	Adenovirus
Pneumocystis jiroveci	Epstein-Barr (Epstein-Barr-encoded RNA)
Pseudallescheria boydii	Actinomycetes
Zygomycoses (genus only)	*Actinomyces israelii*
Sporothrix schenckii	*Actinomyces naeslundii*
Trichosporon	*Arachnia propionica*

Immunohistochemical Methods

A large number of immunostains are available that can be helpful in the identification of microorganisms (Table 2.4).[13] Many of these are commercially available and currently enjoy wide popularity in diagnostic pathology laboratories. Others are available reliably only at highly specialized centers, such as the Centers for Disease Control and Prevention. Development of new immunohistochemical techniques can be a worthwhile but labor-intensive task. Because there is wide cross-reactivity among fungal species and among bacteria, it is critical to establish the potential cross-reactivity of any new antibody and its relative specificity.[14] Nevertheless, it may at times be possible to limit the use of a reagent to a narrow range of differential diagnostic possibilities (e.g., to distinguish *Aspergillus* spp. from *Pseudallescheria boydii* infection).

Molecular Diagnostics

Molecular techniques continue to dramatically reshape clinical microbiology practice. Currently, molecular techniques involving the identification of microbial nucleic acids are critical to the management of a growing number of infectious agents, most importantly the chronic viral infections, including human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C virus. HIV management is a prototype for the implementation of molecular medicine because the diagnosis can be made using reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of viral RNA, antiviral therapy regimens can be adjusted based on serial RT-PCR viral load measurements, and resistance mutations can be detected by sequencing of the viral genes targeted by current drugs (protease and reverse transcriptase inhibitors).

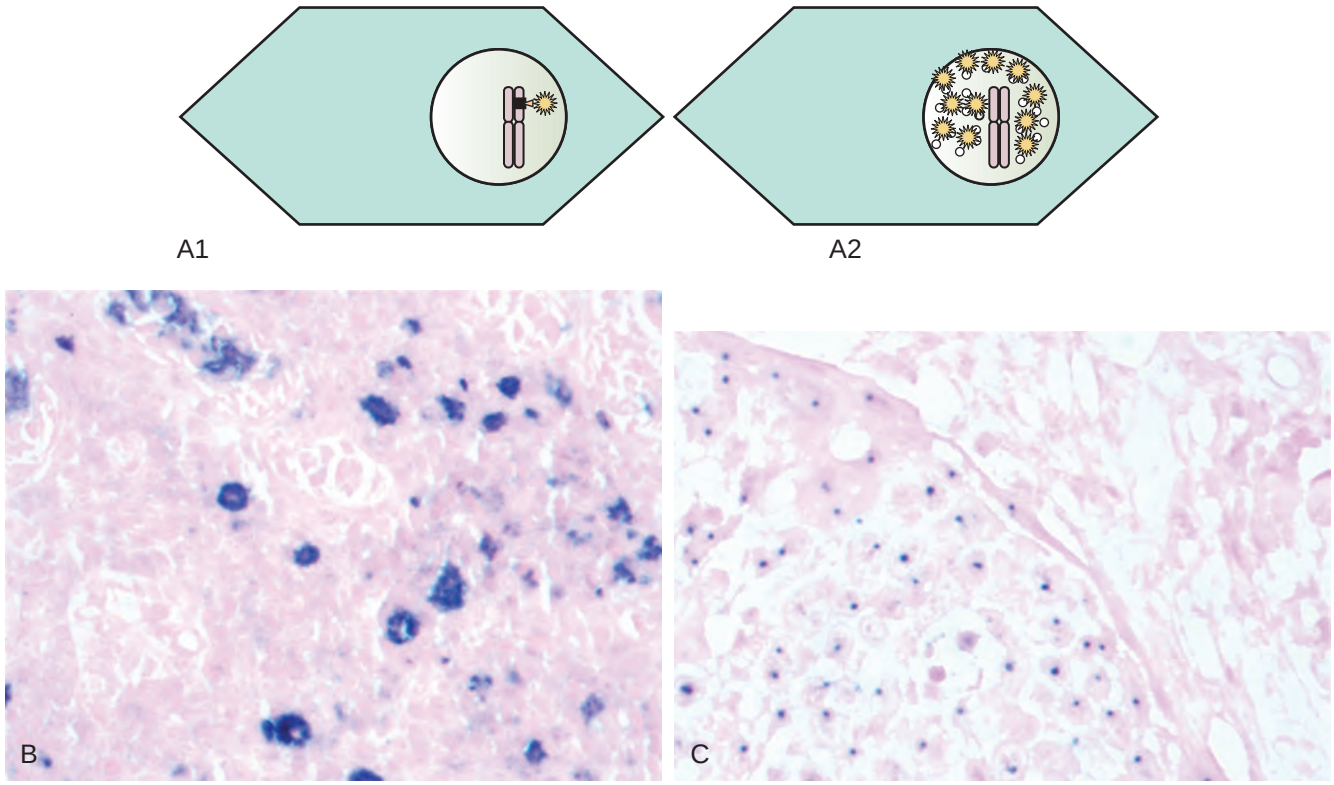
In the setting of surgical pathology a role is beginning to be developed for molecular techniques in the pathologic assessment of infection. The most relevant techniques in the setting of tissue diagnosis are in situ microbial detection using nucleic acid probes (in situ hybridization [ISH]) and PCR using nucleic acids purified from tissue sections. These two techniques can allow for diagnosis when special stains and immunohistochemical stains are insensitive (e.g., low antigen expression), and in some instances they can allow for speciation when microbes are identified with those techniques. In addition, molecular identification can accelerate definitive diagnosis with organisms that grow slowly or not at all in culture (e.g., fastidious organisms such as mycobacteria).

In Situ Hybridization

ISH is a technique that uses fluorescent or radiolabeled nucleic acid probes to recognize specific microbial sequences in tissue sections. The probes contain RNA or DNA sequences complementary to the target genetic elements and allow for specific localization of microbes in tissue or within cells. Depending on the sequence, some ISH probes can also bind specifically to nucleic acids from individual species, allowing for differentiation of organisms with variable virulence. Although there is great specificity with many ISH probes, immunohistochemistry is preferred, if possible, because of ease of incorporation into the modern automated pathology laboratory.

Most critical to the surgical pathologist is the identification of human papillomavirus (HPV) infection in cervical cancer screening, and this is most often accomplished with the use of PCR or similar techniques from liquid Pap smear specimens. ISH can also be very effective for definitive detection of high-risk HPV subtypes in cervical biopsy analysis for dysplasia and in defining HPV status of oropharyngeal squamous cell carcinomas.[15] Immunohistochemistry for HPV antigens has not proved sensitive enough (although p16 positivity is a reasonable surrogate marker of HPV infection). The HPV genome is present as episomes in low-grade lesions, so ISH reveals diffuse and intense staining (Fig. 2.22). In high-grade lesions and invasive carcinomas, the HPV genome integrates into the host genome, and ISH reveals a punctate nuclear signal.

ISH is also useful in the detection of Epstein-Barr virus (EBV) in lymphocytes, including in lymphoproliferative disorders, because the EBV-encoded RNAs (EBER1 and EBER2) are expressed at very high levels. Commercial EBER ISH assays have been developed and are automatable.



• **Figure 2.22** **A,** Human papillomavirus (HPV) infection is manifested by viral genomes present as A1 episomes or as A2 integrated DNA. Therefore in situ hybridization (ISH) probes give diffuse *(lower right)* or discrete *(lower left)* staining patterns in HPV-positive tissue. Actual ISH is shown in two oropharyngeal squamous cell carcinomas, with episomal signals **(B)** and integrated virus **(C)**. (Photographs courtesy of Dr. Jennifer Hunt, Cleveland Clinic Department of Pathology.) (×250).

In 2016, a novel chemical variant of DNA called peptide nucleic acids (PNAs), consisting of nucleoside bases joined by a peptide backbone rather than a sugar backbone, has been used to detect microbial genetic material. PNA probes offer the advantage of chemical stability and higher sensitivity and specificity. These properties offer the opportunity of developing probes that can differentiate species in situ. Such probes have proved successful in differentiating tuberculous from nontuberculous mycobacterial infections by targeting the 16S ribosomal RNA; others have detected *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida* species.[16-19]

Polymerase Chain Reaction

PCR amplification to detect infectious agents in surgical pathology specimens is now so common that a basic description of the technique is unnecessary.[20,21] PCR is without a doubt the most sensitive detection method available, and because it can be performed on archived formalin-fixed, paraffin-embedded samples, important diagnoses can be rendered even if cultures were not obtained from tissue biopsies at the time of processing (e.g., lung wedge resections for tumor that later reveal necrotizing granulomas). Common applications of PCR to surgical samples are listed in Table 2.5.

However, central to PCR is the requirement that the exact RNA or DNA sequences to be amplified must be known. Abundant microbial sequence data are publicly available, so that any equipped laboratory can analyze the most known pathogens.

TABLE 2.5 Applications of the Polymerase Chain Reaction to Microbial Detection in Tissues

Organism	Pathologic Process
Aspergillus	Invasive aspergillosis[21]
Human papillomavirus	Cervical HSIL and LSIL, HPV-positive squamous cell carcinoma of the oropharynx
Herpes virus	Herpes encephalitis (CSF or brain biopsy)
Mycobacteria	Necrotizing granulomatous inflammation
Bartonella	Cat-scratch disease or bacillary angiomatosis
Enterovirus, adenovirus, influenza A virus	Viral myocarditis[20]

CSF, Cerebrospinal fluid; *HPV*, human papillomavirus; *HSIL*, high-grade squamous intraepithelial lesion; *LSIL*, low-grade squamous intraepithelial lesion.

There are a number of well-conserved genes in microorganisms, such as the ribosomal 16S gene and the heat shock protein/chaperonin HSP60/65 (or GroEL), that are excellent targets for PCR. Analysis of the16S ribosomal RNA gene in bacteria by PCR and subsequent sequencing is especially informative because there are well-conserved sequences that can be used as binding sites for

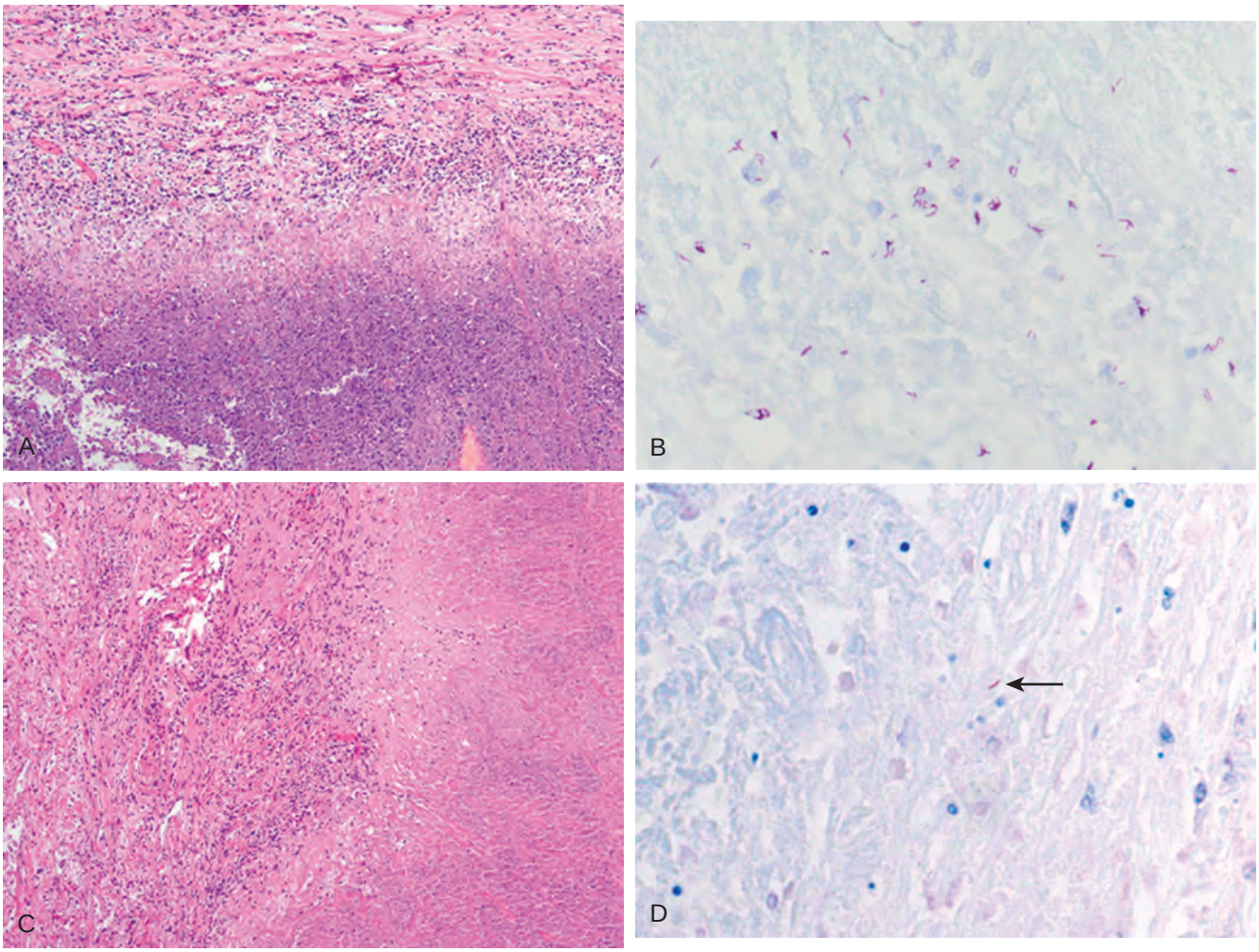
universal PCR primers adjacent to variable sequences and then analyzed and compared with databases of known sequences (Fig. 2.23).

This sensitivity of PCR is not without its problems. Many of the most important infectious agents seen in general practice are found in the environment and can contaminate surgical specimens. For example, *Aspergillus* and mycobacterial species are normal denizens of the clinical laboratory; if samples are not kept sterile, they may come in contact with these species during tissue processing. Even more vexing, such species also can cause opportunistic infections, and so their identification cannot always be dismissed as clinically irrelevant.

Branch Chain In Situ Hybridization

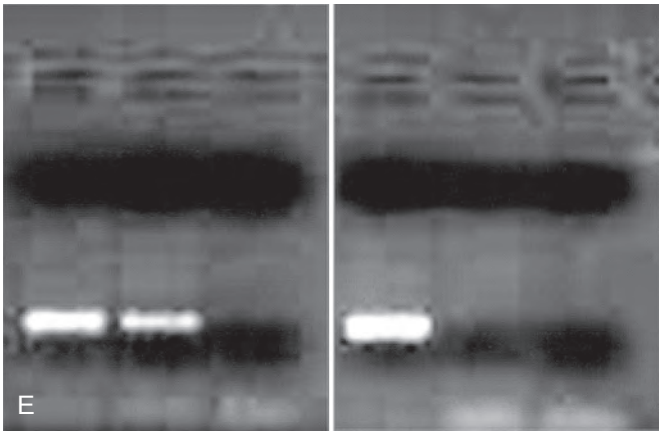
A branch chain in situ hybridization (bISH) assay is yet another platform for detecting infectious organisms in formalin-fixed, paraffin-embedded tissue (Fig. 2.24).[22] This technology uses a branched DNA structure for signal amplification, but, unlike PCR, the target is not amplified. The signal amplification greatly increases the sensitivity of the assay, although it is significantly lower than PCR-based techniques. Multiple tiling probes for a gene are hybridized to the target RNA, and these are typically distributed throughout the genome of interest, greatly increasing the sensitivity of the assay. A signal amplification structure is created by a series of hybridization steps. The final steps involve hybridization of multiple alkaline phosphatase–conjugated label probes, and the eventual branched structure has approximately 400 binding sites for the alkaline phosphatase–labeled probe, which can be visualized as a red chromogen with a bright field microscope. The signal is typically seen as dots, although with highly expressed transcripts individual dots are no longer visualized. These assays are commercially available and can be performed on clinical grade automated immunohistochemistry instruments.

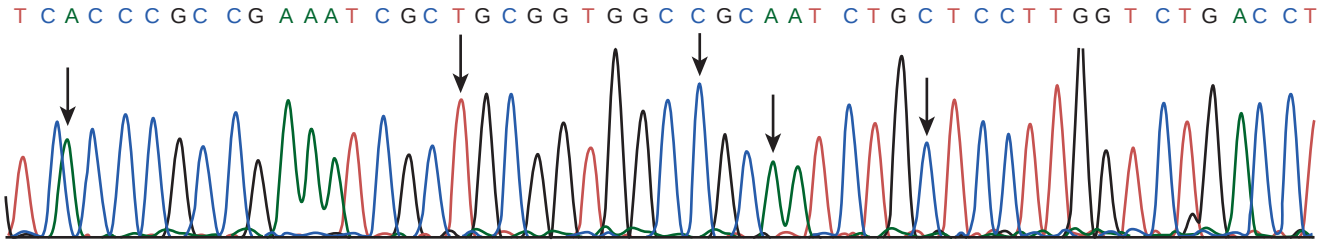
Although it is theoretically possible to develop probes against virtually any organisms, the bISH assay is generally successful in cases in which the target is highly expressed, such as EBV, cytomegalovirus (CMV), and high-risk (HR) HPV infections. The bISH assay detects E6 and E7, transcripts that are highly expressed



• **Figure 2.23** Two cases of mycobacterial infection. **A,** *Mycobacterium avium-intracellulare* complex (MAC) infection in a lung specimen with **(B)** numerous acid-fast bacteria (AFB). **C,** Lung specimen with *Mycobacterium tuberculosis* complex (MTb) infection with rare AFB (*arrow* in **D**) (×200).

Continued





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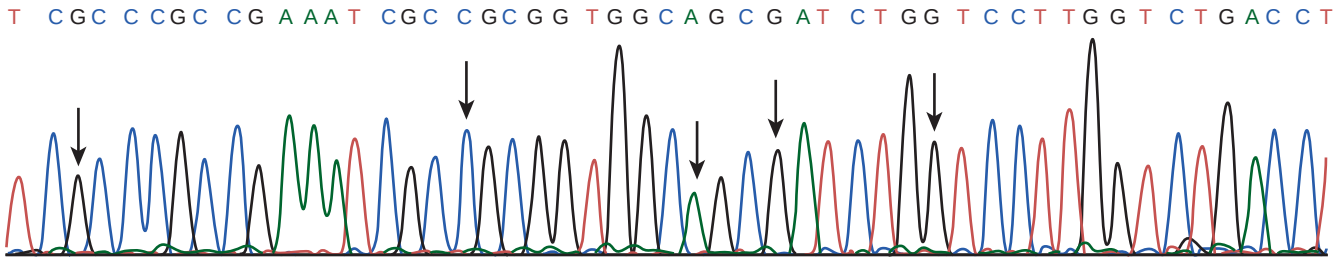
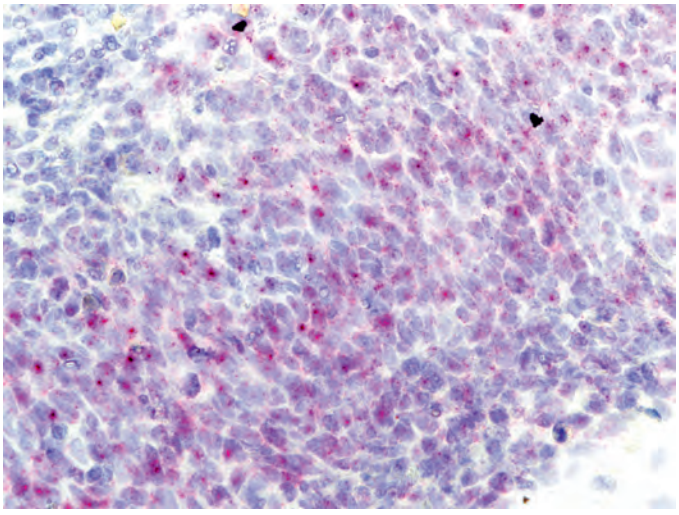


G

• **Figure 2.23, cont'd** DNA was isolated from formalin-fixed, paraffin-embedded tissue and amplified with primers to the *HSP65* gene, showing positive bands for the MTb sample (**E,** *lane 1*) and the MAC sample (**E,** *lane 2*). An MTb-specific gene polymerase chain reaction assay (*IS6110* gene) reveals a band with only MTb (**E,** *lane 4*). Water-only control lanes show no band in either reaction (**E,** *lanes 3 and 6*). The *HSP65* gene products were subject to DNA sequencing with the bacterial sequence from the MTb sample in **F** and from the MAC in **G**. Alignment of the two sequences reveals numerous sequence differences *(arrows)* in the region, which can be used to differentiate the two species.



• **Figure 2.24** This bISH RNA assay for high-risk HPV targets HPV-16 E6 and E7 transcripts. The red chromogen in this head and neck squamous cell carcinoma is noted within both the nucleus and the cytoplasm.

in HPV-related squamous cell carcinoma. The sensitivity of the RNA-based assay for HR HPV is higher than DNA-based assays.[23] Furthermore, the RNA assay is biologically more relevant because it detects transcripts key to oncogenic transformation.

The limitations of the bISH RNA assay need to be recognized. Nonspecific staining, although generally fewer than 1 dot per 10 cells, could be significantly higher, making it impossible to distinguish true from nonspecific signal. Poor RNA quality could undermine the assay, and rapid formalin fixation is often necessary for optimal preservation of RNA. Finally, although in theory the assay is capable of detecting a single transcript, most successful clinical-grade bISH assays express several hundred target RNA transcripts per cell.

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3

Biopsy in the Diagnosis of Infection: Clinical Approach

JAY A. FISHMAN

Overview: Biopsy
Assessing the Benefit of Invasive Testing
Contraindications to Biopsy
Approach to the Patient: General Concepts
Biopsy in the Immunocompromised Host
Timetable of Infection
Summary

Overview: Biopsy

The practice of clinical infectious disease management has undergone a revolution with the advent of newer technologies, such as organism-specific molecular or protein-based diagnostic tools and, more recently, high throughput sequencing technologies on microbial diagnosis and the management of infection.[1] Coupled with a variety of newer antimicrobial therapies, such as those for fungal and viral infections, significant shifts have occurred in clinical care. Despite these advances, a specific and timely diagnosis is often not achieved to guide therapy. In some conditions in which the disease process is self-limited or the therapies are broadly active or relatively nontoxic, a specific diagnosis may not be essential. Thus in community-acquired pneumonia a specific diagnosis is made in fewer than half of cases, but common therapies (e.g., with an antimicrobial macrolide or fluoroquinolone agent) have an antimicrobial spectrum broad enough to "cover" the common pathogens or the patient gets better on his or her own (i.e., viral syndrome). By contrast, in rapidly progressive infection in an immunocompromised host or infections of uncertain etiology, early and specific diagnosis allows targeted antimicrobial therapy with the best opportunity for success and with minimization of toxic side effects of such interventions. Specimen management and the selection and performance of appropriate assays are also critical to the success of interventional diagnosis. Cytologic or histopathologic diagnosis is a valuable adjunct to culture-based techniques because these may allow the interpretation of the significance of microbial detection (e.g., for herpesviruses in respiratory specimens).

Assessing the Benefit of Invasive Testing

Whether or not to obtain a biopsy depends on the condition of the patient and the urgency for diagnosis. Many factors will affect the decision process. Common considerations include whether the patient will tolerate the procedure. Complex patients often have multiple coexisting processes that increase clinical risk. What is the likelihood of success—can we reach the affected site and obtain an adequate sample?

Added considerations in biopsy-driven diagnostic procedures include the following:

- Urgent diagnosis before the patient becomes "too sick" for invasive procedures or in the face of rapidly progressive disease
- In patients in whom various therapies carry significant or unacceptable toxicities
- Distinguishing between infectious and noninfectious etiologies (e.g., inflammatory or cancer), notably in patients with atypical presentations of disease
- Sampling of a likely infection for microbiologic analysis (i.e., culture and susceptibility testing)
- Detection of organisms for which microbiologic culture techniques may be imprecise or unavailable (parasitic infections)
- Microbiologic and histopathologic evaluation in the patient with a lesion failing to respond to "appropriate therapy" (Fig. 3.1) or slow to respond
- To help to resolve infection (excisional biopsy)

In general, a biopsy is utilized in patients in whom a specific diagnosis is critical to optimal management. The timing of the biopsy procedure is important. Some patients will predictably become "sicker" or less able to tolerate complications of a biopsy (bleeding tendency, significant cardiac disease, pneumothorax, infection, bowel perforation) later in the course of their disease process. These include patients undergoing cancer chemotherapy, organ or stem cell transplantation, or planning travel to medically underserved regions. These conditions may mitigate towards early "definitive" diagnosis. Underlying clinical conditions will often define the biopsy approach used; transjugular liver biopsies are used in patients with bleeding tendencies (the patient may bleed but into the vasculature from which the biopsy was obtained). Transjugular biopsies tend to be adequate for diagnosis less often than percutaneous biopsies (87% vs 69%), but in a retrospective study the difference was not significant.[2,3] Transjugular biopsies require cardiac monitoring for arrhythmias induced by the catheterization procedure. The samples obtained are often fragmented

• **Figure 3.1** Forearm lesion in renal transplant recipient at site of prior intravenous catheter. Swab cultures grew methicillin-susceptible *Staphylococcus aureus*. The lesion failed to heal after multiple courses of antibacterial therapy. Biopsy revealed *Cryptococcus neoformans*. Healing occurred on antifungal therapy.

but generally adequate for histologic diagnosis. The yield of a biopsy often depends on sampling and the skill of the pathologist interpreting the specimen, notably in certain conditions such as in hepatitis C infection of liver allografts.[4,5] It is essential that an adequate tissue sample be obtained if a biopsy procedure is performed. This may be limited by the patient's clinical condition and ability to tolerate complications (low platelet count, mechanical ventilation, and encephalitis). However, an inadequate sample may necessitate repeat biopsies. Thus the specific biopsy procedure selected depends on the urgency for diagnosis, the tissue being sampled, and the likely pathogens in a given host. Notably, in the immunocompromised host with a wide array of potential pathogens, the handling of the specimen must be determined in advance so as to ensure the best chance of microbial diagnosis.[6]

An example of the decision-making process is in the evaluation of diffuse pulmonary infections. Such processes may reflect multiple lung injuries, including noninfectious (e.g., fibrosis, radiation injury, heart failure, hemorrhage) as well as infectious. In acquired immunodeficiency syndrome (AIDS) patients, bronchoalveolar lavage (BAL) often provides a diagnosis, whereas BAL with transbronchial biopsy may be preferred in the non-AIDS immunocompromised host. This reflects the higher organism burden and slower progression of common infectious processes in AIDS (pneumocystis pneumonia, mycobacterial infection) than in the immunosuppressed or neutropenic individual. Similarly, a small tissue sample from fine-needle aspiration (FNA) may be adequate for diagnosis of a focal process (abscess) or transjugular biopsy for a diffuse process or if molecular amplification techniques can be used (viral hepatitis). A larger sample (core biopsy) is needed for patchy processes (BK polyomavirus infection of the transplanted kidney). FNA and percutaneous biopsies may have the disadvantage of tracking of infection or cells along the biopsy path. Thus video-assisted thoracoscopic surgical (VATS) or open (thoracotomy) excision of lung lesions in immunocompromised hosts (e.g., for focal *Aspergillus*-species pneumonia) may be more likely to provide adequate tissue for microbial diagnosis and, with disease debulking, allow the initiation of otherwise potentially fatal chemotherapy. Advantages of VATS biopsy include larger sample size, better selection of the biopsy site, and direct visualization of the biopsy site, which allows better control of any bleeding encountered. Radiologic techniques are often used to guide the biopsy procedure directly (under ultrasound or computed tomography [CT] scan) or generally, as in patchy processes affecting the lungs.

Contraindications to Biopsy

Clinical conditions (bleeding diathesis, fragile condition), lack of radiologic or technical support at the center, and epidemiologic considerations may affect the choice to proceed with biopsy. Biopsies should generally not be obtained if an epidemiologic history suggests the possibility of echinococcal cystic disease, due to the risk of dissemination and anaphylaxis associated with cyst leakage, or for a perivascular process with a significant bleeding hazard. The clinical laboratory must be notified if fungal infections including the agents of mucormycosis are suspected—such specimens must be diced rather than ground for culture and staining. *Brucella* species, *Francisella tularensis*, *Bacillus anthracis*, Q fever *(Coxiella burnetii)*, and *Coccidioides immitis* are biohazards for technicians in the lab and require similar notification.

Approach to the Patient: General Concepts

The individual with infection will often present in an ambulatory setting. The evaluation of the patient depends on a series of questions that provides clues to management, including the need for hospitalization and selection of antimicrobial agents. Subsequently, clinical data provide a basis for adjusting antimicrobial therapy. These questions include the following:

1. **Is the process life-threatening?**
   - What is the time course of the process? Is it rapidly progressive or gradual?
   - Does the patient need to be admitted to the hospital?
   - **Is there time to delay therapy or diagnostic procedures?**
   - Will therapy alter the yield of diagnostic testing?
2. **Does the patient have immune deficits?** Could the process be underestimated based on the absence of normal inflammatory responses? Will the patient clear infection without specific therapy?
3. **History:** Are there clues to a specific etiology of infection and to the severity of the illness?
   - Underlying clinical conditions.
   - Epidemiologic history (i.e., travel, contacts, exposures, social history, vaccines, medications, prior infections or hospitalizations): Has the patient traveled or does the patient have any hobbies (gardening, hiking, and cooking) that might provide an epidemiologic clue?
   - Symptoms: rate of progression, other systemic signs.
4. **Physical examination:** Skin lesions (e.g., rash attributed to drug allergy vs disseminated fungal infection), lymph nodes (symmetrical or regional), retinal examination, perirectal abscess, chest wall or spinous tenderness, and neurologic disease (pulmonary–brain syndromes) are often ignored but may provide valuable clinical clues.
5. **Basic laboratory data:** Many systemic processes are reflected in abnormalities of blood counts, urinalysis, and routine blood chemistries.
6. **Radiology:** No radiographic findings are specific enough to define the microbial origin of a given process. One approach can be illustrated best in the evaluation of pulmonary processes using both the rate of symptomatic progression, as well as the radiologic appearance of the lesion (Table 3.1).

TABLE 3.1 Differential Diagnosis of Fever and Pulmonary Infiltrates in the Organ Transplant Recipient According to Roentgenographic Abnormality and the Rate of Progression of the Symptoms

	ETIOLOGY ACCORDING TO THE RATE OF PROGRESSION OF THE ILLNESS	
Chest Radiographic Abnormality	Acute*	Subacute-Chronic*
Consolidation	Bacterial (including legionnaires'), thromboembolic, hemorrhage (pulmonary edema)	Mold, *Nocardia*, Mycobacteria, viral (drug-induced, radiation, pneumocystis, tumor)
Peribronchovascular	Pulmonary edema (leukoagglutinin, reaction, bacterial)	Viral, pneumocystis (fungal, *Nocardia*, tumor, mycobacteria)
Nodular infiltrate	(Bacterial, pulmonary edema)	Mold, *Nocardia*, tuberculous *(Pneumocystis)*

*An acute illness develops and requires medical attention in a matter of relatively few hours. A subacute-chronic process develops over several days to weeks. (Note that unusual causes of a process are in parentheses.)

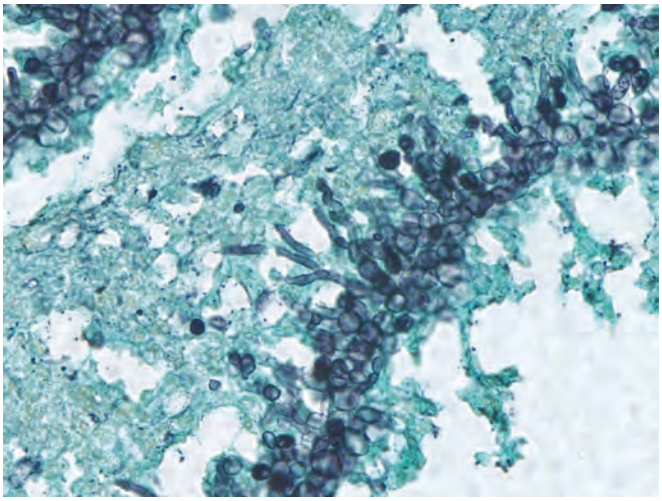
- Define the radiographic pattern as lobar or segmental consolidation, patchy bronchopneumonia, nodules (large, small, or miliary), or an interstitial process. Many large round pulmonary densities in a renal transplant recipient suggest *Nocardia* infection rather than pneumocystis pneumonia, whereas in a heroin addict with cough, fever, and pleuritic chest pain, such densities suggest acute right-sided endocarditis rather than pneumococcal pneumonia.
- Compare with prior radiographs: Is the process old or new? Are there multiple processes? Has the patient had surgery in the intervening period? Is the spleen enlarged or absent?
- Confounding variables: Is it too early in the process to detect radiologic changes (first 18 to 24 hours)? Is the patient neutropenic (early viral or fungal pneumonitis) or otherwise immunocompromised (pneumocystis pneumonia is often with minimal or no findings on plain chest radiographs)? Dehydration is commonly cited as a cause of false-negative radiographs—but, in general, this concept is probably overrated.
- CT scanning is sensitive to changes unrecognized in plain radiographs and is useful in guiding invasive procedures.

7. **Examination of clinical specimens** (appropriately stained smear of sputum or pleural fluid, blood buffy coat, skin lesions, throat swabs) often provides a provisional diagnosis. Examination of an appropriately stained smear of sputum can provide a shortcut to diagnosis if the findings are reasonably definitive. Special staining methods provide additional data, including Kinyoun and modified acid-fast stains for mycobacteria, *Actinomyces*, or *Nocardia* species; Wright-Giemsa or a variant or direct fluorescent antibody staining of induced sputum samples for *Pneumocystis carinii* or *Legionella pneumophila*.
   - Culture of sputum or blood or other bodily fluids may provide a specific etiologic diagnosis when evaluation of a sputum smear has not supplied a provisional diagnosis.
   - In some patients an etiologic diagnosis can sometimes be made by alternative means: for example, urinary antigen tests for *Legionella* or *Histoplasma* infections, antigenemia, molecular, or serologic assays for viral or other less common processes. This may avoid invasive procedures. Screening tests are highly useful for respiratory viruses (urinary antigen or nasal swab coupled with immunofluorescence). Induced sputum examinations have a high yield for *Pneumocystis* and mycobacteria.
8. **How can a diagnosis be achieved most expeditiously?** Which invasive procedures are performed well at my institution?
   - Invasive diagnostic procedures: In patients who are critically ill or unlikely to tolerate invasive infections (immunocompromised hosts, recent major surgery, heart failure, chronic obstructive pulmonary disease), it is reasonable to consider more invasive diagnostic procedures early in the clinical course. In such patients, only specific etiologic diagnoses can direct appropriate therapy and limit attendant toxicities. However, this observation illustrates the tension between empiric therapies and the risks inherent to invasive tests. Empiric antimicrobial therapies carry the risk of obscuring a specific microbiologic diagnosis, as well as drug-associated toxicities. Invasive diagnostic procedures are used to obtain uncontaminated lower respiratory tract secretions or pulmonary tissue for microbiologic and histologic analysis.
   - The selection of such procedures should be based on the nature of the illness and the likelihood of success for each procedure at the institution. Important considerations include the type and location of the lesion, the ability of the patient to cooperate with the required manipulations, the presence of coagulopathies, and experience at the particular hospital in performing each of the procedures.
   - Histologic and microbiologic data from biopsies should be reviewed by the medical teams caring for each patient to optimize the use of each specimen (microbiologic testing, histologic analysis).

Antimicrobial therapy: In practice, initial therapy is empiric and based primarily on available clinical clues. The selection of drug(s) for empiric therapy depends on the clinical setting and on the gravity of the pulmonary process.

Biopsy in the Immunocompromised Host

By the time a biopsy is considered for infectious lesions, unless time pressure for a diagnosis is critical, noninvasive diagnostic approaches should have been exhausted. The risks of the procedure are balanced against the toxicities, costs, and availability of appropriate empiric therapies. For example, in the case illustrated, a woman underwent lung transplantation after years of immunosuppression for dermatomyositis. Immunosuppression was intensified given poor initial lung function. An incidental nasal swab culture grew *Scedosporium prolificans*, which was treated



• **Figure 3.2** Eye enucleation specimen of patient with disseminated *Scedosporium prolificans*.

• BOX 3.1 Factors in the Net State of Immune Suppression

Immunosuppressive therapy: dose, duration, temporal sequence
Underlying immune deficiency: autoimmune disease, functional immune deficits
Mucocutaneous barrier integrity: catheters, epithelial surfaces, devitalized tissue, fluid collections
Neutropenia, lymphopenia
Metabolic conditions
- Uremia
- Malnutrition
- Diabetes
- Alcoholism with cirrhosis

Viral coinfection
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis B and C
- Human immunodeficiency virus

with prophylactic voriconazole and nebulized amphotericin. After extensive rehabilitation, she returned for evaluation of pain with decreased vision in the left eye. Vitreal tap was unrevealing. Retinal biopsy was performed based on the likelihood that this could be a disseminated mold infection of the eye and despite the potential risk of visual loss, given multiple areas of retinal detachment. The retinal biopsy revealed invasive fungal infection, and enucleation was performed (Fig. 3.2). Fungal cultures revealed *S. prolificans* with resistance to voriconazole and susceptibility to a combination of amphotericin, posaconazole, and terbinafine. Although the patient had preexisting renal dysfunction, based on the available data it was considered unlikely that the patient would survive without therapy that included all potentially effective antimicrobial agents, including liposomal amphotericin B. This decision would not have been possible without the results of the biopsy procedure. Immunosuppression was also reduced. She continues to survive while receiving triple antifungal therapy, despite disseminated infection.

In the normal host a careful medical history including epidemiologic exposures and prior procedures will generally indicate the nature of likely pathogens. Unusual infections occur most often in association with anatomic defects, unusual epidemiologic exposures, or unrecognized immune deficits. These individuals will mount an inflammatory response that will serve to slow disease progression and indicate on physical exam or radiology, the location of the infectious process.

By contrast, the immunocompromised host has muted signs and symptoms of inflammation and is susceptible to infection by a broad array of pathogens, and infection will often be asymptomatic until quite advanced. Dissemination of infection (e.g., *Nocardia* or *Cryptococcus* species) outside the site of initial infection (the lungs) is common, and morbidity is increased. The key to successful management of the immunocompromised host is the ability to obtain the specimens required for microbiologic diagnosis before the initiation of empiric therapies which will obscure subsequent diagnosis. As for any patient, the risk of infection is determined by the interaction of two factors: the potential pathogens to which the individual is exposed (epidemiologic exposures) and a measure of the individual's susceptibility to infection, termed the "net state of immunosuppression" (Box 3.1). **Epidemiologic exposures** of importance to the immunocompromised patient can be divided into two general categories: those occurring within the community and those occurring within the hospital. Exposures within the community will vary based on such factors as geography and socioeconomic status. Thus community-acquired opportunistic pathogens include the geographically restricted systemic mycoses (blastomycosis, coccidioidomycosis, and histoplasmosis), *Mycobacterium tuberculosis*, *Strongyloides stercoralis*, *Leishmania donovani*, *Trypanosoma cruzi*, *P. carinii (jiroveci)*, *Legionella* species, and community-acquired respiratory viral infections (e.g., influenza, respiratory syncytial virus, metapneumovirus, and parainfluenza). Common viral agents may include herpes simplex virus, cytomegalovirus (CMV), varicella zoster, hepatitis B virus (HBV), and hepatitis C virus (HCV). Within the hospital, excessive environmental exposures can occur on the hospital unit where the patient is housed or when the patient is transported to contaminated operating rooms, radiology suites, or catheterization laboratories for procedures. When the air, food, equipment, or potable water supply is contaminated with pathogens, such as *Aspergillus* species, *Legionella* species, or vancomycin-resistant enterococci, clustering of cases of infection will be observed.

Specific immune deficits tend to predispose to specific types of infection (Table 3.2). The **net state of immunosuppression** (see Box 3.1) is a concept that describes all of the host factors that contribute to infectious risk. The additive factors include: the dose, duration, and temporal sequence in which immunosuppressive drugs are deployed; injuries to the primary mucocutaneous barrier to infection (e.g., indwelling catheters, gastrointestinal or bronchial anastomoses in organ transplant patients); neutropenia or lymphopenia; underlying immune deficiency; pulmonary aspiration injury; metabolic problems including protein-calorie malnutrition, uremia, and perhaps hyperglycemia; the presence of devitalized tissues, and fluid collections (hematoma, effusions, ascites); and infection with immunomodulating viruses (CMV, Epstein-Barr virus [EBV], HBV, HCV, and the human immunodeficiency viruses, HIV-1 and -2), which predispose to other opportunistic infections, as well as to graft rejection or graft-versus-host disease. In general, more than one factor is present in each host; the identification of the relevant factors, and correction when possible, is central to the prevention and treatment of infection in these hosts. The "spectrum" of susceptibility to infection

TABLE 3.2 Infections Associated With Specific Immune Defects

Defect	Common Causes	Associated Infections
Granulocytopenia	Leukemia, cytotoxic chemotherapy, AIDS, drug toxicity, Felty syndrome	Enteric GNR, *Pseudomonas*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, streptococci, *Aspergillus*, *Candida* and other fungi
Neutrophil chemotaxis	Diabetes, alcoholism, uremia, Hodgkin disease, trauma (burns), lazy leukocyte syndrome, CT disease	*S. aureus, Candida*, streptococci
Neutrophil killing	CGD, myeloperoxidase deficiency	*S. aureus*, *Escherichia coli*, *Candida*, *Aspergillus*, *Torulopsis*
T-cell defects	AIDS, congenital, lymphoma, sarcoidosis, viral infection, organ transplants, steroids	Intracellular bacteria (*Legionella Listeria*, mycobacteria), HSV, VZV, CMV, EBV, parasites *(Strongyloides, Toxoplasma)*, fungi *(Pneumocystis carinii, Candida, Cryptococcus)*
B-cell defects	Congenital and acquired agammaglobulinemia, burns, enteropathies, splenic dysfunction, myeloma, ALL	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Salmonella*, and *Campylobacter* spp., *Giardia lamblia*
Splenectomy	Surgery, sickle cell, cirrhosis	*S. pneumoniae*, *H. influenzae*, *Salmonella* spp., *Capnocytophaga*
Complement	Congenital and acquired defects	*S. aureus*, *Neisseria* spp., *H. influenzae*, *S. pneumoniae*
Anatomic	IV/Foley catheters, incisions, anastomotic leaks, mucosal ulceration, vascular insufficiency	Colonizing organisms, resistant nosocomial organisms

AIDS, Acquired immunodeficiency syndrome; *ALL,* acute lymphocytic leukemia; *CGD,* chronic granulomatous disease; *CMV*, cytomegalovirus; *CT,* connective tissue; *EBV*, Epstein-Barr virus; *GNR,* gram-negative rods; *HSV,* herpes simplex virus; *IV,* intravenous; *VZV,* varicella zoster virus.

is a *continuum* from individual deficits (e.g., viral upper respiratory infection that paves the way for bacterial superinfection) to multiple simultaneous deficits (e.g., the organ transplant recipient).

Timetable of Infection

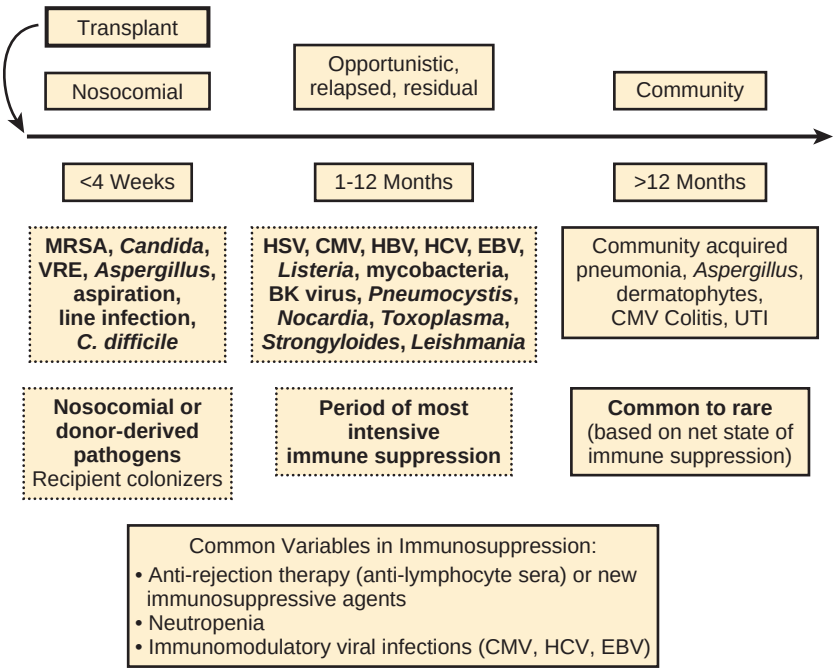
In the broad spectrum of immunocompromised hosts, the risks of infection may be *relatively stable* over time, as in the diabetic patient with vasculopathy and neuropathy who is prone to skin and soft tissue infections. The risks of infection may be *time limited*, as in the postsurgical patient without complications or in the autologous bone marrow transplantation recipient with effective engraftment. The risk of infection may be *cumulative and progressive,* as in the AIDS patient, in whom infection is a function of declining immunity (without therapy), falling $CD4^+$ lymphocyte counts, rising viral loads, and the effects of other persistent infections (CMV). In these individuals the occurrence of new infections suggests the progression of immune compromise. The risks may also be *progressive but not cumulative.* Thus the risks of infection in the recipient of allogeneic stem cell or solid organ transplant *change predictably with time* as a function of the evolving condition of the patient. For example, in the early phase after hematopoietic stem cell transplantation (HSCT), infection is often the result of nosocomial exposures during neutropenia. Subsequently, during treatment for acute and chronic graft-versus-host disease, susceptibility to infection is a function of immune suppression and mucosal injuries (possibly from chemotherapy, radiation, or infections such as *Clostridium difficile* colitis).

With standardized immunosuppressive and chemotherapeutic regimens, specific types of infections often occur in a predictable pattern ("timeline") as a reflection of the specific risk factors (surgery, immune suppression, acute and chronic rejection, reemergence of underlying diseases, viral infections; Fig. 3.3) present at each phase of the posttransplantation course. The patterns have been altered by the availability of a broader range of immunosuppressive and chemotherapeutic agents, the use of stem cells instead of marrow for transplantation, and antimicrobial prophylaxis. However, the general concepts and major determinants of infection remain the same—the exogenous immune suppression or chemotherapy and any additional immune suppression used to treat either graft rejection or graft-versus-host disease. Superimposed viral infections will enhance the risk of infection at any point along the timeline.

Because each risk factor renders the patient susceptible to infection by new groups of pathogens, infections occurring with the "wrong" pathogen or at the wrong time suggest an undiscovered "immune deficit" (fluid collection, neutropenia) or an unusual epidemiologic exposure. The occurrence of specific infections can be prevented by the use of antimicrobial prophylaxis, vaccines, and behavioral modifications (e.g., no raw vegetables or digging in gardens without masks). This will result in a "shift to the right" of the timeline—infections generally observed later in the course of disease or therapy will be observed at the appropriate time in the absence of infections that tend to occur earlier but have been prevented by a variety of preventive measures.

Summary

Invasive diagnostic procedures have been coupled with advanced detection techniques (immunostaining, molecular diagnostics) to enhance the specificity of clinical diagnoses. The early and appropriate deployment of invasive procedures will often reduce the exposure of patients to unnecessary antimicrobial agents and associated toxicities. The clinician must be familiar with the available techniques at each institution and work closely with pathologists to exchange clinical information and optimize patient care.



• **Figure 3.3** The timeline of common infections after solid organ transplantation. *CMV,* Cytomegalovirus; *EBV,* Epstein-Barr virus; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HSV,* herpes simplex virus; *MRSA,* methicillin-resistant *Staphylococcus aureus*; *VRE,* vancomycin-resistant enterococci.

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4

Cytopathology of Infectious and Inflammatory Diseases

VICKI J. SCHNADIG

Introduction

Processing of Cytologic Samples for Infectious and Inflammatory Diseases

Culturing of Fine-Needle Aspirations for Microorganisms

Inflammatory Patterns and Associated Pathogens
- Purulent Inflammatory Response
- Eosinophils and Allergic Mucin
- Granulomatous Inflammation
- Granulomatous Inflammation Admixed With Neutrophils

Unusual Host Reactions to Infections in the Immunocompromised Patient
- Patients With Neutropenia or Defective Neutrophils
- Severely Impaired Cell-Mediated Immunity and Diffuse Macrophage Infiltration
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Conclusion

Appendix

Introduction

In the previous edition, this chapter stressed the importance of infectious disease cytology to the education of pathology trainees and practitioners. Within the contents of the chapter, are illustrated cases intended to demonstrate the value of the following:

1. A cytopathologist with experience and interest in infectious disease.
2. Rapid onsite evaluation (ROSE) to provide immediate presumptive diagnoses and ensure proper use of cultures and/or molecular testing.
3. Workhorse (hematoxylin and eosin [H&E] and Romanowsky) stains, as well as judicious use of so-called special stains, performed in a fashion suitable for cytologic preparations.
4. Last but far from least, the importance of close communication between microbiologists and cytopathologists.

It is my opinion that, equally essential to quality patient care, is a pathologist who involves himself/herself with clinical medicine and interacts with patients as well as clinicians, radiologists, and endoscopists, thus becoming part of an integrated team of physicians devoted to providing the best patient care. Especially in a teaching hospital, an onsite pathologist has the opportunity to review images and differential diagnoses with the radiologist (or endoscopist), as well as obtain important information directly from patients.

There are caveats that need to be addressed. In response to much-needed efforts to cut medical expenses, we risk throwing the baby out with the bathwater. Some believe that cytopathologist participation in ROSE is unnecessary and ROSE should be used for adequacy assessment only. My response is that, particularly for infectious disease diagnosis, adequacy is inadequate. ROSE is essential for triage, aimed at distinguishing infectious and/or inflammatory conditions from neoplasia and ensuring proper processing of samples. Pathologists should guide the radiologist toward procurement of material required for definitive diagnosis, such as cultures, molecular studies, core biopsies, and flow cytometry, and should work in partnership with clinical microbiologists to ensure optimal material is submitted for culture and/or molecular studies. This mode of practice should be taught to pathology trainees. There should be a "culture" of communication between cytopathologist, radiologist/endoscopist, clinical microbiologists, and clinicians.[1,2]

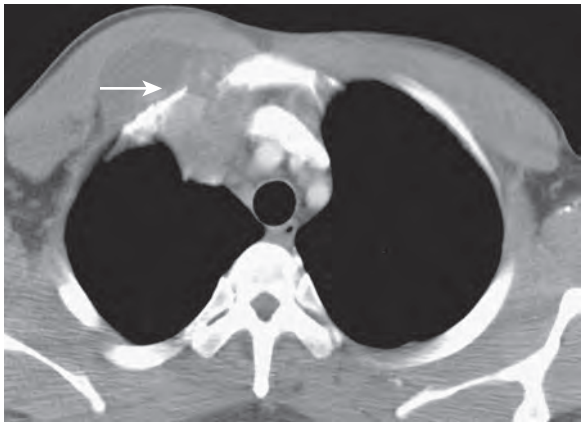
During the period between the publication of the first and second editions of this book, it was determined that our cytopathology laboratory should no longer perform its own Gram, acid-fast, and fungal stains and that a limited number of stains should be performed by the standard histologic techniques used in surgical pathology. The Gram-Weigert stain, which we found very useful for actinomycetes and nonviable or treated gram-positive organisms, is no longer performed. Although this decision was likely made to streamline and cut costs, it is my belief that special stains for cytology should be performed by methods suited to air-dried (postfixed in methanol) or ethanol-fixed smears and cytospin preparations. Relying on histologic techniques may not be optimal for smears and may delay turnaround time for reporting. For this reason I have decided to leave the appendix to this chapter in place for those who would like to take advantage of it. Note that all of the cytology illustrations given in this chapter are from stains performed within the cytopathology laboratory.

Finally, to be in compliance with the zeitgeist, the spelling of the Romanovski (stain) has been changed to Romanowsky, the usual spelling used in the United States. One should acknowledge

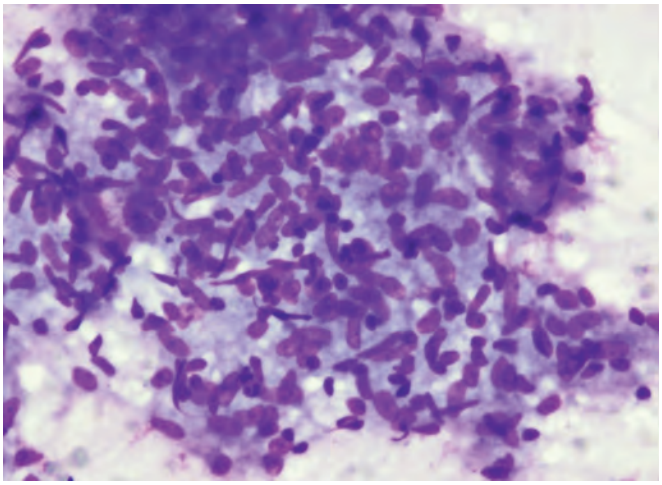
that Romanowsky (stain) was the name of a Russian physician; the Cyrillic alphabet spelling is Романовский, and the name can be spelled in several fashions using the Latin alphabet.

Despite its potential benefits to patients, infectious disease cytology is often relegated to the pathology education back burner. This is unfortunate because accurate diagnosis of infectious and inflammatory diseases (IIDs) by cytology can be lifesaving and involves noninvasive or minimally invasive procedures. ROSE not only can guide in the procurement of appropriate cultures but can allow for instigation of empiric therapy when needed.

Infectious diseases not infrequently manifest with signs that mimic those of neoplasia, and an experienced cytologist should be as knowledgeable about the clinical and morphologic features of infectious diseases as he or she is familiar with the morphologic patterns of neoplasia. For example, in Fig. 4.1 the following gloomy prognostication was rendered on the basis of computed tomography (CT): "Large malignant neoplasm destroys the first rib and sternum and extends and invades the mediastinum and the pectoralis muscles." However, a fine-needle aspiration (FNA) of the pectoralis mass (Fig. 4.2) led to a presumptive diagnosis of caseating granuloma on the basis of onsite evaluation. An aliquot of aspirated material was then submitted to the microbiology laboratory for bacterial, mycobacterial, and fungal cultures, which identified *Mycobacterium tuberculosis*. Follow-up CT, after appropriate therapy, showed significant improvement with marked decrease in the size of the mass.



• **Figure 4.1** Computed tomogram of the chest. A large, partially necrotic mass *(arrow)* involves the right lung, mediastinum, and thoracic soft tissue, with destruction of the rib.



• **Figure 4.2** Fine-needle aspiration of right chest wall mass from the same patient as in Fig. 4.1. An aggregate of epithelioid macrophages admixed with lymphocytes is seen. Caseation (also present, focally) is not illustrated in this image (Romanowsky stain, ×400).

To accurately diagnose infectious diseases by cytology, one must do the following:

1. First and foremost, think about the possibility of infection.
2. Have adequate knowledge of the patient's clinical presentation, immune status, and results of any imaging studies performed. At our institution, trainees typically review the electronic medical record prior to both palpation- and image-guided FNA procedures; this is considered an essential part of trainee education.
3. Be able to recognize inflammatory patterns by cytology and know what types of infection to suspect based on the type of inflammatory cell reaction, combined with the clinical and radiologic findings.
4. Be able to appropriately culture and request indicated special stains.

Some infections, such as *Strongyloides stercoralis* hyperinfection, are medical emergencies that necessitate immediate action. Others, such as tuberculosis, are communicable diseases that, when suspected, require notification of infection control personnel and the primary caregivers, as well as appropriate cultures or molecular studies to establish or rule out infection by *M. tuberculosis*. Scrape or touch preparations of cutaneous ulcers, stained by Romanowsky method, can provide onsite diagnosis of infectious processes and provide material for microbial cultures, thus precluding necessity for biopsy. FNA and touch or scrape preparations are highly useful, albeit underused, stratagems for diagnosing infectious diseases in the autopsy suite.[3-5] Their use obviates the necessity for, and potential contamination of, the cryostat[6,7] and helps to confirm or exclude communicable disease in the preliminary autopsy diagnosis.

We have diagnosed infections and unusual nonneoplastic conditions from all types of samples submitted to the cytopathology laboratory, including sputum, bronchial brushings and washings, body cavity and cerebrospinal fluids, bronchoalveolar lavage (BAL) samples, transbronchial FNA, both bronchial and gastrointestinal endoscopic ultrasound, and percutaneous FNA. Most infections can be presumptively diagnosed, or at least suspected, by using a combination of the two workhorse cytology colorants, the Papanicolaou and Romanowsky stains. These two stains make an excellent team for diagnosis of IIDs, as well as neoplasia. The advantage of these two dye combinations over the so-called special stains is that they allow one to assess the type of tissue reaction found in the sample, as well as recognize the presence of infectious organisms. The tissue reaction is of great importance and is discussed later in this chapter. Detailed discussions of the stains used for diagnosis of IIDs are given in other references.[8-11] Briefly stated, the Papanicolaou stain is similar to the workhorse of histopathology, the H&E stain, with the following exceptions. It is performed on preparations that are wet-fixed in alcohol, most commonly 95% ethanol. This technique coarsens and sharpens nuclear chromatin and produces a translucency to the stained preparation that allows one to examine relatively thick cell preparations at various levels of focus. As with the H&E preparation, the nuclear stain is hematoxylin that has been alkalinized to give a bluish color to the cell nuclei. In contrast to H&E,

there are three cytoplasmic stains: eosin, light green or a lead-free equivalent, and orange G. Moreover, the Papanicolaou stain often colors mucin a purple-to-red color, which is useful for recognition of *Cryptococcus* and allergic mucin (see later discussion).

The Romanowsky stains comprise a number of eosin-thiazine metachromatic stains, including Wright, Giemsa, May-Grünwald, and the rapid variants commercially available as Diff-Quik, Quik-Dip, and so on. Romanowsky stains are usually performed on air-dried slide preparations that are postfixed in methanol. Because of the air-drying, the nuclear and cytoplasmic texture is not as crisp; however, because cells are more thinly spread out over the slide and have more surface area in contact with the slide, there is less cell loss. The Romanowsky stain supplements the Papanicolaou by highlighting features that are not well seen with the former. Cell chromatin pattern and cytoplasmic texture are best seen in the Papanicolaou stain, making it advantageous for cancer diagnosis. Certain fungi, nematode larvae, and ova are also best seen with the Papanicolaou stain. Romanowsky is ideal for characterization of leukocytes, and it is an excellent stain for bacteria and certain other fungi, such as *Histoplasma* and *Pneumocystis* (see later discussion). Mucin, colloid, and cytoplasmic granules are best distinguished with this stain. Romanowsky stains can also be performed on slides wet-fixed in ethanol; nuclear features are better visualized; however, cells will appear smaller, and there will be greater cell loss.

A small number of ancillary special stains are useful for the diagnosis of infectious agents. Prior to 2015 at the University of Texas Medical Branch Cytology Laboratory, most of these were performed in the cytopathology rather than the histopathology laboratory. With the exception of the Grocott-Gomori methenamine silver (GMS) stain, the methods used in cytopathology are designed for smear and cytocentrifuge preparations rather than paraffin-embedded sections.

For suspected bacterial infections, Gram staining is performed on air-dried, heat-fixed, very thinly prepared smears or on cytocentrifuge preparations, using techniques adapted to the microbiology laboratory.[12] Classically, the air-drying and heat-fixation was the preferred method for Gram stain preparation; however, postfixation in methanol has been reported to result in less cell loss and better clarity.[13] Air-drying prior to fixation is still recommended.

For suspected *Nocardia* or *Actinomyces* infections, we previously used a modified Gram technique, the Gram-Weigert stain.[14,15] The Gram-Weigert was designed as a tissue fibrin stain and is rarely used today, but it is an excellent stain for gram-positive bacteria, especially actinomycetes. The latter are often very faintly colored by the standard Gram stain. In addition, even senescent gram-positive bacteria retain their blue color. As an added bonus, *Pneumocystis* cyst forms and *Histoplasma capsulatum* yeast forms are well stained by the Gram-Weigert technique, which substitutes a mixture of aniline and xylene for acetone alcohol as a decolorant. The obvious disadvantage of the Gram-Weigert stain is the necessity to decolorize under a chemical hazard hood. In addition, it is not good for the identification of gram-negative bacteria.

For suspected mycobacterial infections, we used a cold Kinyoun stain[15] rather than the standard Ziehl-Neelsen acid-fast stain. The Kinyoun stain does not require heat, uses a higher concentration of fuchsin and phenol, and colors *M. tuberculosis* and mycobacteria other than tuberculosis (MOTT). It is easily modified for *Nocardia* by shortening of the decolorization time and substitution of a 1% sulfuric or 1% hydrochloric acid solution for acid alcohol.

Fluorochrome stains[12] are typically used in microbiology laboratories and are ideal for large-volume, low-power screening for mycobacteria. Auramine orange has been used to assess FNA for some mycobacteria.[16] A disadvantage of fluorochrome dyes is that they require a fluorescent microscope. In addition, some MOTT, especially *Mycobacterium fortuitum*, may not be seen with fluorochrome stains.[9]

Nonspecific fluorescent stains, such as Calcofluor white,[12,17-19] have been used for wet preparation and cytologic identification of *Pneumocystis* and other fungi. Specific immunofluorescent techniques have also been used with varying amounts of enthusiasm.[18-20]

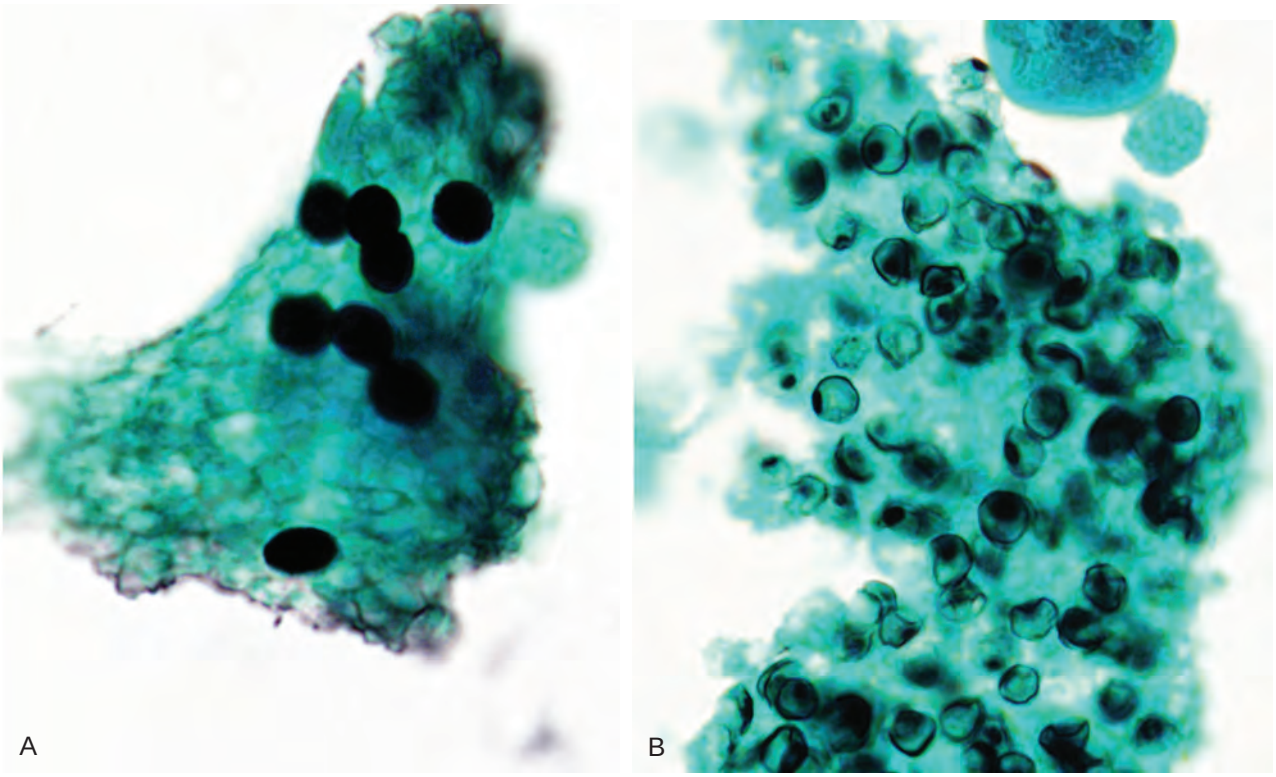
The GMS, the classic stain for fungi, including *Pneumocystis*, is much beloved and demanded by clinicians and formerly loathed by histotechnologists.[21] Microwave histotechnology greatly simplified this rather cumbersome procedure.[22] Automated staining machines are currently widely used in histology for special infectious agent stains; however, automation may limit one's ability to modify staining techniques to accommodate different types of specimens. Although automation can provide time-efficient, high-quality, standardized staining for surgical pathology and for Papanicolaou-stained cytology samples, whether or not quality of automated special stains for infectious agents on cytology samples compares well to stains designed for air-dried and ethanol-fixed smears is not clear. We have satisfactorily used the manual microwave modification of the GMS technique, with the caveat that a good-quality GMS is a joy to interpret, whereas a poorly performed GMS is nearly useless. In reality, GMS is not always the best choice among the cytologic fungal stains, as discussed in later sections of this chapter. There is no substitute for an experienced technologist who understands the characteristics of a good-quality GMS, whether it be performed by hand or machine. There must be a culture of cooperation and communication between histotechnologist and pathologist to ensure good-quality staining.

An overstained and a correctly stained GMS are illustrated in Fig. 4.3. Our former protocols for GMS and processing of both BAL samples and induced sputa are provided in the appendix of this chapter.

We used commercial or homemade smear preparations as controls for all of our special stains, with the exception of the GMS, for which we used paraffin-embedded sections of lung with *Pneumocystis*. The latter is used mainly out of convenience and did not seem to be problematic. Either spit or a buccal scrape makes an excellent control for the Gram or Gram-Weigert stain. Commercial smear preparations containing both acid-fast and non–acid-fast bacteria are used for our Kinyoun and modified Kinyoun stain controls. A blood smear is a good control for the Romanowsky stain.

There are a few histology stains that we occasionally use for diagnosis. These include the following:

1. Mayer mucicarmine, a mucin stain that is useful for confirmation of encapsulated forms of *Cryptococcus*.[23]
2. Fontana-Masson melanin stain for paucicapsular *Cryptococcus*[24,25] and dematiaceous fungi (pigmented fungi associated with chromoblastomycosis, phaeohyphomycosis, and allergic fungal sinusitis).
3. Periodic acid–Schiff (PAS) with diastase, a nonspecific mucopolysaccharide stain that can be useful in the diagnosis of pulmonary alveolar proteinosis (PAP) and malakoplakia. Because there may be loss of material from the slide during diastase digestion, we prefer to submit material for PAS-diastase staining on charged slides.



• **Figure 4.3** Bronchoalveolar lavage samples with *Pneumocystis*. **A,** Sample is overstained. The cysts appear dense and homogeneously black. **B,** Properly stained sample demonstrates the presence of a small, dotlike thickening in the cyst wall (Grocott-Gomori methenamine silver stain, ×1000).

Immunohistochemistry techniques for *Pneumocystis* are also available.

Processing of Cytologic Samples for Infectious and Inflammatory Diseases

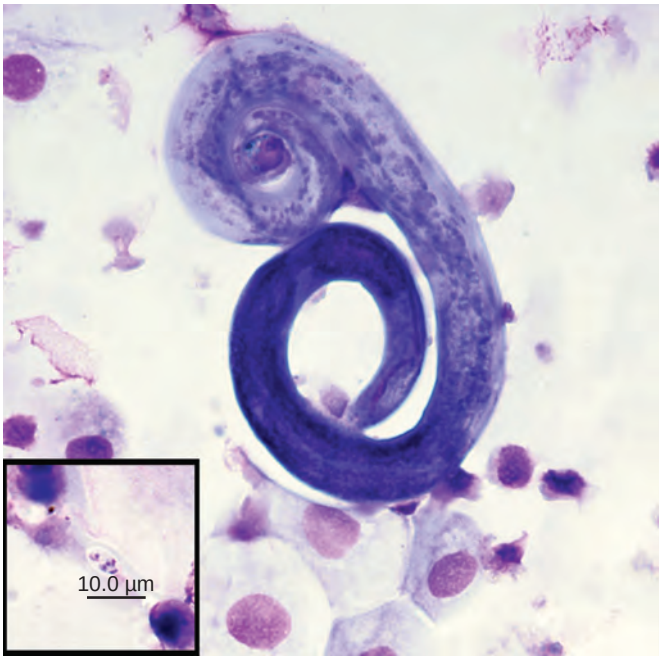
Samples of brushings, washings, lavage fluids, and FNA are prepared in the same way as for routine cancer diagnosis. For brushing slides, a portion of the slide material can be retained unstained for special staining; however, acid-fast, GMS, and Gram-Weigert stains can be performed over the Papanicolaou after rehydration of the slides back to distilled water. It is not necessary to decolorize the nuclear stain. We have not had sufficient time to assess whether restaining or staining of smears by automated methods is as reliable as by our previously used methods. Restaining is the more effective method if there is only a limited number of slides, because the quality of material present on different slide preparations may vary. We prefer to perform special stains on slide preparations already shown to have inflammatory material suspicious for infection. Cytocentrifuge preparations performed on FNA needle rinses or on centrifuged body fluids are also excellent for staining. The former requires that there be adequate inflammatory material in the rinse fluid. If the cytocentrifuge preparations are paucicellular, restaining of smear preparations is preferable. Cytocentrifuge preparations have the advantage of yielding thin, evenly distributed cellular material. A very thin slide preparation is essential for a good-quality Gram stain.

Induced sputum samples from immunocompromised patients are sometimes submitted to the cytopathology laboratory to be evaluated for *Pneumocystis jiroveci*. We process sputa by first adding a mucolytic agent, 10% dithioreitol, centrifuging the sample, and then preparing cytocentrifuge preparations including Papanicolaou, Romanowsky (Giemsa), and GMS-stained slides. We currently use a rapid Romanowsky technique rather than the more cumbersome standard Giemsa stain. (Note that Diff-Quik is a brand name for one of many commercial rapid Romanowsky stains rather than a generic type of metachromatic dye). Cytocentrifuge preparations of concentrated sputa, fluids, and BAL samples provide well-concentrated, thin, circular sample preparations that can be evaluated for inflammation, fungi (including *Pneumocystis*), and other microorganisms. Fig. 4.4 illustrates a cytocentrifuged, Romanowsky-stained sputum sample from an acquired immune deficiency syndrome (AIDS) patient that contained both *Strongyloides* larvae and scanty *Pneumocystis*. Note that the intracystic bodies, not the cyst, are stained by the Romanowsky method. Our protocol for processing sputa for IIDs is given in more detail in the appendix to this chapter.

Culturing of Fine-Needle Aspirations for Microorganisms

In general, only FNA material is submitted to the microbiology laboratory by the cytopathologist, so the discussion here is limited to methods for submitting FNA samples for microbial cultures.

When FNA yields several milliliters of white-yellow creamy or granular material, one suspects purulent inflammation, caseous necrosis, or coagulative necrosis. Purulent inflammation consists of a mixture of neutrophils, macrophages, and necrotic debris.



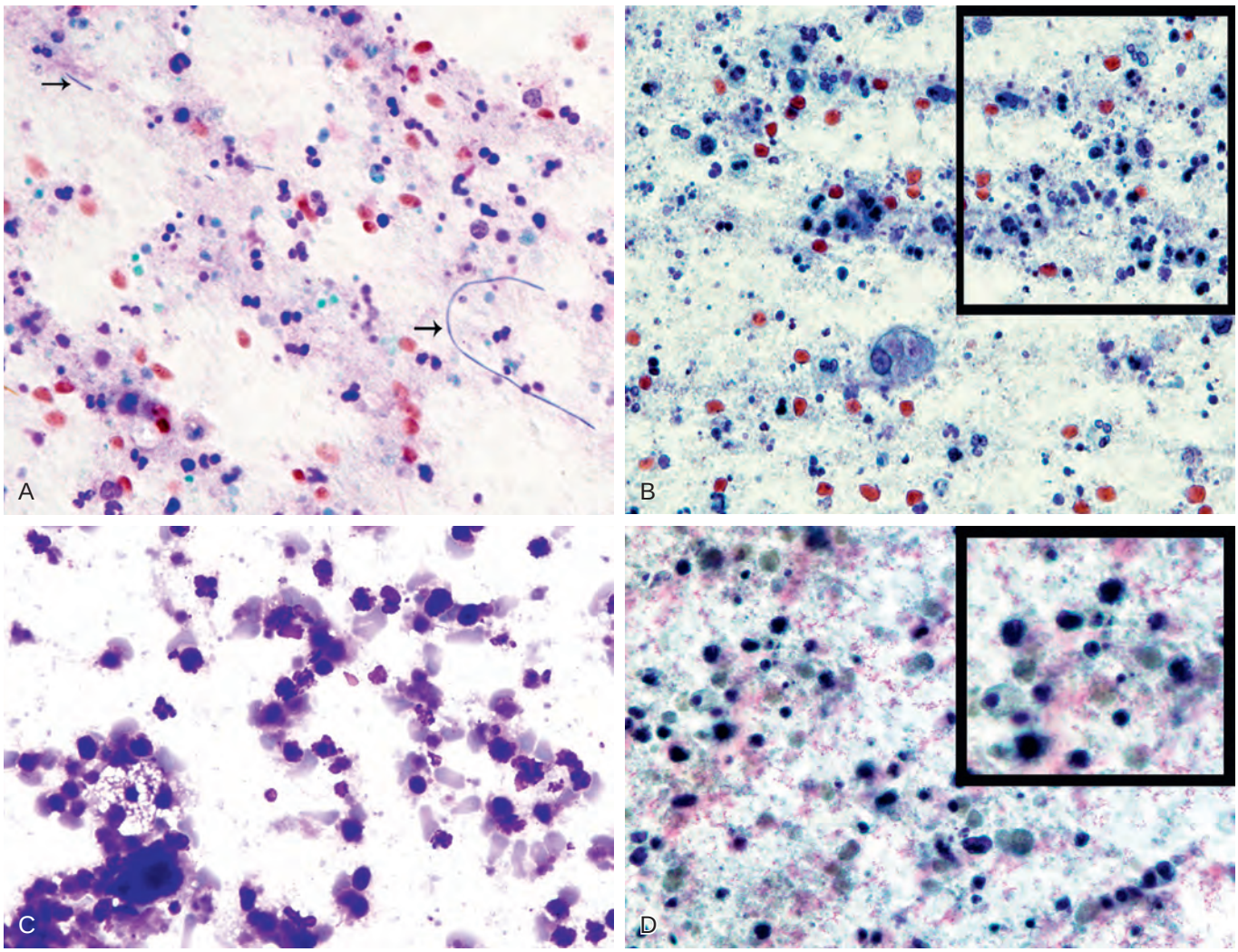
• **Figure 4.4** Cytocentrifuge preparation of bronchoalveolar lavage sample containing a nematode larva compatible with *Strongyloides stercoralis* (Romanowsky stain, ×400). A nonstaining cyst containing tiny intracystic bodies typical of *Pneumocystis* is seen in the *inset* (Romanowsky stain, ×1000).

Caseous necrosis (sometimes called bland necrosis) often has a more crumbly dry or granular appearance, and coagulative necrosis, often associated with keratinizing squamous cell carcinoma (SCC) or high-grade lymphoma, may yield material that is grossly indistinguishable from purulent inflammation. ROSE can help to exclude cancer-associated coagulative necrosis and eliminate the need for cultures. Fig. 4.5 illustrates the difference between necrosis from a bacterial abscess (*Salmonella enteritidis* was cultured) and coagulative necrosis and apoptosis from a necrotizing large B-cell lymphoma. Compare the neutrophil-rich inflammation with presence of bacilli in Fig. 4.5A and B with the presence of cell outlines of coagulative necrosis and apoptotic bodies in Fig. 4.5C and D. The former was sent for cultures, and the latter was shown to be predominantly B-lymphocytes by immunochemistry and confirmed as large B-cell lymphoma by biopsy. If in doubt, it is best to err on the side of caution and submit an aliquot of FNA from suspected infections for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures. If limited material is procured, one should use ROSE to guide the sample processing. The cytologist should collaborate with the microbiology laboratory personnel. Culture techniques and methods should be discussed and should be mutually acceptable to professionals working in both areas.

The cytopathologists and radiologists who submit material for culture must discipline themselves to provide adequate clinical information to aid the microbiologist. The exact site from where the specimen came, the types of culture requested, and the pertinent history should be provided on the request form. It is important to notify the laboratory of any specific organisms suspected, because processing of the sample may vary if unusual organisms are suspected. For example, if one sees evidence of *Actinomyces* infection, the laboratory will incubate the anaerobic culture broth longer than usual before reporting the culture as negative. If organisms whose culture forms are potentially hazardous to microbiology personnel, such as *Coccidioides immitis*, are seen, it is considerate and professional to inform the laboratory.

Culture of FNA for bacteria requires either the proper type of transport medium or immediate inoculation of culture broth onsite. If one chooses to use transport medium, various tactics must be used to submit samples for aerobic and anaerobic cultures. We prefer, whenever possible, to perform or request that the radiologist perform a dedicated pass for microbial cultures after ROSE confirms inflammatory material in aspiration site. If 5 mL or more of purulent-appearing material is aspirated, several drops of sample should be expelled into a vial of anaerobic transport medium. There are several commercially available types of anaerobic transport kits, and it is best to obtain and use the type that is used by the microbiology laboratory. The remaining material can be expelled from the syringe into a sterile specimen cup and submitted to the laboratory for aerobic, fungal, and mycobacterial cultures. Swab transport kits are also available for submission of material for aerobic culture; however, swabs should not be used to send samples for either mycobacterial or fungal cultures.

If scanty material is aspirated, one can rinse the needle in approximately 5 mL of sterile saline and process as described. However, for pathologist-performed FNA, we do not use swabs or anaerobic transport media for bacterial cultures; we prefer to use a pair of aerobic and anaerobic blood culture bottles for bacterial cultures. Our microbiology laboratory uses the automated Bactec system (BD Diagnostic Systems, Franklin Lakes, New Jersey). Approximately 0.5 to 1 mL of aspirated pus or needle rinse material is injected into each of the two (aerobic and anaerobic) Bactec bottles, using the sterile aspirating needle and syringe. This is an efficient method for immediately placing the sample into nutrient culture media with aerobic and anaerobic environments. One can also submit an air-dried smear to the clinical microbiology laboratory for Gram stain. Previously, Gram stain was performed in our cytopathology laboratory on material directly smeared from aspirate onto a glass slide. This technique sometimes identified bacteria not identified by cultures and allowed us to include gram stain results in our final report. In a review of 14 years of cytology-culture correlation, we found 19 out of 204 (9%) cases of non-actinomycete bacteria found by cytology with negative cultures. Three out of 16 cases of *Actinomycetales* infection had positive cytology and negative cultures.[1] It is our belief that, although definitive identification of bacteria is not possible by cytomorphology, reporting the presence of bacteria (bacilli, cocci, actinomycetes) provides important information to the clinician. Using clinical and morphologic information, one can provide a reasonable differential diagnosis, as will be illustrated in case studies to follow. We have found the blood culture bottle method to be easy and effective and have successfully cultured *Staphylococcus, Streptococcus*, gram-negative bacilli, and anaerobes, including *Actinomyces* spp. from blood culture–bottled FNA samples. Our high correlation of directly observed bacteria with culture results and our success with culture of anaerobic organisms lead us to believe that the blood culture bottle method is effective. (Correlation of culture and cytology results should be just as much a part of cytology quality assurance as histology-cytology correlation.)[1] Two studies that examined the efficiency of the Bactec system for direct culture of body fluids found that it performed as well or better than the conventional methods.[26,27] After an aliquot of material is placed in the blood



• **Figure 4.5** Fine-needle aspirations of a gram-negative bacterial abscess (**A** and **B**) and a large cell lymphoma (**C** and **D**). **A,** Neutrophil-rich exudate contains some bacilli *(arrows)* (Romanowsky stain, ×400). **B,** Neutrophils and macrophages are seen (Papanicolaou stain, ×400, *inset* ×1000). **C,** Smudgy cells, larger than the neutrophils seen in **A**, represent coagulative necrosis and apoptosis (Romanowsky stain, ×400). **D,** Coagulative necrosis, apoptosis, and degenerated blood (Papanicolaou stain, ×400, *inset* ×1000).

culture bottles, the remainder of the dedicated IID pass should be submitted in a sterile specimen cup for mycobacterial and fungal cultures.

Recognizing the different types of inflammatory and tissue reaction patterns by cytology and being able to construct a differential diagnosis based on those patterns is essential to cytodiagnosis of IIDs. An important concept to grasp in understanding the histopathology and cytopathology of infection is that the type of tissue response observed is highly dependent on the host's immune status and the manner in which the microorganism interacts with the host. As knowledge of immunology increases, so does awareness of the complexity and variability of the host reaction to infection. The variety of clinical disorders caused by, and types of host response to, *Aspergillus* spp. alone would be sufficient to fill an entire volume. In an admittedly simplified manner, inflammatory patterns and their differential diagnoses are discussed in the following sections. Formation of a differential diagnosis involves not only recognition of the inflammatory pattern but knowledge of the host's immune status and the clinical and radiologic findings. A summary of inflammatory patterns, organisms, and preferred stains is given in Table 4.1.

Inflammatory Patterns and Associated Pathogens

Purulent Inflammatory Response

If a predominance of neutrophils, macrophages, and fibrin is seen in a sample, one should include the following in the differential diagnosis: pyogenic bacteria (commonly *Staphylococcus aureus, Streptococcus* spp., gram-negative bacilli); actinomycetes (*Actinomyces* and *Nocardia* spp.); and some fungi (*Candida, Aspergillus,* and zygomycetes). The clinical history and findings narrow the range of suspects, as does the cytopathologist's evaluation of the Papanicolaou- and Romanowsky-stained slides. The following two case studies illustrate commonly encountered diagnostic issues.

Text continued on p. 34

TABLE 4.1 Summary of Inflammatory Patterns, Likely Organisms, and Stains of Value in Diagnosis

Inflammation	Organisms	Ideal Stains for Microorganism in Cytology	Other Useful Special Stains	Comments
Purulent	1. Pyogenic 2. Actinomycetes 3. *Aspergillus*/Zygomycetes	1. Romanowsky or Gram 2. Gram-Weigert/Gram 3. Pap	1. None 2. Modified Kinyoun 3. GMS *(Aspergillus)*	2. Modified Kinyoun is useful for distinguishing between *Nocardia* spp. or *Mycobacterium fortuitum* and *Actinomyces* spp.
Allergic mucin	1. *Aspergillus*/ dematiaceous fungi	1. Romanowsky or Pap	1. GMS 2. Fontana-Masson (melanin)	—
Granuloma	1. Mycobacteria 2. *Histoplasma* 3. *Cryptococcus*	1. Kinyoun or Ziehl-Neelsen 2. GMS 3. GMS	1. Modified Kinyoun — —	2 & 3. In well-formed granulomas, *Histoplasma* and *Cryptococcus* tend to be scant in number and nonviable *(Histoplasma)* or paucicapsulated *(Cryptococcus)*; GMS is the preferred stain in this situation.
Mixed granuloma/ purulent	1. *Blastomyces* 2. *Coccidioides* 3. *Paracoccidioides* 4. Chromoblastomycosis and phaeohyphomycosis agents 5. Sporotrichosis	1. Pap 2. Pap 3. Pap 4. Pap 5. GMS	— — — 4. Fontana-Masson (melanin) —	5. *Sporothrix* is usually very scant in cytologic and histologic samples and poorly stained by Pap unless a SHP is seen; in GMS, the yeast forms have elongated, cigar-shaped blastoconidia.
Diffuse macrophage	1. Mycobacteria, especially MAC 2. *Histoplasma* 3. *Leishmania*	1. Romanowsky and Kinyoun/Ziehl-Neelsen 2. Romanowsky 3. Romanowsky	— 2. GMS —	1. MAC and other mycobacteria often have a negative stain/refractile red appearance with Romanowsky stain.
Scant to none	1. *Pneumocystis* 2. *Cryptococcus* 3. *Aspergillus*/Zygomycetes (neutropenic patients) 4. *Candida* (neutropenic patients)	1. Romanowsky/GMS 2. Romanowsky 3. Pap 4. Romanowsky	— 2. Mucin 3. GMS *(Aspergillus)* 4. Gram	1. *Pneumocystis* can usually be presumptively diagnosed on Pap-stained slides.

GMS, Grocott-Gomori methenamine silver stain; *MAC*, *Mycobacterium avium* complex; *Pap*, Papanicolaou stain; *SHP*, Splendore-Hoeppli phenomenon.

CASE 1

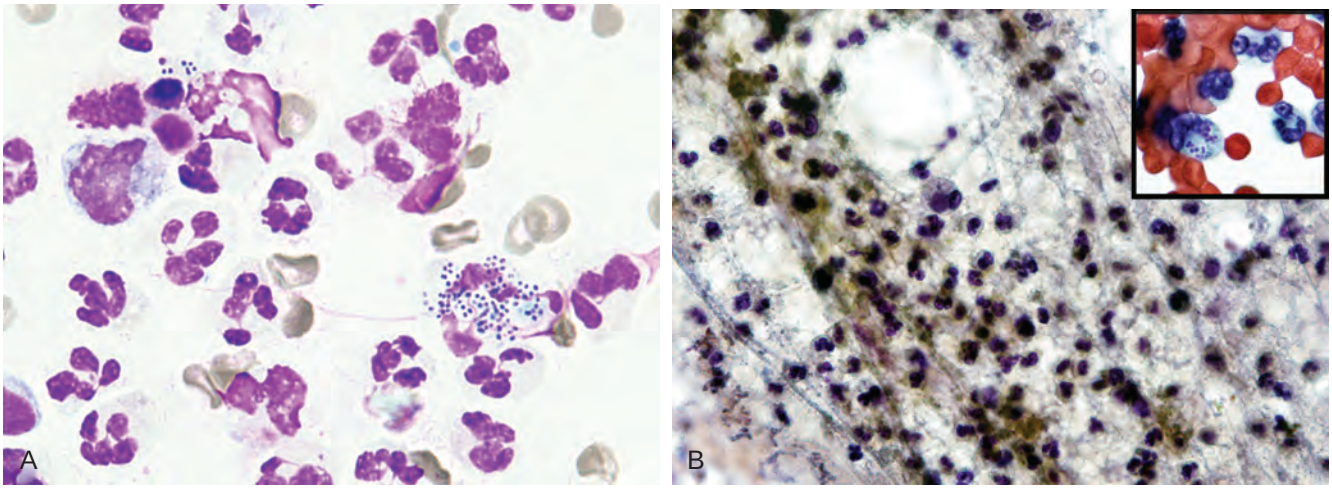
Elicitors of the Not-So-Laudable Pus

A 59-year-old man presented with sudden onset of a painful swelling in the neck. A tender, soft, fluctuant, and slightly erythematous, 5 × 4 cm mass was palpated in the level II region of the left neck. Two smaller, soft, and tender nodules were noted along the cervical lymph node chain. Teeth were in good condition, and no recent dental procedures were reported. The patient was a prison inmate and a smoker, and the clinicians were concerned about tuberculous lymphadenitis and SCC. An FNA was performed and yielded approximately 3 mL of creamy, white-gray material. The Romanowsky- and Papanicolaou-stained slides are shown in Fig. 4.6. Because the mass was aspirated by the cytopathologists, the patient was both interviewed and examined by the team that was to process and interpret the FNA results. Having the opportunity to talk with and examine the patient is optimal for high-quality FNA interpretation. The clinical findings of relatively rapid onset, erythema, and tenderness were suggestive of infection rather than neoplasia, and a pyogenic bacterial infection was favored. The rapid Romanowsky-stained slide performed onsite (see Fig. 4.6A) confirmed the presence of a purulent exudate composed largely of neutrophils and macrophages. Dark blue cocci, both intracellular and extracellular, were seen. Because of these findings, an aliquot of the exudate was submitted for bacterial cultures.

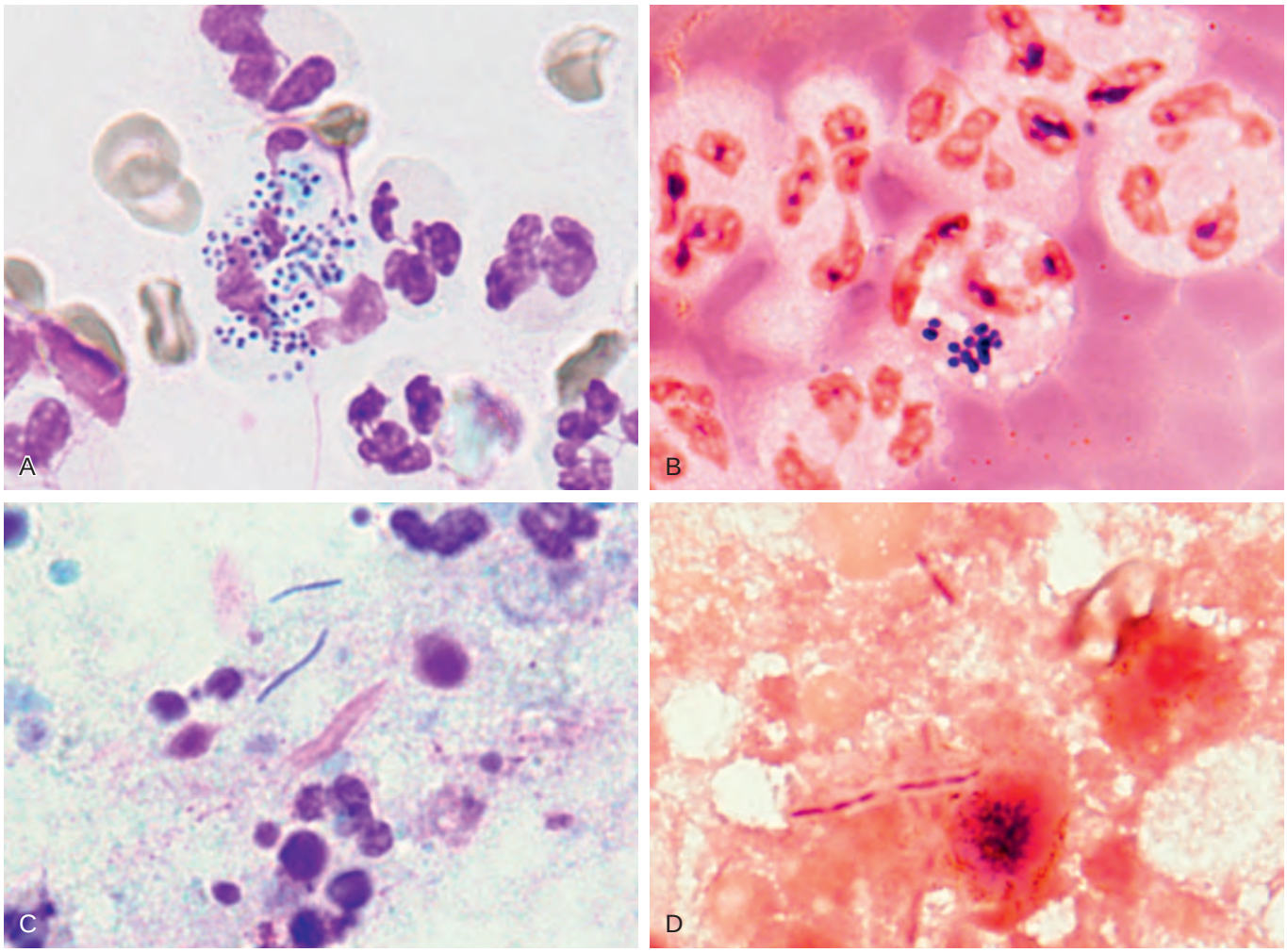
Even if infection with *Staphylococcus* or possibly other aerobic pyogenic cocci is suspected, abscesses should always be cultured for both aerobic and anaerobic bacteria. In addition, if there is adequate material, we habitually submit material (as described earlier) for fungi and acid-fast bacilli determinations. Although it might be argued that this is not cost effective, we believe that it is better, especially if the FNA was obtained under image guidance, to err on the side of a relatively inexpensive overkill. Neutrophils may predominate in some fungal infections, as well as mycobacterial infections, in an immunocompromised host (as discussed later). Culture of the FNA from this patient grew methicillin-resistant *S. aureus* (MRSA). Culture with request for sensitivity testing of bacterial infections is essential because often the antibiotic sensitivity spectrum cannot be predicted on the basis of either morphology or isolated species. Fibrinopurulent exudate is also seen in the Papanicolaou-stained slide (see Fig. 4.6B). Bacteria can often be seen as red- or gray-colored structures in Papanicolaou-stained slides (see Fig. 4.6B, inset); however, the contrast between the organisms and the background is not as great as with Romanowsky-stained preparations. By Gram stain—and again we prefer the classic method as used by microbiology laboratories over the tissue Gram stains—one can discriminate between gram-positive and gram-negative bacteria, as demonstrated in Fig. 4.7.

The differential diagnosis of FNA yielding turbid fluid containing neutrophils includes noninfectious conditions, and the cytopathologist must be aware of these. Two common mimics of infectious lymphadenitis are

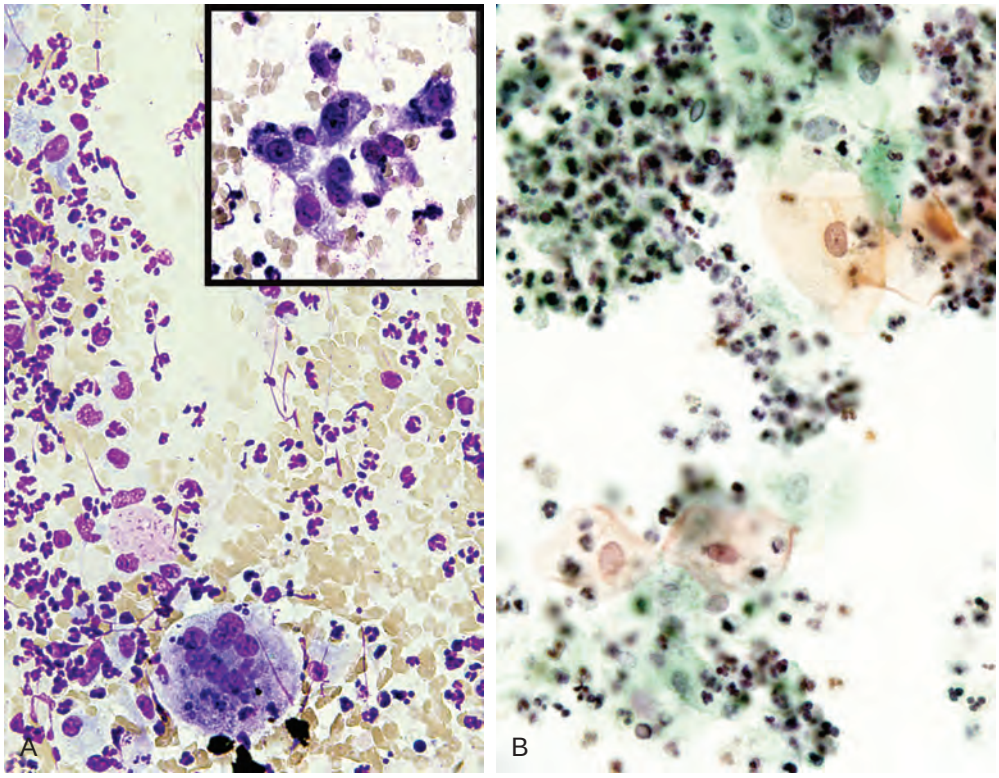
Continued



• **Figure 4.6** Fine-needle aspiration of neck mass. **A,** Exudate composed predominantly of neutrophils with a single macrophage containing blue-colored cocci that are readily distinguished from the more purple-colored cell nuclei (Romanowsky stain, ×1000). **B,** Fibrinopurulent exudate (Papanicolaou stain, ×400) and macrophage containing small, dark cocci, also shown in the *inset* (Papanicolaou stain, ×1000).



• **Figure 4.7** Fine-needle aspirations of two different cervical neck abscesses, with culture-proven *Staphylococcus aureus* (**A** and **B**) and *Salmonella enteritidis* (**C** and **D**). **A,** Large blue cocci within macrophage (Romanowsky stain). **B,** Cocci are gram-positive (Gram stain). **C,** Blue-colored bacilli admixed with degenerating inflammatory cells (Romanowsky stain). **D,** Gram-negative bacilli are seen (Gram stain). All magnifications are ×1500.



• **Figure 4.8** Fine-needle aspirations of two different neck masses: metastatic squamous cell carcinoma **(A)** and branchial cleft cyst **(B)**. **A,** Purulent exudate and presence of a giant cell; a cluster of atypical, poorly preserved cells is seen in the *inset* (both Romanowsky stain, ×400). **B,** Neutrophil-rich inflammatory exudate with presence of benign-appearing squamous cells (Papanicolaou stain, ×400).

CASE 1

Elicitors of the Not-So-Laudable Pus—cont'd

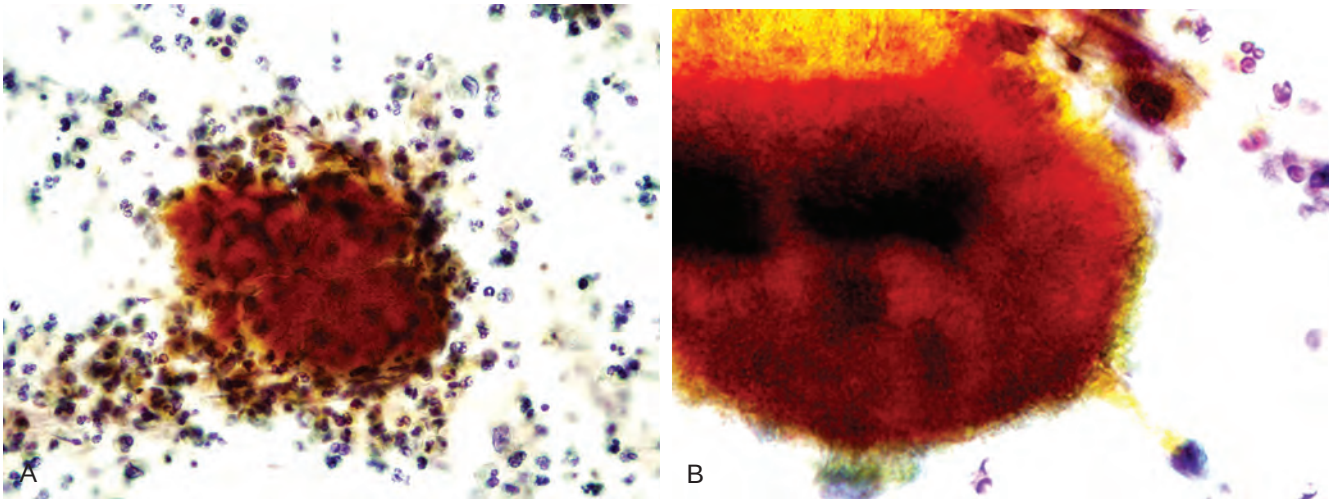
developmental cysts of the neck and cystic degeneration within metastatic SCC. Fig. 4.8 illustrates these conditions, from two patients with level II neck masses. In Fig. 4.8A, rapid Romanowsky-stained FNA, there is inflammation composed of both neutrophils and giant cell macrophages. Focally (inset), atypical cells are seen. This process may be misinterpreted as an abscess. In Papanicolaou-stained slides, malignant keratinizing cells were seen, and metastatic SCC was diagnosed. Fig. 4.8B shows Papanicolaou-stained FNA from the second neck mass. Neutrophilic inflammation and benign-appearing squamous cells are seen, and the case was interpreted as consistent with an inflamed branchial cleft cyst, which was later confirmed by histology.

Although, in our experience, bacterial cultures are typically negative, neutrophils may be abundant in developmental cyst fluid, particularly with branchial cleft cysts. Metastatic SCC, especially from primary in the tonsil or other parts of Waldeyer ring, is often cystic in nature,[28] and neutrophils may be seen in necrotic SCC from any site. A clue to the correct diagnosis is an abundance of coagulative necrosis that should stimulate a search for neoplastic cells. A prominent granulomatous inflammatory reaction, especially in keratinizing SCC, may also be seen, as demonstrated by the giant cell reaction in Fig. 4.8A.

CASE 2

A More Indolent Fibrinopurulent Infection That Goes Bump in the Neck

A 25-year-old man with no significant medical history came to the otolaryngology clinic complaining of a swelling in the right jaw. He had undergone extraction of his right third mandibular molar 7 months previously and had noticed progressive swelling in that region thereafter. The patient was a nonsmoker and had recently tested HIV seronegative. A prior CT of the neck showed a $3 \times 2 \times 1$ cm, necrotic-appearing mass in the left neck in the region of the hyoid bone, and physical examination revealed a 4×3, firm, mildly tender, right-sided mass just inferior to the angle of the mandible. Approximately 2 mL of thick, white, creamy material was aspirated. Neutrophils were seen in an onsite rapid Romanowsky–stained slide, and material was submitted for cultures, as described previously. Again, the clinical findings are useful: the patient's age and the lesion's temporal relation to tooth extraction are evidence against neoplasia, and he had no known immunosuppressive disease. Papanicolaou-stained preparations showed a neutrophilic exudate and several interspersed, grainlike structures (Fig. 4.9A). On high-power magnification, the grains were found to consist of aggregates of bacteria; individual, filamentous bacilli could be seen extending outward from the granules (see Fig. 4.9B). At the



• **Figure 4.9** Fine-needle aspiration of cervical actinomycosis. **A,** A granule is seen surrounded by neutrophils (Papanicolaou stain, ×200). **B,** At higher magnification, hairlike filaments and a yellow-colored band are seen at the periphery of the granule (Papanicolaou stain, ×400).

CASE 2

A More Indolent Fibrinopurulent Infection That Goes Bump in the Neck—cont'd

interface between some of the granules and the inflammatory exudate, there was a yellow-colored band surrounding the granule. This band is a Splendore-Hoeppli phenomenon (SHP).[29,30]

The SHP is seen in H&E staining as club-shaped or bandlike eosinophilic structures surrounding either microorganisms or foreign bodies. Its composition and etiology have been debated but are thought to represent a host reaction including glycoprotein and, in some instances, antigen-antibody complexes. SHP coats the bacteria at the interface and forms club-shaped structures that appear yellow in Papanicolaou stain and dark pink in H&E (Fig. 4.10). The combination of granules of filamentous bacteria and SHP is highly suggestive of an actinomycotic mycetoma, and anaerobic culture is indicated. In such cases it is mandatory to communicate this suspicion to the microbiology laboratory personnel, so that the anaerobic culture bottle will be incubated for at least 1 week before being discarded. Mycetomas are infections that manifest as tumorlike masses and are characterized by the presence of grainlike or granulelike structures composed of tangled aggregates of microorganisms.[31] Mycetomas can be caused by actinomycetes, other types of bacteria (botryomycosis), or fungi (eumycotic mycetomas).[30,31] Like other types of mycetoma, actinomycotic mycetomas are chronic lesions, and draining sinuses and extensive fibrosis are common.

The following two caveats should be considered:

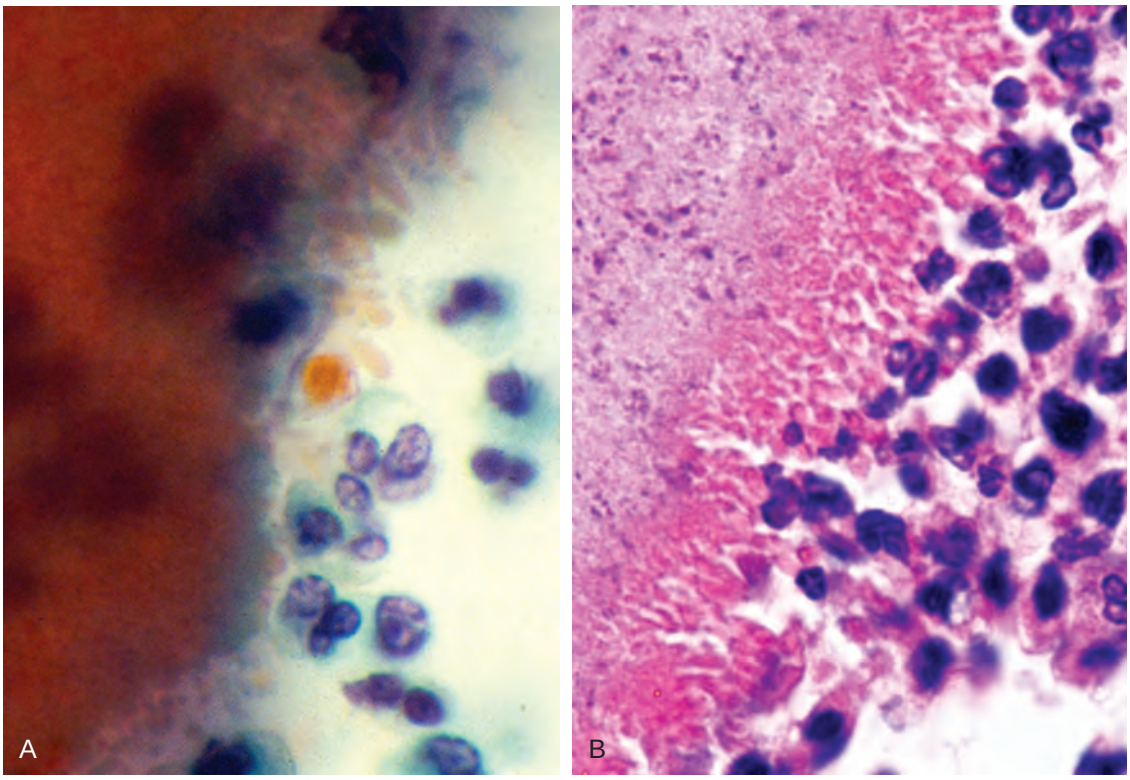
1. Fibrosis may predominate over purulent exudate and may prevent successful diagnosis by FNA.
2. Proliferating fibroblasts and reactive squamous atypia may result in misdiagnosis as malignancy.

In the case of this patient, both *Actinomyces meyeri* and *Actinomyces odontolyticus* were cultured from FNA material placed into an anaerobic blood culture bottle. In our experience, actinomycetes, like mycobacteria, are not easily recognized in Romanowsky-stained preparations. We usually recognize actinomycosis by the granules present in the Papanicolaou-stained material. Gram stain, especially the Gram-Weigert, is important for confirmation that the granule is composed of branching gram-positive bacilli (Fig. 4.11). It should be noted that actinomycetes and other bacteria can be seen in GMS-stained material; however, bacterial staining may require a longer time devoted to the silver deposition step and could result in increased nonspecific background staining.

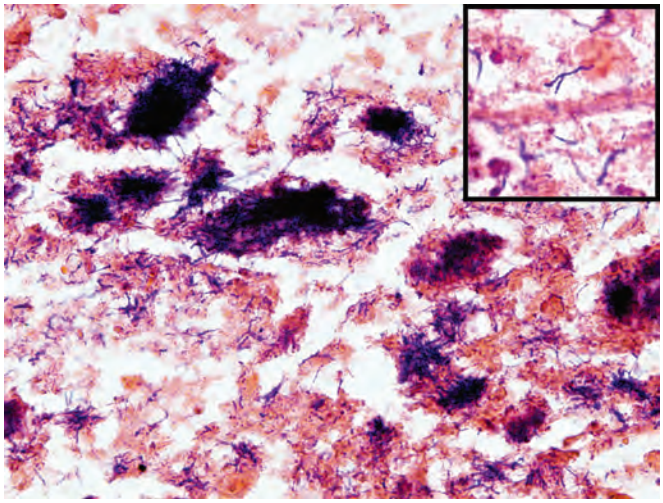
The clinical and cytologic findings described in this case are greatly in favor of actinomycosis; however, botryomycosis and nocardiosis are part of the differential diagnosis. Gram staining helps to rule out the former, and a modified acid-fast stain, the latter. Although nocardial mycetomas are common in tropical countries, in the United States, nocardiosis typically manifests as a necrotizing pneumonia in an immunosuppressed patient (Fig. 4.12). *M. fortuitum* complex (MFC) infections in AIDS patients may manifest as necrotizing, neutrophil-rich cervical neck abscesses, and the morphology of MFC is difficult or impossible to differentiate from that of *Nocardia* species.[32] On Gram stains, both appear as branching, beaded, gram-positive bacilli and are at least weakly acid fast. MFC may not fluoresce with routine auramine fluorochrome staining[9,32] and, like *Nocardia*, sometimes prefers a modified stain to show off its acid-fast qualities (Fig. 4.13).

Neither the pseudoactinomycotic radiate granules (PAMRGs) seen in cervical Papanicolaou (Pap) tests from pregnant women[33,34] nor the cocklebur- or rhomboid-shaped crystals of hematoidin are indicative of actinomycosis, and these should not be confused with mycetomas (Fig. 4.14). Although PAMRGs, like *Actinomyces*, have been described in people with intrauterine devices, our experience since the 1990s is that these are predominantly seen in pregnant women, as described by Zaharopoulos and colleagues.[33] PAMRGs have features similar to the Splendore-Hoeppli reaction[34,35]; however, they are not associated with an underlying microorganism. Hematoidin, a golden-brown, cocklebur- or rhomboid-shaped structure, has been found to be a byproduct of erythrocyte degradation and consists of golden pigment, akin to bilirubin, and lipid.[36] Hematoidin is distinct from both actinomycosis and PAMRG and is often found in cavities containing old blood. In our experience, aspirates from pancreatic pseudocysts and organizing hematomas are good places to find hematoidin crystals (Fig. 4.15). We have rarely seen them in cervicovaginal smears. PAMRGs are far more common in the latter.

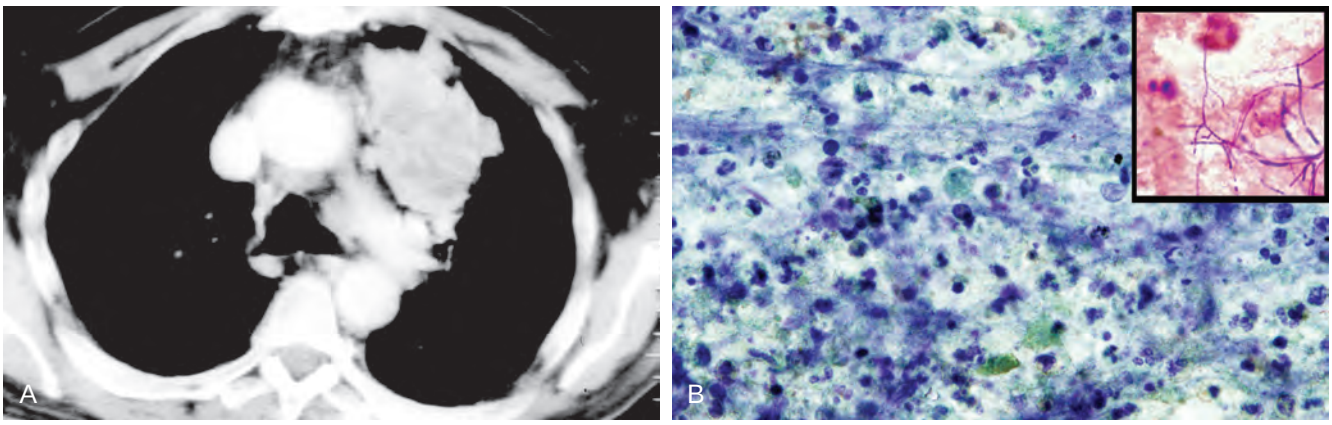
Neutrophils are an important first line of defense against certain fungi,[37] including *Candida*, *Aspergillus*, and zygomycetes (mucormycosis agents). In nonneutropenic patients, invasive infections with these fungi elicit a neutrophil-rich inflammatory cell reaction. Because of the importance of granulocytes as a defense mechanism, invasive candidiasis, zygomycosis, and aspergillosis are potential complications of severe neutropenia. All three have propensities to invade blood vessels, and infarcts with coagulative necrosis are common complications of the invasive forms of these fungal infections. The cytologic features of these fungi are discussed in the sections dealing with cytodiagnosis of infections in immunocompromised patients.



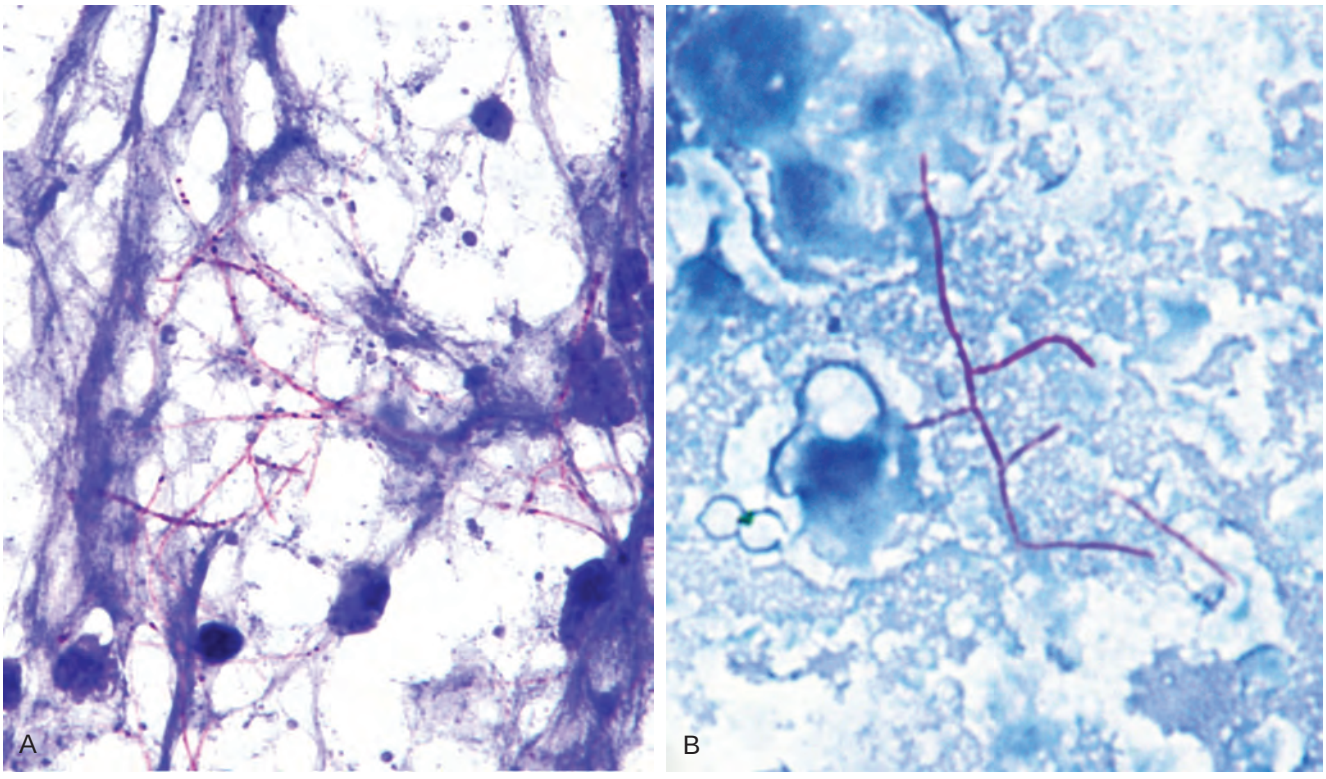
• **Figure 4.10** High-power images of fine-needle aspiration (FNA) and histologic section of actinomycosis with Splendore-Hoeppli phenomenon. **A,** FNA of neck mass shows club-shaped structures at the interface between the granule and the neutrophilic exudate (Papanicolaou stain, ×1000). **B,** Autopsy section from fatal pulmonary actinomycosis demonstrates a bright eosinophilic band and clubs at the microbe-exudate interface (hematoxylin and eosin stain, ×1000).



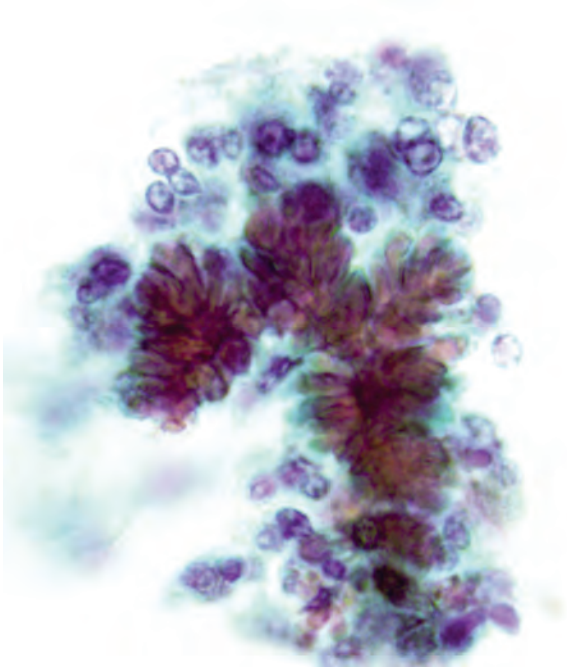
• **Figure 4.11** Fine-needle aspiration of cervical actinomycosis. Clumps of gram-positive bacilli are seen (Gram-Weigert stain, ×400), with branching form seen in *inset* (Gram-Weigert stain, ×1000).



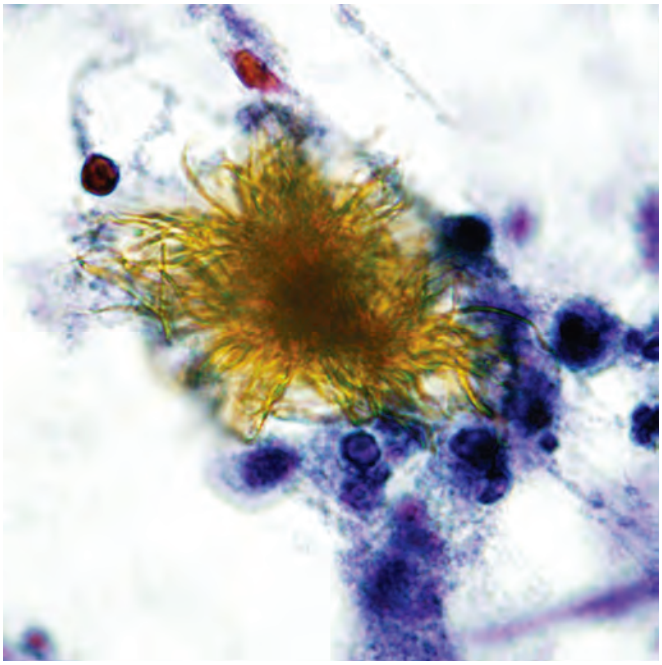
• **Figure 4.12** Pulmonary nocardiosis in a heart transplant recipient. **A,** Chest computed tomography (CT) shows large left upper lobe infiltrate with areas of necrosis. **B,** CT-guided fine-needle aspiration of infiltrate demonstrates the presence of a necrotizing, fibrinopurulent inflammation (Papanicolaou stain, ×400). In the *inset*, branching gram-positive filamentous bacilli are revealed by a Gram stain (Gram-Weigert stain, ×1000).



• **Figure 4.13** *Nocardia* sp. **(A)** and MFC **(B)** in modified acid-fast stains. **A,** Branching, beaded filaments. **B,** In this image, MFC appears slightly thicker and has shorter branches than *Nocardia*; however, these features are not adequate for definitive distinction from *Nocardia* spp. (Kinyoun stain, ×2000).



• **Figure 4.14** Cervical liquid-based Papanicolaou test from a pregnant woman with pseudoactinomycotic radiating granules. Note the similarity to actinomycotic Splendore-Hoeppli phenomenon. However, no bacterial filaments are present (Papanicolaou stain, ×1600).



• **Figure 4.15** Fine-needle aspiration of a subcutaneous fistula derived from pancreatic pseudocyst. A cockleburr-like, golden-yellow crystal typical of hematoidin is seen (Papanicolaou stain, ×1600).

Eosinophils and Allergic Mucin

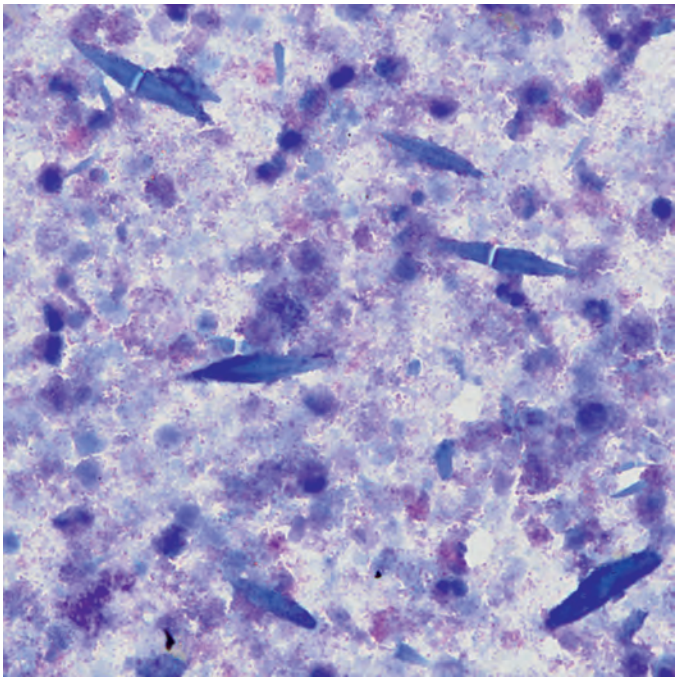
Inflammatory exudates with a predominance of eosinophils are found in asthma and other allergic conditions, including noninvasive allergic fungal diseases; parasitic infections; and eosinophilic pneumonia, and as a nonspecific finding in chronic pleural effusions of varying etiologies including, but not limited to, pneumothorax. One should remember that where there are Charcot-Leyden crystals, there are eosinophils, and where there are eosinophils, one must look for allergic disease and parasites. Charcot-Leyden crystals are elongated, dipyramidal to rectangular crystals with pointed or raggedy ends, quite variable in size. They are composed of lysolecithin acylhydrolase, which has lysophospholipase, an enzyme important in the antiparasitic, antineoplastic, and immune functions of eosinophils.[38] In Romanowsky-stained preparations, they have a light blue color, and in Papanicolaou stain, they may be orange or turquoise. Cases that exemplify eosinophil-rich disease processes diagnosed by cytology, visceral larva migrans–associated eosinophilic hepatic pseudotumor, and allergic fungal disorders are described here. It should also be noted that eosinophils may be found in inappropriate abundance in certain fungal infections (discussed later), and this finding may portend disseminated disease with poor prognosis.

CASE 3

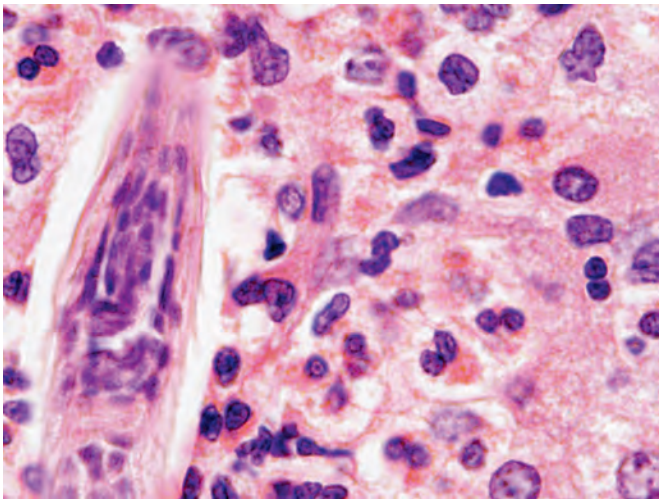
The Hepatocellular Carcinoma That Was Not

CT monitoring for hepatocellular carcinoma in a Cambodian man with hepatitis C disclosed a liver mass that was sampled by FNA. Fig. 4.16 illustrates the findings in an onsite rapid Romanowsky stain. ROSE revealed the presence of necrosis and a large number of reddish granules, consistent with degenerating eosinophils, and many Charcot-Leyden crystals. For this reason, cancer was preliminarily rejected, and suspicion turned to parasites. The preliminary diagnosis was hepatic eosinophilic pseudotumor, and visceral larva migrans was included in the final differential diagnosis. Subsequent serotesting for *Toxocara* was positive, and the nodule regressed with therapy.

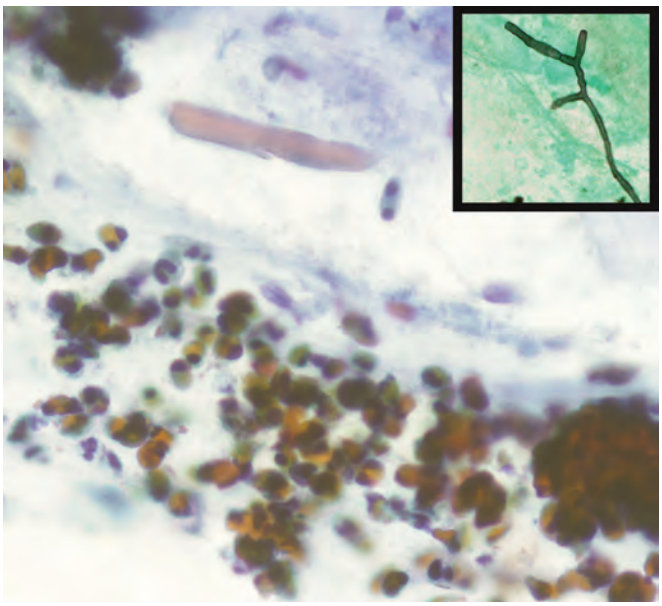
Necrosis, necrotic eosinophils, and Charcot-Leyden crystals in a solitary liver nodule, especially from a child or from an adult with recent foreign travel, makes one suspect visceral larva migrans.[39] Nonhuman ascarid larvae are the most common culprits; however, the actual nematode larva is rarely seen, even in histologic preparations.[40] A portion of larva surrounded by abundant eosinophils is seen in Fig. 4.17. This is a histologic section taken from another case of visceral larva migrans–associated hepatic pseudotumor that was resected without presurgical biopsy or FNA. Nodular collections of eosinophils can also be found in Hodgkin disease and other lymphomas, hypereosinophilic syndrome, and carcinomas, most notably gastric adenocarcinoma.[40-42] Again, careful attention to patients' clinical history and laboratory findings are essential.



• **Figure 4.16** Fine-needle aspiration of a hepatic eosinophilic pseudotumor. Abundant degenerating eosinophils, extracellular eosinophilic granules, and blue-colored Charcot-Leyden crystals are seen (Romanowsky stain, ×600).



• **Figure 4.17** Excisional biopsy of liver mass in a case of visceral larva migrans. Abundant eosinophils are seen surrounding a portion of nematode larva. Dotlike internal structures are often seen in cytologic and histologic preparations of nematodes (hematoxylin and eosin stain, ×1000).



• **Figure 4.18** Fungal mucus plug in a bronchial brushing slide. Within strands of pale blue-colored mucin, there are sheets of eosinophils in the lower half of the image and a Charcot-Leyden crystal in the upper center (Papanicolaou stain, ×400). In the *inset,* branching hyphae are seen in silver-stained preparation (Grocott-Gomori methenamine silver stain, ×400).

CASES 4, 5, AND 6

If There Is Allergic Mucin, the Fungi Are Not Invasive

Fig. 4.18 illustrates the bronchial brush findings from an elderly woman who had a pulmonary infiltrate and a history of therapy for uterine cancer. Eosinophils and abundant mucin were found, and a GMS stain was performed over one of the Papanicolaou-stained slides. Mucin, eosinophils, and fungi are consistent with an allergic fungal mucus plug, the differential of which is discussed in the following paragraphs. Fig. 4.19 illustrates an aspiration of sphenoid sinus contents from a 6-year-old boy with a history of allergic rhinitis and multiple paranasal sinus opacifications and cranial nerve palsies. Note the presence of granular debris, necrotic eosinophils, and Charcot-Leyden crystals. Elsewhere in the preparations, fungal hyphae were found, and the patient proved to have a noninvasive condition known as allergic fungal sinusitis. Fig. 4.20 is an aspirate from an ethmoid sinus of

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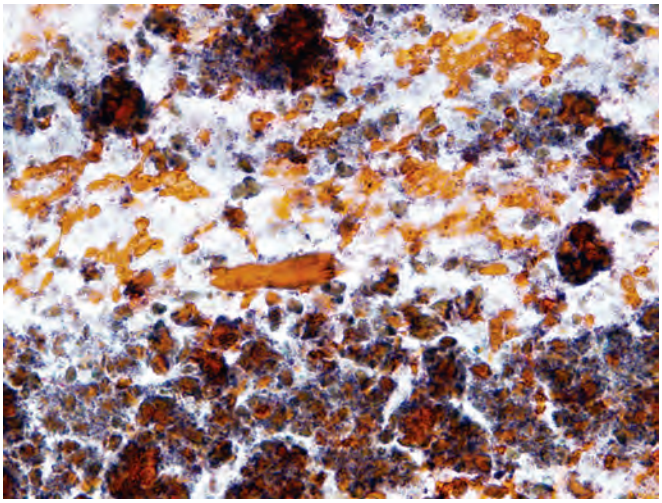
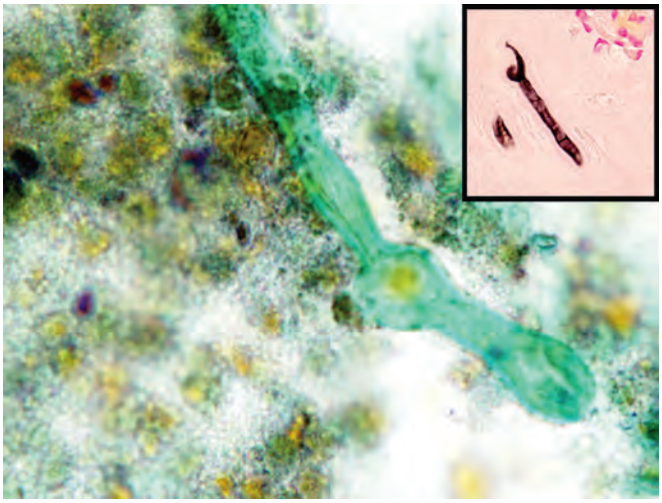


• **Figure 4.19** Aspiration of sphenoid sinus material from a patient with allergic fungal sinusitis. Aggregates of degenerating eosinophils are seen in lower and upper portions, and a Charcot-Leyden crystal is present in the center (Papanicolaou stain, ×200).



• **Figure 4.20** Aspiration of ethmoid sinus from another case of allergic fungal sinusitis. There are necrotic eosinophils and a strand of cyanophilic fungal hyphae (Papanicolaou stain, ×1600). The fungi did not appear brown on Papanicolaou stain; however, they were shown to be dematiaceous by melanin stain of surgical curettage (*inset:* Fontana-Masson stain, ×400).

CASES 4, 5, AND 6

If There Is Allergic Mucin, the Fungi Are Not Invasive—cont'd

another patient with allergic fungal sinusitis. Note the presence of hyaline, cyanophilic hyphae admixed with degenerating eosinophils. Elsewhere in the sample, mucin admixed with relatively intact eosinophils were seen. The fungi proved to be dematiaceous by melanin stain (see Fig. 4.20, inset).

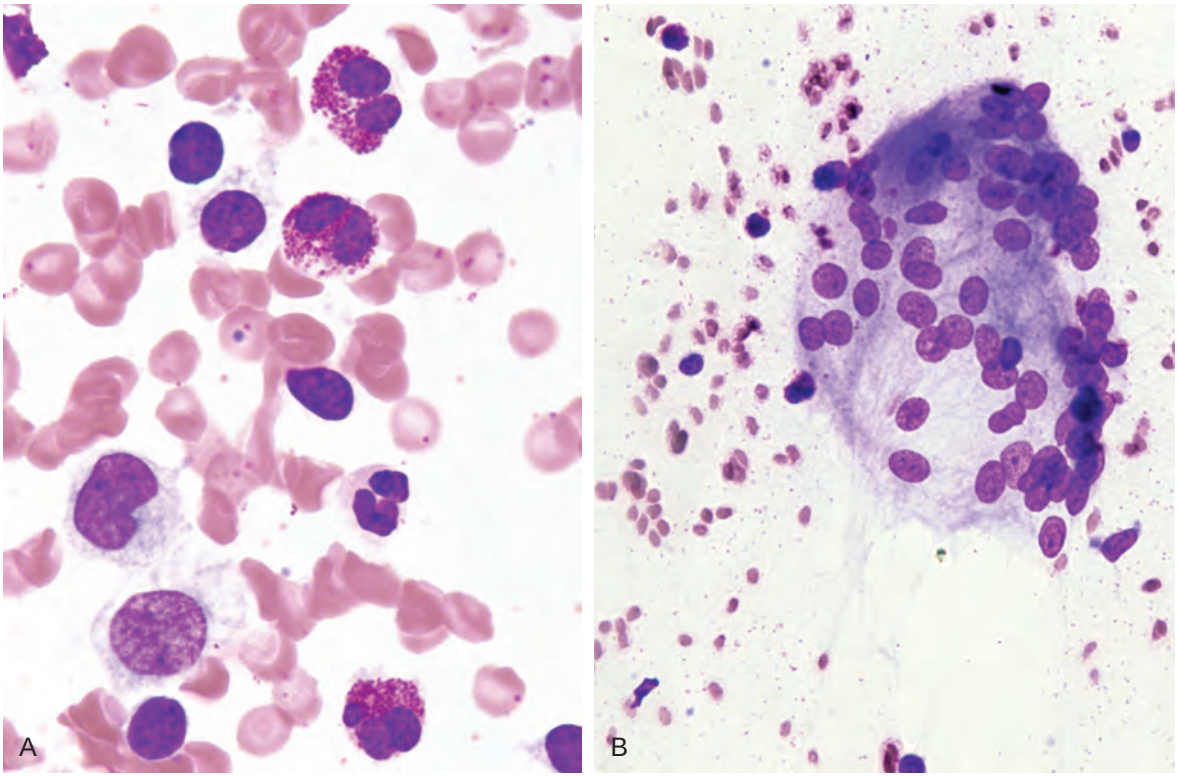
These three cases illustrate the diagnostic significance of allergic mucin. Allergic mucin, composed of a mixture of mucin, cellular debris, eosinophils, and Charcot-Leyden crystals, is found within the conducting airways in allergic airway diseases. The hallmark of *Aspergillus* (and other fungal-associated) noninvasive allergic disease is allergic mucin.[43,44] If one sees allergic mucin and fungi in an FNA or bronchial brushing (see Fig. 4.18), as in the case of the elderly woman, allergic bronchopulmonary aspergillosis (ABPA) must be ruled out, and communication with the clinical team is recommended. In 2014, ABPA was redefined by clinical parameters that include history of asthma or cystic fibrosis with deterioration of lung function, immediate *Aspergillus* species skin test reactivity, increased serum immunoglobulin E (IgE) ≥1000 ng/mL, increased *Aspergillus* species–specific IgE and IgG antibodies, and chest radiographic infiltrates. Peripheral blood eosinophilia, mucus plugs containing fungus, central bronchiectasis, and *Aspergillus* serum precipitins can be considered additional criteria.[45]

Bronchial mucoid impaction that demonstrates the presence of allergic mucin and fungi but lacks the other criteria for ABPA may also occur. In the case of the elderly woman, no fungus-specific antibodies were found. Allergic mucin, containing fungal organisms, most commonly dematiaceous fungi, is also seen in allergic fungal sinusitis. This condition is similar to ABPA in that the fungi are noninvasive. Allergic fungal sinusitis symptoms are caused by massive mucus impaction within the paranasal sinuses. The impaction may cause compression of cranial nerves and symptoms suspicious for neoplasia.[46] Evacuation of contents of involved sinuses typically yields grumous material that has been described as foul-smelling and having the consistency of peanut butter.[47] Cytologists should be familiar with the microscopic features of allergic mucin to help to establish the diagnosis of allergic fungal disease and rule out both neoplasia and invasive fungal disease.[48]

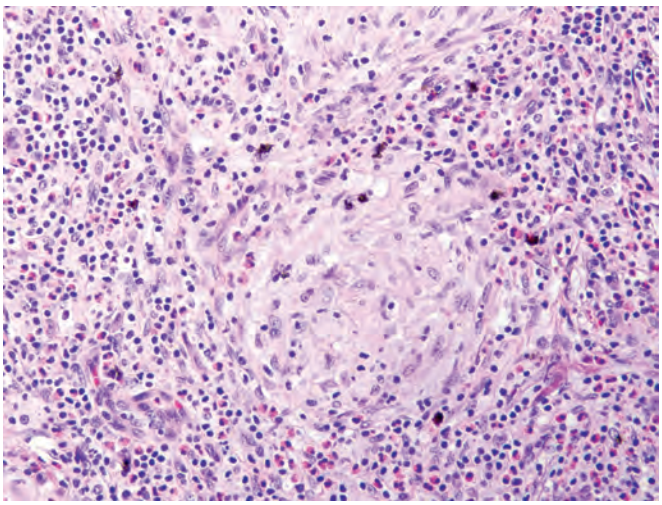
Peripheral blood eosinophilia and eosinophils admixed with granulomatous inflammation can be associated with chronic, disseminated coccidioidomycosis and may portend poor prognosis.[49,50] Figs. 4.21 and 4.22 demonstrate the FNA and lymph node biopsy findings, respectively, in a young man from Mexico who presented with lower extremity lymphedema, eosinophilia, and a history of an unknown type of fungal infection. Granulomatous inflammation and abundant eosinophils were seen, with very focal fungal spherules (not illustrated) present in the biopsy sample. Cultures of lymph node grew *C. immitis*. Special attention should be paid to the patient's history, to the country of origin or travel, and always to the culture results when inflammation is present.

Granulomatous Inflammation

Granulomatous inflammation is a type of chronic inflammation characterized by the presence of immunologically modified macrophages that have increased amounts of cytoplasm and cytoplasmic organelles that form in response to persistent microbial infections or large antigenic particles.[51] The term *granuloma* is used to describe the aggregation of these modified macrophages into nodular aggregates. Lymphocytes and plasma cells are also seen, usually at the periphery of granulomas. In cytologic preparations the transformed macrophages of granulomas have elongate oval- to boomerang-shaped nuclei and abundant, finely granular cytoplasm that is pink in H&E-stained slides and cyanophilic in Papanicolaou. The cytoplasmic borders are not well defined, and adjacent cells blend into one another. The cells bear some resemblance to epithelial cells and are called *epithelioid macrophages*. Multinucleated giant cells are often seen but not essential for the diagnosis of granuloma. Two FNA preparations showing noncaseating granulomatous inflammation are illustrated in Fig. 4.23. The Papanicolaou-stained sample



• **Figure 4.21** Fine-needle aspiration of inguinal lymph node from a case of disseminated coccidioidomycosis with marked peripheral blood eosinophilia. **A,** Mixed inflammatory cell reaction with presence of eosinophils (Romanowsky stain, ×1000). **B,** Giant cell macrophage (Romanowsky stain, ×400).



• **Figure 4.22** Lymph node biopsy specimen from the same patient as in Fig. 4.21. Well-formed granulomas are surrounded by an eosinophil-rich inflammatory exudate (hematoxylin and eosin stain, ×200).

derives from a cervical lymph node in a 33-year-old man who had sarcoidosis. The Romanowsky-stained preparation was taken from a lymph node FNA in a woman with tuberculosis and widespread lymphadenopathy. Noncaseating granulomas found in a lymph-node or visceral-organ FNA from an asymptomatic young person frequently indicates sarcoidosis; however, one must not be complacent. One cannot distinguish between sarcoidosis and infectious causes of noncaseating granulomas on the basis of morphology. Always culture the sample, and be sure that the clinician is aware of the patient's clinical and radiologic findings. Fig. 4.24 is taken from a tonsil biopsy of the man with sarcoidosis. It is easy to see why the term "epithelioid cells" is appropriate!

Caseating granuloma refers to the presence of epithelioid macrophages surrounding granular amorphous material that, by gross evaluation, has the appearance of crumbly white cheese. In Papanicolaou-stained cytologic preparations, caseating necrosis has a granular, gray-green appearance (Fig. 4.25). Entrapped, degenerated cells are seen, and, with luck, some epithelioid macrophages may be found. It is very important to recognize caseation in a cytologic preparation because acid-fast and fungal staining are indicated. In Romanowsky-stained preparations, caseous material is more faintly stained, usually rather pink, but the gross appearance of the material expelled from the FNA needle often provides a clue. It is creamy, somewhat granular, and often drier in appearance than pus. If the Romanowsky stain (see Fig. 4.25, inset) fails to demonstrate neutrophils and abundant amorphous material is seen in a compatible clinical setting, one should suspect caseation and request mycobacterial and fungal cultures. Epithelioid cell aggregates without caseation may be found in aspirates from chronic coccidioidomycosis, blastomycosis, and histoplasmosis. A presumptive diagnosis of sarcoidosis and therapy with steroids can prove deadly in patients with disseminated tuberculosis or fungal infection.

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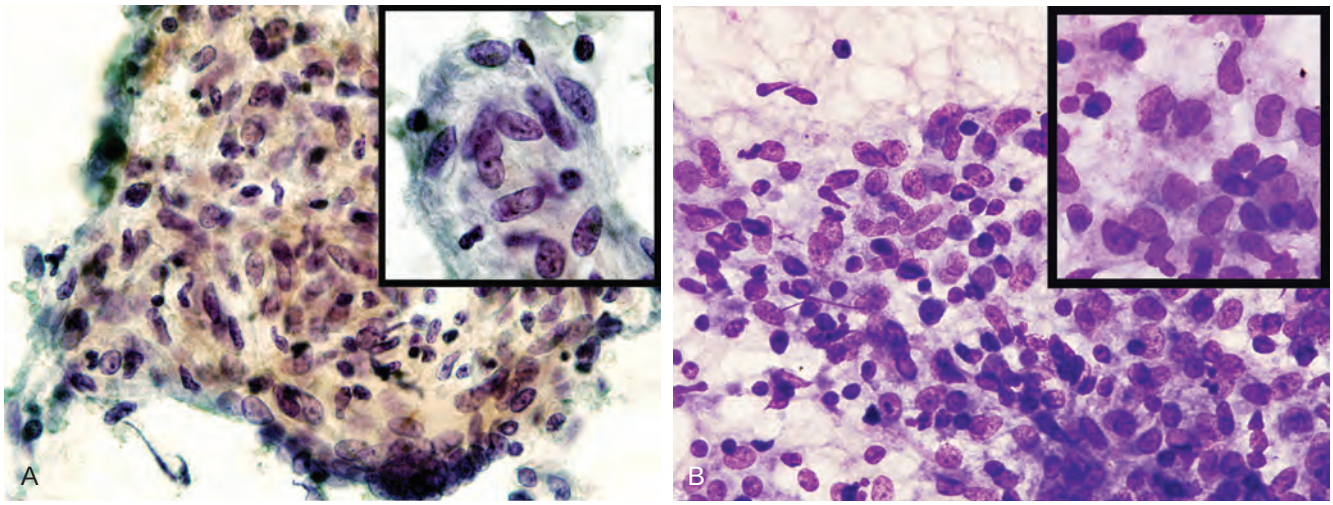
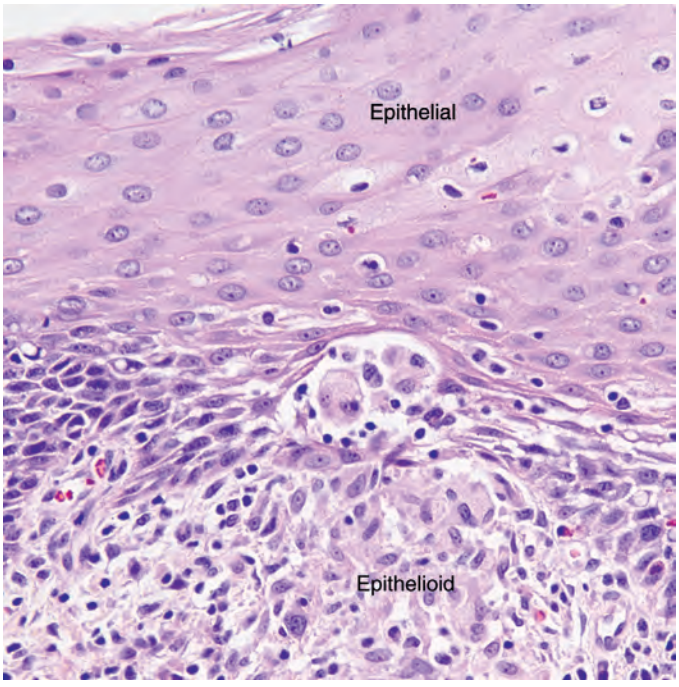
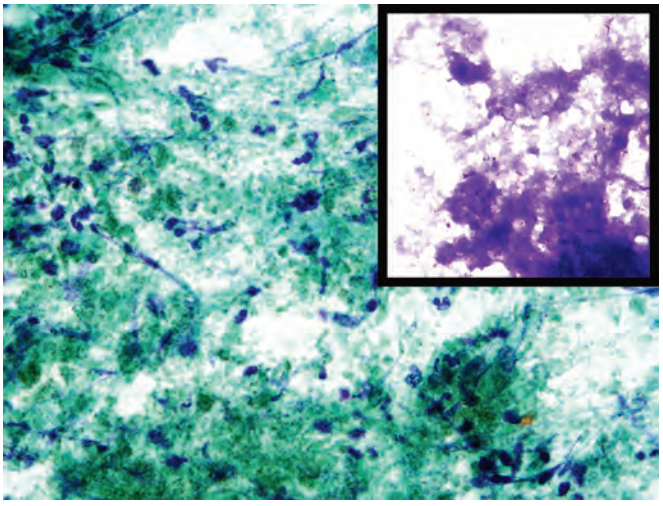


• **Figure 4.23** Fine-needle aspiration of lymph nodes that had noncaseating granulomas. **A,** Cervical lymph node from a patient with sarcoidosis shows aggregates of epithelioid macrophages with elongated to boomerang-shaped nuclei and poorly defined cytoplasmic borders (Papanicolaou stain, ×400; *inset* ×1000). **B,** Cervical lymph node from a woman with tuberculosis shows similar features (Romanowsky stain, ×400; *inset* ×1000).



• **Figure 4.24** Tonsil biopsy from a patient with sarcoidosis. Note the squamous epithelium above and the aggregates of epithelioid macrophages below (hematoxylin and eosin stain, ×400).



• **Figure 4.25** Fine-needle aspiration from caseating granuloma. Amorphous granular, green material is admixed with few cell nuclei (Papanicolaou stain, ×400). The material appears less granular and equally amorphous in Romanowsky stain (*inset:* Romanowsky stain, ×400).

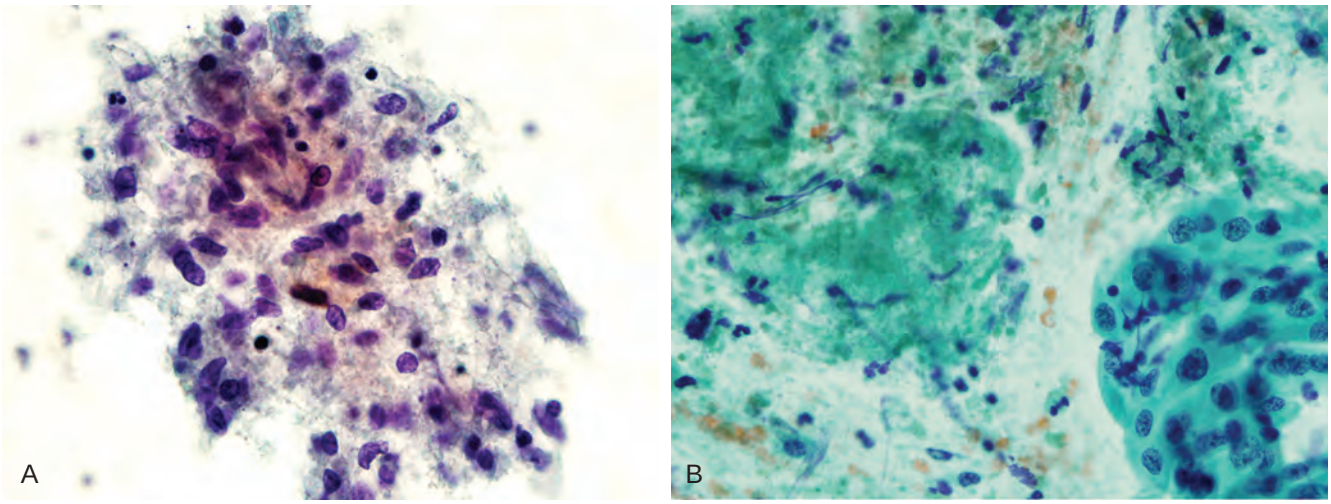
CASES 7 AND 8

A Tale of Two Tubercles

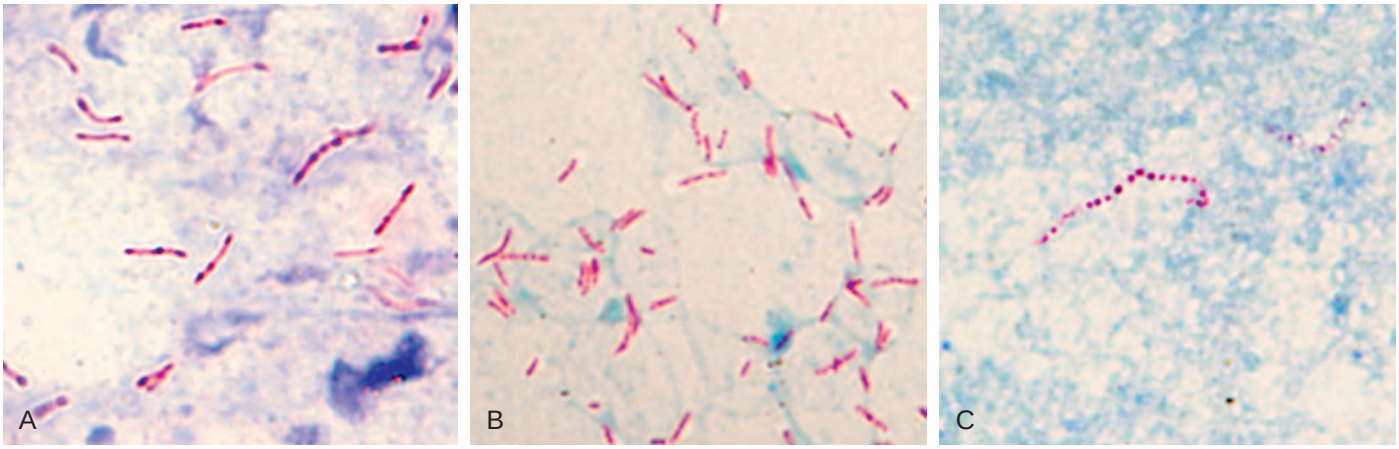
A 73-year-old man had complained of weight loss and cough. By CT, he was found to have a thick-walled cavitary mass in the right upper lung and two smaller masses near the pleura. Clinicians were concerned about cavitary SCC. A bronchial brushing slide contained the findings illustrated in Fig. 4.26A. Note the loosely formed epithelioid cells with interspersed granular material, indicative of caseating granuloma. Scanty acid-fast bacilli were seen on a Kinyoun stain performed over a Papanicolaou-stained brushing slide. Culture of the respiratory material from bronchoscopy grew *Mycobacterium avium* complex (MAC), and the cavity wall markedly thinned after therapy; however, 1 year later, a dependent, bulbous mass appeared within the base of the cavity. FNA revealed the presence of branching septate hyphae, and the diagnosis rendered was fungus ball arising in a preexisting cavity.

A 41-year-old woman also presented with cough and chest CT interpreted as suspicious for bronchogenic carcinoma. Abundant caseous material with reactive squamous metaplasia was seen (see Fig. 4.26B). Acid-fast bacilli were seen on Kinyoun stain performed over Papanicolaou-stained slides of bronchial brushing and bronchial washing samples, but culture of bronchoscopic material was not performed. One must keep in mind that mycobacterial infections can mimic neoplasia and that granular amorphous material in an FNA, bronchial brush, or transbronchial fine needle aspiration (TBNA) sample should lead one to request acid-fast (and fungal) stains as well as cultures. Caseation may, at times, be difficult to distinguish from coagulative tumor necrosis; however, outlines of necrotic neoplastic cells can usually be seen in the latter. Note that had ROSE been performed during bronchoscopy and caseous-like necrosis or granuloma been identified, the clinicians would have been instructed to culture bronchial brushing and washings. The following two caveats should be kept in mind:

1. Reactive squamous metaplasia can lead to misdiagnosis of SCC in cases of infections. We have seen markedly atypical squamous metaplasia associated with tuberculosis and actinomycosis.
2. MAC cannot be accurately distinguished from *M. tuberculosis* complex (MTC) by acid-fast stain, and MAC infections are not confined to severely Tcell–immunosuppressed patients.[52-55] Culture is required for identification of MOTT, and culture or presumptive identification by molecular methods, such as RNA amplification for MTC. *Mycobacterium kansasii* has a distinct morphology that can allow for presumptive diagnosis in some cases,[56,57] and MFC closely resembles *Nocardia* spp., as mentioned previously. The comparative acid-fast staining features of MTC, MAC, and *M. kansasii* are shown in Fig. 4.27. All slides were taken from cytologic samples.



• **Figure 4.26** Caseating granulomata diagnosed by bronchial brushings. **A,** A loosely formed aggregate of epithelioid macrophages admixed with amorphous granular material is seen (Papanicolaou stain, ×400). **B,** Amorphous granular material with entrapped nuclei is seen on the left, and reactive squamous metaplasia on the right. The latter can result in misdiagnosis of neoplasia (Papanicolaou stain, ×400).



• **Figure 4.27** Mycobacteria are seen in Kinyoun stain from cytologic preparations. **A,** *Mycobacterium tuberculosis* complex. **B,** *Mycobacterium avium* complex. **C,** *Mycobacterium kansasii* (Kinyoun stain, ×1000).

CASE 9

Willowy Macrophages and AIDS

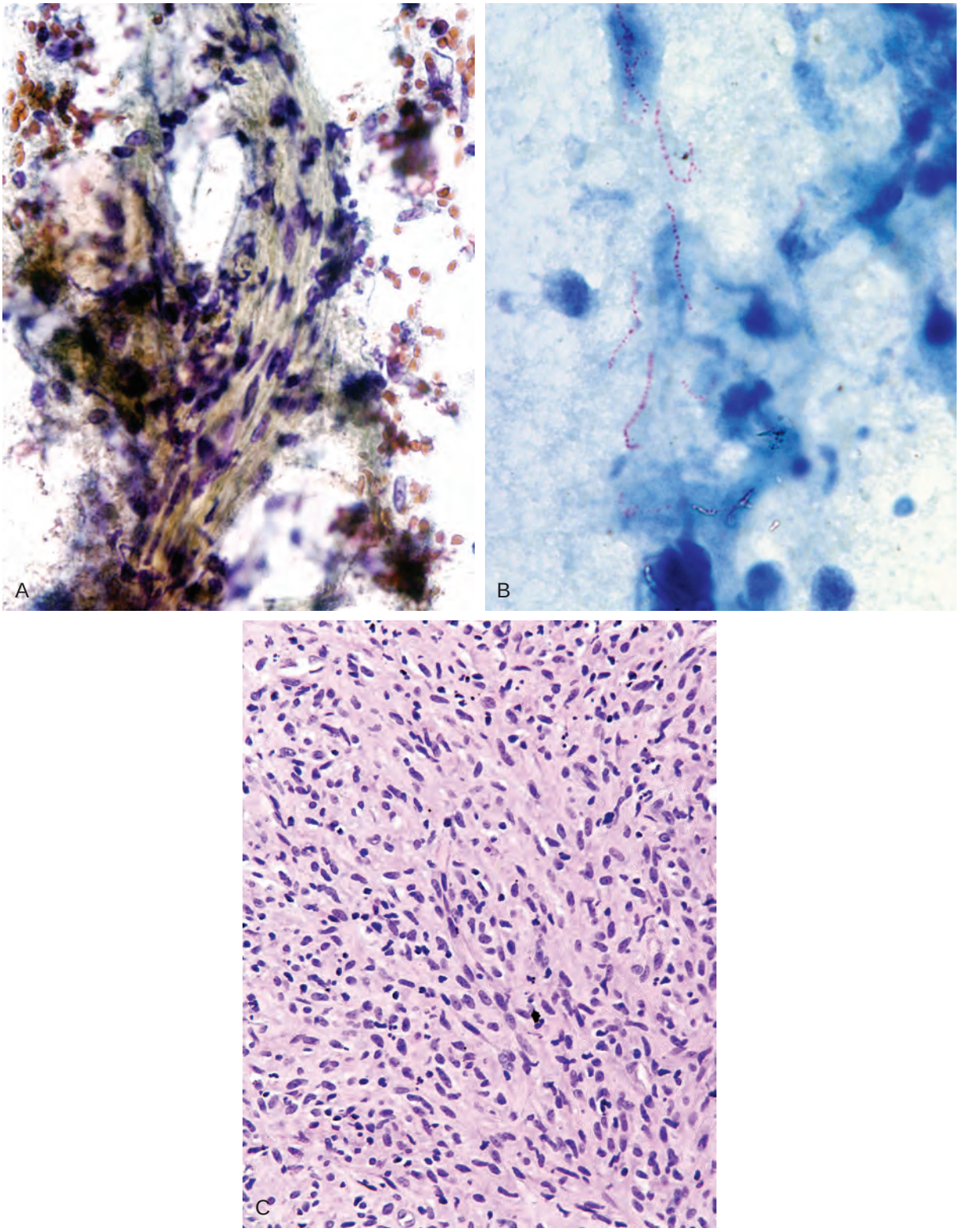
Fig. 4.28 illustrates the TBNA and transbronchial biopsy findings from a man with AIDS and mediastinal lymphadenopathy. Lymphoma was suspected. Note the presence of spindle-shaped cells that could be misinterpreted as an Epstein-Barr–associated smooth muscle tumor[58] or Kaposi sarcoma.[59] The spindle cells in this type of pseudotumor have immunohistochemical features of macrophages[60]; in an AIDS patient, they are highly suspicious for mycobacterial infection. MTC, MAC, and *M. kansasii* have all been isolated from patients with so-called spindle cell granulomas.[57,61] In this case, very long, coarsely beaded, acid-fast bacilli with curved ends are seen. Their shape has been compared to a shepherd's crook, although a Christmas candy cane might be a better simile. This is the classic appearance of *M. kansasii* and is the only type of mycobacteria for which we can usually make a presumptive species identification on the basis of morphology. MFC also has a relatively unique appearance; however, as previously stated, differentiation from *Nocardia* is problematic.

CASE 10

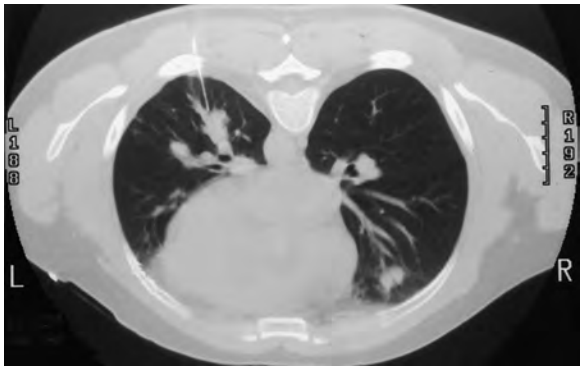
Granulomatous Inflammation With Vesiculose Macrophages

A 37-year-old HIV-seronegative man was referred for evaluation of an abnormal chest radiograph. CT showed multiple bilateral pulmonary opacities 1 to 4 cm in diameter (Fig. 4.29). An atypical infectious process was favored, and a CT-guided FNA was performed. Findings are illustrated in Fig. 4.30. This patient was immunocompetent, and epithelioid macrophages, giant cells, lymphocytes, and plasma cells were present, consistent with granulomatous inflammation; however, the macrophage cytoplasm had a vesiculated rather than granular appearance. To best see the intracytoplasmic structures, the substage condenser must be closed. This gives fungi a refractile appearance and makes them much easier to see.[9] On high-power examination, one sees relatively round structures that are clear to slightly pink. The fungi lack abundant capsular material, as is common in infections with this pathogen in immunocompetent or mildly immunosuppressed patients. Note the considerable variation in size, a very important clue to the diagnosis! These organisms typically vary from 5 to 20 μm in diameter. This is well demonstrated in the GMS stain (Fig. 4.31). Also note the thin cell wall and round shape, which are typical of *Cryptococcus*. *Cryptococcus* infection in T cell–immunosuppressed patients is discussed in the next section.

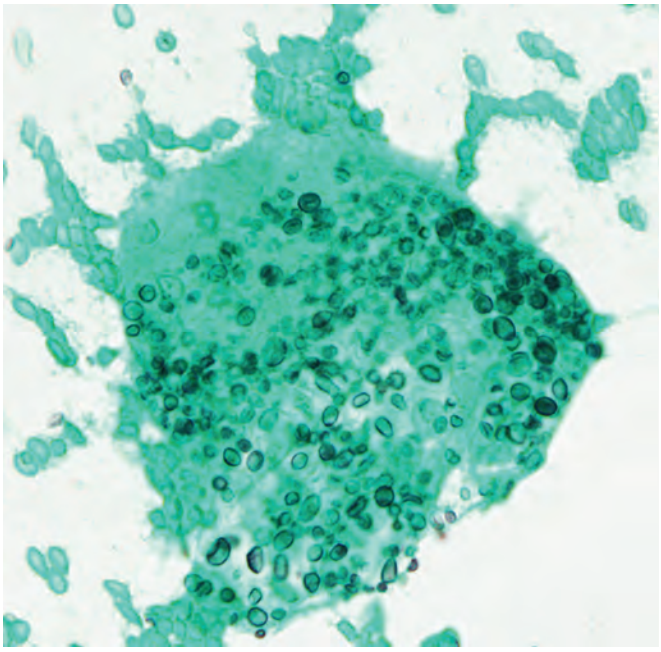
One should be aware that cryptococci are easily overlooked in H&E- and Papanicolaou-stained preparations. They may also be mistaken for phagocytized erythrocytes or lipid (Fig. 4.32). Remember to close the substage condenser! Paucicapsular forms of *Cryptococcus* often stain weakly with mucicarmine. Fontana-Masson melanin stain is recommended in these cases.[24-25] Remember, the marked variation in diameter of cryptococcal yeast forms helps one to distinguish this fungus from both erythrocytes and other yeastlike pathogens.



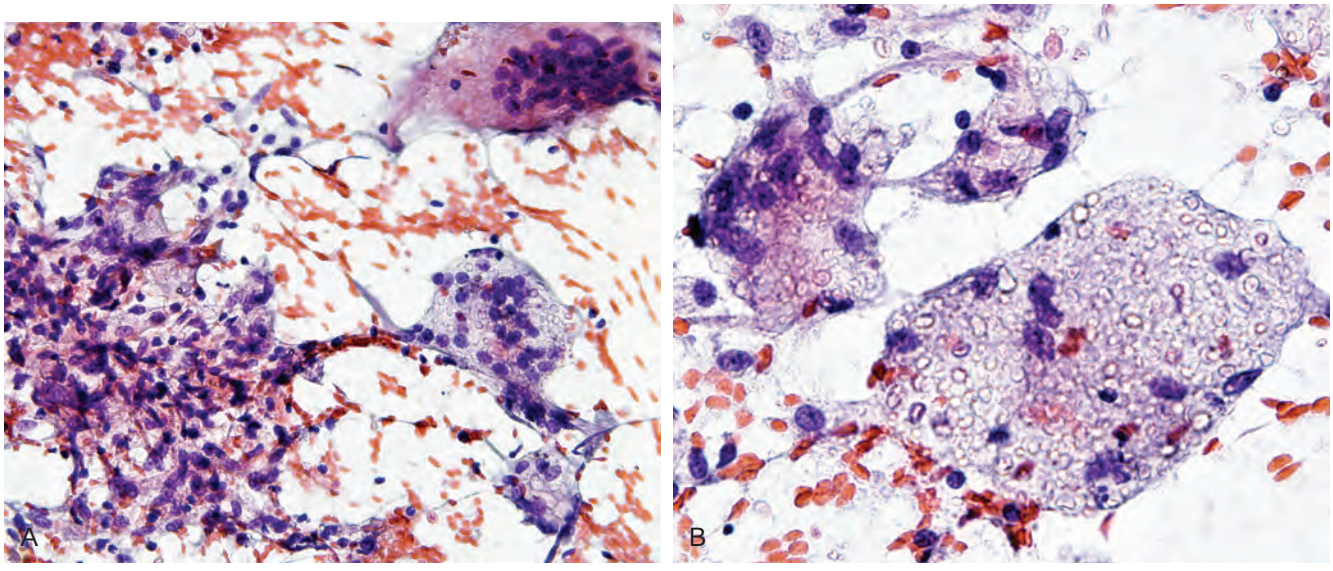
• **Figure 4.28** *Mycobacterium kansasii* spindle-cell granuloma in an acquired immune deficiency syndrome patient. **A,** Transbronchial fine-needle aspiration (FNA) shows a group of spindly cells (Papanicolaou stain, ×600). **B,** Long, coarsely beaded bacilli are seen in an acid-fast stain of the FNA sample (Kinyoun stain, ×1600). **C,** Transbronchial biopsy from the same patient (hematoxylin and eosin stain, ×600).



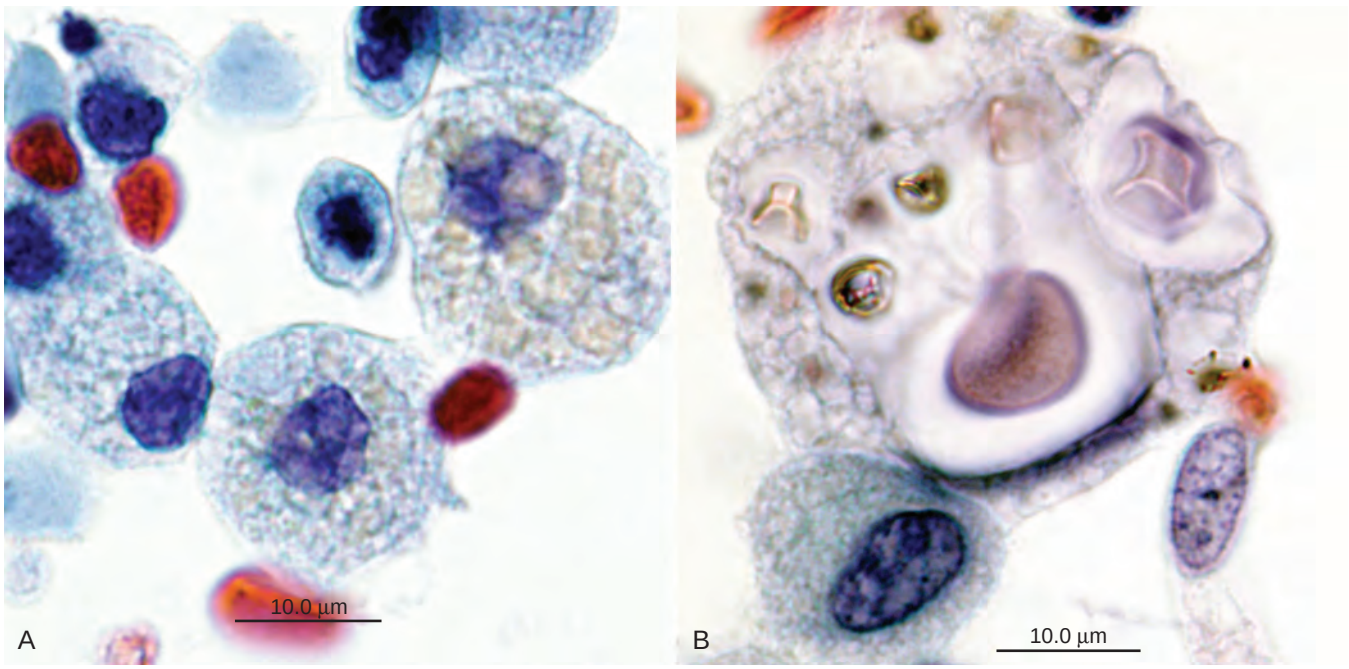
• **Figure 4.29** Chest computed tomogram showing multiple, bilateral pulmonary opacities, one of which was sampled by percutaneous fine-needle aspiration (see Fig. 4.30).



• **Figure 4.31** Grocott-Gomori methenamine silver (GMS)-stained slide from the same patient as in Figs. 4.29 and 4.30, showing round shape and variability in size of yeast forms (GMS stain, ×400).



• **Figure 4.30** Fine-needle aspiration of the pulmonary lesions illustrated in Fig. 4.29. **A,** An aggregation of macrophages and several multinucleated giant cells are seen (Papanicolaou stain, ×200). **B,** Multinucleated cells contain refractile, round yeast forms (Papanicolaou stain, ×400).

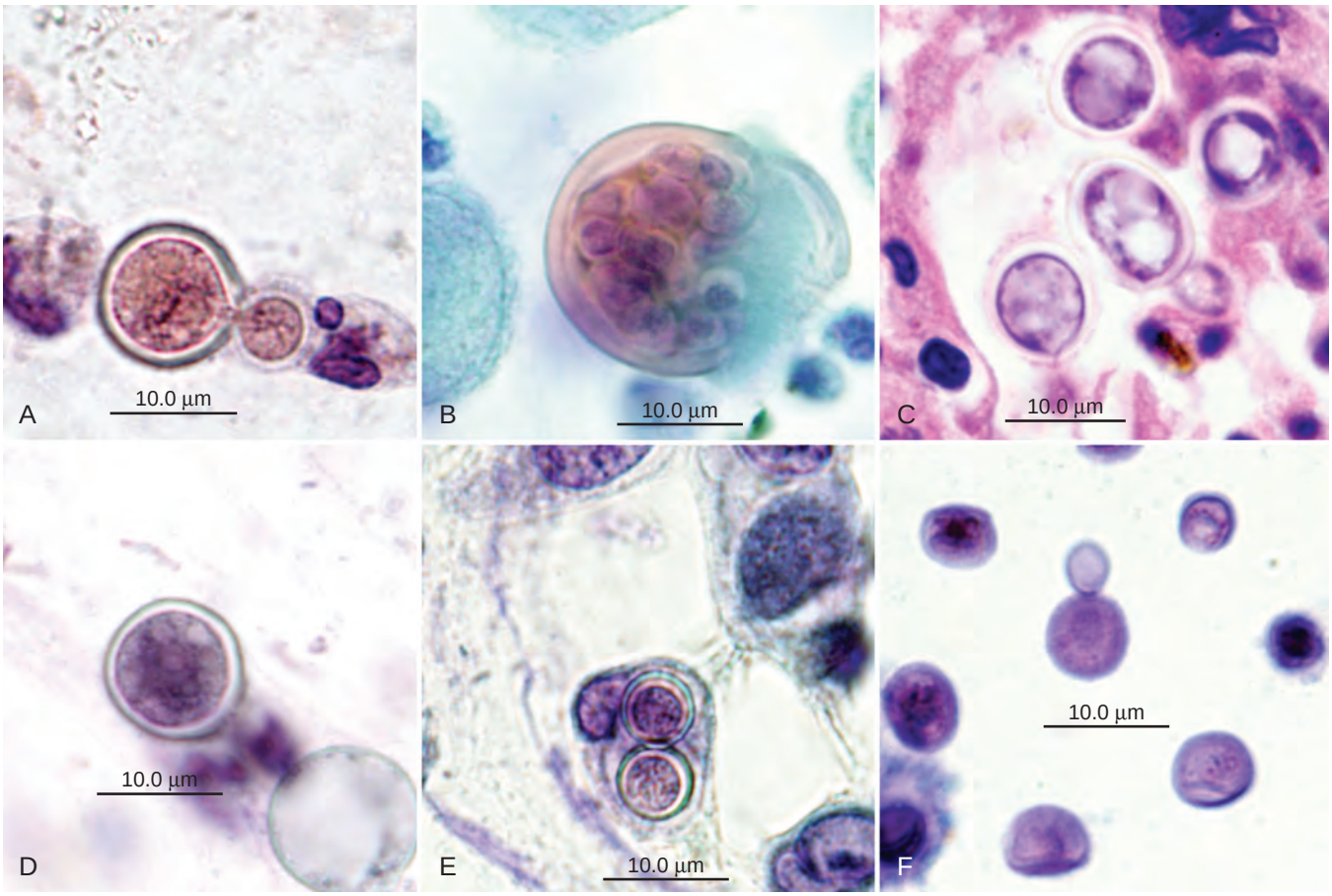


• **Figure 4.32** Comparison of lipid- and erythrocyte-laden macrophages to *Cryptococcus*. **A,** Intraoperative aspiration of a Rathke cleft cyst of the brain. Macrophages containing lipidlike vacuoles and degenerating erythrocytes are seen (Papanicolaou stain, ×2000). **B,** Bronchoalveolar lavage specimen containing macrophages with phagocytized *Cryptococcus neoformans*. Note marked variation in size of yeast forms and the presence of refractile granular material within some yeast (Papanicolaou stain, ×2000).

Granulomatous Inflammation Admixed With Neutrophils

A mixture of epithelioid macrophages and neutrophils is the typical inflammatory response to many of the dimorphic fungi, including *C. immitis, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Sporothrix schenckii,* and the dematiaceous fungi associated with chromoblastomycosis and phaeohyphomycosis.[30] Neutrophils are rarely seen in cases of histoplasmosis or cryptococcosis. *H. capsulatum* and *Cryptococcus neoformans* usually elicit either a granulomatous, diffuse macrophage reaction or, in the case of *Cryptococcus*, scant to absent inflammation. Diffuse macrophage infiltration, typically found in patients with deficient Th1 cell-mediated immunity, is discussed in the following section. In dimorphic fungal infections, the proportion of epithelioid macrophages to neutrophils varies with the host's ability to interact with the pathogen. In chronic, indolent infections, one sees a predominance of granulomatous inflammation. In fulminant infections, neutrophils predominate. In advanced AIDS, abundant necrosis admixed with myriad fungi and varying numbers of neutrophils is typical.

It is important that the cytologist know which stains best demonstrate the features of the dimorphic fungi. Although GMS is often considered the gold standard and is very sensitive, it is not the best stain for assessment of distinctive fungal features. *C. immitis, B. dermatitidis,* and *P. brasiliensis* are best seen on H&E or Papanicolaou stain. These stains allow one to scrutinize the internal features of the organisms, including cell wall, endoplasm, and nuclei. In the case of *C. immitis*, endospore formation can be seen. Agents of chromoblastomycosis and phaeohyphomycosis, dematiaceous fungi, are also usually well seen on H&E and Papanicolaou stains. Chromoblastomycosis pertains to infections localized to the epidermis and dermis and is characterized by presence of multiseptate bodies or Medlar bodies. Phaeohyphomycosis pertains to subcutaneous or visceral infections.[62] In phaeohyphomycosis, hyphae and moniliform pseudohyphae usually predominate; however, we have seen multiseptate bodies admixed with hyphae in an FNA in one case of disseminated phaeohyphomycosis caused by *Fonsecaea pedrosoi*.[63] Fig. 4.33 shows the comparative features of *B. dermatitidis, C. immitis,* and *P. brasiliensis*, which can easily be confused in cytologic or histologic preparations. Note the pseudococcioidoid, nonbudding *Blastomyces* in Fig. 4.33D and the paired pseudoblastomycoid *Coccidioides* immature spherules in Fig. 4.33E. *Cryptococcus* rarely is confused with these in H&E- or Papanicolaou-stained material because it lacks a thick, double refractile cell wall, but an image is included (see Fig. 4.33F) to illustrate the contrast between *C. neoformans* and the others. In GMS-stained preparations, differentiation of yeastlike fungi is more problematic. *Sporothrix schenkii*, usually a localized skin pathogen, can infrequently cause pulmonary or disseminated infections and may be difficult to distinguish from other dimorphic fungi by GMS stain and difficult to visualize by routine stains. In GMS, it is usually 3 to 5 µm in width and may demonstrate elongated, "cigar-shaped" blastoconidia (See Appendix).

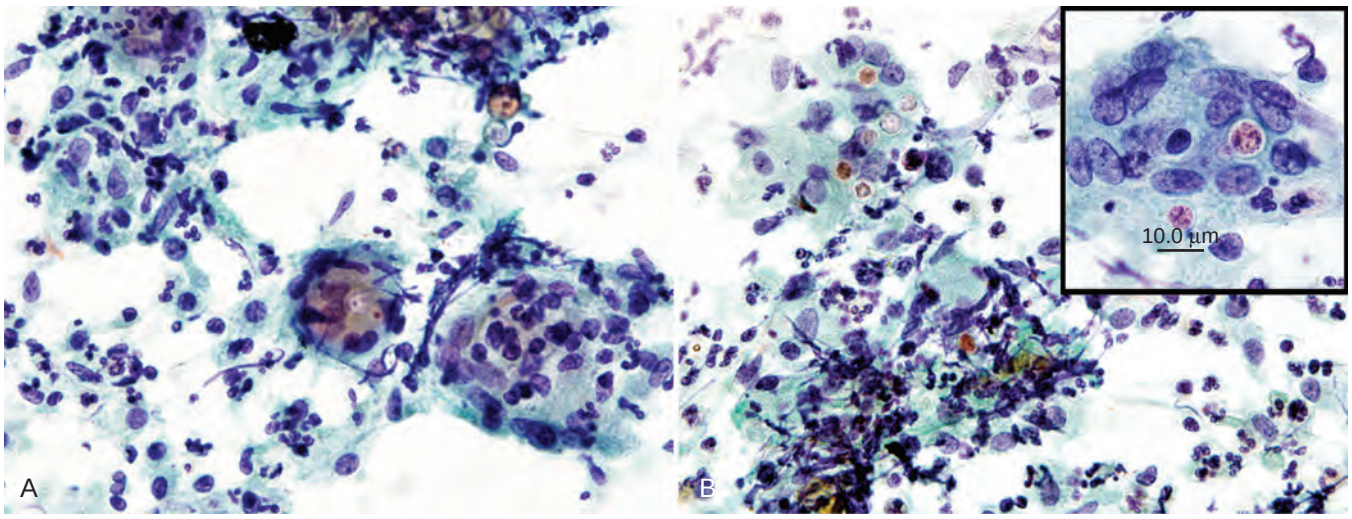


• **Figure 4.33** Comparison of *Blastomyces dermatitidis, Coccidioides immitis, Paracoccidioides brasiliensis,* and *Cryptococcus neoformans*. **A,** *B. dermatitidis*. The yeast form is approximately 12 µm in diameter, with a broad-based bud and a thick, refractile cell wall. A narrow clear zone separates the fungal protoplasm from the cell wall. **B,** Mature spherule of *C. immitis*. The spherule has a round shape, is relatively large, has a thick cell wall, and contains multiple round endospores. **C,** Yeast forms of *P. brasiliensis* lack the classic "ship's wheel" budding. Yeasts are more variable in size than *Blastomyces* but are easily confused with both *Blastomyces* and with immature forms of *Coccidioides*. **D,** Nonbudding form of *Blastomyces*. **E,** Closely apposed immature spherules of *Coccidioides immitis* imitating *Blastomyces*. **F,** Yeast forms of *Cryptococcus neoformans* (**A, B,** and **D-F,** Papanicolaou stain, ×2000; **C** and **E,** hematoxylin and eosin stain, ×2000).

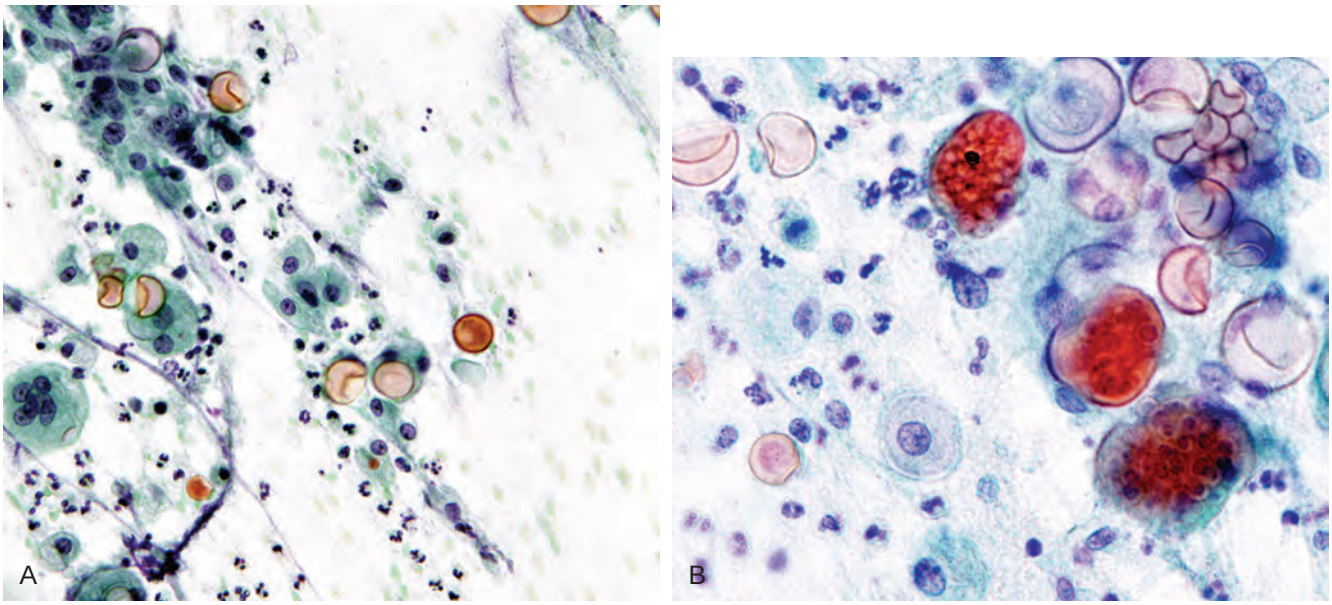
CASE 11

The Community-Acquired Pneumonia That Wasn't

A 21-year-old male student from eastern Louisiana underwent bronchoscopy because of a right lower lung infiltrate, chills, and fever that failed to respond to therapy for community-acquired pneumonia. The man had no history of alcohol or drug abuse and was HIV negative. His bronchial brushing material is illustrated in Fig. 4.34. Note the combination of epithelioid macrophage aggregates and neutrophils (see Fig. 4.34A). This type of inflammatory pattern and the clinical history should generate a search for dimorphic fungi. In Fig. 4.34B, one sees the characteristic features of *B. dermatitidis*. In tissue, *B. dermatitidis* is seen as yeastlike cells that are 8 to 15 µm in length and relatively uniform in size (in contrast to *Cryptococcus, Coccidioides* spherules, and *Paracoccidioides*); the cell wall is thick and double contoured, and the endoplasm is typically retracted so as to leave a clear zone between the cell wall and the internal structures (see Fig. 4.33A and D). Under very high magnification, multiple nuclei can sometimes be seen as tiny dots, but these may be difficult to discern in Papanicolaou and H&E stains. Broad-based budding is typical of *Blastomyces* and is useful if found (see Fig. 4.33A). The main differential includes *P. brasiliensis* and *C. immitis* (see Fig. 4.33). Because these organisms have different geographic distributions,[30] knowing the travel history or region of birth of the patient is extremely helpful. *P. brasiliensis* is thick-walled like *B. dermatitidis*, but it has narrow-necked budding, and multiple buds are common. The characteristic "ship's wheel" budding pattern is lovely and diagnostic if you can find it, but it may be difficult to find. A very useful differentiating feature is the marked variability in size that is found in *P. brasiliensis* compared with *B. dermatitidis*. The greatest diagnostic pitfall is the immature spherule of *C. immitis*, which may closely resemble *B. dermatitidis* and *P. brasiliensis* (see Fig. 4.33). The *Paracoccidioides* yeast forms illustrated here derive from a lung section rather than a cytologic specimen and are shown for the sake of comparison.



• **Figure 4.34** Bronchial brushing from a case of pulmonary blastomycosis. **A,** An inflammatory exudate composed of neutrophils, epithelioid cells, and giant cells is seen (Papanicolaou stain, ×400). **B,** Yeast like cells are seen within giant cell macrophages (Papanicolaou stain, ×400). Higher magnification of yeast forms is seen in the *inset* (Papanicolaou stain, ×1000).



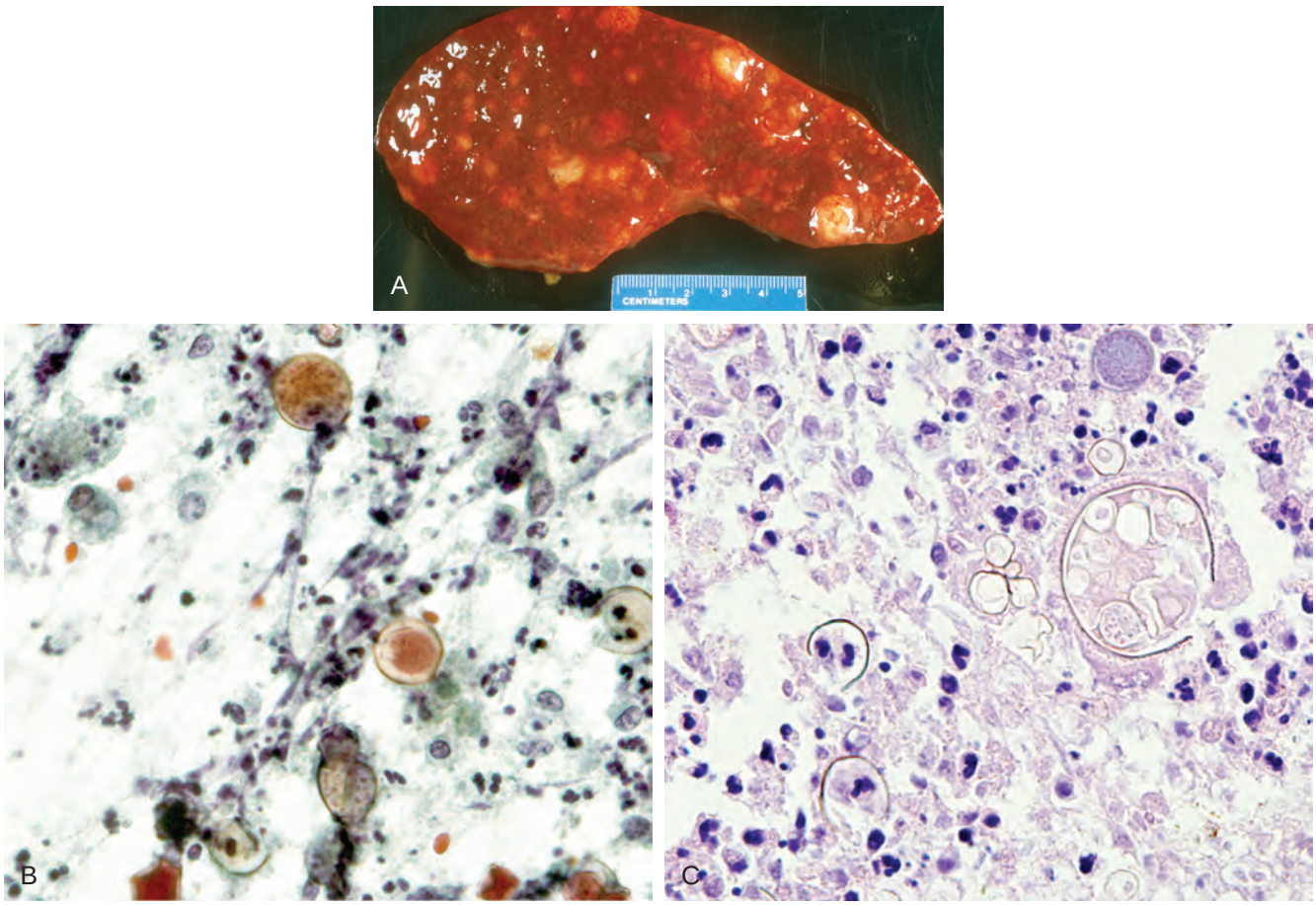
• **Figure 4.35** Fine-needle aspiration of subcutaneous coccidioidomycosis. **A,** A mixture of neutrophils, epithelioid macrophages, and giant cells is seen, along with empty and collapsing spherules (Papanicolaou stain, ×200). **B,** Mature endosporulating spherules are seen, along with empty and collapsed forms (Papanicolaou stain, ×400).

CASE 12

Fluctuant and Full of Fungi

A 26-year-old man who had been working in the Texas Rio Grande Valley presented with fever and several fluctuant, subcutaneous masses. Chest radiography revealed diffuse interstitial infiltrates. FNA was performed from a 3 × 3 cm subcutaneous mass overlying the fifth lumbar vertebra. Several milliliters of gray-colored, thick material were aspirated. A Papanicolaou-stained slide of aspirate is illustrated in Fig. 4.35. Spherules of markedly varying sizes are seen in a background of necrosis, giant cells, and neutrophils. Endosporulation is seen within some of the spherules. Note that immature and empty spherules that lack endosporulation can readily be mistaken for pollen grains, *Blastomyces*, or *Paracoccidioides*. Cutaneous and subcutaneous lesions of *Blastomyces, Paracoccidioides,* and *Coccidioides* typically represent disseminated pulmonary disease; however, in some cases, pulmonary lesions are not apparent. This was a fulminant case, and neutrophils and necrosis are prominent, although epithelioid macrophages and giant cells are also seen.

This patient tested negative for HIV. In patients with coccidioidomycosis and advanced AIDS, extensive necrosis, myriad organisms, and variable numbers of neutrophils and macrophages are seen. Granulomas are absent (Fig. 4.36). Similar histologic reactions to *Paracoccidioides, Blastomyces,* and *Sporothrix* have been reported in AIDS patients.[64-67]

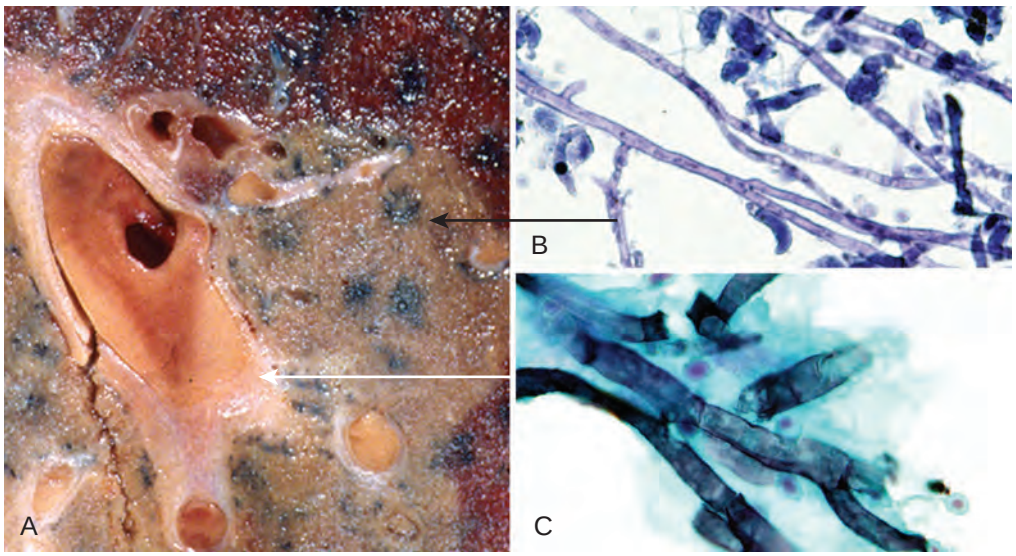


• **Figure 4.36** Disseminated coccidioidomycosis in a patient with AIDS. **A,** Gross photograph of spleen at autopsy. **B,** Postmortem scraping of one of the splenic lesions shows necrotic debris, scanty macrophages and neutrophils, and abundant spherules (Papanicolaou stain, ×400). **C,** Postmortem section of spleen shows the presence of abundant necrosis and spherules and relatively scanty inflammation (hematoxylin and eosin stain, ×400).

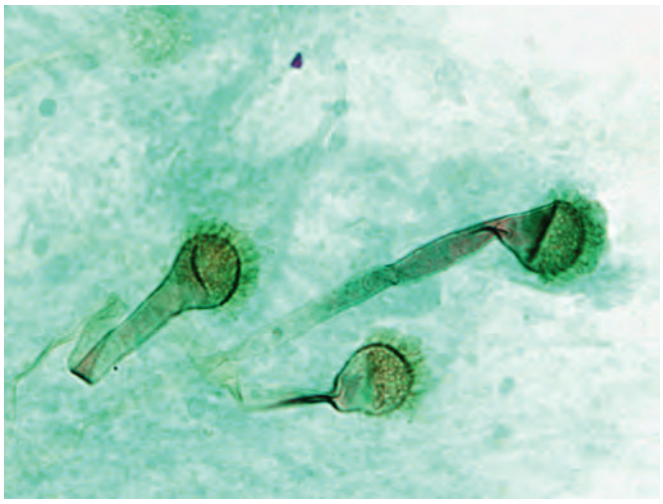
Unusual Host Reactions to Infections in the Immunocompromised Patient

Patients With Neutropenia or Defective Neutrophils

Neutropenic patients are susceptible to a number of infections, including pyogenic bacteria, invasive aspergillosis, candidiasis, and mucormycosis. In these patients, one sees abundant organisms but not the expected purulent inflammatory reaction. Patients with adequate numbers of neutrophils but defective neutrophil function (e.g., in diabetes) also are at risk for invasive infections with these fungi. Vascular invasion is typical of invasive aspergillosis, candidiasis, and mucormycosis, although this phenomenon is usually appreciated on histologic sections rather than in the cytology laboratory. However, we have demonstrated fungi within rounded infarcts and thrombosed blood vessels by postmortem cytology.[5] Fig. 4.37 shows the gross and scrape preparation findings from the autopsy of a severely neutropenic patient with multiple round pulmonary infarcts and vascular thromboses. Fungal hyphae were found within the infarcts and blood vessels by cytology. Examination of sections confirmed the presence of invasive fungal disease. Postmortem cultures grew *Aspergillus glaucus*. Normally, the clinical history and radiologic features are needed to differentiate between aggressive, invasive fungal disease and more indolent invasive disease, such as chronic necrotizing aspergillosis[68] or intrabronchial zygomycosis.[69,70] However, using the clinical and radiologic information, the cytologist can and should discuss the possibilities with the clinician. The variety of diseases associated with *Aspergillus* alone is mind boggling and includes noninvasive fungus ball, ABPA and mucoid impaction, chronic necrotizing aspergillosis, ulcerative bronchitis, and aggressive invasive aspergillosis, to name a few.[68,71,72] It is important to note that there are cases of chronic necrotizing zygomycosis that are analogous to chronic necrotizing aspergillosis. These tend to occur in patients with mild neutrophil dysfunction as occurs in diabetes mellitus. Allergic disease can be diagnosed on the basis of allergic mucin, as discussed previously; however, the other potential diagnoses require careful clinical correlation. By tissue morphology alone, *Aspergillus* cannot be definitively differentiated from other hyalohyphal fungi unless conidia-bearing fruiting heads are seen. Fig. 4.38 demonstrates conidiophores and fruiting heads of *Aspergillus fumigatus* found in a bronchial brushing from a patient with endobronchial aspergillosis. This finding so mesmerized the cytopathologist that the cells deriving from small cell carcinoma, also present in the preparation, were overlooked. Zygomycetes, as a group, can be differentiated from other mycelia-producing fungi (Fig. 4.39); however, the genus cannot be



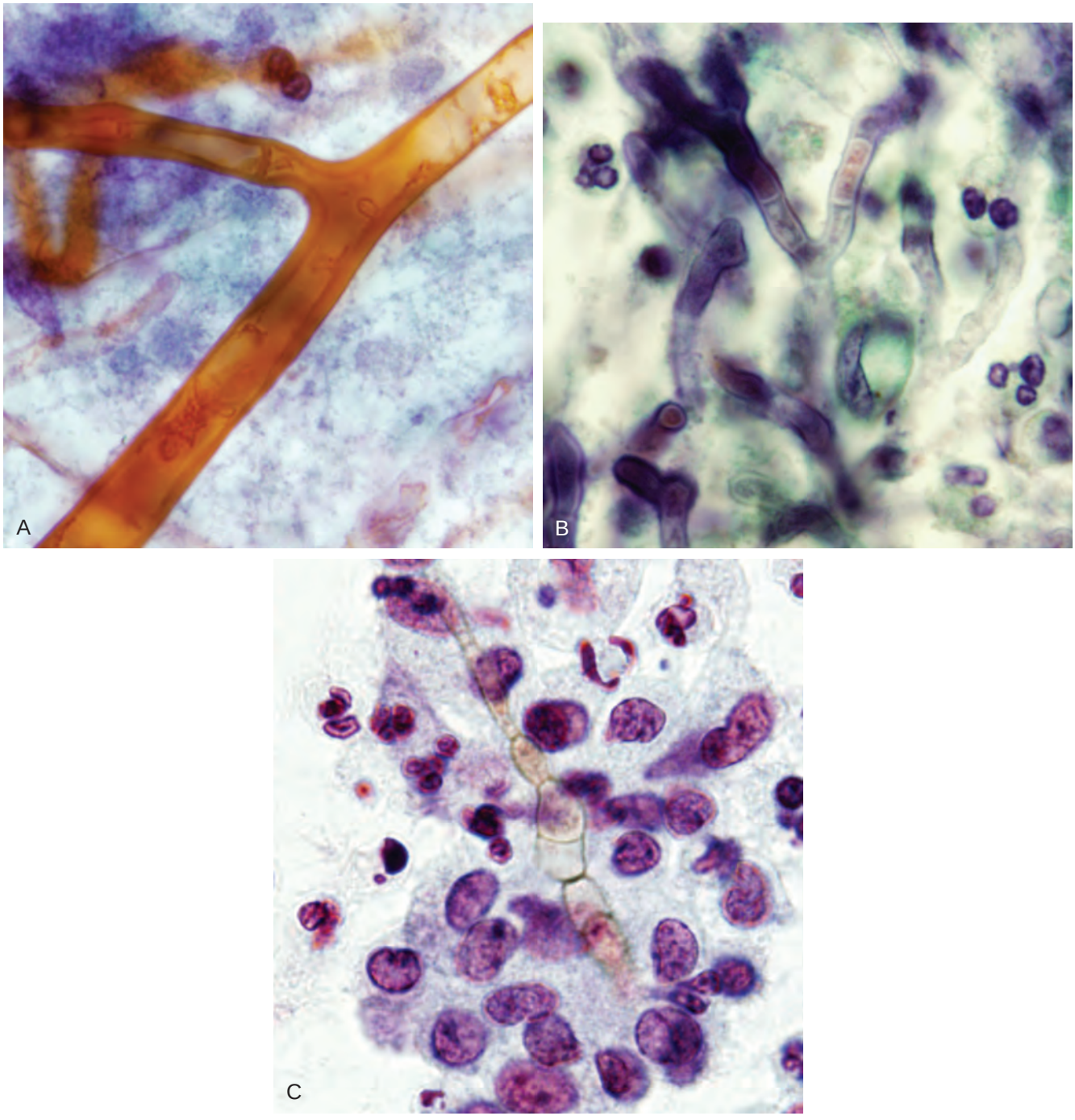
• **Figure 4.37** Invasive pulmonary aspergillosis in a severely neutropenic patient. **A,** Cross-section of lung taken at autopsy shows the presence of round, pale regions and vascular thromboses. **B,** Scraping of pale round area indicated by black arrow. Fibrin and fungal hyphae with narrow angle branching are seen (Papanicolaou stain, ×1000). **C,** Scraping of intravascular thrombus indicated by white arrow. Fungal hyphae are present (Grocott-Gomori methenamine silver stain, ×1500).



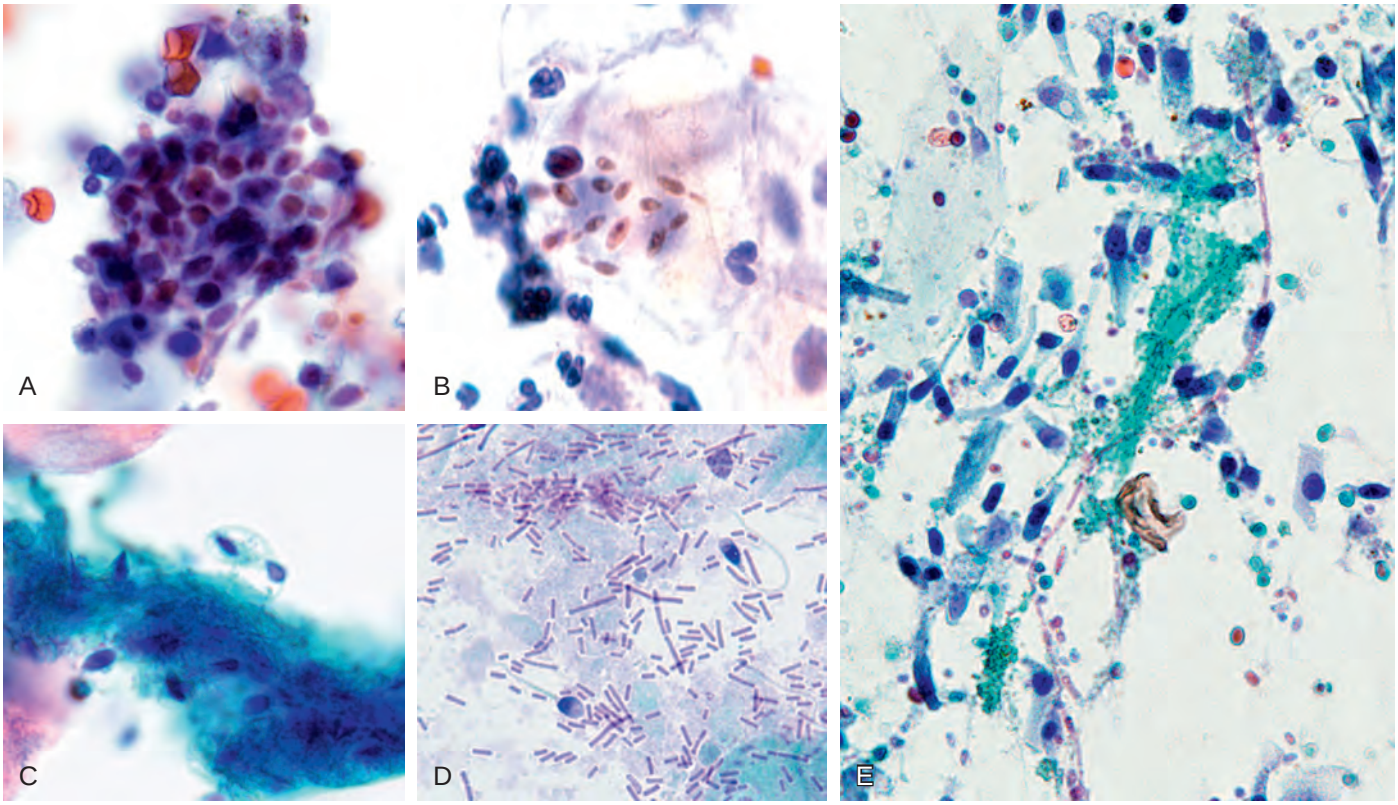
• **Figure 4.38** Bronchial brushing from endobronchial aspergillosis with formation of fruiting heads. Conidiophores contain the typical fruiting heads of *Aspergillus fumigatus* (Grocott-Gomori methenamine silver stain, ×1500).

identified in the absence of fruiting heads. Although the hyphal structures of zygomycetes and aspergillus (or other non-zygomycete hyalohyphal fungi) can be confused in tissue sections, it has been our experience that confusion is less likely in cytologic preparations, owing to the greater thickness and transparency of Papanicolaou-stained smears and the dense, often yellow-orange staining of zygomycetes (Fig. 4.39A). Dematiaceous fungi responsible for phaeohyphomycosis can often be differentiated from hyalohyphal fungi by the presence of light brown pigmented cell walls, presence of moniliform pseudohyphae, and positive melanin staining; however, neither genus nor species can be determined by tissue morphology. Fig. 4.39C illustrates the FNA findings from a case of subcutaneous phaeohyphomycosis.

Candida infections (Fig. 4.40A,B, and D) can be superficial and limited to the epithelium of mucous membranes, as in thrush and vulvovaginitis, or they can be locally invasive or aggressive, with vascular invasion and hematogenous dissemination. *Candida* found in cervical Pap test slides usually derives from a superficial infection, and aggregates or stacks of squamous cells are seen entangled in pseudohyphae, often with scanty inflammation. Vaginal candidiasis is discussed elsewhere in this text. *Candida* vaginitis is rarely misdiagnosed as another type of infection, but we have seen a couple of instances of spermatozoa misinterpreted as *Candida* in liquid-based preparations. In liquid-based cervical Pap tests, *Candida* yeast forms usually have a reddish homogeneous staining and budding can be demonstrated because spermatozoa heads tend to have a two-toned, blue-clear appearance and occasionally a tail can be seen (Fig. 4.40C and D). Invasive candidiasis cannot be distinguished from upper tract contamination or superficial candidiasis on the basis of brushings, washings, or BAL; however, the finding of blastoconidia and pseudohyphae in a visceral organ aspirate or an ulcerative lesion with pseudomembrane formation should suggest invasive fungal infection in the proper clinical setting. Fig. 4.40E illustrates the postmortem scrapings from the rectum of a severely neutropenic patient with ulcerative, pseudomembranous lesions of the esophagus and rectum. In the Papanicolaou-stained sample, note the fibrinous exudate associated with presence of colonic-type columnar epithelium and fungal pseudohyphae. Cytologic evaluation of ulcer scrapings allowed for a preliminary diagnosis of candidiasis and excluded other causes of ulcerative mucosal lesions (e.g., herpesvirus, cytomegalovirus). On the basis of the gross appearance, at least superficial invasive disease was suspected. Candidiasis was confirmed by histologic sections examined subsequently. Papanicolaou- and Romanowsky-stained esophageal endoscopic scrapings are routinely used at our institution for presumptive diagnosis of esophageal candidiasis and to rule out Herpes esophagitis. The only fungus likely to be confused with *Candida* is *Trichosporon*, which can cause invasive disease in immunosuppressed hosts. Differentiation is by culture and the slightly larger size, greater pleomorphism, and presence of arthroconidia in GMS-stained preparations of *Trichosporon*.[73]



• **Figure 4.39** Comparison of fungal hyphae in Papanicolaou-stained preparations. **A,** Zygomycete (*Rhizopus* sp. by culture) with easily seen broad hyphae. **B,** Culture-proven *Aspergillus* sp. showing more narrow hyphae. **C,** Dematiaceous fungus (*Phialophora verrucosa* by culture) showing brown cell wall and moniliform pseudohyphae (Papanicolaou stain, ×2000).



• **Figure 4.40** Bronchoalveolar lavage. **A,** Cluster of *Candida* yeast forms. Cervical Papanicolaou (Pap) smear. **B,** *Candida* yeast forms with budding. Cervical liquid-based Pap test **(C)** Cluster of ovoid spermatozoa heads admixed with seminal vesicle material. Cervical Pap smear **(D)** Spermatozoa admixed with large bacilli consistent with lactobacilli. Postmortem scrape preparation of *Candida albicans* pseudomembranous proctitis **(E)**. Colonic columnar epithelial cells and fungal blastoconidia and pseudohyphae are seen admixed with fibrin (all Papanicolaou stain, ×400).

CASE 13

Diabetes and Intrabronchial Zygomycosis With Indolent Course

A 37-year-old diabetic man presented with isolated solitary pulmonary and renal lesions. Sputum, urine, and kidney FNA contained zygomycete fungi. Urine and kidney FNA findings are seen in Fig. 4.41. Note the presence of neutrophils and broad, well-stained, ribbonlike hyphae typical of zygomycetes. This patient proved to have locally invasive intrabronchial zygomycosis with an isolated mycotic renal abscess. Cultures of both sites grew *Rhizopus* spp. This case illustrates an unusual, indolent presentation of zygomycosis in a nonneutropenic patient who had diabetes mellitus.[70] Cytologic examination could only diagnose zygomycosis. Histologic studies of the resected lung lesion demonstrated chronic, intrabronchial zygomycosis, a condition analogous to chronic necrotizing aspergillosis. The kidney lesion was also resected, and no evidence of other infected sites was found subsequently.

Severely Impaired Cell-Mediated Immunity and Diffuse Macrophage Infiltration

Patients with severe defects in cell-mediated immunity, such as occurs in HIV infection, have defective T lymphocyte- and macrophage-mediated killing of fungi and *Mycobacteria*. Neither effective killing of microbes nor granuloma formation occurs in these patients. Instead, in some infections, one sees a diffuse infiltration of macrophages containing massive numbers of microorganisms. Several different types of microbes, including *Mycobacteria* (especially MAC), *Leishmania*, and *Histoplasma*, elicit granulomas in immunocompetent patients and diffuse macrophage infiltration in patients with defective cell-mediated immunity. The host reaction to infection with *C. neoformans* varies with the immune status of the individual and the amount of cryptococcal encapsulation.[74,75] Alveolar macrophages and T cell–mediated immunocompetence are of major importance in the defense against this organism.[74] Although neutrophils are believed to be important as a second line of defense, they are rarely seen in histologic or cytologic preparations from cases of cryptococcosis. The inflammatory response to *Cryptococcus* varies from well-formed granulomas, as found in chronic, localized infections, to disseminated infection with absence of granuloma formation and minimal inflammation, predominantly macrophages. *Histoplasma* and *Mycobacteria* elicit some interesting inflammatory patterns in T cell–immunodeficient patients, and these are illustrated in the following cases.

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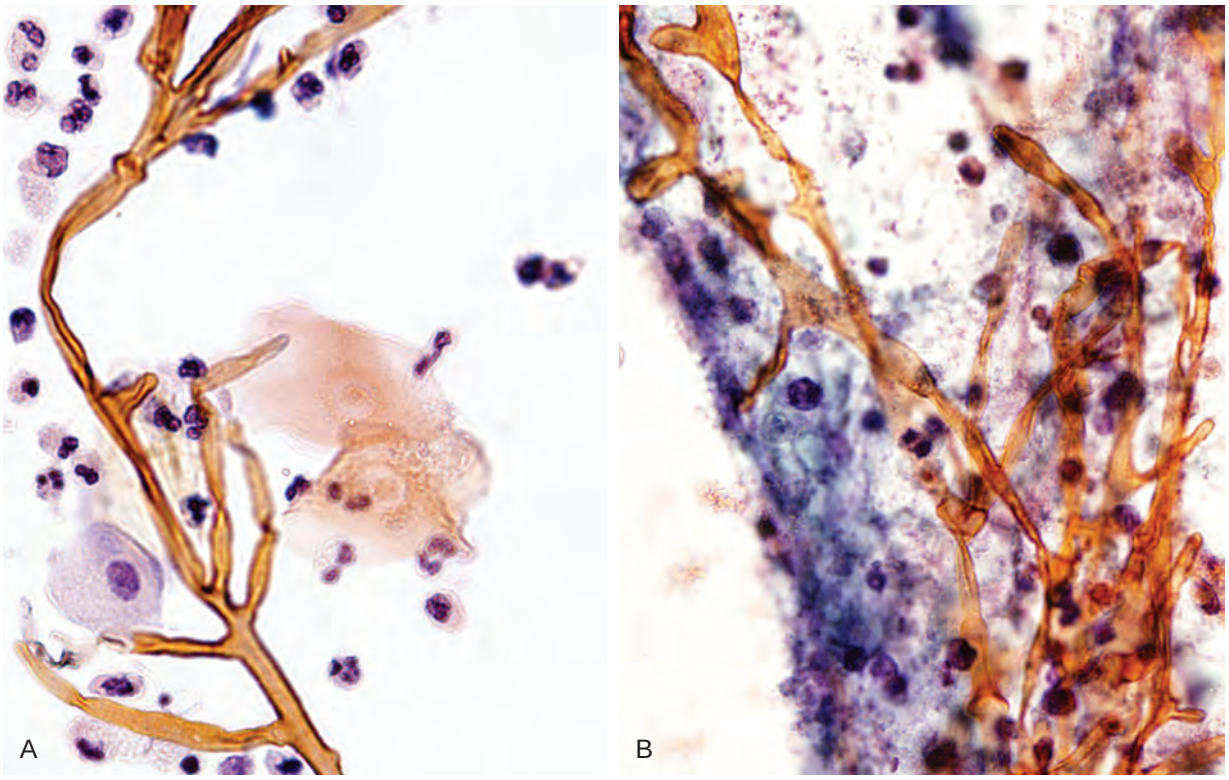


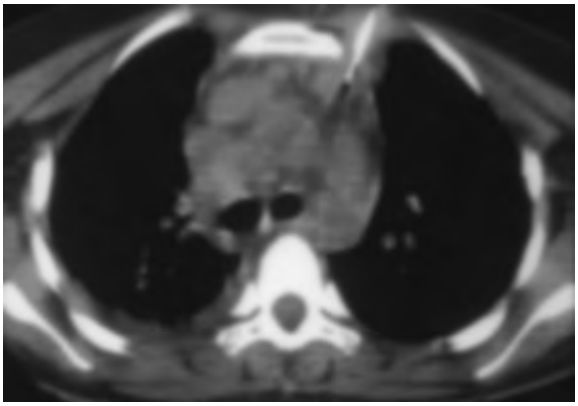
• **Figure 4.41** Urine **(A)** and kidney fine-needle aspirate **(B)** from a patient with renal and pulmonary zygomycosis. **A,** Large, ribbonlike hyphae are seen admixed with neutrophils. **B,** Necrosis, degenerating inflammatory cells, and broad hyphae are seen (Papanicolaou stain, ×400).

CASE 14

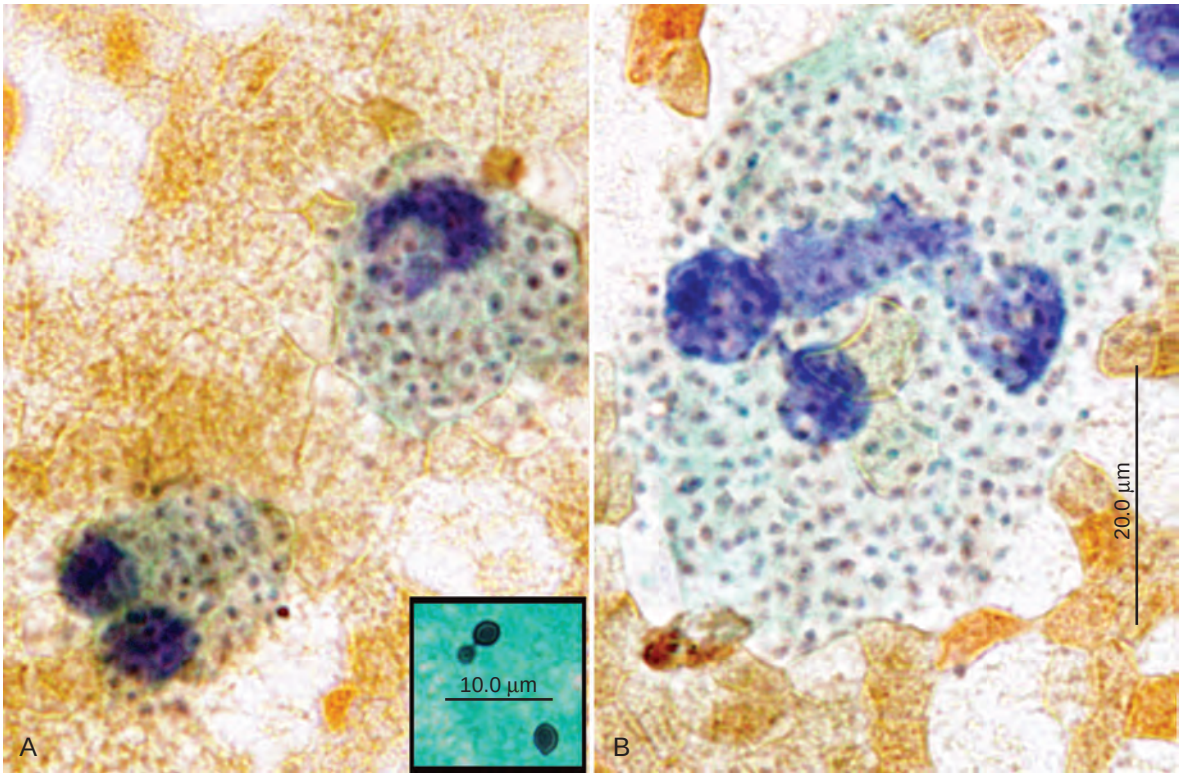
The Young Lady With the Polka-Dotted Macrophages

A 21-year-old woman presented with chills, fever, weight loss, and an abnormal chest radiograph. CT of the chest (Fig. 4.42) demonstrated anterior mediastinal lymphadenopathy with necrosis, findings suspicious for lymphoma. CT-guided FNA of a mediastinal lymph node was performed, and a Papanicolaou-stained sample is seen in Fig. 4.43. One sees macrophages containing dark, oval "polka dots" surrounded by narrow halos. These oval structures are approximately 3 μm in length. Findings are very suggestive of *H. capsulatum*. The GMS-stained slide contained abundant small yeast with narrow-necked buds, one of which is seen in the inset of Fig. 4.43A. Yeast forms are slightly larger in GMS preparations because of precipitation of silver around the organism. Culture confirmed *H. capsulatum*. Based on the cytologic findings of abundant yeast forms and a diffuse macrophage reaction, HIV testing was suggested. HIV serology was positive, and the patient was diagnosed with AIDS. Although the finding of intracellular, small yeast forms of 3 to 5 μm with narrow-necked buds on GMS is suggestive of *H. capsulatum*, the morphology on Romanowsky stain is much more specific, as is seen in a BAL sample from a 41-year-old HIV-seropositive man who presented with bilateral, mixed interstitial and alveolar pulmonary infiltrates and air bronchograms on chest radiographs (Fig. 4.44). Abundant, 2- to 3-μm, oval, intracellular yeast forms are seen. These have a characteristic magenta-colored cap of protoplasm, and the remaining internal structure appears light blue or has a clear vacuole. Many yeast forms are surrounded by a narrow halo. This represents retraction of the yeast endoplasm from a very thin cell wall rather than an actual capsule. Nonetheless, the name *H. capsulatum* has persevered. In Romanowsky-stained preparations the organism most resembles the amastigote stages of *Leishmania* spp. and *Trypanosoma cruzi*.

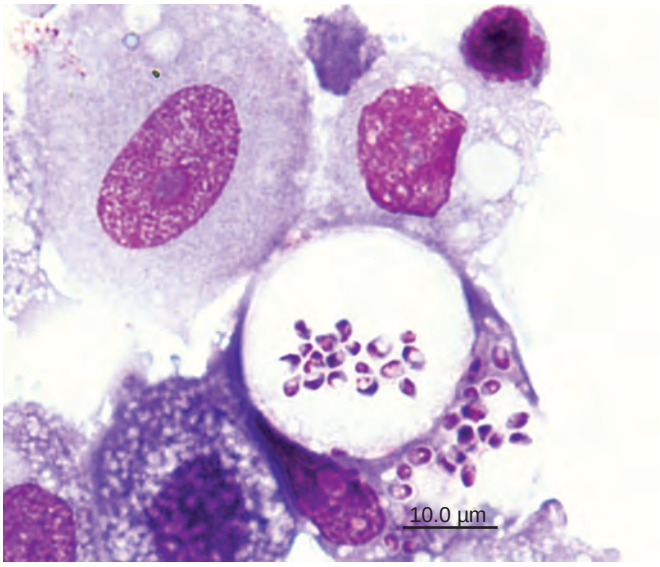
A comparison of *H. capsulatum* and *Leishmania* is seen in Fig. 4.45. Another cause of polka-dotted macrophages, albeit rare in Western countries, is *Penicillium marneffei*, a pathogenic dimorphic fungus endemic to southeastern and far eastern Asia (see later discussion). *Candida* yeast forms also bear some resemblance to *H. capsulatum* in Romanowsky-stained preparations, especially *Candida glabrata*, which does not form pseudohyphae; however, *Candida* is slightly larger and lacks the magenta-colored cap. *Candida* also has a pseudocapsule caused by the presence of a thin, nonstaining cell wall. Single dotlike structures consistent with single nuclei are seen within some of the organisms in Fig. 4.46. In this figure the pathogen is *Candida albicans*, and both yeast forms with blastoconidia and pseudohyphae are seen.



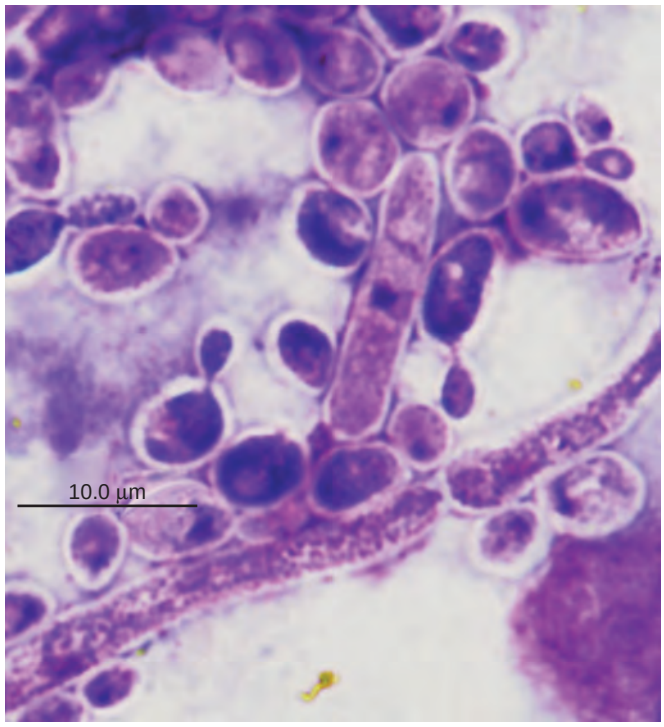
• **Figure 4.42** Chest computed tomogram demonstrating image-guided fine-needle aspiration of mediastinal lymphadenopathy.



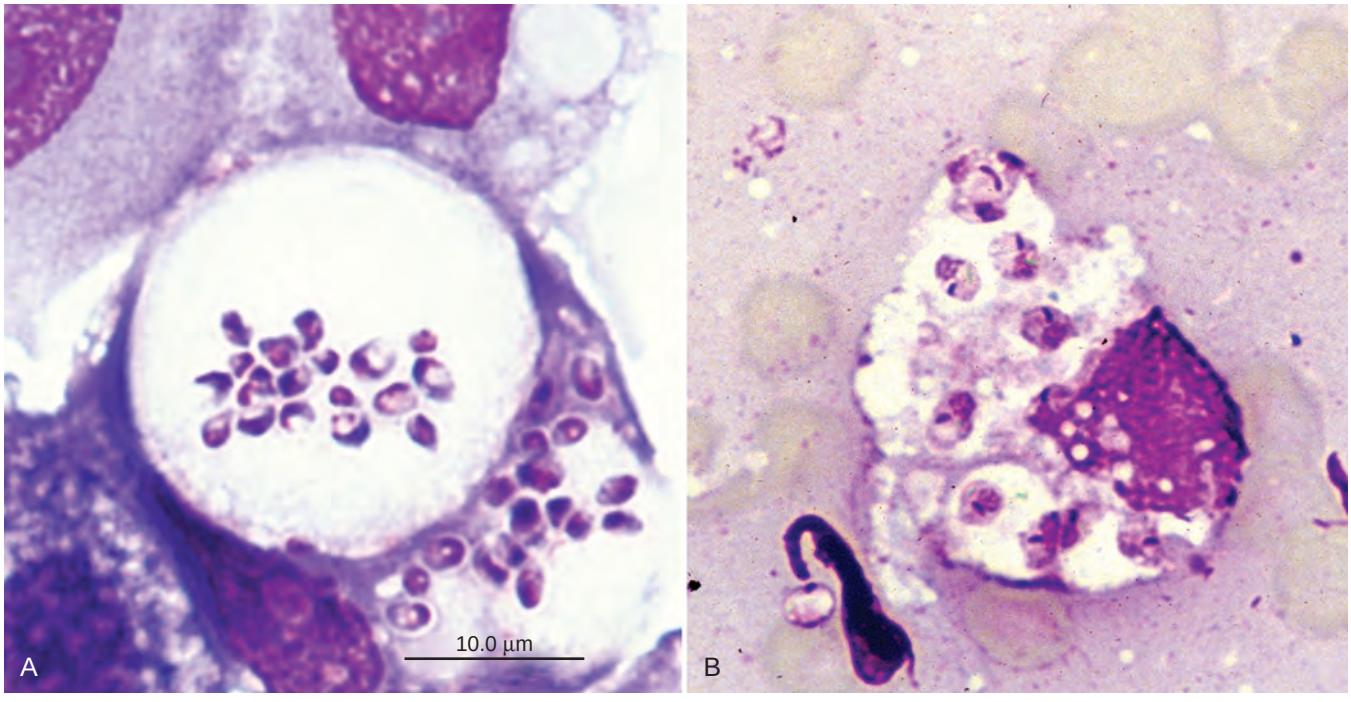
• **Figure 4.43** Fine-needle aspiration of mediastinal lymph node from the same patient as in Fig. 4.42. **A** and **B,** Macrophages containing dark oval structures surrounded by narrow halos are seen (Papanicolaou stain, ×2000). Budding yeast form is seen in the *inset* in **A** (Grocott-Gomori methenamine silver stain, ×1500).



• **Figure 4.44** Bronchoalveolar lavage sample from a patient with AIDS and histoplasmosis. Alveolar macrophages and small, intracytoplasmic oval yeast forms with a magenta-colored cap are seen. Some contain an intracytoplasmic vacuole (Romanowsky stain, ×1200).



• **Figure 4.46** Postmortem esophageal brushing demonstrates candidiasis. Blastoconidia and pseudohyphae are seen (Romanowsky stain, ×2500).



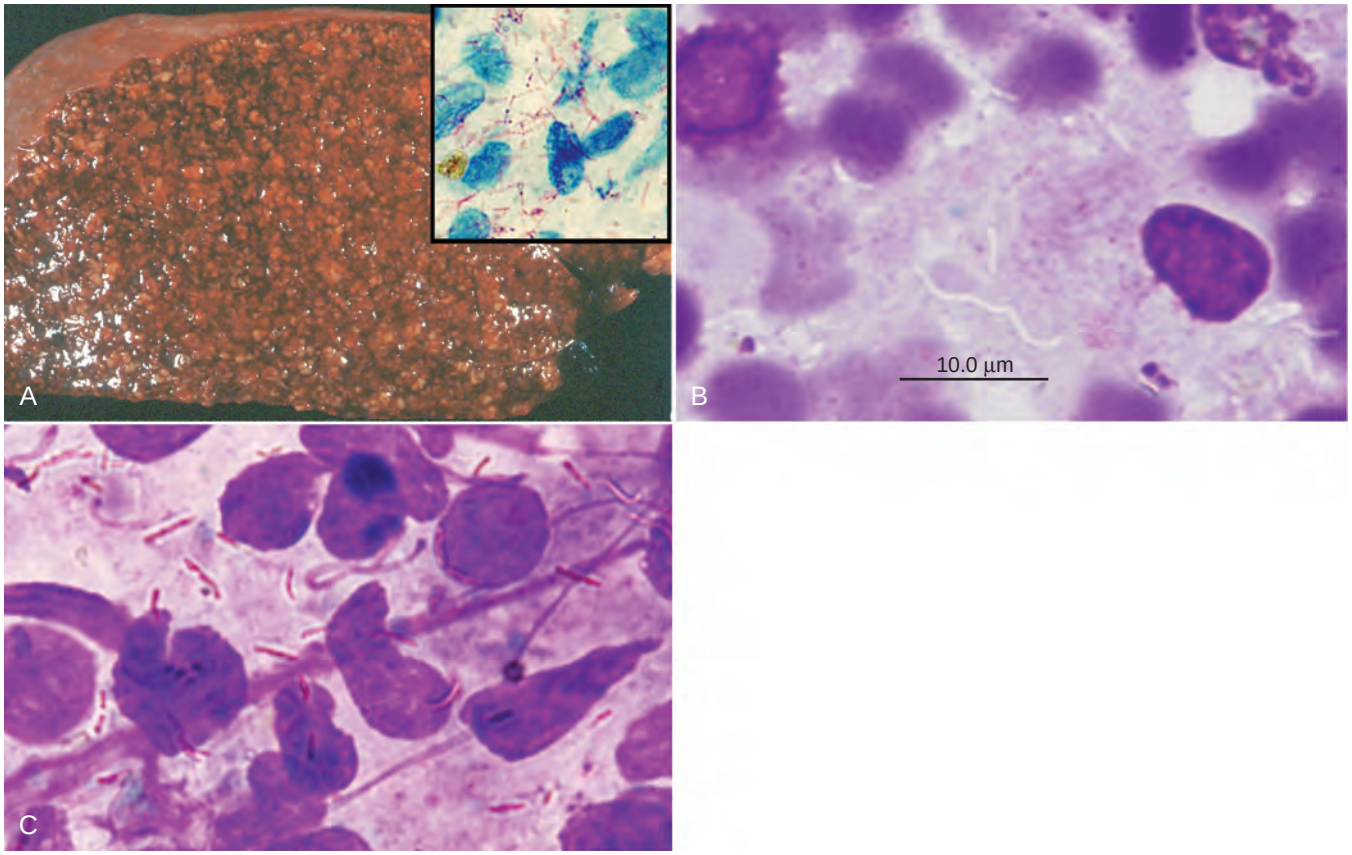
• **Figure 4.45** Comparison of *Histoplasma capsulatum* and *Leishmania* spp. **A,** *H. capsulatum* with magenta-colored cap and halo. **B,** *Leishmania* sp. with kinetoplast (Romanowsky stain, ×2000).

CASE 15

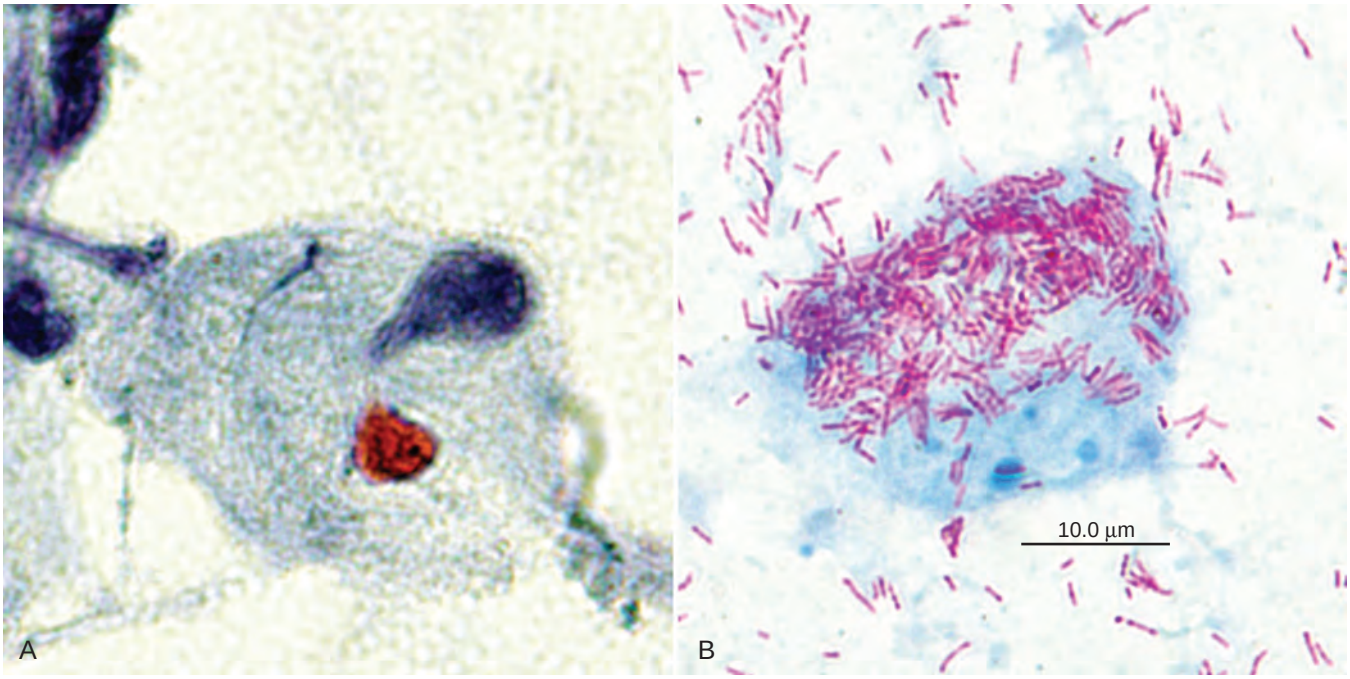
The Macrophage Wore Pinstripes

A 33-year-old man died in hospice. At autopsy, he was found to have hepatosplenomegaly and diffuse subcentimeter nodules throughout his liver and spleen (Fig. 4.47A). During autopsy, touch preparations of spleen were taken. A rapid Romanowsky–stained preparation is seen in Fig. 4.47B and C. In Fig. 4.47B one can see many white linear structures that represent negatively stained bacilli. Fig. 4.47C, taken at a different level of focus, shows refractile reddish-colored bacilli. The macrophages' cytoplasm is not well visualized. This negative staining and refractile appearance is typical of a mycobacterial infection, most commonly MAC.[76] In AIDS patients the pathogen is usually MAC; however, other *Mycobacteria* species may be associated with a diffuse macrophage reaction. Although they are reportedly gram-neutral, it has been our experience that some mycobacteria, especially *M. fortuitum* and sometimes MTC, retain the gentian/crystal violet dye and appear as beaded, gram-positive bacilli in both the classic Gram and Gram-Weigert stains. It has been suggested that mycobacteria were more likely to stain blue in Gram stains using older versions of gentian violet.[9] In the case presented here, cultures grew MAC. On Papanicolaou stain, intracytoplasmic grayish stripes can sometimes be seen associated with diffuse macrophage infiltration secondary to mycobacteriosis. Pinstriped macrophages lead one to suspect infection and to perform an acid-fast stain, as seen in Fig. 4.48, taken from an FNA of an enlarged axillary lymph node in an HIV-positive patient. Note that the macrophages are stuffed full of bacilli and have a striated appearance. In acid-fast stained preparations, one might refer to these macrophages as flagellated or flogged. Others have referred to Papanicolaou-stained, mycobacteria-laden macrophages as pseudo-Gaucher cells.[77]

An interesting phenomenon associated with AIDS, highly active antiretroviral therapy (HAART), and MAC, as well as other infectious organisms, is immune reconstitution syndrome.[78] AIDS patients with therapy-induced CD4 count recovery may develop lesions with florid granuloma formation or necrotizing inflammation. Immune reconstitution syndrome should be considered in patients with marked granuloma formation or other type of inflammatory response, a high CD4 count, and negative cultures despite the presence of visible microorganisms on direct examination. Cytologists should be aware of this condition and should routinely correlate cytologic findings in AIDS patients with patients' concomitant or most recent CD4 count.



• **Figure 4.47** Postmortem case of disseminated *Mycobacterium avium* complex in an AIDS patient. **A,** Gross image of spleen at autopsy. Acid-fast bacilli are seen in the *inset* in **A** (Kinyoun stain, ×1000). Scrape preparations of spleen demonstrate rod-shaped structures with negative staining **(B)** and refractile, red staining bacilli **(C)** at a different level of focus (Romanowsky stain, ×1200).



• **Figure 4.48** Fine-needle aspiration of axillary lymph node from a patient with disseminated *Mycobacterium avium* complex. **A,** A macrophage containing intracytoplasmic striations is seen (Papanicolaou stain, ×2000). **B,** Abundant intracellular and extracellular bacilli are seen (Kinyoun stain, ×2000).

CASE 16

Overstuffed Macrophages With Lysosome Indigestion

A 33-year-old HIV-seropositive man presented with cough. Bilateral pulmonary infiltrates with cavitation in the left upper lobe were found on chest radiography. He had a medical history of *Rhodococcus* and *M. kansasii* infections. BAL findings are shown in Figs. 4.49 and 4.50. The alveolar macrophages appear to be stuffed with globules and vacuoles of varying sizes (see Fig. 4.49A and B). Focally, vacuoles containing beaded coccobacilli are seen (see Fig. 4.49C); also focally, round, laminated intracytoplasmic inclusions are present (see Fig. 4.49D). Intracytoplasmic, gram-positive coccobacilli can be seen on a Gram-Weigert–stained slide (see Fig. 4.50). There are narrow halos around some of the organisms. The findings are consistent with malakoplakia, and the organisms seen are suggestive of *Rhodococcus* spp.

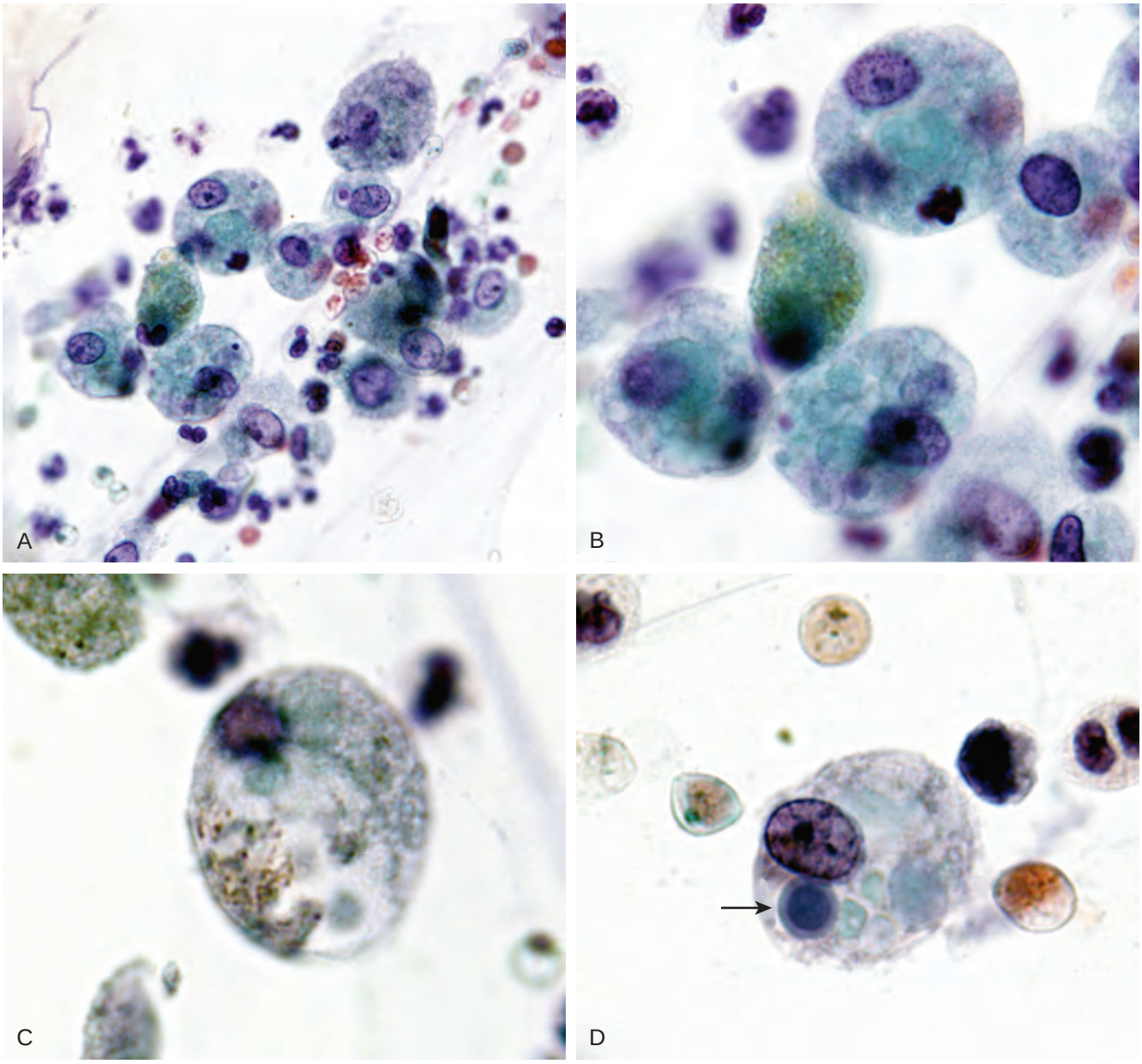
Pulmonary malakoplakia is a known complication of *Rhodococcus equi* pneumonia in AIDS patients.[79] The bacteria seen here are morphologically consistent with *Rhodococcus* spp. They are coccoid to diphtheroid and beaded in appearance and can easily be mistaken for streptococci on Gram stain. A narrow halo suggestive of a capsule is seen around some of the bacteria. BAL culture grew *R. equi*. Although its pathogenesis is still controversial, malakoplakia is believed to represent a defect in lysosome-mediated killing of intracellular bacteria and is most commonly found associated with *Escherichia coli* and *Klebsiella* infections of the genitourinary tract.[80] Malakoplakia is characterized by sheets of macrophages containing globules or vacuoles of varying size, some of which may contain bacteria. These globules have been found to be phagosomes, giant lysosomes, and phagolysosomes. Bacteria can often be identified within some of the phagocytic vacuoles.[80,81] Pathognomonic findings are these overstuffed macrophages and the presence of intracytoplasmic, laminated Michaelis-Gutmann bodies that contain traces of iron and calcium. The latter are approximately 3 to 7 µm in diameter and are thought to represent lysosomal calcifications that may form in reaction to undigested bacteria.[82,83]

Descriptions of *Rhodococcus*-associated malakoplakia diagnosed by FNA and BAL have been published.[84,85] We have seen *Rhodococcus* infections in cytologic preparations from FNA of an arm lesion, BAL samples, and in TBNA of a mediastinal lymph node, all in AIDS patients. One BAL sample, illustrated in Fig. 4.49D, had typical Michaelis-Gutmann bodies. The TBNA sample contained coccobacilli and macrophages containing large globules; however, Michaelis-Gutmann bodies could not be found.

Fig. 4.51 shows PAS, iron, and modified acid-fast stains of lung taken at autopsy from a patient who died of *R. equii* pneumonia. The macrophages contain PAS-diastase–resistant globules (see Fig. 4.51A), and iron stain (see Fig. 4.51, inset) shows a faintly iron-positive Michaelis-Gutmann body. *Rhodococcus* is sometimes, but not invariably, acid-fast when a *Nocardia* modification is used (see Fig. 4.51B). Positive staining of cytologic preparations using Fite stain has been reported.[86]

The overstuffed macrophages of malakoplakia bear some resemblance to the foamy macrophages found in amiodarone therapy; however, the clinical histories and presentations are likely to be quite different. Therapy with amiodarone, an antiarrhythmic agent, is associated with phospholipidosis of the lung and other tissues.[87,88] Some patients develop diffuse alveolar damage secondary to amiodarone toxicity.[88,89] Foamy macrophages are only an indication of amiodarone therapy effect, not toxicity; however, absence of foamy macrophages would militate against amiodarone toxicity.[87] It has been debated whether toxicity is directly related to phospholipidosis or a hypersensitivity reaction.[90] Israel-Biet et al. found that BAL samples from patients receiving amiodarone, regardless of the presence or absence of toxicity, typically contained macrophages with both clear and dense vacuoles.[90] The dense vacuoles were found to represent phagolysosomes containing phospholipid.

We have also noted two types of vacuoles in BAL from patients receiving amiodarone. Fig. 4.52 illustrates BAL findings in a patient who had been



• **Figure 4.49** Bronchoalveolar lavage sample from an AIDS patient with *Rhodococcus*-associated pulmonary malakoplakia. **A,** Macrophages containing intracytoplasmic globules are seen (Papanicolaou stain, ×800). Higher magnification illustrates globular cytoplasm **(B)**, intracytoplasmic bacilli **(C)**, and a Michaelis-Gutmann body **(D)** *(arrow)* (Papanicolaou stain, ×2000).

CASE 16

Overstuffed Macrophages With Lysosome Indigestion—cont'd

treated with amiodarone. Macrophages containing clear and cyanophilic globules are seen. When we receive a BAL sample with notice of possible amiodarone toxicity, we consider that our main role is to rule out other causes of respiratory failure. One expects to see the foamy macrophages; however, they represent therapy effect, not toxicity, and we usually look for evidence of diffuse alveolar damage. If neutrophils, fibrin, or hyperplastic pneumocytes or fibroblasts are present, we suspect diffuse alveolar damage. This is definitively diagnosed by histologic examination. Fig. 4.53 shows an open biopsy from the patient whose BAL result was illustrated in Fig. 4.52. Interstitial fibrosis, lymphoplasmacytic inflammation, and foamy macrophages are seen. Bronchiolitis obliterans (not illustrated) was also noted. No specific infection or other causative agents were found, and the findings were considered consistent with amiodarone toxicity.

Pulmonary alveolar proteinosis syndrome (PAP) is, like malakoplakia, a disease related to macrophage dysfunction, most commonly, defective granulocyte-macrophage colony stimulating factor (GM-CSF) signaling. The

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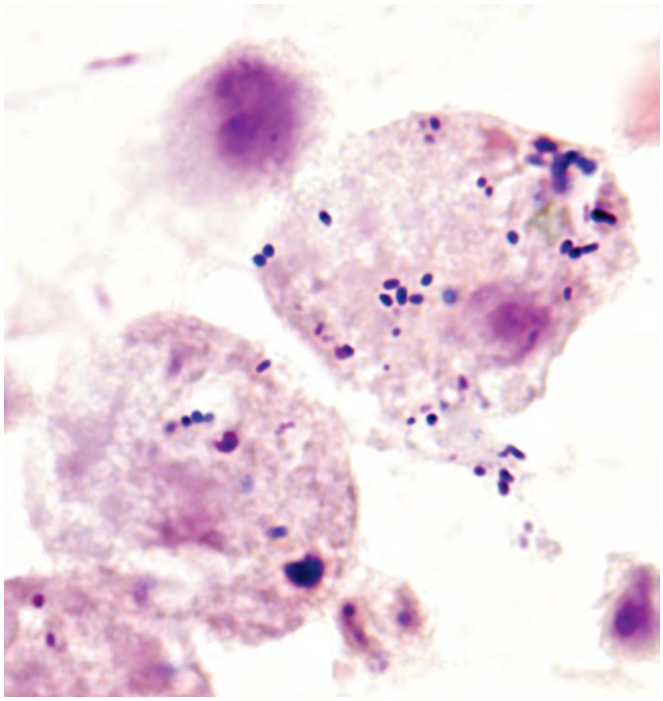
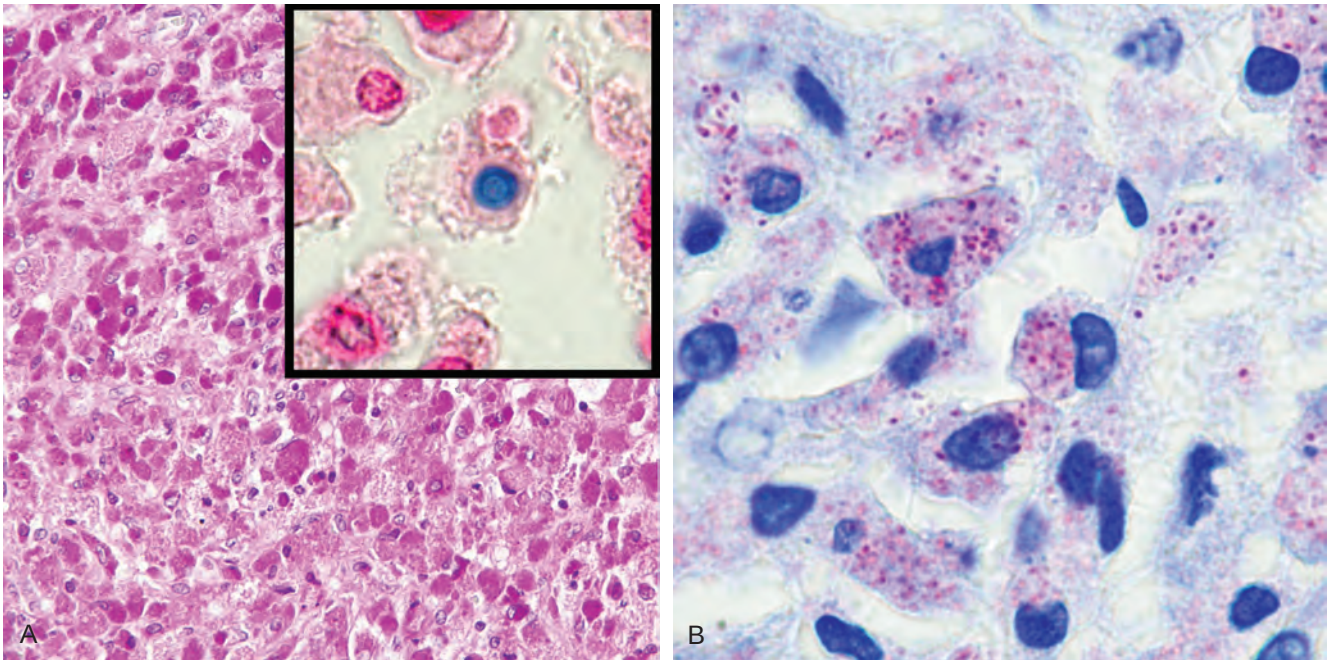
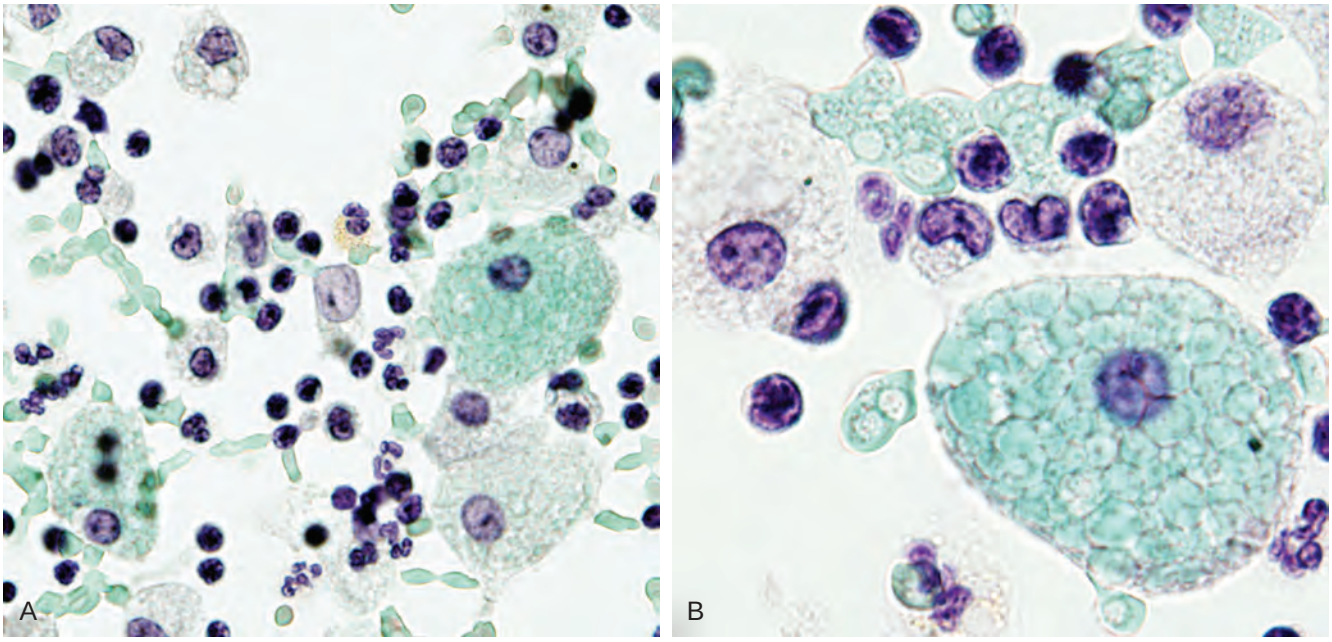


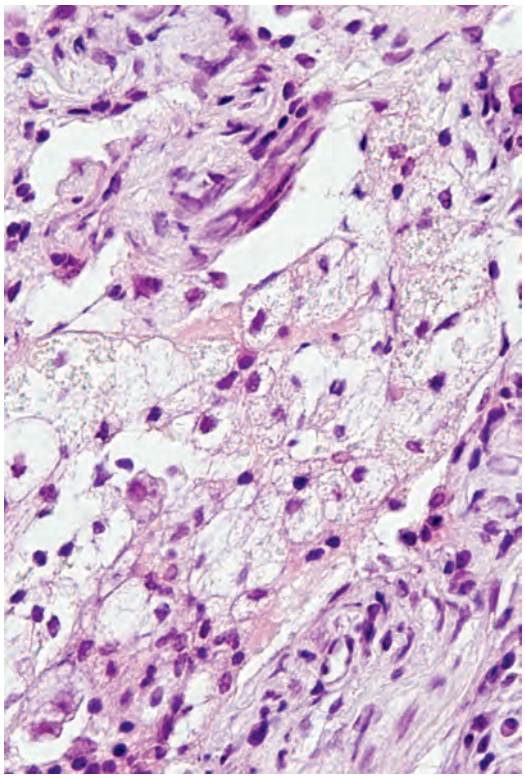
• **Figure 4.50** Bronchoalveolar lavage sample from the same patient as in Fig. 4.49, showing macrophages that contain gram-positive coccoid and bacillary bacteria surrounded by narrow halos (Gram-Weigert stain, ×2000).



• **Figure 4.51** Autopsy sections of lung from a patient with *Rhodococcus* pulmonary malakoplakia. **A,** Sheets of macrophages containing pink material (periodic acid–Schiff with diastase, ×400). *Inset* shows a Michaelis-Gutmann body (Prussian blue stain, ×1000). **B,** Intracellular acid-fast coccobacilli (Fite stain, ×1000).



• **Figure 4.52** Amiodarone effect in bronchoalveolar lavage fluid. **A,** There are some macrophages containing clear vacuoles; others have cyanophilic vacuoles (Papanicolaou stain, ×600). **B,** High power illustrates the intracytoplasmic vacuoles (Papanicolaou stain, ×1000).



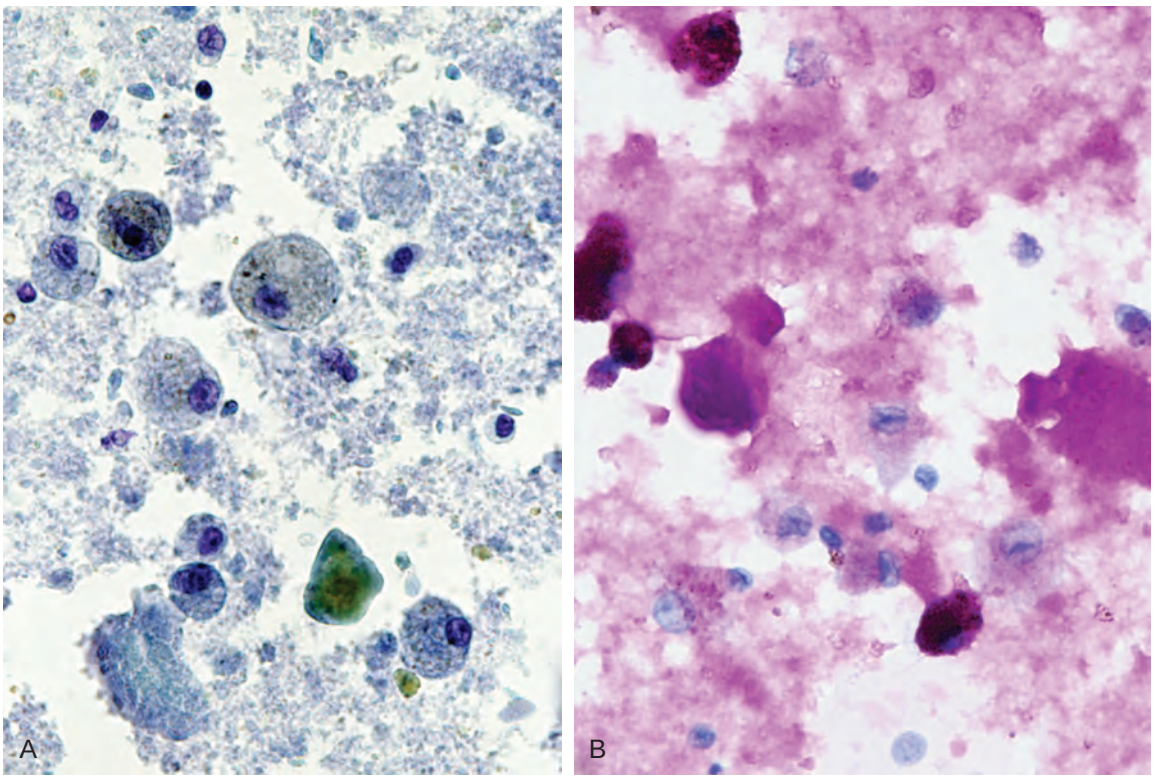
• **Figure 4.53** Open lung biopsy from patient with suspected amiodarone toxicity showing fibrosis and presence of foamy macrophages (hematoxylin and eosin stain, ×400).

CASE 16

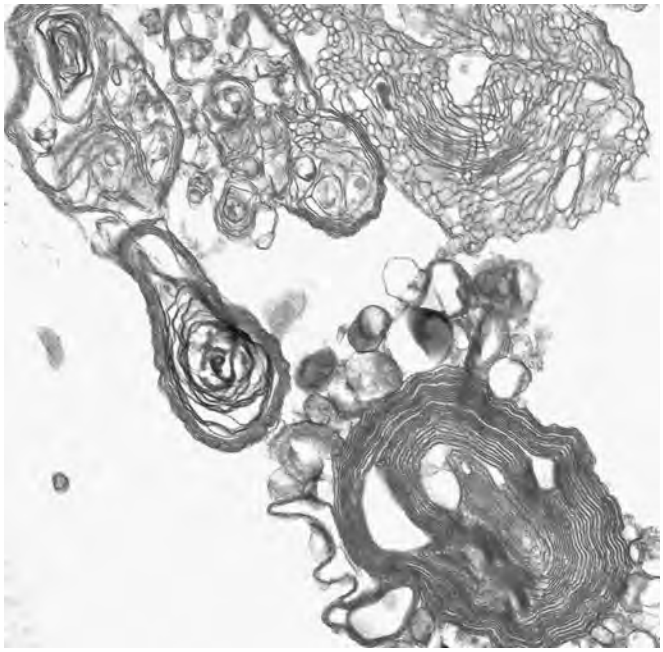
Overstuffed Macrophages With Lysosome Indigestion—cont'd

most common form of PAP (autoimmune PAP) has been found to be associated with autoantibodies directed against GM-CSF and subsequent defective surfactant catabolism by alveolar macrophages.[91] As with malakoplakia and amiodarone effect, foamy macrophages are present in BAL samples from patients with PAP; however, in PAP, there is generally an abundance of extracellular granular and globular material and a relative paucity of macrophages (Fig. 4.54).[92,93] If this pattern is seen in a BAL sample, one immediately asks to see the centrifuged BAL fluid. The fluid typically has 1 to 2 cm of precipitate in the bottom of the tube. The pulmonologist usually has noted that the BAL fluid was markedly turbid when extracted from the bronchus. The granular intracellular and extracellular material seen on microscopic examination of the BAL represents abnormal surfactant that has not been degraded by alveolar macrophages. The intra-alveolar material is largely composed of phospholipid and is PAS positive, diastase resistant. Fine granules and larger globules are seen. The gross appearance of the fluid, the clinical and radiologic findings, and the presence of abundant PAS-positive extracellular material are usually sufficient evidence on which to make a presumptive diagnosis of PAP. In primary PAP, anti–GM-CSF autoantibodies are typically present and can be used to confirm diagnosis.[91] Electron microscopic examination of the BAL precipitate in cases of PAP demonstrates abnormal surfactant accumulation, including the presence of multilamellated structures and, occasionally, abnormally formed tubular myelin lattices.[94,95] In a few cases of PAP, we have submitted an aliquot of BAL sediment for transmission electron microscopy. Fig. 4.55 is a transmission electron micrograph derived from a case of acquired PAP in a renal transplantation patient, showing concentrically laminated structures and abnormal tubular myelin lattice formation.

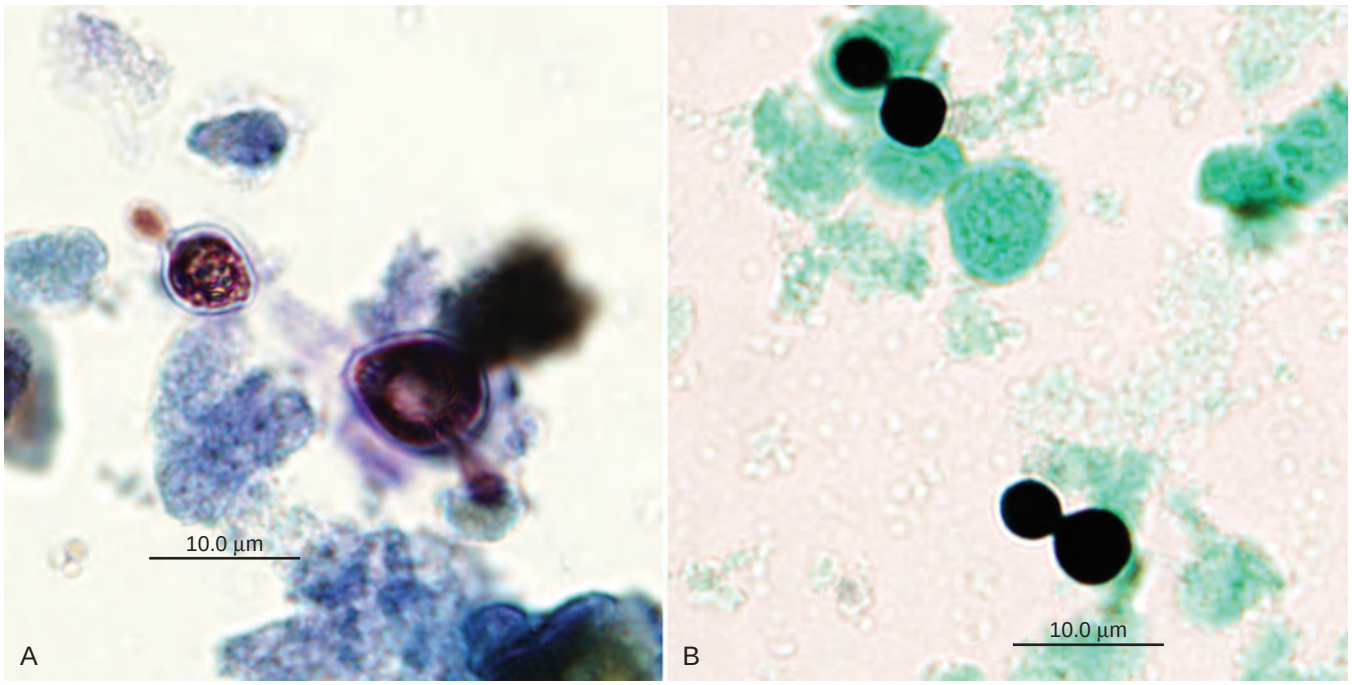
PAP is associated with dysfunctional macrophages, and patients with acquired PAP are susceptible to a number of opportunistic infections, including MAC, pneumocystosis, and nocardiosis. It is a good idea to culture and perform special stains for these organisms on BAL sediment. Fig. 4.56 illustrates a case of PAP in which fungi consistent with *Cryptococcus* were found in BAL fluid, and *C. neoformans* was cultured subsequently.



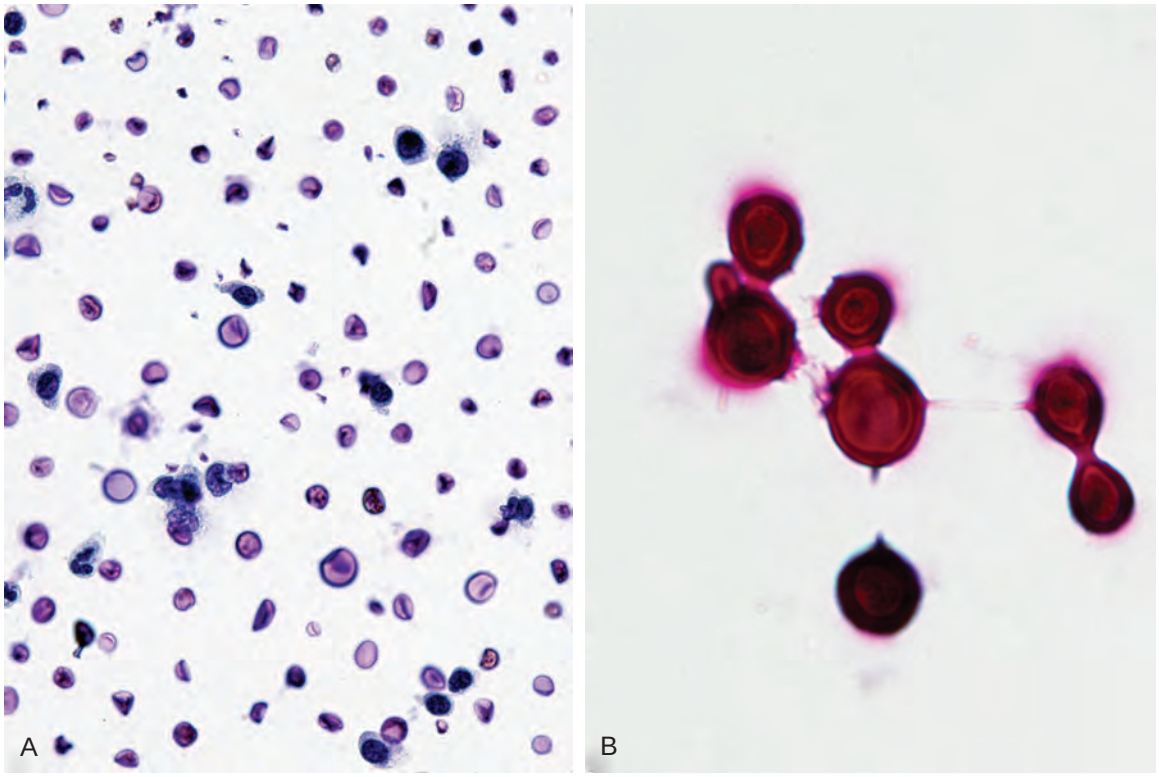
• **Figure 4.54** Bronchoalveolar lavage sample from a patient with pulmonary alveolar proteinosis. **A,** Abundant granular and globular material with relative paucity of macrophages (Papanicolaou stain, ×400). **B,** Extracellular and intracellular material is periodic acid–Schiff (PAS) positive, diastase resistant (PAS with diastase stain, ×400).



• **Figure 4.55** Transmission electron micrograph of bronchoalveolar lavage fluid sediment from a case of pulmonary alveolar proteinosis. A typical, concentrically laminated multilamellate body is seen in the lower right, and lattice formation of atypical tubular myelin in the upper right (×15,000). (Electron micrograph image courtesy Julie Wen.)



• **Figure 4.56** Bronchoalveolar lavage sample from a patient with acquired pulmonary alveolar proteinosis and *Cryptococcus* infection. **A,** Budding yeast forms are seen admixed with granular material (Papanicolaou stain, ×2000). **B,** Budding yeast forms (Grocott-Gomori methenamine silver stain, ×2000).

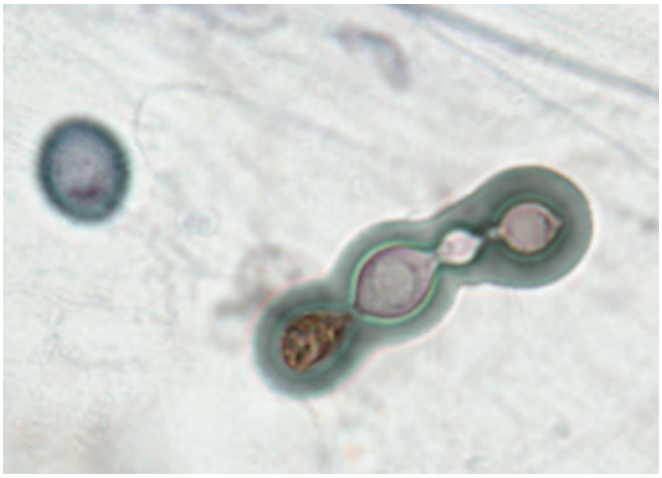


• **Figure 4.57** Cerebrospinal fluid from a patient with AIDS and *Cryptococcus* meningitis. **A,** Round yeast forms of varying size (Papanicolaou stain, ×400). **B,** Yeast forms are bright red, and blastoconidia are connected to parent yeast by a narrow tube (mucicarmine stain, ×1000).

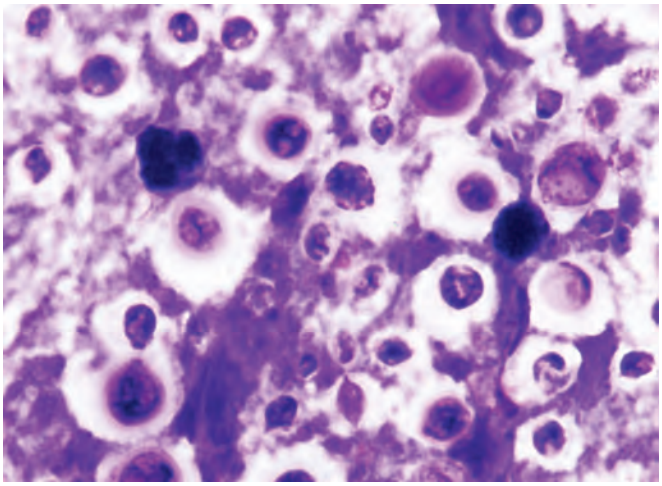
Organisms That Elicit Scanty to No Inflammation in Patients With Impaired Cell-Mediated Immunity

Very scanty to no inflammation is seen with *Cryptococcus* and *Pneumocystis* infections in patients with severely impaired cell-mediated immunity. *Cryptococcus*, as mentioned earlier, is associated with granuloma formation in nonimmunocompromised and mildly immunocompromised hosts. *P. jiroveci* (formerly called *Pneumocystis carinii*) was originally described in conjunction with plasma cell pneumonia in malnourished children during and after World War II[96]; *P. jiroveci* pneumonia is currently most commonly seen in patients with AIDS and transplant recipients.[97] In T cell–immunocompromised patients the inflammatory response to the organism is minimal, usually consisting of macrophages, lymphocytes, and plasma cells.

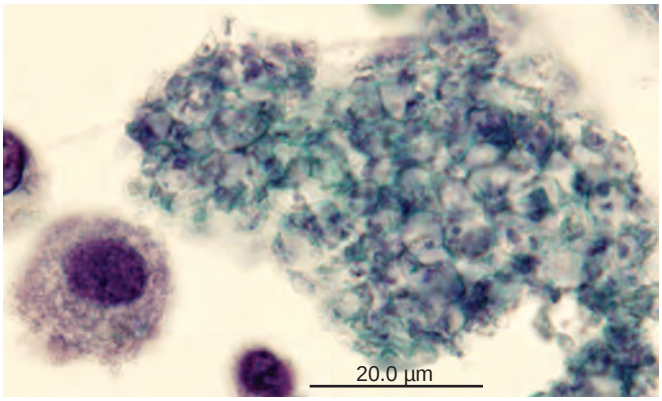
C. neoformans infection in a severely immunocompromised patient is seen in Fig. 4.57. The figure shows Papanicolaou- and mucicarmine-stained cerebrospinal fluid samples from a patient with AIDS. Fig. 4.58 shows a sputum sample from another AIDS patient who had both a massive cryptococcal pneumonia and meningitis. Note the absence of inflammatory cells, the round yeast cell shape, and the marked variation in size. The yeast forms often appear purple to red in Papanicolaou stain; however, sometimes they are not well colorized and are difficult to find. Closing the substage condenser helps a great deal. Focally, there is crystalline-like material within some of the yeast cells. This is typical of *Cryptococcus* in Papanicolaou stain. Note also the abundant capsular material, which is best illustrated in the mucicarmine stain of cerebrospinal fluid (see Fig. 4.57B) and in the Papanicolaou stain of sputum (see Fig. 4.58). Pseudohyphae formation is also common in immunodeficient patients with fulminant infections (see Fig. 4.58). In AIDS patients, symptomatic pulmonary disease is relatively common, and we have seen massive vascular dissemination with involvement of multiple organs at autopsy. Abundant mucin within the cryptococci causes a pink or clear halo around the organism. In Romanowsky stains the cell wall appears bright pink or purple and the capsule clear to pink (Fig. 4.59). Mucicarmine staining is usually not necessary



• **Figure 4.58** *Cryptococcus* forming pseudohyphae in sputum sample from a patient with AIDS. Note thick capsule and crystalline material in the yeast cell in the lower center (Papanicolaou stain, ×1000).



• **Figure 4.59** Autopsy scrape preparation of spleen from patient with disseminated cryptococcosis (Romanowsky stain, ×1000).

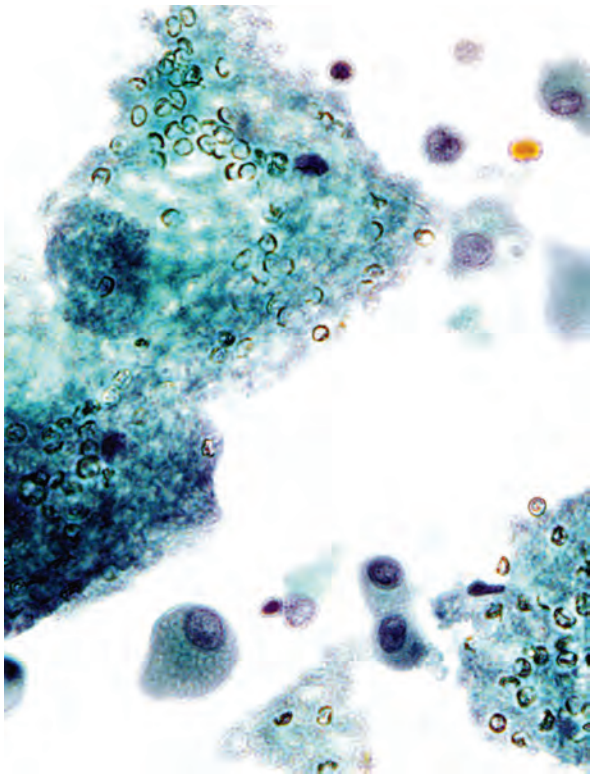


• **Figure 4.60** Bronchoalveolar lavage sample from an AIDS patient with pneumocystosis. A macrophage is seen on the left and foamy material containing dark dots typical of *Pneumocystis* on the right (Papanicolaou stain, ×1000).

for diagnosis, but it can be performed if the identity of the fungus is in doubt. Immunosuppressed patients typically have encapsulated forms of the organism, and mucicarmine is usually strongly positive.

Although polymerase chain reaction (PCR) may become a standard technique for diagnosis of pneumocystosis, the cytologist should still have knowledge of the cytomorphologic features of this infection, and thus the following information is provided. *P. jiroveci* may be found in cytologic preparations of adequately induced sputum and in BAL fluid. We recommend the addition of a mucolytic agent, followed by centrifugation and cytocentrifugation. This concentrates the sample and provides a small, round area that facilitates examination. Our technique for processing sputum is given in the appendix. Gill et al. also recommended use of mucolytic agents and cytocentrifugation for the processing of sputum for *Pneumocystis* diagnosis.[98] This group found peak recovery of organisms by cytocentrifugation at 1200 rpm for 10 minutes. (This would correspond to 163 Xg using a Thermo Shandon Cytospin 4.) We have found that a combination of Papanicolaou, GMS, and Romanowsky stains is usually adequate for evaluation in samples that are concentrated as described. As previously stated, others have touted fluorescent antibody immunostaining and autofluorescence. Toluidine blue O, a thiazine dye, is also still used by some. The take-home message is that laboratory personnel must choose the methods that work best for their individual laboratory. Variations in type of stains used, processing of samples, and expertise of observers with different stain techniques make published studies difficult to compare.

Fig. 4.60 shows *P. jiroveci* in a Papanicolaou-stained sample of BAL fluid. Note the bubbly appearance of the "exudate." Of great importance, also note the small, dark dots within the bubbles. In our experience the latter are very characteristic of *P. jiroveci* in Papanicolaou-stained material and possibly represent the dense area in the cyst wall seen on GMS. The so-called foamy exudate is actually not an exudate but rather a massive collection of cysts and extracystic forms of the fungus. With the substage condenser closed, these bubbles appear refractile, as do yeast forms of many other fungi (Fig. 4.61). In a properly performed GMS stain, *P. jiroveci* cysts appear as round structures approximately 6 to 7 µm in diameter with a light gray center containing a black dot representing a thickened area in the cell wall. Under very high power, this area is seen actually to be two small dots (Fig. 4.62). Fibrinous

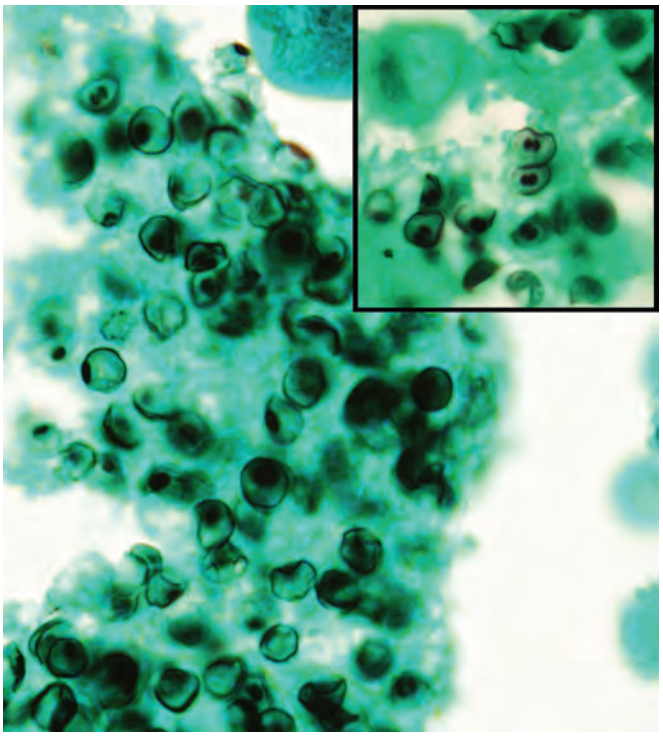


• **Figure 4.61** *Pneumocystis* in bronchoalveolar lavage fluid, viewed with substage condenser diaphragm closed (Papanicolaou stain, ×600).

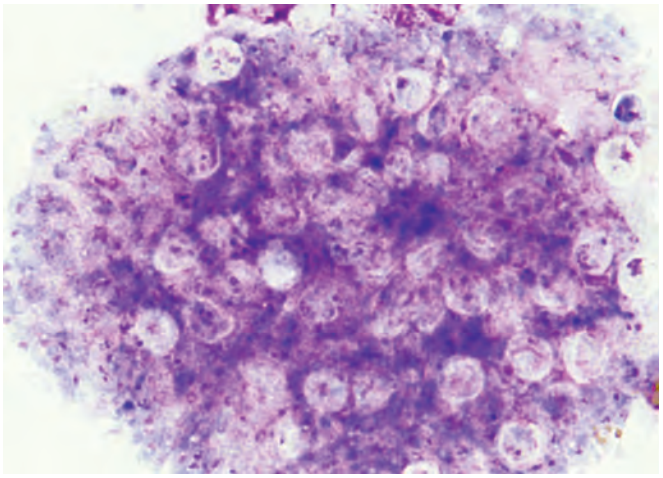
debris, as may be observed in the exudative stage of diffuse alveolar damage or organizing pneumonia, appears stringy and lacks the bubbly appearance of *P. jiroveci*. It should be noted that pneumocystosis may be accompanied by the presence of fibrinous debris and hyperplastic pneumocytes, along with clinical findings consistent with acute respiratory distress syndrome.[97,99] In Papanicolaou stain, degenerating erythrocytes also appear bubbly and can resemble *P. jiroveci*; however, erythrocytes lack the dots (Fig. 4.63). Romanowsky stains color only the intracystic and extracystic bodies; the cyst walls appear clear (Fig. 4.64). The organisms bear a resemblance to malarial trophozoites, although *P. jiroveci* is now classified as a fungus. Evaluation of a Romanowsky stain for

Pneumocystis requires high-power magnification and a good deal of experience, and that may explain its lack of popularity. Nonetheless, in some hands, it is an excellent stain for *P. jiroveci* diagnosis.[19] Individuals who are both accustomed to routine use of Romanowsky stain for *P. jiroveci* detection and not reluctant to use high-power magnification are likely to do better than those who are more used to GMS and low power. We have found that, particularly in treated patients, the cysts may stain poorly or not at all with GMS, although one can still see the extracystic stages in Romanowsky-stained preparations. The combined use of both Romanowsky and GMS is likely to yield the maximum benefit.

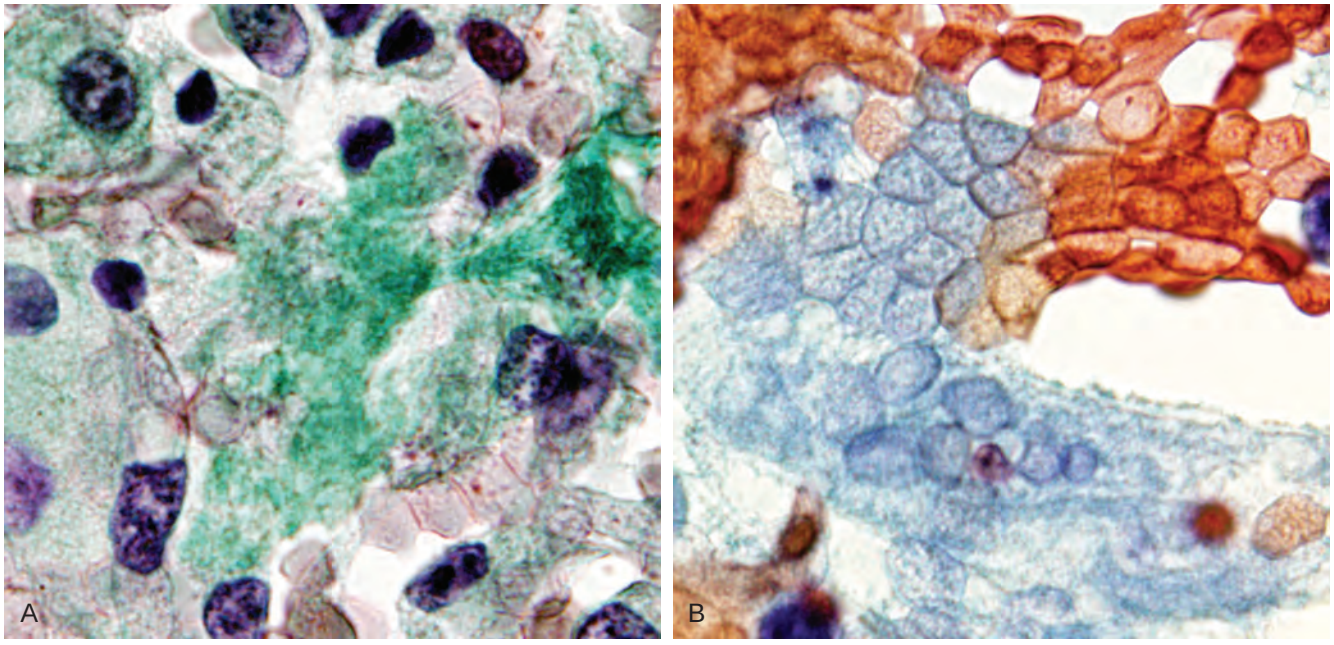
Although *P. jiroveci* can potentially be confused with *H. capsulatum*, particularly in overstained GMS preparations, the two organisms appear completely different in Romanowsky stains (Fig. 4.65). *P. marneffei* may also be confused with both *H. capsulatum* and *Pneumocystis* in GMS preparations. *P. marneffei* endemicity is limited to southeastern and far eastern Asia and is predominantly an infection of the immunosuppressed, mainly patients with AIDS. The disease caused by *P. marneffei* is remarkably similar to disseminated histoplasmosis.[100,101] *P. marneffei* differs from both



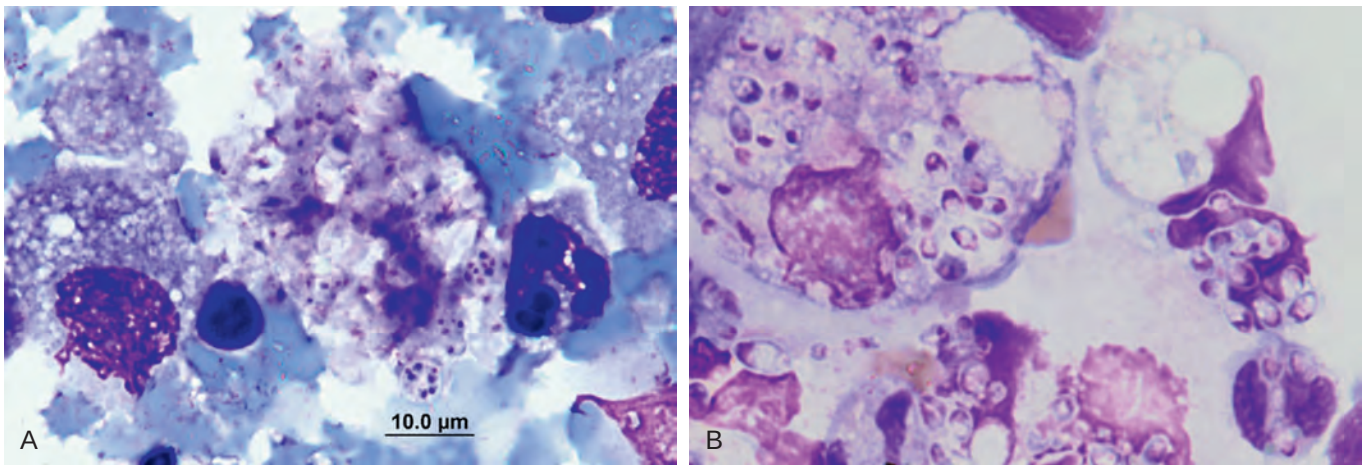
• **Figure 4.62** *Pneumocystis* in silver-stained bronchoalveolar lavage sample, showing small dots believed to be thickened areas in cyst walls. The *inset* shows the presence of two small dots within each of two small cysts (Grocott-Gomori methenamine silver stain, ×1000).



• **Figure 4.64** Induced sputum containing *Pneumocystis* is seen in Romanowsky stain. Cysts are clear, and intracystic and extracystic bodies have bluish cytoplasm and magenta-colored nuclei (Romanowsky stain, ×1000).



• **Figure 4.63** Bronchoalveolar lavage samples with fibrin **(A)** and ghosts of erythrocytes **(B)**, findings that could be confused with *Pneumocystis* in Papanicolaou stain (Papanicolaou stain, ×1500).



• **Figure 4.65** Comparison of *Pneumocystis* **(A)** and *Histoplasma* **(B)** in bronchoalveolar lavage fluid (Romanowsky stain, ×1000).

H. capsulatum and *Pneumocystis* by its division via fission. In GMS stain, the presence of a single transverse septum is characteristic of *P. marneffei*; this feature is not seen in either *Histoplasma* or *Pneumocystis*. In FNA samples, *P. marneffei* organisms appear as intracytoplasmic, yeastlike cells that very closely mimic *H. capsulatum* in Papanicolaou and Romanowsky stains. The presence of division by transverse septa, best seen on GMS, and the absence of budding are important differential characteristics. In Romanowsky-stained preparations the transverse septa of *P. marneffei* appear clear.[102] *P. marneffei* is also somewhat larger and more variable in size than *Histoplasma* and more elongate than *Pneumocystis*. Again, in Romanowsky-stained material, *Pneumocystis* can be readily distinguished from *Histoplasma* and *P. marneffei*. Comparative illustrations of GMS-stained, yeastlike fungi are provided in the Appendix.

Cytodiagnosis of Viral Infections

Cytodiagnosis of viral infections is limited to those viruses that produce a characteristic cytopathic effect (CPE), and identification hinges on recognizing that effect. The accompanying inflammatory pattern, or lack thereof, is generally not useful for diagnosis. Although viral infection can be detected by recognition of the CPE, the clinical relevance of the virus cannot necessarily be predicted on the basis of cytology. The site at which the viral CPE is found and the clinical findings are very important in determining the significance of the infection. The main viruses that are identified by CPE in cytology include human papillomavirus (HPV), herpes simplex virus (HSV)/varicella-zoster (VZ), cytomegalovirus (CMV), adenovirus, molluscum contagiosum (a poxvirus), and human polyomavirus. Formerly known as koilocytosis, the CPE of a productive HPV infection is discussed ad infinitum in cytopathology texts and will not be dealt with further here. Respiratory syncytial virus (RSV) and measles also cause a CPE that is associated with multinucleated giant cells and with intracytoplasmic inclusions in RSV and both intracytoplasmic and intranuclear inclusions in measles. These CPEs have infrequently been described in cytologic preparations of respiratory samples.[103,104]

The HSV/VZ CPE is best seen in an alcohol wet-fixed, Papanicolaou-stained preparation. Despite the age-old tradition of sending an air-dried smear for Romanowsky staining (the Tzanck smear), wet fixation in 95% ethanol followed by Papanicolaou staining has unequivocal advantages over an air-dried Romanowsky-stained preparation. With the latter, one must look for multinucleated giant cells with molded nuclei, although these findings are nonspecific. The characteristic feature of HSV/VZ—the complete margination of nuclear chromatin that leaves a clear, glassy nucleoplasm—is not seen in the air-dried Romanowsky slide. Neither are intranuclear Cowdry type A inclusions or intranuclear dispersed particles easily seen in an air-dried preparation. The artifactual chromatin coarsening that occurs with wet ethanol fixation provides much better appreciation of the HSV/VZ CPE.

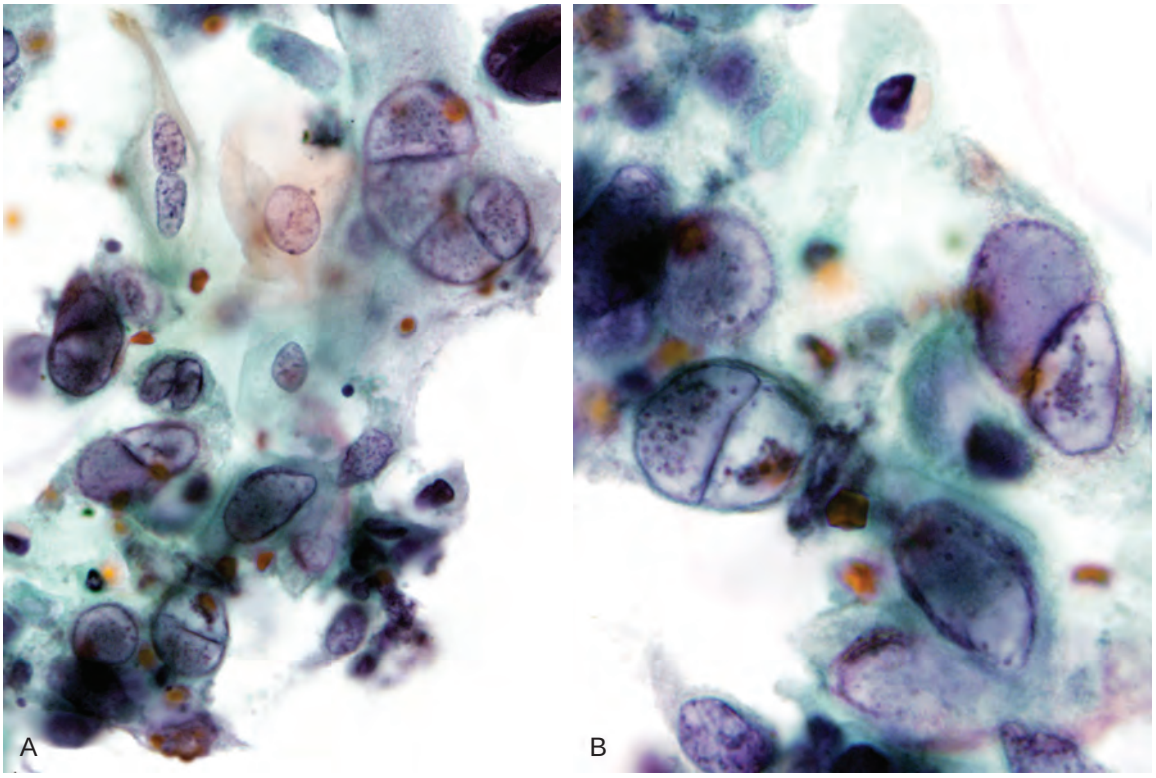
Text continued on p. 69

CASE 17

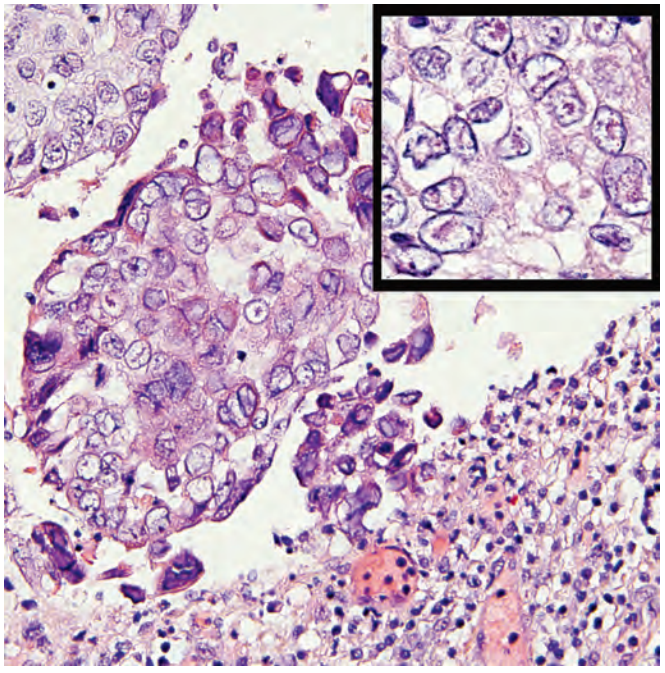
Intensive Care Unit Vocal Cord Lesion

A 62-year-old man died of *S. aureus* endocarditis. He had been intubated and had received prolonged mechanical ventilation. Autopsy revealed the presence of a large mitral valve vegetation, septic emboli, and myocardial abscesses. A 0.5-cm, white, roughened area was found on the right vocal cord. An early neoplasm was suspected, and the pathologist made a scraping of the lesion and wet-fixed the slides in 95% ethanol. The cytologic findings in Papanicolaou-stained slides are seen in Fig. 4.66. The typical CPE of HSV/VZ is seen. Multinucleation with nuclear molding, complete margination of the nuclear chromatin, dispersed intranuclear viral material, and intranuclear Cowdry type A inclusions are seen. The histology of the lesion is seen in Fig. 4.67. Sections of lung showed the presence of diffuse alveolar damage. Neither hemorrhagic pneumonitis nor unequivocal viral CPE was found in the lungs. Herpes simplex reactivation is known to occur in patients undergoing prolonged mechanical ventilation; the clinical

Continued



• **Figure 4.66** Postmortem vocal cord scraping with herpesvirus cytopathic effect. **A,** Multinucleation, nuclear molding, and Cowdry type A inclusions are present (Papanicolaou stain, ×1000). **B,** Dispersed viral material and Cowdry type A inclusions are seen (Papanicolaou stain, ×1500).



• **Figure 4.67** Histologic section of ulcerated vocal cord lesion from the same patient as in Fig. 4.66 (hematoxylin and eosin stain, ×400; *inset* ×800).

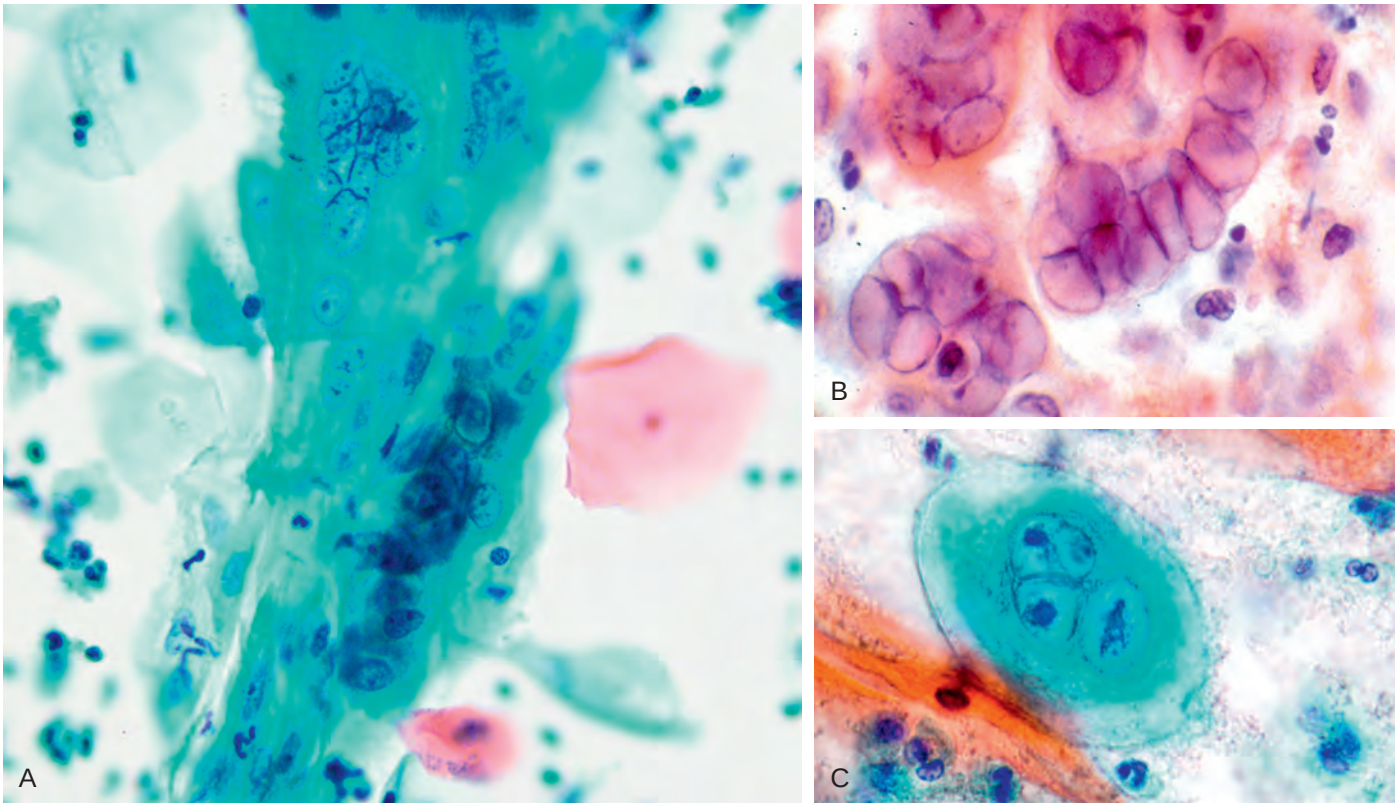
CASE 17

Intensive Care Unit Vocal Cord Lesion—cont'd

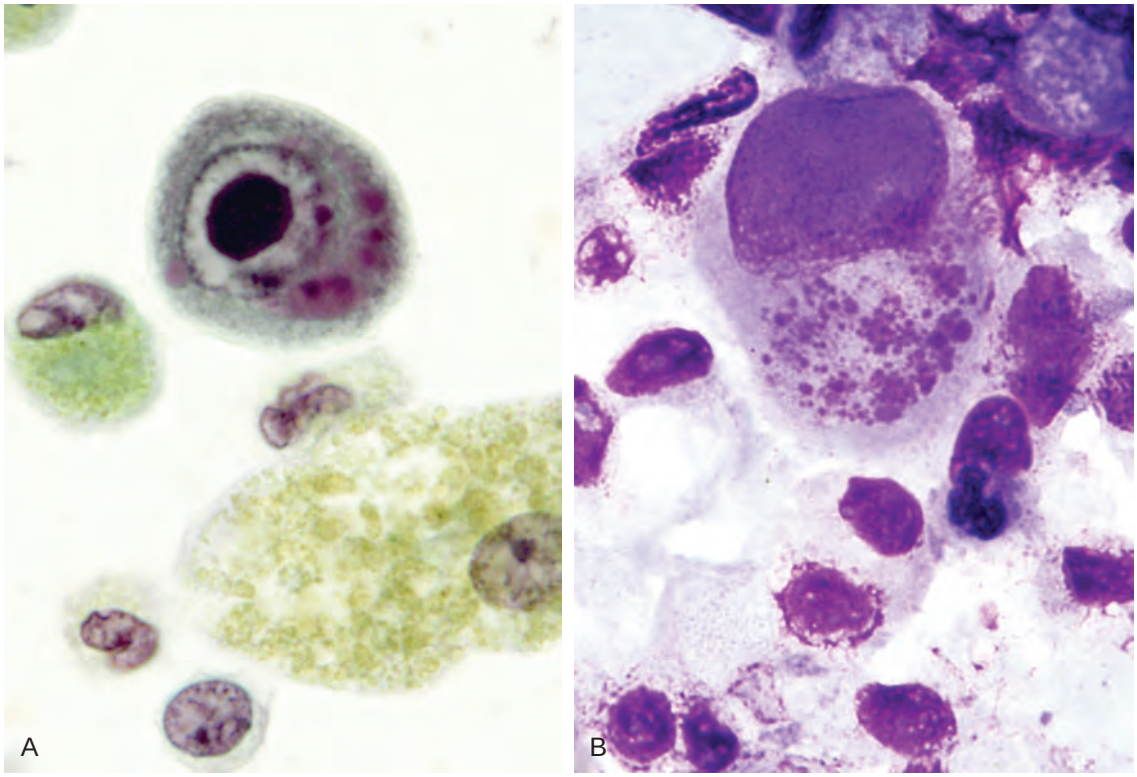
significance of HSV reactivation in tracheobronchial material is still under investigation; however, reactivation may worsen prognosis.[105] The cytopathologist can report the presence of viral CPE, but correlation with the clinical and anatomic pathologic findings is essential for determining its significance.

When sampling an ulcerative or vesicular lesion for cytologic evaluation for suspected viral infection, it is best to remove the cap of the vesicle and gently scrape the base of the ulcer at the junction of the ulcer crater with the intact epithelium. The CPE is present in the squamous cells at the edge of the ulcer (see Fig. 4.67). If one samples the central portion of the ulcer, there may be no epithelium and no CPE. HSV/VZ CPE must be differentiated from squamous regenerative changes associated with nonspecific ulceration. This can be problematic in the interpretation of cervical Pap tests, as illustrated in Fig. 4.68. Note that the intranuclear inclusion of herpesvirus CPE is much larger than the prominent nucleolus found in regenerative changes. We have used direct scrapings of vesicular lesions in burn patients to provide rapid turnaround presumptive diagnoses of HSV/VZ; however, this technique is less sensitive and specific than PCR or immunofluorescence. The latter are needed to distinguish between HSV and chickenpox vesicles, both of which we have seen in children in our burn unit. Herpes simplex infection of the cervix must also be distinguished from trophoblast and keratinizing SCC, and immunostaining and molecular techniques have been used.[106]

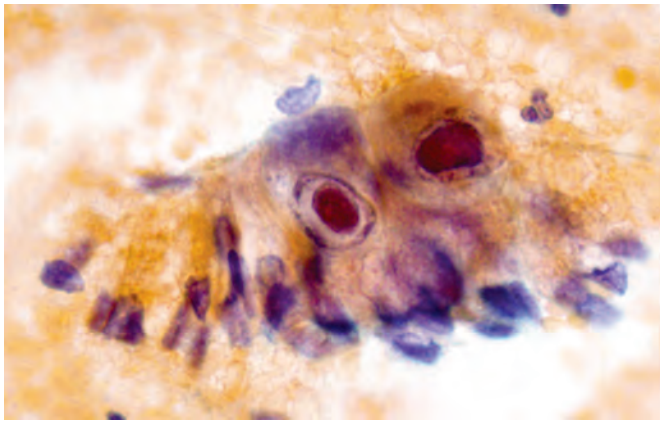
CMV cytopathic effect is beautifully and diagnostically seen in Papanicolaou-stained preparations. As in histologic preparations, the infected cells are enlarged and uninucleated and have single, large, red intranuclear (Cowdry type A) inclusions that are much larger than a nucleolus. The nuclear chromatin is marginated, leaving a clear space between the inclusion and the nuclear membrane. Intracytoplasmic inclusions are characteristic of CMV CPE; however, these are often not seen in Papanicolaou-stained material, or they appear as fuzzy, blue-gray colored areas. The intracytoplasmic inclusions are much more readily apparent in Romanowsky-stained, air-dried preparations, in which they appear as multiple, small, round balls, whereas the intranuclear inclusions are less well seen. BAL samples containing CMV CPE in Papanicolaou and Romanowsky stains are shown in Fig. 4.69. Early infections may lack cells with typical CMV CPE, and immunostaining, in situ hybridization, and other molecular techniques may be useful for documenting infected cells in biopsy or BAL samples if clinically significant infections are suspected.[107] On the other hand, CMV CPE may be found in cytologic preparations from patients lacking clinically significant manifestations of CMV disease, and clinical correlation is mandatory. CMV, like HSV/VZ, may colonize the conducting airways or other organs without causing overt disease. CMV CPE has been reported in cervical Papanicolaou tests,[108,109] but this finding is not necessarily indicative of symptomatic disease. Although rarely seen in Papanicolaou tests, CMV shedding has been demonstrated by PCR in a large number of women infected with HIV-1.[109] We have seen CMV CPE twice in Pap smears, one from a woman with advanced AIDS and disseminated CMV (Fig. 4.70), and the other from a healthy, asymptomatic woman. In cervical Pap tests, CMV CPE is usually found within the endocervical gland cells rather than the squamous cells, where one typically sees HSV CPE.



• **Figure 4.68** Regenerative changes compared with herpesvirus cytopathic effect in cervical Papanicolaou tests. **A,** Regenerative changes are characterized by preservation of fine chromatin and presence of small nucleoli (Papanicolaou stain, ×400). **B** and **C,** Herpesvirus cytopathic effect features multinucleation, chromatin margination, and nuclear molding **(B)** and intranuclear inclusions **(C)** (Papanicolaou stain, ×1000).



• **Figure 4.69** **A** and **B,** Bronchoalveolar lavage fluid containing cytomegalovirus cytopathic effect (**A,** Papanicolaou stain; **B,** Romanowsky stain; both ×1500).



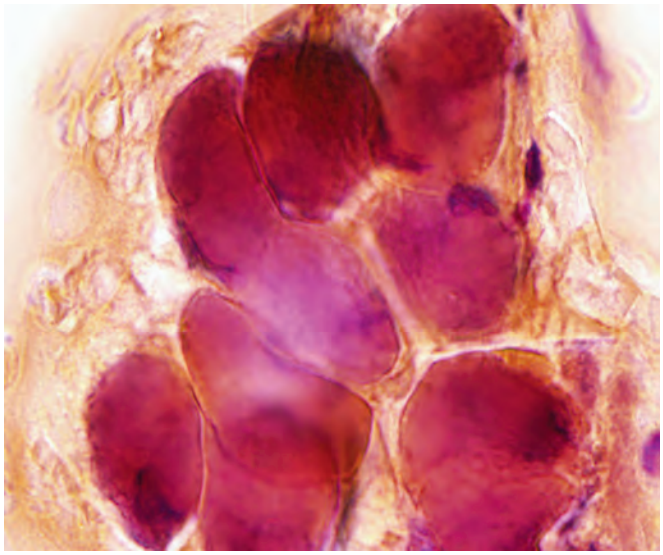
• **Figure 4.70** Cervical Papanicolaou smear demonstrates the presence of cytomegalovirus cytopathic effect in endocervical columnar cells (Papanicolaou stain, ×1000).

CASE 18

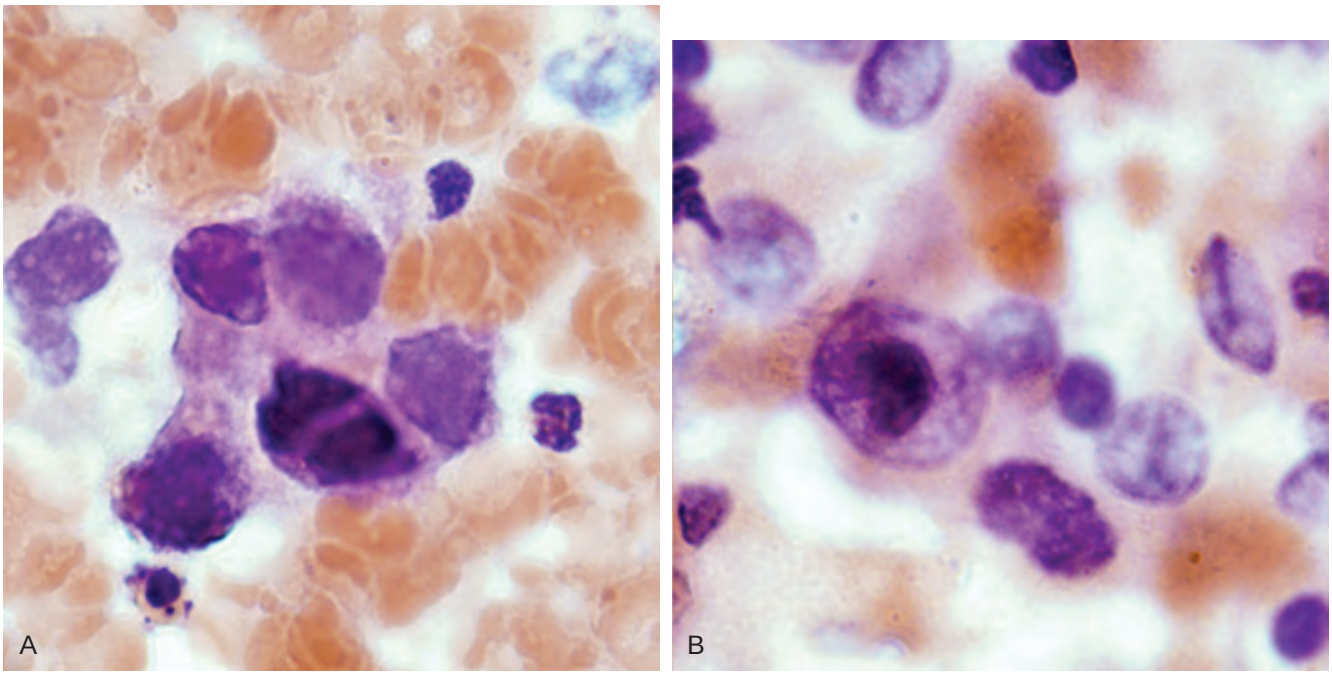
Is It Herpes or Molluscum?

Ethanol-fixed scrapings were prepared from erythematous, roughened, and focally vesicular lesions that followed a dermatome pattern on the chest of an adolescent male with Wiskott-Aldrich syndrome and a history of VZ infection. The pediatric oncologists wanted to know whether the lesions represented recurrent HSV/VZ or molluscum contagiosum. The Papanicolaou stain findings are seen in Fig. 4.71. Anucleated keratin and foci of large, smudgy, oval inclusions are seen. The cell nuclei cannot be seen. These smudgy structures are in fact intracytoplasmic inclusions that fill essentially the entire cytoplasm. The cell nuclei are pushed to the side and are not seen in the cytologic preparations. The findings are typical of the CPE caused by the poxvirus responsible for molluscum contagiosum and are not compatible with HSV/VZ. In this case the vesicular and eczematoid gross appearance of the skin lesions and the history of VZ necessitated ruling out herpes viral infection.

One should be aware that adenovirus infection may be confused with CMV or herpes. All three viruses can produce severe interstitial pneumonia in immunosuppressed patients. Fig. 4.72 illustrates a postmortem lung FNA from a patient with Kartagener syndrome who had undergone lung transplantation and died of fulminant adenovirus pneumonia. Note the lack of cell enlargement and the presence of smudge cells and intranuclear inclusions. Adenovirus was confirmed by culture and immunostaining.



• **Figure 4.71** Skin scraping from a case of molluscum contagiosum with prominent intracytoplasmic inclusions (Papanicolaou stain, ×1000).



• **Figure 4.72** Postmortem fine-needle aspiration of lung from a case of adenovirus pneumonia. **A,** Smudge cells with focal margination of chromatin. **B,** Intranuclear inclusion (Papanicolaou stain, ×1500).

CASE 19

To Immunosuppress or Immunoenhance; That Is the Question!

A urine sample was received from a 45-year-old kidney transplant recipient with acute renal failure. Fig. 4.73 illustrates a Papanicolaou-stained cytospin slide preparation of a centrifuged urine sample. Note the presence of degenerated cellular debris. The sample is hypercellular and contains atypical, enlarged, and pleomorphic epithelial cells that are worrisome for neoplasia; however, it should also be noted that many of the cells appear highly degenerated. They have granular cytoplasm and anisonucleosis. In some cells, the nuclei appear smudgy and have margination of the chromatin. Within other images, the chromatin appears grossly and irregularly clumped. Large intranuclear inclusions with clearing of the surrounding nuclear chromatin are seen in some cells, and focally mitotic figures and binucleation are also seen. In this case, there was positive nuclear immunostaining for BK virus large T antigen, and electron microscopy, performed on urine sediment for teaching purposes,

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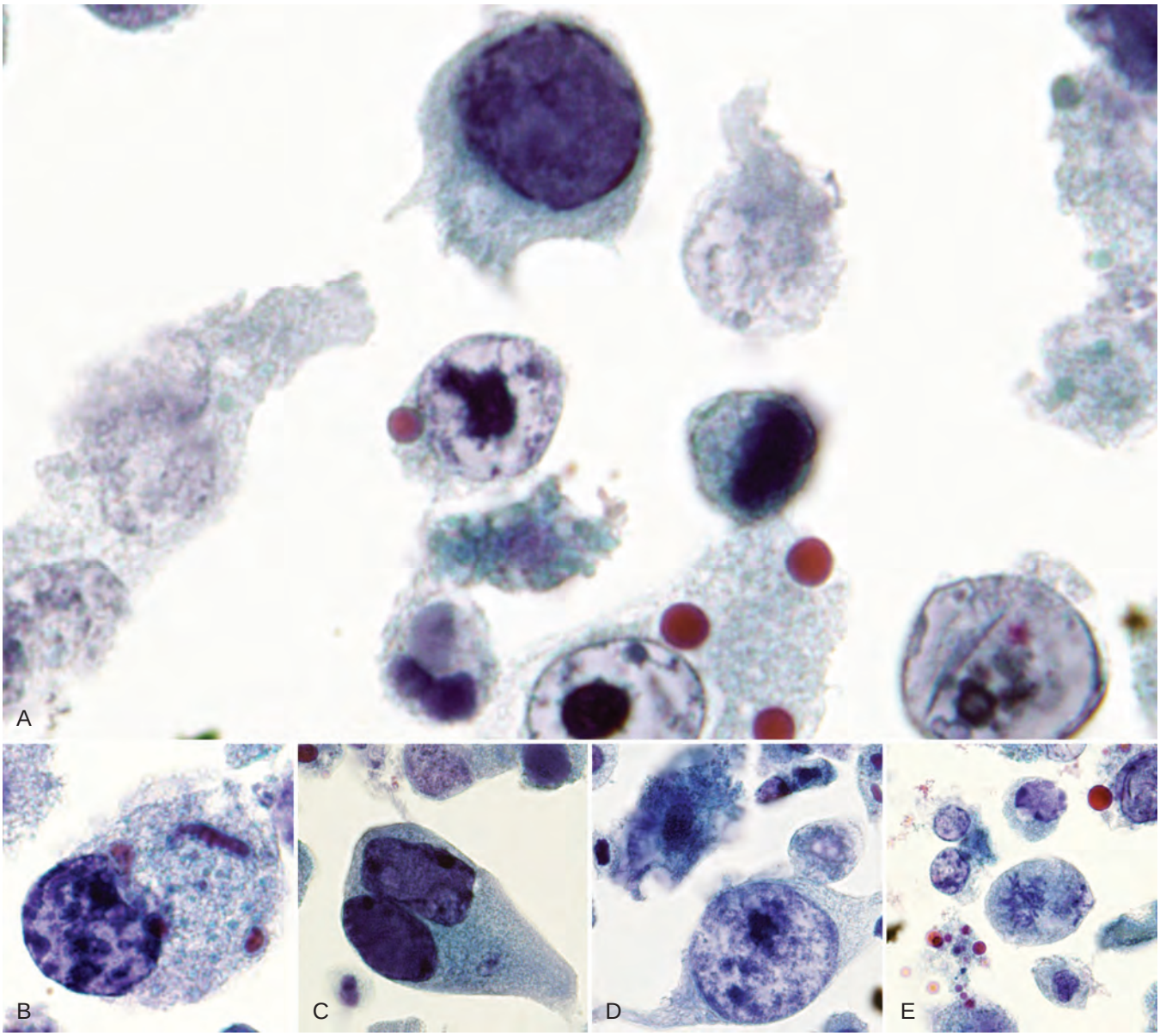


• **Figure 4.73** Voided urine sample from a case of BK virus nephropathy in a renal transplantation patient. **A,** Hypercellular sample with granular debris and atypical cells. Margination of chromatin and intranuclear inclusions are seen. **B,** Cell with coarsely clumped chromatin. **C,** Smudged chromatin. **D,** Cell with coarsely clumped chromatin. **E,** Mitotic activity (Papanicolaou stain, ×1500).

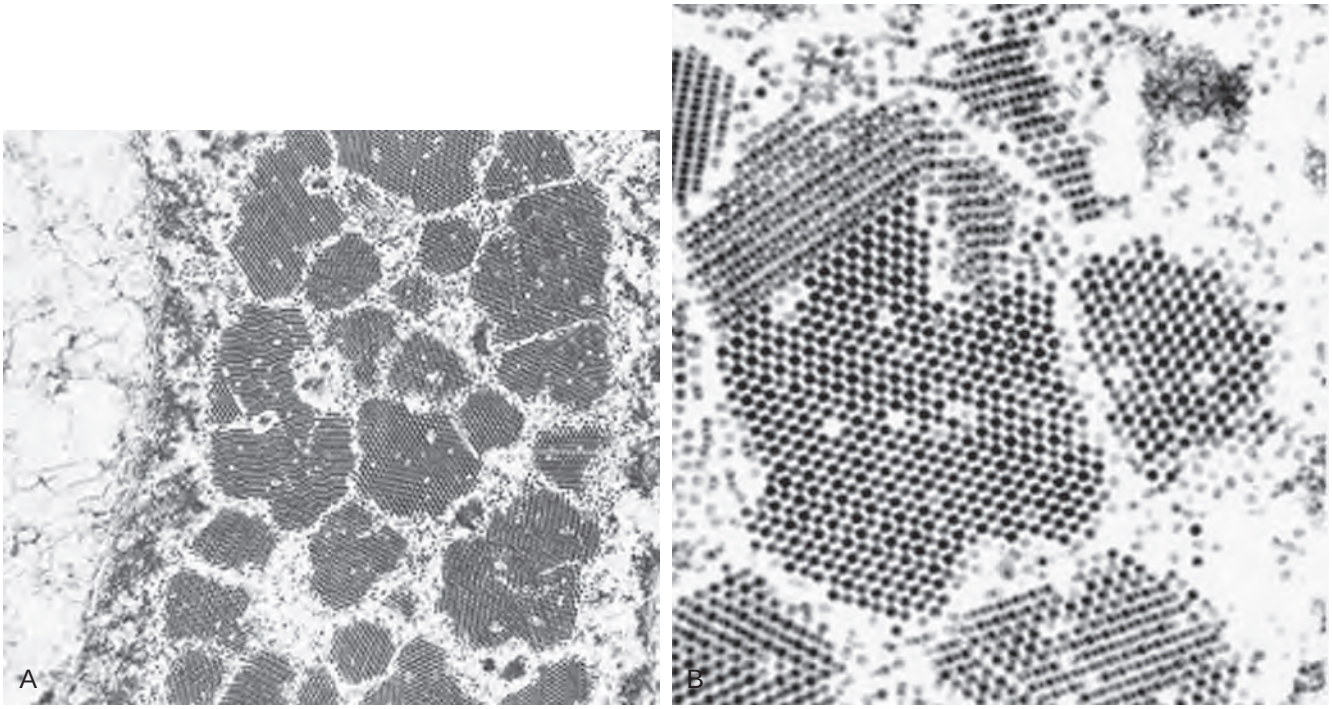
CASE 19

To Immunosuppress or Immunoenhance; That Is the Question!—cont'd

demonstrated typical intranuclear polyomavirus particles (Fig. 4.74). Renal biopsy demonstrated the presence of interstitial nephritis and abundant intranuclear inclusions within the renal tubules.

Widely known in the cytology vernacular as decoy cells, courtesy of a senior cytotechnologist at Memorial Hospital during the 1950s,[110] urothelial cells with polyomavirus CPE are notorious mimics of malignancy. The urine cytopathology of BK virus infection has been described in detail.[110,111] Urine cytology has been found to be a sensitive test for detection of BK viral infection; however, it is again important to point out that the presence of viral inclusions in the urine does not necessarily indicate BK virus nephropathy. Distinguishing between polyomavirus-induced renal failure and acute rejection is essential because the former is treated by decreasing, and the latter by increasing, immunosuppression. Renal biopsy represents the gold standard for confirming polyomavirus nephropathy.[112] Currently, real-time PCR, rather than urine cytology, is used for BK virus screening and quantification in renal transplant patients; however, cytologists should be aware of the CPE of BK virus and its potential to mimic neoplasia.

The take-home message for those diagnosing viral infections by cytology is that viral disease may occur without CPE, and CPE indicative of active viral infection may occur without symptomatic disease! Clinical and, in some instances, histologic correlation may be required. Although molecular testing for viral infections has become standard, owing to its greater sensitivity and relatively rapid turnaround time, cytology has the potential to detect unsuspected viral infections, and cytologists should vigilantly screen for viral CPE when evaluating samples.

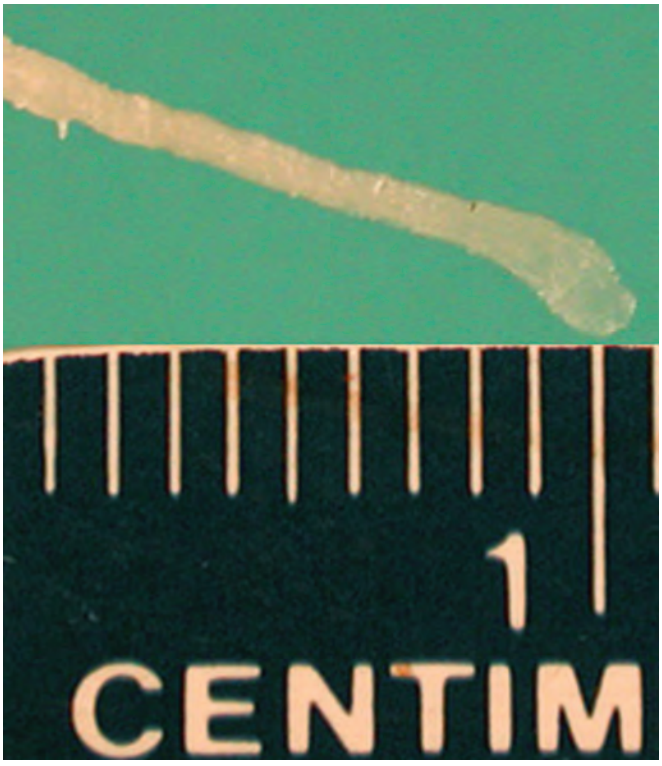


• **Figure 4.74** Transmission electron micrograph of urine sediment from the same patient as in Fig. 4.73, showing intranuclear icosahedral viral capsids (**A,** ×15,500; **B,** ×39,000). (Electron photomicrographs courtesy of Julie Wen.)

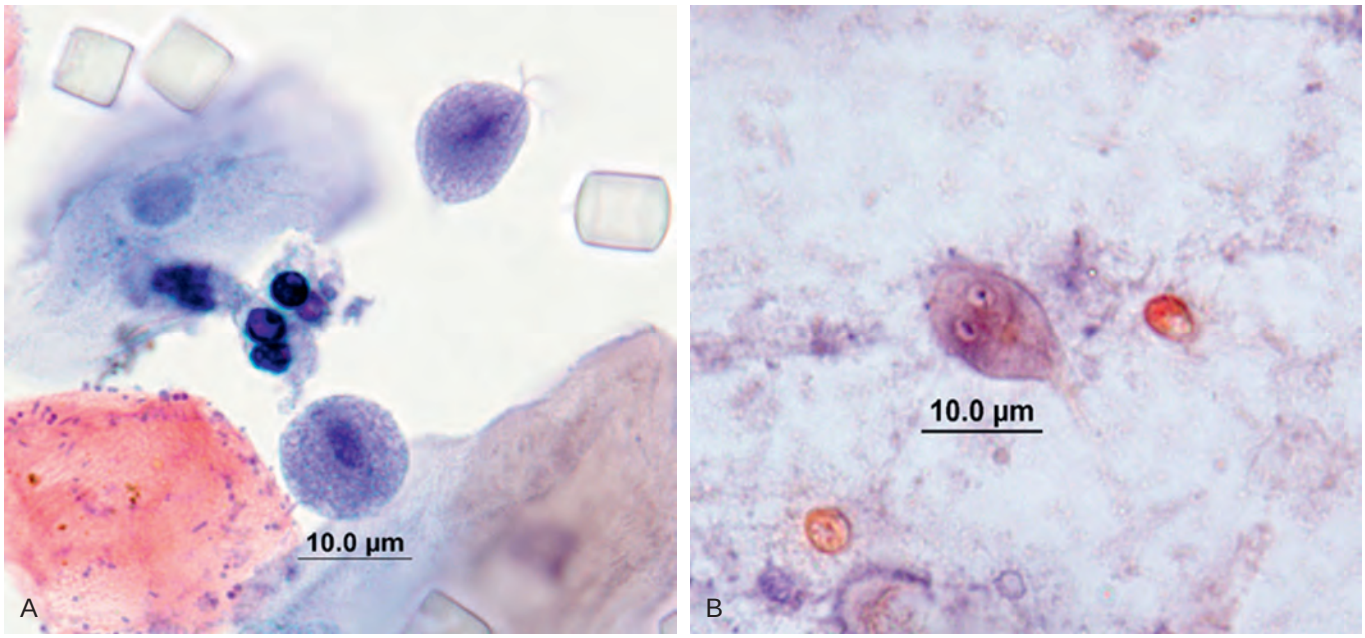
Parasitic Disease in Cytology

In parts of the world where protozoan and helminthic diseases are common, parasite infections are more frequently found in cytologic preparations than in northern climates; however, one should never be complacent about diagnosis of parasitic disease by cytology, and knowledge of the basic morphology of some of the more common pathogens is useful. In addition, one should always be on the lookout for something new and different. To paraphrase a line from the movie *Forrest Gump*, examination of an FNA is like a box of chocolates: you never know what you are going to get. Fig. 4.75 illustrates a portion of a cestode larva—a sparganum, to be more precise—that emerged from the FNA puncture site of a thigh nodule found in an elderly woman from rural Louisiana. It appeared that the newly materialized stuff was gauze or mucus, so the faculty pathologist decided to pull on the structure, and out came approximately 3 cm of cestode larva. This was duly placed in a cell-block preparation and proved, by histologic sections, to be consistent with a sparganum, the larval form of *Spirometra* spp. This adventure resulted in resolution of the thigh nodule and no surgery for the happy patient and disappointed surgeon. Inflammation and a few laminated structures consistent with calcareous corpuscles were seen in FNA slides.

The parasite most commonly seen in cytology in the United States is *Trichomonas vaginalis*; this protozoan is discussed in the chapter on cervical pathology. Although cervical Pap test is the usual source, it may also be found in urine from both men and women (Fig. 4.76A). *Entamoeba histolytica* has also been diagnosed by cytology in areas where the protozoan is endemic.[113] Numerous individual case reports of cytodiagnosis of parasitic infections can be found in the literature, and discussion of them all is beyond



• **Figure 4.75** Gross photograph of *Sparganus*, a cestode larva extracted by fine-needle aspiration from a thigh mass.



• **Figure 4.76** Urine **(A)** containing two *Trichomonas vaginalis* trophozoites and some calcium oxalate crystals. A solitary, small, oval nucleus can be seen within trophozoite in lower center, and flagella are seen in trophozoite in upper right. Duodenal brushing **(B)** containing *Giardia lamblia* trophozoite (both Papanicolaou stain, ×1000).

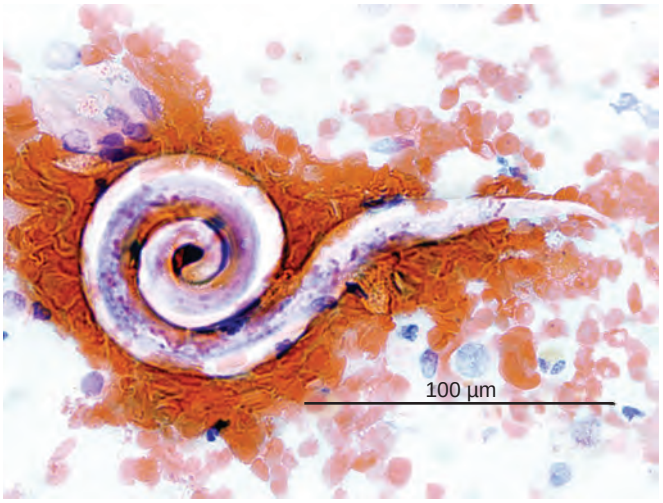
the scope of this chapter. Instead, we briefly cover a few of the infections more commonly found in the United States, with a couple of zebras thrown in for fun.

Giardia lamblia (also known as *G. intestinalis* and *G. duodenalis*) is a ubiquitous protozoan that is common in the United States and throughout the world. In the United States it is usually transmitted via drinking water contaminated with human waste. Although *Giardia sp.* are also found in domestic and wild animals, the role of zoonotic transmission is currently unclear.[114] The pear-shaped trophozoite stage of *G. lamblia* has a ventral concave surface and a convex dorsal surface. It is found in the small intestine, where it lies along the surface of the epithelium. Therefore, *G. lamblia* is readily picked up in duodenal brushings (see Fig. 4.76B); however, because of its small size, approximately 15 µm in length, it can easily be missed. High-power magnification reveals a pear-shaped, flagellated organism with two prominent nuclei that give the trophozoite the appearance of a face or "Mardi Gras mask."

S. stercoralis is an important nematode for the cytology professional to remember. The life cycle of this nematode is reviewed in parasitology texts. However, the following two aspects of *S. stercoralis* are important to emphasize here:

1. Subclinical infections with *S. stercoralis* may persist for 30 to 40 years or even longer.
2. Immunosuppression (most importantly treatment with corticosteroids and infection with human T-lymphotropic virus I) and severe malnutrition can lead to a life-threatening, often fatal, hyperinfection.[115-117]

During hyperinfection, literally millions of worms pass through the lungs. This massive worm migration leads to pulmonary hemorrhage and bronchopneumonia. Infectious-stage (filariform) larvae can be found in sputum, bronchial washings, brushes, and lavage fluid. Fig. 4.77 illustrates a *Strongyloides* larva found in a bronchial brushing sample. We regard this finding as a medical emergency and notify the clinician immediately if nematode larvae are found in respiratory samples. The patients at highest risk for life-threatening infections are those receiving corticosteroids, and screening for persons at risk for strongyloidiasis has been recommended for such patients.[116,117] Usually, only the filariform larval stages are found in the lung; however, rarely, adolescents, adults, and eggs have been found. We have seen only one such case, in a patient treated with steroids for chronic obstructive pulmonary disease, who had first-stage larvae, an adult, and eggs in cytologic preparations of his sputum (Fig. 4.78).



• **Figure 4.77** Bronchial brushing from patient with *Strongyloides stercoralis* hyperinfection. Larva admixed with blood is seen (Papanicolaou stain, ×400).

Text continued on p. 75

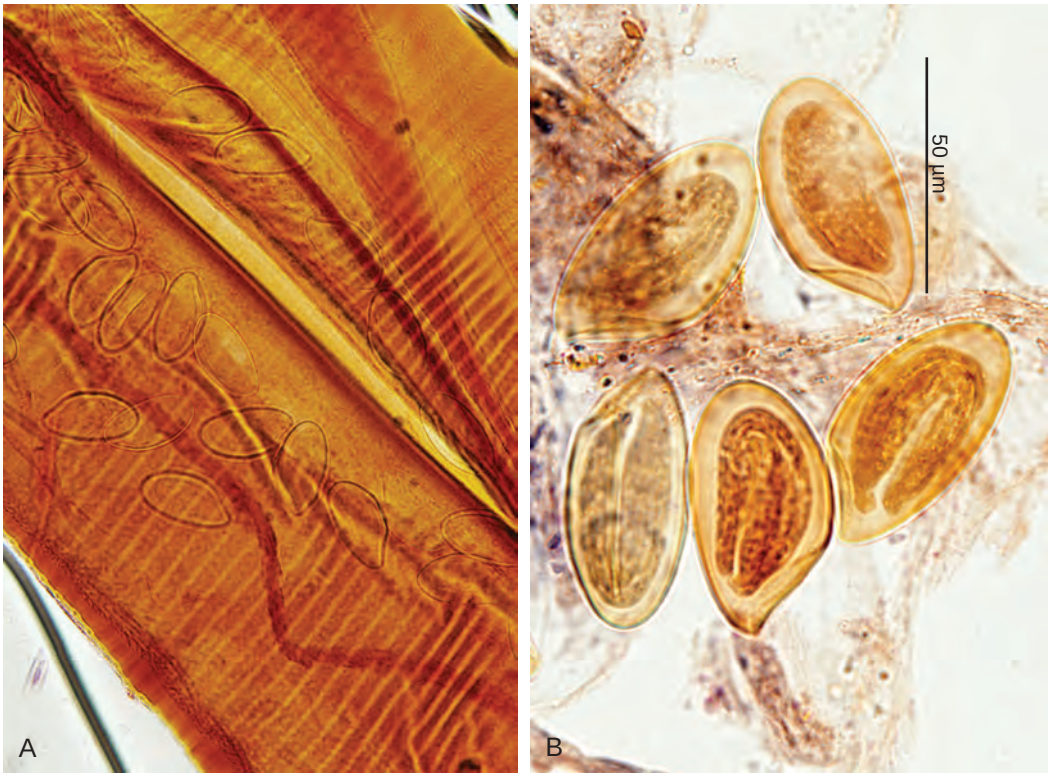
• **Figure 4.78** Sputum sample from a patient with *Strongyloides* hyperinfection shows adult worm and eggs *(inset)* in respiratory material (Papanicolaou stain, ×100; *inset* ×1000).

CASE 20

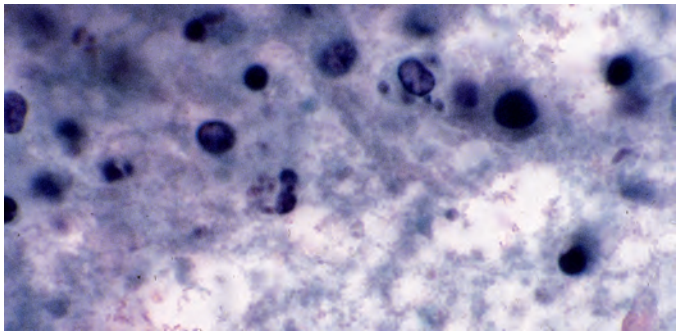
A Worm With the Wanderlust

A conventional Pap smear is performed on a young woman as part of a well-woman examination. The findings are seen in Fig. 4.79, in which one sees a portion of nematode with a striated cuticle. Within the worm are oval eggs that have a convex surface and a somewhat more flattened opposing surface. Fig. 4.79B, taken from another part of the smear and external to the nematode, shows eggs, approximately 50 μm in length. Some are fully embryonated. The nematode is *Enterobius vermicularis*, also known as pinworm. *E. vermicularis* is an extremely common and ubiquitous parasitic nematode, especially in children who attend school or daycare. The worms dwell in the large intestine, where they mate. The gravid female crawls out onto the perineal skin, usually at night, where she deposits embryonated, and hence infectious, eggs. The female occasionally gets the urge to ramble and can wander into the vaginal canal and even up into the uterus, fallopian tubes, or peritoneal cavity.[118] The adult female is approximately 1 cm in length, and the entire worm or fragments of disintegrating worm can be picked up in Pap smears. The morphology of the egg is characteristic and diagnostic. Usually, this parasite is a harmless, albeit itchy and irritating, nuisance; however, intraperitoneal granulomas and pelvic abscesses have been associated with pinworm infections, with occasional diagnosis by cytology.[119]

As previously stated, it is beyond the capacity of this chapter to cover all parasitic infections that can be or have been diagnosed by cytology nor to cite every report. *Toxoplasma* tachyzoites and bradyzoites have been occasionally reported cytologic preparations.[120-122] Bradyzoites (cysts) can be identified in Papanicolaou- and H&E-stained cytology and histology preparations. Tachyzoites are best seen in smears or cytospin preparations and are not readily seen in histologic preparations. Immunohistochemistry or immunofluorescence allows better visualization of tachyzoites and confirmation of infection. We have rarely diagnosed toxoplasmosis by cytology. Fig. 4.80 illustrates *Toxoplasma* tachyzoites in a Papanicoloaou-stained squash preparation of a brain procured at autopsy from a patient with advanced AIDS. Microsporidia, also rarely reported in cytologic preparations,[123] can be identified as intracellular bodies by Gram stain. Do not be complacent! One should always be vigilant for interesting and unusual infections and be willing to learn new information about infectious diseases and their diagnoses.



• **Figure 4.79** Cervical Papanicolaou smear containing portion of *Enterobius vermicularis* female and eggs. **A,** Nematode cuticle and intrauterine eggs (Papanicolaou stain, ×200). **B,** Embryonated eggs (Papanicolaou stain, ×400).



• **Figure 4.80** Squash preparation of brain taken at autopsy. Mononuclear cells containing small oval-to-elongate Toxoplasma tachyzoites are seen (Papanicolaou stain, ×1000).

CASES 21 AND 22

Parasite Infections Seen in Postmortem Cytology

Smear and FNA preparations taken at autopsy have provided us with some of our most interesting cases, and postmortem cytology has proved excellent for IID diagnosis.[5] The postmortem cytology findings from two interesting parasitic infections found at autopsy are described here.

At autopsy, an enlarged, slate-gray spleen and severe cerebral edema were found in a patient who had died en route home from West Africa. Fig. 4.81 shows a Romanowsky-stained scrape preparation of spleen. Note the presence of abundant dark golden pigment and a banana-shaped structure containing pigment in the center. The pigment is consistent with malarial pigment (hemozoin). In the inset, one sees cuboidal-shaped asexual forms that have single, eccentrically placed red-dot nuclei and blue-colored cytoplasm. These findings led to a diagnosis of malaria; the clinical findings and banana-shaped structure, morphologically typical of a gametocyte, were consistent with *Plasmodium falciparum*. Cerebral malaria was confirmed by histologic sections of brain.

Fig. 4.82 illustrates a scraping of brain from an immunosuppressed patient who died with severe pneumonia. Necrotizing bronchopneumonia and hemorrhagic cerebral lesions were found at autopsy. Histologic sections

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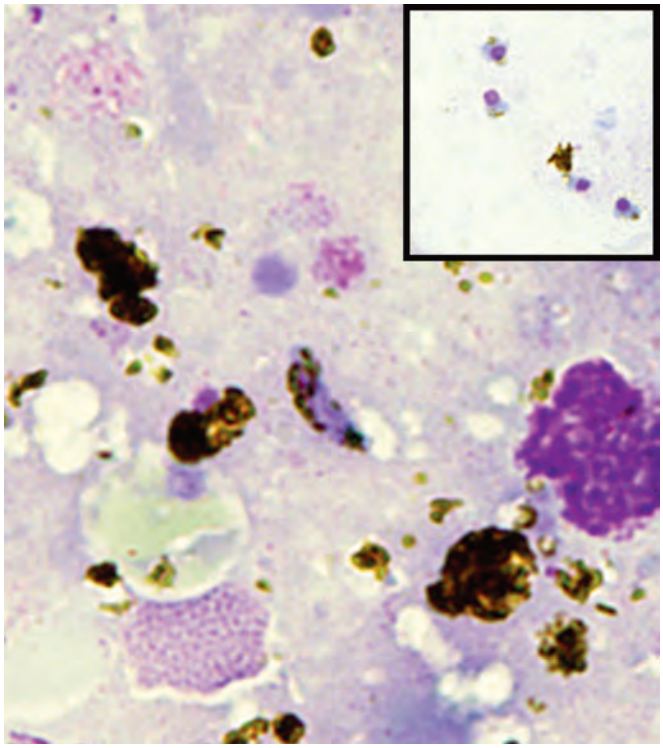
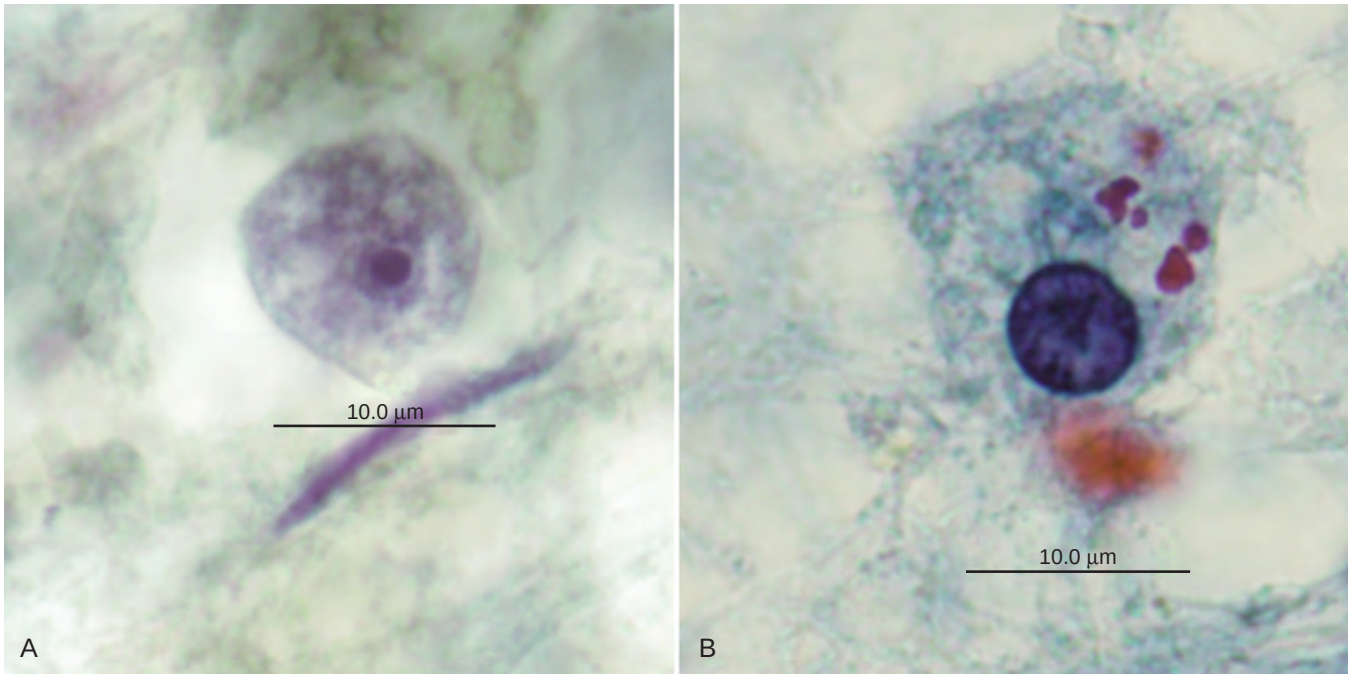


• **Figure 4.81** Scrape preparation of spleen from a patient with falciparum malaria. Abundant hemozoin pigment and a gametocyte are present (Romanowsky stain, ×2500). *Inset* shows asexual forms (Romanowsky stain, ×3000).



• **Figure 4.82** Touch preparation of brain from a patient with *Acanthamoeba* pneumonia and encephalitis. **A,** Free-living ameba with large intranuclear karyosome. **B,** Macrophage with larger nucleus and absence of karyosome (Papanicolaou stain, ×3000).

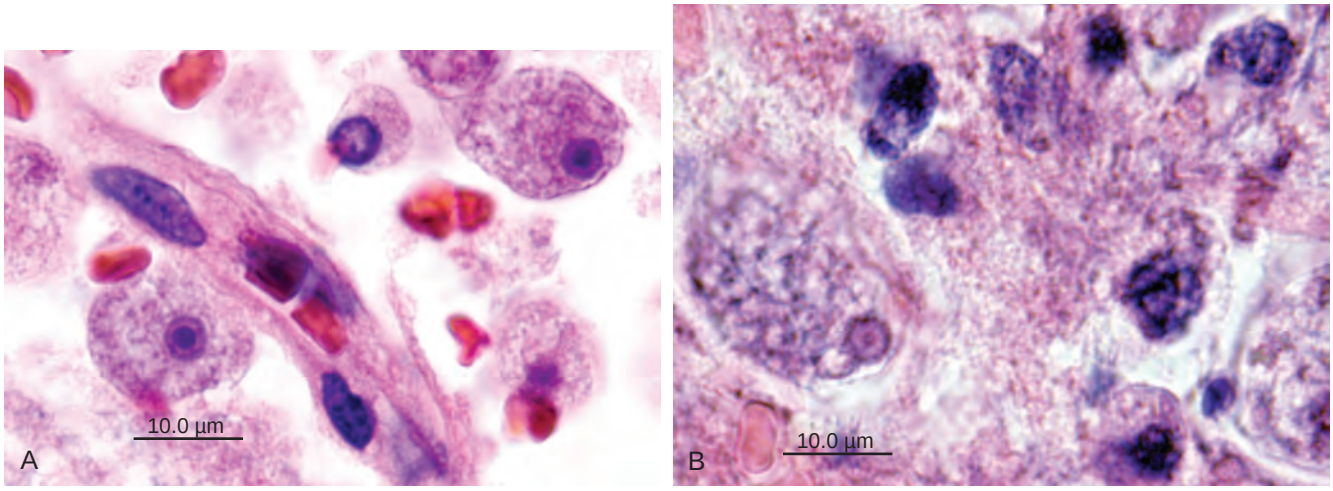
CASES 21 AND 22

Parasite Infections Seen in Postmortem Cytology—cont'd

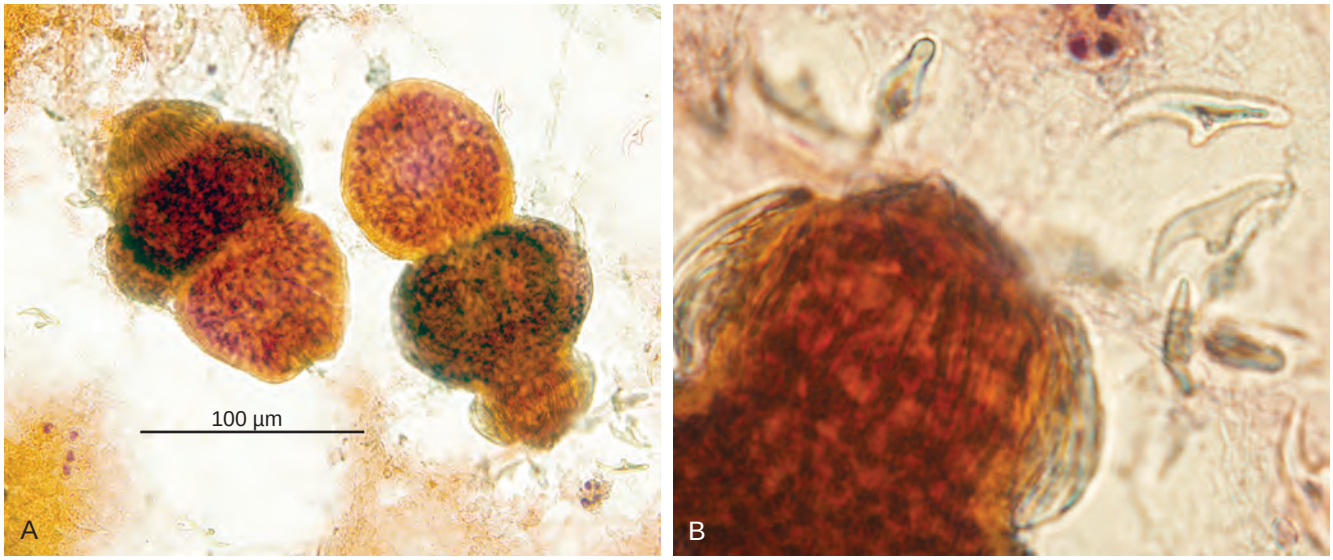
established a diagnosis of free-living amebic pneumonia and encephalitis.[124] In this case the primary diagnosis was not made by cytology; however, the cytologic preparations of brain provided useful teaching material. In Fig. 4.82A, one sees a small, rounded structure, approximately 12 μm in diameter. It has a small nucleus containing a large karyosome similar to that seen in *Entolimax nana*. This was a free-living ameba, later identified as a species of *Acanthamoeba*. Compared with the macrophage (see Fig. 4.82B), with which these organisms can be confused, the ameba has a much smaller nucleus, and a vacuole surrounds the cytoplasm. Fig. 4.83A is an H&E-stained section of the brain showing two free-living amebic trophozoites gathering around a capillary. For comparison, a section of *E. histolytica* from a rectal ulcer biopsy is shown in Fig. 4.83B. The sizes are similar; however, the *E. histolytica* karyosome is much smaller and often is difficult to see in sections. Both organisms have a much smaller nucleus-to-cytoplasm ratio than do macrophages. Free-living amebas are well-known causes of keratitis in contact lens wearers and in patients who have sustained ocular trauma.[125] Impression smear cytology has been used to diagnose amebic keratitis in a limited number of cases.[126]

Cestode larvae that are pathogenic for humans include mainly *Echinococcus granulosus* (hydatid cyst), *Echinococcus multiceps*, and *Taenia solium* (cysticercus cellulosae). Cestode larvae have occasionally been aspirated by FNA. Fig. 4.84 illustrates the scoleces of a hepatic hydatid cyst. Note the hooklets arranged around the rostella of the scoleces and the free-lying hooklets.

There are certainly other parasites that may be found by cytodiagnosis; here we have only attempted to discuss some of the more common pathogens and a few unusual and interesting cases. For discussions of trematodes (flukes) and ectoparasites, the reader is referred to parasitology textbooks.



• **Figure 4.83** Histologic sections of *Acanthamoeba*, from the same patient as in Fig. 4.82 **(A)**, and *Entamoeba histolytica* proctitis **(B)** (hematoxylin and eosin stain, ×1500).



• **Figure 4.84** Fine-needle aspiration of hepatic echinococcal hydatid cyst. **A,** Two scoleces are present (Papanicolaou stain, ×400). **B,** Rostellum of scolex with many hooklets is present (Papanicolaou stain, ×1000). (Glass slide donated courtesy Dr. A. Joe Saad.)

Conclusion

One cannot diagnose infectious diseases if one does not think about them. Infections are often treatable and curable, and skillful diagnosis by the cytopathologist can be of enormous benefit to both patient and clinician by providing a rapid and accurate preliminary diagnosis and material for appropriate cultures with minimally invasive techniques. IID should share the front burner of cytopathology education with neoplasia. In addition, the pathologist should work in tandem with clinical microbiologists, radiologists, endoscopists, and clinicians, using acquired knowledge of microbiology, pathology, and clinical medicine to guide his or her colleagues toward optimal patient care. ROSE, including onsite personal interaction with patients, and knowledge of proper staining and culture methods are essential to this goal. Although PCR, in situ hybridization, and immunohistochemistry are emerging as sensitive and valuable diagnostic techniques, their use should not discourage the cytopathologist from developing knowledge and skill in infectious disease diagnosis because every day is a diagnostic adventure, and you never know what you are going to get.

Appendix: Further Information

Fig. 4.85 illustrates the comparative morphology of some yeastlike fungi, as seen in GMS-stained preparations. Note that with GMS, often only the yeast-cell size and uniformity, or lack thereof, offer information regarding fungus identification. Cell wall thickness and internal structures are not seen as clearly as with Papanicolaou and Romanowsky methods. Small size and cell uniformity of *Histoplasma* helps to differentiate this fungus from *Cryptococcus* and *Coccidioides*. *Blastomyces* is usually larger and relatively uniform in size; however, small forms exist. In Fig. 4.85C, *Cryptococcus* could be confused with *Paracoccidoides* (Fig. 4.85G), owing to presence of double budding. The thin cell wall, typical of *Cryptococcus*, is easily appreciated in GMS. Ship's wheel budding, although considered a classic feature of *Paracoccidioides*, may be very difficult to find. When scanty organisms are present, it is difficult to distinguish many of these fungal pathogens from each other by GMS. Having access to Romanowsky and Papanicolaou (or H&E) and good clinical information, as well as GMS stain, is optimal. Figs. 4.85D, F, and G are taken from histologic preparations.

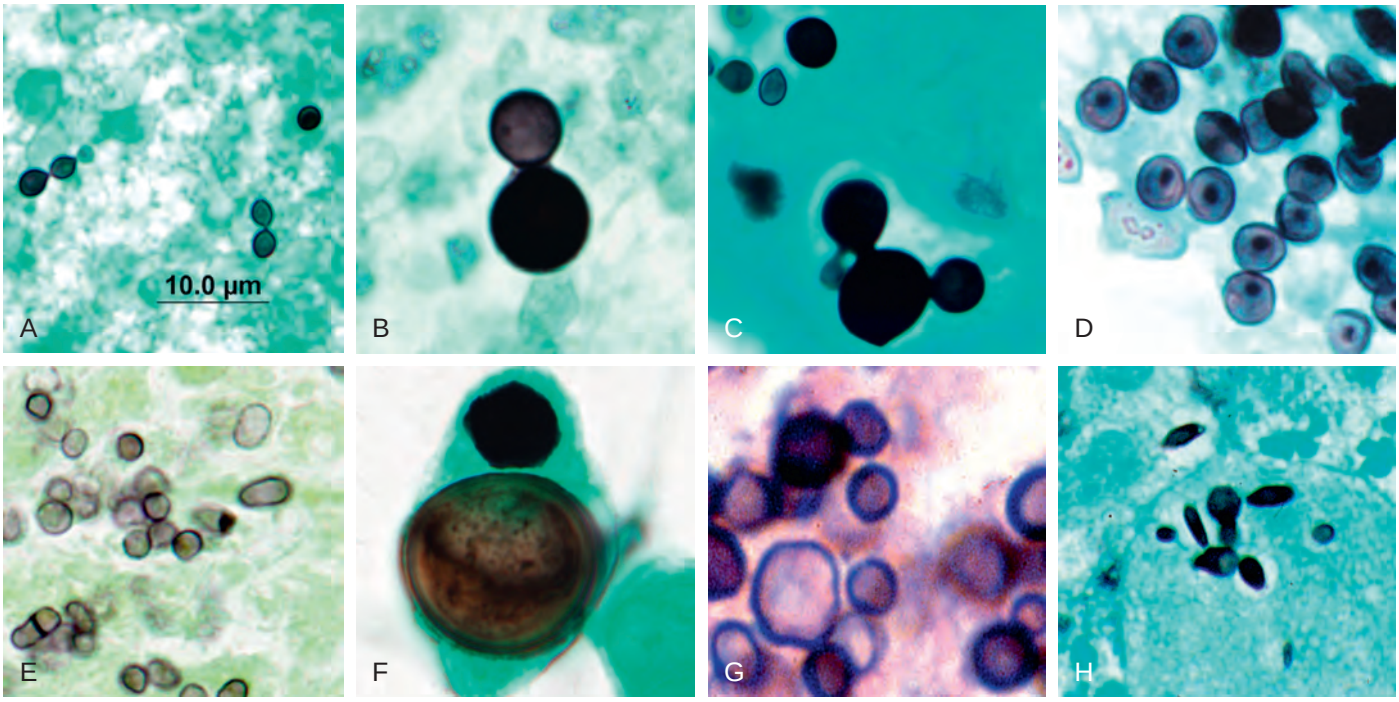
Sample Preparation and Staining for Diagnosis of Infectious and Inflammatory Diseases Including *Pneumocystis*

This section provides a few modifications of sample preparation and staining procedures that have been found useful for diagnosis of IIDs. The author wishes to acknowledge Steven Burke, BS, for his assistance with composition of the Appendix.

Bronchoalveolar Lavage and Induced Sputum Evaluation for Pneumocystis

Staining for *P. jiroveci* is performed on induced sputum samples or BAL samples.

BAL samples usually contain 10 to 20 mL of fluid. The sample is first centrifuged at a relative centrifugal force (RCF) of 600 *g*



• **Figure 4.85** Comparative images of *Histoplasma* **(A)**, *Blastomyces* **(B)**, *Cryptococccus* **(C)**, *Pneumocystis* **(D)**, *Penicillium marneffei* **(E)**, *Coccidioides* **(F)**, *Paracoccidioides* **(G)**, and *Sporothrix* **(H)** in Grocott-Gomori methenamine silver (GMS)-stained preparations. Note double budding form of *Cryptococcus* could be confused with *Paracoccidioides* with this stain. Cell wall and internal features are not well visualized (GMS stain, ×1000)

for 5 minutes. The supernatant is drawn off with a pipette or by decanting, leaving 1 to 2 mL of fluid for resuspension of the sediment. Two to four drops of suspension, depending on the turbidity of the suspension, is placed into each of four cytocentrifuge specimen holders (Shandon Scientific Limited, Cheshire, UK); two drops of normal saline are added to the chambers, and the specimens are cytocentrifuged for 2 minutes at 1200 rpm (163 Xg). Three of the slide preparations are fixed in 95% ethanol for Papanicolaou and GMS staining, and one is air dried for Giemsa staining.

It is our policy to routinely prepare two additional ethanol-fixed cytospin preparations, one Kinyoun and one Gram-Weigert, for BAL samples from severely immunosuppressed patients, to evaluate for bacterial, actinomycete, and mycobacterial infections.

Preparation of Sputum Samples

Sputum samples for detection of *Pneumocystis* should be induced by trained respiratory therapy personnel. In the laboratory a mucolytic agent is added to make the specimen adequately fluid for centrifugation and cytocentrifugation.

Procedure

Reagents

- Sputum sample, approximately 2 to 10 mL, usually received in clean specimen cup
- Dithiothreitol: Mucolyse (Pro-Lab Diagnostics, Ontario, Canada)
- Normal saline

Technique

1. Approximately 10 mL of distilled water is added to the dithiothreitol powder vial and agitated until the powder is dissolved. The contents of the vial are then added to a container of water to make a final volume of 100 mL.
2. The mucolytic agent solution is then added to the sputum container at a ratio of 1 to 3 times the volume of sputum, placed in a 50-mL conical centrifuge tube, and vortexed for 15 to 30 seconds. The solution is then allowed to stand at room temperature for 1 to 3 hours or until the sputum sample appears liquid and nonviscous. (Fifteen minutes has been recommended by the manufacturer for culture purposes; however, we have found that more than 1 hour is required for optimal cytocentrifuge preparations. In addition, Gill et al.[98] reduced the mucolysis period to 3 to 5 minutes by incubating in a 37°C waterbath.)
3. The sample is then centrifuged at 600 RCF for 5 minutes. The supernatant is decanted, and the sediment is resuspended in approximately 5 mL of normal saline.
4. Four cytocentrifuge preparations are made as described earlier. One is allowed to air dry, and the others are wet–fixed in 95% ethanol. Cytocentrifuge preparations are made on clear, ionized (charged) slides to minimize sample loss.

Staining. Standard staining for sputum with request for *P. jiroveci* is as follows:

1. Two ethanol-fixed cytocentrifuge preparations stained by nongynecologic Papanicolaou method.
2. One air-dried cytocentrifuge preparation stained by Giemsa Romanowsky method.
3. One ethanol-fixed cytocentrifuge preparation stained by microwave modification of GMS stain as described in the following section.

Microwave Modification of Grocott-Gomori Methenamine Silver Stain for Fungi Including Pneumocystis

Types of Samples

- Formalin—fixed, paraffin-embedded sections, air dried, or fixed with 95% ethanol
- Fixed smears
- Cytocentrifuge preparations

Procedure

Reagents

- 5% CHROMIC ACID*
  Chromic acid: 5 g
  Distilled water: 100 mL
  Chromic acid is an irritant and should be stored in an acid cabinet. Coplin jars with 5% chromic solution are stored under a chemical fume hood.
- 5% SILVER NITRATE SOLUTION*
  Silver nitrate: 5 g
  Distilled water: 100 mL
- 3% METHENAMINE SOLUTION
  Hexamethenamine USP: 3 g
  Distilled water: 100 mL
- 5% BORAX SOLUTION
  Borax: 5 g
  Distilled water: 100 mL
- STOCK METHENAMINE SILVER NITRATE SOLUTION
  5% Silver nitrate solution: 5 mL
  3% Methenamine solution: 100 mL

  Note: A white precipitate will form when reagents are first combined. This will dissolve with agitation. Store stock solution in the refrigerator at 5°C to 10°C in a dark or opaque, sealed bottle. Although this is supposed to be fairly stable, it is best not to make up more than stated or more than can be consumed in 2 weeks.
- WORKING METHENAMINE SILVER NITRATE SOLUTION
  5% Borax: 2 mL
  Distilled water: 25 mL
  Methenamine silver nitrate stock solution: 25 mL

  Note: *This must be made up fresh with every run.* Make up this solution just before initiation of the staining procedure.
- 1% SODIUM BISULFITE SOLUTION†
  Sodium bisulfite: 1 g
  Distilled water: 100 mL
- 2% SODIUM THIOSULFATE SOLUTION†
  Sodium thiosulfate: 2 g
  Distilled water: 100 mL
- 2% GOLD CHLORIDE
- STOCK LIGHT GREEN SOLUTION
  Light green, S.F: 0.2 g
  Distilled water: 100 mL
  Glacial acetic acid: 0.2 mL
- WORKING LIGHT GREEN COUNTERSTAIN
  Stock light green solution: 10 mL
  Distilled water: 50 mL

*Reagents may be doubled or tripled and water added to make up 200- or 300-mL aliquots, if desired.[5]

†Reagent can be made in large quantities, if desired.

General Comments. A *Pneumocystis* control slide should be run whenever this stain is performed for detection of *Pneumocystis*. Paraffin sections are used for this purpose. The section should be deparaffinized in xylene for 10 minutes or longer. Some controls can be kept in xylene if the stain is run frequently. The control must then be rehydrated through 100% ethanol and 95% ethanol to distilled water before running the stain. Alcohol-fixed cytocentrifuge preparations are placed in distilled water before running. Preparations for GMS staining over Papanicolaou should be placed in xylene to remove coverslip and mountant, then rehydrated up to distilled water. Decolorization of hematoxylin with dilute hydrochloric acid is not necessary.

Technique

1. Loosely place cap on chromic acid jar without test slides or control, and microwave on high for 30 seconds. Place slides in heated chromic acid jar for 1 minute. Overheating or boiling of chromic acid may result in unsatisfactory staining. Always replace hot acid under the fume hood immediately after removal from the oven.
2. Remove slides from chromic acid and place in a Coplin jar filled with distilled water. Rinse slides with distilled water to remove excess chromic acid.
3. Place slides in 1% sodium metabisulfite for 30 to 60 seconds to clear.
4. Place slides back in Coplin jar, and rinse in distilled water.
5. Heat methenamine silver working solution in microwaveable Coplin jar for 33 seconds on high. Immediately place slides in heated methenamine silver solution. Ideally the solution should be clear to faintly gray when removed from the oven. Watch carefully for control specimen color change. Remove slides to a Coplin jar of distilled water. This may vary with laboratory and characteristics of water and solutions. Sometimes the solution must turn a dark black color before adequate staining has occurred. In other instances a dark color may be indicative of overstaining. Therefore slides should be removed before the solution turns an opaque black, rinsed in distilled water, and checked under the microscope for staining of control slide.
6. Rinse the control slide in distilled water and examine microscopically for black staining (or brownish red before clearing in gold chloride) of the *Pneumocystis* cysts.
7. If necessary, the time in silver solution can be extended by replacing slides in the warm methenamine solution. If the sample has turned opaque black but no staining has occurred, there is a problem with the reagents, and additional staining will not help. Usually, the problem is that the working silver nitrate methenamine solution is old and must be made up fresh or that the sample was overheated during the microwaving in chromic acid.
8. If staining of the control is adequate, place slides in gold chloride solution for 30 to 60 seconds. Rinse in distilled water.
9. Place slides in Coplin jar filled with 2% sodium thiosulfate for 2 minutes.
10. Rinse in distilled water.
11. Cover with light green working solution for 2 to 5 minutes. Rinse in 95% alcohol, and dehydrate to 100% ethanol. Clear with xylene and coverslip.

Results. The *Pneumocystis* cyst wall should stain black with a gray, more lucent center. Often, one or two dark dots are seen within the cyst, representing a thickening in the cyst wall. If the cysts appear solid black without a lucent center or if the background is black, overstaining has occurred, and slides may be difficult to interpret. This should be avoided. Fungi also stain black. When overstained, leukocyte granules, bacteria, and reticulin may also appear black and can lead to misinterpretation.

Problems. Overheating of chromic acid may result in loss of material or nonstaining of organisms. In addition, although stock methenamine is fairly stable, we have found that it should be stored in a dark container and should be made up fresh after a couple of weeks or if nonstaining occurs with no other valid explanation.

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5

Ultrastructural Diagnosis of Infection

ALTON B. FARRIS III, MARTIN K. SELIG, G. PETUR NIELSEN

Introduction

Electron Microscopy Technique

Prions

Viral Infections
- Herpesvirus
- Varicella Virus
- Adenovirus
- Influenza
- Paramyxovirus
- Smallpox
- Hepatitis Viruses
- Parvovirus B19
- Enterovirus
- Viral Gastroenteritis
- Polyomaviruses
- Rabies Virus
- Eastern Equine Encephalitis Virus
- Human Immunodeficiency Virus
- Ebola Virus
- Zika Virus

Bacterial Infections
- Gram-Positive Bacteria
- Gram-Negative Bacteria
- Mycobacteria
- Other Important Bacteria

Fungal Infections
- *Histoplasma capsulatum*
- *Pneumocystis jiroveci*

Parasitic Infections
- *Acanthamoeba*
- *Toxoplasma gondii*
- *Cryptosporidium parvum*
- *Giardia lamblia*
- *Cystoisospora belli*
- Microsporidia
- *Trypanosoma cruzi*

Introduction

Electron microscopy (EM) can assist in the identification of infectious organisms in surgical and clinical pathology specimens. However, since the advent of immunohistochemical, molecular (e.g., in situ hybridization, polymerase chain reaction), and serologic techniques, EM has been less often used to identify infectious pathogens. EM can still be useful, particularly in the identification of specific infections and the classification of novel organisms.[1-7] In addition, EM is advantageous in situations in which most viral particles are present as empty virions devoid of nucleic acid. This chapter is not intended to be an exhaustive compendium of the EM characterization of all infectious diseases; rather, this chapter focuses on those infections in which EM plays a critical role[1-4] (Table 5.1).

Electron Microscopy Technique

EM is best conducted on freshly fixed tissue. Surgical specimens (from biopsies or resections), cytology specimens, fecal samples in the case of gastrointestinal episodes, fluids or smears from skin lesions, cerebrospinal fluid, nasopharyngeal washes, saliva, tears, and urine are examples of samples that may be used for EM. In our laboratory, we typically use a modified Karnovsky fixative consisting of 2.0% glutaraldehyde, 2.5% formaldehyde, and 0.025% CaCl in a 0.1 M sodium cacodylate buffer pH 7.4. Fixation times vary with tissue type. Skin biopsies require long fixation times, sometimes longer than 3 hours, whereas cell suspensions or tissue-cultured cells can be adequately fixed in as little as 15 minutes. Postfixation with osmium tetroxide is necessary for good ultrastructural preservation because it preserves lipids that are typically lost in formalin-fixed, paraffin-embedded tissues. For a more comprehensive overview of our tissue processing method, see Table 5.2. Sometimes tissue for conventional light microscopy that has been fixed in conventional buffered formaldehyde or paraffin-embedded tissue is the only material available. This tissue can also be used for EM, although significant artifact is typically present. Tissue remaining in buffered formaldehyde after fixation gives better results than tissue embedded in paraffin or tissue fixed in Bouin, Zenker, or B-5 fixative.[1-4]

Although it is not routinely performed in our laboratory, rapid diagnosis of negatively stained viral particles by EM may be achieved within 10 minutes of receiving a sample. Negative staining methods usually use staining with a heavy metal aqueous salt solution, such as 2% uranyl acetate, 5% ammonium molybdate, or 1.0% potassium salt of phosphotungstic acid (PTA).[5] Samples are dried onto thin-film carbon-coated grids, washed, stained,

TABLE 5.1 Comparative Sizes of Important Pathogens

Pathogen	Approximate Size
Prion	12-230 nm[31]
Virus	0.02-0.22 μm
Bacteria	
Staphylococcus aureus	0.7 μm
Escherichia coli	0.5 μm
Fungal Hyphae	
Histoplasma	3 μm
Cryptococcus	20 μm
Red blood cell	7 μm
Parasite	
Worm eggs	16-80 μm
Worms	≥300 μm

Modified from Hawley LB. *High-Yield Microbiology and Infectious Diseases.* Philadelphia: Lippincott Williams & Wilkins; 2000.

air-dried, and taken directly to the microscope. Viral inactivation is usually accomplished through drying and then irradiation with ultraviolet light. In our laboratory, we create agar cell blocks from samples otherwise too small to process. Briefly, in 5.0 mL Eppendorf tubes, samples (from fine-needle aspiration, bronchial lavage, urine, ascites fluid, or tiny tissue fragments) are fixed in standard fixative, centrifuged at 1000 rpm, resuspended in warm (liquid) 2% agar, and centrifuged again. The agar is then allowed to cool and harden. The centrifuge tube tip containing material is then cut off with a sharp razor blade, and the resulting tissue-containing agar block is processed routinely.[4,8,9]

Transmission electron microscopy (TEM) is typically the technique used to examine surgical and clinical pathology specimens. With this technique, electrons are passed through samples and then imaged. Samples must be quite thin (80 to 100 nm) for the electrons to be able to pass through the specimen.[1-3]

Prions

Most research related to diseases thought to be mediated by prions (small proteinaceous infectious particles) indicates that prions are protease-resistant proteins that do not contain nucleic acid. Prions are difficult to isolate and too small to be visualized by standard EM techniques. Typically, only vacuolated nervous tissue and reactive astrocytosis are seen histologically, and EM shows vacuoles in neurons and neuropils, as well as altered plasma membranes.

TABLE 5.2 Routine Automatic Processor Program for Electron Microscopy

Vial #	Contents	Time (min)	Temperature (°C)	Agitation?
1	Na cacodylate buffer	5	10	No
2	Na cacodylate buffer	5	10	No
3	1.3% Osmium teroxide, aqueous	90	10	Yes
4	Na cacodylate buffer	10	10	No
5	25% Ethanol (aq.)	15	15	No
6	50% Ethanol (aq.)	15	15	No
7	3% Uranyl acetate in 70% ethanol	90	RT	Yes
8	95% Ethanol (aq.)	15	RT	No
9	95% Ethanol (aq.)	15	RT	No
10	100% Ethanol (absolute)	15	RT	No
11	100% Ethanol (absolute)	15	RT	No
12	100% Ethanol (absolute)	15	RT	No
13	Propylene oxide (100%)	10	RT	No
14	Propylene oxide (100%)	10	RT	No
15	PPO/epoxy resin (70/30)	90	25	Yes
16	PPO/epoxy resin (30/70)	90	30	Yes
17	Epoxy resin (100%)	180	30	Yes
18	Epoxy resin (100%)	180	30	Yes
19	Empty vial	—	—	—
20	Empty	—	—	—

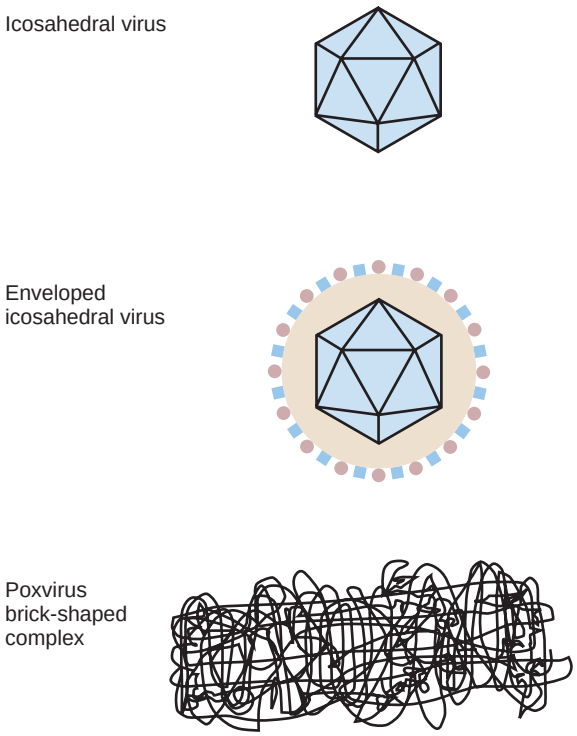
aq, Aqueous; *min*, minutes; *Na*, sodium; *PPO*, poly(phenylene oxide) (PPO); *RT*, room temperature.

Recent research has focused on using immunologic methods, such as prion protein (PrP) immunohistochemistry and cryo-immunogold EM, to identify prions. It is hoped that such techniques will yield insights into disorders in which prions are considered to be critical, such as Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome in humans and bovine spongiform encephalopathy (BSE) in cattle—and even other neurodegenerative disorders, such as Alzheimer, Parkinson, and Huntington diseases.[10-13]

Viral Infections

Viruses can be recognized as organized structures (Fig. 5.1) smaller than cells and may be intracellular or extracellular, ranging from 20 to 300 nm in diameter. Viruses typically have one or more layers surrounding an electron-dense core consisting of DNA or RNA, referred to as the nucleoid (Table 5.3), and are surrounded by a single or multilayer outer coating or shell, the capsid.[1,14]

Size, shape, and organization of virions are useful characteristics in identifying viruses. Sizes range from small (e.g., papovaviruses) to intermediate (e.g., adenoviruses, herpesviruses) to large (e.g., poxviruses). Virions may be organized into latticelike arrangements (e.g., adenoviruses), which vary from regular to random (e.g., papovavirus).[1,14] Because of these characteristic differences, EM can be quite helpful in characterizing viruses.[6,7,15-18] Although the morphology and size are useful in identifying viruses, there is

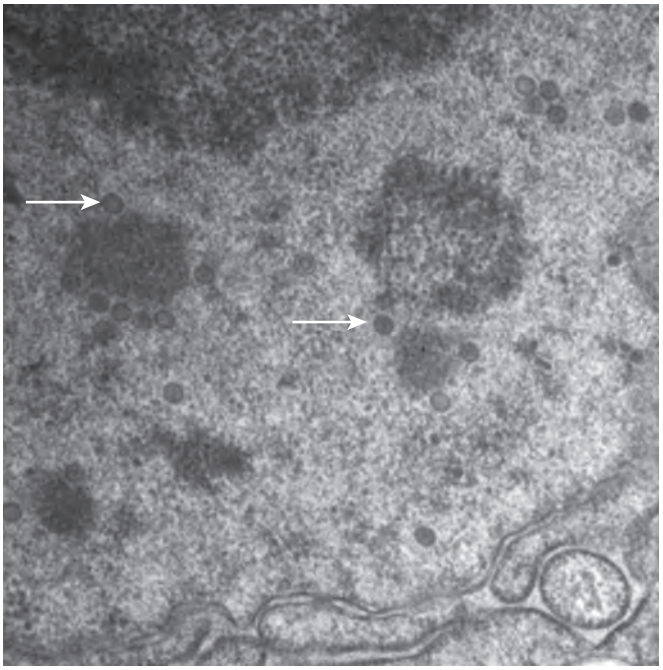


• **Figure 5.1** Basic structure of viruses.

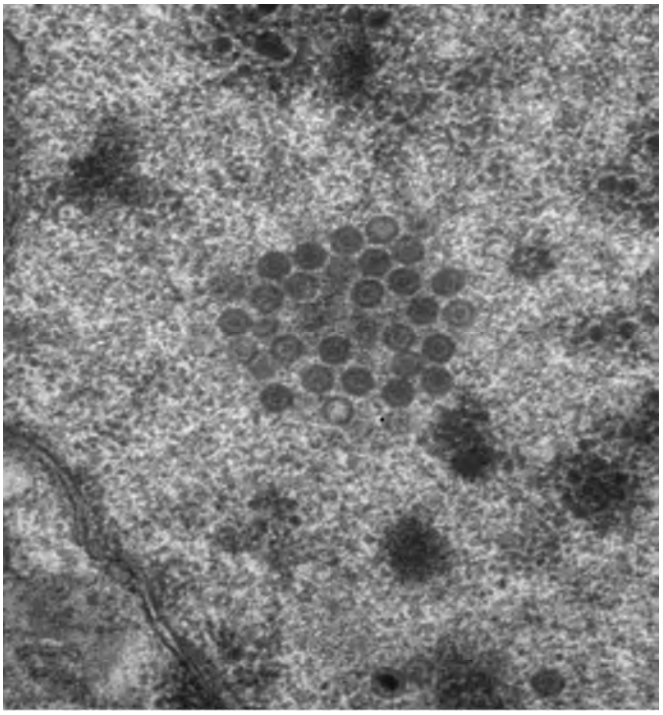
TABLE 5.3 Important Viruses Confronted by Electron Microscopy and Their Important Features

Virus Family	Size* (nm)	Nucleic Acid Material	Shape
Parvoviridae	18-26	Naked ss DNA	Icosahedral
Hepadnaviridae	22-27	Enveloped ds circular DNA	Icosahedral
Papovaviridae	45-55	Naked ds circular DNA	Icosahedral
Adenoviridae	80-110	Naked ds DNA	Icosahedral
Herpesviridae	150-200	Enveloped ds circular DNA	Icosahedral
Poxviridae	140-260 × 220-450	Coated ds DNA	Complex
Picornaviridae, Astroviridae, Caliciviridae	27-38	Naked ss RNA	Icosahedral
Reoviridae	60-80	Naked ds segmented RNA	Icosahedral
Togaviridae	50-70	Enveloped ss RNA	Icosahedral
Flaviviridae	45-60	Enveloped ss RNA	Complex
Arenaviridae	50-300	Enveloped ss segmented RNA	Complex
Coronaviridae	80-220	Enveloped ss RNA	Complex
Retroviridae	80-100	Enveloped ss diploid RNA	Complex
Bunyaviridae, Orthomyxoviridae	80-120	Enveloped ss segmented RNA	Helical
Paramyxoviridae	150-300	Enveloped ss RNA	Helical
Rhabdoviridae	60-80 × 130-220	Enveloped ss RNA	Helical, bullet-shaped
Filoviridae	80 × 1,000	Enveloped ss RNA	Helical

*Size is expressed as diameter or diameter × length.
ds, Double-stranded; *nm*, nanometers; *ss*, single-stranded.
Data from references 1, 13, 20, and 49.



• **Figure 5.2** Herpesvirus (original magnification, ×28,000). Numerous clusters of intranuclear viral particles *(arrows)* are seen in a sample from the cerebrum of a patient with herpes simplex encephalitis.



• **Figure 5.3** Herpesvirus (original magnification, ×44,000). Round to hexagonal or "stop sign"-shaped, targetoid viral particles are seen.

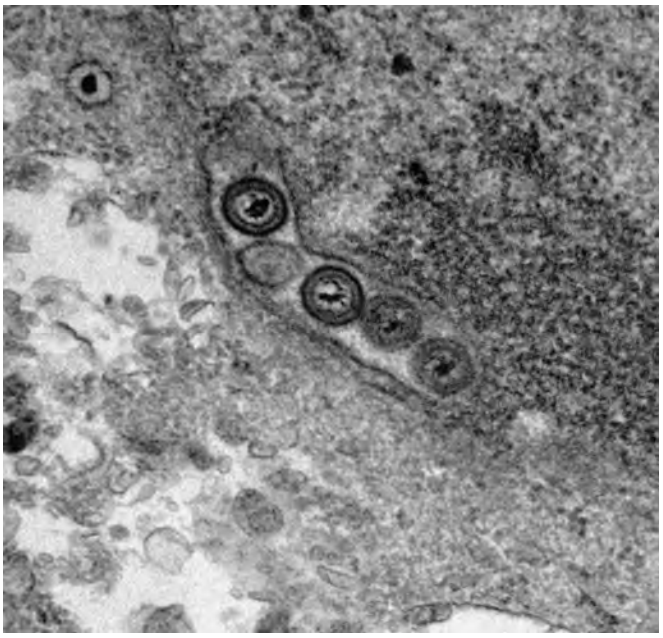
overlap, and complementary techniques to EM are often needed, including immunohistochemistry, in situ hybridization, Southern blot analysis, and polymerase chain reaction for viral DNA.[1,14]

Herpesvirus

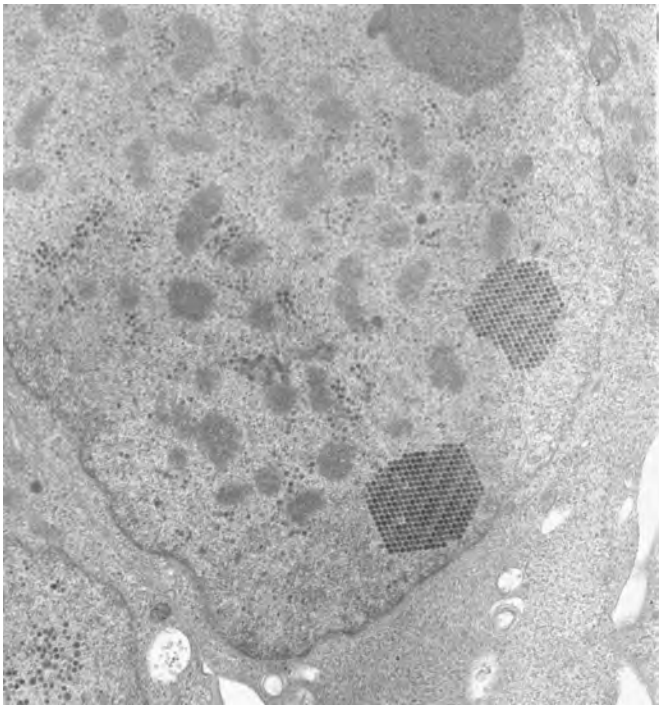
Herpesvirus virions (Figs. 5.2 and 5.3) are surrounded by limiting lipid bilayers, giving the virions a "fried egg" look. The envelopes serve both a functional and a structural role. The virions have a hexagonal outline (icosahedral morphology) and are covered with 152 tubular capsomeres. The complete herpes viral structure with envelope is 150 to 180 nm in diameter, and the internal portion of all herpes group viruses is approximately 100 nm in diameter. Viral inclusion bodies of herpesvirus are composed of lattice arrangements of mature viral particles (virions). Other members of the herpesvirus family (i.e., herpes simplex, cytomegalovirus, and Epstein-Barr virus) have a similar appearance, and all are 100 to 120 nm in size. They all replicate in the nucleus of the host cell.[6,13,19]

Varicella Virus

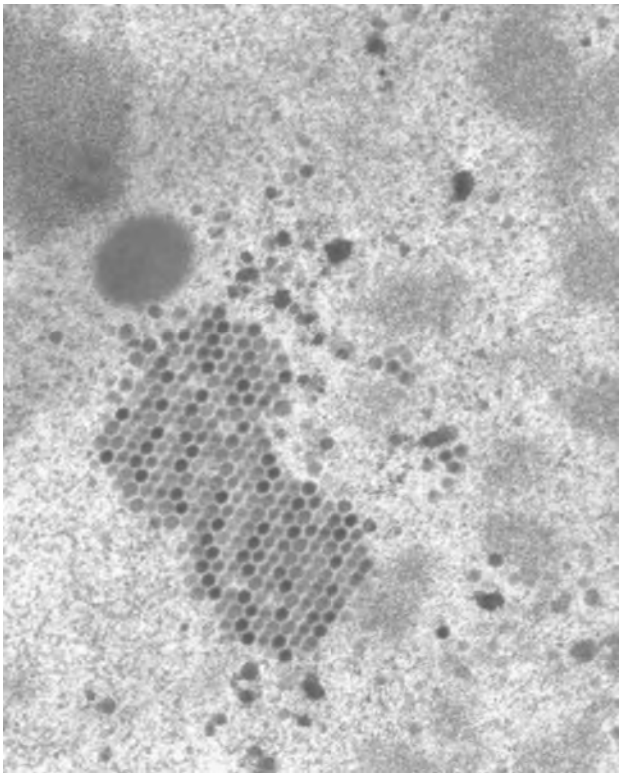
Varicella virus, the causative agent of chickenpox (also known as human herpesvirus 3) is usually a benign disease of childhood. Ultrastructurally (Fig. 5.4), viral nucleocapsids can be identified in the nucleus and budding through the nuclear envelope. Virions may be present in cytoplasmic vacuoles and are sometimes abundant. Herpes zoster (shingles) is a reinfection of the varicella virus that commonly manifests in the skin, often as vesicular eruptions in a dermatomal distribution along sensory nerves. Virus is produced in lower quantities than in the primary chickenpox infection.[13]



• **Figure 5.4** Varicella virus (original magnification, ×57,000). Round to hexagonal or "stop sign"-shaped, targetoid viral particles are seen in cell culture. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



• **Figure 5.5** Adenovirus (original magnification, ×11,000). Numerous packets of virions in a regular latticelike arrangement are seen in cell culture. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



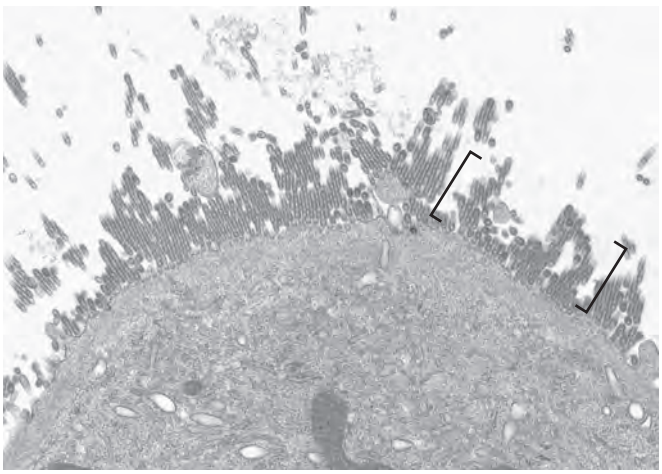
• **Figure 5.6** Adenovirus (original magnification, ×34,000). Numerous packets of virions in a regular latticelike arrangement are seen. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)

Adenovirus

Adenovirus (Figs. 5.5 and 5.6) commonly infects the respiratory and enteric tracts. Rarely, the liver and pancreas may be involved. Adenovirus is contained as numerous packets of virions in a paracrystalline array, often in the nucleus of cells when newly synthesized and sometimes in the nucleolus as an inclusion body. In early infection, incoming virus is seen in the cytoplasm. Individual virions have a hexagonal (icosahedral) shape with 20 sides and 12 vertices. Surrounding the core of the virus are 252 pentagonal capsomeres, each with a filamentous projection (fiber). Cells may become large (cytomegaly) with large eosinophilic inclusions appreciable by light microscopy. Granular aggregates in the nuclei of cells infected by adenovirus are made up of various viral-induced proteins.[1,13,20]

Influenza

Influenza virus (Figs. 5.7 and 5.8), an orthomyxovirus, is a pleomorphic virus consisting of round-to-spherical, kidney-shaped, or elongated virions located at and beneath the cell surface. Virions are 80 to 120 nm in diameter, and filamentous forms may be several micrometers in length. Viruses typically enter respiratory tract epithelium by attaching to and fusing with the cell membrane, mediated by hemagglutinin binding to sialic acid residues in a ligand-receptor interaction. Hemagglutinin and neuraminidase spikes project 8 to 10 nm from the viral envelope, and the nucleocapsid, 9 to 15 nm in diameter, has helical symmetry. Infected cells may have an increased density of nucleoli during active protein synthesis. Hemagglutinin proteins form in the cytoplasm and may be present in excess as long fibers. Dense protein lattices surrounded by small dense ribosomes

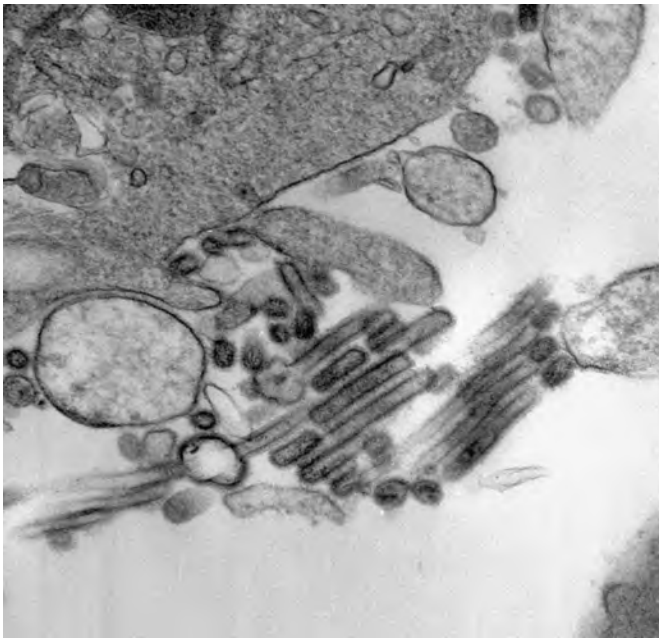


• **Figure 5.7** Influenza A (original magnification, ×15,000). Round and elongate virions at the cell surface are seen in cell culture *(brackets)*. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)

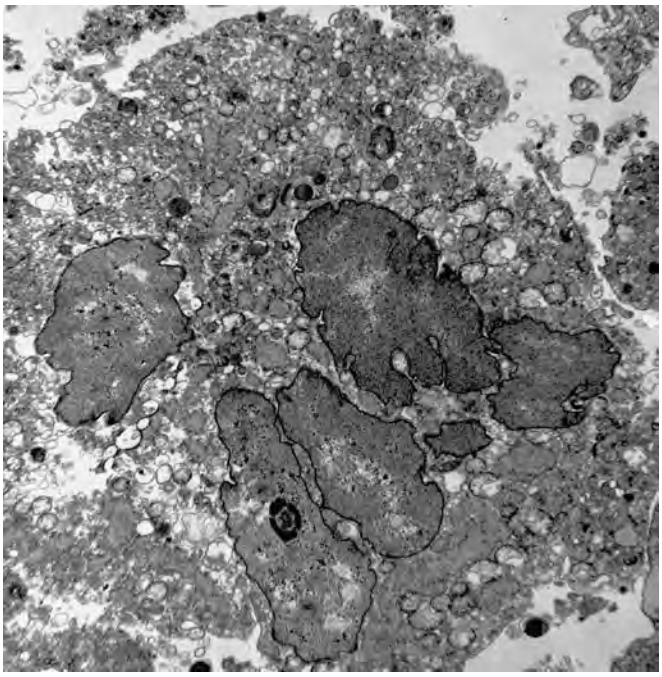
are present. Virions emerge by budding from the host cell plasma membrane.[1,13,20]

Paramyxovirus

Members of the *Paramyxoviridae* family measure 120 to 300 nm in size. There are many members of *Paramyxoviridae*, including mumps, parainfluenza (Figs. 5.9 and 5.10), *Morbillivirus* species

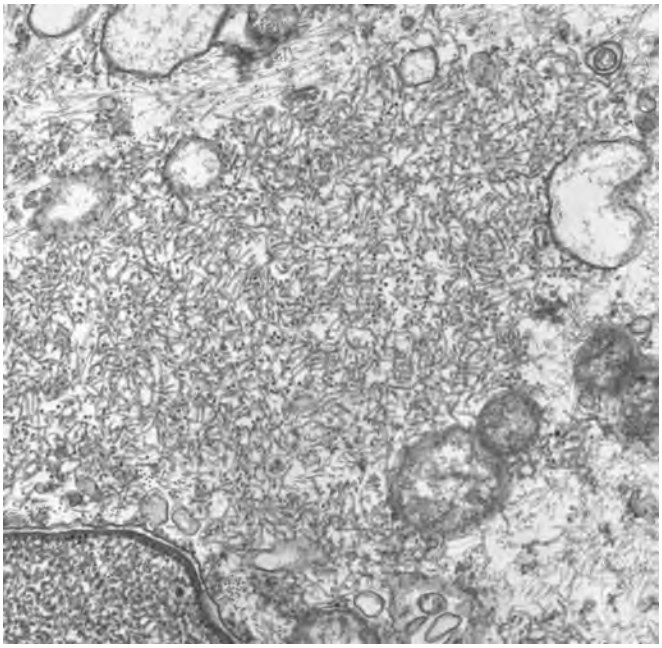


• **Figure 5.8** Influenza A (original magnification, ×45,000). Round and elongated virions are seen. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



• **Figure 5.9** Parainfluenza virus (original magnification, ×3400). Multiple nuclei with a ground glass texture are seen in a sample of lung. Measles can have a similar appearance.

(including measles), and *Pneumovirus* species (including respiratory syncytial virus). One common place to identify paramyxovirus is in type II pneumocytes, which may be multinucleated and may have a ground-glass appearance. The virions are diffusely dispersed in the cell and have a filamentous protein nucleocapsid protecting the viral nuclei acid, giving the virus a "herringbone" appearance.[1,13,19]



• **Figure 5.10** Parainfluenza virus (original magnification, ×19,000). Virions are diffusely dispersed in the cytoplasm in a sample of lung. Measles can have a similar appearance.

Smallpox

Variola virus, the agent of smallpox, is often recognized from material collected from skin lesions. The slightly rounded, brick-shaped virions measure approximately 270 by 350 nm. The organisms have a complex surface structure and may be recognized in one of two types: a mulberry form with 10- to 20-nm diameter and a beaded short-tubular surface and a capsular form with a 30-nm membrane.[4,6,9]

Hepatitis Viruses

Hepatitis A Virus

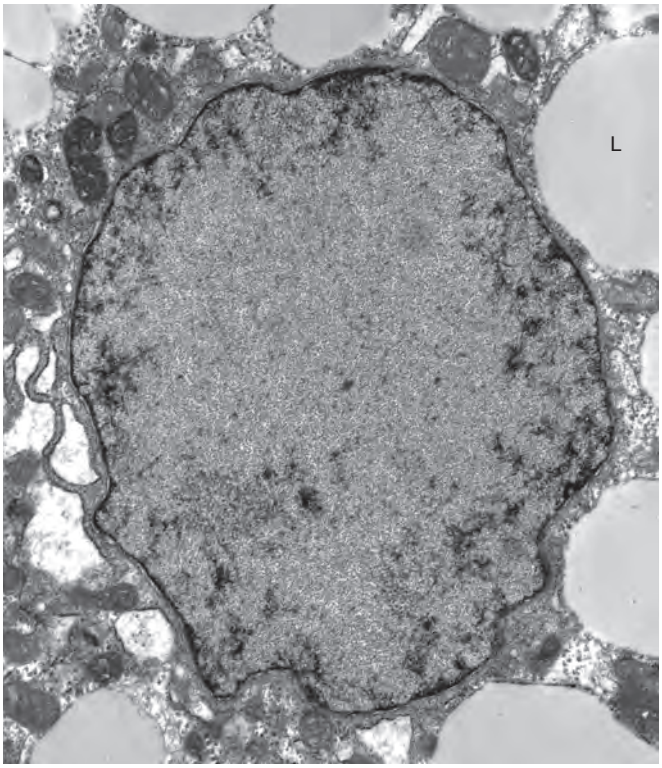
Hepatitis A virus (enterovirus 72) is a member of the picornavirus family, 24 to 30 nm in diameter, and roughly spherical. Cytopathic effect leads to an increase in intracellular ribosomes and large polyribosomes.[20]

Hepatitis B Virus

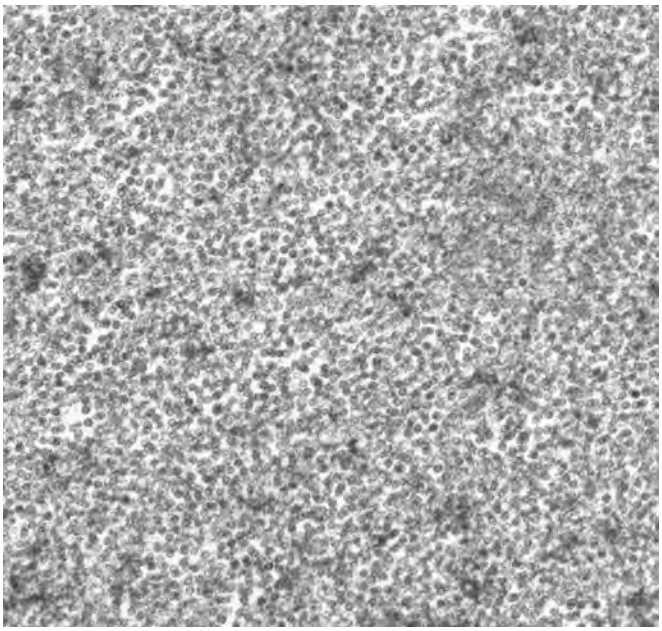
Hepatitis B virus, a hepadnavirus (Figs. 5.11 and 5.12), is 22 to 27 nm in diameter and consists of a central core of double-stranded DNA with nucleocapsid DNA. An envelope containing the hepatitis B surface antigen (HBsAg) is 7 nm wide and contains a 27-nm diameter central core, also referred to as the Dane particle, which contains the hepatitis B core antigen (HBcAg) and e antigen (HBeAg). The viral particles may form spherules and tubules.[1,13,19]

Parvovirus B19

Parvovirus B19 is a small, round, nonenveloped icosahedral virus, 18 to 26 nm in diameter, without distinctive surface features. They may be confused with ribosomes. At high magnification, they have a roughly hexagonal outline. Virus is assembled in the nucleus and appears either "empty" or "full." It may be recognized in blood serum by EM.[19,20]



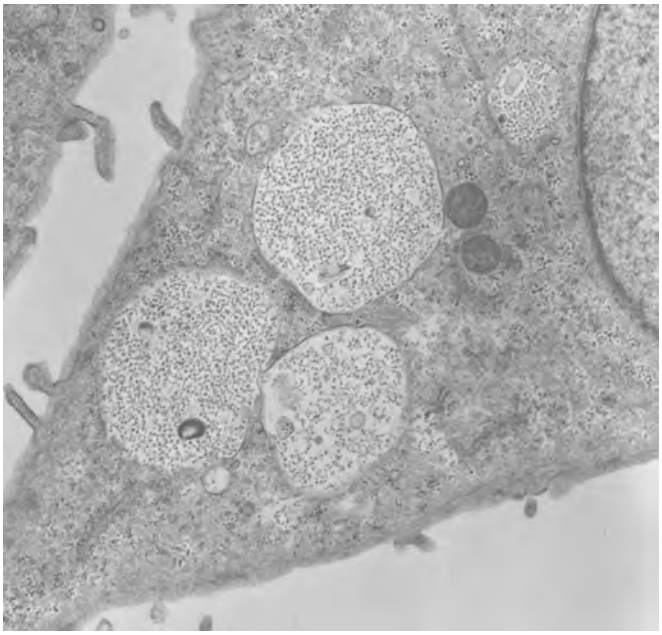
• **Figure 5.11** Hepatitis B virus (original magnification, ×9100). An infected hepatocyte is seen with numerous intranuclear viral particles and surrounding lipid droplets *(L)*.



• **Figure 5.12** Hepatitis B virus (original magnification, ×56,000). Numerous granular viral particles are seen.

Enterovirus

Enterovirus (Figs. 5.13 and 5.14) is a member of the picornavirus family, which also includes poliovirus, coxsackievirus, echovirus, hepatitis A, rhinovirus, and aphthovirus (the causative agent of foot-and-mouth disease), among others. The enterovirus genus includes poliovirus, coxsackievirus A and B, echovirus, enterovirus,



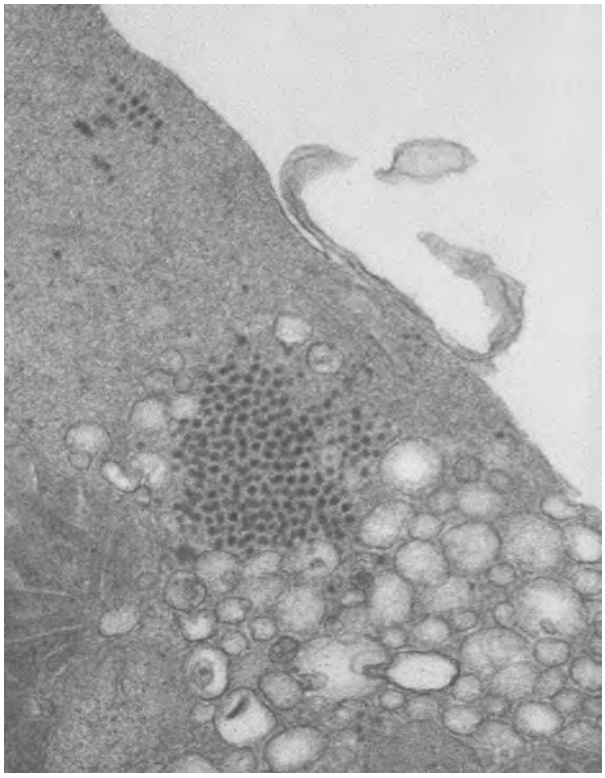
• **Figure 5.13** Enterovirus: Coxsackie A (original magnification, ×15,000). Membrane-bound vesicles containing numerous virions are seen in a tissue culture sample. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)

and others. Cells infected by enterovirus have innumerable spherical virions, some of which are enclosed in membrane-bound vesicles. The vesicles may be appreciated on lower magnification. Enterovirus typically infects the gastrointestinal tract but may extend to the brain, where neurons may be infected, producing cell swelling, chromatolysis, and cell death.[1,13]

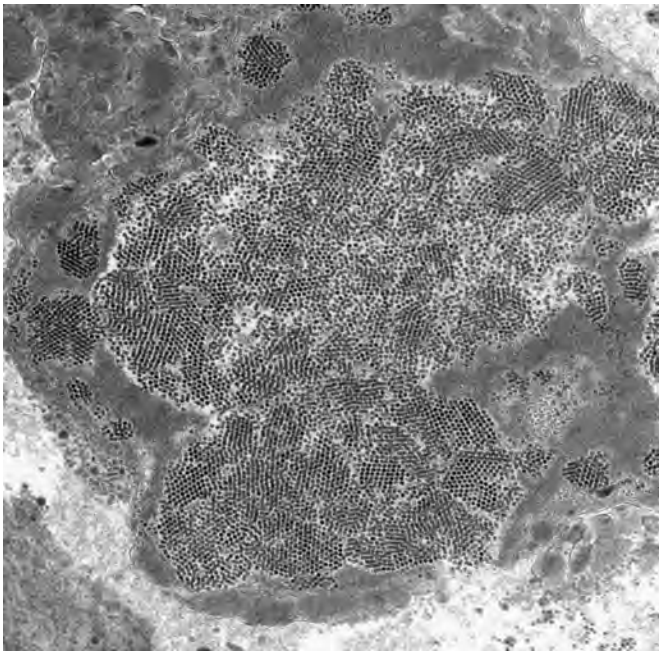
Poliovirus replicates in enteric mucosa and is present in large quantities in the feces. It targets the nervous system, infecting vascular endothelium of the brain. Viruses may replicate in circulating leukocytes. Virions are the size of ribosomes and are often difficult to identify. Coxsackieviruses are enteroviruses that can cause enteric, respiratory, meningeal, and cutaneous infections. Infections resemble the EM changes of poliovirus. Infection with echovirus, which derives its name from "enteric cytopathic human orphan," is usually subclinical but can manifest with febrile illness and meningitis, often in newborns, who may develop fatal disseminated infections. The EM findings are similar to those of poliovirus.[1,13]

Viral Gastroenteritis

Rotavirus belongs to the reovirus family. The reovirus name refers to *r*espiratory *e*nteric *o*rphan viruses. Rotavirus has linear double-stranded RNA with 11 genome segments. Rotavirus causes severe diarrheal illness, particularly in infants and young children. Enzyme-linked immunosorbent assay (ELISA) kits are available for the detection of rotavirus, but new strains may not be recognized by these kits, which recognize only those strains that have an antigen common to the rotavirus that was used in making the antiserum for the kit. EM may be useful in identifying other rotaviruses and causes of viral gastroenteritis, particularly those for which kits are not available. The EM appearance of viruses can be diagnostic in many of these circumstances. For example,



• **Figure 5.14** Enterovirus: echovirus (original magnification, ×45,000). Numerous round virions can be seen in a tissue sample. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)

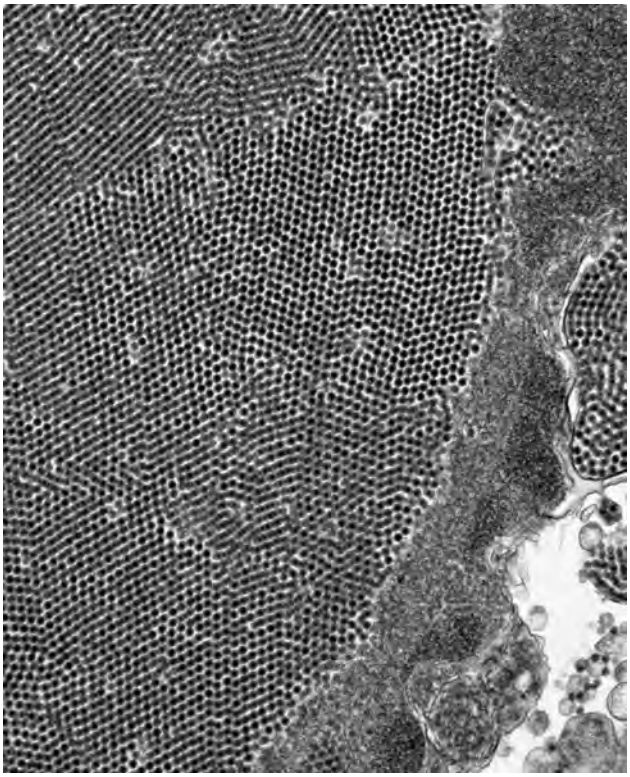


• **Figure 5.15** Polyomavirus in a case of BK nephropathy (original magnification, ×19,000). Numerous virion packets of polyomavirus can be seen embedded in renal tubular debris.

rotavirus may be identified on EM of stool specimens as a 70-nm viral particle with three protein layers and a characteristic "wheel" shape (hence the prefix *rota,* which means "wheel" in Latin).[6,9,21,22]

Polyomaviruses

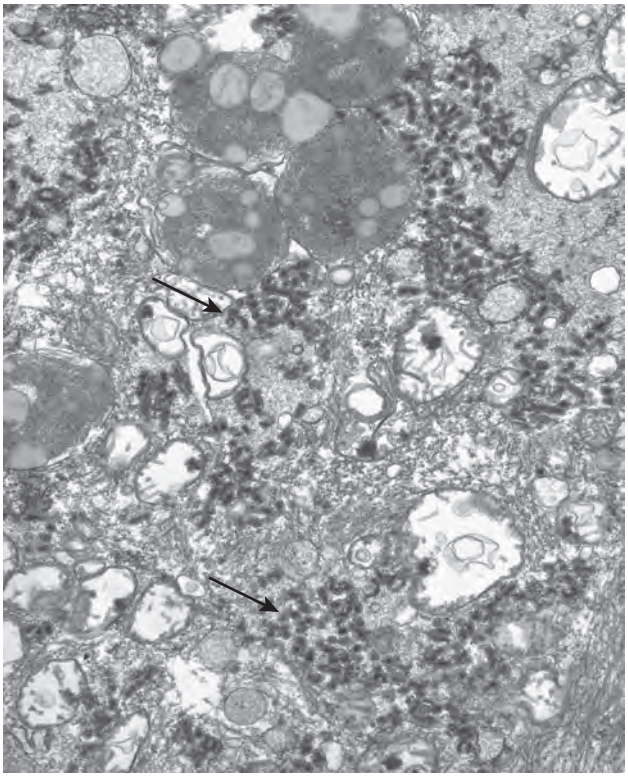
The *Papovaviridae* (Figs. 5.15 and 5.16) derive their name from the first two letters of the viruses in the papovavirus family: papilloma, polyoma, and vacuolating agents. Virions measuring approximately 50 nm in diameter are present in packets and are found in oligodendrocytes in cases of progressive multifocal leukoencephalopathy (PML). JC polyomavirus, a member of the papovavirus family, is the causative agent of PML. BK polyomavirus is a common inhabitant of the genitourinary tract that may cause a nephropathy in immunocompromised transplant recipients (renal transplant recipients in particular). In cases of human papillomavirus (HPV)–related disease, there may be keratohyaline granules, which are seen by EM as large, dense cytoplasmic granules.[13,20] Research has indicated that EM may be useful in the identification of urinary polyomavirus in cases of BK nephropathy. One method involves the identification of so-called urinary Haufen, which are three-dimensional, castlike urinary polyomavirus aggregates.[23,24]



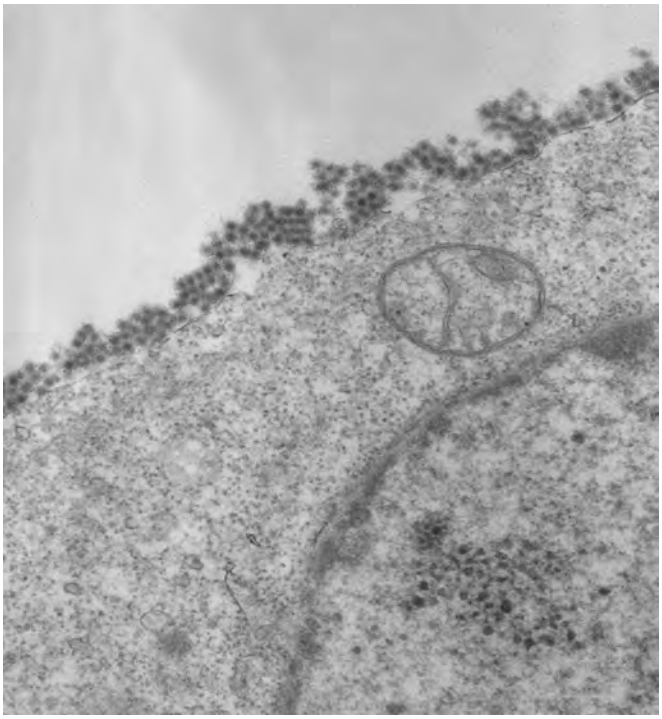
• **Figure 5.16** Polyomavirus (original magnification, ×25,000). Numerous virion packets of polyomavirus with a relatively regular organization in groups, also referred to as a paracrystalline arrangement, can be seen.

Rabies Virus

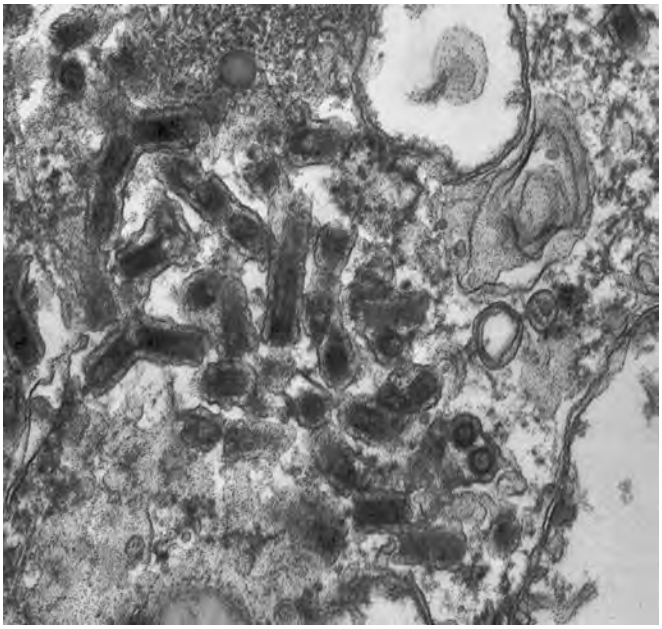
Rabies virus (Figs. 5.17 and 5.18), a member of the *Rhabdoviridae* family and *Lyssavirus* genus, is a bullet-shaped virus that aggregates around or in electron-dense cytoplasmic material. This correlates to the Negri bodies seen in neurons in rabies virus infections,



• **Figure 5.17** Rabies virus (original magnification, ×11,000). Bullet-shaped viral particles *(arrows)* can be appreciated within the cytoplasm of a cerebellar Purkinje cell from an autopsy specimen.



• **Figure 5.19** Eastern equine encephalitis virus in a cell culture sample (higher magnification, ×25,000). Many virions can be appreciated at the cell surface and within the nucleus. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



• **Figure 5.18** Rabies virus from an autopsy specimen of cerebellum (original magnification, ×25,000). Bullet-shaped viral particles are seen.

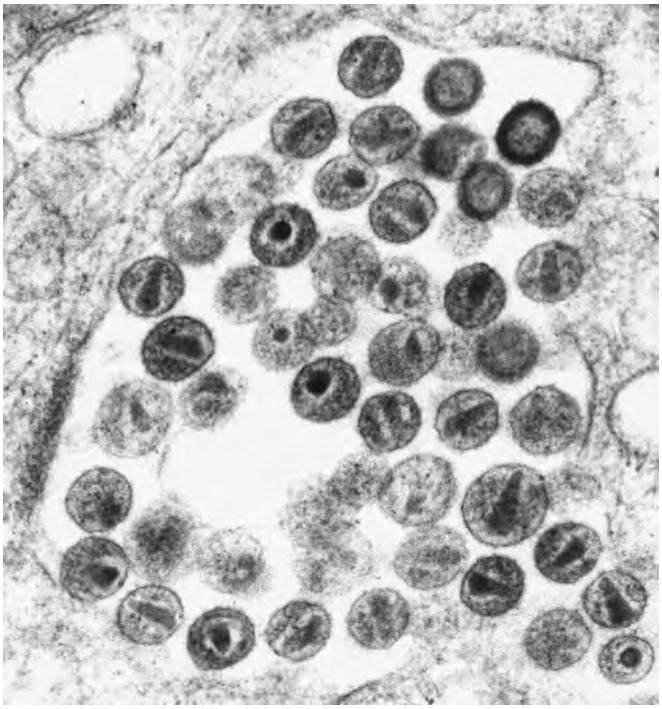
notably in hippocampal pyramidal cells and less frequently in cortical neurons and Purkinje cells of the cerebellum. A process of budding off of membrane-bound organelles leads to formation of individual virions.[1,12]

Eastern Equine Encephalitis Virus

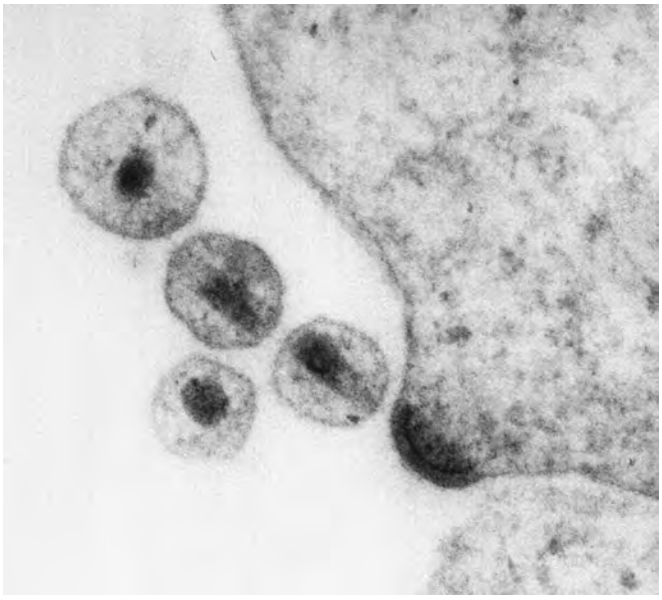
The eastern (Fig. 5.19) and western equine encephalitis viruses, members of the *Togaviridae* family and *Alphavirus* genus, are transmitted to humans, horses, and other animals. In fatal infections, neuronal degeneration and inflammation are present in the cerebral cortex, basal ganglia, hippocampus, and medullary nuclei, with relative sparing of the cerebellum and spinal cord. Viruses are 55 to 58 nm in diameter and typically have a dense, 28- to 30-nm core surrounded by a spherical translucent layer. There may be projecting subunits. Viruses are seen in the cytoplasm and associated with membranous structures. The outer envelope typically comes from budding from the plasma membrane but may also come from endoplasmic reticulum. Dense particles may be seen in the nucleus late in infection and are thought to be a possible early phase of the viral life cycle.[13,20]

Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) (Figs. 5.20 and 5.21), a retrovirus and member of the *Lentivirus* genus, is recognized by its conical- or rod-shaped dense nucleoid, which forms from cytoplasmic membrane and vacuolar membrane of lymphocytes and cells in the monocyte–macrophage lineage. Host cell membranes

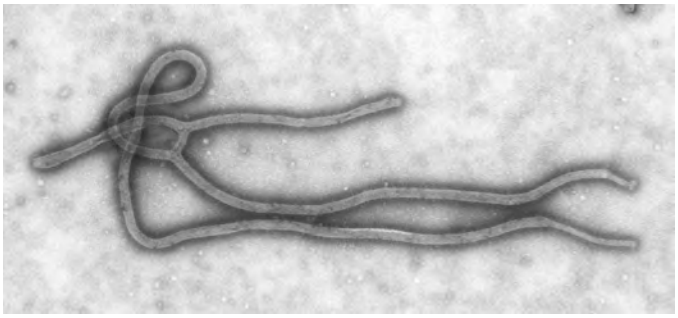


• **Figure 5.20** Human immunodeficiency virus: virions within a macrophage vacuole (from cell culture). Virions are ring-shaped, and some have conical nucleoids (×7500). (Photographs courtesy Jan M. Orenstein, MD, Department of Pathology, George Washington University Medical Center.)



• **Figure 5.21** Human immunodeficiency virus: virions bud from the plasma membrane (from cell culture, ×10,000). (Photographs courtesy Jan M. Orenstein, MD, Department of Pathology, George Washington University Medical Center.)

form the outer membrane of the virus, and the immature virus forms surface spikes during budding. HIV can form a crescent-shaped budding profile as it buds off cells, and it can then form a doughnut-shaped configuration as it is released from the cell.[1,6]



• **Figure 5.22** Ebola virus in a negative stain preparation. (Courtesy Cynthia S. Goldsmith, Centers for Disease Control and Prevention.)

Ebola Virus

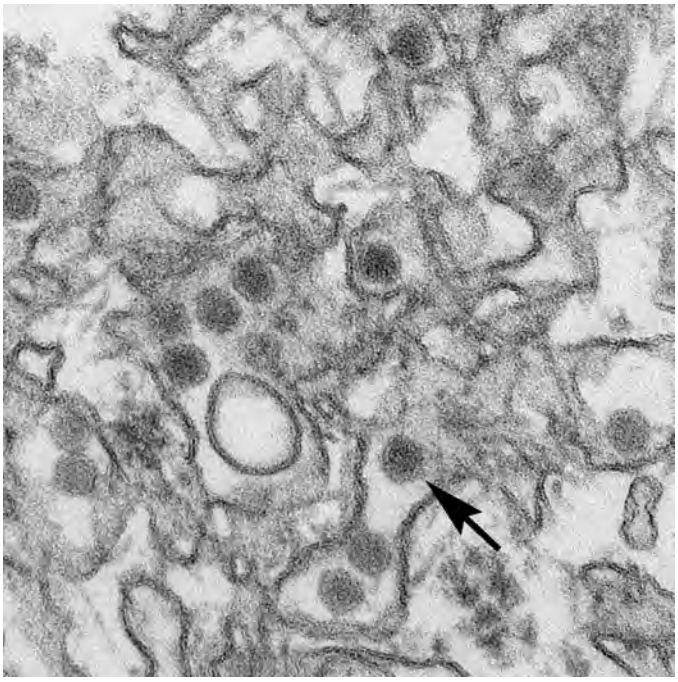
Ebola virus (Fig. 5.22) belongs to the *Filovirus* genus, which also includes Marburg virus. Both Ebola and Marburg viruses can cause serious febrile hemorrhagic disease with case fatality rates of 50% to 90% for Ebola and 24% to 88% for Marburg. Both *Filoviridae* belong to the order *Mononegavirales*, which also includes *Paramyxoviridae* and *Rhabdoviridae*. Filoviruses are composed of long particles of variable length up to 14,000 nm and approximately 80 nm in diameter, having a pleomorphic morphology that may be filamentous, branched, circular, six-shaped, or U-shaped. Ebola virus is reported to typically have a length of 805 to 1200 nm; and Marburg virus reportedly typically has a length of 665 to 860 nm. Filovirions have a negative-sense, single-stranded RNA genome and are composed of a helical nucleocapsid. Culture fluids can be examined by EM to search for the Filoviruses. Immunoelectron microscopy has also been developed to identify filovirus.[6,25-33]

Zika Virus

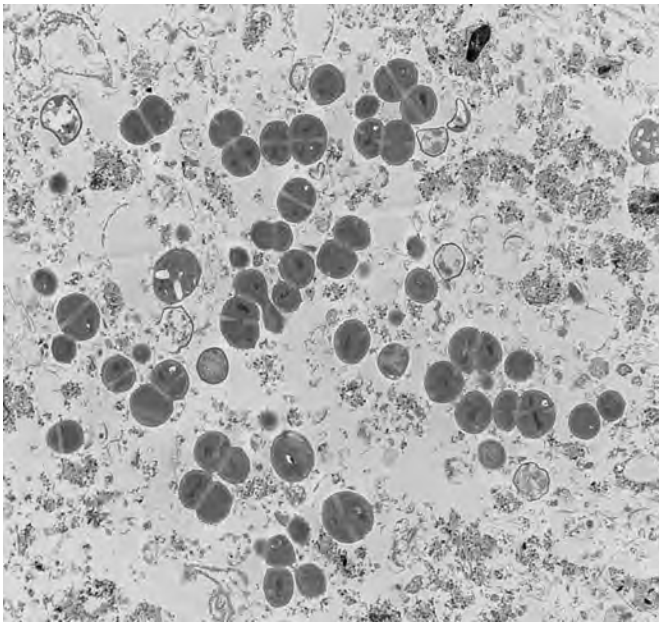
Zika virus (Fig. 5.23) is a flavivirus, belonging to the family *Flaviviridae*. *Flaviviridae* are positive-strand RNA viruses that include such human pathogens as the West Nile virus, yellow fever virus, mosquito-transmitted dengue virus, Japanese encephalitis virus, and tick-borne encephalitic virus. Zika virus particles are 40 nm in diameter and have an outer envelope and an inner dense core. Zika virus circulates in a sylvatic transmission cycle among nonhuman primates and Aedes mosquitos, and the virus eventually enters a human-mosquito-human transmission cycle in which the *Aedes aegypti* mosquito transmits the virus.[34-37] It has typically most often been recognized that Zika causes a febrile or exanthematous illness; however, it has come to the attention of the public health community that Zika can lead to serious human birth defects, particularly microcephaly. This makes Zika virus the first major infectious disease to be linked to human birth defects in more than half a century.[34]

Bacterial Infections

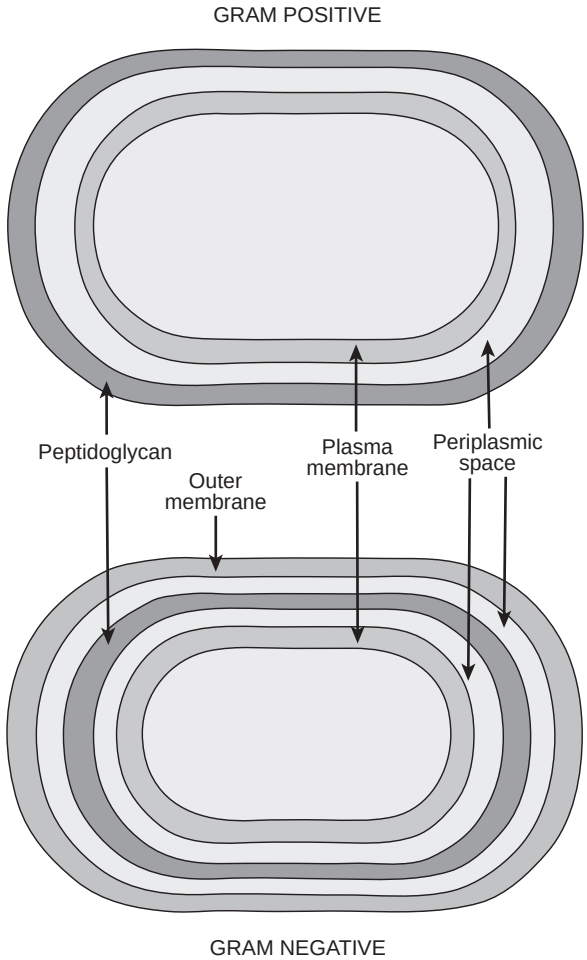
As prokaryotic single-celled organisms, bacteria (Fig. 5.24) have a simple internal structure without a membrane-bound nucleus, membrane-bound organelles, endoplasmic reticulum, or mitochondria. Bacteria have an outer cell wall (except for *Mycoplasma*), an inner cell membrane, dense cytoplasm composed primarily of ribosomes, and vesicles formed from the inner cell membranes (known as mesosomes), storage vacuoles, and endospores. They



• **Figure 5.23** Zika virus *(arrow)* is shown in a thin section preparation. (Courtesy Cynthia S. Goldsmith, Centers for Disease Control and Prevention.)



• **Figure 5.25** Gram-positive bacteria (original magnification, ×5700). Numerous gram-positive bacteria are appreciable at relatively low electron microscopic magnification after retrieval from a paraffin block.



• **Figure 5.24** Basic structure of bacteria.

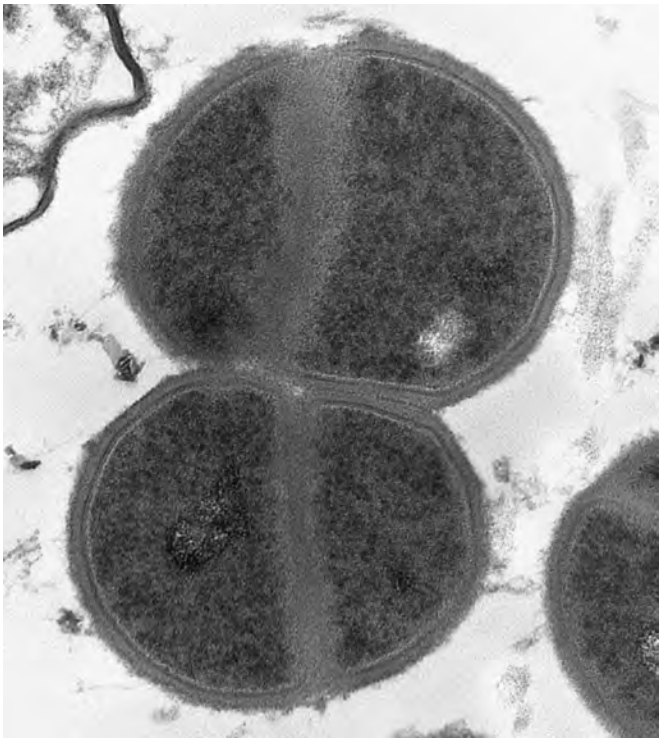
may have a loose, fuzzy extracellular capsule (glycocalyx), flagella, pili (fimbria), and a non–membrane-bound nucleus. Gram-positive bacteria have a thicker outer wall compared with gram-negative bacteria. Although bacteria may be visible by light microscopy, smaller bacteria are much more readily visualized with EM, particularly *Legionella pneumophila*. Bacteria have a variety of shapes, including cocci; coccobacilli; and straight, curved, or branched rods. The size of bacteria ranges from less than 1 µm in diameter and up to 1 to 6 µm long.[1] EM can be used to recognize bacteria when tissue integrity is compromised and when bacteria are difficult to distinguish from artifact.[38]

Gram-Positive Bacteria

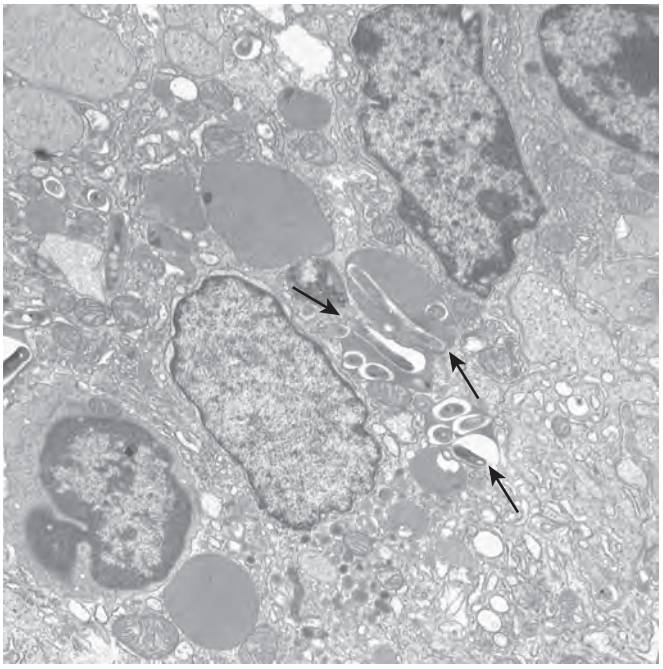
Gram-positive bacteria (Figs. 5.25 and 5.26) are characterized by their thick walls, which are composed of peptidoglycans and mucocomplexes containing muramic acid and stain prominently with osmium as 20- to 50-nm thick, electron-dense layers surrounding the plasma membrane. Gram positivity is due to the thick peptidoglycan layer, which gives shape, strength, and rigidity to the cell wall. An ultrastructural Gram technique replaces the crystal violet–iodide complex with a complex platinum salt–containing compound.[13,39-41]

Gram-Negative Bacteria

Gram-negative bacteria have a rigid peptidoglycan layer that separates outer and inner membranes. The outer membrane is often undulant. Gram-negative bacteria also may have outer membranes made of phospholipids, glycolipids, lipopolysaccharides (LPSs), and proteins. A periplasmic space separates the outer membrane from the cell membrane. LPSs may be visualized as long, tubular complexes or as spherical disks. LPSs, along with other membrane components, such as proteins and phospholipids, help to form endotoxin.[13,41]



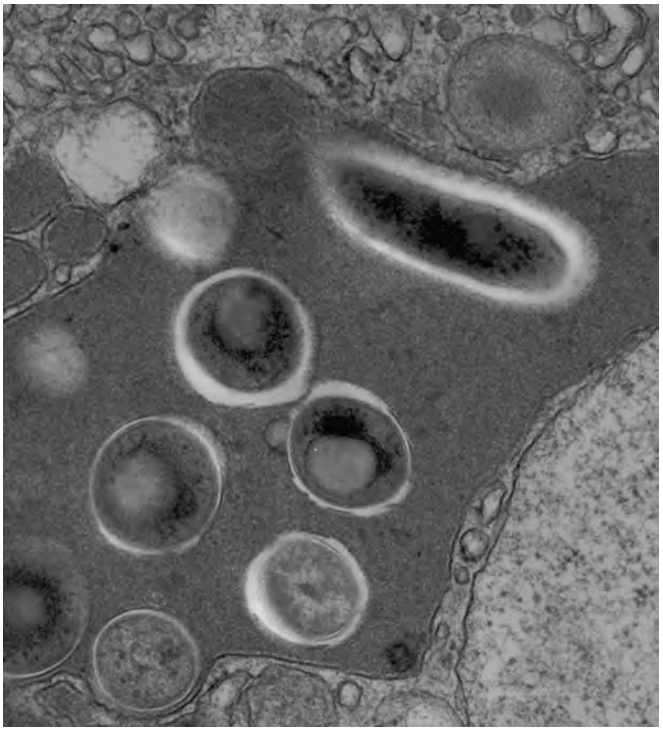
• **Figure 5.26** Gram-positive bacteria (original magnification, ×71,000). Gram-positive bacteria have a plasma membrane and a thick peptidoglycan layer. By contrast, gram-negative bacteria have an additional, undulant outer membrane, a plasma membrane *(inner membrane)*, and a narrow, irregular peptidoglycan layer.



• **Figure 5.27** *Mycobacterium avium-intracellulare* (original magnification, ×3400). Mycobacteria can be seen *(arrows)* in the lamina propria of the duodenum with surrounding chronic inflammatory cells.

Mycobacteria

Mycobacteria (Figs. 5.27 and 5.28) are coccobacillary or filamentous, acid-fast bacterial organisms that may be present as straight or curved rods and aggregate in branching forms, approximately 0.3 to 0.6 µm wide and 1 to 4 µm long. They have special cell wall lipids that form a waxy coating, together with polysaccharides, glycerides, and high-molecular-weight alcohols, and they are present in macrophages as facultative intracellular organisms. There is a variable-thickness capsule in close contact with a cell wall. The cell wall can be identified as having a high-density outer portion surrounding an inner, electron-opaque, fibrillar portion (containing mycosides) and nuclear region; the cytoplasm has lipid storage bodies and polyphosphates present as dense granules. *Mycobacterium tuberculosis* may be present in lysosomes of macrophages, and these macrophages typically aggregate, forming a tubercle.[13]

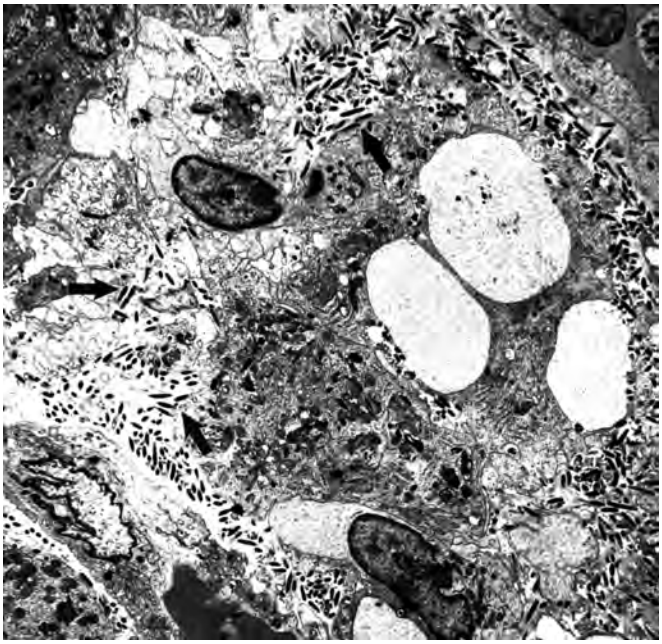


• **Figure 5.28** *Mycobacterium avium-intracellulare* (original magnification ×25,000). Mycobacteria are seen within histiocytic secondary lysosomes in a sample of duodenum.

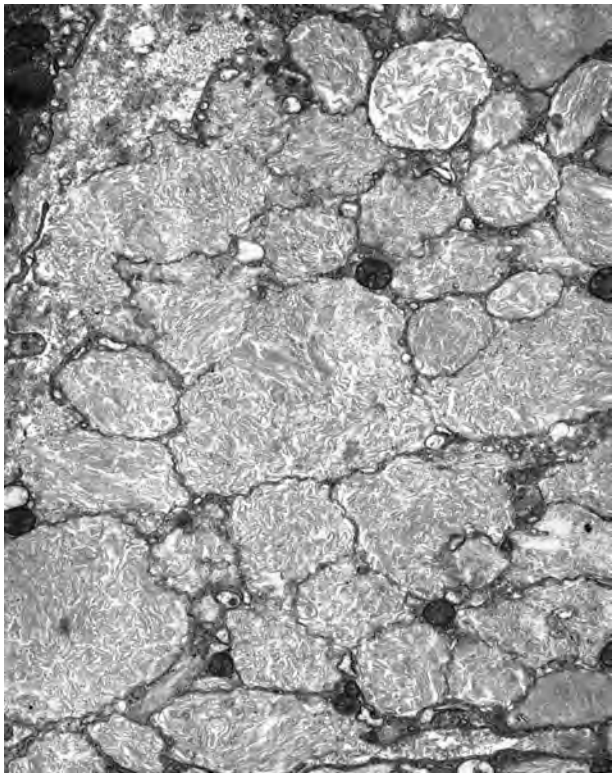
Other Important Bacteria

Tropheryma whippeli

T. whippeli (Figs. 5.29 to 5.31), the causative organism of Whipple disease, is a small (1 to 2 µm long, 0.25 µm in diameter) organism in which the rods are typically found free within macrophages of the intestine. The bacilli have a thick (approximately 20 nm) outer cell wall with a trilaminar membrane. Bacteria replicate extracellularly but are typically ingested by macrophages and digested into intracellular myelin figures, in which case histiocytes are seen distended with lysosomes that are filled with serpiginous membranes (see Fig. 5.29).[1,19]



• **Figure 5.29** Whipple disease (original magnification, ×1900). Whipple disease of the jejunum in which extracellular bacilli *(arrows)* can be seen.



• **Figure 5.30** Whipple disease (original magnification, ×7100). Serpiginous bodies in a case of Whipple disease of the cardiac ventricle in which only the confluent bacterial membrane remnants can be seen (material retrieved from paraffin).

Chlamydia trachomatis

C. trachomatis (Figs. 5.32 and 5.33), formerly thought to be a virus until it was determined to be a bacterium, has an outer membrane and is present within cytoplasmic inclusion vacuoles, which contain numerous chlamydia in infected host cells. It is present as elementary and reticulate bodies with intermediate forms. Elementary bodies enter the host cell by endocytosis and phagocytosis. Inclusion bodies undergo lysis and exit the cell through endocytosis.[1]

Helicobacter pylori

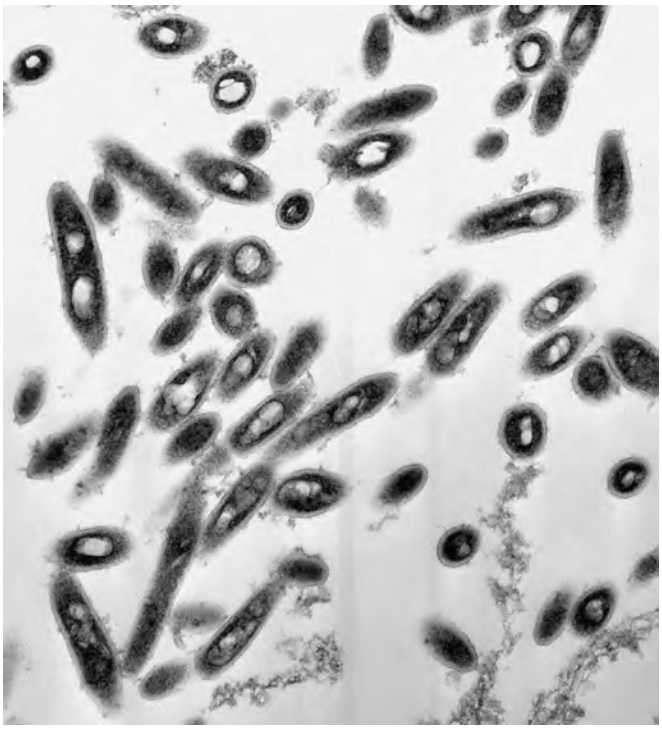
H. pylori is a 2- to 6-µm long, 0.5-µm wide, gram-negative organism that has a surface flagellar sheath; the sheath is thought to provide acid resistance in its typical acidic gastric environment, along with the ureases the organism produces. *H. pylori* attaches to the gastric mucus layer and cell surface microvilli and is the causative agent of many cases of gastritis and gastric ulcers. Numerous *Helicobacter* organisms have been identified. One example is *Helicobacter felis*, a tightly spiraled bacterium that colonizes cats and dogs and has characteristic periplasmic fibrils.[13,19]

Legionella pneumophila

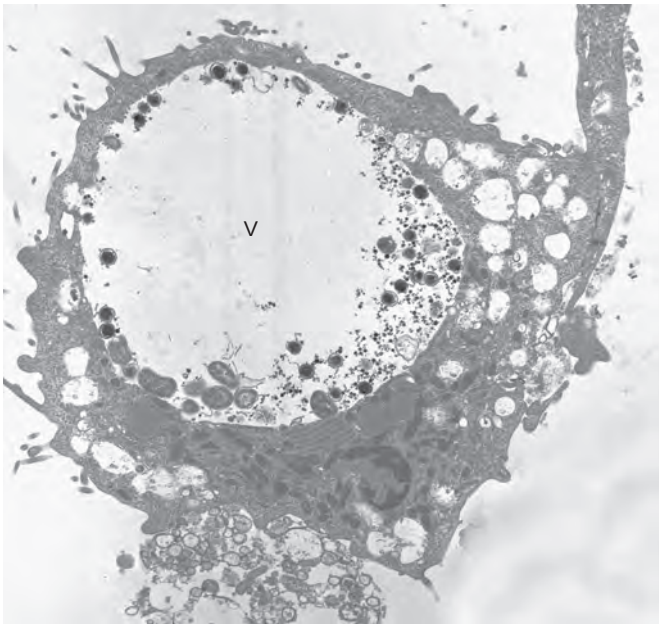
L. pneumophila (Figs. 5.34 and 5.35) is a gram-negative coccobacillus and a facultative intracellular pathogen that replicates in pulmonary macrophages and causes acute fibrinopurulent bronchopneumonia. Large numbers can be found in phagolysosomes and rough endoplasmic reticulum of macrophages or free in cellular debris. Pseudopodia may be found surrounding bacterial cells, leading to rapid macrophage ingestion of bacteria, a process called "coiling phagocytosis."[13,42-44]

Fungal Infections

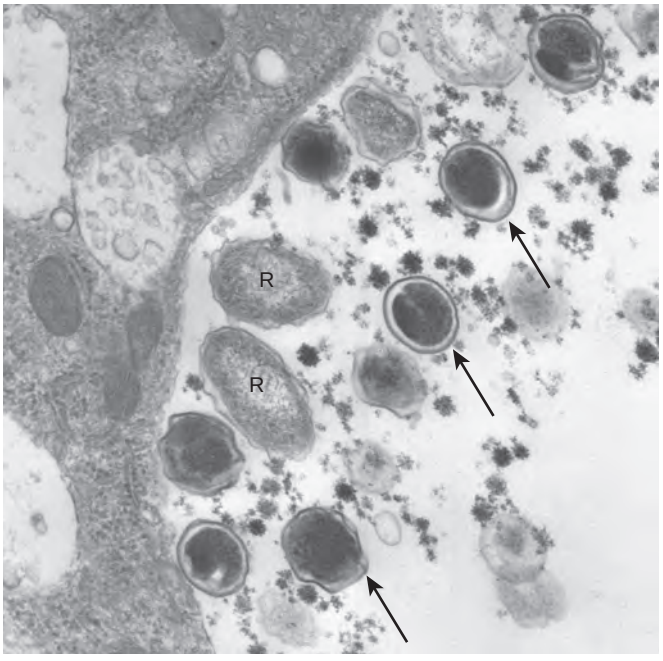
EM can be used to recognize a number of features of fungal organisms, including their interaction with their microenvironment.[45-48]



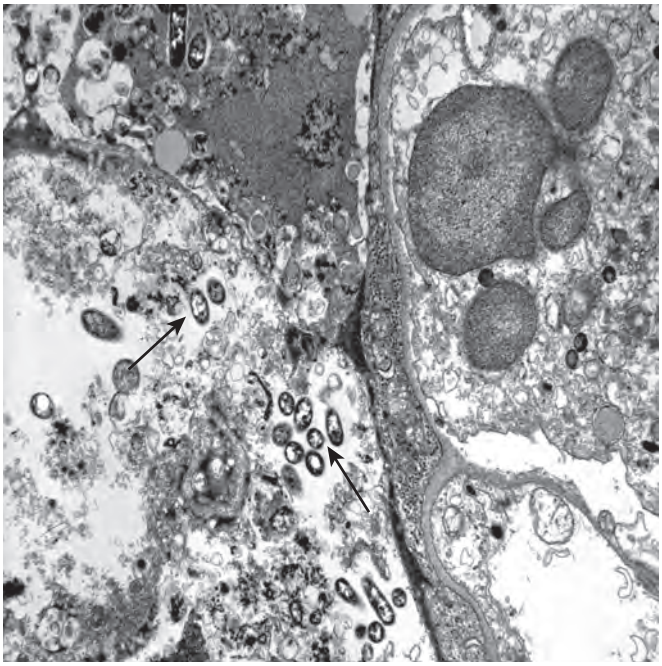
• **Figure 5.31** Whipple disease (original magnification, ×19,000). Whipple disease of the jejunum in which extracellular bacilli with cytoplasmic vacuoles can be seen.



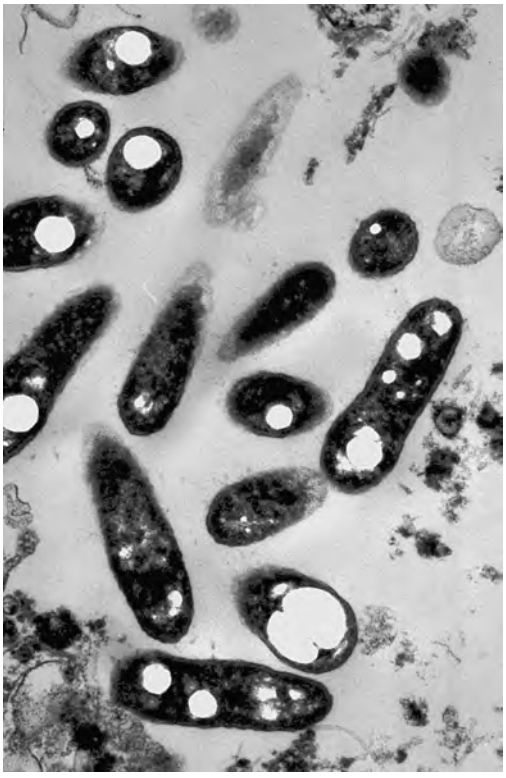
• **Figure 5.32** *Chlamydia trachomatis* (original magnification, ×3400). Chlamydial organisms in various stages can be seen in a large cytoplasmic inclusion vacuole *(V)* from cell culture. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



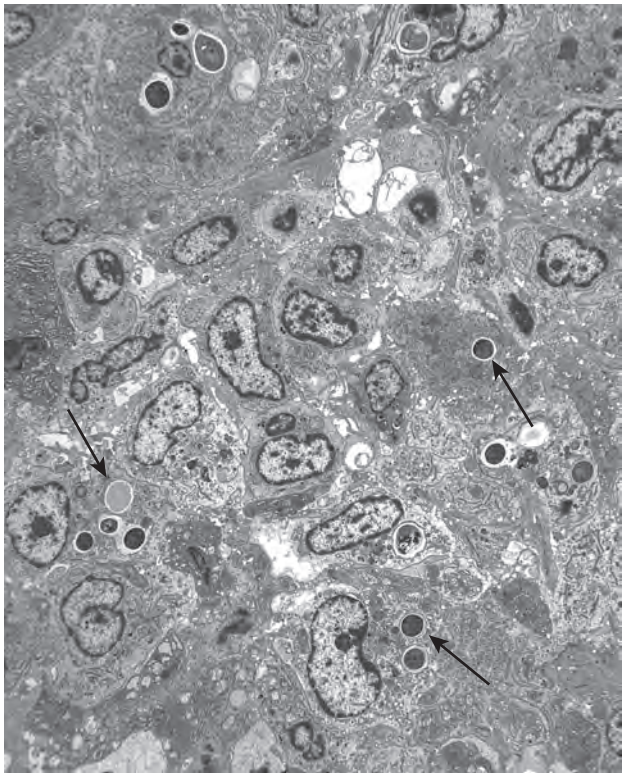
• **Figure 5.33** *Chlamydia trachomatis* (original magnification, ×19,000). Higher-power image of chlamydia showing elementary *(arrows)* and reticulate *(R)* bodies. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



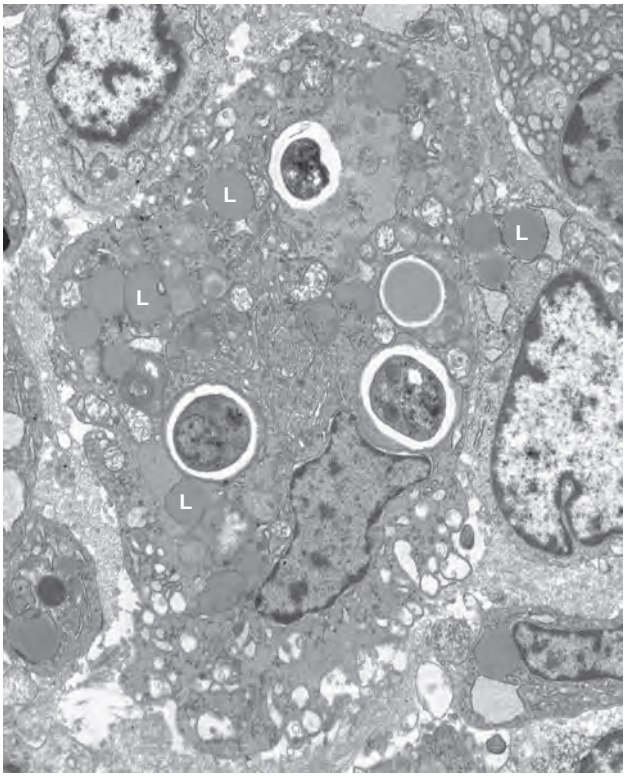
• **Figure 5.34** *Legionella pneumophila* (original magnification, ×1900). Bacterial rods *(arrows)* are seen in alveolar exudate.



• **Figure 5.35** *Legionella pneumophila* (original magnification, ×34,000). Bacterial rods that are largely degenerative are seen. The outer cell membrane and cytoplasmic vacuoles can be appreciated (retrieved from paraffin).



• **Figure 5.36** *Histoplasma* yeast from supraclavicular regional lymph node (original magnification, ×1900). Numerous round yeast forms *(arrows)* can be seen within the cytoplasm of histiocytes. Clear halos surround the organisms underneath their cell membrane.



• **Figure 5.37** *Histoplasma* yeast from supraclavicular regional lymph node (original magnification, ×3400). Clear halos surround the organisms underneath their cell membrane. The histiocyte containing the organism has numerous lipid vacuoles *(L)*.

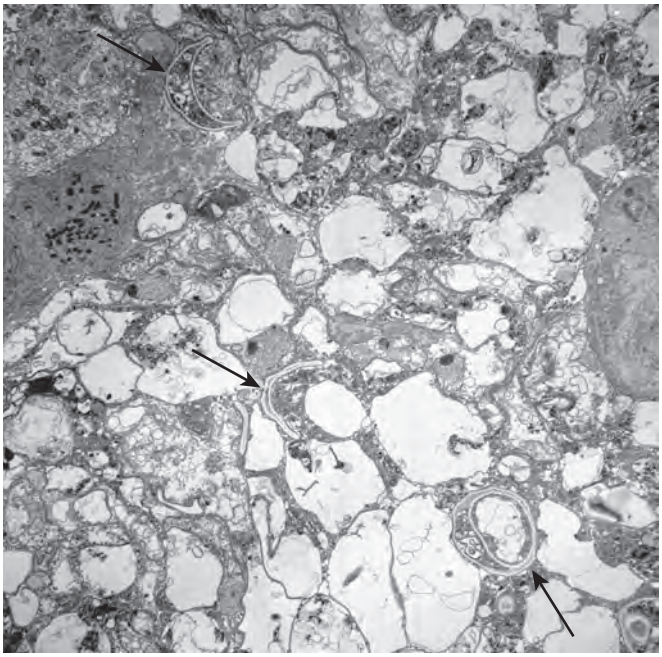
For example, EM has been used to examine the interaction of *Candida* with host, where it may act as a commensal in the mucosa and skin.[46,47]

Histoplasma capsulatum

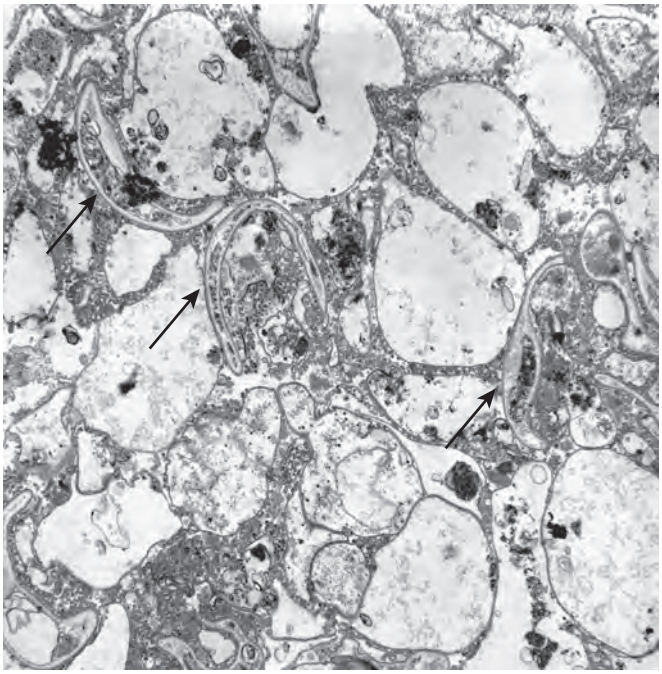
H. capsulatum (Figs. 5.36 and 5.37) consists of both intracellular and extracellular oval yeast forms, 2 to 4 μm in diameter, with no true capsule, a thin cell wall, and a single nucleus. Intracellularly they are found in the cytoplasm of histiocytes. The yeast form may have heavier wall components composed of chitin. There is typically a clear cytoplasmic halo at the periphery of the organisms, which may be appreciated on histologic sections, particularly when stained with silver impregnation or periodic acid–Schiff (PAS) stains. Histiocytes typically contain several organisms, lipid droplets, and organelles within a copious amount of cytoplasm. Breakdown of organism cell walls occurs in macrophage phagolysosomes containing lysosomal acid hydrolases. After the organisms have been killed in chronic infections, cell walls and electron-dense material may remain.[1,13]

Pneumocystis jiroveci

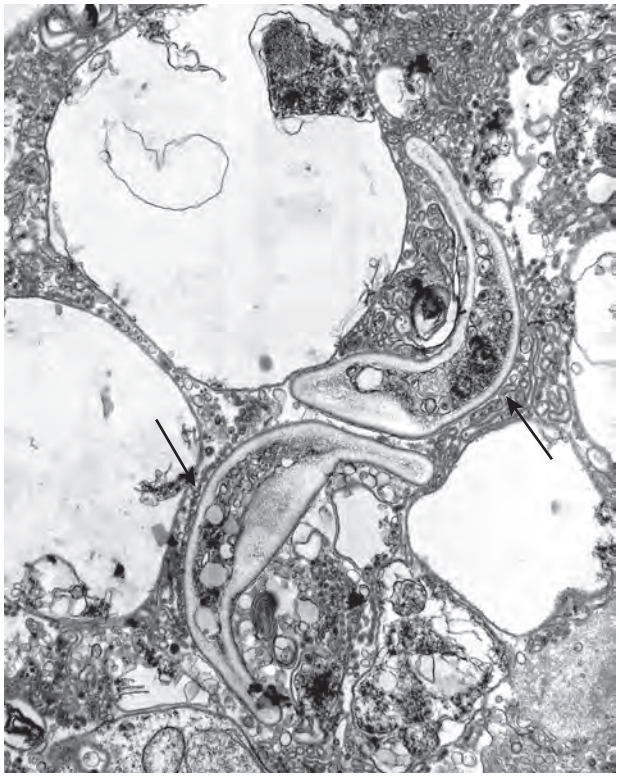
P. jiroveci (Figs. 5.38 to 5.42), once thought to be a protozoan and formerly named *Pneumocystis carinii*, is now believed to be a fungus with a close relationship to ascomycetes, based on molecular biologic studies.[49] *P. jiroveci* consists of thick-walled, spherical intact cysts and crescentic collapsed cysts surrounded by trophozoite processes and vacuoles. The cysts are 4 μm in diameter and



• **Figure 5.38** *Pneumocystis jiroveci* (original magnification, ×1900). Crescent-shaped cysts *(arrow)* of the *Pneumocystis* organism can be seen in a pulmonary alveolar exudate.



• **Figure 5.39** *Pneumocystis jiroveci* (original magnification, ×3400). Crescent-shaped cysts of the *Pneumocystis* organism *(arrow)* can be seen in a pulmonary alveolar exudate.

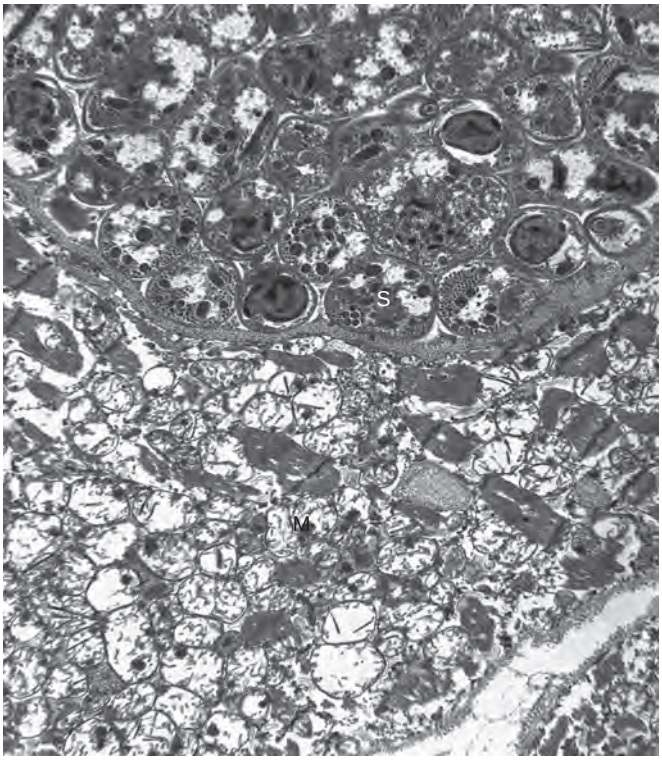


• **Figure 5.40** *Pneumocystis jiroveci* (original magnification, ×7100). Two collapsed crescent-shaped cysts of the *Pneumocystis* organism *(arrows)* can be appreciated.

• **Figure 5.41** *Pneumocystis jiroveci* (original magnification, ×15,000). *Pneumocystis* organism is seen in a pulmonary alveolar exudate with outer dense and inner less dense layers.

• **Figure 5.42** *Pneumocystis jiroveci* (higher magnification). Higher power reveals the ultrastructural characteristics of the elaborately folded, whorled organismal membrane.

• **Figure 5.43** *Acanthamoeba* in acanthamoeba keratitis (original magnification, ×7900). A double-walled cyst with intracystic structures is seen. (Courtesy Norman Michaud at the Massachusetts Eye and Ear Infirmary.)



• **Figure 5.44** Toxoplasmosis (original magnification, ×3400). Numerous *Toxoplasma gondii* sporozoites *(S)* are seen within parasitophorous vacuoles in heart muscle in an autopsy specimen from a patient with human immunodeficiency virus infection. The heart muscle has numerous mitochondria *(M)*.

have an outer dense layer and an inner less dense layer. They may be empty or may enclose sporozoites. Trophozoites are 1.5 to 12 µm long, pleomorphic, intracystic or extracystic, and have a nucleus, cytoplasm, and extensively folded plasmalemmas.[1,50,51]

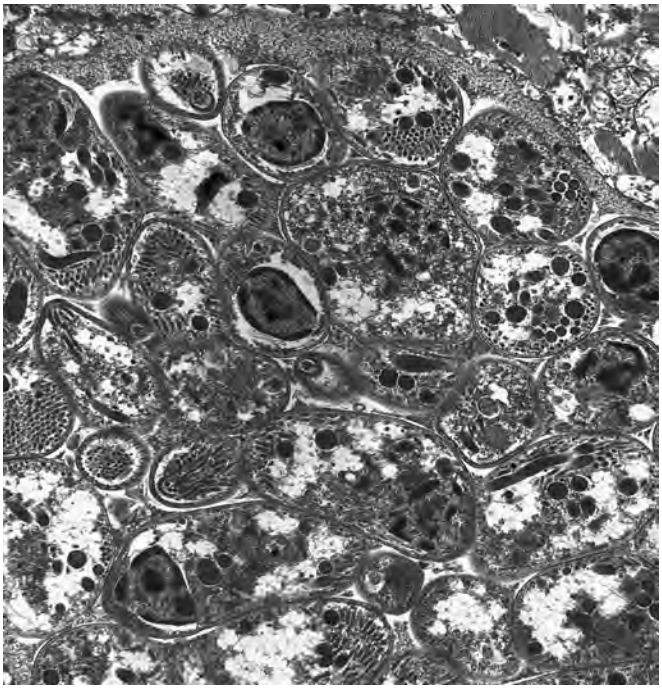
Parasitic Infections

Acanthamoeba

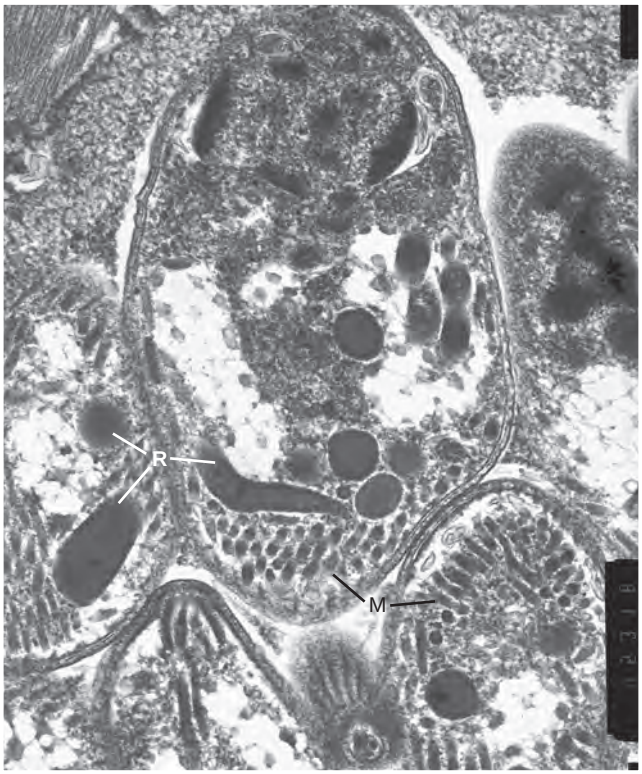
Acanthamoeba (Fig. 5.43) is a pathogenic protozoan that is classified as an ameboflagellate, like *Naegleria fowleri*. *Acanthamoeba* is most commonly seen in humans as the causative agent of keratitis. *Acanthamoeba* may also cause chronic granulomatous lesions, notably chronic amebic encephalitis, in which the organisms typically enter the brain through the cribriform plate. Infections of the respiratory tract and skin may also occur and particularly affect the immunosuppressed. Healthy individuals may be affected by *Acanthamoeba* keratitis, particularly after trauma to the cornea or exposure to contaminated water, contact lenses, or contact lens solutions. The trophozoite, measuring 15 to 30 µm, is the invasive, infective form.[13,52,53]

Toxoplasma gondii

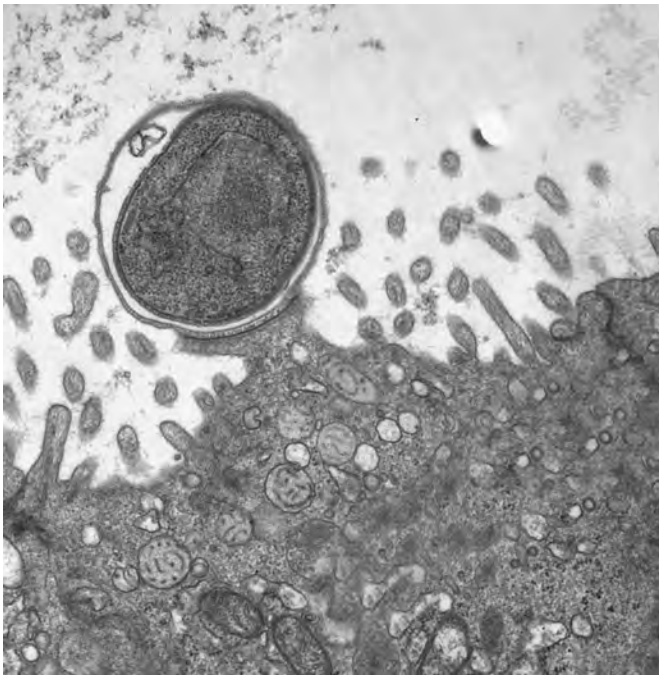
T. gondii (Figs. 5.44 to 5.46) consists of ovoid-, elliptical-, or teardrop-shaped parasites (approximately 3 × 2 µm) within vacuoles in macrophages. *Toxoplasma* has a single posterior nucleus, a triple-layered cell membrane, and organelles that are considered specific and nonspecific. At the anterior end of the organism, there are organelles that have a conoid (conical opening) and 30 to 50 micronemes (secretory tubules in convoluted groups). At the anterior end are rhoptries (club-shaped storage sacs), which empty by ducts into the conoid. EM is useful for helping to distinguish *Toxoplasma* from other sporozoites in the *Apicomplexa* genera, such as *Trypanosoma cruzi*. *T. cruzi* have kinetoplasts, and



• **Figure 5.45** Toxoplasmosis (original magnification, ×7100). Intermediate magnification of heart muscle from the same case as in Fig. 5.44 shows numerous *Toxoplasma gondii* sporozoites.



• **Figure 5.46** Toxoplasmosis (original magnification, ×25,000). *Toxoplasma gondii* sporozoites are seen within heart muscle with characteristic rhoptries *(R)* and micronemes *(M)*.



• **Figure 5.47** Cryptosporidia (original magnification, ×11,000). Spherical microorganism is located along the mucosal surface, attached to the mucosal epithelium.

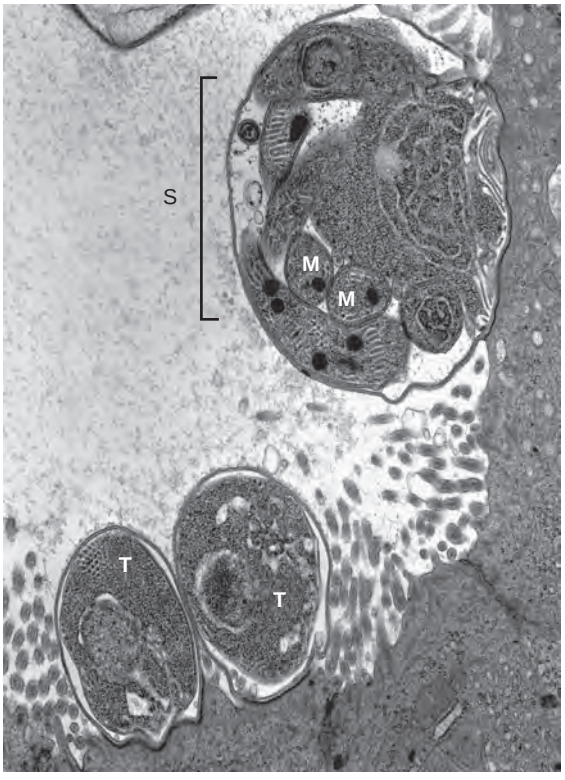
Toxoplasma does not. *Toxoplasma* has conoids and endogenous division (internal budding of daughter cells), and trypanosomes do not.[1]

Cryptosporidium parvum

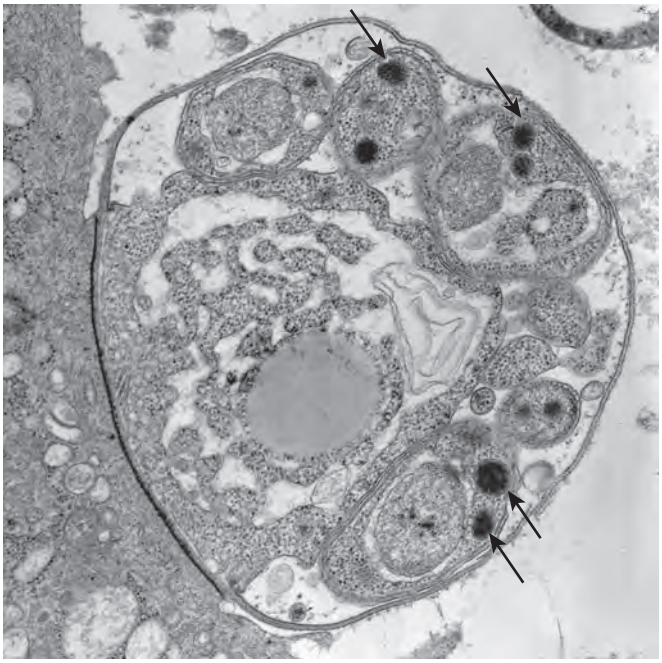
C. parvum (Figs. 5.47 to 5.49), consists of 3- to 4-µm spherical structures that are located along the gastrointestinal epithelium, specifically the small intestine. At light microscopic levels, the spherical structures are often seen attached to the epithelial cell surface. The attachment site usually lacks microvilli and is dense and raised. The parasites have different morphologies based on the phase of their life cycle (i.e., microgamete, merozoite, schizont, trophozoite). Secondary lysosomes (phagosomes) are frequently seen in the apical cytoplasm of epithelial attachment sites.[1]

Giardia lamblia

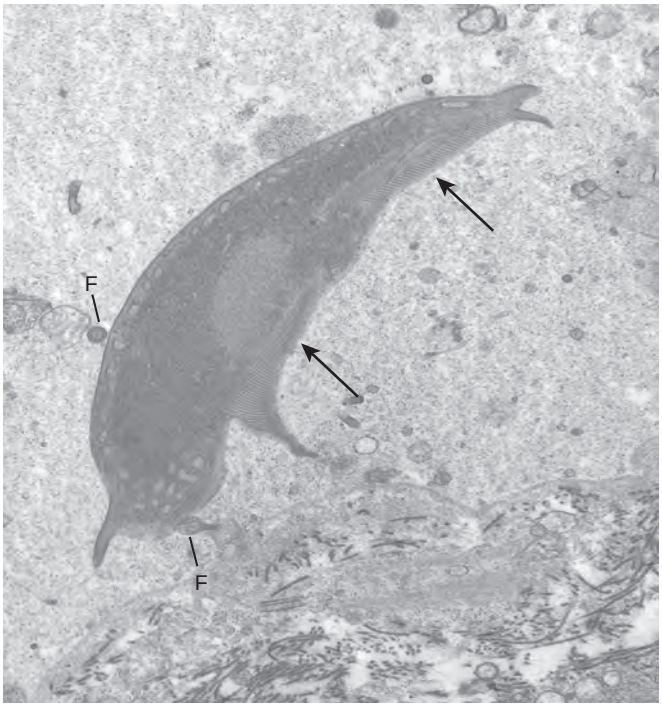
G. lamblia (Figs. 5.50 and 5.51) consists of pear-shaped trophozoites, alternatively convex dorsally and concave ventrally, measuring 9.5 to 21 µm long and 5 to 15 µm wide, that are normally present free in the small intestine and in cysts in the large intestine. *Giardia* is binucleate, with symmetrically spaced nuclei and a prominent karyosome, and has four pairs of flagella adjacent to an adhesion disc. *Giardia* has a rigid cytoskeleton with evenly spaced microtubules linked by microribbons. It does not have mitochondria, peroxisomes, endoplasmic reticulum, or nucleoli. Morphologic types of *Giardia* can be distinguished ultrastructurally, as can the stage of the life cycle (i.e., free trophozoites in the



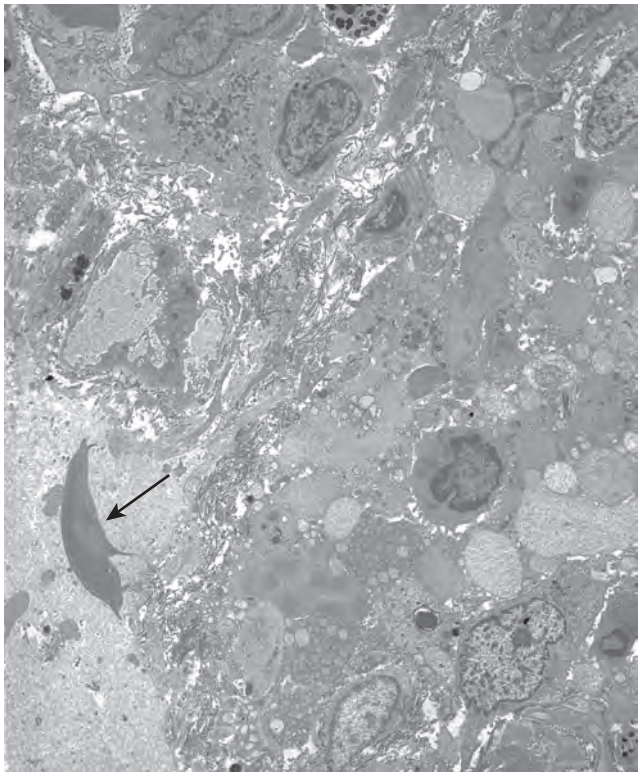
• **Figure 5.48** Cryptosporidia (original magnification, ×11,000). Numerous spherical microorganisms are located along the mucosal surface, attached to the mucosal epithelium with trophozoites *(T)* and an appreciable schizont *(S)* containing merozoites *(M)*.



• **Figure 5.49** Cryptosporidia (original magnification, ×19,000). Higher-power image of a gamete shows dense granules *(arrows)*.



• **Figure 5.51** *Giardia* (original magnification, ×9100). Convex trophozoite with visible intracellular structures including flagellum *(F)* and adhesion disc *(arrows)*. (Epon blocks courtesy William Taylor, MD, and Lynne A. Farr, BA, Rhode Island Hospital, Providence, Rhode Island.)



• **Figure 5.50** *Giardia* (original magnification, ×1900). Convex trophozoite *(arrow)* is seen at relatively low electron microscopic magnification. (Epon blocks contributed by William Taylor, MD, and Lynne A, Farr BA. Rhode Island Hospital, Providence, Rhode Island.)

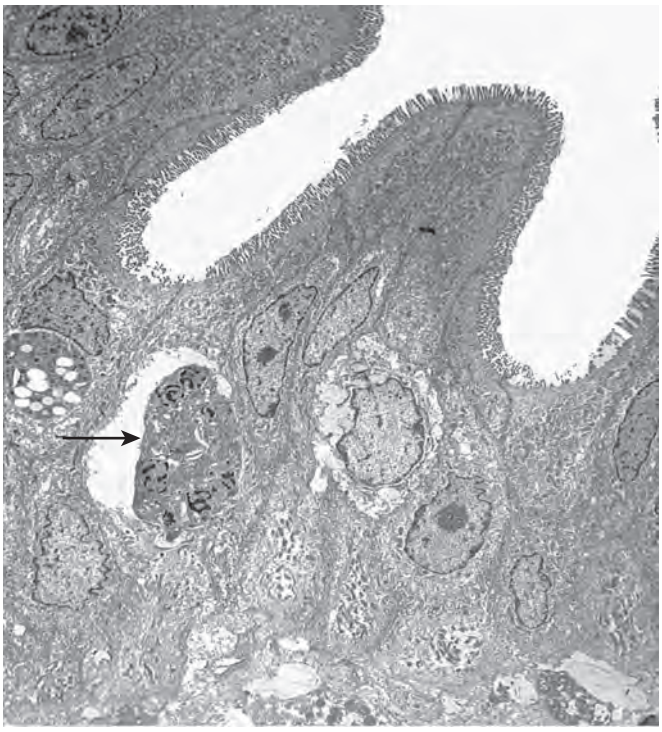
small bowel and cysts in the large bowel and feces). Mature cysts are 8 to 12 µm long and 7 to 10 µm wide and have four nuclei, a dense wall, dense cytoplasm with ribosomes and glycogen granules, and peripheral vacuoles or tubules.[1]

Cystoisospora belli

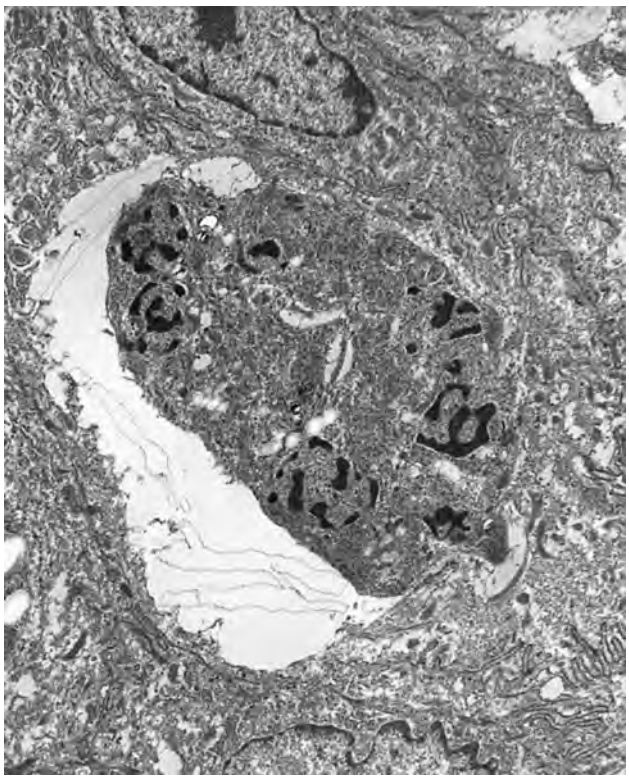
C. belli (Figs. 5.52 and 5.53) belongs to a genus of the pathogenic coccidia that includes other organisms, such as *Cyclospora* and *Eimeria*; and the morphology of *Cystoisospora* is quite similar to *Eimeria*.[13] *C. belli* is the new term for *Isospora belli*.[54] Coccidiosis caused by these organisms involves invasion of intestinal epithelial cells by sporozoites, which form through sporogony from oocysts (the organismal zygotes). In the schizony or growth phase, sporozoites form blastophores, which rupture at the cell surface, releasing merozoites; the merozoites infect more cells and form microgametes in the sexual phase. These then form more oocysts, completing the cycle. In human cystoisosporiasis, the condition may be chronic, with villous atrophy and a lymphoplasmacytic reaction in the lamina propria. The organism may be recognized in all life cycle phases. Unsporulated oocysts are shed in the feces. Encysted zoites may be identified in the lamina propria of the intestinal villi and, rarely, in lymph nodes.[13]

Microsporidia

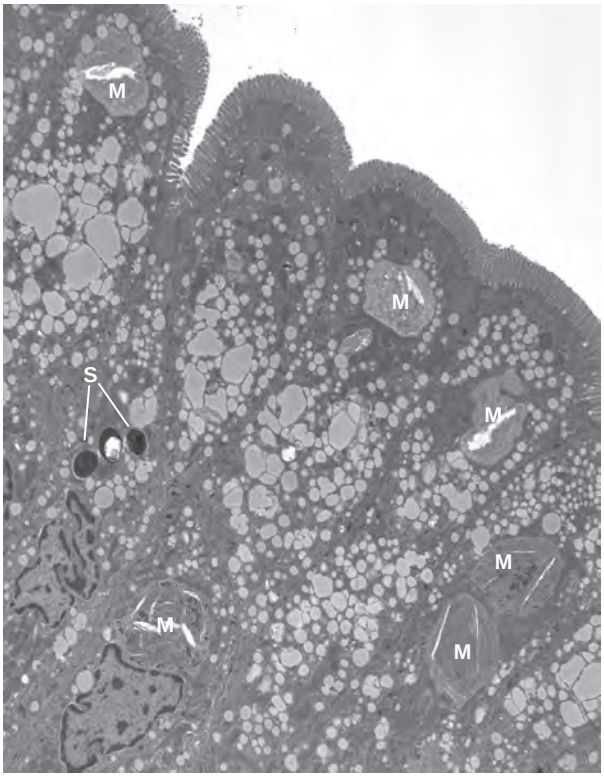
Microsporidia (Figs. 5.54 to 5.57) are obligate intracellular parasites in the phylum *Microspora*.[3] The spores of this organism are 0.5 to 5 µm in diameter. The most common species in humans is *Enterocytozoon bieneusi*. Organisms are identified in fecal material or in routinely processed tissue biopsies. The major



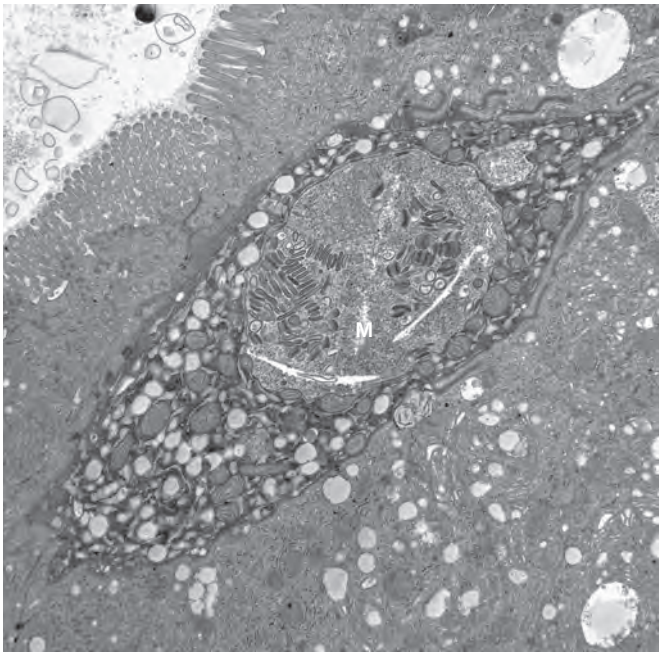
• **Figure 5.52** *Cystoisospora belli* (original magnification, ×2500). *Arrow* indicates *Isospora belli* organism with lobated nuclei in the intestinal lamina propria (retrieved from paraffin).



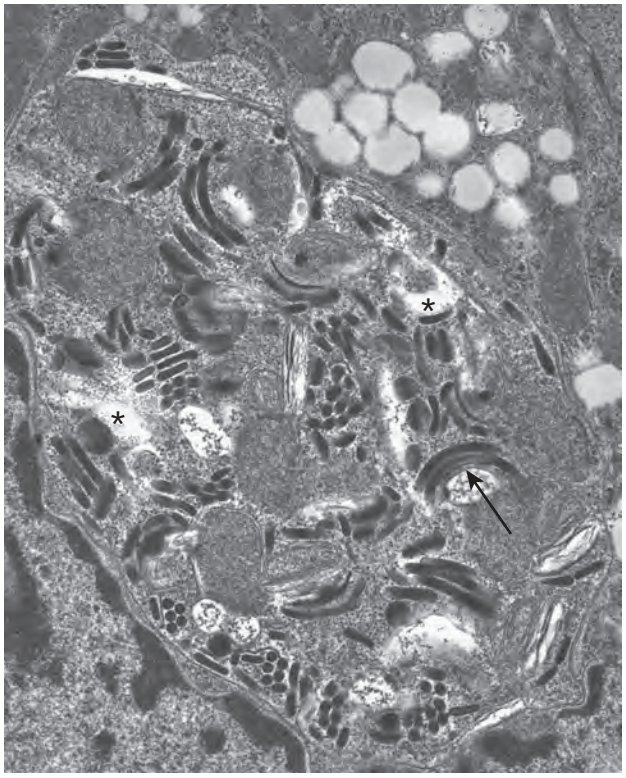
• **Figure 5.53** *Cystoisospora belli* (original magnification, ×5700). Higher magnification shows the lobated nuclei of *Isospora belli* organism (retrieved from paraffin).



• **Figure 5.54** A microsporidian, *Enterocytozoon bieneusi* (original magnification, ×1900). Enterocytes filled with numerous microsporidia spores *(S)* and meronts *(M)* are seen in a sample of duodenum.



• **Figure 5.55** Meront *(M)* of the microsporidian, *Enterocytozoon bieneusi*, in sample of duodenum (original magnification, ×5700).

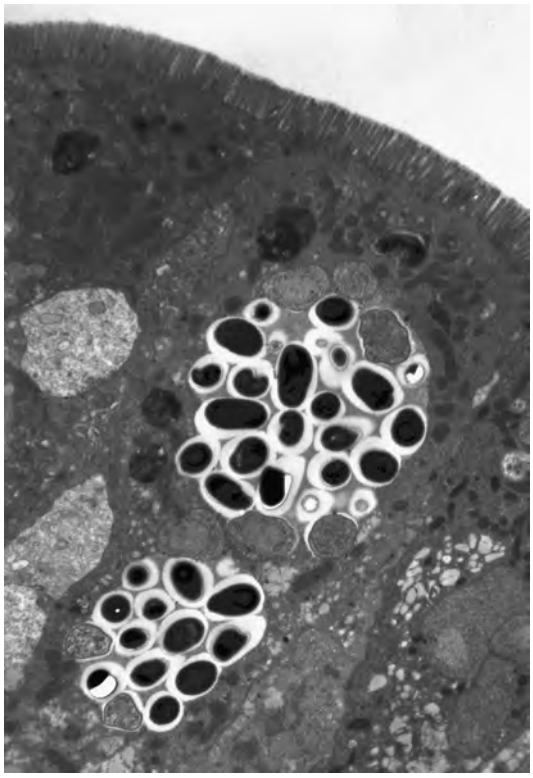


• **Figure 5.56** A microsporidian, *Enterocytozoon bieneusi* (original magnification, ×19,000). High magnification of a meront in a sample of duodenum shows clear clefts *(*)* and electron-dense discs *(arrow)*.

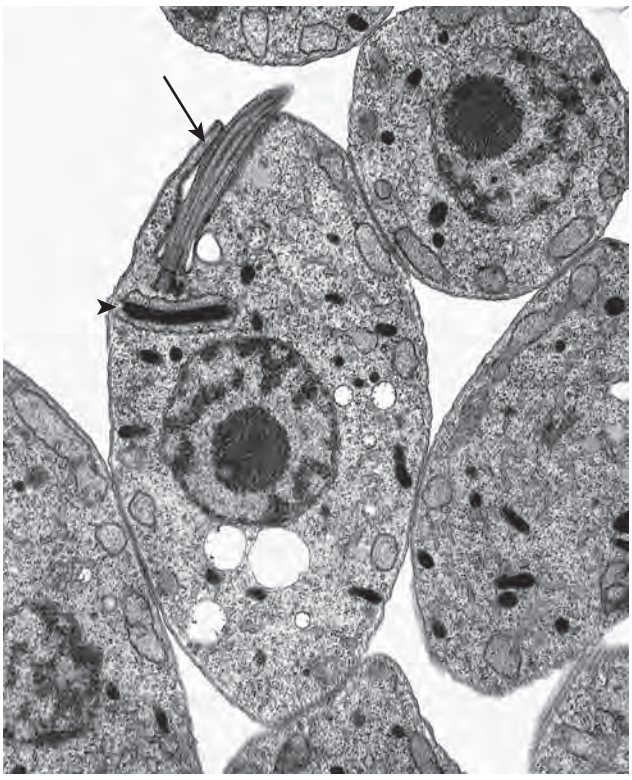
ultrastructural features used for typing microsporidia are the characteristic size and shape, the characteristic nuclei in spores and developmental stages, the host-parasite interface, and the number of coils in the tubular extrusion apparatus (sometimes present as single-coiled polar tubes in the spores). In *E. bieneusi*, cross-sections of the coiled polar tube are seen as a double row in the posterior half of the spore, whereas in the microsporidian *Septata intestinalis* there is a single row. *E. bieneusi* may be seen as spores in direct contact with enterocytes. Enterocytes may be seen infected with microsporidia spores and meronts. In *S. intestinalis*, spores are grouped with septae surrounding individual spores in parasitophorous vacuoles in the supranuclear cytoplasm of enterocytes.[19,55-62] EM aided with the diagnosis in cases of disseminated microsporidiosis after organ transplantation.[63-66]

Trypanosoma cruzi

T. cruzi (Fig. 5.58) consists of oval and elliptical organisms, 10 to 30 μm in diameter, with a characteristic kinetoplast and flagellum in addition to the usual nucleus and cytoplasmic organelles. At the base of the basal body of the flagellum is a kinetoplast, which consists of a curved, electron-dense structure containing DNA. *T. cruzi* has a single, long mitochondrion just underneath the cell membrane, extending from the kinetoplast to the posterior compartment of the cytoplasm, and there may also be a free flagellum outside the cell membrane. The kinetoplast position and the flagellum position and length vary based on the life cycle stage of the organism (e.g., promastigote, trypomastigote, amastigote, epimastigote).[1]



• **Figure 5.57** A microsporidian, *Septata intestinalis*, in a sample of duodenum (original magnification, ×25,000). Organismal spores are present in the supranuclear cytoplasm of an enterocyte within parasitophorous vacuoles with vaguely defined septae surrounding individual spores.



• **Figure 5.58** *Trypanosoma cruzi* from a tissue culture sample. Oval/elliptical trypanosomes are seen with a nucleus and numerous cytoplasmic organelles and a portion of the kinetoplast *(arrowhead)* and flagellum *(arrow)* structures.

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6

New Technologies for the Diagnosis of Infection

NICOLE PECORA, DANNY A. MILNER JR.

Protein-Based Identification

Nucleic Acid–Based Techniques
- Probe-Based Assays
- Singleplex or Limited Target Assays
- Multiplexed Systems

Platforms and Technologies
- FilmArray (BioFire/BioMérieux)
- BD Max (BD Diagnostics)
- Luminex

Sequencing of Ribosomal RNA for Pathogen Identification

Next-Generation Sequencing

Conclusion

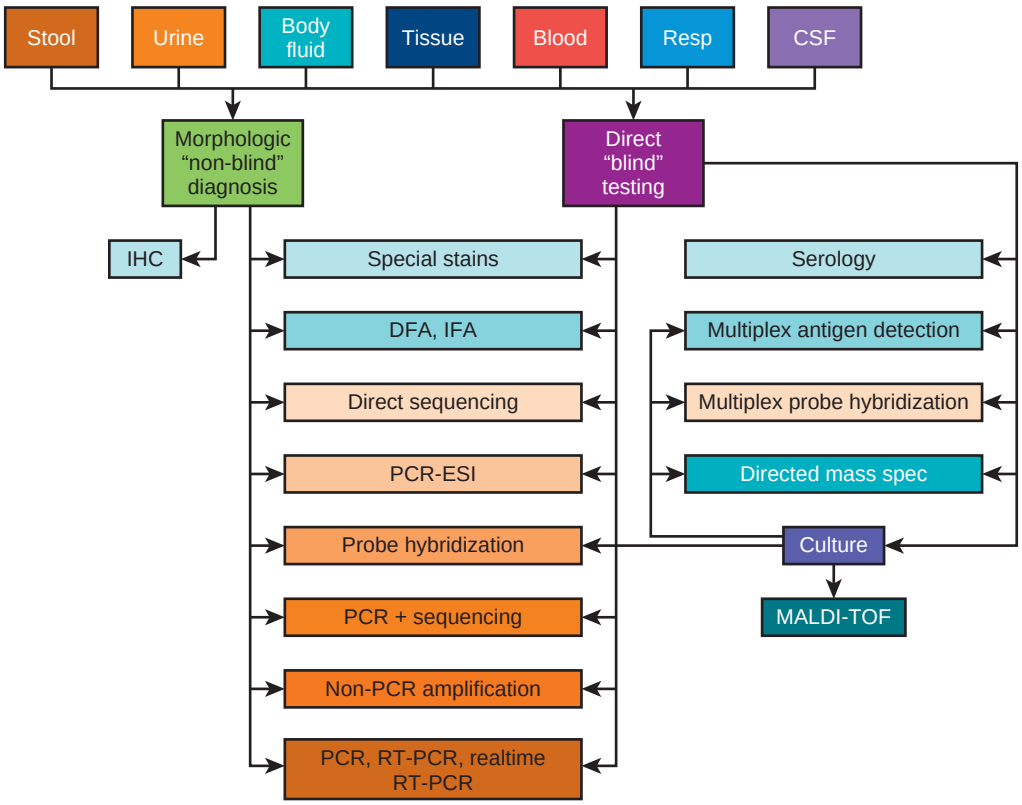
The clinical microbiology laboratory is constantly evolving and progressing. New technologies have revolutionized the characterization and diagnosis of pathogens. The purpose of this chapter is to review the features, benefits, and limitations of some of the key new methodologies in infectious disease diagnostics. The chapter will be organized by technology. New technologies have the most impact in the fields of bacteriology and virology, but there are also promising new developments in mycology and parasitology.

The mainstay of bacterial and mycologic diagnosis continues to be culture by traditional techniques, although increasingly these are automated (Fig. 6.1). After an organism is isolated, however, a host of newer diagnostics have enhanced, and in some cases replaced, the biochemical test algorithm that has historically defined clinical bacteriology. The advent of matrix-assisted laser desorption time of flight (MALDI-TOF) mass spectrometry is one of the main forces behind this shift, allowing a cultured organism to be identified in minutes instead of hours to days or even weeks in the case of very slow-growing organisms, such as mycobacteria. Although still not in routine clinical use, the application of MALDI-TOF to direct patient samples, such as blood and urine, and applications such as antibiotic resistance are exciting developments. Beyond bacteria, MALDI-TOF is becoming established for the identification of yeasts and, with the development of more extensive and higher quality libraries, is expected to also do so for other fungi.

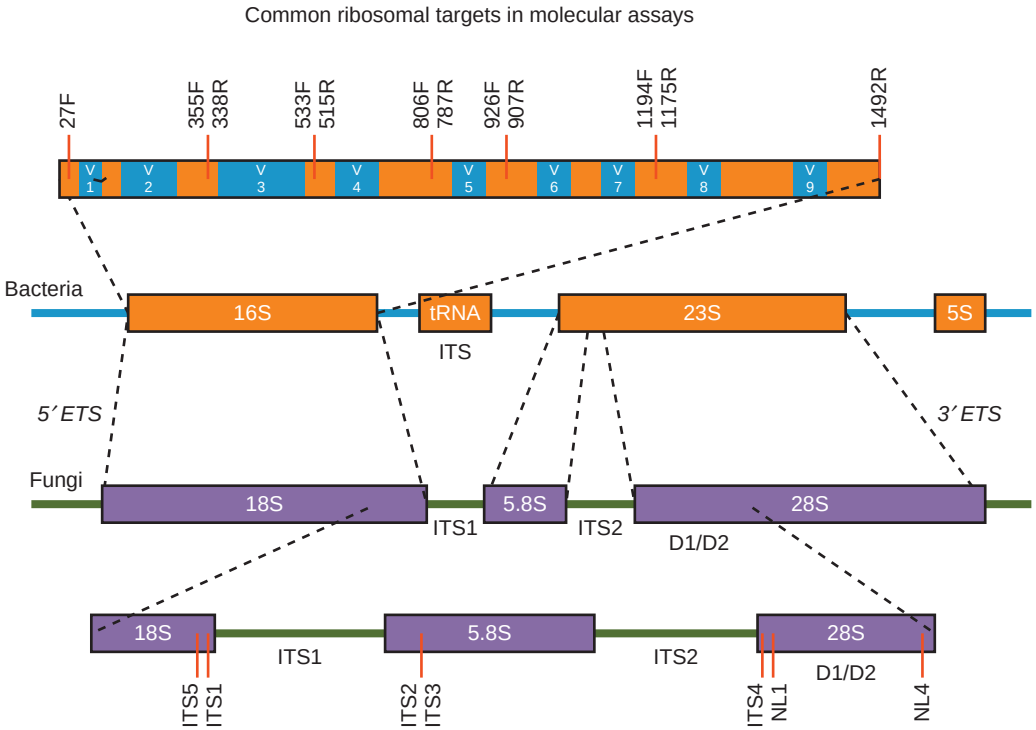
Nucleic acid–based technologies have also had a big impact. In particular, there are a growing number of test platforms that provide rapid diagnostics directly from primary specimens. Many of these are multiplexed to look at several pathogens at one time. The sequencing of the regions of ribosomal RNA (rRNA), such as the 16S rRNA gene in bacteria, already considered the gold standard for bacterial identification, is entering more and more into clinical diagnostics (Fig. 6.2). Similarly, the routine sequencing of the intergenic transcribed spacer (ITS) and 28S rRNA regions in fungi is a welcome addition to current laborious and morphology-heavy techniques for diagnosing fungal illnesses. The entry of next-generation sequencing (NGS) into clinical microbiology has also had a dramatic effect on both the profiling of individual specimens (i.e., members of a potential outbreak or highly drug resistant strains) as well as communities (i.e., microbiome analysis in health and disease). Finally, the emerging technology of polymerase chain reaction (PCR)-electrospray ionization (ESI) mass spectrometry holds great promise for all major phyla of pathogens.

Protein-Based Identification

Antigen-Based Identification uses rapid assessment for the presence of microbial antigens and provides a "point of care" solution for diseases for which immediate answers impact patient care decisions. The principle of all of these assays is that a microbial antigen is present at sufficient quantity for which an antibody has been generated for detection. Common platform types include lateral flow assays (antibodies bound to a matrix through which the patient sample containing antigen passes and results in a colorimetric readout; examples are BinaxNow for Malaria, Remel Xpect for *Clostridium difficile* assays, p24 antigen detection in human immunodeficiency virus [HIV]) and fluorescent immunoassays (similar to lateral flow concept but with automated detection and fluorescently labeled antibodies; examples are Sofia system for bacteria and viruses).[1,2] These assays may be available commercially to consumers or used in microbiology laboratories as inexpensive tools to screen for common diseases (e.g., seasonal influenza, Streptococcal antigens, diarrhea in acutely ill patients). In many laboratories, these rapid tests are confirmed by a second test which may include additional protein testing or nucleic acid



• **Figure 6.1** Algorithm of techniques for diagnosis of pathogens. *CSF,* Cerebrospinal fluid; *DFA,* direct fluorescent antibody; *ESI,* electrospray ionization; *IFA,* indirect fluorescent antibody; *IHC,* immunohistochemistry; *MALDI-TOF,* matrix-assisted laser desorption time of flight; *mass spec,* mass spectrometry; *PCR,* polymerase chain reaction; *resp,* respiratory; *RT,* reverse transcriptase; *RT-PCR,* real-time polymerase chain reaction.



• **Figure 6.2** Common ribosomal targets in molecular assays. *Red lines* indicate commonly used primer binding sites. *ETS,* External transcribed sequence; *ITS,* intergenic transcribed spacer. (Modified from Lafontaine DL, Tollervey D. The function and synthesis of ribosomes. *Nat Rev Mol Cell Biol*. 2001;2:514-20.)

testing. Immunohistochemistry (IHC) in anatomic pathology is based on the same principle as these rapid tests and has a wide range of uses for the detection and sometimes speciation of organisms in formalin-fixed, paraffin-embedded tissue (FFPE) sections. Organisms commonly identified this way include spirochetes, mycobacteria, DNA viruses, *Aspergillus, Candida,* and *Toxoplasma*, although special reference laboratories (e.g., The Infectious Disease Pathology Branch at the Centers for Disease Control and Prevention) have a wide range of antibodies for common to exotic pathogens for tissue confirmation.

MALDI-TOF, a mass spectrometry-based method, allows for a rapid (minutes) assessment of the characteristic complement of ribosomal proteins in an isolate, providing a species-level identification in many cases. After a microbial colony forms, it can be spotted onto a MALDI plate in matrix compound and loaded onto the mass spectrophotometer. This topic has been reviewed in great detail.[3-5] Briefly, a laser is focused onto the sample/matrix spot, causing sublimation, ionization, migration through a time of flight tube, and detection by a mass spectrometer (MS).The readout is a spectrum of peaks representing mass to charge ratios *(M/Z)* of different analytes. The mass range (approximately 2 to 20 kDa) is highly enriched for ribosomal proteins, which make up a large share of the typical MALDI spectra on clinical instruments. Spectra are compared with curated databases and the result is returned along with a metric describing the strength of the identification.

This technology has been widely adopted in Europe and is gradually entering clinical microbiology labs in the United States. There are currently two US Food and Drug Administration (FDA)-approved systems: the Vitek MS (BioMérieux, Durham, North Carolina) and the MALDI Biotyper (Bruker Daltonics Inc., Billerica, Massachusetts). Although the technology behind these two systems is essentially equivalent, they differ in their curated libraries, software, identification algorithms, and scoring systems.[5] For example, the Vitek MS reports isolates with a confidence value of 1% to 100%, whereas the Biotyper returns a score that can range from 0 to 3.000. For most applications a score of 2.000 or greater is necessary for a species level identification. Because of this, it can be difficult to directly compare results between the two systems. There have been several studies using both platforms back to back. One study on a large collection of 642 strains of bacteria, yeasts, and molds demonstrated comparable performance, although noting that the library coverage of the test cohort was greater with the Biotyper (572 strains) than the Vitek MS (406) and that the Vitek was more likely to output a misidentification for strains not represented in the library.[6]

For FDA-approved applications, both the Vitek MS and the Bruker may be used for a large library of aerobic gram-negative and gram-positive organisms, as well as several anaerobes and yeast. In general, performance has been reported to equal or exceed traditional biochemical systems, although it seems to be stronger for gram-negative than gram-positive organisms.[5,7] Lackluster performance, in particular, has been noted for several gram-positive rods. In a study with the Biotyper, the system was unable to identify several isolates of *Nocardia, Tsukamurella, Kocuria,* or *Gordonia*, although 89.7% of *Listeria* and 80% of *Rhodococcus* were correctly classified.[8] Members of *Corynebacteria, Lactobacillus,* and some *Listeria* also seem to present some difficulty, both in terms of difficulty discriminating between species and in requiring a lowered score cutoff (1.7) for species-level identification.[9] In similar studies using the Vitek platform, there were also reported problems in speciation of *Listeria*.[10] The misidentification of a *Kocuria* as a *Corynebacterium* has also been reported.[11] Among both gram-positive and gram-negative organisms, MALDI-TOF has several well-characterized difficulties discriminating certain species and complexes, including *Streptococcus pneumoniae/Streptococcus mitis, Escherichia coli/Shigella,* and members of the *Enterobacter cloacae* complex.[3] Finally, it is important to note that the Biotyper requires a Security-Relevant Library for the identification of such organisms as *Francisella tularensis, Burkholderia pseudomallei,* and *Brucella* spp.[12]

MALDI is rapidly becoming the method of choice for identifying anaerobic bacteria.[13] Several studies using large, diverse collections of anaerobes have demonstrated strong performances by both the BioTyper and the Vitek MS.[5] In one recent multicenter trial bringing together 651 anaerobic isolates for analysis by the Vitek MS 2.0, 91.2% of the isolates were correctly identified to species level.[14] The most notable difficulty was in identifying *Fusobacterium nucleatum* (42.9% identified correctly). The system also showed lower performance for certain species of *Bacteroides, Actinomyces,* and *Prevotella*. Similarly, in a study using the Biotyper platform to identify 197 anaerobes isolated from blood cultures, 86.8% of the strains were correctly identified to the species level (using a score of 2) and 94.9% to the genus level.[15] *Fusobacterium* spp. was again problematic, with only 23% being correctly identified to the species level. With formic acid/ethanol pretreatment, this number increased to 76.9%. In addition, 20.8% of the non-*fragilis Bacteroides* spp. was misidentified as other *Bacteroides*, with high confidence scores, and only 52.9% of the gram-positive anaerobic cocci were correctly identified to the species level. The latter was mostly a function of poor performance on several species of *Peptoniphilus*.[15] Interestingly, in a dedicated study looking at the performance of the Biotyper (2.0) on a collection of 277 isolates of *Bacteroides* spp., 97.5% were correctly identified to the species level.[16]

The identification of yeasts is an FDA-approved application for both the Biotyper and Vitek MS. Both systems have demonstrated strong performance in multiple studies, reviewed by Cassagne et al.[17] One recent study with the Biotyper, for example, used a library of 303 clinical isolates representing a diverse array of yeasts and found an agreement with standard methods in 84.8%.[18] In an additional 26 isolates the Biotyper returned a result that was confirmed as true by sequencing in 21. The Biotyper failed to make identification in an additional 20 isolates. Bader et al. used an even larger cohort of clinical yeast isolates (1192) to compare the performance of traditional methods with the Bruker Biotyper and the Vitek SARAMIS (research use only database). They found an overall agreement between the methods of 95.1% and noted not only that both MALDI methods were able to discriminate species that traditional methods could not, but that the savings in time and money were substantial.[19] More recently, Chao et al. analyzed a collection of 200 clinical yeast isolates with both the Biotyper and the Vitek MS.[20] They found that the Biotyper slightly outperformed the Vitek MS (92.5% vs 79.5% correctly identified to the species or complex level). This compared with rates of 89% and 74% for two common phenotypic methods (Phoenix 100 YBC and the Vitek 2 Yeast ID, respectively). This trend was also observed by Mancini et al., who used a collection of 197 clinical yeast isolates, which included 157 *Candida* or related species and 40 non-*Candida* species. They found that the Biotyper identified 89.8% of isolates, whereas the Vitek MS identified 84.3% using the standard commercially available database. Importantly, the Vitek MS had a much higher rate of misidentification (12.1% vs 1%).[21] In general, it has been found that a formic acid/ethanol

extraction method and lowered identity cutoffs (i.e., 1.7 on the Biotyper) are optimal for the analysis of yeasts.[22]

Although not an FDA-approved application, MALDI has tremendous promise for the identification of mycobacteria and is already the preferred identification method of choice for these organisms in some clinical laboratories.[5] Caveats include the need for enhanced sample preparation methods for efficient cell breakage for biosafety reasons and to ensure high-quality spectra.[3,23,24] Several studies have shown promising results. Using the Biotyper equipped with the mycobacterial library version 1.0 and the Vitek MS, 84.7% to 93.8% of isolates were identified correctly to the species level.[24,25] Bruker has subsequently released a new version of the mycobacterial library, version 3.0, containing 149 different species. It reportedly demonstrated high performance (>95% correct identification to species level) in a group of 1045 clinical samples.[26] Version 3.0 of the Biotyper mycobacterial library was also described by Rodriguez-Sanchez et al., who found 91.7% identification to species level on a group of 109 non-tuberculosis mycobacteria.[27]

There is a great deal of interest in the identification of fungi other than yeast by MALDI, also not an FDA-approved application. Concerns with this group include not only efficient cell lysis but also the need for higher-quality reference databases.[17] Few studies have assessed the Biotyper or Vitek MS platforms on fungal isolates using only the commercially available databases. Iriart et al. analyzed a group of 236 clinical isolates with the Vitek MS (192 yeast and 44 *Aspergillus*) and found that 93.2% of the isolates were correctly identified, including 81.8% of the *Aspergillus*.[28] This compared with 94.1% and 88.6%, respectively, by routine laboratory methods. When the authors limited the study to only those species that were present in the database, 100% of the *Aspergillus* isolates were correctly identified.[28] In another study the Vitek MS was able to correctly discriminate closely related *Aspergillus* species, *Aspergillus fumigatus* and *Aspergillus lentulus*.[29] Schulthess et al. did a prospective study on 200 isolates using the Biotyper/Filamentous Fungi Library 1.0. They achieved a 79% species and 83.5% genus level identification. Particularly poor performance was noted for *Mucor* and *Scopulariposis*.[30] Chen et al. evaluated 50 clinical mold isolates with the Biotyper and found that several were not identified or identified with a low score (<1.7).[31] Twenty-eight isolates of *Penicillium marneffei* were not identified due to the lack of reference spectra in the database; however, the rate of identification went up to 85.7% after a single *P. marneffei* spectrum was added to the database. This result is consistent with a number of studies indicating high performance of MALDI on fungal isolates using custom databases.[32-37]

Although the use of MALDI-TOF in the microbiology lab has been confined, for the most part, to cultured organisms, there have been several studies exploring the feasibility of its use in primary samples. For example, the use of MALDI directly on positive blood cultures is attractive for both its rapidity and the breadth of detection possible with an open system. Sample processing directly from blood is not trivial and not yet FDA approved, but each major platform has a dedicated process and the results are promising with high clinical accuracy (80% to 90%).[3,38,39] Other promising applications include direct analysis of urine and cerebrospinal fluid (CSF), although, as with blood, additional screening and processing steps are required.[3,40-43]

In summary, MALDI-TOF in the clinical microbiology lab has been developed to complement and in many cases replace the traditional biochemical diagnosis of bacteria and yeasts and can shorten turnaround time (TAT) by hours to days (depending on the organism in question).[5,7] Other benefits to MALDI in routine clinical microbiology include low costs of reagents and less production of waste materials (reduced consumables). There are a few shortcomings of this technology. For the most part, MALDI is limited to cultured isolates, so although it offers tremendous TAT advantages it is inherently limited by the time to growth of the organism. Sparse database coverage over certain phyla (e.g., filamentous fungi, some anaerobes) presents an issue in some cases, although commercially available libraries continue to be expanded. Another major caveat of MALDI is that it is not yet a practical method for assessing antibiotic resistance. Direct detection of resistance-conferring enzymes is not practical within the assay's limits of detection; however, some promising studies have been published looking at the ability of MALDI to detect breakdown products after incubation in the presence of antibiotic.[3] However, resistance testing is unlikely to be incorporated into a standard MALDI microbial identification platform in the near future.

Mass spectroscopy has been applied to human tissues, including FFPE, for a variety of purposes, but, to date, there is not a commercially available or routine method for using MS in the detection of organisms in FFPE.[44,45] With the advent of rapid and inexpensive nucleic acid methods for FFPE, this field largely lies dormant, although incredible potential for detection and other data extraction from FFPE through MS may be possible in the future.

Nucleic Acid–Based Techniques

For more than 30 years, a wide spectrum of assays based on the detection of nucleic acids has been developed for the diagnostics of infectious disease. They range from simple probes to qualitative and quantitative target amplification to sequencing. For example, the first nucleic acid probe-based assay was launched in 1985 to test for legionnaires' disease (Gen-Probe). Real-time platforms, such as the Smartcycler and the Lightcycler, have an established place in both detection and quantification of pathogens, although the major impact has mostly been in virology, where the nucleic acid–based techniques were quickly adopted to overcome the difficulties inherent in viral culture. For example, hepatitis C virus (HCV) and HIV viral loads are routine applications of these technologies. There have been a number of newer nucleic acid–based technologies that have had a tremendous impact on microbial diagnostics, many of which are also geared towards bacterial pathogens. These include highly sensitive probes for use in direct specimens, to alternative amplification methods, rapid assays of single targets, and multiplexed systems that allow for the detection of many organisms in one assay. Sequencing assays are becoming more commonplace, with targets ranging from 16S rRNA to whole genomes and even metagenomic studies looking at the make-up of complex microbial ecosystems within the human host. In this section the technologies behind several of the most important new assays will be briefly introduced, followed by a discussion of their application to clinical syndromes, such as respiratory and gastrointestinal (GI) disease, bloodstream infections, meningitis, and reproductive health/sexually transmitted disease.

Probe-Based Assays

Probe hybridization assays were one of the earliest diagnostic techniques using nucleic acids as a target. The most commonly used probes are marketed by Hologic Gen-Probe Inc. (Marlborough,

Massachusetts) and consist of single-stranded DNA probes tagged with acridinium esters that hybridize to rRNA. Tests are read in a luminometer. Because there is no amplification, probe-based assays generally suffer low sensitivity and are reserved for culture confirmation, where organisms are present in high numbers. AdvanDX (Woburn, Massachusetts) has produced a series of probe-based assays using peptide nucleic acid (PNA) probes tagged with fluorescent markers.[46] PNA probes have several advantages over traditional DNA probes, such as increased stability and ability to penetrate the bacterial cell wall. Fluorescent tags have the added advantage of allowing for multicolor detection and the creation of limited panels. These are rapid assays (less than 1 hour) and are mostly targeted towards rapid diagnostics of positive blood culture bottles. The AdvanDX systems (PNA fluorescence in situ hybridization [FISH] and QuickFISH) offer a variety of testing options, such as *Staphylococcus* (*Staphylococcus aureus* [SA] vs coagulase-negative staphylococci [CNS]), *Enterococcus* (*Enterococcus faecalis* vs *Enterococcus faecium* or other enterococci), and gram negatives (*E. coli* vs *Klebsiella pneumoniae*, vs *Pseudomonas aeruginosa*) that can be chosen based upon the Gram stain results.[47-50] PNA probes have been used for rapid identification of organisms, such as fungi, bacteria, and mycobacteria, in human tissues sections for fresh, frozen, and FFPE.[51-54] In situ hybridization (ISH) of Epstein Barr encoding region for confirmation of Epstein-Barr virus infection on FFPE is routinely used in cancer diagnostics, but few, if any, other common viral infections are confirmed this way, with IHC being preferred. Application of RNA-ISH to *Aspergillus* and *Candida* in FFPE showed less sensitivity than real-time PCR with sequencing (gold standard), although some FISH-positive, PCR-negative cases with obvious fungal elements were seen, suggesting refinements of this technique may be valuable for rapid identification of these common organisms, especially if mucormycosis is in the differential.[55]

A unique probe-based approached to nucleic acid detection is the RNA hybridization and digital counting technology offered by NanoString (Seattle, Washington). This method uses a capture probe (usually a specific 50-mer) and a reporter probe (a second, adjacent, specific 50-mer attached to a unique molecular color barcode) to bind up individual molecules of RNA in a sample, capture them on a solid state matrix, and use digital imaging to count the barcodes in the sample. The current version can measure up to 500 independent capture:reporter pairs. The technology can be applied to any RNA target (human or microbial), and the great advantage in infectious disease is the extremely low input quantities that can be detected without interference from other RNA. Because of the counting algorithm, with proper probe selection in a given mixture, accurate quantification of RNA is possible without amplification. One pitfall to this technology is that the presence of any given target in a disproportionate ratio to other targets will flood the digital analysis (i.e., consider hemoglobin relative to cytokines in peripheral blood). Relative to sequencing, the technology remains expensive, but the benefit of quantification of extremely limited sample with minimal processing makes this an attractive target for future test development. For microorganisms, broad panels using signature genes and/or focused panels using genes in antibiotic metabolism pathways can be created. Studies in a range of diseases have shown that RNA from any source, including FFPE, is sufficient for NanoString analysis,[56-58] In one study, quantitative expression of *Plasmodium falciparum* schizonts from human tissue (both frozen and FFPE) were measured and, using imputation, the entire expression profile for the organisms determined.[59]

Singleplex or Limited Target Assays

There are a variety of focused assays in the clinical microbiology lab and anatomic pathology samples that query one or a handful of genes by amplification of the target. The majority of these relies on the PCR and includes both qualitative and quantitative assays, such as those run on real-time PCR platforms (i.e., the Lightcycler [Roche Diagnostics, Indianapolis, Indiana] and the Smartcycler [Cepheid, Sunnyvale, California]). Target detection is typically by fluorescent probes, such as TaqMan.[60] Single-target PCR has been applied to fresh, frozen, and FFPE in anatomic pathology for a large range of microorganisms but largely through home-brew or in-house assays and/or performance by large specialty reference labs, due to the challenges of control tissue, validation, and complexity of interpretation.

Recent developments in this area are based on the same technology but are innovative in their convenience, detection method, and, in some instances, sensitivity. For example, using standard real-time PCR technology, FDA-approved assays, such as the Prodesse kits (Hologic Gen-Probe), provide convenient detection of small sets of respiratory viruses. The Xpert system (Cepheid) has had a large impact on the clinical lab due to its ease of use (sample-to-result platform), excellent performance, and rapid TAT (approximately 1 hour). Detection is by melt curve analysis rather than fluorescence. This platform offers a variety of cartridges (e.g., *C. difficile*, MRSA, MTB/RIF) focused on quick answers to major clinical decision points.[61-64] Newer assays from Cepheid are in development for FFPE, which will include microbiologic applications.

For novel detection methods and increased sensitivity, the T2MR platform (T2 Biosystems, Lexington, Massachusetts) is one of the most innovative technologies. It is a complete sample-to-result platform that relies on detection of pathogen-specific amplicons by hybridization with tagged superparamagnetic particles.[65] The T2Candida Panel (T2 Biosystems) is the only FDA-approved system that directly analyzes blood (without culturing) and has demonstrated impressive results in clinical trials (91% sensitivity with a 4.2-hour TAT).[66] T2 Biosystems is currently developing a bacterial panel as well, which is reported to target *Acinetobacter baumannii, E. faecium, K. pneumoniae, P. aeruginosa,* and SA.[67]

Although PCR remains the most common means of nucleic acid amplification, other technologies have been developed that address the presence of single targets. Transcription-mediated amplification (TMA) has been applied to mycobacteria, HCV, *E. coli*, and *Listeria*, where RNA is bound by a target oligo and then acted upon, in sequence, by reverse transcriptase (RT) (to produce cDNA), RNase H (to degrade RNA template), RT again to produce a double-stranded DNA promoter sequence, and then RNA polymerase to produce many copies of single-stranded RNA—then the process repeats itself. Another isothermal reaction occurs in loop-mediated isothermal amplification, which uses a group of four to six primers and *Bst* DNA polymerase, with the creation of complimentary sites to generate exponentially amplified loop structures. These assays have been developed for a wide range of targets and produce larger quantities of DNA than traditional PCR in a matter of minutes to hours. This technique has been applied to viruses, bacteria, protists, fungi, and mycobacteria in a point of care manner.[68-72] LAMP techniques are limited by the challenges of complex primer design, limited ability to multiplex, and common inhibitors of the enzymes in certain human samples, including blood. A last isothermal amplification is

accomplished by the introduction of helicase, which unwinds DNA, and single-stranded DNA binding proteins, which keep the templates separated. This is followed by primer hybridization and amplification to produce more single-stranded DNA and thus a continuous reaction. Assays to measure herpes simplex virus (HSV)-1 and HSV-2 using helicase have been developed with similar performance to traditional molecular methods and assays are available for *Bordetella*, group A *Streptococcus*, *Trichomonas*, and malaria.[73] Commonly used platforms that use these technologies include the Tigris and Panther systems by Gen-Probe, which are based on TMA, SmMIT-LAMP based on LAMP, and Ampli-Vue based on helicase. Use of these assays in FFPE tissue has shown some promise with improved performance over PCR for some organisms (such as HCV in TMA), equivalent performance to PCR (such as human papillomavirus [HPV] in head and neck cancers with LAMP), and detection of limited signatures (such as microcarcinomas in small biopsies with helicase assays).[74-76]

Multiplexed Systems

The ability to detect multiple organisms in primary samples using PCR-based panels has both provided clinicians with tremendous diagnostic opportunities and presented new challenges.[77] Major platforms include FilmArray (BioMérieux), Verigene (Nanosphere), xTAG/xMAP (Luminex, Austin, Texas), with technologies that range from real-time PCR to solid and liquid phase arrays. For a comprehensive listing of platforms see www.captodayonline.com/productguides. For an in-depth discussion of the major technologies see the articles included in the reference list.[78,79] These systems are generally geared towards syndromic testing, with panels directed towards respiratory, GI, and sexually transmitted diseases, as well as meningitis and bloodstream infections. After discussing the specific challenges of each of the major syndromes, this section will focus on commonly used FDA-approved panels that are currently available from a number of manufacturers and that scan for a wide array of microbes, encompassing viral, bacterial, and fungal organisms.

Panels aimed at respiratory pathogens are focused most heavily on viruses but also incorporate several bacterial agents.[80,81] Commonly covered organisms include *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Bordetella* species. *M. pneumoniae and C. pneumoniae*, both very common causes of community-acquired pneumonia, are particularly well-suited to nucleic acid amplification tests because they are difficult to culture.[82] Although less common, *Bordetella pertussis* is considered to be the most common vaccine-preventable disease in the United States, is increasing in incidence, and is also difficult to culture.[83] The "bread and butter" of respiratory panels (RPs) focus on viruses with influenza A (and B), adenovirus, and respiratory syncytial virus (RSV) being always including, along with a mixture of other coronavirus, enterovirus, metapneumovirus, and the parainfluenza group. These panels are extremely powerful from the clinician's point of view but also provide a larger range of diagnostics for respiratory viruses than can be found in most anatomic pathology labs, which are largely limited to the DNA viruses. Although not all of these viral diagnoses are chemotherapeutically actionable, confirmation of a viral cause is valuable for prognosis and resource allocation.

Diarrheal diseases are a global public health concern. Although these diseases are a common cause of illness and hospitalization in the United States, they impose an enormous toll in the developing world, particularly on children. The most common bacterial pathogens include *E. coli* and *Shigella, Salmonella, Campylobacter,* and *Clostridium perfringens*. Typical viral pathogens included in these panels are adenovirus, norovirus, and rotavirus, representing the three most common symptomatic viral diarrheas. Diagnosis is moving more and more towards multiplex panels that cover major bacterial pathogens, as well as some parasitic and viral agents.[84-86] In addition to standard agents of infectious diarrhea, there are special considerations when testing for disease caused by *C. difficile*. These panels are, again, extremely valuable because the tools to diagnose these disease in anatomic pathology are often very limited and too invasive for most patient situations.

Parasitic enteric infections represent a major challenge in developing countries but remain rare in countries in which most new technologies are being developed. Despite this disparate situation, efforts have been made to approach parasitic infections, and thus the inherent challenges have become apparent. Extraction from stool for suspected parasite infections requires that protists (ciliates, ameoba, flagellates, coccidia), helminth larvae (nematodes), and helminth eggs (nematode, trematode, and cestode) be lysed sufficiently to release DNA efficiently. This challenge has led to eight or more different extraction protocols with a range of preparation requirements, including spin columns, automated magnetic bead assays, and "bead beating" methods. Molecular targets for subsequent detection assays as specific single targets across this range of organisms has included 18S rDNA, ITS, COX 1, pOV-46, 5.8S rDNA, Segment A, COWP, actin, HDP2, DL1, and 28S rDNA.[87] In commercially available, multiplex PCR assays, the majority of the platforms target only *Cryptosporidium, Giardia, Entamoeba histolytica,* and, rarely, *Cyclospora*. Because these first three represent 80% to 99% of parasites isolated by all methods from stool in developed nations, additions of other much less common pathogens is not common. Inclusion of nonpathogenic ameba is common in these assays but of questionable value.

Genitourinary samples (fluid from cervical samples, urethral swabs) are another sample that benefits from multiplex testing as public health screening of sexually transmitted diseases are extremely valuable for individual patient diagnosis, as well as epidemiologic survey, especially with the inclusion of HIV. *Neisseria gonorrhea, Chlamydia trachomatis,* and HPV in the Hologic Panther system can be performed on a single sample, and this system also can test for *Trichomonas*. The FilmArray from BioFire (Salt Lake City, Utah) detects *Chlamydia, Neisseria,* syphilis, *Trichomonas, Mycoplasma/Ureaplasma*, herpes simplex, and chancroid in a single sample.[88]

Although relatively uncommon, meningitis and encephalitis (both nosocomial and community-acquired) carry high morbidity and mortality.[89-91] Time is of the essence when diagnosing a causative agent, particularly when trying to determine whether it is a bacterial pathogen that needs immediate antibiotic coverage along with herpes simplex, which needs immediate antivirals. Common bacterial pathogens include *S. pneumoniae, Streptococcus agalactiae, Neisseria meningitidis, Haemophilus influenzae,* and *Listeria monocytogenes*, although there are also several other, less frequently isolated, pathogens. Viral pathogens in these panels include the primary actionable viruses (*Cytomegalovirus*, herpes simplex, varicella zoster virus), as well as other common viruses (*Enterovirus, Parechovirus*, and human herpesvirus (HHV)-6). Platforms also may include *Cryptococcus* species of fungi, which is very valuable in immunosuppressed populations.[92] Current diagnostics are heavily focused on CSF biochemical profile (glucose, protein, lactate), cell count, and Gram stain. The latter is the most specific, but the limit of detection is approximately 10(4)

organisms per milliliter.[90] There is a great need for rapid molecular diagnostics in this area. In anatomic pathology, sample with culture and/or morphology with special stains and/or IHC can resolve many of these infections; however, optimal patient care and best outcomes occur in those patients who can be diagnosed early with these panels and not require an invasive biopsy.

Sepsis continues to be a tremendous cause of mortality, ranking as one of the top 10 causes of death in the United States.[93] Organisms commonly associated with bloodstream infections include SA, *E. coli, Enterococcus* spp., *K. pneumoniae,* CNS, *P. aeruginosa, Candida albicans, E. cloacae,* and *Serratia marcescens.*[94] The current gold standard for diagnosis is culture, with several automated systems (such as the BacT/Alert [BioMérieux Inc.]) available to clinical laboratories.[95] Although these systems are quite sensitive, approximately only one-third of patients with sepsis have positive cultures.[96] Some of this lack of sensitivity may be due to pretreatment with antibiotics, and some of it may be due to the low numbers of organisms present in many clinically significant bacteremias.[97] In addition to sensitivity, speed is also a major issue. The rapid diagnosis of bloodstream infections is critical for patient outcomes, with estimates of a 7.6% decrease in survival for every hour that appropriate antimicrobials are delayed after the onset of hypotension.[98] The need for sensitive, fast detection, ideally accompanied by some assessment of antibiotic resistance determinants, has inspired a number of new technologies geared towards improved speed and sensitivity of blood culture diagnostics. Several of these have been reviewed.[99-101]

Platforms and Technologies

FilmArray (BioFire/BioMérieux)

The FilmArray system consists of nested PCR followed by high-resolution melt curve analysis.[102] All steps of the assay, from cell lysis to the final analysis, take place within a pouch containing freeze-dried reagents that can be stored at room temperature. FilmArray has a short TAT of approximately 1 hour. Disadvantages of the system include the relatively high price of the pouches and restriction of the platform to one test at a time. Laboratories must purchase multiple FilmArray platforms if they desire to run tests in parallel.

The FilmArray panel was the first FDA-approved RP to include bacterial pathogens, covering *B. pertussis, C. pneumoniae,* and *M. pneumoniae*, along with 18 common respiratory viruses.[102] For GI testing, the FilmArray is the most comprehensive of the current FDA-approved panels, covering an array of 22 bacteria, viral, and parasitic targets, including the common agents listed above, as well as *Plesiomonas shigelloides, Yersinia enterocolitica*, and several species of *Vibrio.* It can also differentiate between enteroaggregative, enteropathogenic, enterotoxigenic, Shiga toxin-producing, and enteroinvasive *E. coli* (EIEC). Studies have shown a sensitivity of 95.9% to 100% and a specificity of 96.6% to 100% for bacterial pathogens.[103,104] In many cases the FilmArray detected pathogens in samples that were negative and was far more likely to diagnose mixed infections than standard techniques.[104,105] For viral pathogens the FilmArray GI panel has shown value in the younger age groups (patients younger than 12 years) for most tested pathogens (sensitivity: 95.5% to 100%; specificity: 99.1% to 99.9%), whereas Norovirus appears to be valuable across all age groups (sensitivity: 94.5%; specificity: 98.8%).[103] Performance for parasitic pathogens in this panel is equally high for *Cryptosporidium, Cyclospora,* and *Giardia* (sensitivity: 100%; specificity: 99.5% to 100%), but, as has been common with many panels and individual tests, laboratories have difficulty obtaining natural clinical cases of *E. histolytica*.[103]

Currently, the only FDA-approved multiplex assay for agents of meningitis and encephalitis is the FilmArray meningitis panel. It covers 14 pathogens, including the following bacteria: *E. coli K1, H. influenza, L. monocytogenes, N. meningitides, S. agalactiae,* and *S. pneumoniae.* Although the panel was only recently approved by the FDA (October 2015), there are a few reports of its performance. The pre-FDA evaluation was conducted both on archived samples and prospectively on a multicenter collection of 1560 samples of CSF. Among 235 archived samples (32 with bacteria), the percent positive and negative agreement was 100% for bacterial targets. Among the 1560 prospective samples, there were only eight with bacterial pathogens (none with *L. monocytogenes* or *N. meningitides*). Of those that were present, the FilmArray ME panel did not identify the only *S. agalactiae*. The archived arm of the evaluation included two *S. agalactiae* samples, both of which were correctly identified.[106] Since FDA approval, one US study has been published on the performance of the panel in several Texas medical centers. In that study of 48 patients with community-acquired meningitis and a negative Gram stain, the FilmArray detected two samples with bacterial pathogens, both *S. pneumoniae*. Culture detected only one of these, although the other one was positive for streptococcal urinary antigen.[107]

Finally, the FilmArray blood culture identification (BCID) panel tests for an array of 19 bacterial targets, including: *Enterococcus, L. monocytogenes,* SA, *Streptococcus* (multiple), *A. baumannii, P. aeruginosa, E. coli,* and *K. pneumoniae*. It also tests for five species of *Candida* and three bacterial resistance genes: *mecA, vanA/B,* and *kpc*. It has performed well on both monomicrobial and polymicrobial specimens in several clinical studies.[108,109]

BD Max (BD Diagnostics)

The BD Max is a sample-to-result real-time PCR–based platform. Amplicons are detected via fluorescent probes. A distinguishing feature is the capability to run laboratory-developed tests alongside FDA-approved panels. Current FDA-approved assays include GBS (for group B Streptococci), CT/GC/TV (for vaginitis), MRSA XT (for extended MRSA coverage), Cdiff (for C. difficile), and StaffSR (for Staphylococcus aureas and MRSA). The bacterial panel includes *Salmonella* spp., *Campylobacter* spp. (*jejuni*/*coli*), Shigellosis disease-causing agents (*Shigella* spp. and EIEC), as well as Shiga-toxin producing *E. coli*. It has demonstrated high sensitivity for the organisms in its library in several studies.[110-112] The parasite panel includes the three most common protist parasites in humans in developed countries, including *Giardia lamblia, Cryptosporidium,* and *E. histolytica*, but has the difficult challenge of very low population prevalence relative to the bacterial assays, making cost a challenge over other methods for uptake.[113]

Luminex

Verigene

Verigene is another widely used multiplex PCR platform incorporating PCR of multiple viral and bacterial targets followed by hybridization to a solid-phase microarray. Detection occurs via sandwich hybridization with another analyte-specific probe bound to a gold nanoparticle. The signal is amplified via deposition of colloidal silver that is then detected by light diffraction. Verigene

has a TAT of approximately 2 hours and has the advantage of offering flexible pricing by which the laboratory is charged only for the tests they request, instead of the entire panel. Disadvantages include the need to select the appropriate cartridge based on Gram stain results for bloodstream diagnostics. Respiratory, GI, and bloodstream panels are available.

The Verigene platform can detect 13 respiratory viruses with high sensitivity (including multiple types of influenza, parainfluenza) and three *Bordetella* species (*B. pertussis, Bordetella parapertussis/Bordetella bronchiseptica,* and *Bordetella holmesii*),[114,115] although one advantage of this system is the flexibility to test limited panels based on a given population. The GI panel is somewhat more limited than the FilmArray, covering 22 targets, including 13 bacteria, 5 viruses, and 4 parasites. Bacteria include the *Campylobacter* group, *Salmonella* spp., *Shigella* spp., *Vibrio* group, and *Y. enterocolitica*, as well as Shiga toxin. In one study of 725 stool samples the Verigene demonstrated a 98.5% agreement rate with culture.[116] For the diagnosis of bloodstream infections, the Verigene system has separate cartridges for gram-positive and gram-negative organisms, necessitating an accurate Gram stain to pick the appropriate test and making polymicrobial testing a bit more complicated. Each panel assesses a few extra organisms than the FilmArray single panel. In addition, the gram-negative panel queries several more resistance targets than the FilmArray, including CTX-M, IMP, NDM, OXA, and VIM.[117] Verigene in RPs has excellent performance for the influenza viruses (A and B), including the ability to subtype viruses with perfect agreement with gold standard methods (molecular); however, mixed infections with non-influenza viruses dampen performance.[118] Head-to-head comparisons of the limited Verigene panel (influenza A and B, RSV) with FilmArray RP showed equivalent detection of the three major pathogens at a slightly reduced cost for the Verigene; thus analysis of the clinical population and the desires of the local clinical team for reported virus and actionable diagnoses may help in choosing between these technologies.[114]

xTAG

The xTAG assay system from Luminex is a liquid array based on multiplex PCR followed by amplicon extension using a target-specific primer. The extension step incorporates a hybridization tag onto the amplicon, along with a biotin label. Elongated, tagged amplicons are then incubated with polystyrene microbeads of various fluorescent profiles, each coated with a specific anti-tag sequence. Bead/amplicon pairs are then stained with another streptavidin-linked fluorophore. The beads are analyzed by a system of two lasers on the xMAP instrument. The first determines which beads are bound to amplicons by way of the streptavidin-fluorophore. The second laser indicates which specific target was amplified by way of the unique fluorescence of that bound bead.[119] Although the Luminex platforms generally have the potential to detect the greatest number of targets, drawbacks include the need for a separate nucleic acid extraction step and longer TAT (4 hours or longer) then the other multiplex platforms.

There are two xTAG platforms focused on respiratory testing: the original xTAG platform, restricted to viral pathogens, and the next-generation system (NxTAG), which included several bacterial pathogens as well, including *Legionella pneumophila*.[120] The xTAG Gastrointestinal Pathogens Panel (GPP) tests for nine bacterial, three viral, and three parasitic targets. Among bacterial loci, the Luminex xTAG covers *Campylobacter*, *C. difficile* toxin A/B, *E. coli* O157, enterotoxigenic *E. coli* (ETEC) LT/ST, Shiga toxin-producing *E. coli* (STEC) stx1/stx2, *Salmonella, Shigella, Vibrio cholerae,* and *Y. enterocolitica*. In one study comparing the xTAG with the FilmArray and conventional techniques, the two platforms behaved comparably, with the xTAG slightly trailing the FilmArray in the diagnosis of mixed infections (27% for FilmArray vs 14.1% for xTAG vs 8.3% for routine testing).[104] The performance of xTAG for viral pathogens when compared with realtime RT-PCR and multiplex realtime RT-PCR, showed a wide variation in sensitivity across viruses for xTAG (20% to 100%), with performance for RSV-A, RSV-B, H1N1, and influenza B being 13.3%, 47.3%, 54.2%, and 20%, respectively.[121] In head-to-head comparisons of FilmArray, eSensor RVP, and xTAG, the three methods were essentially equivalent, with xTAG having the best performance for influenza A.[122]

Some of the caveats of the xTAG system are addressed with newer Luminex platforms, such as the Aries, which is a sample-to-result platform for testing primary clinical specimens. The assay consists of real-time PCR using the Luminex MultiCode technology. This system uses primers that incorporate isobases, isoC and isoG, which, when incorporated, can pair only with each other. One isobase is tagged with a fluorescent reporter, and the other is tagged with a quencher. Upon the successful generation of product, both isobases are incorporated and fluorescence decreases. A subsequent melt curve analysis is performed, and fluorescence is restored. Unlike the xTAG technology, MultiCode can be run on either commercially available real-time PCR systems or within the sample-to-result Aries platform from BD. There is only one FDA-approved test for this panel, an HSV-1 and HSV-2 panel for use in testing cutaneous and mucocutaneous lesions.

Sequencing of Ribosomal RNA for Pathogen Identification

Although all of the nucleic acid–based systems described above demonstrate high levels of sensitivity and specificity, they are all inherently limited to a select group of organisms based on the probe/primer sets they contain. Open assays using broad-range primers that target nearly universal sequences are nearly all based on the 1977 discovery by Carl Woese that the 16S rRNA subunit could be used to determine the phylogenetic structure of the domains of life.[123] Although this idea represented a fundamental shift in bacteriology, it took many years for it to be widely accepted, and even longer for the use of 16S rRNA sequencing (16Sseq) to be used as a standard means for identifying bacteria in the clinical laboratory.[124] However, since 2000, 16Sseq has become the gold standard for determining bacterial species, and its analysis has led to a great deal of restructuring and renaming among phyla.[125] Briefly, the 16S gene encodes a portion of the small ribosomal (30S) subunit and is composed of nine variable regions alternating with nine conserved regions (Fig. 6.2). The gene is sequenced using primers that bind to the highly conserved regions and then compared with a reference database to identify an isolate.

The choice of database is typically the most challenging part of the assay. There are a number of databases that can be used to identify the sequence, ranging from large, public, uncurated or partially curated databases, such as Genbank and the Ribosome Database Project (RDP, University of Michigan) to commercial, highly curated collections with a much more limited scope. Identification algorithms include Ripseq (Pathogenomix, Santa Cruz, California), the Integrated Database Network System

(IDNS) (SmartGene, Raleigh, North Carolina), and MicroSEQ (Applied Biosystems, Thermo-Fisher, Waltham, Massachusetts). The first two products are solely bioinformatic reference solutions. The last includes both reagents and access to a curated database. None of these products are FDA approved. The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for the identification of organisms by nucleic acid sequencing in which the recommended percentage identity to make a match and guidance for organisms requiring additional targets (e.g., *rpoB, hsp65*) for accurate speciation are laid forth.[126]

The use of 16Sseq in the clinical microbiology laboratory has been shown to be particularly useful in such applications as the identification of unusual and/or slow-growing organisms and the identification of organisms from specimens in which culture is not possible or has failed, such as in specimens from antibiotic-treated patients and fixed specimens.[127-130] For example, the identification of mycobacterial species identified by acid-fast staining of FFPE is a commonly used application of 16S sequencing, although additional targets, such as IS6110 and *hsp65*, are often needed to differentiate species.[131,132] Specimen types most successfully analyzed by direct 16Sseq include heart valves, CSF, and synovial fluid.[133-138]

Fungal pathogens are also identified using rRNA sequencing, although it is a less established practice than it is for bacterial targets. The fungal rRNA 18S corresponds to bacterial 16S, although it has fewer hypervariable regions and is generally not as useful for isolate discrimination.[139] The most common regions for fungal identification are the ITS regions 1 and 2, as well as the D1 and D2 hypervariable regions of the 28S rRNA large subunit component (Fig. 6.2).[139-141] When using these targets to identify an isolate, criteria required for percentage identity and percentage difference between competing matches are not as clear for fungi as they are for bacteria.[126]

The lack of high-quality databases has been a major difficulty in fungal sequencing, with estimates of up to 20% of the entries in GenBank being incorrectly identified down to species level.[142] To address this, several of the private databases that are available for bacterial sequencing also include curated or partially curated fungal databases (IDNS and RipSeq).[143] The MicroSEQ system also includes a fungal component, but it is limited to the D2 region of 28S. Recently, several public databases have become available with high-quality, curated content. Databases that are most focused on species of medical importance include the International Society for Human and Animal Mycology (ISHAM) ITS database contains 3200 sequences representing 524 human/animal pathogenic fungal species and is a major resource for clinical laboratories.[144] It can be searched via a web-based platform (its.mycologylab.org). The National Center for Biotechnology Information RefSeq ITS database contains 3060 sequences representing 270 families from 39 classes. The smaller 28S RefSeq database contains approximately 500, representing greater than 100 families from 21 classes.[145] This technique is particularly promising for identifying fungi from fixed tissue.[55,146,147]

Important caveats of rRNA sequencing include high cost and complexity, issues with uncurated databases (described above), and the potential for false positives. This is particularly the case when broad-range sequencing is applied to primary specimens to "fish" for an infectious agent in the absence of any corroborating culture or gram stain. Furthermore, rRNA should not be used to determine whether an infection has "cleared," due to the potential for lingering nucleic acid even in a successfully treated infection. Analysis should be restricted to normally sterile sites for tests using Sanger sequencing, which is generally unable to assess polymicrobial infections, although some analysis software packages have algorithms to deconvolute traces of 16Sseq from two or three different organisms.[148]

Next-Generation Sequencing

NGS is a high-throughput method that generally refers to a massively parallel process that generates tremendous quantities of raw sequence reads. There are a number of commercially available platforms that vary in the quantity, quality, and length of the reads that they produce. The majority of these include a step for clonal amplification of the template and produce short (50 to 400 bp) reads. For excellent reviews see articles provided in the reference list.[149,150] The advent of NGS was a groundbreaking event in clinical medicine and is a key component of initiatives for "precision medicine" for cancer. Beyond human genomic analysis, NGS has also had a tremendous influence on clinical microbiology that extends from infectious disease testing and epidemiology to analysis of the human microbiome in health, disease, and intervention.[151-153]

Within the diagnostic clinical microbiology laboratory, NGS of microbial whole genomes has been applied as an alternative to traditional methods, such as pulsed-field gel electrophoresis, for clonality analysis in outbreak tracking.[154] The refinement made possible with total chromosomal single-nucleotide polymorphism analysis, for example, allowed one group to assess the spread of variants of a colonizing population of carbapenem-resistant *K. pneumoniae* from one patient to several others in multiple transmission events. These variants were differentiated from each other by fewer than a dozen nucleotides.[155] Other applications that are becoming more and more routine include the characterization of unusual pathogens, the tracking of antibiotic resistance genes, and even the characterization of the plasmids that carry them through horizontal gene transfer.[156-159] Although not yet routine, NGS has been used to diagnose infections directly from primary samples in cases in which all conventional techniques have failed.[160] In one report, after 4 months of progressive neurologic symptoms and a thorough but unrevealing infectious disease workup, NGS was finally able to diagnose a case of neuroleptospirosis directly from the CSF of a 14-year-old boy.[161]

In addition to the detection and characterization of pathogens, NGS has also proven to be an invaluable tool for the characterization of the human microbiome. The influence of the microbiome on human health and disease has become progressively more established. Examples are many and include the development of obesity, the metabolism of therapeutic drugs, and the development of inflammatory bowel disease.[162-164] The two most common ways of assessing the microbiome are by 16S rRNA or shotgun metagenomics.

NGS 16S analysis entails sequencing one portion of 16S rRNA from a mixed community (e.g., stool sample) with subsequent clustering of sequences either against a reference dataset or into operational taxonomic units (OTUs). It gives a picture of taxonomic diversity in a sample and allows for the analysis of changing taxonomic composition in the face of various perturbations and interventions, such as antibiotic administration. Shotgun metagenomics refers to the sequencing of total DNA from a mixed community. This technique allows for more refined organism identification and an analysis of biochemical pathways, resistance genes, etc. but requires a greater amount of raw sequence.[165,166]

In FFPE in which an organism has been seen but not identified (such as bacteria, fungal elements, viral cytopathic or necrotic effect, or unidentified parasitic elements), NGS has enormous potential to rectify theses diagnoses but is currently limited by cost per sample and the enormous amounts of raw human data in comparison with the relatively small amount of the organism's sequence. As costs fall for sequencing and rapid bioinformatic methods for elucidating causative sequence emerge, NGS will become a primary tool for confirmation of microbial infections.

One last technology to consider is the hybrid system, PLEX-ID by Abbott, which combines a PCR step to amplify through a set of primers (PLEX-ID Viral IC, PLEX-ID BAC Spectrum BC, or PLEX-ID Flu) a broad range of organisms and then detects the PCR products through mass spectroscopy. This approach combines amplification of many sample types, including body fluids, tissue, FFPE, culture isolates, and blood, with a highly sensitive detection system to make subtle species determinations across organism types, including bacterial, fungal, viral, and some parasitic.[167-170] As the database of spectra improves and the multiplex primers improve, this system will gain popularity, although it is still limited by initial organism burden and procedure time (up to 8 hours).

Conclusion

The microbiology laboratory remains the most important companion for anatomic pathologists who have a potentially infectious diagnosis under the microscope. Anecdotal and small studies have demonstrated that the yield from molecular tests that are performed blindly (without visualization) versus with pathologist's review is miniscule and extremely expensive. Therefore anatomic pathologists and their microbiologic colleagues must have a constant line of communication from the moment the specimen is collected until final reporting to ensure interpretation of all modalities is maximally beneficial to patient care. This dialogue should also include discussions of molecular modalities that are applied to clinical and anatomic pathology specimens and changes, advantages, pitfalls, and benefits of those modalities in an ever-evolving manner. As with any approach to medicine, evidence-based use of the most efficient and inexpensive method that produces the highest-impact result for the patient is the constant goal.

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7

Ear, Nose, and Throat Infections

MATTHEW M. JOHNSON

Bacterial Rhinosinusitis
Otitis Media
Tonsillitis
Peritonsillar Abscess
Rhinoscleroma
Actinomycosis
Botryomycosis
Syphilis
Tuberculosis
Leprosy
Oral Candidosis
Fungal Rhinosinusitis
Paracoccidioidomycosis
Sporotrichosis
Blastomycosis
Coccidioidomycosis
Cryptococcosis
Histoplasmosis
Rhinosporidiosis
Mucocutaneous Leishmaniasis
Epstein-Barr Virus
Herpes Simplex Virus
Human Papillomavirus
Human Immunodeficiency Virus
Mumps

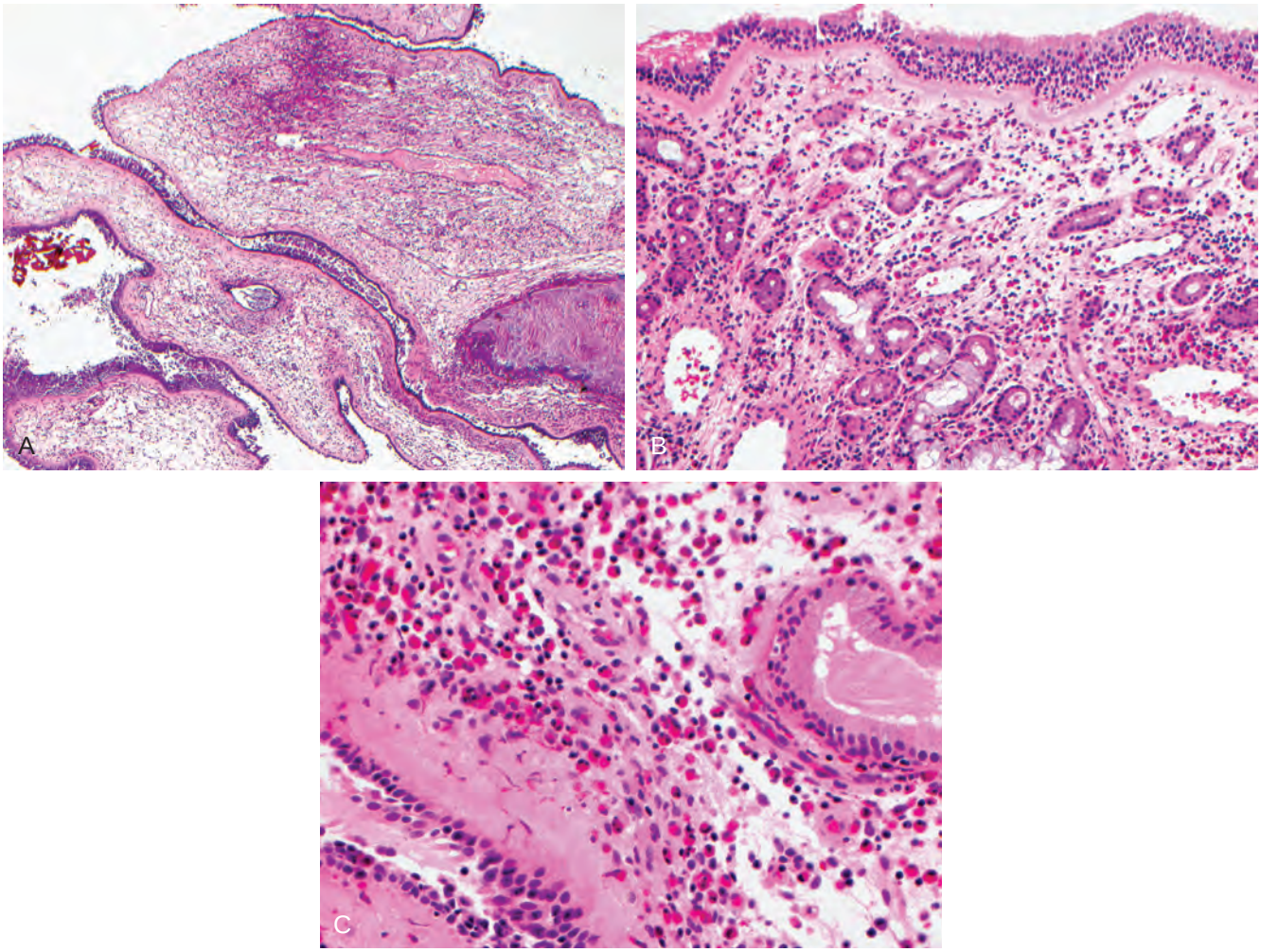
The head and neck area is arguably the most complex area of the human body; with its many physical connections to the outside environment, it plays host to myriad infectious processes. The aim of this chapter is to provide the surgical pathologist with important information that will aid in establishing an accurate diagnosis of infection. Because many of the infectious processes affecting the head and neck involve multiple sites, the chapter is organized around causative organisms rather than specific locations in the head and neck.

Bacterial Rhinosinusitis

Acute bacterial rhinosinusitis is an uncommon complication of acute viral rhinosinusitis, and it is caused by a variety of organisms, including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus,* streptococci, and various anaerobes. Signs and symptoms include purulent nasal discharge, unilateral facial pain and tenderness, and worsening symptoms, often after an initial improvement.[1] The rare surgical specimen from a patient with acute bacterial rhinosinusitis demonstrates nonspecific findings, including congestion, edema, and subepithelial mixed inflammation showing mainly neutrophils, with chronic inflammation and fibrosis being absent.

By definition, chronic rhinosinusitis persists for at least 12 weeks despite therapy and manifests with mucopurulent drainage, nasal obstruction, facial pain or pressure, and decreased sense of smell.[2] Chronic rhinosinusitis can be divided into three clinicopathologic syndromes: chronic rhinosinusitis with polyposis or without polyposis, and allergic fungal rhinosinusitis.[3] The pathophysiology of chronic rhinosinusitis is complex and incompletely understood, but bacteria contribute to all forms of chronic rhinosinusitis through failed resolution of acute infection, by the formation of biofilms on the sinus mucosa, or by the stimulation of enterotoxin-specific immunoglobulin E (IgE) antibodies, as in chronic rhinosinusitis with nasal polyposis and *S. aureus* colonization.[4,5] In contrast to acute bacterial rhinosinusitis, surgical specimens from patients with chronic rhinosinusitis are commonly seen, and histologic evaluation reveals a wide range of pathologic changes.

Gross examination may show polypoid sinonasal mucosa with varying amounts of mucus and fragments of swollen or scarred sinonasal mucosa admixed with bone fragments. The inflammatory response includes predominantly lymphocytes and plasma cells, with smaller numbers of eosinophils and neutrophils (Fig. 7.1). A predominance of eosinophils should prompt consideration of allergic fungal rhinosinusitis, although this may also be seen as a component of chronic rhinosinusitis with or without polyposis. Depending on chronicity, stromal fibrosis may be sparse or marked, with hyperplasia of seromucinous glands. The respiratory mucosa varies from attenuated to hyperplastic, particularly in polypoid fragments, which typically show stromal edema, basement membrane thickening, inflammation, and fibrosis. Bone fragments may appear reactive and sclerotic or unremarkable.



• **Figure 7.1** Chronic rhinosinusitis. **A,** Polypoid edematous respiratory mucosa (×40). **B,** Submucosal mixed inflammation including lymphocytes, plasma cells, and eosinophils (×200). **C,** Prominent eosinophils should prompt consideration of fungal rhinosinusitis. Gomori methenamine silver stain was negative in this case. Note thickened basement of respiratory epithelium (×400).

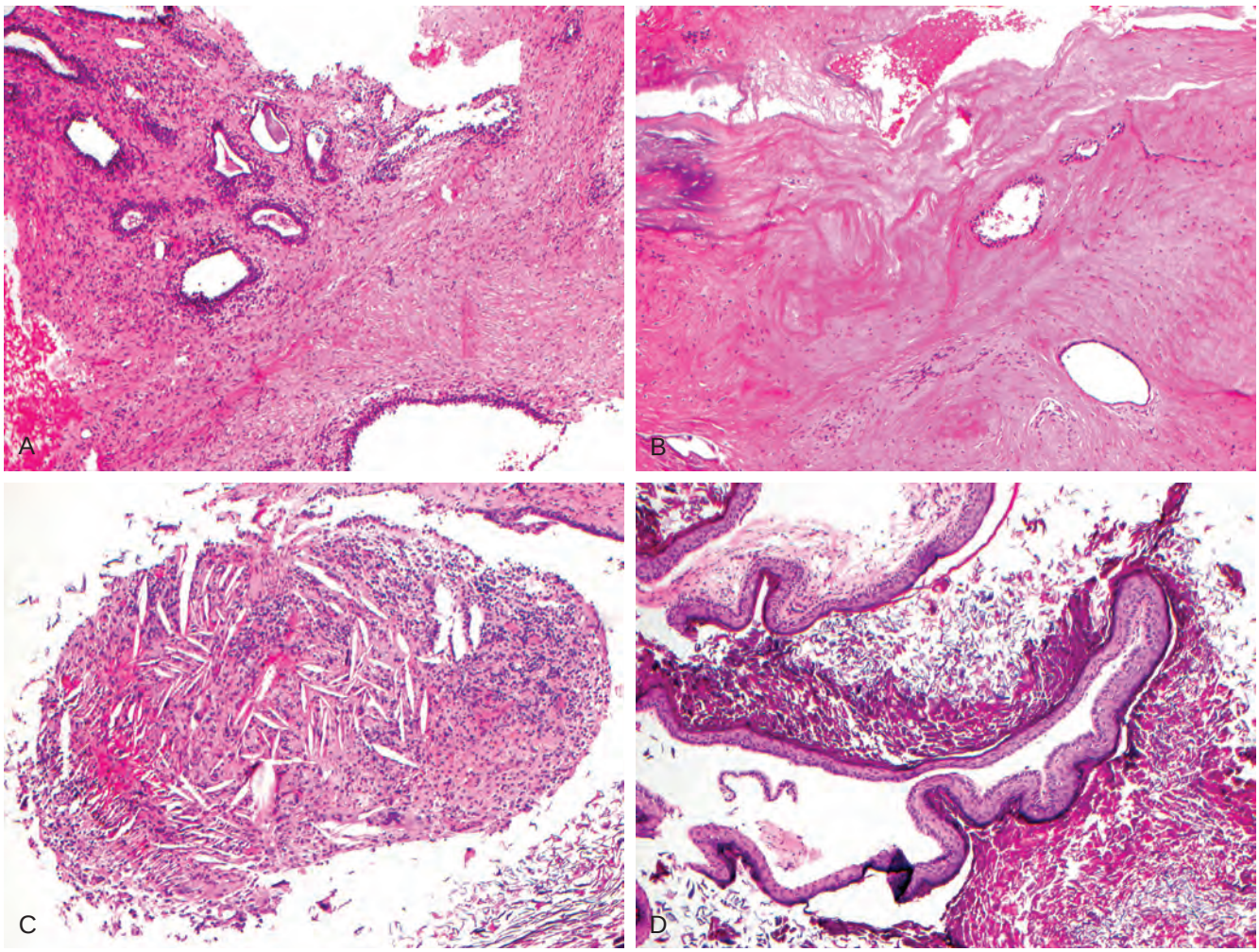
Otitis Media

Otitis media, infection of the middle ear, may be acute or chronic. Acute otitis media is the most common disease of childhood and is usually caused by bacteria, such as *S. pneumoniae, H. influenzae,* and *M. catarrhalis*.[6] The most common presenting symptom is earache, although patients may also demonstrate sore throat, night restlessness, or fever.[7] Examination typically reveals a bulging opaque tympanic membrane with decreased mobility. Recurrent infections lead to chronic otitis media, which may eventually require myringotomy and tympanostomy tube placement.[8]

The histologic findings in acute and chronic otitis media are nonspecific, and pathologists do not generally evaluate specimens from patients with acute otitis media. In patients with chronic otitis media, specimens from corrective surgery may reveal connective tissue with a mixed chronic inflammatory infiltrate, granulation tissue, metaplastic glands, and fibrosis, as well as evidence of tympanosclerosis in the form of hyalinization, calcification, or ossification of the fibrous tissues (Fig. 7.2). Polypoid granulation tissue with mixed chronic inflammation and a respiratory-type or metaplastic squamous surface lining is often seen, constituting the otic or aural polyp. A giant cell reaction to cholesterol (cholesterol granuloma) is a common feature of chronic otitis. Occasionally an associated cholesteatoma is present, characterized by a cyst lined by keratinizing squamous epithelium, containing abundant acellular keratin debris, and surrounded by fibrosis, granulation tissue, and a mixed inflammatory infiltrate, often with giant cells.

Tonsillitis

Tonsillitis is a common disease of the head and neck, and it can be acute or chronic, with a variety of bacteria and viruses as the usual etiologic culprits. Acute pharyngotonsillitis is not usually treated with surgery. It is most often attributable to infection with a respiratory virus, such as adenovirus, influenza virus, parainfluenza virus, rhinovirus, or respiratory syncytial virus, but in 30% to 40% of cases a bacterial infection is the cause, most commonly group A β-hemolytic streptococci.[9] Recurrent infection results in chronic tonsillitis, often with tonsillar enlargement that can cause obstruction. One study identified *S. aureus* (30.3%), *H. influenzae* (15.5%), and *Streptococcus pyogenes* (14.4%) as the most common isolates in chronic tonsillitis, and *H. influenzae* (31.4%), *S. pyogenes* (24.2%), *S. aureus* (22.9%), and *S. pneumoniae* (12.6%) as the most common isolates in tonsillar hypertrophy.[10]



• **Figure 7.2** Chronic otitis media. **A,** Fibrosis, chronic inflammation, and scattered respiratory-type, mucosa-lined tubular glands (×100). **B,** Tympanosclerosis, characterized by hyalinized fibrous tissue with focal calcification and several adjacent metaplastic glandular structures with surrounding fibrosis (×100). **C,** Cholesterol granuloma, with lymphoplasmacytic and granulomatous inflammation surrounding cholesterol clefts (×100). **D,** Cholesteatoma, demonstrating abundant keratin debris, keratinizing squamous epithelium, and subjacent fibrosis (×100).

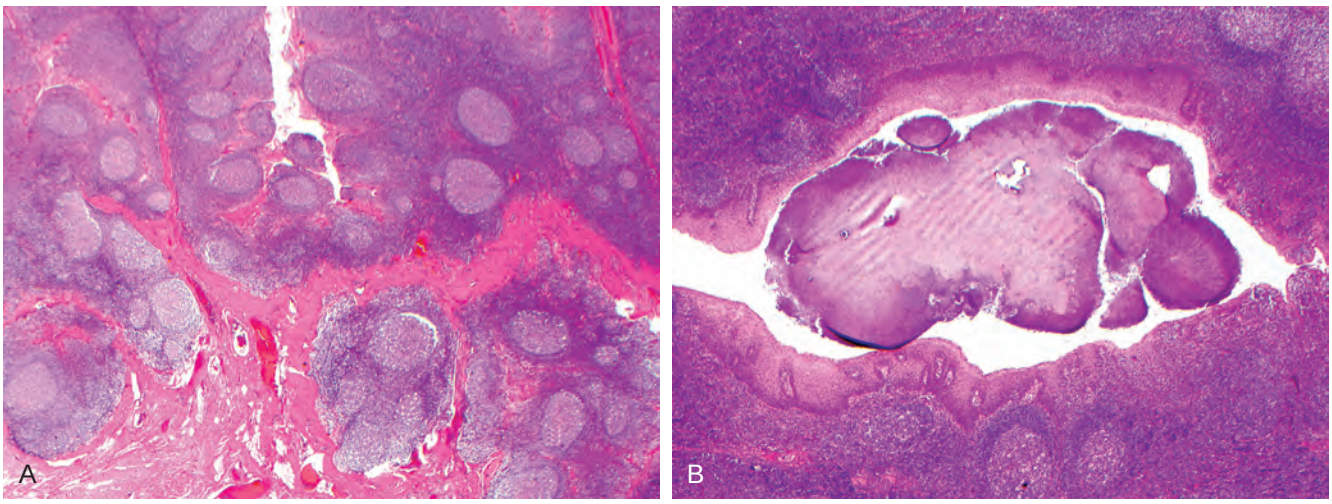
For the most part, unless there is clinical concern for malignancy, adenoids and tonsils removed for chronic tonsillitis or hypertrophy are not processed for histologic examination.[11] Microscopically, one sees reactive lymphoid hyperplasia, often with associated fibrosis, accentuating the interlobular fibrous septae (Fig. 7.3).[12] Acute inflammation is usually absent, and occasionally the commensal "sulfur granules" of *Actinomyces* can be identified between crypts.[13] Epstein-Barr virus (EBV) and herpes simplex virus (HSV) can manifest particular histologic findings in the tonsil that are identical to those seen in lymph nodes, as discussed later in this chapter and described in detail in Chapter 12.

Peritonsillar Abscess

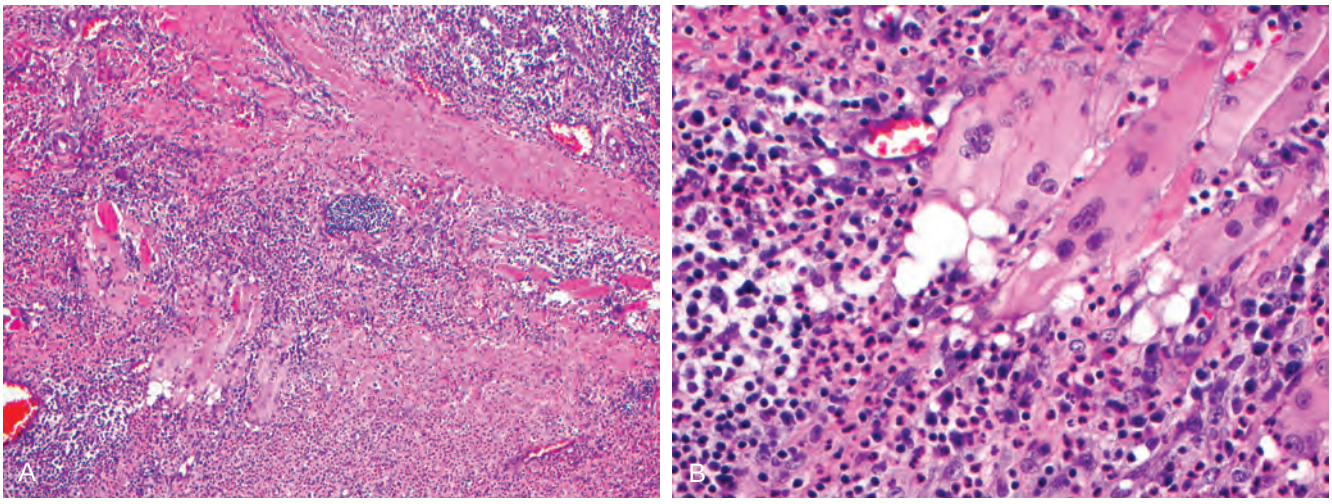
Peritonsillar abscess, or quinsy, is the most common deep infection of the head and neck. It occurs as a complication of tonsillitis and cellulitis and is a polymicrobial infection associated with a wide variety of bacteria, both aerobic and anaerobic (most commonly *S. pyogenes* and *Fusobacterium* spp., respectively).[14] The disease affects mainly young adults during November to December and April to May, when streptococcal pharyngitis and exudative tonsillitis are at their highest incidence. Abscess forms in the area between the palatine tonsil and its capsule, causing such signs and symptoms as fever, malaise, sore throat, dysphagia, otalgia, trismus, drooling, swelling, muffled "hot potato" voice, rancid breath, and lymphadenopathy.[15]

Lemierre syndrome (postanginal sepsis, necrobacillosis), a rare but severe complication of peritonsillar abscess most often associated with *Fusobacterium necrophorum*, consists of the development of internal jugular vein suppurative thrombophlebitis, which leads to septicemia and metastatic infection in the lungs and joints.[16]

Diagnosis of peritonsillar abscess is usually made on clinical grounds with or without imaging or needle aspiration for direct examination and culture, and treatment includes antibiotic therapy and drainage.[14] Surgical specimens demonstrate abscess formation and acute inflammation of peritonsillar connective tissues (Fig. 7.4).



• **Figure 7.3** Chronic tonsillitis. **A,** Reactive lymphoid follicular hyperplasia with fibrotic accentuation of interlobular septae (×20). **B,** Commensal "sulfur granule" of *Actinomyces* (×40).

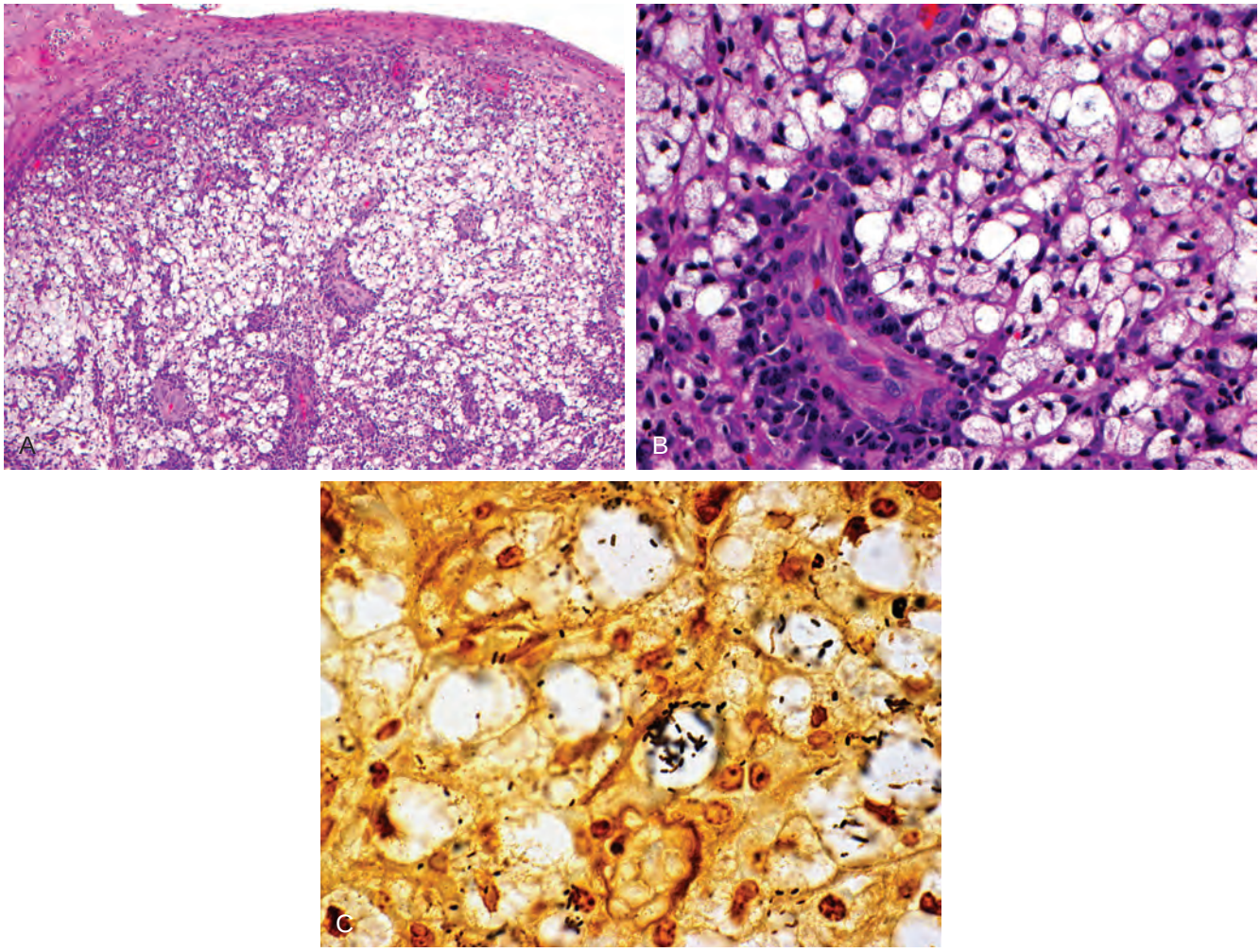


• **Figure 7.4** Peritonsillar abscess. **A,** Abscess formation is seen at the bottom of the image, adjacent to the peritonsillar skeletal muscle (×100). **B,** Neutrophils infiltrating degenerate-appearing peritonsillar skeletal muscle (×400).

Rhinoscleroma

Previously referred to as "scrofulous lupus," rhinoscleroma (scleroma) is a chronic, indolent disease that primarily affects the upper aerodigestive tract (although tracheobronchial disease may occur) and is caused by a gram-negative coccobacillus, *Klebsiella rhinoscleromatis*. Rare in the United States, the disease is not uncommon in endemic areas, including Central and South America, Egypt, India, and central and eastern Europe, where it affects patients in the third and fourth decades and is related to poor hygiene, overcrowding, and malnutrition, with human leukocyte antigen (HLA) research suggesting a genetic predisposition.

The disease is characterized by three distinct phases: a catarrhal (also called atrophic or rhinitic) phase, consisting of mucopurulent rhinitis lasting months to years; a proliferative or granulomatous phase, with formation of multiple firm nodules that obstruct and deform the nasal passages; and a cicatricial phase, characterized by mucosal sclerosis.[17,18] Diagnosis is usually established in the proliferative phase. Microscopic findings in this phase include a submucosal mixed inflammatory infiltrate of lymphocytes and plasma cells with scattered Russell bodies, a prominent granulomatous component, and numerous clusters of foamy histiocytes known as Mikulicz cells that contain bacterial organisms. The organisms can at times be seen on hematoxylin and eosin (H&E) stain but are more readily visualized with silver impregnation stain (e.g., Warthin-Starry) (Fig. 7.5). Immunohistochemical methods for bacterial detection have also been developed.[19] The overlying respiratory mucosa may demonstrate hyperplasia, squamous metaplasia, or ulceration. The cicatricial phase is marked by extensive submucosal fibrosis with a paucity of Mikulicz cells. One group developed a diagnostic model for confirming or excluding a diagnosis of rhinoscleroma in the absence of Mikulicz



• **Figure 7.5** Rhinoscleroma. **A,** Attenuated nasal vestibule mucosa with submucosal lymphoplasmacytic infiltrate and numerous foamy-to-clear histiocytes (Mikulicz cells) (×100). **B,** Perivascular accentuation of lymphoplasmacytic infiltrate and adjacent cluster of Mikulicz cells (×600). **C,** Warthin-Starry stain, highlighting intracytoplasmic coccobacilli (×1000).

cells, using a combination of various clinical and histopathologic parameters.[20]

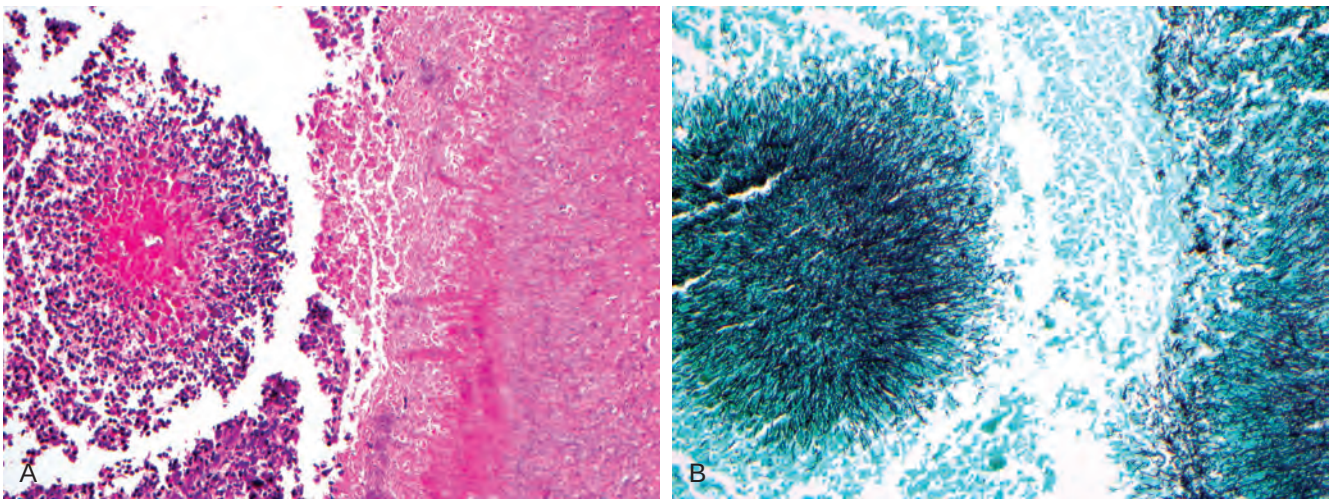
Actinomycosis

Actinomycosis is a polymicrobial bacterial infection of the orocervicofacial region caused by gram-positive anaerobic or microaerophilic filamentous oral flora of the genus *Actinomyces*, most commonly *Actinomyces israelii*.[21] Infection follows mucosal injury, often related to dental manipulation, caries, or trauma, and usually manifests as a chronic, progressively enlarging, fluctuant purple mass at the border of the mandible, although it may also manifest as a mass or ulcer affecting the tongue, larynx, paranasal sinuses, salivary glands, or buccal mucosa, with lymph node involvement developing late in the disease course.[22-24] In chronic lesions, central necrosis and abscess formation occur, and sinus tracts and fistulas may eventually develop and drain foul-smelling, purulent material containing characteristic yellow granular colonies of bacteria known as sulfur granules.[25]

Histologic evaluation reveals mixed granulomatous and purulent inflammation with abscess formation and clusters of bacteria with central basophilia and a peripheral rim of eosinophilic, club-like projections composed of immunoglobulin, fibrin, and tissue debris; this is known as the Splendore-Hoeppli phenomenon (Fig. 7.6).[26] Organisms are highlighted on Gomori methenamine silver (GMS) and tissue Gram stains and are often seen in the crypts of routine tonsillectomy specimens removed for hypertrophy, where they most likely exist as saprophytes (see Fig. 7.3).[27] *Actinomyces* may be confused histologically with *Nocardia*, which, in contrast to the former, affects predominantly immunocompromised patients, is weakly acid-fast, and is only rarely associated with orocervicofacial disease.[28,29]

In addition to routine histology, diagnostic methods include fine-needle aspiration and molecular methods, as well as culture, which is typically of low yield.[30,31] Treatment generally consists of prolonged antibiotic therapy, usually with penicillin.[32]

The two other main forms of actinomycosis, pulmonary and abdominal disease, are covered in other chapters. In addition, whereas past literature suggested a role for *Actinomyces* in the pathogenesis of osteoradionecrosis and bisphosphonate-related osteonecrosis of the jaw, it is currently held that the bacteria identified in such cases represent a secondary infection of necrotic bone.[33]



• **Figure 7.6** Actinomycosis. **A,** Small cluster of *Actinomyces* with surrounding mixed inflammation and Splendore-Hoeppli phenomenon, with a larger cluster of organisms at right (×200). **B,** Gomori methenamine silver stain of the same field, highlighting the filamentous forms (×200).

Botryomycosis

Botryomycosis is caused by a chronic bacterial infection and is analogous to mycetoma but unrelated to fungi or *Actinomyces* spp. It most commonly involves the subcutis but can also affect mucosal or visceral sites in rare cases.[34] Cutaneous infections are caused most commonly by *S. aureus*, with *Pseudomonas aeruginosa* as the second most common pathogen, although reported cases suggest that *P. aeruginosa* may be the most common pathogen at sinonasal sites.[35,36] Although the pathophysiology is incompletely understood, the disease is most commonly seen in patients with some degree of immunosuppression.[37]

In general, botryomycosis manifests as a firm nodule or mass associated with a sinus draining granular material. Microscopic examination reveals the botryomycotic granules consisting of bacterial aggregates best appreciated with tissue Gram or silver stains, with clublike projections of amorphous eosinophilic material radiating from them (Splendore-Hoeppli phenomenon). The botryomycotic granule is surrounded by a polymorphic infiltrate of neutrophils, lymphocytes, and histiocytes. Well-formed granulomas with central necrosis may be seen, together with edema and fibrosis.[38] Surgical management is usually necessary because these infections are resistant to medical therapy.

Syphilis

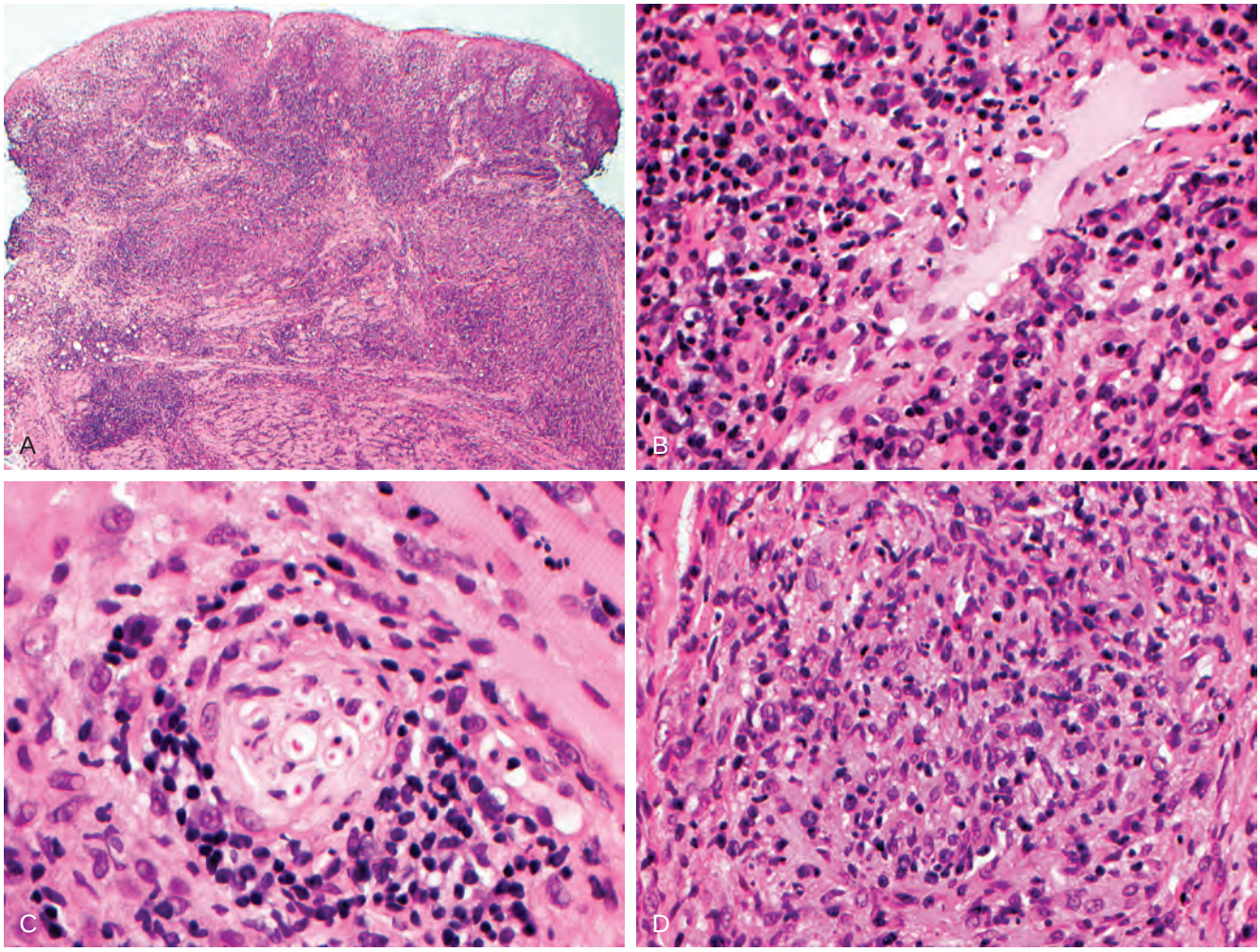
Since reaching an all-time nadir in 2000, the incidence of primary and secondary syphilis in the United States has increased, largely driven by cases among men who have sex with men.[39,40] It remains a significant cause of morbidity in this country. The oral cavity is the most common extragenital site of infection,[41] and the disease may affect virtually any region of the head and neck, with clinical manifestations in the primary, secondary, and tertiary stages, as well as in congenital infections.

After an incubation period of 9 to 90 days, primary syphilis manifests as one or more painless ulcers or chancres at the inoculation site, which can include the oral mucosa and tongue, associated with painless lymphadenopathy.[42] In addition, primary infection may manifest as painless tonsillar enlargement.[43] Histologic examination of the chancre reveals a diffuse inflammatory infiltrate of lymphocytes and plasma cells, with scattered histiocytes, neutrophils, and eosinophils (Fig. 7.7). The inflammatory infiltrate shows a tendency to surround small blood vessels, producing endothelial proliferation and obliterative endarteritis; poorly formed granulomas, plasma cell neuritis, and focal necrosis can also be seen.[44] Lymph node findings are discussed in Chapter 12.

Secondary syphilis manifests 6 to 8 weeks after spontaneous recovery from the primary infection, producing flulike symptoms and a rash that often affects the palms and soles. Tonsils may be affected by highly infectious, gray-white, necrotic mucous patches with surrounding erythema, which may extend to the buccal mucosa, tongue, uvula, and soft palate. Maculopapular lesions, moist ulcers, and irregular linear "snail track" erosions can be seen, histologically characterized by dense subepithelial inflammation composed predominantly of plasma cells.[45]

"Lues maligna," or ulceronodular syphilis, is a rare aggressive form of secondary syphilis that most commonly affects individuals coinfected with human immunodeficiency virus (HIV). It is characterized by papulopustular skin lesions that rapidly enlarge and evolve into sharply demarcated ulcers with a central rupioid crust with associated constitutional symptoms, and it may show involvement of the mucous membranes of the mouth and nose.[46] The histopathology is similar to that of other lesions of secondary syphilis. If untreated, patients pass into the asymptomatic latent phase of disease and lose infectivity before developing signs and symptoms of tertiary disease 4 to 8 years later.

Tertiary syphilis is characterized by multiorgan involvement and is sometimes referred to as "the great imitator" because of its protean manifestations. The gumma is the hallmark lesion of tertiary syphilis. It manifests as a painless swelling of the palate or tongue or less commonly the alveolus or parotid gland. Lesions swell and coalesce, eventually forming areas of ulceration, with possible bone destruction, palatal perforation, or oronasal fistula formation.[47] Gummas are characterized histologically by obliterative endarteritis with necrosis and mixed inflammation consisting of plasma cells (which may be scarce), lymphocytes, epithelioid histiocytes that often form loose granulomas, and giant cells. Syphilitic leukoplakia of the tongue may also be seen and may predispose to the development of squamous cell carcinoma.[48] The



• **Figure 7.7** Primary syphilis. **A,** Tongue biopsy from a patient with an indurated and ulcerated lesion of the tongue, showing dense deep inflammation involving the skeletal muscle fibers (×40). **B,** Perivascular accentuation of plasma cells and reactive-appearing endothelial cells (×400). **C,** Neurotropism of mixed inflammatory infiltrate (×600). **D,** Vaguely granulomatous inflammatory component (×400).

lesions appear as homogeneous white plaques on the dorsum of the tongue; histologically they show psoriasiform hyperplasia and parakeratosis of the mucosa with infiltrates of lymphocytes, plasma cells, and histiocytes. Interstitial glossitis also occurs.[49] Spirochetal sensorineural hearing loss is an infrequent late consequence of acquired syphilis.[50]

Congenital syphilis occurs after transplacental spread during pregnancy and can result in numerous abnormalities in surviving affected progeny. Early signs of congenital infection typically occur between 1 month and 2 years of age and include rhinitis ("snuffles"), vesiculobullous eruptions of the mucous membranes, and radial perioral skin lesions ("rhagades"). Late signs occur after 2 years and include keratitis (often leading to blindness) and eighth nerve deafness, as well as more typical signs of tertiary syphilis. Such patients are additionally affected by typical orofacial stigmata, including tooth abnormalities (Hutchinson incisors, Fournier "mulberry" molars, and Moon molars), saddle nose deformity (caused by destruction of the vomer), frontoparietal bossing, and maxillary hypoplasia ("bulldog jaw").[51,52]

The diagnosis of syphilis may be accomplished in several ways. In the primary and secondary stages, as well as in early congenital disease, lesions contain numerous spiral-shaped treponemal organisms, often within the surface epithelium, surrounding small vessels and within macrophages, and they can be visualized with the Warthin-Starry stain, immunohistochemistry, or darkfield microscopy. However, microscopic findings should be interpreted with caution, because spirochetes are part of the normal flora of the oral cavity. Serologic techniques are currently the mainstay of diagnosis and include both treponemal (specific) and nontreponemal (nonspecific) antibody tests. Nonspecific tests include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) tests, which are rapid and inexpensive and traditionally have served as screening tests. Specific tests, such as the fluorescent treponemal antibody-absorbed (FTA-ABS) assay, should be performed for confirmation of the diagnosis. No single test should be used alone in making a diagnosis of syphilis.[43]

Tuberculosis

Tuberculosis, one of the most important global infectious causes of morbidity and mortality, is most commonly caused by the acid-fast aerobic bacterium, *Mycobacterium tuberculosis*, which is estimated to infect one-third of the world's population, and less commonly by the other members of the *M. tuberculosis* complex,

which includes *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canetti,* and *Mycobacterium microti*.[53,54] Although it most commonly manifests as pulmonary disease, tuberculosis is a systemic infection and can affect virtually any part of the body, including the head and neck. Extrapulmonic manifestations are more common in immunosuppressed patients, particularly those with HIV.[55,56]

Tuberculosis of the head and neck most commonly manifests as cervical lymphadenopathy, although many other sites may be involved, either with and without pulmonary involvement, including the larynx, pharynx, middle ear, salivary glands, tongue, sinonasal region, thyroid gland, and oral cavity.[57-71] Lesions take on a variety of clinical appearances depending on site of involvement, and the patient may present with a parotid mass, a mouth ulcer, a soft tissue abscess, a retropharyngeal space swelling, otorrhea, or many other signs and symptoms.[72-82] Material for pathologic examination is vital and may be obtained by incisional biopsy or fine-needle aspiration.

Histologic evaluation reveals caseating granulomatous inflammation, including well-formed granulomas with scattered Langhans-type giant cells and surrounding lymphocytes and fibroblasts (Fig. 7.8). Organisms are typically sparse, and acid-fast staining techniques are required for visualization of the bacilli. In addition to traditional diagnostic tests, such as sputum smear and tuberculin skin testing, newer methods have become available, including nucleic acid amplification and interferon-release assays.[83]

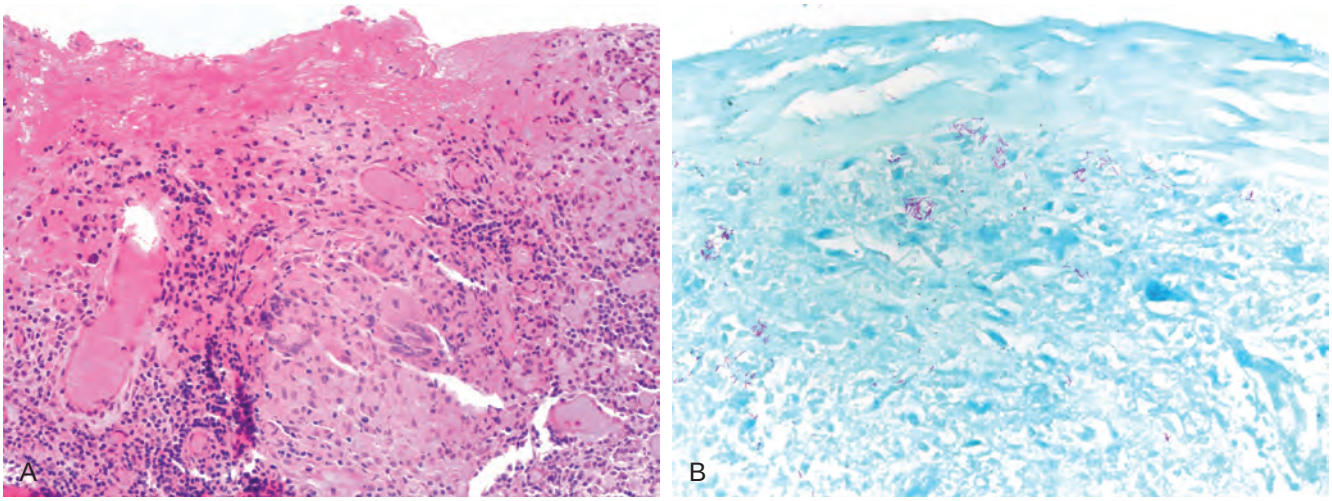
Leprosy

Leprosy is an infection caused by *Mycobacterium leprae*, also known as Hansen bacillus, which produces a chronic systemic granulomatous disease that may affect the skin, mucous membranes, peripheral nerves, skeleton, and viscera. Although it is infrequently encountered in the United States, leprosy continues to be a significant health problem worldwide. Transmission of the disease is incompletely understood, but current research suggests that contacts of untreated, affected individuals are at risk for infection through respiratory exposure to organisms shed into the environment from skin and nasal mucosa, with salivary anti-*M. leprae* IgA levels correlating with protection.[84,85] Recent research has identified *Mycobacterium lepromatosis* sp. nov. as a second species responsible for clinical leprosy.[86]

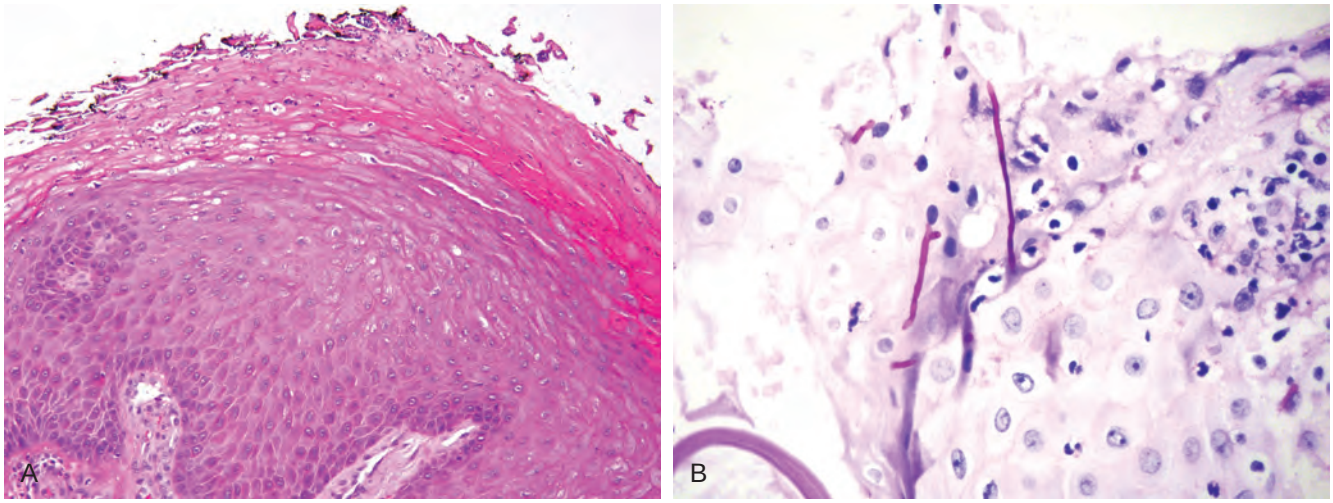
The two main clinical forms are tuberculoid leprosy (the paucibacillary form, representing intact cell-mediated immunity) and lepromatous leprosy (the multibacillary form, representing relative anergy); there are additional intermediate clinical forms depending on the degree of cell-mediated immunity.[87] Mucosal lesions usually are present in the chronic lepromatous form, and they consist of multiple nodules (lepromas) or ulcers affecting the palate and nasal mucosa, and less commonly the tongue, lips, and gums, with destruction of the anterior maxilla and loss of teeth.[88,89] Histologic analysis of nasal and palatal lesions reveals mucosal hyperplasia or ulceration with a dense submucosal inflammatory infiltrate consisting of lymphocytes, as well as foamy histiocytes containing numerous acid-fast bacilli (lepra cells). However, histologic findings, particularly in oral lesions, may consist of nonspecific chronic inflammation without associated organisms. Well-formed granulomas and giant cells are infrequent but may be seen in intermediate forms of the disease, as well as in clinically normal mucosa of patients with lepromatous leprosy.[90] In contrast, in the tuberculoid form, well-formed granulomas are often identified in the skin, but the mucosa remains relatively unaffected by disease, both clinically and histologically. Because mucosal lesions of lepromatous disease often show nonspecific inflammatory changes, the diagnosis is usually based on smear or biopsy of cutaneous lesions in conjunction with clinical findings. Molecular methods of diagnosis are also available.[91]

Oral Candidosis

Candida albicans is a dimorphic fungus that frequently colonizes the oral cavity, and it is also the most common fungal pathogen isolated from this location, causing opportunistic infection in the immunosuppressed, the elderly, and the very young.[92] Other *Candida* species, including *Candida glabrata* and *Candida tropicalis*, are less commonly isolated. Oral infection is classified as primary or secondary. Primary oral candidosis includes acute forms (e.g., pseudomembranous candidosis [thrush] and erythematous candidosis), chronic forms (e.g., erythematous candidosis



• **Figure 7.8** Tuberculous otitis. **A,** Well-formed granuloma with surrounding granulation tissue and mixed inflammatory infiltrate (×200). **B,** Acid-fast stain, highlighting scattered bacilli within degenerate connective tissue (×400).



• **Figure 7.9** Oral candidosis. **A,** Biopsy of a tongue lesion, showing mucosal hyperplasia, hyperparakeratosis, spongiosis, several intraepithelial neutrophils, and fungal elements consisting of superficial spheroid yeast forms and scattered pseudohyphal forms present within the parakeratotic layer (×200). **B,** Periodic acid–Schiff stain with diastase digestion, highlighting the intraepithelial pseudohyphae (×600).

and hyperplastic candidosis), and several *Candida*-associated lesions (e.g., denture stomatitis, angular cheilitis, and median rhomboid glossitis). Secondary oral candidosis includes oral disease associated with systemic infection, usually as a result of congenital or acquired immunodeficiency.[93] Patients may be asymptomatic, or they may report burning or dysgeusia, and on clinical examination they demonstrate a variety of lesions, depending on the form of disease.

The histologic appearance of lesions also varies but often demonstrates epithelial hyperplasia with hyperparakeratosis, spongiosis, and scattered intraepithelial neutrophils, as well as a submucosal mononuclear cell infiltrate (Fig. 7.9). Fungal pseudohyphae are seen within the superficial parakeratotic layer, and ovoid yeast forms may also be present more superficially. GMS stain and periodic acid–Schiff (PAS) with diastase can aid in the identification of organisms, which are gram positive on tissue Gram stain. Exfoliative cytology is a useful diagnostic tool, and immunohistochemical and molecular methods are also available.[94,95]

Fungal Rhinosinusitis

Sinonasal mycotic disease is classified based on clinical, radiologic, and histologic findings and includes both tissue invasive and noninvasive processes. The invasive forms of disease are acute fulminant invasive fungal sinusitis, chronic invasive fungal sinusitis, and granulomatous invasive fungal sinusitis; the noninvasive forms are sinus fungal ball, allergic fungal rhinosinusitis, and saprophytic fungal infestation (Table 7.1).[96]

Acute fulminant invasive fungal sinusitis occurs almost exclusively in immunocompromised patients and constitutes a medical emergency. It is referred to as mucormycosis, zygomycosis, or fulminant invasive sinusitis, depending on the pathogen, but because the clinical syndromes are virtually identical, the term *acute fulminant invasive fungal sinusitis* is currently favored.[97] This disease is most commonly caused by molds of the class Zygomycetes, including *Mucor, Rhizopus,* and *Absidia* spp., but can also result from infection with *Aspergillus* spp., *Fusarium* spp., and *Pseudallescheria boydii*.[98] Clinical signs and symptoms include fever, headache, cough, epistaxis, nasal discharge or crusting, facial swelling, and mental status changes. The presence of orbital or cranial nerve symptoms at presentation is more often related to *Mucor* than to *Aspergillus* species.[99]

Examination may reveal a palatal ulcer or eschar, as well as necrosis of nasal mucosa. Gross surgical débridement specimens typically consist of necrotic, hemorrhagic soft tissue and occasionally bone fragments. Microscopic evaluation reveals hemorrhage, necrosis, and mixed acute and chronic inflammation that may have a granulomatous component (Fig. 7.10). Fungal hyphal forms are usually evident on routine H&E staining but are best appreciated with GMS. Tissue invasion occurs with striking angiotropism and angioinvasion, often occluding vessel lumens and resulting in thrombosis with surrounding necrosis. *Mucor* also has a propensity for perineural involvement.[100] Hyphal forms of *Aspergillus* spp. are septate with acute-angle branching, whereas *Mucor, Rhizopus,* and similar species produce pauciseptate, ribbonlike hyphae that tend to branch at right angles. Immunohistochemistry for mucin 5B (MUC5B) expression is positive in most cases of *Aspergillus* infection and generally absent in order *Mucorales* sinusitis.[101] Given the urgent nature of acute fulminant invasive fungal sinusitis, intraoperative evaluation of frozen sections may be requested, and antibiotic therapy should be initiated before culture results are available.

Chronic invasive fungal sinusitis, a rare disease, is usually seen in diabetic patients and is defined as sinusitis of at least 4 weeks' duration with compatible radiologic findings and histologic evidence of tissue invasion by fungal hyphal forms.[102] Patients may develop the orbital apex syndrome, which consists of ocular immobility and loss of vision, owing to the presence of a mass in the superior part of the orbit, caused by fungal spread from the ethmoid sinus. Chronic invasive fungal sinusitis is characterized histologically by vascular, bone, and soft tissue invasion by fungal hyphae, most commonly *Aspergillus fumigatus*, with necrosis and a mild associated chronic inflammatory infiltrate. Fungal organisms are abundant and resemble a sinus fungal ball, a finding that has led some to postulate that the two diseases exist along a

TABLE 7.1 Classification of Sinonasal Fungal Disease

Classification	Immune Status	Causative Organisms	Clinical Presentation	Microscopic Findings	Management
Invasive Disease					
Acute fulminant invasive fungal sinusitis	Compromised	Class Zygomycetes, *Aspergillus* spp., *Fusarium* spp.	Fever, headache, discharge, epistaxis, mental status changes	Necrosis, hemorrhage, acute and chronic inflammation, fungal tissue and angioinvasion	Emergent débridement, antibiotic therapy
Chronic invasive fungal sinusitis	Compromised (usually diabetics)	*Aspergillus fumigatus*	Fever, headache, discharge, epistaxis, mental status changes	Necrosis, chronic inflammation, fungal invasion, abundant organisms resembling fungal ball	Emergent débridement, antibiotic therapy
Granulomatous invasive fungal sinusitis	Competent	*Aspergillus flavus*	Slowly progressive sinusitis, headache, unilateral proptosis	Granulomatous inflammation, fungal tissue invasion	Débridement, aeration, antibiotic therapy
Noninvasive Disease					
Sinus fungal ball	Competent	*A. fumigatus*	Facial pain, headache, nasal obstruction and discharge, cacosmia, visual changes	Matted fungal hyphae, mixed sinus inflammation, absence of tissue invasion	Débridement, aeration
Allergic fungal rhinosinusitis	Competent	*Aspergillus, Fusarium, Curvularia, Bipolaris,* and *Pseudallescheria* spp.	Chronic pansinusitis, nasal polyps, history of atopy	Polypoid sinus mucosa with stromal edema and mixed inflammation with many eosinophils, allergic mucin, sparse fungal forms	Débridement, aeration, corticosteroid therapy
Saprophytic fungal infestation	Competent	Various	Asymptomatic	Fungal spores within mucus crusts	Crust removal, irrigation

spectrum.[98] Like acute fulminant invasive fungal sinusitis, chronic invasive fungal sinusitis has a poor prognosis, requiring aggressive management.

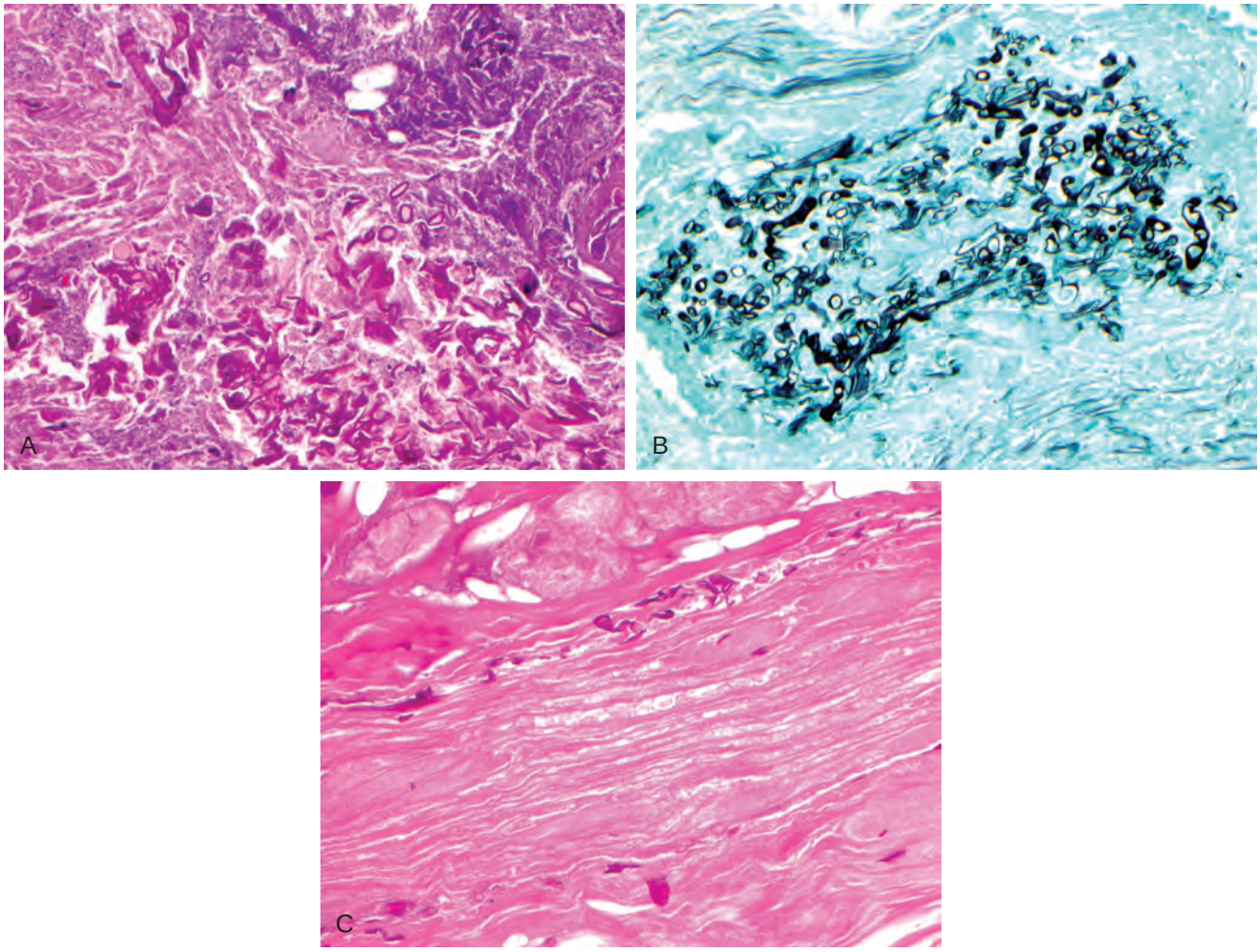
Granulomatous invasive fungal sinusitis is a subtype of chronic invasive fungal sinusitis that shows a few key differences. The disease is most commonly reported in northern Africa, where it affects apparently immunocompetent individuals and is caused almost invariably by *Aspergillus flavus*.[97] Also known as indolent fungal sinusitis or primary paranasal granuloma, granulomatous invasive fungal sinusitis is characterized clinically by slowly progressive sinusitis with headache and unilateral proptosis.[103] Pathologically, it shows florid granulomatous inflammation with tissue invasion by fungal hyphae. Noncaseating granulomata with giant cells and occasional central eosinophilic microgranulomata, as well as fibrinoid necrosis, vasculitis, vascular proliferation, and perivascular fibrosis, have also been described.[104,105] Fungal forms may be sparse and within giant cells or profuse and mass forming.[98,106] Prognosis is better than in other invasive fungal sinus infections, and patients tend to respond to débridement, aeration, and antifungal therapy, with recurrence being common.

Sinus fungal ball, frequently (and inappropriately) referred to as mycetoma or aspergilloma, is a dense conglomeration of fungal hyphae that forms a mass within a sinus cavity without invading the surrounding host tissue.[107] Patients are immunocompetent and usually present with facial pain because the maxillary sinus is most commonly involved, but they may also experience headache, nasal obstruction and discharge, cacosmia, and visual disturbances.[108] Radiologic findings include unilateral sinus opacification, often with associated flocculent calcifications owing to calcium oxalate deposition by the fungus. Examination of sinus contents reveals mucopurulent, caseous, or claylike material.[109] The most common fungus recovered is *A. fumigatus*, although culture is often negative.[110]

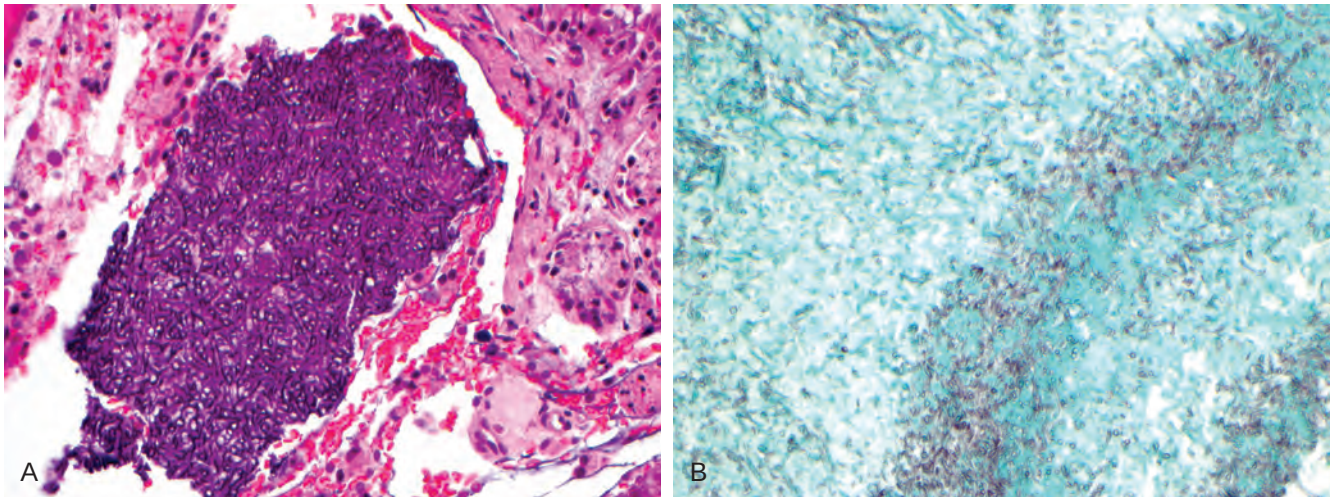
Histologic examination of sinus contents reveals matted fungal hyphae that are evident on H&E stain but best delineated with GMS (Fig. 7.11). Intact hyphae are identified at the periphery of the mass but are often densely packed and difficult to recognize centrally. Tissue invasion is absent, but fragments of sinus mucosa may demonstrate mixed inflammation consisting of neutrophils, lymphocytes, plasma cells, mast cells, and eosinophils. Neither the presence of allergic mucin nor a predominance of eosinophils is a feature of sinus fungal ball, and these findings suggest a concomitant allergic fungal rhinosinusitis.[109,111]

Therapy consists of débridement and aeration, and antifungal medication is not required. It has been proposed that another form of noninvasive fungal sinusitis exists, so-called chronic erosive sinusitis, that is characterized by radiologic evidence of bony erosion without histologic evidence of tissue invasion; however, it is presently unclear whether this represents a separate entity or more extensive involvement by sinus fungal ball. Possibly it represents an intermediary form of disease between sinus fungal ball and chronic invasive fungal sinusitis.[107,112]

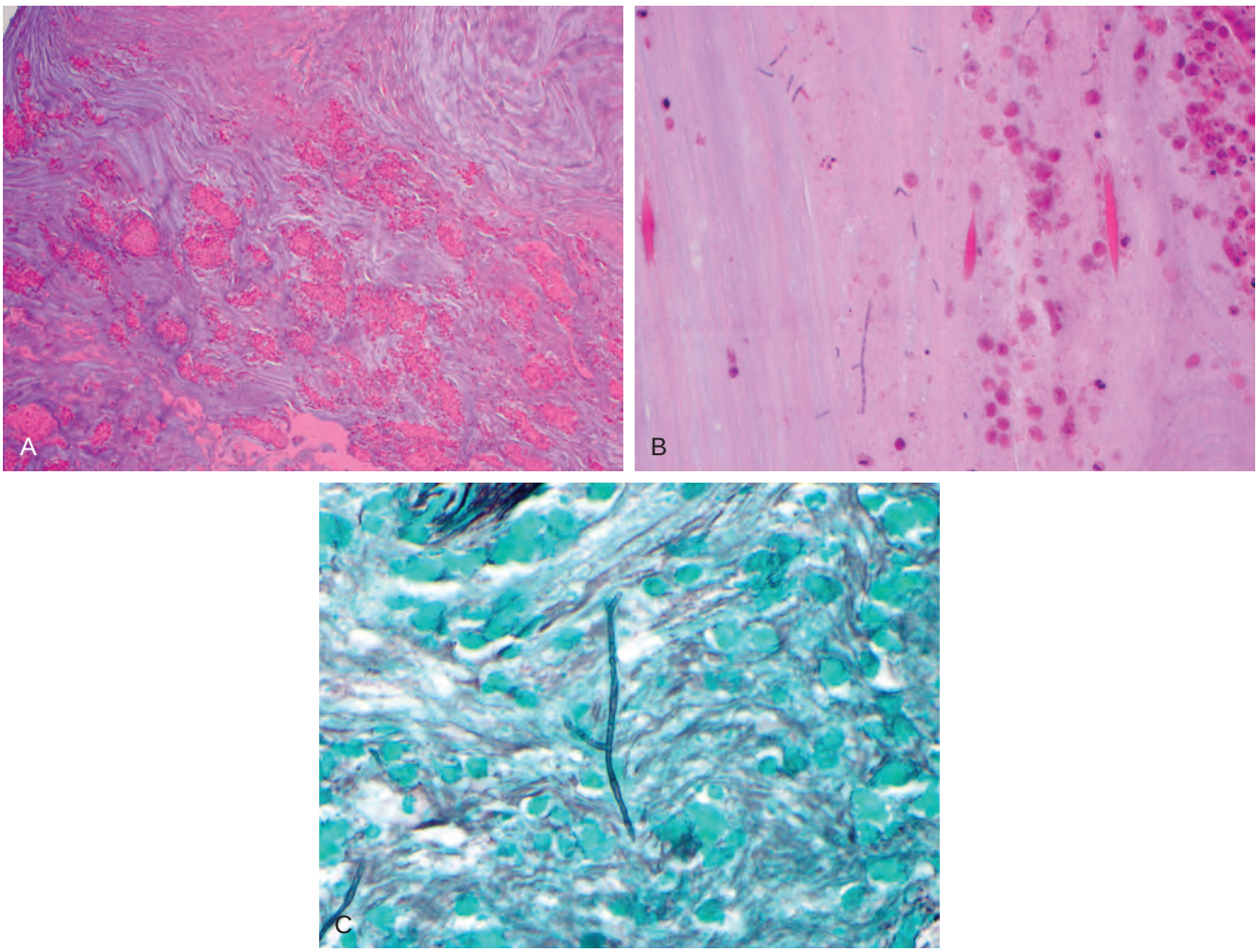
Allergic fungal rhinosinusitis is currently thought to represent a hypersensitivity reaction to fungus within the sinus cavity, and



• **Figure 7.10** Acute fulminant invasive fungal sinusitis. **A,** Tissue invasive *Mucor* sp. characterized by eosinophilic irregular, ribbonlike hyphae with surrounding necrotic fibrous tissue (×400). **B,** Gomori methenamine silver stain highlighting angioinvasive *Mucor* sp. (×400). **C,** Scattered hyphae of *Mucor* sp. surrounding a necrotic nerve (×400).



• **Figure 7.11** Sinus fungal ball. **A,** Matted hyphae of *Aspergillus* sp. removed during endoscopic sinus surgery with adjacent submucosal glands (×400). **B,** Gomori methenamine silver stain highlighting the matted hyphae (×400).



• **Figure 7.12** Allergic fungal sinusitis. **A,** Allergic mucin, characterized by clusters of eosinophils imbedded in acellular mucus (×100). **B,** Slender bipyramidal Charcot-Leyden crystals and several sparse hyphal forms, here identifiable on routine hematoxylin and eosin stain (×400). **C,** Gomori methenamine silver stain, highlighting a septate hyphal form with acute-angle branching, surrounded by allergic mucin. (×600).

the proposed diagnostic criteria include the presence of allergic mucin and fungal hyphae within a sinus, radiographic findings confirming sinusitis, absence of tissue invasion by the fungus, and absence of predisposing immunodeficiency or immunosuppression.[113] Hyaline molds, such as *Aspergillus* and *Fusarium,* as well as pigmented dematiaceous fungi, such as *Curvularia, Bipolaris,* and *Pseudallescheria*, are commonly implicated.[106] With regard to identification of the fungus, it has been suggested that histologic evidence is preferable to culture isolation because research has revealed the frequent presence of fungus in cultured nasal lavage specimens of normal individuals, and culture carries an inherent risk of contamination.[114,115] However, speciation by histology is not possible. Because fungus is ubiquitous in the environment and is present in the nasal passages of apparently normal individuals, it is believed that disease manifestation is caused not by the fungus per se but rather by an exaggerated immune response against it. Allergic fungal sinusitis is an area of intense study, and, although the pathology is incompletely understood, there is evidence to support both IgE- and cell-mediated processes.[116-119] Current terminology reflects this uncertainty and includes such terms as eosinophilic fungal rhinosinusitis (a term used to stress the eosinophil-mediated nature of the disease) and eosinophilic mucin rhinosinusitis (proposed to represent a disease unrelated to fungus).[120-122]

The typical patient with allergic fungal sinusitis presents with chronic, often intractable, pansinusitis and associated nasal polyposis, with a history of atopy and multiple sinus operations.[98] Examination of surgical specimens of allergic mucin reveals chalky gray to black or brown tenacious mucus that microscopically appears as thick, gray-green to pink, lamellated material containing mucin, serum, abundant eosinophils with scattered neutrophils and Charcot-Leyden crystals, cellular debris, and sparse, often fragmented, fungal hyphae, usually visualized only with special stains, such as GMS (Fig. 7.12). Sinonasal mucosa is often polypoid and usually demonstrates underlying mixed inflammation consisting primarily of eosinophils with admixed lymphocytes, plasma cells, mast cells, and scattered neutrophils, together with associated stromal edema. Although tissue invasion is not a feature of allergic fungal sinusitis, the collection of allergic mucin may be large enough to cause adjacent bony erosion, cranial nerve palsies,

sinus perforation, and disease extension into the orbit or anterior skull base, sometimes mimicking neoplasia; however, despite this potentially aggressive behavior, most patients respond to débridement, aeration, and corticosteroid therapy.[123-126]

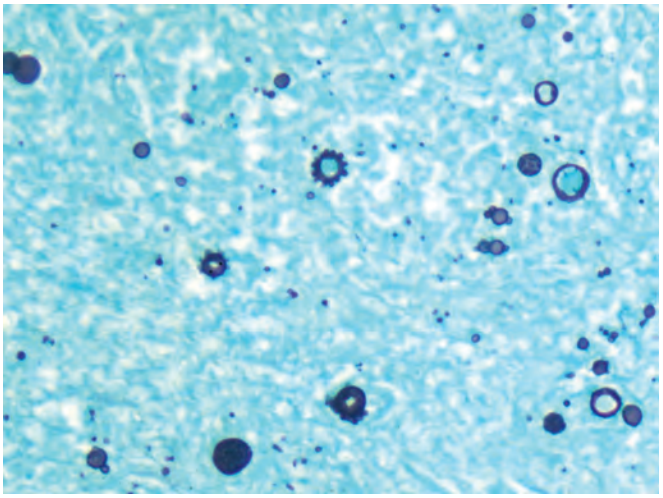
Saprophytic fungal infestation is the finding of fungal spores on mucous crusts of respiratory passages. Its natural history and role in sinonasal pathology are uncertain, but the condition is considered distinct from that of asymptomatic individuals with positive fungal cultures alone.[96] Patients typically respond to crust removal and home irrigation,[96] and, because crusts are usually removed in a clinic setting, specimens are not typically submitted for histologic evaluation.

Paracoccidioidomycosis

Paracoccidioidomycosis (also known as South American blastomycosis or Lutz-Splendore-Almeida disease) is a systemic granulomatous disease caused by the dimorphic fungus *Paracoccidioides brasiliensis*, which is endemic to Latin America, where approximately 10 million people are infected, 2% of whom manifest disease.[127] Primary infection typically occurs in the second decade of life but can occur at any age, with a lower female incidence in pubertal years, owing to the inhibitory effect of estrogen against fungal transition from mycelia to yeast form.[128,129] Infection is thought to occur through inhalation of conidia, with the two main clinical forms of disease being the acute/subacute (juvenile) form and the more common chronic (adult) form; these affect primarily the reticuloendothelial system and the lungs, respectively, although any organ may be involved.[130]

Head and neck manifestations commonly occur in the chronic form of the disease, usually in oral sites, including the alveolar process, gingiva, tongue, lip, buccal mucosa, palate, and floor of mouth, as well as the larynx and, uncommonly, the nasal mucosa.[131-136] Patients present with stomatodynia, hoarseness, odynophagia, sore throat, and dyspnea. On examination, they may exhibit characteristic muriform lesions, which are reminiscent of a mulberry and are characterized by a fine granular surface, as well as ulcers, plaques, tooth loss, thickening of the lips, and, rarely, palatal perforation.[137-140] Infection may mimic carcinoma both clinically and histologically, with chronic and progressive lesions that histologically demonstrate pseudoepitheliomatous hyperplasia.[141]

Microscopic examination reveals intraepithelial abscesses and subepithelial granulomatous inflammation with scattered multinucleate giant cells, as well as admixed neutrophils, lymphocytes, plasma cells, and eosinophils (Fig. 7.13).[142] Well-formed granulomas are composed mainly of macrophages and CD4-positive T lymphocytes and contain fewer yeast particles than poorly formed granulomatous areas, possibly reflecting a more effective host immune response.[143] The characteristic polygemulating yeast forms appear as double-contoured structures 6 to 30 µm or more in diameter with multiple surrounding budding forms, often likened to a captain's wheel (although single budding may also be seen), as well as smaller yeast forms that may cause confusion with *Histoplasma capsulatum*.[144] Organisms are usually visible on routine H&E staining, although they are better appreciated by GMS staining. Exfoliative cytology has proved to be a useful rapid diagnostic tool, and immunohistochemical and molecular methods of identification have also been developed.[145-151] Antifungal therapy with sulfonamides or azoles is effective in treating infection, with fibrotic sequelae being a common and sometimes disfiguring complication in healed lesions.[130,152]



• **Figure 7.13** Paracoccidioidomycosis, highlighted on Gomori methenamine silver stain as scattered, double-contoured yeast forms with multiple surrounding budding forms (×400).

Sporotrichosis

Sporotrichosis is caused by *Sporothrix schenckii*, a dimorphic fungus with worldwide distribution that thrives in tropical and temperate climates, where it exists as a common saprophyte found in plant materials, soil, and a variety of animals.[153] The disease usually manifests as lymphocutaneous or fixed cutaneous disease or, uncommonly, as disseminated disease.[154] There are several case reports of sporotrichosis involving the nasal passages, paranasal sinuses, or oral cavity, and it appears that many of the affected patients suffered from some form of immunocompromise.[155-162] The histologic findings are identical to those seen in cutaneous disease.

Blastomycosis

Blastomycosis (sometimes referred to as North American blastomycosis) is a chronic granulomatous and suppurative disease caused by *Blastomyces dermatitidis*, a dimorphic fungus endemic to the south-central and Midwestern United States.[163] There are five categories of blastomycosis occurring in humans: primary pulmonary disease, single organ system disease, generalized multiorgan disease, chronic cutaneous disease, and inoculation blastomycosis.[164] It is generally accepted that most cases of cutaneous disease occur after lymphohematogenous spread from a primary pulmonary infection, even in the absence of clinical pulmonary disease.[165] Blastomycosis has been reported in the nasal passages, paranasal sinuses, mouth, and parotid gland, often manifesting as a mass and mimicking a neoplasm.[166-180] The histologic findings are identical to those seen in cutaneous disease.

Coccidioidomycosis

Coccidioidomycosis, commonly referred to as valley fever, is caused by *Coccidioides immitis* and *Coccidioides posadasii*, two morphologically identical but genetically distinct dimorphic fungi endemic to the southwestern United States and Mexico, as well as parts of Central and South America.[181-183] The five main clinical manifestations of coccidioidal infection are acute pneumonia, chronic progressive pneumonia, pulmonary nodules and cavities,

extrapulmonary nonmeningeal disease, and meningitis.[184] Head and neck manifestations are rare and are usually associated with disseminated disease.[185] Reported sites of involvement include the tongue, retropharyngeal space, deep neck, temporal bone, and thyroglossal duct cyst.[186-188]

Cryptococcosis

Cryptococcosis is caused by the ubiquitous yeast of the *Cryptococcus* species complex, which includes *Cryptococcus neoformans* (further divided into var. *neoformans* and var. *grubii*) and *Cryptococcus gattii*.[189] *Cryptococcus* can cause disease in any organ of the body but most commonly affects the lungs and central nervous system, particularly in immunocompromised patients.[190] Involvement of various head and neck sites, including the oral cavity, thyroid gland, paranasal sinuses, and salivary glands, has rarely been reported and typically occurs in the setting of immunocompromise with disseminated disease.[191-200]

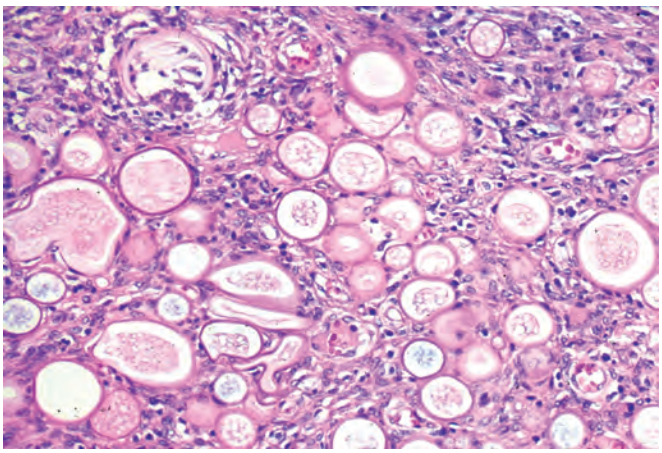
Histoplasmosis

Histoplasmosis, also known as Darling disease, is caused by *H. capsulatum* var. *capsulatum*, a dimorphic fungus that is found worldwide and is highly endemic to the Ohio and Mississippi River valleys.[201-203] Clinical manifestations include pulmonary, mediastinal, inflammatory, and disseminated syndromes and are dependent on the size of the inoculum and the underlying health and immune status of the patient.[204] Involvement of head and neck sites is uncommon and usually occurs in the setting of disseminated disease in HIV and acquired immune deficiency syndrome (AIDS).[205-236]

Rhinosporidiosis

Rhinosporidiosis is a rare chronic granulomatous disease that most commonly affects the nasal and nasopharyngeal mucosa but also can involve various other sites, including the oropharynx, conjunctiva, skin, trachea, and urethra, occasionally manifesting as disseminated disease.[237-239] The causative agent is generally believed to be *Rhinosporidium seeberi*, which was once thought to be a fungus but is currently categorized as a protist of class *Mesomycetozoea*, which comprises mostly uncultivable aquatic microbes at the animal-fungal boundary.[240] It has also been suggested that the causative organism is a cyanobacterium.[241] The disease is encountered in the Americas, Europe, Africa, and Asia and has its highest incidence in Sri Lanka and southern India, usually affecting but not restricted to adult men.[242] The mode of transmission is unknown, but water and soil are believed to be reservoirs for infection, and the incidence of disease is higher among sand workers, paddy cultivators, and people bathing in stagnant waters.[243]

Presenting symptoms include nasal obstruction, epistaxis, and viscid nasal discharge. Examination reveals a friable vascular polyp studded with tiny white dots, giving the characteristic "strawberry" appearance.[244] Histologic examination reveals hyperplastic mucosa with an underlying dense lymphoplasmacytic infiltrate and scattered intraepithelial and subepithelial, large (up to 350 µm or larger), thick-walled (up to 5 µm), cystic structures called sporangia, which contain multiple small (2 to 10 µm), round endospores, both of which stain with H&E, GMS, mucicarmine, and PAS (Fig. 7.14).[243,245] In addition to the lymphoplasmacytic infiltrate, neutrophils, histiocytes, and occasional giant cells may be seen, as well as submucosal hypervascularity and foci of necrosis.[246] Fine-needle aspiration cytology has proved useful in diagnosis.[247] The histologic differential diagnosis includes *C. immitis*, which is smaller and negative for mucicarmine, and myospherulosis, a foreign body response to petroleum-based material. The mainstay of treatment is surgical removal of lesions, occasionally with dapsone as adjunct therapy, and recurrence is a known complication.[248,249]

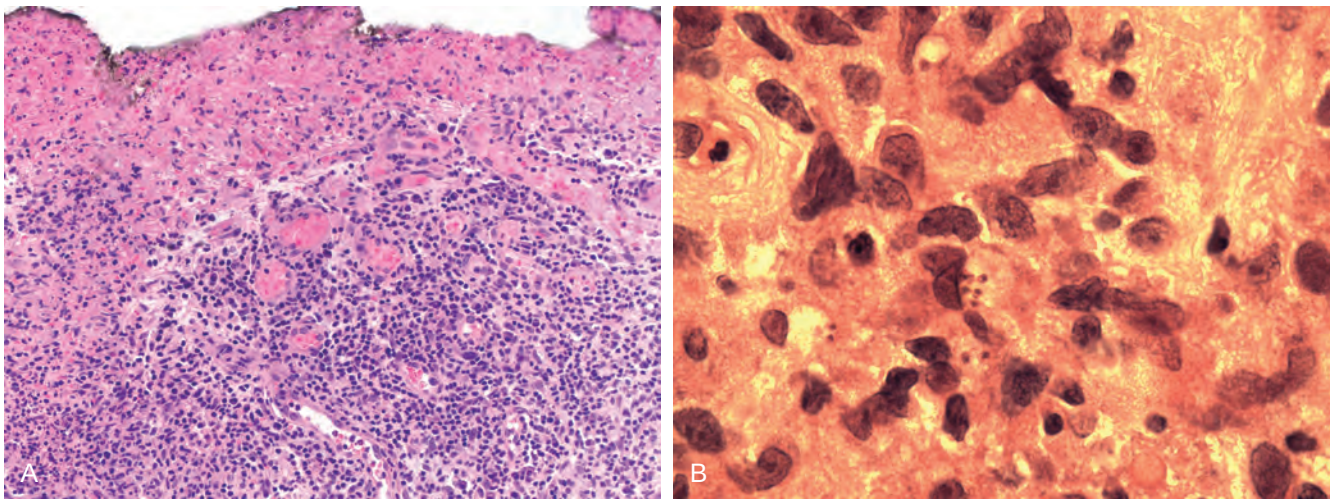


• **Figure 7.14** Rhinosporidiosis, characterized by multiple, thick-walled sporangia of variable diameter containing multiple endospores, with surrounding lymphoplasmacytic inflammation (×200).

Mucocutaneous Leishmaniasis

Leishmaniasis is a vector-borne parasitic disease caused by any of more than 25 species of the hemoflagellate *Leishmania*. It is transmitted by the bite of an infected sandfly and is prevalent in at least 88 countries worldwide.[250] The four forms of disease generally recognized are localized cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, mucocutaneous leishmaniasis (also known as espundia), and visceral leishmaniasis (also known as kala-azar). The mucocutaneous form is most commonly observed in South America, where *Leishmania brasiliensis* is the major cause.[251-253]

Mucocutaneous leishmaniasis predominates in adults, with mucosal lesions occurring concomitantly with, or years after, cutaneous manifestations and involving the nose, lips, mouth, pharynx, and larynx in the form of progressively enlarging swellings or nodules with associated granulation tissue that can result in severe disfigurement if left untreated.[254] Histologically, lesions may show pseudoepitheliomatous hyperplasia or ulceration and associated granulation tissue, with an underlying mixed inflammatory infiltrate consisting of lymphocytes, plasma cells, and histiocytes, as well as occasional giant cells containing amastigotes, which are visible on routine H&E, Giemsa, or immunohistochemistry staining as 2- to 4-µm, round to oval, intracytoplasmic bodies (Fig. 7.15).[255-257] In addition to biopsy, lesional smear preparation is a routine method of amastigote identification, but, because lesions may contain few organisms, other diagnostic methods, including culture and molecular analysis, should also be considered.[258,259] Treatment options include meglumine, amphotericin B, and pentamidine, with a cure rate of approximately 88%.[260]



• **Figure 7.15** Leishmaniasis. **A,** Nasal septal biopsy specimen from a patient with chronic infection, showing surface ulceration, underlying granulation tissue, and mixed inflammation (×200). **B,** Organisms appear as intracytoplasmic bodies within histiocytes (×1000).

Epstein-Barr Virus

EBV is an enveloped DNA herpesvirus that infects more than 90% of people worldwide and is associated with a variety of head and neck infections, notably infectious mononucleosis and oral hairy leukoplakia (OHL), as well as numerous neoplastic processes, both epithelial and hematogenous, including nasopharyngeal carcinoma, Hodgkin lymphoma, nasal type NK/T-cell lymphoma, Burkitt lymphoma, and lymphoepithelial carcinoma of the salivary glands.[261-263]

Infectious mononucleosis, commonly referred to as the "kissing disease," is one of the most common diseases of adolescence. It is transmitted primarily through saliva and typically produces fever, malaise, pharyngitis, lymphadenopathy, and an atypical lymphocytosis that reflects the virus's infection of B lymphocytes.[264] Diagnosis relies on a combination of clinical findings and serologic studies, the most common being heterophile antibody detection (as in the monospot test), which is used to identify the presence of EBV-induced antibodies that cross-react with horse, sheep, and bovine erythrocytes.[265] Other organisms associated with an infectious mononucleosis–like syndrome (heterophile-negative infectious mononucleosis) include cytomegalovirus, HIV, HSV, human herpesvirus 6 (HHV-6), *S. pyogenes*, adenovirus, and *Toxoplasma gondii*.[266] Additional serologic evaluation of antibodies to various viral protein complexes, including early antigen (EA), viral capsid antigen (VCA), and nuclear antigen (EBNA), is useful for patients in whom a falsely heterophile-negative infection (common in young children and older adults) is suspected or to document prior EBV infection.[267] Tissue diagnosis is not usually required except in cases with atypical or incomplete presentations (e.g., asymptomatic tonsillitis).[268,269]

Histologic findings in tonsillar tissue mirror those seen in the lymph nodes and are described in detail in Chapter 12. Infectious mononucleosis usually follows a benign, self-limited course, although some patients may develop chronic active infection characterized by recurrent bouts of infectious mononucleosis–like symptoms, hepatosplenomegaly (rarely encountered in acute infection), and an abnormal pattern of anti-EBV antibodies.[270]

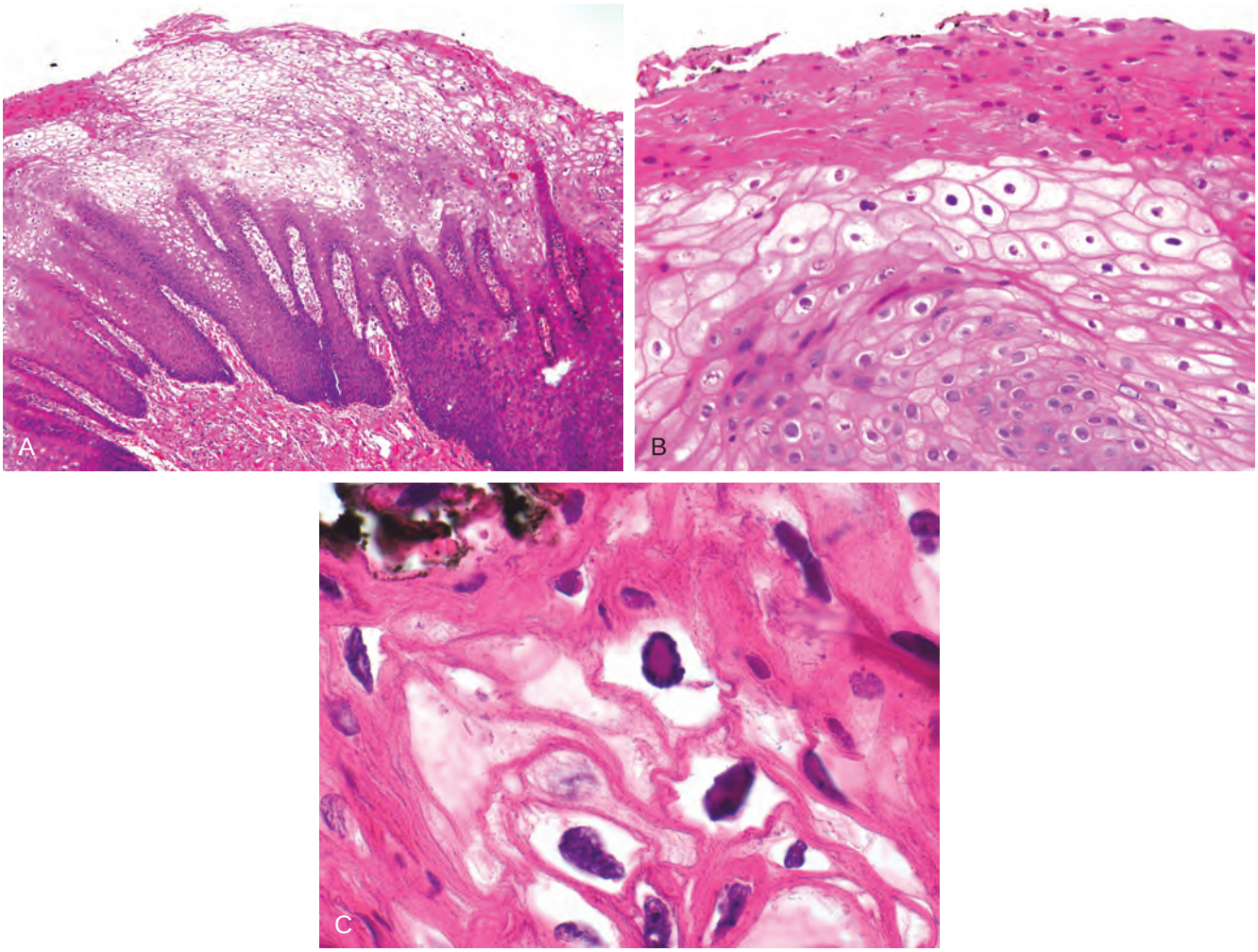
OHL is a benign condition seen almost exclusively in immunocompromised patients, particularly HIV patients, but also in the setting of transplantation, leukemia, and chemotherapy; it manifests on the lateral and dorsolateral tongue as white vertical folds or ridges that cannot be scraped off.[271] Long-term use of a steroid inhaler for chronic respiratory conditions has also been associated with OHL.[272] The histologic features include ballooning degeneration of keratinocytes of the upper stratum spinosum (sometimes with Cowdry type A inclusions or peripheral chromatin beading), epithelial hyperplasia with hyperparakeratosis, and mild to absent submucosal chronic inflammation (Fig. 7.16).[273,274] Immunohistochemistry, in situ hybridization, and polymerase chain reaction are all available as diagnostic aids.[275] Therapy is not necessary unless lesions are symptomatic, and antiviral therapy is generally effective treatment.[276]

Herpes Simplex Virus

HSV types 1 and 2 are closely related neurotropic, double-stranded DNA viruses of the *Herpesviridae* family that gain entry to the body through the skin and mucous membranes, producing chronic recurrent mucocutaneous disease during the active "lytic" infectious phase and lying dormant in sensory neurons during the "latent" phase.[277] Although HSV types 1 and 2 are considered to be "oral" and "genital" types, respectively, they are not restricted to these sites with regard to causing disease.[278] Primary infection is usually subclinical, although rare infected individuals may present with acute herpetic gingivostomatitis or pharyngotonsillitis.[279]

The most common head and neck manifestation of HSV infection is recurrent herpes labialis, commonly referred to as a cold sore or fever blister, which represents reactivation of dormant virus and is characterized by burning or tingling in the affected site, followed several hours later by the formation of vesicles that rupture and coalesce to form larger, irregular, crusted erosions.[280] Recurrent herpes stomatitis is much less common than recurrent herpes labialis and may be mistaken clinically for recurrent aphthous stomatitis or shingles. In immunocompromised patients, recurrent herpes stomatitis can be extensive and severe.[281]

Diagnosis is usually made clinically, although a number of laboratory methods are available for use when the diagnosis is not straightforward, including cytology (Tzanck smear), biopsy, culture, serology, and viral DNA detection.[282] The Tzanck method



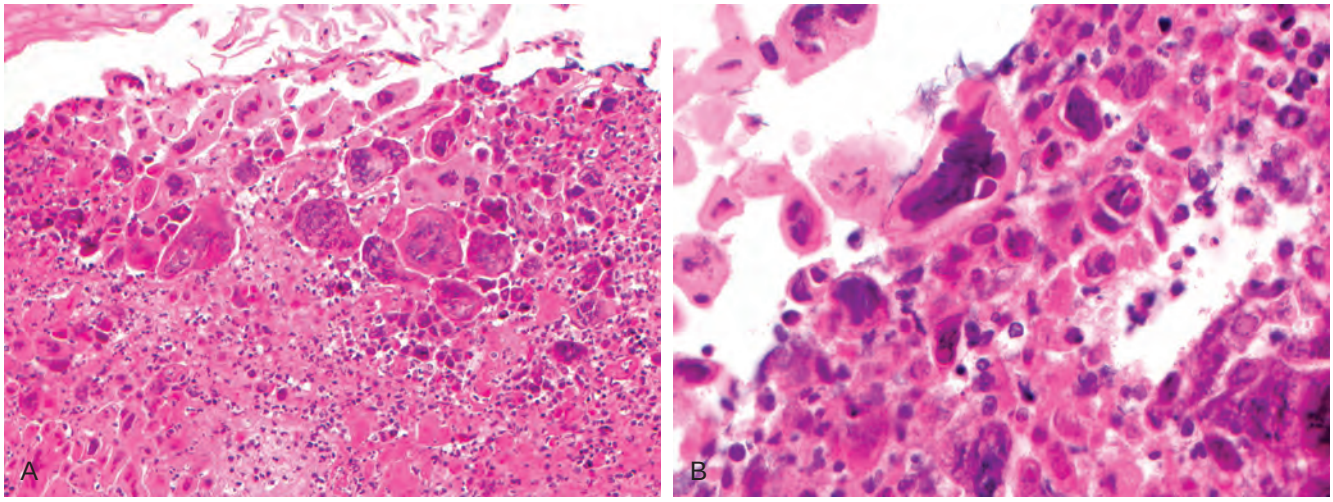
• **Figure 7.16** Oral hairy leukoplakia. **A,** Irregular hyperplasia with parakeratosis and superficial cytoplasmic clearing (ballooning degeneration) (×100). **B,** Ballooning degeneration in the upper spinous layer in a patient with a concomitant candidal infection (×400). **C,** Balloon cell with a Cowdry type A inclusion, characterized by a glassy intranuclear inclusion and peripheral chromatin margination (×1000).

involves direct examination of lesional material for the presence of the Tzanck cell, an epithelial cell containing numerous nuclei, demonstrating nuclear molding and viral inclusions that give the nuclei a homogeneous, glassy appearance and can be seen in HSV, herpes zoster, varicella, and the pemphigus group of autoimmune blistering diseases.[283,284] Although biopsy is not usually performed, it reveals intraepithelial vesicles with epithelial ballooning degeneration, acantholysis, intranuclear inclusions, and multinucleate giant cells with characteristic nuclear molding and positive staining by immunohistochemistry (Fig. 7.17). Treatment is aimed at reducing symptom duration, as well as the frequency and severity of attacks, and consists of antiviral therapy.[285]

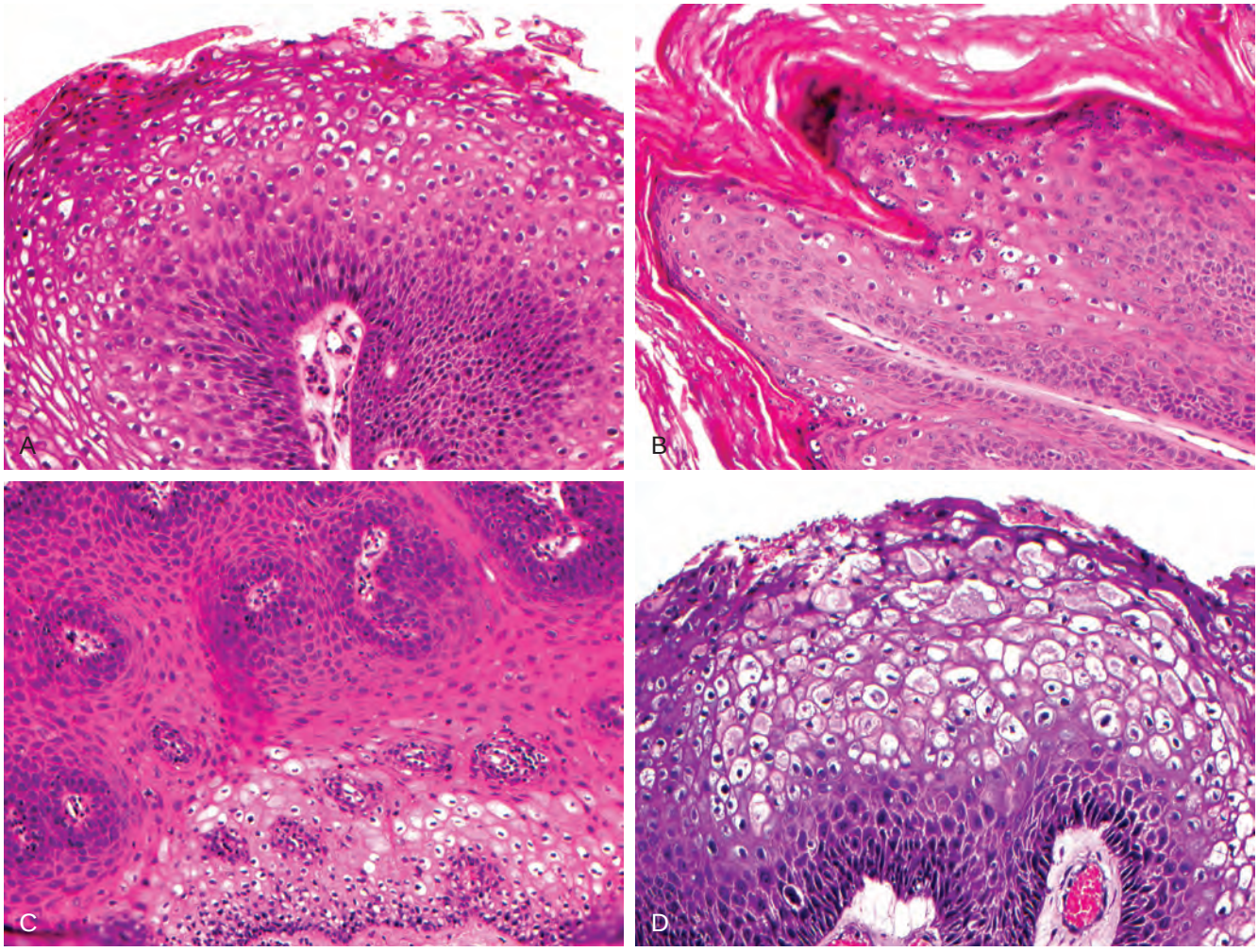
Human Papillomavirus

The human papillomavirus (HPV) family comprises a heterogeneous group of epitheliotropic, double-stranded DNA viruses that cause verrucae and cervical cancer, as well as a variety of reactive and neoplastic processes affecting the head and neck, including 25% to 33% of oropharyngeal cancers, which are attributable to infection with the high-risk anogenital subtypes 16 and 18.[286] Oral squamous papillomas, oral verruca vulgaris, oral condyloma acuminatum, focal epithelial hyperplasia (also known as Heck disease), and fungiform sinonasal Schneiderian papillomas are all associated with HPV infection to some degree, and they share the common histologic feature of epithelial koilocytosis, characterized histologically by a nucleus that appears shrunken, dense, and irregular (often referred to as "raisinoid") surrounded by a sharp rim of perinuclear clearing (the "halo") (Fig. 7.18).[287-289] Verruca vulgaris and condyloma acuminatum are described in detail in Chapter 20.

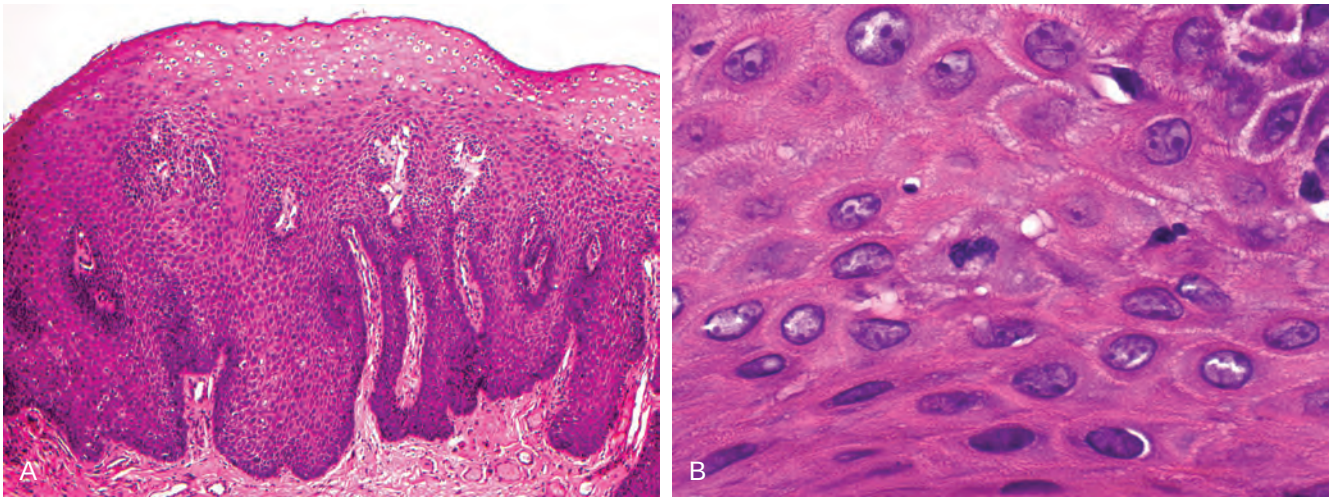
Focal epithelial hyperplasia is an uncommon benign, contagious HPV infection (usually caused by HPV type 13 or 32) that affects mainly Native Central and South American children, particularly those who express HLA-DR4. It causes multiple, asymptomatic, 1- to 10-µm exophytic papules or nodules on the oral mucosa, gingiva, tongue, lip, and palate.[290,291] Biopsy reveals abrupt exophytic acanthosis and hyperparakeratosis of the mucosa with confluent, widened, club-shaped rete ridges that extend to the same depth as adjacent normal rete, with scattered koilocytes and mitosoid bodies (irregular nuclei resembling mitotic figures) (Fig. 7.19).[292] Immunohistochemistry and hybridization techniques



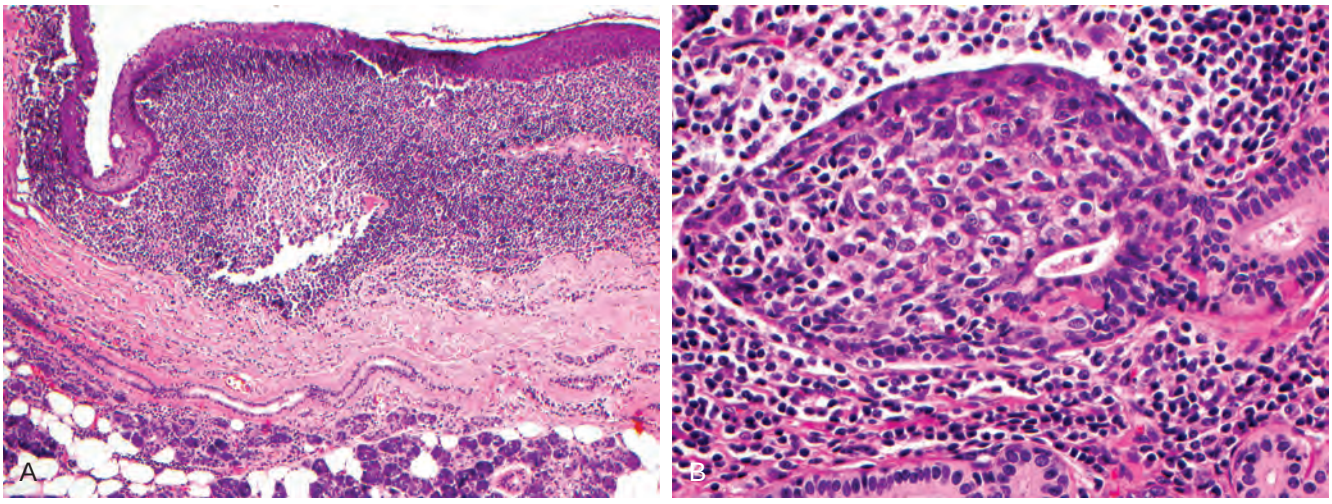
• **Figure 7.17** Herpes labialis. **A,** Severe infection characterized by necrosis, acantholysis, and numerous intracellular inclusions (×100). **B,** Typical herpes simplex virus inclusion, demonstrating multinucleation and molding (×600).



• **Figure 7.18** Human papillomavirus effect (koilocytosis). **A,** Oral squamous papilloma (×200). **B,** Nasal vestibular verruca vulgaris (×200). **C,** Oral condyloma acuminatum (×200). **D,** Fungiform sinonasal Schneiderian papilloma (×200).



• **Figure 7.19** Focal epithelial hyperplasia. **A,** Acanthotic mucosa with parakeratosis, irregular rete, and superficial koilocytes (×100). **B,** Characteristic mitosoid body (×1000).



• **Figure 7.20** Human immunodeficiency virus–associated lymphoepithelial cyst. **A,** Squamous-lined cyst with surrounding follicular lymphoid infiltrate and fibrosis (×100). **B,** Characteristic lymphoepithelial lesion, showing squamous metaplasia of the affected duct (×400).

are available to confirm the presence of HPV; in cases in which lesions do not regress spontaneously, topical interferon, cryosurgery, or laser treatment may be beneficial.[293,294]

Human Immunodeficiency Virus

HIV is a lentivirus that is responsible for the clinical syndrome of AIDS; it infects CD4-positive T lymphocytes and other cells in the human body and results in disease manifestations relating directly to the virus, as well as a variety of opportunistic infectious agents.[295] Specific head and neck manifestations (excluding opportunistic infections) include acute retroviral syndrome, infection of extranodal lymphoid tissues, and HIV salivary gland disease. Acute retroviral syndrome manifests 2 to 6 weeks after exposure, with nonspecific symptoms and laboratory findings that can include fever, fatigue, pharyngitis, weight loss, night sweats, lymphadenopathy, myalgias, headache, nausea, diarrhea, leukopenia, thrombocytopenia, and mild transaminitis, as well as an infectious mononucleosis–like syndrome that may provide an important clue to the diagnosis.[296]

Infection of the extranodal lymphoid tissues of Waldeyer ring results in tonsillar enlargement with histologic changes identical to those seen in nodal tissue, with the additional finding of virus-harboring, Langhans-type multinucleate giant cells that concentrate at the lymphoepithelial junction and have also been identified in HIV-associated lymphoepithelial cysts of salivary gland tissue.[297] Such cysts affect the parotid gland, with infection of periparotid nodal tissue causing gland enlargement and histologic findings similar to those seen in affected lymph nodes, along with cystic dilation of squamous-lined ductal structures that demonstrate infiltration by lymphoid cells and a variety of metaplastic changes (lymphoepithelial lesions) (Fig. 7.20).[298] Lymphoepithelial cysts of the salivary gland are not limited to HIV patients, and there is evidence to suggest that, at least in some cases, they arise not from lymphoid hyperplasia but from chronic sialadenitis.[299]

Mumps

Mumps is caused by a single-stranded RNA virus of the paramyxovirus family that is transmitted through respiratory droplets and commonly affects the salivary glands of unvaccinated children.[300] In recent years, several outbreaks have led to reconsideration of current vaccination practices to account for waning immunity.[301] After an incubation period of 16 to 18 days, patients experience a prodrome of headache, anorexia, malaise, myalgias, and fevers followed by unilateral or bilateral parotid swelling and tenderness, sometimes with associated otalgia, difficulty speaking, or trismus.[300] Although the diagnosis is usually made on clinical findings, histologic features of the affected gland include hemorrhage, necrosis, and a chronic inflammatory infiltrate composed of lymphocytes and plasma cells. Therapy is generally not required, because the infection is self-limited.

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8

Pathology of Pulmonary Infection

RICHARD L. KRADIN, EUGENE J. MARK

Introduction
- Approach to Sampling for Infection
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- Fine-Needle Aspiration Biopsy
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Handling Lung Biopsies

Pulmonary Injury in Infection
- Pulmonary Host Response
- Diffuse Alveolar Damage
- RNA Viruses
- DNA Viruses
- Other Atypical Pneumonias
- Bacterial Infections
- Mycobacterial Infection
- Fungal Infection Due to Yeasts
- Hyphate Fungi
- Dematiaceous (Pigmented) Fungi
- Parasites

Microbes Associated With Bioterrorism
- Anthrax
- *Yersinia pestis* (Plague Pneumonia)
- *Francisella tularensis* (Tularemia Pneumonia)

Pleural Infection

Introduction

In its role as a portal between the ambient environment and the internal milieu, the lung is also the most frequent site of serious infection. A variety of factors predispose to pulmonary infection, including distortions in lung anatomy, decreased mucociliary clearance, and abnormal cellular and humoral immunity. Iatrogenic immunosuppression and that resulting from human immunodeficiency virus type 1 (HIV-1) infection have led to the emergence of opportunistic infections that can present diagnostic challenges for the surgical pathologist.

Because a variety of microbes can potentially infect the lung and the histopathology of noninfectious conditions frequently mimic infection, the differential diagnosis of pulmonary infection is often broad. Although in many cases the clinical history, radiographic findings, and the noninvasive sampling of secretions can establish the cause of infection, lung biopsy is required at times. How to approach the sampling of the lung in infection is an area of some complexity, so that it is incumbent upon both clinicians and pathologists to recognize the advantages and limitations of the currently available methods.

Approach to Sampling for Infection

The optimal approach to sampling the lung for infection depends primarily on whether disease is localized or diffuse (Box 8.1). In immunosuppressed patients, diffuse pulmonary infiltrates due to infection can often be diagnosed by sputum induction or bronchoalveolar lavage (BAL). This is particularly the case when the microbial burden is also large. Noninvasive approaches are less sensitive than biopsy in diagnosing localized infections, and they cannot distinguish a colonizing commensal from an invasive pathogen.[1] In addition, the lung biopsy affords an opportunity to evaluate host immunocompetence with accuracy that cannot be achieved by noninvasive or minimally invasive methods. Lung biopsy may also be required to exclude infection definitively and to establish noninfective diagnoses (e.g., acute lung injury due to chemotherapy).

Pathologists prefer to examine generous samplings of lung because diagnoses based on such biopsies are more accurate, yield a greater degree of information with respect to host immunity, and may reveal other potentially treatable disorders.[2]

Transbronchial Biopsy

The lung has approximately the surface area of a tennis court; thus sampling error is an unavoidable pitfall in diagnostic pulmonary pathology. The transbronchial biopsy (TBB) preferentially samples peribronchiolar lung tissue, yielding tissue fragments of 1 to 3 mm in diameter.[3] However, peripherally located lung lesions often cannot be accessed by this approach.

TBB is generally adequate for diagnosing diffuse pulmonary infections and peribronchiolar granulomatous diseases, such as sarcoidosis and lymphangitic spread of tumor or tuberculosis, but at times the findings of a TBB can be nonspecific and misleading. For example, "organizing pneumonia" in a TBB may represent a nonspecific reaction adjacent to focus of infection or malignancy, a nonspecific manifestation of chemotherapy effect, aspiration, or cryptogenic disease. For this reason, the findings gleaned from a TBB must always be thoughtfully correlated with clinical and radiographic findings.[4]

• BOX 8.1 Approach to the Isolation of Pulmonary Microorganisms

Expectorated sputum
Induced sputum
Bronchoalveolar lavage
Fine-needle aspirate (1 mm)
Bronchial biopsy (1-3 mm)
Transbronchial biopsy (1-3 mm)
Transbronchial needle biopsy (1 mm)
Video-assisted thoracoscopic biopsy (2-3 cm)
Open-lung biopsy (2-3 cm)
Surgical lobectomy
Autopsy

Fine-Needle Aspiration Biopsy

Computed tomography (CT)-guided fine-needle aspiration biopsies have a high yield in the diagnosis of peripheral nodular infiltrates.[5] Biopsies can be semiliquid or include a 1-mm core of tissue. When performed with the assistance of a cytotechnologist, rapid diagnoses can be proffered by examining stained smears directly at the bedside. Fine-needle aspirates are useful in diagnosing localized infections, and cytopathologists can also suggest the pattern of inflammation based on the types of inflammatory cell subsets in the sample and the presence or absence of necrosis.

Transbronchial Needle Aspiration Biopsy

Transbronchial needle aspiration biopsy of regional lymph node groups is often a low-yield procedure because nonspecific reactive regional lymphadenitis is common in the presence of pulmonary infection.[6] The procedure is prone to artifacts that may present diagnostic difficulties for the surgical pathologist. However, when adopted judiciously, this approach may be adequate for the diagnosis of infection, as in one series in which approximately 50% of cases of tuberculous lymphadenitis were accurately diagnosed.

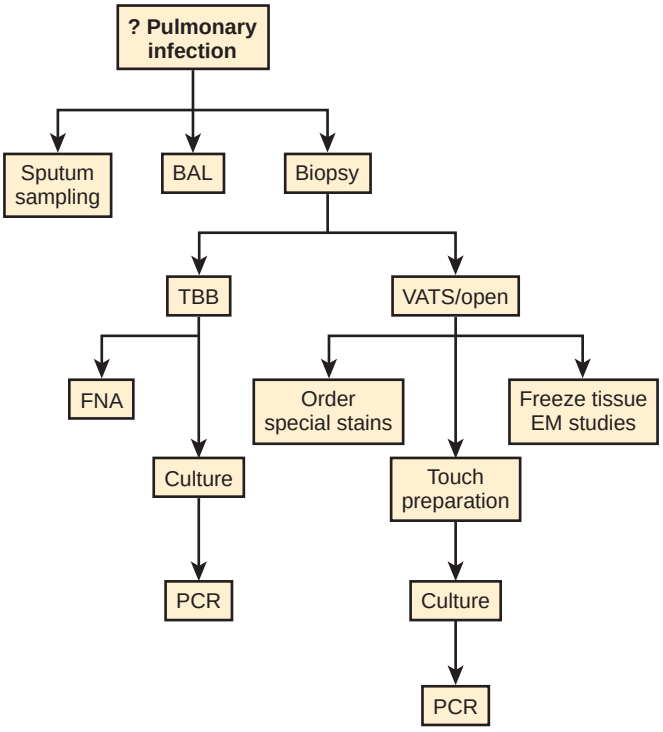
The technique of endobronchial ultrasound-guided transbronchial and needle biopsies has become standard procedure in many centers. This approach increases the diagnostic yield for localized lesions and allows for mediastinoscopic evaluation. It may be particularly helpful in patients with lung tuberculosis with mediastinal adenopathy.[7]

Video-Assisted and Open Thoracoscopic Biopsy

Video-assisted thoracoscopic surgical (VATS) lung biopsy has replaced open thoracotomy biopsy as the optimal approach for obtaining large samples of lung.[8] The procedure is associated with modest and acceptable morbidity, has the advantage of allowing direct access to widely separated lung segments, and provides generously sized wedge biopsies of 2 to 3 cm. Consequently, VATS should be considered a first line approach when a timely accurate diagnosis is essential.

Handling Lung Biopsies

Appropriate handling of the lung biopsy is essential for obtaining the highest diagnostic yield[9] (Fig. 8.1). Sampling the lung for microbiologic culture should ideally take place under sterile conditions in the operating room, but the pathologist processing the biopsy should try to ascertain that all necessary diagnostic tests have been ordered and be prepared to harvest additional samples for testing that may have been overlooked. When preparing tissue for microbiologic isolation, the lung should be minced rather than crushed because hyphate fungi (e.g., *Zygomycoses* spp.) may fail to grow in culture following maceration.[10] A pathologist should not place a lung biopsy directly into fixative, without first considering a diagnosis of infection. If questions arise as to which tests to order or how best to transport the specimen to the laboratory, discussions with the hospital microbiology laboratory staff or a hospital infectious disease specialist will be of assistance.



• Figure 8.1 Approach to the handling of lung biopsies. *BAL,* Bronchoalveolar lavag; *EM,* electron microscopy; *FNA,* fine needle aspiration; *PCR,* polymerase chain reaction; *TBB,* transbronchial biopsy; *VATS,* video-assisted thoracoscopic surgical.

The examination of touch imprints of lung tissue is a simple and rapid way of identifying pathogens. Touch imprints can be prepared from foci of pulmonary consolidation, necrosis, or suppuration and rapidly stained for bacteria, mycobacteria, and fungi in the surgical pathology suite or the microbiology laboratory. Concomitantly biopsies may be harvested for ultrastructural analysis, polymerase chain reaction (PCR) assays, or research purposes. For large biopsies, it may be possible to inflate the lung with 5% formalin via a small (23 to 25) gauge needle to optimize subsequent histologic examination.

Pulmonary Injury in Infection

Pulmonary Host Response

The diagnosis of infection requires both an interpretation of the morphologic changes evoked by the pathogen and the identification of a pathogen in situ. The pattern of pulmonary inflammation often suggests the route of entry of an infectious agent and may help to narrow the diagnostic possibilities. It is necessary to be

familiar with the multiplicity of response patterns evoked by infection and to recognize that these can vary depending on the route of entry, pathogen load, and the competence of host defenses. For example, whereas herpesvirus 1 can produce a miliary pattern of fibrinoid necrosis in an immunosuppressed patient with viremia, it can also cause ulceration of the tracheobronchial mucosa in a chronically intubated patient.

Microbes are rarely identified randomly or diffusely in infections; rather, they tend to be compartmentalized, so that substantial effort may be wasted in searching for them where they are not likely to be found. Mycobacteria and fungi are usually localized in areas of necrosis; *Rickettsia* spp. and *Bartonella* spp. largely target the microvasculature; viruses tend to attack the airways; thus the surgical pathologist must both be acquainted with the pulmonary microanatomy and with the preferential localization of microbes in pulmonary tissues.

Anatomy of Pulmonary Defense

The lung is an elastic organ composed of dichotomously branching conducting airways terminating in alveolated surfaces.[11] It has a dual blood supply; the pulmonary circulation arises from the cardiac right ventricle and carries deoxygenated blood at low arterial pressures to the alveolar surfaces; the bronchial circulation arises from branches of the aorta and nourishes both the airways and the connective tissue stroma with oxygenated blood. All new growths within the lung, including regions of infective bronchiectasis, lung abscess, and tuberculous cavities, will evoke neoangiogenesis from the bronchial microcirculation.

Two systems of pulmonary lymphatic channels drain the lung either centrifugally towards the hilum, or centripetally along the convexities of the pleural surfaces before coursing to the hilar lymph nodes. From the lymph nodes, organisms can enter the systemic circulation and spread widely throughout the body. For example, group A streptococci spp. will rapidly invade pulmonary lymphatics and course to the pleura to produce an empyema.

Pulmonary Defenses

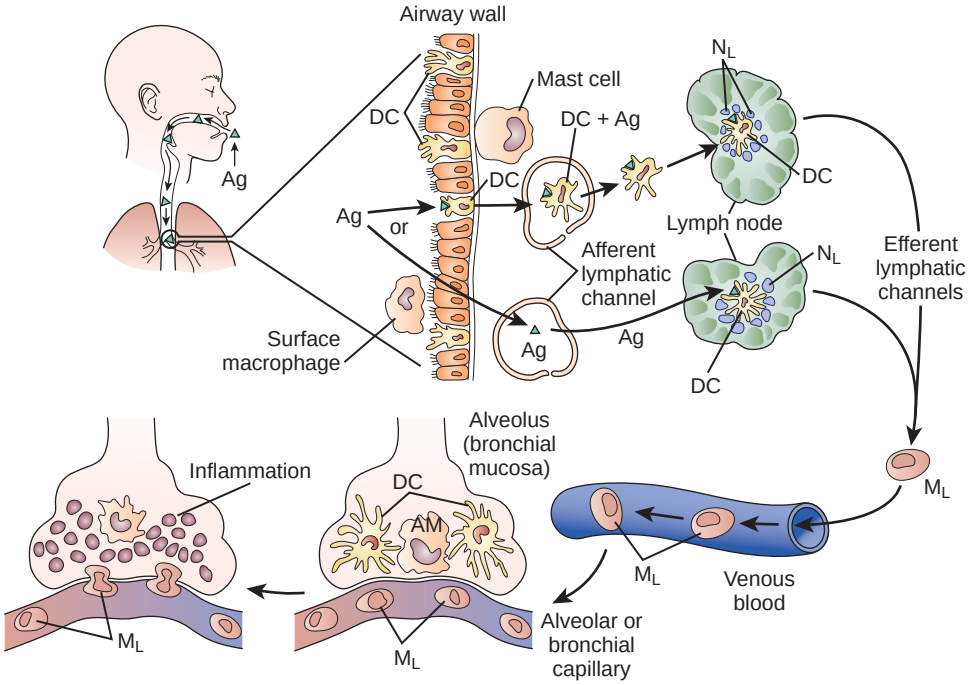
Most microbes are small (<5 μM) and can penetrate to the distal gas-exchanging surfaces of the lung, although the majority are excluded by the defenses of the upper airways or deposit along the conducting airways to be cleared by the mucociliary escalator. Humoral factors, including secretory immunoglobulin A (sIgA) and defensins released by airway cells, limit microbial penetration into tissues. Airway mucosal dendritic cells (DCs) trap microbial antigens and transport them to regional lymph nodes, where they are processed and present to both T and B lymphocytes, evoking adaptive immunity (Fig. 8.2).[12]

Ulceration or thickening of the gas-exchange surface limits diffusion of oxygen and carbon dioxide. For this reason, the alveolus is under normal conditions maintained sterile by resident macrophages that scavenge inhaled particulates and secrete monokines, including interleukin (IL)-10 and transforming growth factor (TGF)-β, that locally suppress inflammation and promote immunotolerance.

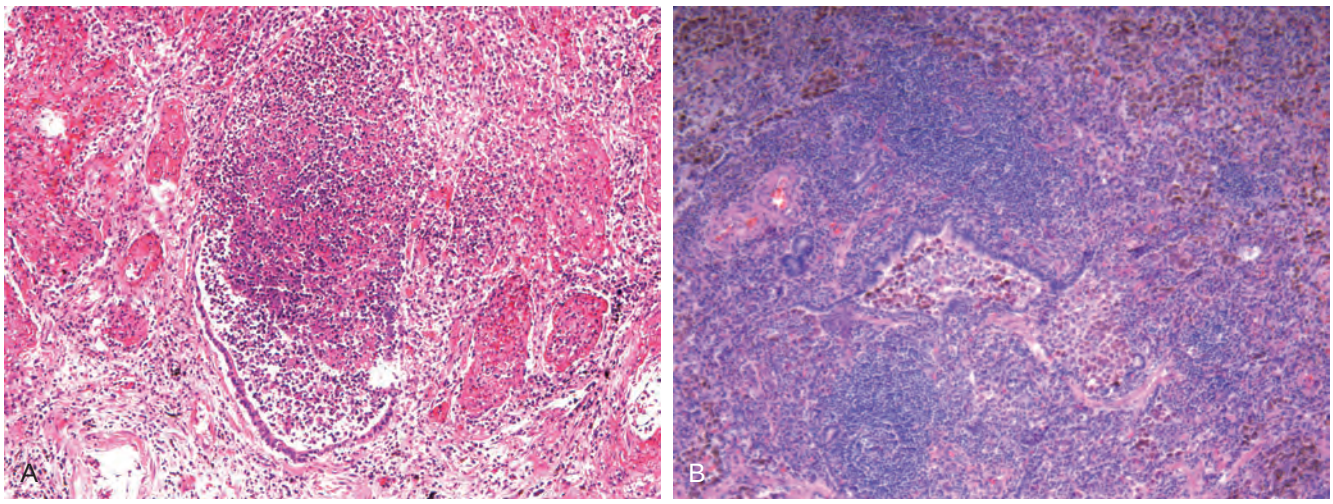
When the alveolar lining is injured or when the number of invading organisms exceeds the phagocytotic capacities of resident macrophages, neutrophils and exudate monocytes are recruited to sites of lung infection.[13] Even small numbers of virulent pathogens can greatly amplify inflammation via the release of chemokines, cytokines, and complement by host immune cells. These defenses promote the clearance of infection but can also damage the lung. Lung biopsies afford the pathologist a unique opportunity to assess these dynamic responses directly, in addition to identifying a causative pathogen.

Patterns of Lung Injury Due to Infection

A number of generic patterns of inflammation may be evoked by infection, but how they are distributed is often specific to the involved tissue (Box 8.2). Clinicians and radiologists have developed classification systems with respect to pulmonary infection



• **Figure 8.2** Pulmonary immune anatomy. *Ag,* Antigen; *DC,* dendritic cells; N_L, lymph node; M_L, macrophage.



• **Figure 8.3** **A,** Acute bronchiolitis showing neutrophilic exudate in the lumen of a small airway **B,** Chronic lymphocytic bronchiolitis.

• BOX 8.2 Injury Patterns Seen in Lung Infection

Tracheobronchitis
Bronchiolitis (acute, chronic, necrotizing)
Bronchiectasis
Bronchopneumonia (acute, chronic, necrotizing)
Eosinophilic pneumonia
Pulmonary hemorrhage
Pulmonary edema
Diffuse alveolar damage
Pulmonary nodules and micronodules
Cavitary pneumonia
Vasculitis
Capillary dissemination
Lymphatic dissemination
Pulmonary hypertension
Pleuritis

that are distinct from those of pathologists. For example, a variety of infectious agents yield a picture that clinicians generically term "atypical" interstitial pneumonia, to differentiate them from "typical" bacterial pneumonias.[14] However, the histopathology of an "atypical pneumonia" may be centered on the lung interstitium, small airways, or the alveolar spaces; and because this text is aimed primarily at surgical pathologists, pathologic schemas of classification will be adopted here, with reference to their clinical counterparts when appropriate.

Tracheobronchitis/Bronchiolitis and Miliary Infection

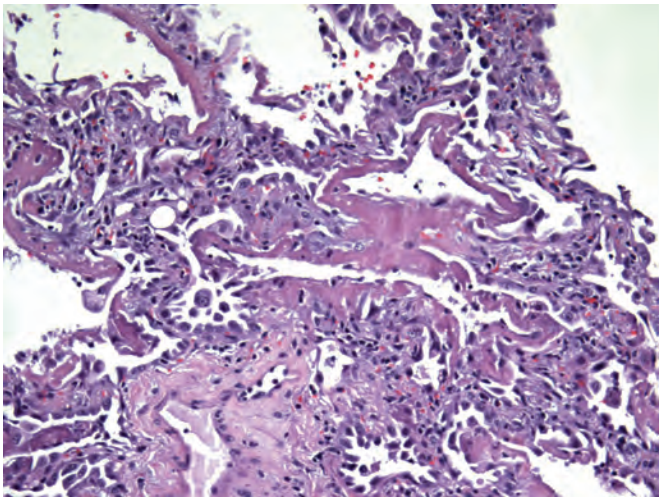
Many pathogens target the conducting airways to produce tracheobronchitis and bronchiolitis. Pathologic changes range from superficial erosion of the lining respiratory epithelium, to ulceration and repair. The type of inflammation will vary from intralumenal neutrophilic exudates (Fig. 8.3A) to airway cuffing by lymphocytes and histiocytes (see Fig. 8.3B), depending on the offending pathogen.

Diffuse Alveolar Damage

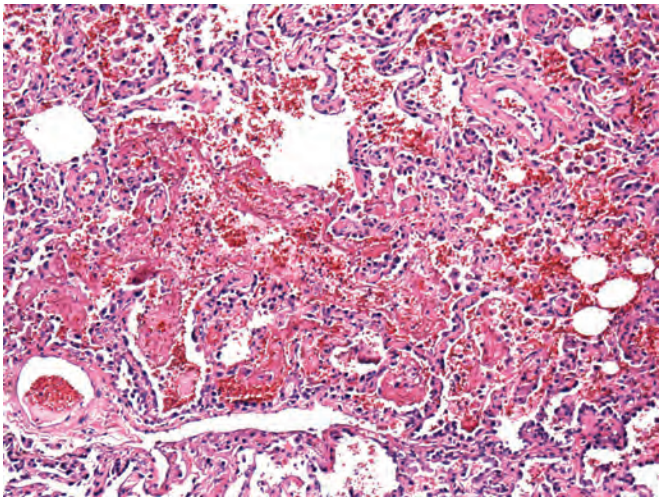
Disease of the gas-exchange alveolar surfaces can show a spectrum of changes, including acute ulceration and septal infiltration by

• **Figure 8.4** Consolidated lung with the beefy red appearance of diffuse alveolar damage in a patient who died of influenzal pneumonia.

chronic inflammatory cells.[15] Diffuse alveolar damage (DAD) represents a global injury to the gas-exchange surfaces due to disruption of the blood–air barrier leading to exudative edema and fibrosis and resulting in severely impaired blood and tissue oxygenation (Fig. 8.4). The sine qua non of DAD is the *hyaline membrane* that is composed of necrotic alveolar lining cell debris and an extravascular fibrin coagulum apposed to an ulcerated alveolar wall, which yields a gel that entraps lung water (Fig. 8.5). Although DAD is the most frequent pathologic cause of the clinical entity, the adult respiratory distress syndrome (ARDS),



• **Figure 8.5** Hyaline membrane lining an alveolar duct in diffuse alveolar damage



• **Figure 8.6** Lung in exudative phase of diffuse alveolar damage with hemorrhage and inflammation. This finding should not be overinterpreted as evidence of infection.

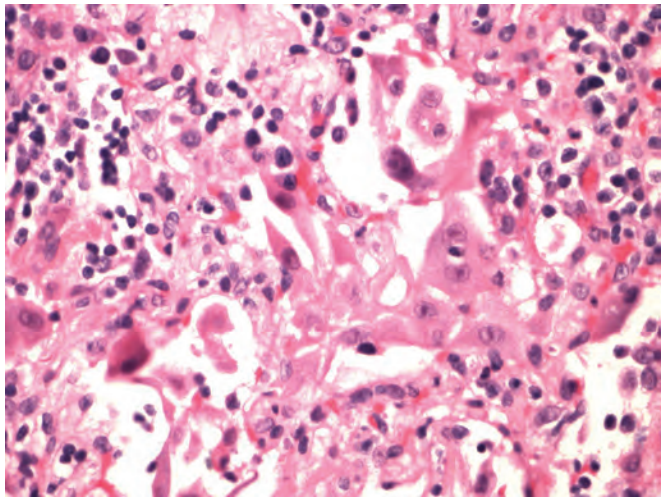
• BOX 8.3 Causes of Diffuse Alveolar Damage

Pulmonary infection
Systemic infection with sepsis
Vasodilatory shock
Aspiration
Drugs
Radiation
Trauma
Accelerated phase of chronic interstitial pneumonia
Idiopathic

other diseases, including extensive bronchopneumonia and acute pulmonary hemorrhage, can also lead to ARDS (Box 8.3). The pathology of the exudative phase of DAD can focally mimic acute bacterial infection, and one must maintain a high threshold for making the diagnosis of acute infection in this setting (Fig. 8.6).

Viruses are the most common infectious cause of DAD, although bacteria, fungi, and parasites also produce diffuse lung injury. DAD can result from sepsis that complicates either pulmonary or extrapulmonary infection. Whenever DAD is present, the surgical pathologist must examine the lung for evidence of viral-induced cytopathic changes. These vary with the type of viral infection, and some viruses do not produce cytopathic changes, so that viral infection is always in the differential diagnosis of DAD (Table 8.1). A common pitfall in diagnosis is to mistake the hyperplastic reparative alveolar type II cells of DAD that can exhibit prominent nucleoli with viral infected cells, particularly when examining rapidly frozen sections, in which these changes may be especially prominent (Fig. 8.7).

Due to the introduction of noninvasive and rapid PCR diagnoses of respiratory viruses from nasal swabs and respiratory secretions, it has become increasingly unusual to encounter these infections in the routine practice of surgical pathology, except at autopsy in lethal cases.

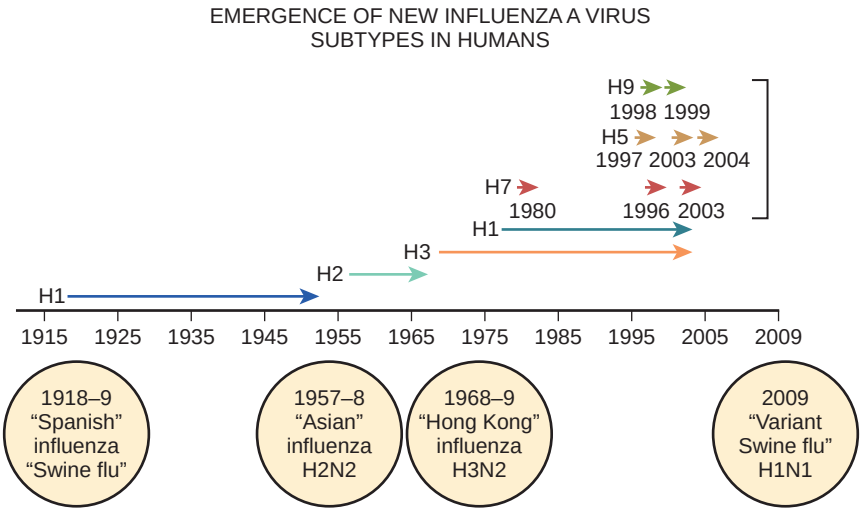


• **Figure 8.7** Lung in reparative phase of acute lung injury showing highly atypical alveolar lining cells with changes that mimic viral infection.

TABLE 8.1 Changes Seen in Virus-Infected Lung Cells

Organism	Cytopathic Change
Influenza	No cytopathic change
SARS (Coronavirus)	No cytopathic change
Respiratory syncytial virus	Polykaryons, inconspicuous cytoplasmic inclusions
Parainfluenza	Polykaryons, intracytoplasmic inclusions
Measles	Polykaryons, intranuclear inclusions
Adenovirus	Intranuclear inclusions (smudge cells)
Herpesvirus	Intranuclear inclusions, polykaryons
Cytomegalovirus	Intranuclear and cytoplasmic inclusions
Varicella-Zoster	Intranuclear inclusions
EBV	No cytopathic change

EBV, Ebstein-Barr virus; SARS, severe acute respiratory syndrome.



• **Figure 8.8** Neuraminidase and hemagglutinin expression of *Influenza* correlates with epidemic outbreaks.

RNA Viruses

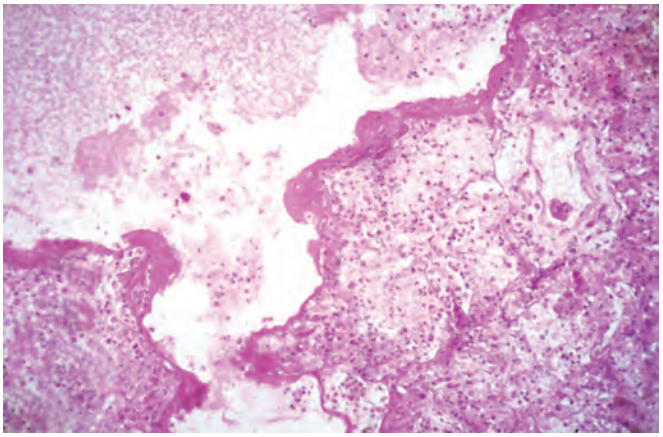
Influenza

Influenza is a rod-shaped RNA virus that can cause either bronchiolitis or DAD without apparent cytopathic changes. Influenza infection recurs each year due to a high incidence of mutation of its hemagglutinin (H) and neuraminidase (N) antigens, and these determine its virulence. When mutations occur concomitantly in both the H and N antigens, pandemics with potentially high degrees of morbidity due to the lack of immunity may ensue (Fig. 8.8).[16] H1N1 influenza was responsible for an epidemic in this country, and sporadic cases have persisted. Patients dying with this infection were often relatively young and their lungs showed DAD with prominent areas of hemorrhage.[17] Currently, epidemiologists are carefully monitoring the evolution of an avian influenza in Southeast Asia for evidence of spread to humans.

Influenza is the most common cause of viral pneumonia, although most cases are subclinical. The virus most commonly causes a diffuse tracheobronchitis/bronchiolitis in which the normal ciliated respiratory epithelium is sloughed.[18] However, when DAD develops, it can carry a high mortality even in the absence of acute bacterial superinfection (Fig. 8.9).[19] The lungs in DAD due to influenza in patients with prolonged survival often develop prominent squamous metaplasia of bronchoalveolar lining cells (Fig. 8.10A). Although these findings are characteristic, they are also nonspecific, so that immunostains, in situ hybridization, electron microscopy, or viral antigen detection may be required to establish the diagnosis (see Fig. 8.10B). Superinfection by pyogenic bacteria, including *Haemophilus influenza*, group A *Streptococcus*, and *Staphylococcus* are a well-recognized complication and may mask evidence of a healing influenza infection.

Severe Acute Respiratory Syndrome

The epidemic of the zoonotic coronavirus infection termed severe acute respiratory syndrome (SARS) fortunately has not recurred, as the virus led to acute respiratory distress with high mortality. The lungs at autopsy showed DAD with scattered multinucleated giant cells of uncertain diagnostic significance. The virus otherwise produced no cytopathic changes and was essentially histologically indistinguishable from DAD due to influenza.[20]



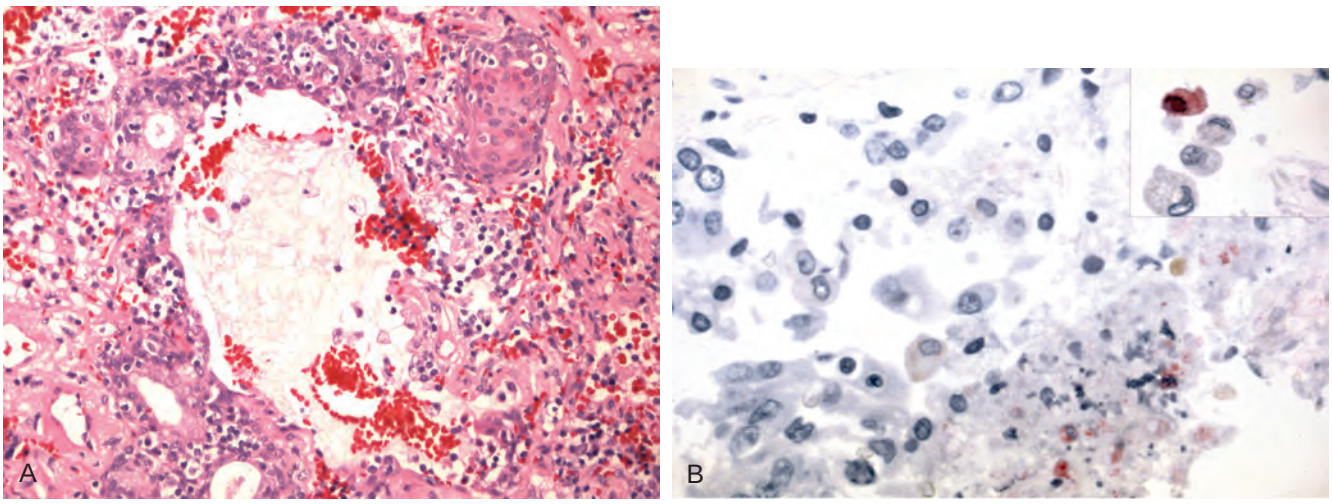
• **Figure 8.9** Lung from a patient who died in the 1918 influenza epidemic showing diffuse alveolar damage with no cytopathic changes.

Middle East Respiratory Syndrome

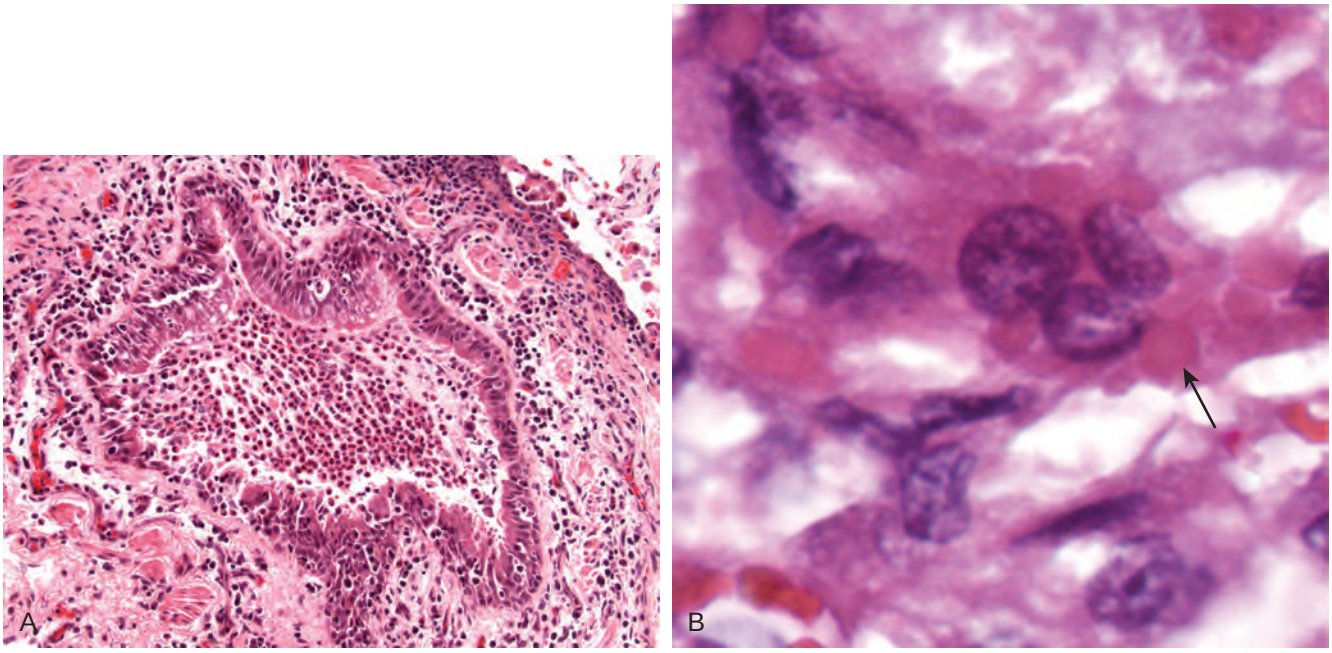
Like SARS, Middle East respiratory syndrome (MERS) is also caused by a coronavirus.[21] The first cases were reported in Saudi Arabia in the fall of 2012, but cases had occurred in Jordan earlier that year. All cases of MERS to date have been linked through travel to or residence in countries in and near the Arabian Peninsula. The largest known outbreak of MERS outside the Arabian Peninsula occurred in the Republic of Korea in 2015 and was associated with a traveler returning from the Arabian Peninsula. MERS-Corona virus (Co-V) spreads from ill people to others via close contact, such as caring for or living with an infected person, and patients have ranged in age from infants to nonagenarians. Unfortunately, the detailed histopathology of patients with MERS has not been reported but is likely to resemble that due to other coronaviruses.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) causes a benign respiratory infection in older children and has been recognized as a cause of adult community-acquired pneumonia, acute bronchiolitis, and



• **Figure 8.10** **A,** Lung in patient with diffuse alveolar damage due to influenza showing prominent squamous metaplasia of terminal airways. **B,** Immunostain confirms the presence of influenza A.



• **Figure 8.11** **A,** Acute bronchiolitis in respiratory syncytial virus (RSV). **B,** Multinucleated epithelial cells in RSV contain inconspicuous eosinophilic cytoplasmic inclusions *(arrow)*.

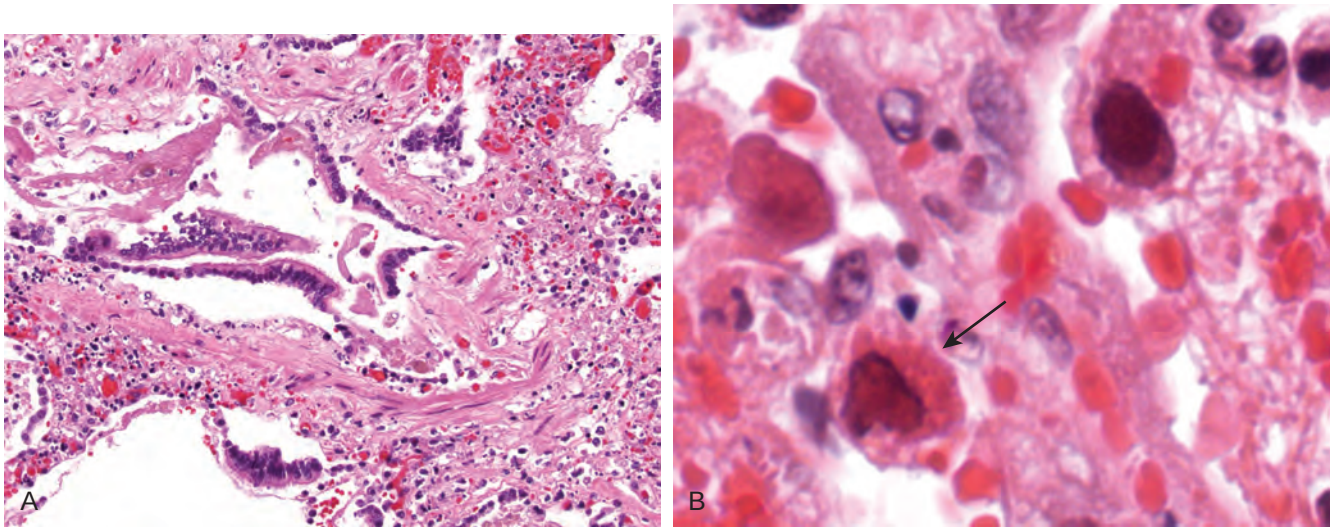
DAD in the immunosuppressed host.[22] The infection targets the respiratory lining epithelium producing syncytial giant cells with nonprominent eosinophilic inclusions (Fig. 8.11). Human metapneumovirus produces changes comparable to RSV and must be included in its differential diagnosis.

Parainfluenza

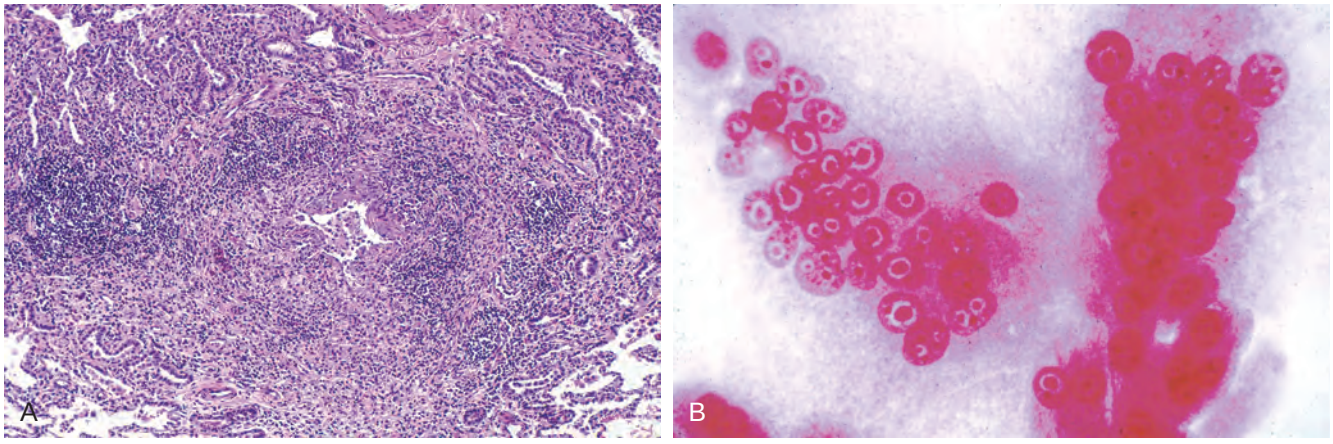
Parainfluenza causes a benign upper respiratory infection in children that rarely progresses to DAD, although severe disease may develop in the immunosuppressed host. Like RSV, parainfluenza produces bronchiolitis and DAD with syncytial giant cells and epithelial cell intracytoplasmic inclusions.[23] However, the latter are both more frequent and larger than those seen in RSV (Fig. 8.12).

Measles

Measles pneumonia is a rare and serious complication of the childhood viral exanthem. The pathology of pulmonary measles infection ranges from bronchiolitis (Fig. 8.13A) to DAD. The virus produces multikaryons with prominent glassy eosinophilic nuclear Cowdry type A inclusions (see Fig. 8.13B).[24] The differential diagnosis of giant cell pneumonia includes RSV and hard-metal pneumoconiosis; however, the giant cells in the latter disorders lack intranuclear inclusions, and hard-metal



• **Figure 8.12** **A,** Bronchiolitis in parainfluenza infection. **B,** Epithelial cell showing eosinophilic inclusions that are both larger and more frequent than in respiratory syncytial virus *(arrow)*.



• **Figure 8.13** **A,** Chronic inflammation involving a small airway in a patient with measles. **B,** Multinucleated cell showing glassy nuclear Cowdry type A inclusions.

pneumoconiosis specifically lacks the exudative features of an acute infection.

DNA Viruses

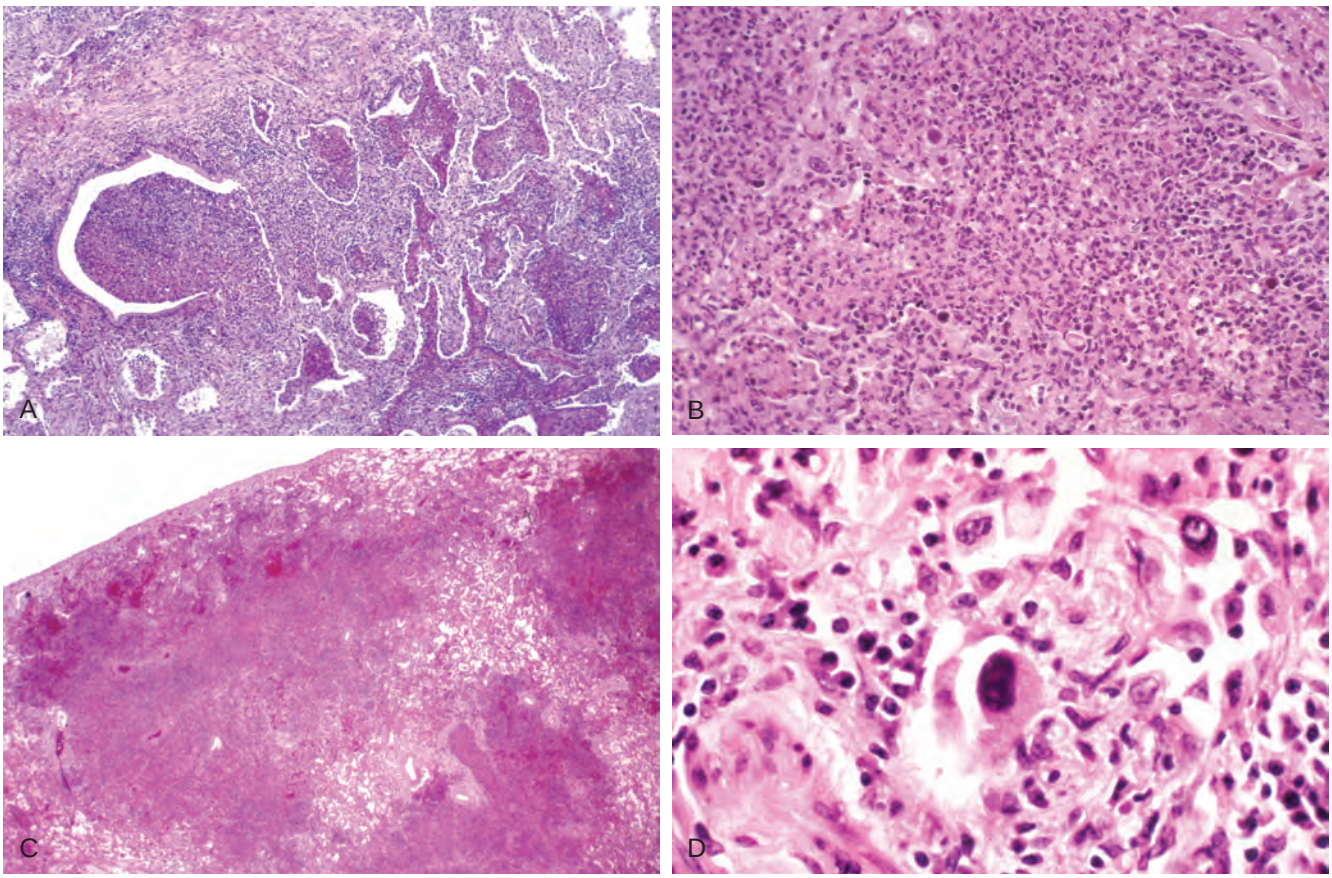
Adenovirus

Adenovirus primarily affects the immunosuppressed host but can produce outbreaks in healthy subjects living at close quarters (e.g., military recruits). Adenovirus typically produces (1) ulcerative bronchiolitis with karyorrhexis (Fig. 8.14A), (2) neutrophilic pneumonia (see Fig. 8.14B), (3) acute intrapulmonary necrosis with hemorrhage (see Fig. 8.14C), or (4) DAD. Infected cells can exhibit amphophilic intranuclear inclusions with perinuclear clearing that mimic *herpesvirus* infection but more characteristically produce "smudge cells" (see Fig. 8.14C), showing hyperchromatic nuclei extruding beyond the confines of their nuclear membranes.[25]

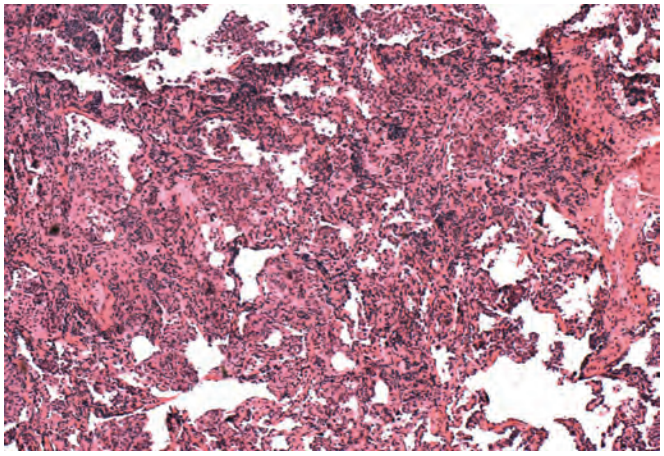
The appearance of "smudge cells" can be mimicked by pulmonary cytotoxic drug injury or by epithelial repair in the early proliferative phase of DAD. For this reason and because of the potential overlap with herpesvirus-induced cytopathic changes, the diagnosis of adenovirus infection should always be confirmed by immunohistochemical staining, ultrastructural examination, or viral isolation.

Cytomegalovirus

Cytomegalovirus (CMV) occurs at the extremes of age or as a result of immunosuppression, and it is a common infection in HIV/acquired immune deficiency syndrome (AIDS).[26] The number of cells showing cytopathic changes can vary considerably and parallels the severity of infection. CMV primarily targets pulmonary macrophages and endothelial cells, but virtually any cell can show cytopathic features. The most common distribution is blood-borne miliary disease (Fig. 8.15), but bronchiolitis and DAD also occur. The diagnostic features of infection are (1) cytomegaly, (2) intranuclear inclusions with characteristic Cowdry type B inclusions (Fig. 8.16A), and (3) ill-defined amphophilic intracytoplasmic inclusions that are seen with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Gomori methenamine silver (GMS) stains (see Fig. 8.16B). In patients receiving prophylactic treatment with antivirals, CMV infection may fail to exhibit cytopathic changes. However, immunostains and in situ hybridization will continue to identify intracellular CMV antigens (see Fig. 8.16C).[27]



• **Figure 8.14** **A,** Ulcerative bronchiolitis in adenovirus infection. **B,** Neutrophilic pneumonia due to adenovirus. **C,** Necrotizing hemorrhagic pneumonitis. **D,** "Smudge cell" showing extrusion of nuclear contents beyond the confines of the nuclear membrane.



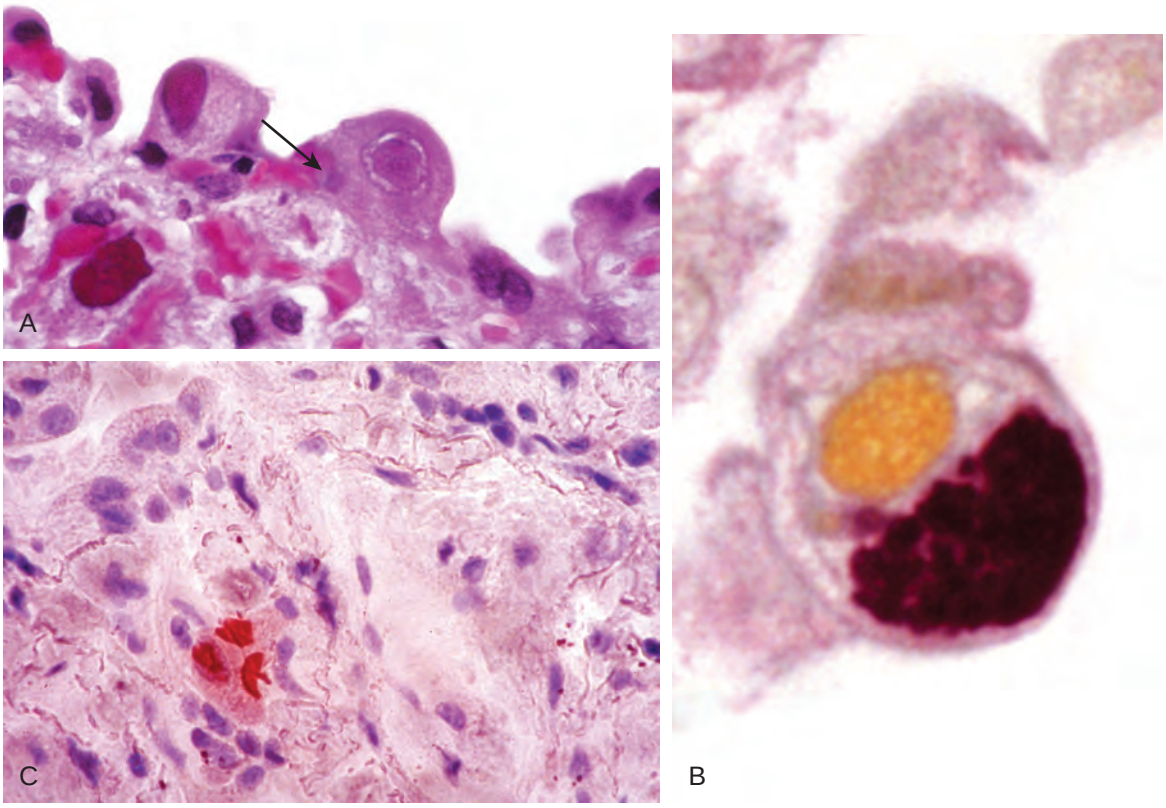
• **Figure 8.15** Focus of miliary infection in cytomegalovirus.

CMV is a frequent copathogen in the immunosuppressed patient and a cause of immunosuppression in its own right. Patients with active pulmonary infections will generally have circulating CMV DNA as judged by PCR assay of peripheral blood. CMV infection can also be seen together with other viral infections, *Pneumocystis jiroveci*, or opportunistic fungal infections (Fig. 8.17).

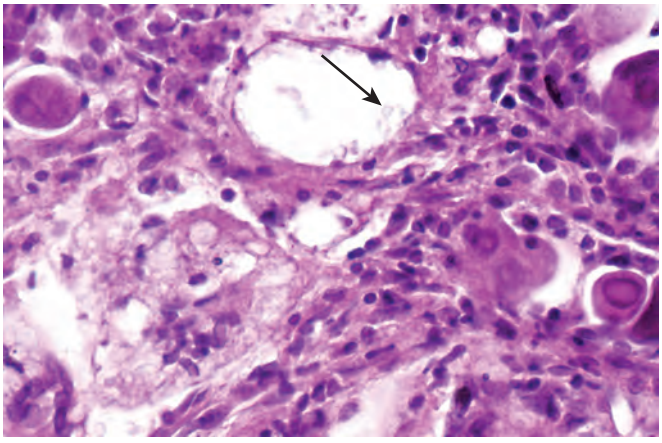
Herpesvirus

Herpesvirus types 1 and 2 both infect the lung.[28] The incidence of herpesvirus infection increases with immunosuppression and when mucosal barrier defenses have been breached. Herpesvirus characteristically elicits a prominent neutrophilic response that mimics pyogenic bacterial infection, but foci of necrosis, cell karyorrhexis, and piled up virus-infected cells with amphophilic nuclei confirm the diagnosis (Fig. 8.18). Diagnostic cytopathic changes include type A or type B Cowdry nuclear inclusions showing molding of adjacent cells with multikaryon formation (Fig. 8.19). Immunosuppressed patients with herpesvirus viremia develop miliary foci of hemorrhagic necrosis with prominent fibrinous exudates (Fig. 8.20A and B).[29] In a recently reported case, herpesvirus type 2 was demonstrated to occur as a superinfection in a patient recovering from a bacterial superinfection of influenza pneumonia (see Fig. 8.20C).[30]

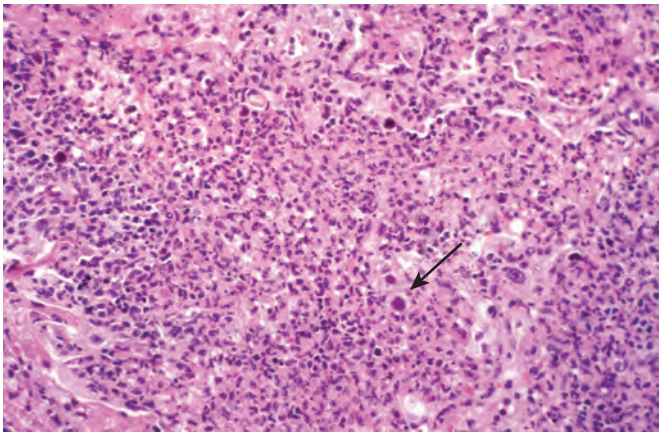
Herpesvirus pulmonary infections also develop in patients with structural abnormalities of the airways or as complications of primary infections of the oropharynx and esophagus. Intubated patients receiving chronic ventilatory support are at increased risk due to local barotrauma from inflated endotracheal tubes. The respiratory mucosa is the primary target (Fig. 8.21). At times, extensive necrosis of an ulcerated airway suggests the diagnosis, but immunostaining for herpes viral antigen can demonstrate high background staining, obscuring the diagnosis.[27] When this is the case, examining paraffin-embedded tissues by electron microscopy can reveal diagnostic virions (Fig. 8.22A to C).



• **Figure 8.16** **A,** Alveolar type II lining cells with prominent cytomegaly, Cowdry type B inclusions, and cytoplasmic inclusions *(arrow)*. **B,** Cytoplasmic inclusions stain positive with Gomori methenamine silver. **C,** Immunostain demonstrates cytomegalovirus antigen in a patient treated with gancyclovir.



• **Figure 8.17** Patient with cytomegalovirus infection and cryptococcal pneumonia *(arrow)* complicating human immunodeficiency virus/acquired immune deficiency syndrome.



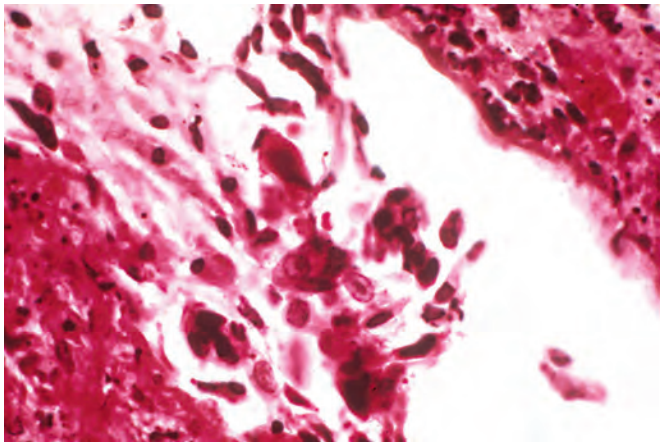
• **Figure 8.18** Neutrophilic bronchopneumonia due to herpesvirus 1. *Arrow* shows viral infected cell.

Varicella Zoster

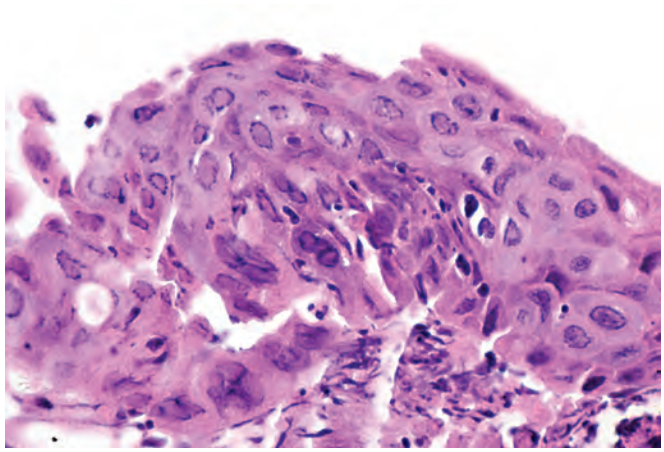
Varicella zoster pneumonia is a rare complication of the childhood chickenpox, and it is more commonly encountered as the consequence of reactivated virus in the immunocompromised host. Following nonlethal pulmonary infections in childhood, the lung shows multiple calcified miliary lesions. Cases coming to biopsy or autopsy show miliary nodules of hemorrhagic necrosis in lung and pleura or DAD (Fig. 8.23).[31] Infected cells with primarily Cowdry type A inclusions may be seen at the edges of the lesion, but they are harder to identify than in herpesvirus pneumonia.

Hantavirus

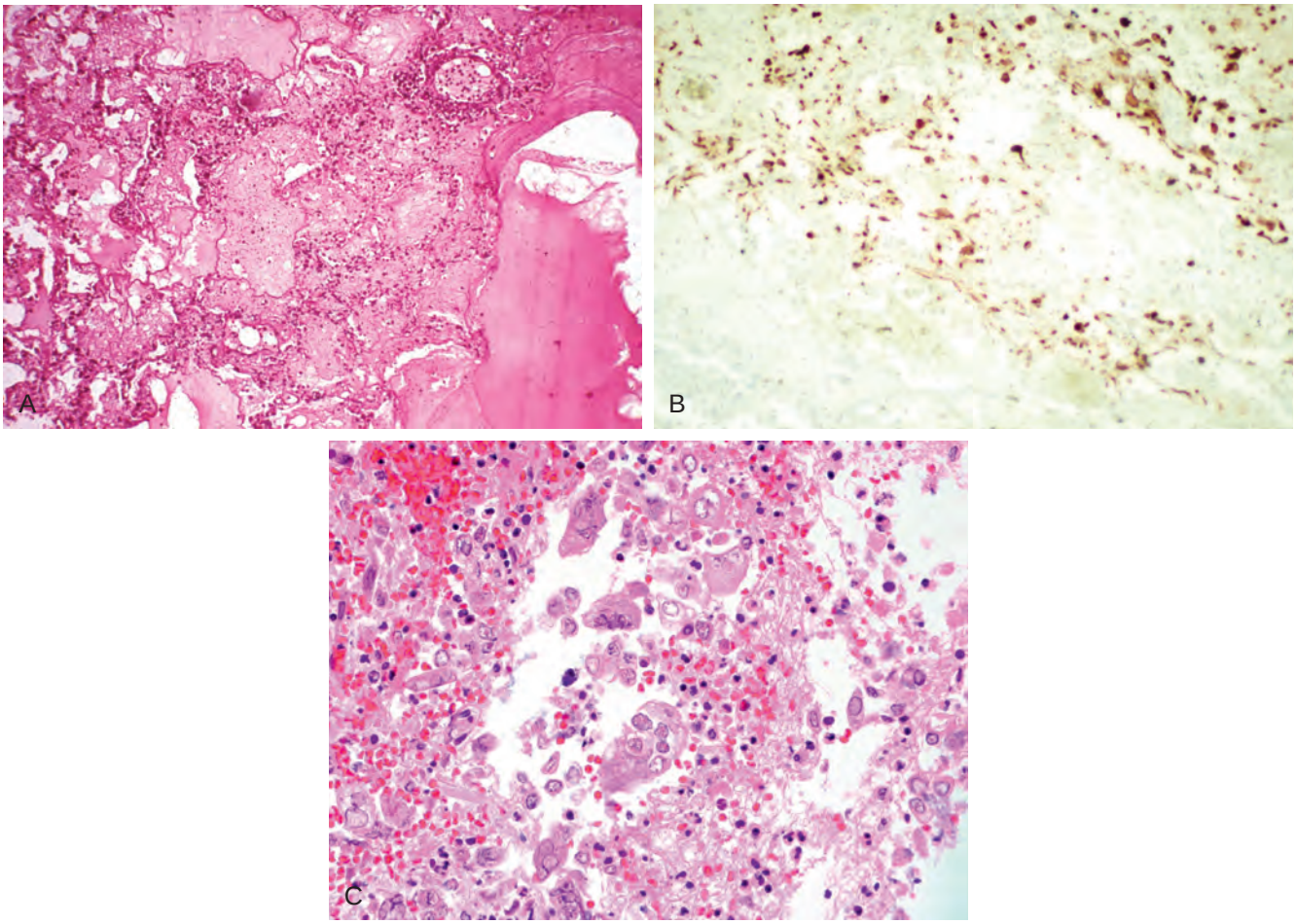
This virus produced an epidemic in the four corners region of the southwestern United States in 1993.[32] The infection is a zoonosis transmitted by infected rodent feces. The most common radiographic presentation is diffuse pulmonary edema with pleural effusions mimicking congestive heart failure. Histologically the lung shows pulmonary edema with scant poorly formed hyaline membranes (Fig. 8.24A), with atypical lymphocytes circulating within the pulmonary vasculature (see Fig. 8.24B).[33] Confirmation of the diagnosis requires specific immunohistochemistry,



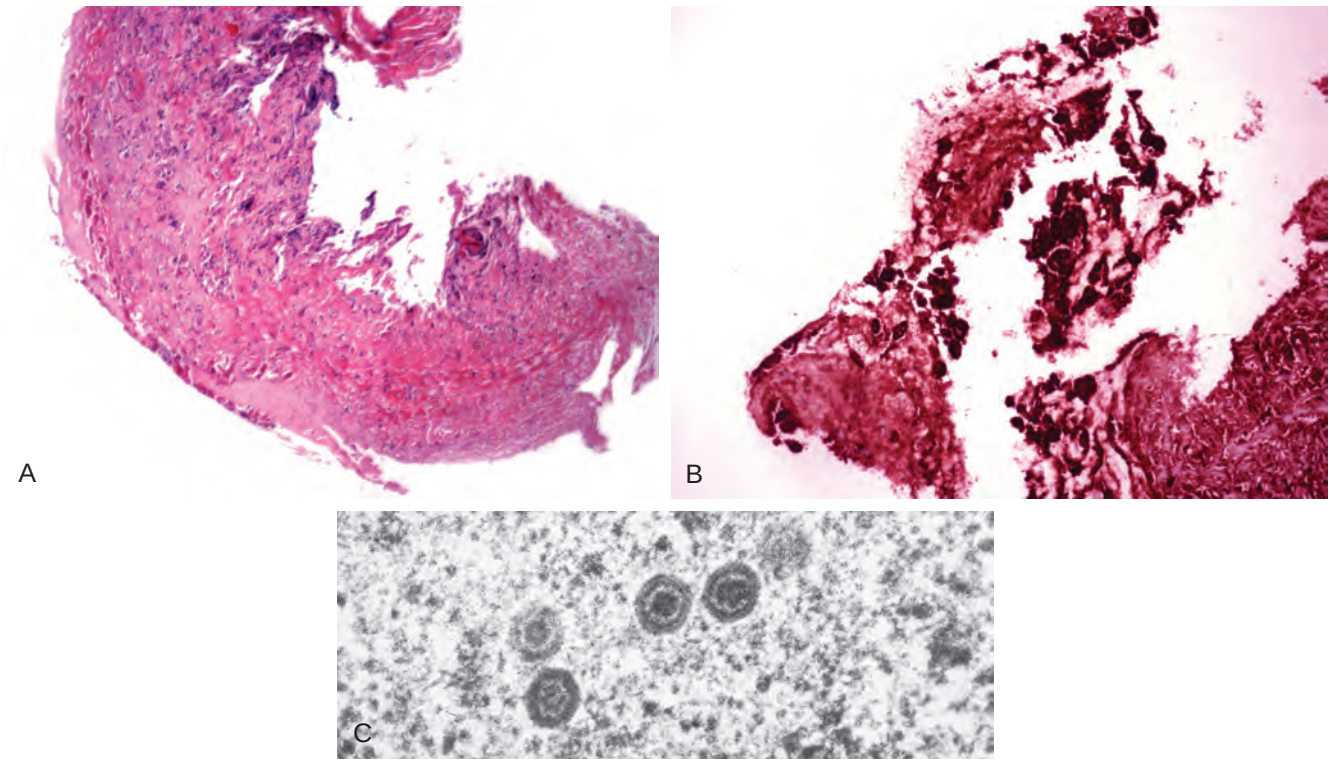
• **Figure 8.19** Nuclear inclusions in herpetic pneumonia.



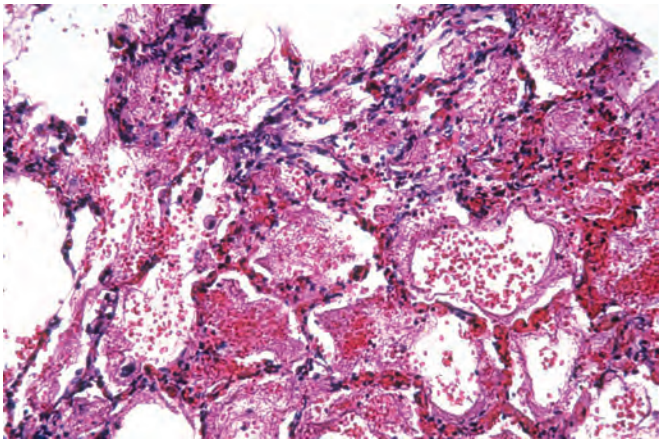
• **Figure 8.21** Herpetic inclusions in squamous respiratory epithelium of a chronically intubated patient.



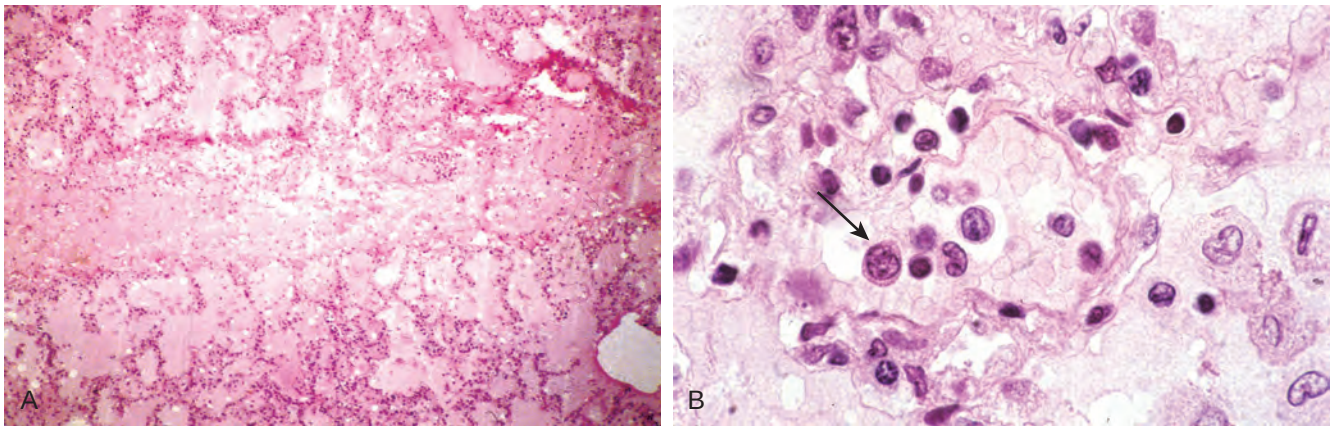
• **Figure 8.20** **A,** Hemorrhagic necrotizing pneumonia in an immunosuppressed patient with herpesvirus 1 viremia. **B,** Multiple infected cells immunostaining for herpesvirus 1. **C,** Herpesvirus type 2 complicating influenza pneumonia with collections of multinucleated cells with nuclear inclusions.



• **Figure 8.22** **A,** Ulcerated tracheal lesion showing **(B)** intense immunostaining for herpesvirus 1 with diagnosis **(C)** confirmed by ultrastructural examination demonstrating diagnostic virions.



• **Figure 8.23** Hemorrhagic pneumonia due to Varicella-Zoster virus.



• **Figure 8.24** **A,** Pulmonary edema in patient with hantavirus infection. **B,** Pulmonary vessel with intraluminal atypical lymphocytes. *Arrow* shows circulating atypical lymphocyte.

serologic evidence of hantavirus-specific IgM, PCR, or ultrastructural identification of the causative virions.

Other Atypical Pneumonias

Mycoplasma Pneumonia

Mycoplasma are the smallest (0.2 to 0.8 μM) free-living bacteria, but they lack a true cell wall. They are facultative anaerobes, except for *Mycoplasma pneumoniae*, the most common pulmonary pathogen, which is a strict aerobe. Mycoplasma pneumonia occurs worldwide with no increased seasonal activity, but epidemics predictably occur every 4 to 8 years. Although primarily an infection of young adults, it can attack older adults. The most common clinical syndrome is tracheobronchitis, with one-third of patients developing a mild but persistent pneumonia.

Mycoplasma pneumonia is rarely biopsied, because positive cold agglutinin and specific complement fixation antigen assays will establish the diagnosis. Biopsied cases show lymphocytic or neutrophilic bronchiolitis with alveolar wall inflammation and fibrinous exudates (Fig. 8.25).[34] Similar changes are seen in both *Chlamydia* (Fig. 8.26) and *Coxiella* pneumonia.

Epstein-Barr Virus

Epstein-Barr virus (EBV) has been implicated in disorders ranging from the mononucleosis syndrome to malignant lymphoid neoplasia.[35] The mononucleosis syndrome includes pharyngitis, lymphadenitis, and hepatosplenomegaly. Pulmonary involvement can occur as part of the syndrome, but it is unusual and rarely biopsied. EBV pneumonia shows patchy peribronchiolar and interstitial polyclonal lymphoid infiltrates with scant interstitial and intra-alveolar fibrin exudates (Fig. 8.27). The diagnosis is generally established serologically by EBV antigen titers but can be confirmed by in situ hybridization.

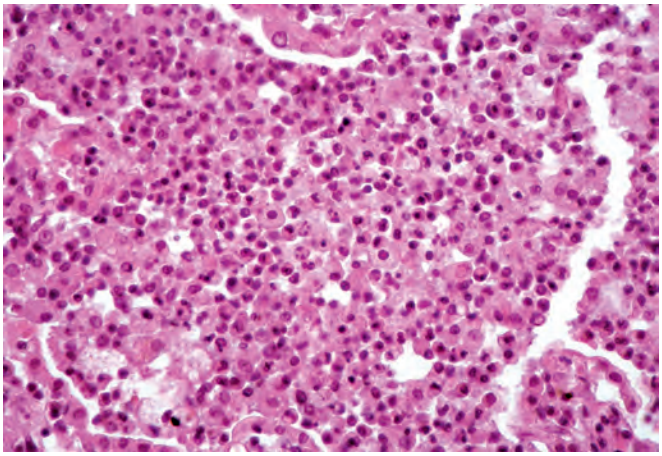
Pneumocystis jiroveci

For years, the organism formerly known as *Pneumocystis carinii* was thought to be a protozoon; however, it is now confidently classified as a fungus. Originally described as a plasma-cell interstitial pneumonia in malnourished children, it was subsequently seen in patients with hematologic malignancies and in those receiving chemotherapy or chronic corticosteroids. In the 1980s pneumocystis pneumonia became a signal infection in establishing the diagnosis of AIDS. It was encountered in epidemic proportions until prophylactic use of trimethoprim-sulfa *(Bactrim)* became a routine aspect of HIV/AIDS management. *P. jiroveci* is currently most often diagnosed by sputum induction, and lung biopsy is reserved for diagnostic challenges.[36]

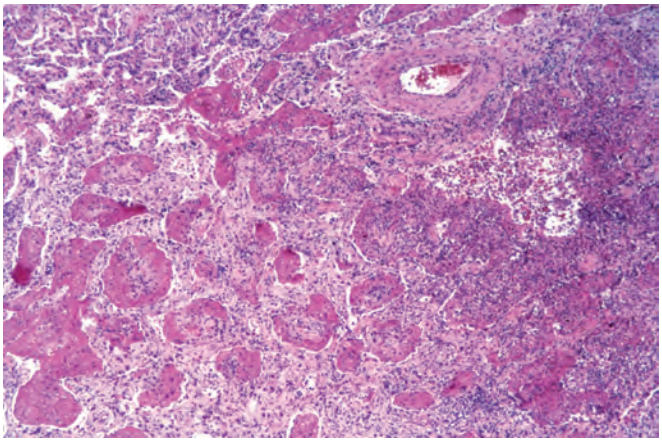
Although the disease presents radiographically as an atypical "interstitial" pneumonia, interstitial inflammation is not its most striking pathologic feature. In pneumocystis pneumonia the alveoli are filled with a frothy eosinophilic exudate that mimics exudative pulmonary edema or alveolar lipoproteinosis (Fig. 8.28A). The pulmonary interstitium shows a mild plasma cell–rich pneumonitis and prominent alveolar type II cell hyperplasia.

The diagnosis is confirmed by the presence of oval-, helmet-, or crescentic-shaped GMS-positive "cysts," 4 to 6 μM in greatest dimension, within the alveolar froth (see Fig. 8.28B).[37] Pneumocystis is distinguished from GMS-positive fungal yeast forms by the absence of budding, pericapsular accentuation, and a so-called intracytoplasmic dot, in the former. However, when these features are absent and when the organism load in the biopsy is low, it may be difficult to confidently exclude *Histoplasma* or *Cryptococcus.* In these cases, specific immunohistochemistry can confirm the diagnosis.

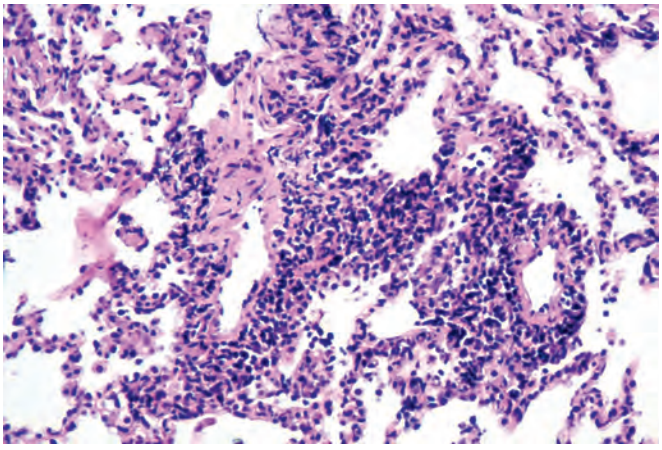
Multiple patterns of unusual host reactions to *P. jiroveci* have been recognized.[38] These include DAD (Fig. 8.29A), solitary necrotizing granulomas (see Fig. 8.29B), miliary infection, lymphoid interstitial pneumonia, and regional lymphadenitis.



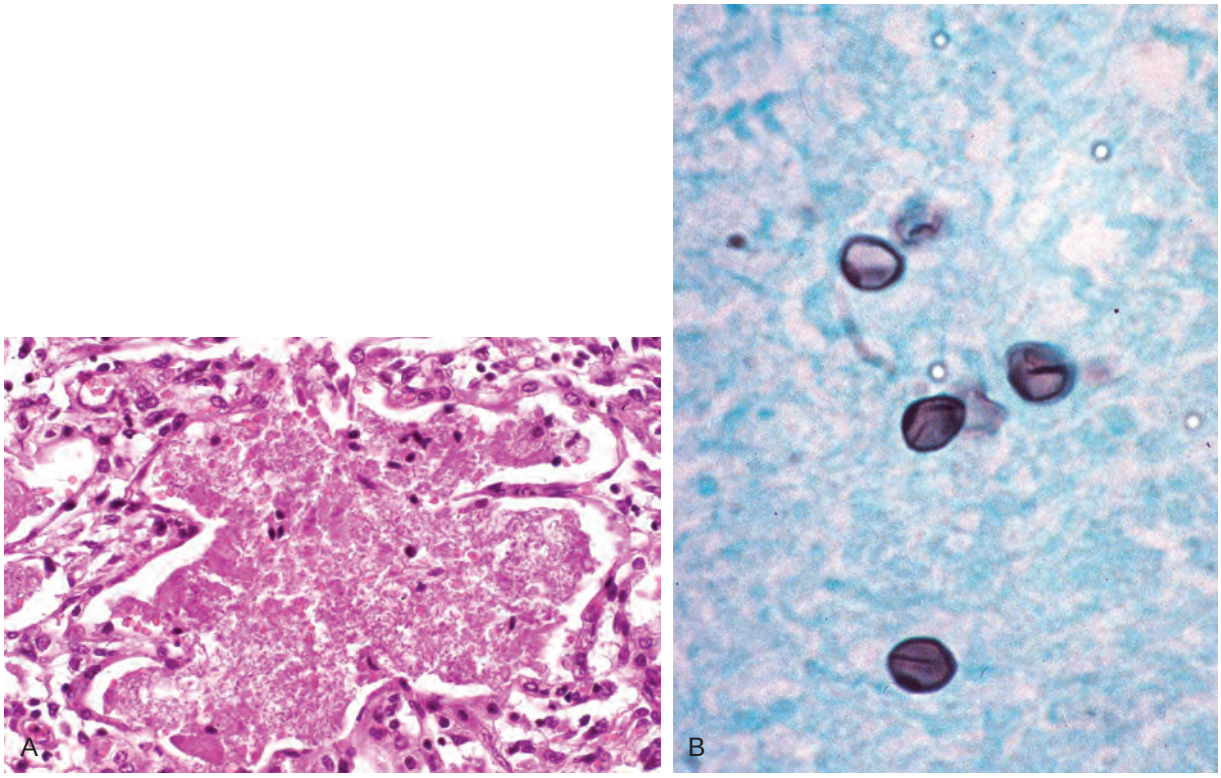
• **Figure 8.25** Histiocytic and fibrinous exudates in Mycoplasma pneumonia.



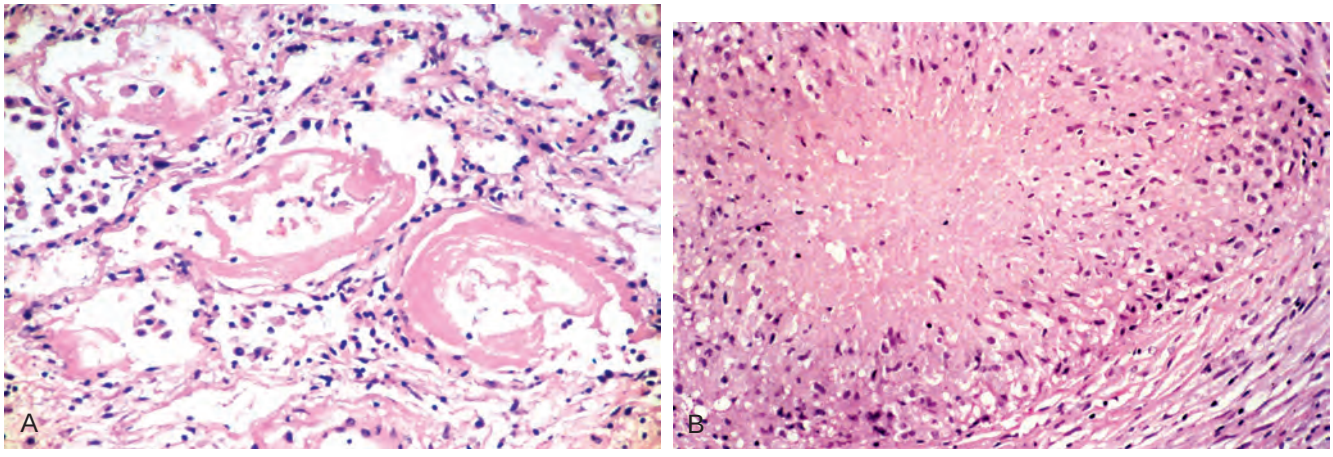
• **Figure 8.26** Prominent fibrinous and histiocytic exudates due to *Chlamydia* spp.



• **Figure 8.27** Dense lymphoid infiltrate in patient with Epstein-Barr virus pneumonia.



• **Figure 8.28** **A,** Alveolar eosinophilic exudate in pneumocystis pneumonia showing **(B)** Gomori methenamine silver–positive cyst forms with pericapsular accentuation.



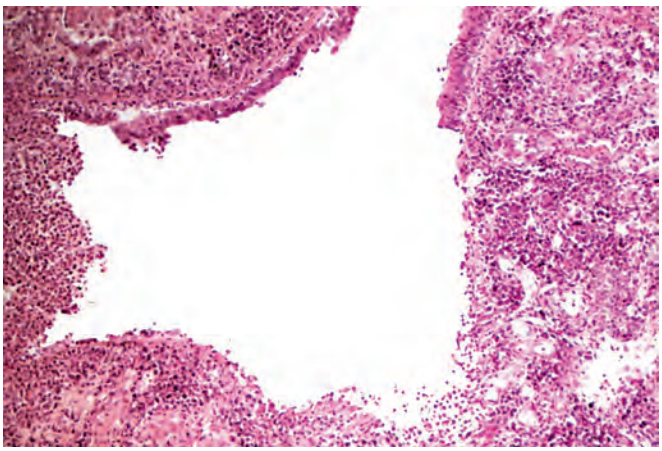
• **Figure 8.29** **(A)** Diffuse alveolar damage and **(B)** Necrotizing granulomatous inflammation due to pneumocystis.

Microcalcifications may be seen in areas of infection, and thin-walled cyst formation may develop.

Bronchiectasis

Bronchiectasis is one of the major manifestations of pulmonary infection. Dilatation and anatomic distortion of conducting airways can result from acute and chronic airway injury from airway infection (wet bronchiectasis) or from adjacent parenchymal scarring ("dry" or traction bronchiectasis). A number of infections can damage the large airways and lead to bronchiectasis. Reversible cylindrical bronchiectasis may appear radiographically in patients, following an acute bacterial pneumonia. Because this change often resolves, biopsies of suspicious bronchiectatic regions should be deferred until a period of weeks has elapsed after an acute infection. Because regions of chronic bronchiectasis are fed by varicose bronchial arteries that often course directly beneath the airways surface, endoscopic biopsies of bronchiectatic areas are generally contraindicated.

Prior to the development of childhood vaccination, viral exanthems and *Bordetella pertussis* were common causes of bronchiectasis (Fig. 8.30). Cystic fibrosis, a genetic disorder of chloride transport, is currently a leading cause of severe bronchiectasis in young adults, as advances in management have resulted in patients surviving beyond childhood (Fig. 8.31).



• **Figure 8.30** Bronchiolitis secondary to *Bordetella pertussis* infection, once a common cause of bronchiectasis.

• **Figure 8.31** The lung in cystic fibrosis showing bronchiectasis and a cavitary abscess.

In bronchiectasis, the airways lose their cartilaginous support due to chronic inflammation and become dilated and prone to repeated bouts of mucoid impaction and infection.[39] The airways develop a spectrum of gross changes, ranging from cystic, varicose, to cylindrical dilatation, associated with distal peribronchiolar inflammation (Fig. 8.32A), and increasing degrees of parenchymal scarring with loss of gas-exchanging alveoli (see Fig. 8.32B). Although the large airways are invariably ectatic, the distal airways in bronchiectasis are generally narrowed by constrictive bronchiolitis.

Recurrent polymicrobial infections with necrotizing bronchopneumonia and abscess formation complicate bronchiectasis. Patients are also prone to develop infections due to antibiotic-resistant mucoid forms of *Pseudomonas*, *Burkholderia* spp., *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* (see Fig. 8.32C)[40] and *Staphylococcus* spp. Some patients with infective bronchiectasis develop allergic bronchopulmonary aspergillosis (ABPA) and superinfection with atypical mycobacteria.[41]

Acute Bronchopneumonia

Acute bronchopneumonia is the most common distribution of pulmonary infection. Gram-positive and gram-negative bacteria, as well some viruses, including herpesvirus and adenovirus, elicit primarily an exudation of neutrophils, whereas the cellular response to other viruses, fungi, mycoplasma, and chlamydia are primarily lymphohistiocytic. Bronchopneumonia generally results from microaspiration of pathogens that have colonized the oropharynx (Fig. 8.33A). At times the aspiration of colonized food particles can carry bacteria and fungi into the lung (see Fig. 8.33B). Terminal episodes of aspiration often show colonies of gram-positive *Aerococci*, previously referred to as "gaffkya," (see Fig. 8.33C) and their appearance is both common and characteristic in autopsy lungs.[42]

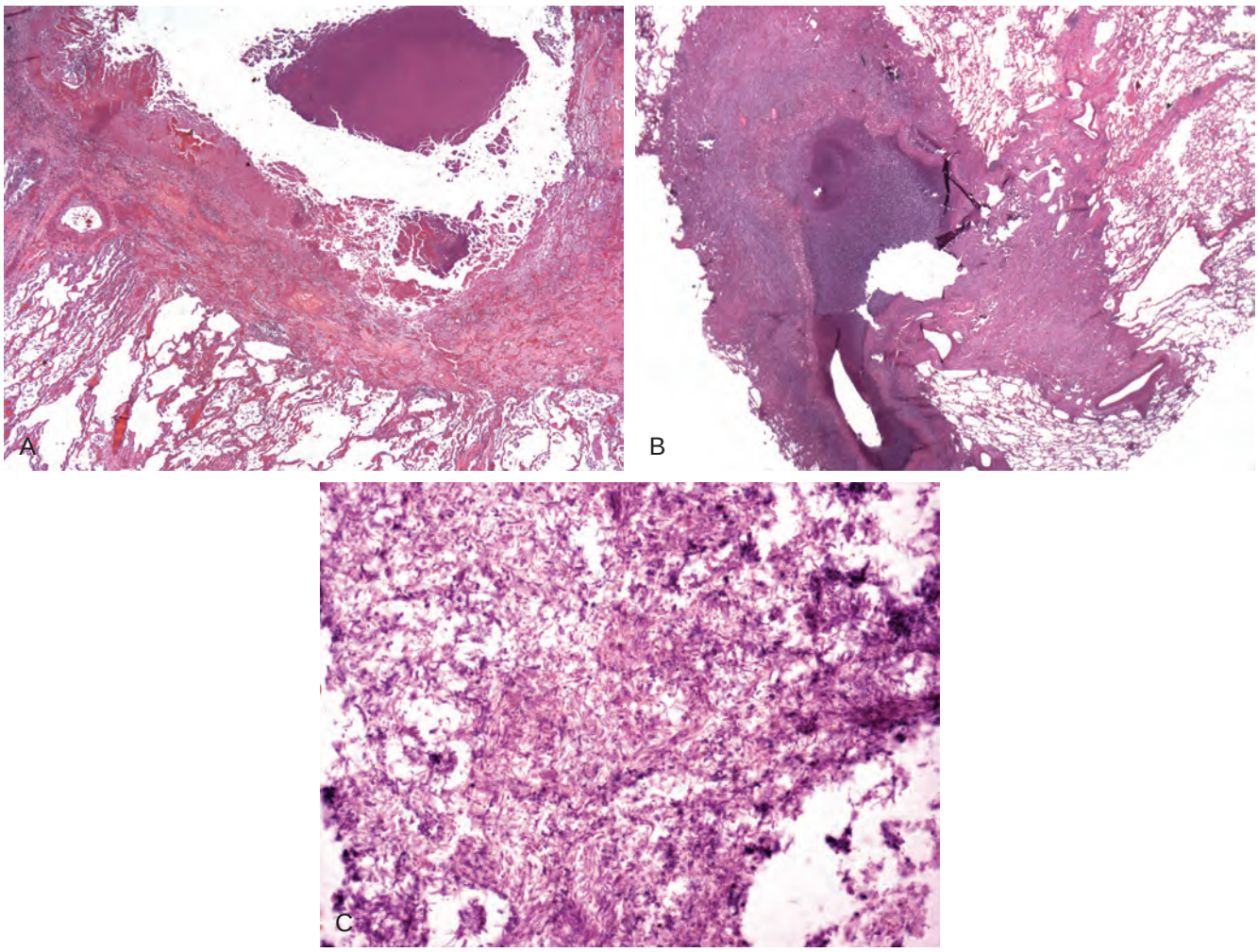
Bacterial Infections

The pyogenic bacteria are distinguished by their propensity to evoke acute neutrophilic inflammation and "pus." Pyogenic infections as well as other acute necrotizing bronchopneumonias progress to *organizing pneumonia*, characterized by a fibrohistiocytic response that obliterates small airways along with inflammation of the surrounding alveolar interstitium (Fig. 8.34). This reaction is nonspecific, and "organizing pneumonia" or "bronchiolitis obliterans with organizing pneumonia" is a rubric for the generic lesion that may be due to infection or noninfective inflammatory disorders or may be idiopathic.[43] It is important for the surgical pathologist to convey clearly to the treating clinicians that a diagnosis of "organizing pneumonia" does not indicate a specific etiology.

Pneumococcal Pneumonia

Streptococcal pneumonia (pneumococcal pneumonia) is a community-acquired pneumonia that classically produces a lobar pneumonia healing by resolution (i.e., without necrosis or scarring).[44] In some cases, pneumococcal pneumonia complicates a resolving viral tracheobronchitis or influenza. In the age of antibiotic treatment, most cases do not progress to lobar involvement and are limited to acute nonnecrotizing bronchopneumonia. A large number of serotypes of streptococcal pneumonia have been isolated, and certain ones (e.g., type 3) can be virulent, producing necrotizing pneumonia, bacteremia, and death, despite prompt antibiotic treatment.

Although rarely biopsied, lobar pneumococcal pneumonia is still seen at autopsy. The early phase of the disease, the *red hepatization*, shows exudation of edema fluid with the diapedesis of red blood cells (Fig. 8.35A). The exudate spreads to fill an entire lung lobe via the pores of Kohn, interalveolar potential channels within the normal lung (see Fig. 8.35B). The acute cellular response is neutrophilic, but this is followed within days by alveolar filling with exudate macrophages that ingest the infected exudate, the *gray hepatization* (see Fig. 8.35C). Despite the extensive inflammatory changes, alveolar necrosis is absent—a finding best assessed by elastic stains—and the lung heals by *resolution* with minimal sequelae. The offending organisms are gram-positive, lancet-shaped cocci growing in pairs (diplococci) and in



• **Figure 8.32** **A,** Bronchiectasis in cystic fibrosis showing dilatation and destruction of airway wall. **B,** Dense peribronchiolar scar in bronchiectasis. **C,** Bronchiectatic airway with colonies of gram-negative bacilli identified by culture as *Burkholderia cepacia*.

short chains, and they can be identified by both tissue Gram and GMS stains (see Fig. 8.35D).

Group A Streptococci

Group A streptococcal pneumonia occurs at the extremes of life or as a complication of resolving influenza infection.[45] It produces a rapidly progressing and life-threatening pneumonia with edema, hemorrhage, abscess formation, empyema, and septicemia. Group A streptococci evoke a brisk increase in pulmonary capillary permeability, yielding what can be mistaken for cardiogenic pulmonary edema on low-power microscopic examination (Fig. 8.36A). However, further scrutiny reveals necrotic macrophages and innumerable gram-positive bacteria in chains (see Fig. 8.36B). Pulmonary hemorrhage and abscess formation are frequently seen. Streptococci have a predilection to course along pulmonary lymphatic channels, recapitulating erysipeloid spread in the skin, to produce an early empyema (Fig. 8.37).

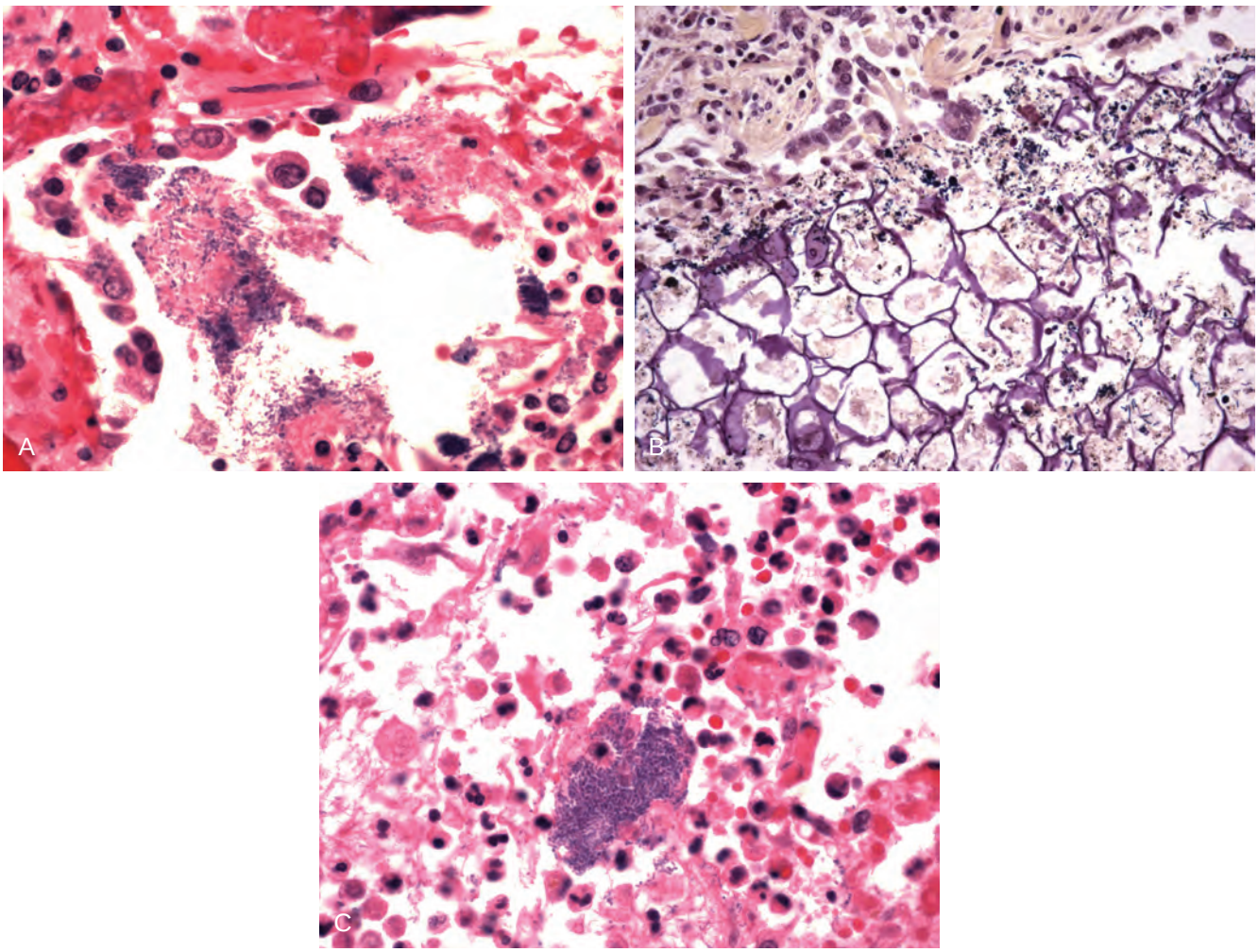
Staphylococcus aureus

S. aureus has emerged as a frequently encountered life-threatening pulmonary pathogen. Previously seen as a community-acquired complication of influenza, staphylococcal infection is now a primary cause of nosocomial pneumonia, and its evolving drug resistance accounts for methicillin-resistant *S. aureus* (MRSA) strains, which are of particular concern for hospital infection control epidemiologists.[46]

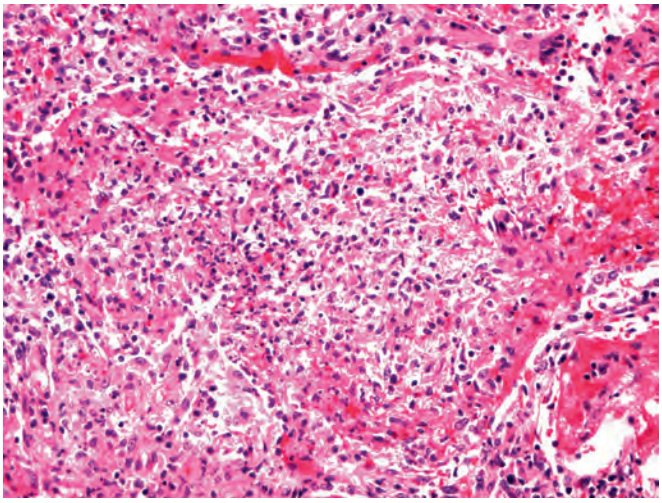
S. aureus spp. produce a necrotizing pyogenic pneumonia with abscess formation (Fig. 8.38A). The organisms grow in clusters as gram-positive microcolonies (see Fig. 8.38B). Infection heals by organization with scarring, and cystic pneumatoceles may develop. Some phage-infected strains of the organism produce an exotoxin that can activate the CD3 receptor to promote the release of the T lymphocyte's complement of lymphokines, leading to a *toxic shock syndrome* with sepsis physiology, DAD, and disseminated intravascular coagulation that leads to death if not treated promptly.

Gram-Negative Bacteria

Most gram-negative bacilli produce a necrotizing bronchopneumonia with hemorrhage and abscess formation. Certain virulent gram-negative species, including *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Burkholderia* spp., have a propensity to infect the pulmonary microvasculature, leading to necrosis, bacteremia, and septic shock.[47] The lung shows fibrinoid necrosis with colonies of



• **Figure 8.33** **A,** Acute bronchiolitis due to *Staphylococcus*. **B,** Lentil aspiration with colonies of gram-positive bacteria. **C,** Terminal aspiration showing intraluminal colony of gram-positive *Aerococcus* spp. (Gaffkya).



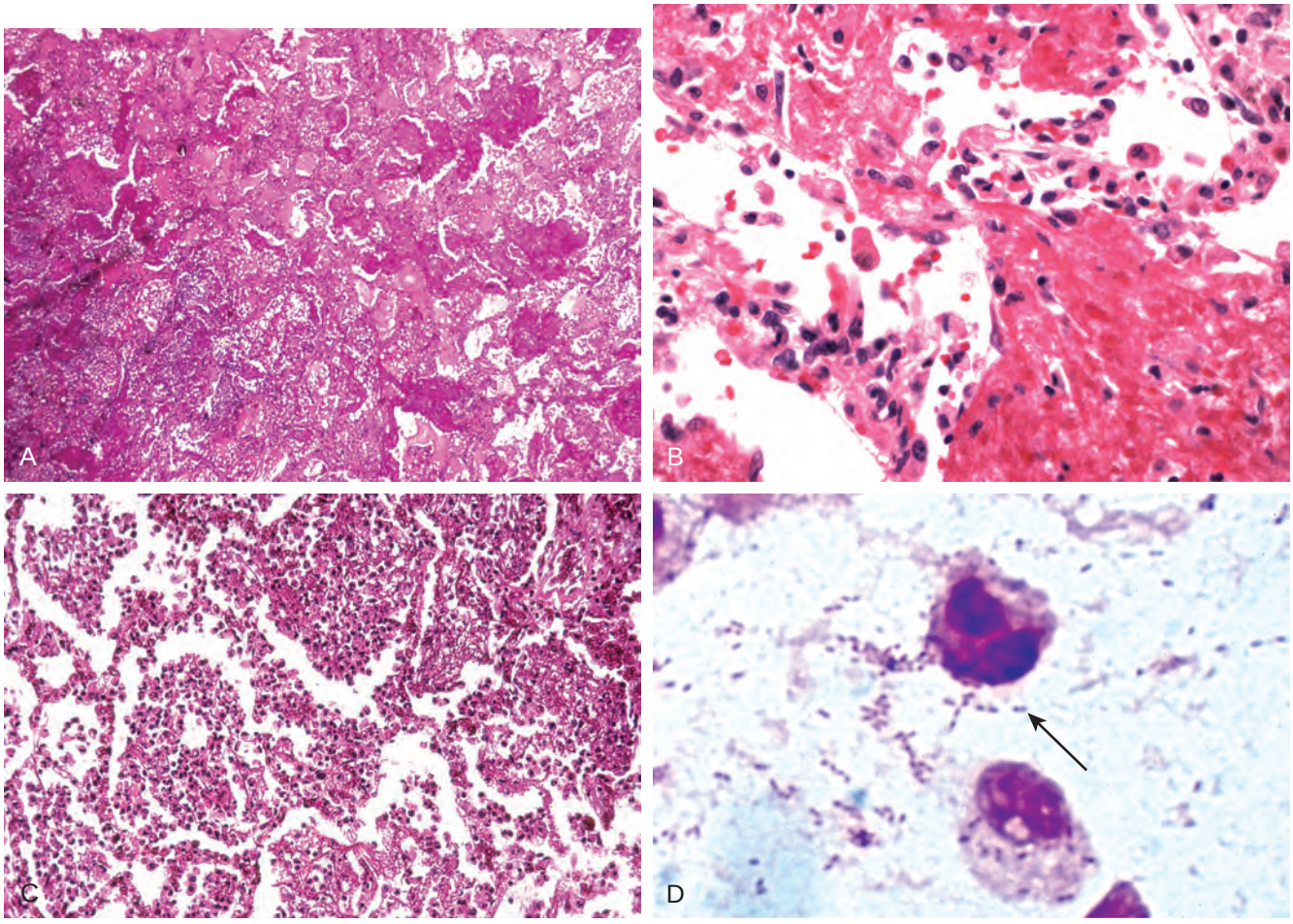
• **Figure 8.34** Organizing pneumonia showing macrophages and early fibrosis in respiratory bronchioles and alveoli.

gram-negative bacilli streaming along the vessel walls, where they create an ill-defined purplish hue in H&E-stained sections (Fig. 8.39A and B)

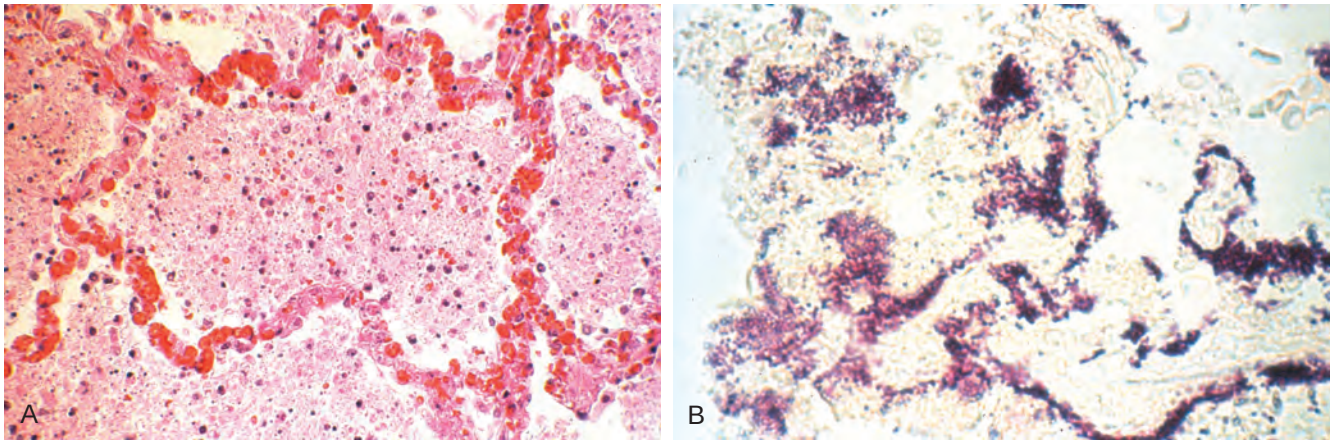
Klebsiella. *Klebsiella* pneumonia generally occurs in patients who are immunocompromised due to age, ethanol abuse, or diabetes mellitus. It is a common cause of ventilator-associated pneumonia (VAP).[48] Like the pneumococcus, *Klebsiella* spp. classically produce a lobar pneumonia with a slimy "mucoid" appearance and an unexplained predilection for the upper lobes (Fig. 8.40A). Infection produces hemorrhagic necrosis, microabscesses, and cavity formation. The organisms are short gram-negative bacilli that can be demonstrated with both tissue gram stain (see Fig. 8.40B) and GMS by virtue of their capsules. Less commonly, *Klebsiella* spp. produce a chronic necrotizing pneumonia with scarring and distortion of the pulmonary anatomy (see Fig. 8.40C).

Lung Abscess Due to Oropharyngeal Aspiration

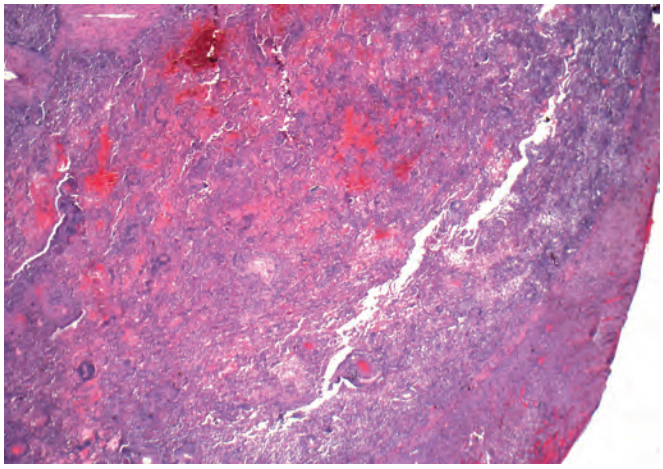
Lung abscess complicates the aspiration of polymicrobial oropharyngeal bacteria. The organisms isolated from a lung abscess include mixed gram-positive and gram-negative aerobic flora, as well as anaerobes. Patients with poor oral hygiene due to dental



• **Figure 8.35** **A,** In the red hepatization phase of pneumococcal lobar pneumonia, alveoli are filled by fibrin with diapedesis of red blood cells. **B,** The infected exudate extends throughout the lobe via the pores of Kohn, potential channels between adjacent alveoli. **C,** In the gray hepatization phase, alveoli are filled by leukocytes that scavenge bacteria and detritus prior to resolution of the infection. **D,** The pneumococcus is a lancet-shaped, gram-positive coccus that grows in pairs (diplococci) *(arrow)* and chains.



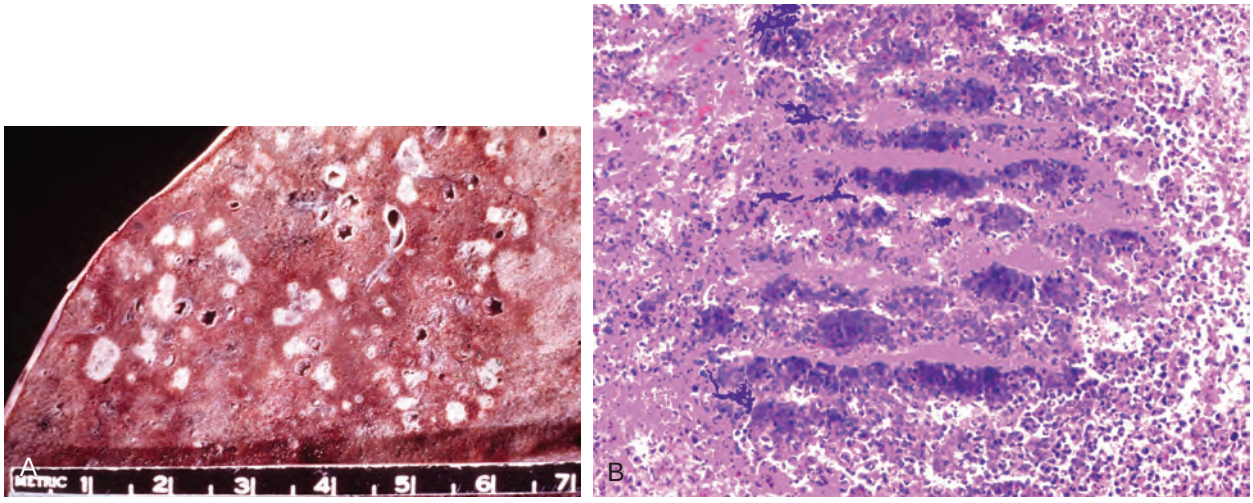
• **Figure 8.36** **A,** The alveolar spaces in streptococcal pneumonia show an eosinophilic exudate that mimics proteinaceous pulmonary edema at low power. **B,** Innumerable gram-positive cocci in chains are seen in the exudates and tend to invade lymphatics.



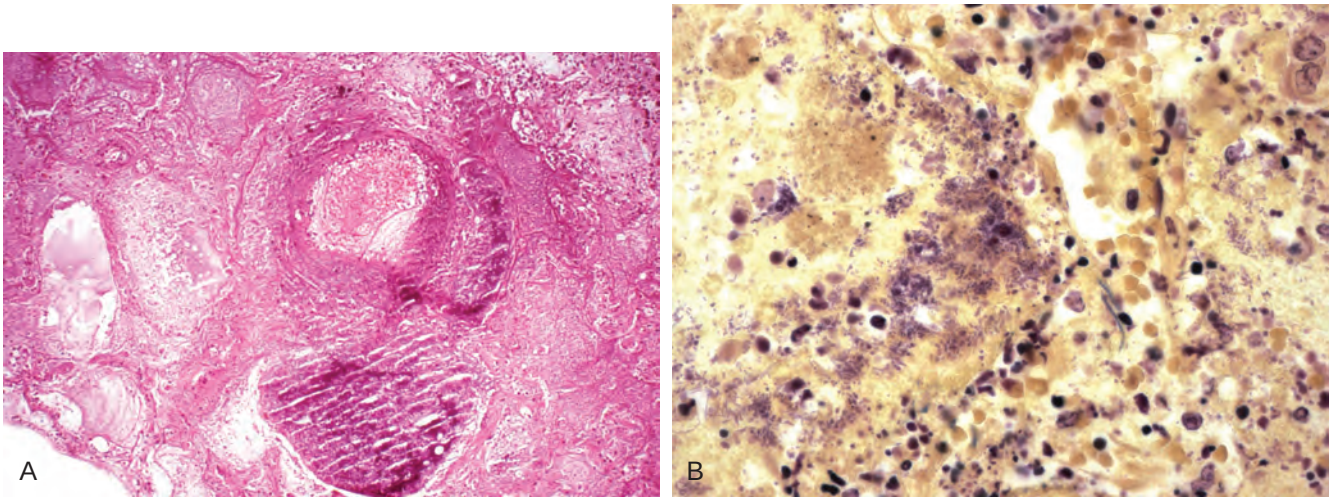
• **Figure 8.37** Abscess formation and empyema occur early in streptococcal pneumonia.

caries, those with gingival and tonsillar disease, and those with disorders that impair either consciousness or normal swallowing (e.g., ethanolism, seizure disorder, and cerebrovascular accidents) are at increased risk for aspiration pneumonia and lung abscess.[49] The disorder begins as a necrotizing bronchopneumonia in a dependent segment of the lung and progresses to produce a cavitary abscess communicating with a feeding adjacent airway. Most lung abscesses are diagnosed and treated noninvasively; however, failure to respond to medical treatment due to poor drainage and closure may prompt surgical excision.

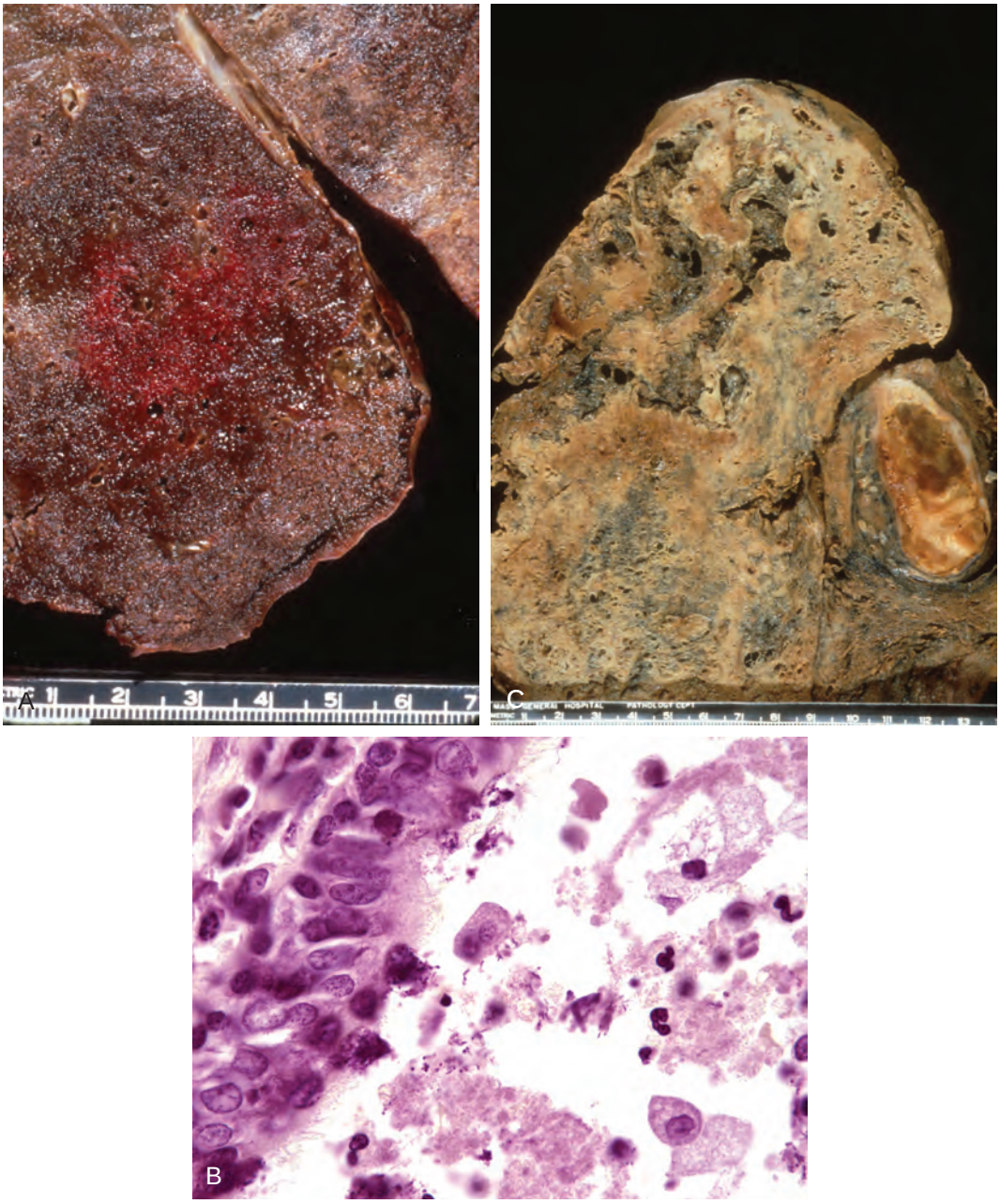
Microscopically the wall of the lung abscess is irregular with a shaggy, fibrinous lining (Fig. 8.41A) that may be difficult to distinguish from a necrobiotic rheumatoid nodule or Wegener granulomatosis. However, palisading granulomatous inflammation is not a prominent finding in lung abscess. The area around the cavity shows acute and organizing bronchopneumonia (see Fig. 8.41B). The activity of a lung abscess may be determined microscopically by examining its lining because actively infected cavities show a squamous epithelial lining, whereas healed cavities are lined by normal respiratory epithelium and may be mistaken for a focus of bronchiectasis.



• **Figure 8.38** **A,** Lung in staphylococcal pneumonia showing bronchopneumonia with abscess formation. **B,** Microabscess with gram-positive *S. aureus* in clusters.



• **Figure 8.39** **A,** Fibrinoid necrosis with dense bacterial growth visible in hematoxylin and eosin sections in Pseudomonas pneumonia. **B,** Clusters of gram-negative bacilli are identified.

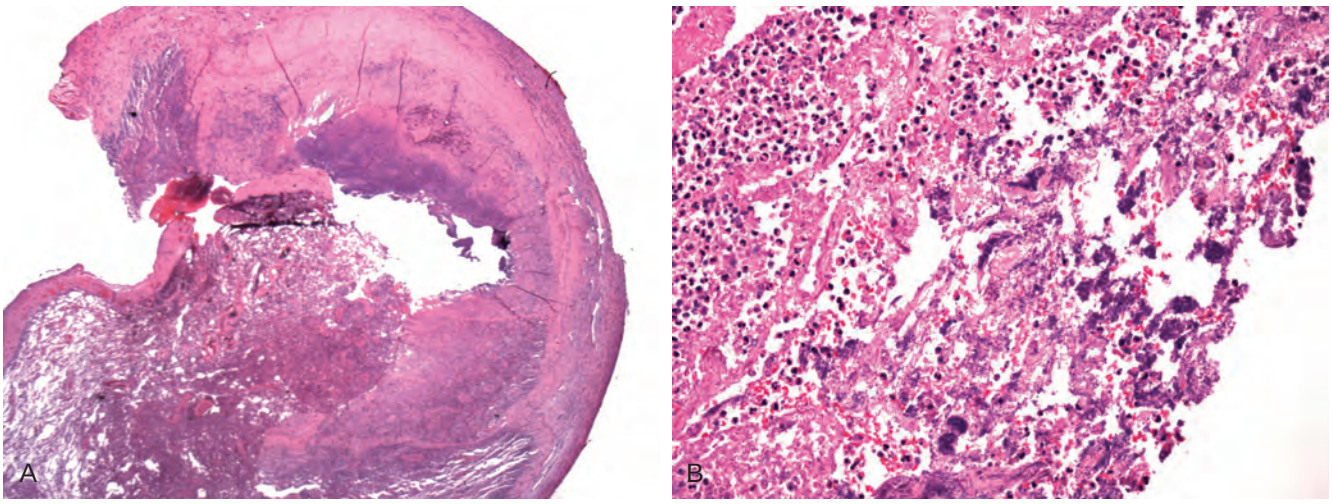


• **Figure 8.40** **A,** Hemorrhagic lobar pneumonia due to *Klebsiella pneumoniae.* The organism has a predilection for the upper lung lobes. **B,** Klebsiella is a small, nonmotile, gram-negative rod that also stains with Gomori methenamine silver. **C,** Chronic necrotizing pneumonia due to *Klebsiella* spp. with extensive scarification of necrotic lung.

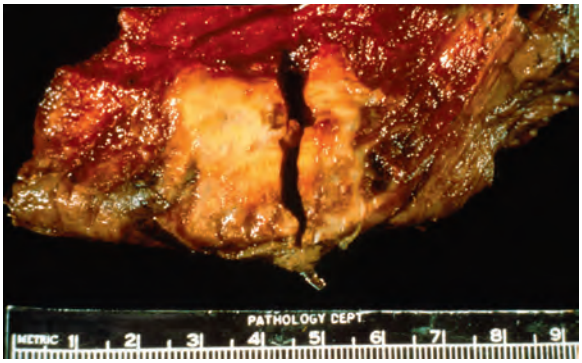
Actinomycosis

Actinomyces spp. are gram-positive filamentous bacteria that cause a chronic distal necrotizing pneumonia with a proclivity towards penetrating into the adjacent soft tissues of the chest wall.[50] *Actinomyces* are aspirated from the oropharynx, where they are commonly part of the tonsillar flora in younger patients or a pathogen related to poor oral hygiene and gingivitis in older adults. The risk factors for actinomycosis are similar to those of lung abscess. The infection extends into the pleura and then forms sinuses within the soft tissues of the chest wall that ultimately exit at the skin surface. The indurated pulmonary lesion may be mistaken clinically for an aggressive peripheral lung malignancy, but its gross appearance at surgery is usually distinct (Fig. 8.42).

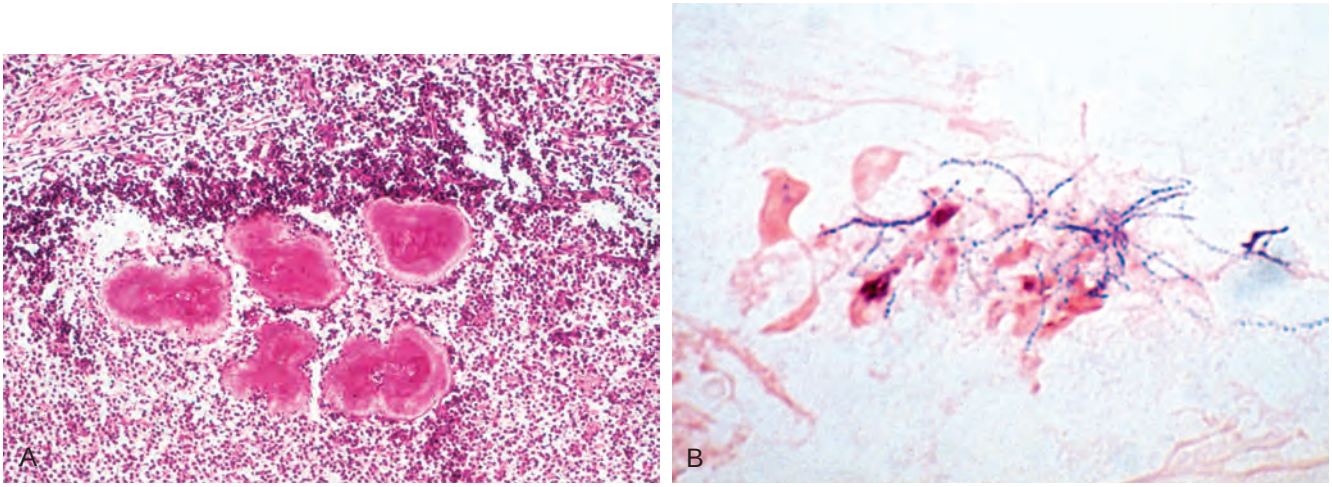
The histologic response evoked by actinomycosis is variegate, with microabscesses, polymorphous infiltrates of lymphocytes, histiocytes, plasma cells, giant cells, and fibrosis. At times, the extent of the fibroinflammatory response can lead one to consider a diagnosis of "inflammatory pseudotumor." The correct diagnosis is established by the presence of "sulfur granules" (Fig. 8.43A)—bright yellow specks seen with the naked eye or with the aid of a hand lens—that microscopically represent colonies of tangled gram-positive, GMS-positive, beaded filamentous bacilli, (see Fig. 8.43B and C) coated by an eosinophilic matrix



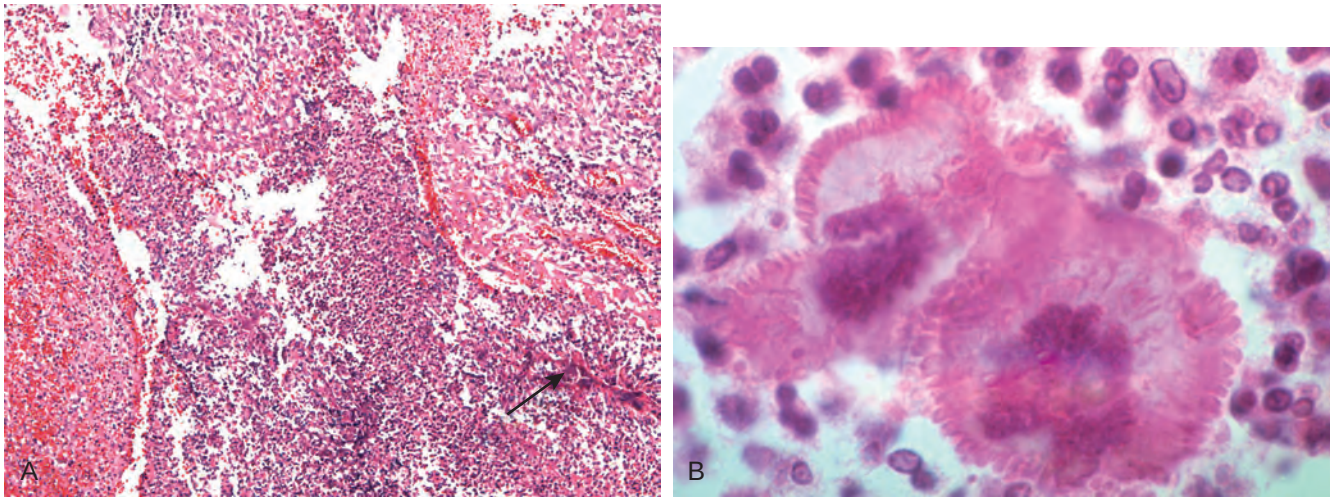
• **Figure 8.41** **A,** Shaggy fibrinous exudate lining wall of lung abscess due to aspiration of oropharyngeal mixed flora. **B,** Necrosis and bacteria in lung abscess.



• **Figure 8.42** Subpleural nodule of actinomycosis resected for suspicion of carcinoma. Note the yellow-tan appearance that suggests inflammation rather than malignancy.



• **Figure 8.43** **A,** Multiple actinomycotic sulfur granules within granulohistiocytic inflammatory response. **B,** Gram stain shows irregularly beaded filaments of *Actinomyces* spp. that also stain with Gomori methenamine silver (GMS). Recall that GMS stains all gram-positive organisms and with no specificity for actinomycosis.



• **Figure 8.44** **A,** Low-power view of botryomycosis in an infected airway. *Arrow* shows focus of botryomycosis at low power. **B,** High power of botryomycotic granule due to *S. aureus*.

of exudate plasma proteins termed the *Splendore-Hoeppli* reaction. Treatment includes long-term antibiotics and surgical resection.

The differential diagnosis of "sulfur granules" in the lung includes *botryomycosis*, a term used to describe a variety infections by colonies of gram-positive cocci, either *Streptococcus* or *Staphylococcus* that grossly appear comparable to the sulfur granules caused by *Actinomyces*.[51] Distinguishing these entities depends on the morphology of the bacteria in the granules (i.e., cocci in botryomycosis versus filamentous bacteria in actinomycosis) (Fig. 8.44A and B).

Nocardia

Nocardia spp. produces pneumonia primarily in the immunosuppressed host.[52] The organism shares morphologic and histochemical staining features with *Actinomyces*, but its clinical and histologic responses are usually easily distinguished. *Nocardia* produces a necrotizing pneumonia with granulohistiocytic inflammation (Fig. 8.45A), and special stains highlight tangles of gram-positive, GMS-positive filamentous organisms showing less beading than *Actinomyces* (see Fig. 8.45B). Sulfur granules are rarely seen in pulmonary infections. As distinct from *Actinomyces*, *Nocardia* are weakly acid fast and can be highlighted with modified Ziehl-Neelsen stains (e.g., Fite-Faraco) (see Fig. 8.45C). Nocardiosis is a recognized complication of pulmonary alveolar lipoproteinosis and has recently been observed in patients receiving therapies that interfere with the activities of tumor necrosis factor (TNF)-α.

Legionella

Legionella spp. produce pulmonary infections ranging in severity from a mild respiratory illness to life-threatening pneumonia. Patients may suffer from a modest degree of immunosuppression due to diabetes and ethanolism. The organism can be inhaled from contaminated water and air conditioning sources, and these have been the sources of point outbreaks of infection.

The diagnosis of *Legionella* infection is currently established noninvasively by immunoassays; however, the disease may be identified histologically in biopsy or autopsy tissues. The infected lung characteristically shows a fibrinohistiocytic response with alveolar filing by fibrin and necrotic macrophages with a sparse neutrophilic component (Fig. 8.46A), although its histology may also be indistinguishable from that of pyogenic infections.[53] It can be mimicked by acute fibrinous organizing pneumonia (AFOP), a recently recognized noninfective pattern of pulmonary injury (see Fig. 8.46B). Although *Legionella* is a gram-negative coccobacillus, it stains weakly with gram stains, and silver impregnation stains are required to blacken the organisms, which are often abundant in situ in the absence of prior treatment (Fig. 8.47A). *Legionella micdadei* is distinguished by its staining with modified Ziehl-Neelsen stains (see Fig. 8.47B).

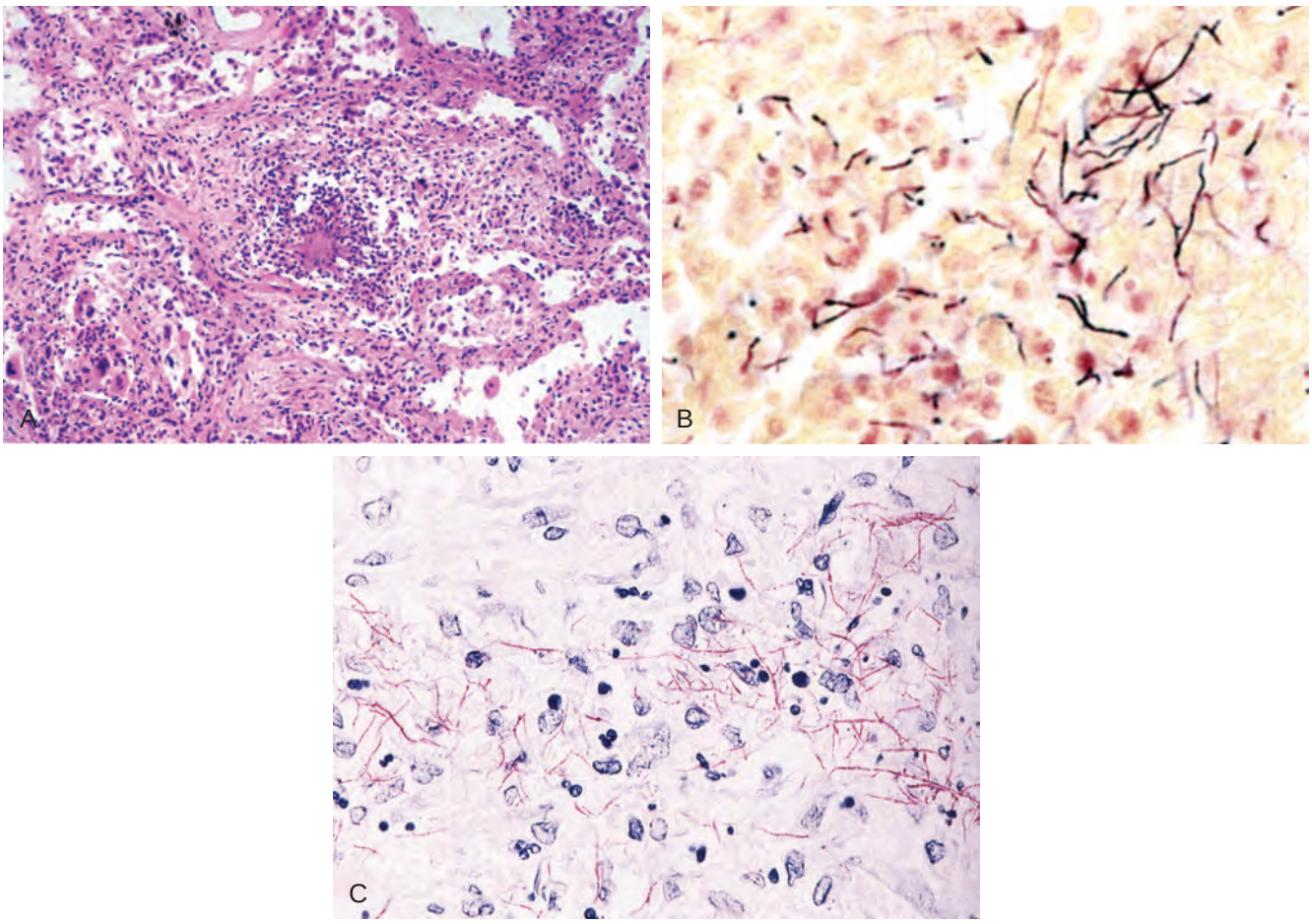
Rhodococcus equi

Rhodococcus pneumonia is a zoonotic infection that causes a nodular histiocytic and cavitary pneumonia in immunosuppressed patients, most commonly with HIV/AIDS (Fig. 8.48).[54] The causative gram-positive cocci are easily identified (Fig. 8.49A) and also stain positive with the modified Ziehl-Neelsen stain (see Fig. 8.49B). The inflammatory response shows *malakoplakia* (Fig. 8.50A), with formation of intracellular calcific concretions termed *Michaelis-Gutmann* bodies that, although nonspecific, are characteristically seen in the infection and can be highlighted by both PAS and iron stains (see Fig. 8.50B).[55]

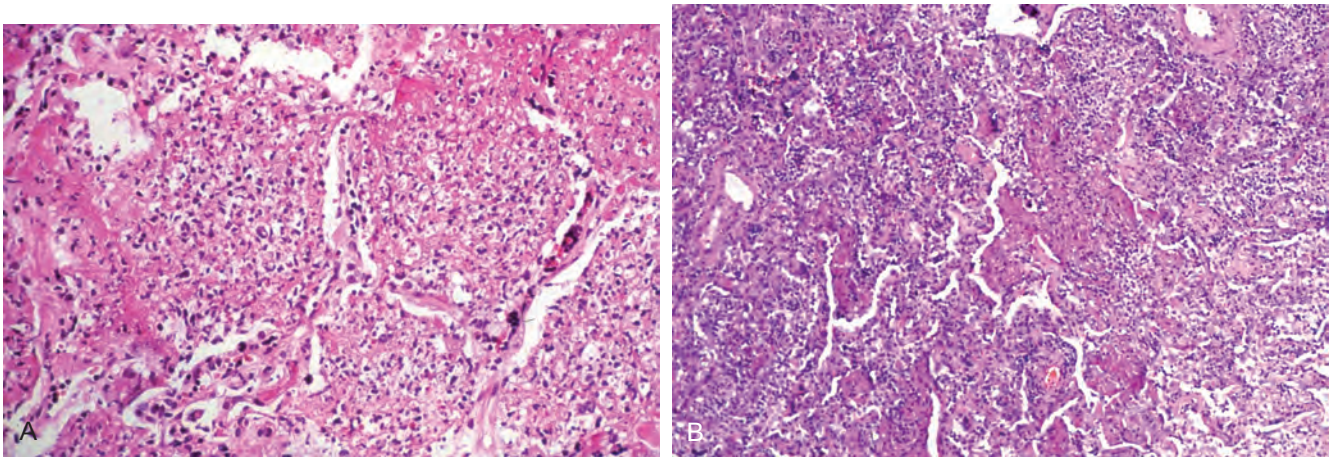
Tropheryma whipplei *(Whipple Disease)*

Whipple disease is a rare disorder caused by the actinomycete *T. whipplei*. Whipple disease most commonly causes intestinal malabsorption, but pulmonary and neurologic disease also occurs. In an examination of the pulmonary microbiome in patients with HIV-1 infection, it was demonstrated that a large percentage of patients are colonized by *T. whipplei*.[56]

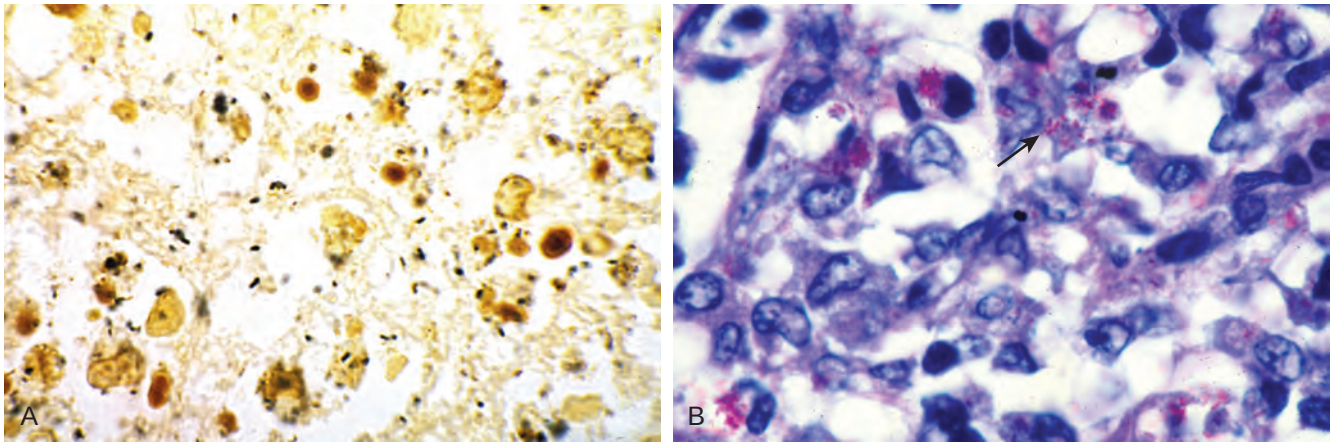
In the lung, Whipple disease can present as interstitial infiltrates, pleural effusions, or pulmonary hypertension.[57] The characteristic change in the disorder is the accumulation of foamy macrophages that show intense staining with PAS (Fig. 8.51). In some cases, microgranulomas that are histologically comparable to those seen in sarcoidosis may confuse the diagnosis (Fig. 8.52), together with the fact that the PAS staining in these granulomas may be equivocal. It is uncertain whether sarcoidosis is associated with Whipple disease or whether the sarcoidal granulomas are evidence of early infection. In these cases a small bowel biopsy will generally establish the diagnosis. Further confirmation can be



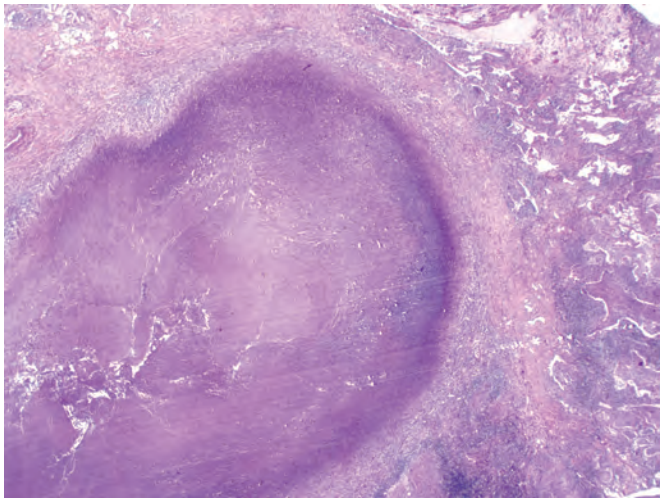
• **Figure 8.45** **A,** Necrotizing pneumonia due to *Nocardia asteroides* shows granulohistiocytic response. **B,** Like *Actinomyces*, *Nocardia* is a gram-positive filamentous bacillary actinomycetes that also stains with Gomori methenamine silver. **C,** Unlike *Actinomyces*, *Nocardia* is weakly acid-fast, and can be demonstrated with the Fite-Faraco stain.



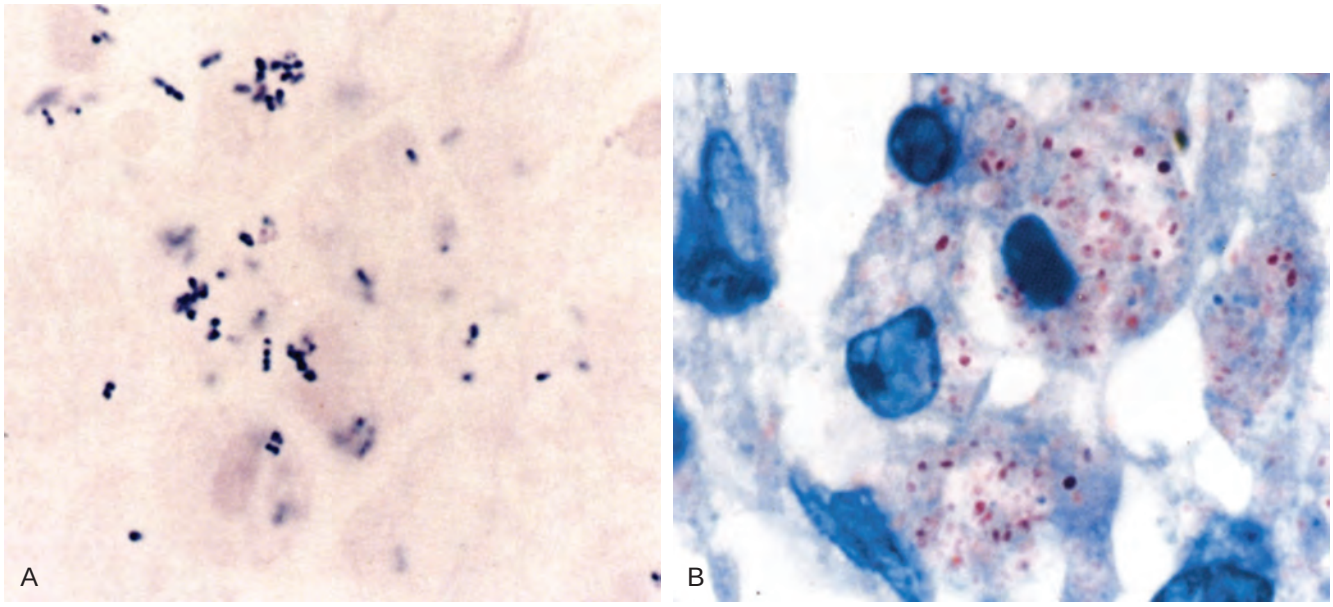
• **Figure 8.46** **A,** *Legionella* spp. characteristically produce a necrotizing bronchopneumonia with alveolar filling by fibrin and histiocytes. **B,** This appearance must be distinguished from acute fibrinous organizing pneumonia, in which the alveolar spaces are filled with fibrin, but this disorder is not due to infection.



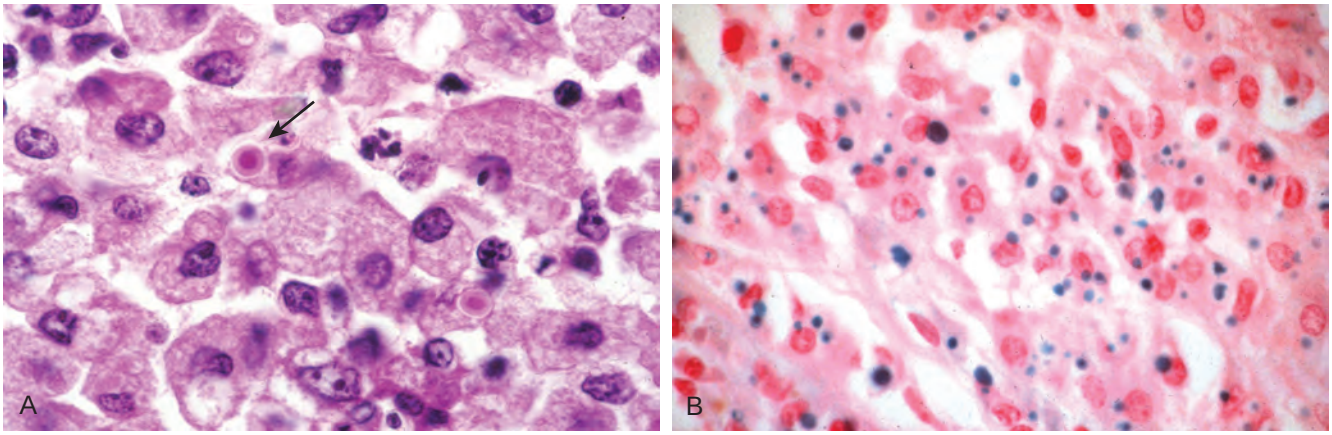
• **Figure 8.47** **A,** *Legionella* spp. are gram-negative coccobacilli but must be demonstrated by silver impregnation. **B,** *L. micdadei*, the Pittsburgh pneumonia agent, also stains with modified acid fast bacillus (AFB) stains. *Arrow* shows AFB-positive bacteria.



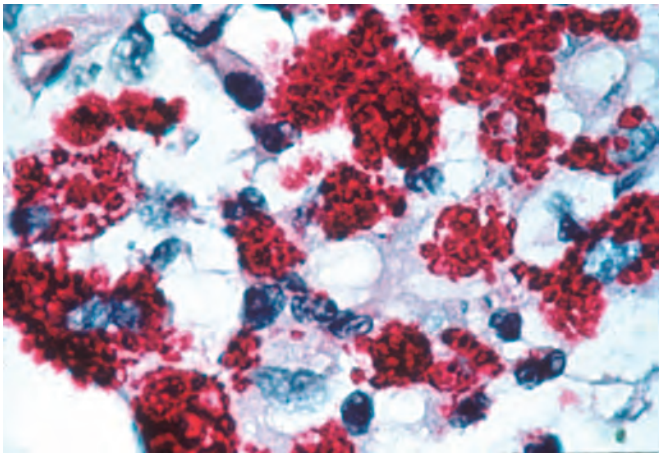
• **Figure 8.48** *Rhodoccocus equi* causes a cavitary nodular pneumonia.



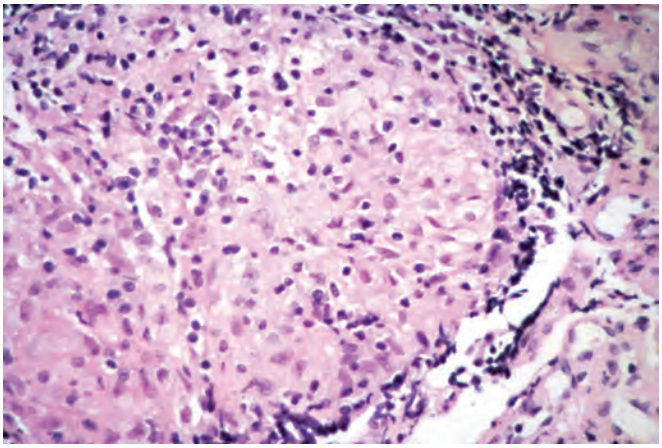
• **Figure 8.49** **A,** The causative gram-positive cocci also **(B)** stain with modified AFB preparations.



• **Figure 8.50** **A,** Malakoplakia with *Michaelis-Gutman* bodies, calcific concretions that are also positive in periodic acid–Schiff- and **(B)** iron-stained sections.



• **Figure 8.51** Macrophages staining intensely periodic acid–schiff-positive in Whipple disease.

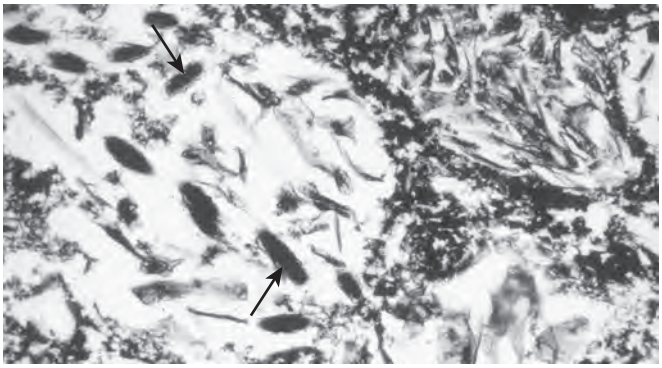


• **Figure 8.52** Nonnecrotizing granulomas mimicking sarcoidosis in Whipple disease.

achieved via ultrastructural examination demonstrating the bacillary organisms (Fig. 8.53) or by specific PCR.

Granulomatous Pneumonia

Pathologists apply the term "granuloma" to a variety of histologic responses, including micronodular collections of tightly knit epithelioid macrophages (tuberculoid granulomas), necrotizing histiocytic reactions (necrotizing granuloma), and diffuse polymorphic infiltrates composed of lymphocytes, histiocytes, and plasma cells (granulomatous inflammation). The organisms that evoke these cellular responses are limited and include actinomycetes, mycobacteria, fungi, and helminths.

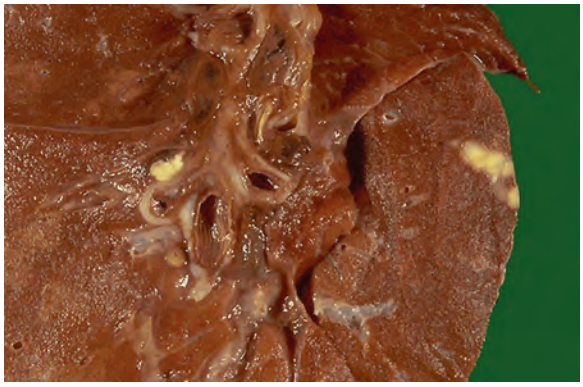


• **Figure 8.53** Ultrastructure demonstrates bacilli of *T. whippelli. Arrows* show "Whipple body" bacterium.

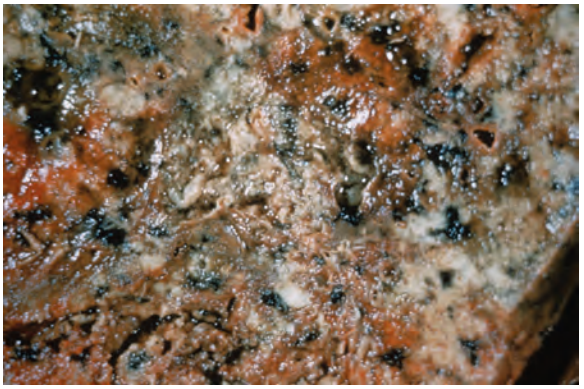
Mycobacterial Infection

Pulmonary necrotizing granulomatous inflammation is most commonly caused by mycobacterial or fungal infections. Tuberculosis is caused by a gram-positive soil actinomycete. The disease was recognized in antiquity, and it continues to represent a major source of global morbidity and mortality, largely due to its recrudescence in the setting of poverty and HIV/AIDS.[58] The term *tuberculosis* is properly limited to infections caused by *Mycobacterium tuberculosis* and its genetically related congeners (e.g., *Mycobacterium bovis*) and is not the appropriate appellation for infections due to nontuberculous or "atypical" mycobacteria.

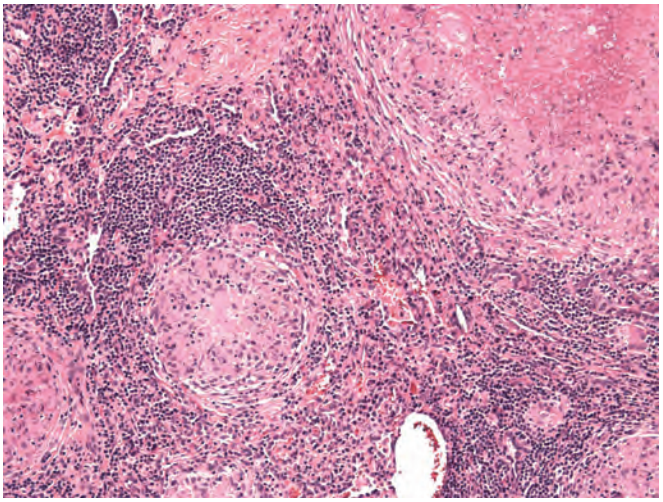
M. tuberculosis can affect the pulmonary airways, parenchyma, or pleura. Inhaled mycobacteria proliferate in the alveolar spaces and are then transported via lymphatics to regional hilar lymph nodes, from which they can enter the systemic circulation to spread to other organs. Progression of infection is limited by the acquisition of effective cell-mediated immunity.[59] The initial pulmonary focus of infection *(Ghon focus)* subsequently heals by fibrosis and may show dystrophic calcification. This Ghon focus together with the accompanying calcified site of infection in a



• **Figure 8.54** The *Ranke* complex includes the intrapulmonary *Ghon* focus of initial infection and the calcified hilar lymph node.



• **Figure 8.56** The cheesy gross appearance of a focus of caseous necrosis.



• **Figure 8.55** The tuberculoid granuloma is a collection of epithelioid histiocytes often with giant cells, surrounded by a lymphocytic infiltrate and fibrosis.

hilar lymph node are termed the *Ranke complex*, (Fig. 8.54) and the foci of primary disseminated infection are termed *Simon foci.* At all sites, the cellular response to *M. tuberculosis* is characterized by nodular collections of epithelioid macrophages with multinucleated giant cells, the tuberculoid granuloma *or tubercle*. These can undergo central necrosis due to a cell-mediated hypersensitivity response. Sites of tuberculous infection may also show neutrophilic and eosinophilic exudates, but these are generally not prominent features.

The mature tuberculoid granuloma is surrounded by a rim of T lymphocytes and contained by an outer zone of fibrosis (Fig. 8.55). The presence of tuberculoid granulomas can be a distinguishing feature clue in differentiating tuberculosis from other forms of necrotizing granulomatous inflammation (e.g., Wegener granulomatosis) in which tuberculoid granulomas are infrequent.

The term *caseous necrosis* properly refers to the "cheesy" appearance of the necrotic lesion on gross inspection. The histologic correlate is destruction of lung tissue (Fig. 8.56) with loss of the underlying reticulin stroma. However, as the latter finding is not apparent in H&E stained sections, it is best, in practice, to term tuberculous lesions as either "necrotizing" or "nonnecrotizing," and to avoid the terms "caseating" versus "noncaseating."

• BOX 8.4 Acid-Fast Bacteria

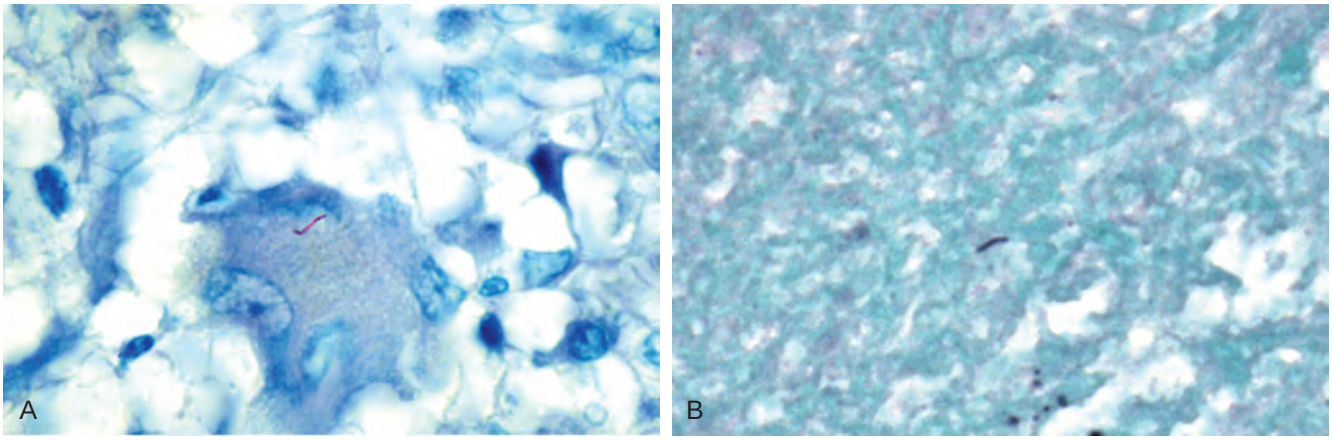
Mycobacteria
Nocardia
Rhodococcus
L. micdadei

Because the response to mycobacterial infection reflects a component of immune hypersensitivity, even small numbers of organism can evoke substantial lung injury, and this complicates the task of identifying mycobacteria in situ. The organisms are best identified by their red color in acid-fast bacillus (AFB)-stained sections, such as with the Ziehl-Neelsen stain or its modifications (e.g., Fite-Faraco). Mycobacteria can show substantial morphologic variability. They are curvilinear, vary in length, and exhibit a characteristic "beaded" appearance attributable to nonhomogeneous uptake of the AFB stain (Fig. 8.57A).

However, there is no reliable way to distinguish *M. tuberculosis* from "atypical" mycobacteria by histochemical staining. Because mycobacteria are weakly gram positive, they can also be demonstrated in GMS-stained sections, a finding that is nonspecific but can at times aid in the identification of the inconspicuous bacilli (see Fig. 8.57B). Because nonmycobacterial organisms can also be AFB positive, differential staining and culture results at times may be required to establish an accurate diagnosis (Box 8.4).

In practice the identification of *M. tuberculosis* by Ziehl-Neelsen staining is relatively insensitive, identifying mycobacteria in approximately 60% of culture-positive cases, so that the diagnosis may depend on isolating organisms in culture. In one study of more than 300 biopsies, mycobacteria were cultured from the biopsy specimens that contained necrotizing granulomas (38.2%), nonnecrotizing granulomas (32.4%), poorly formed granulomas (30.0%), or acute inflammation (15.8%). Tissues with fibrotic or hyalinized granulomas, nonspecific chronic inflammation, nonspecific reactive or reparative changes, no significant histologic abnormality, or malignancy failed to yield positive cultures. This study concluded that biopsy specimens with the latter diagnoses were inappropriate specimens for both mycobacterial culture and histochemical staining. Preliminary results with mycobacterial probes suggest that an in situ Affymetrix technique may increase the yield for detecting mycobacteria in lung biopsies.

Mycobacteria grow slowly in culture, and it can take weeks before they are eventually isolated. Consequently, ancillary



• **Figure 8.57** **A,** Mycobacterial tuberculosis, the "red snapper," is a short beaded bacillus that can be equally well demonstrated with either the Ziehl-Neelsen stain or its weakly acid-fast modifications. **B,** Mycobacteria are apparent in Gomori methenamine silver-stained sections, but this finding is less specific than AFB staining.

• BOX 8.5 Pulmonary Manifestations of Tuberculosis

Exposures with no disease (Ranke complex)
Caseating pneumonia
Acinar-nodose pneumonia
Nodular disease (tuberculoma)
Cavitary pneumonia
Tracheobronchitis
Miliary disease
Lymphadenitis and calcification
Pleuritis
Fibrothorax (late)

methods have been developed with the aim of increasing the likelihood of establishing a diagnosis in a timely fashion. Sensitive fluorescent staining techniques, including the auromine-rhodamine stain, are used routinely in some laboratories in examining smears and tissues. PCR methods have been developed that can identify *M. tuberculosis* and distinguish them from atypical mycobacteria, both in fresh tissues and into paraffin-embedded sections. The sensitivity and specificity of the latter approach is discussed elsewhere in this text.

Spectrum of Pulmonary Tuberculous Infection

Tuberculosis has protean manifestations in the lung (Box 8.5). Following most exposures, the primary infection is limited by the host's cellular immune system and the host remains asymptomatic, showing evidence of previous limited infection only via a positive tuberculin skin test. However, small numbers of mycobacteria can remain potentially viable, and active infection may ensue if cell-mediated immunity is diminished by age, the use of corticosteroids, diabetes, ethanolism, or by concomitant chronic infection. There is a marked increase in tuberculosis in patients with pulmonary silicosis, and establishing the presence of mycobacterial infection in a patient with progressive massive fibrosis due to silicosis can be difficult.

As previously noted, histologic examination of infection can provide an estimation of the adequacy of host immunity. The morphology of giant cells may be an indicator of whether host cell-mediated immunity is adequate in containing tuberculous infection. Prior to the development of effective cell-mediated immunity, many giant cells in the lesions show nuclei that have aggregate towards one pole of the multikaryon (Fig. 8.58A), whereas when infection is effectively contained, Langhans giant cells with peripheral nuclei or giant cells with centrally placed nuclei predominate (see Fig. 8.58B). If cell-mediated immunity is profoundly diminished, granulomas may be poorly formed or absent.

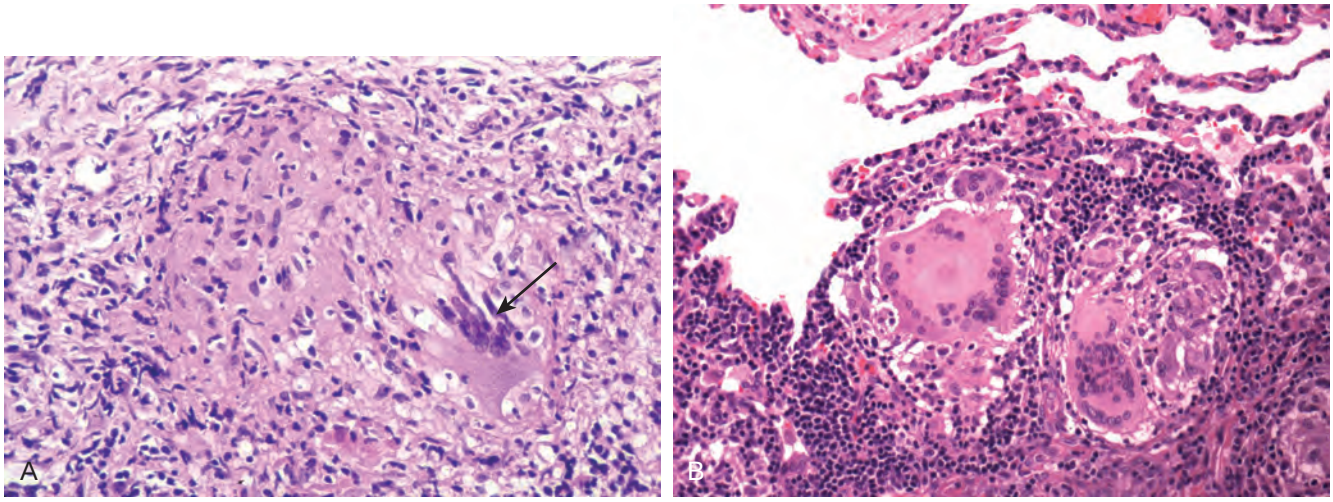
Failure to contain the primary infection leads to its progression. Primary tuberculosis may show necrotizing pneumonia (Fig. 8.59), regionally involved lymph nodes, and a granulomatous pleuritis with lymphocytic effusion. Dissemination of organisms via the bloodstream can yield miliary disease, in which innumerable foci of active infection with poorly formed granulomas are seen (Fig. 8.60 A to C).[60]

Tuberculous acinar-nodose bronchopneumonia results from mycobacterial infection extending along the pulmonary acinus (Fig. 8.61). Tuberculomas are defined foci of nodular tuberculous infection (Fig. 8.62). When these cavitate into an adjacent bronchus, it can lead to discharge of numerous bacilli with cough or expectoration (Fig. 8.63A). Although bacillary counts in cavitary lesions are generally high, even large cavities at times will fail to show a single identifiable organism by histochemical staining, and diagnosis must be made presumptively based on histologic appearance and empiric response to antituberculous medications.[61] The extension of cavitary disease to involve an accompanying pulmonary artery may produce a Rasmussen aneurysm and risk of vascular rupture leading to fatal hemoptysis (see Fig. 8.63B).

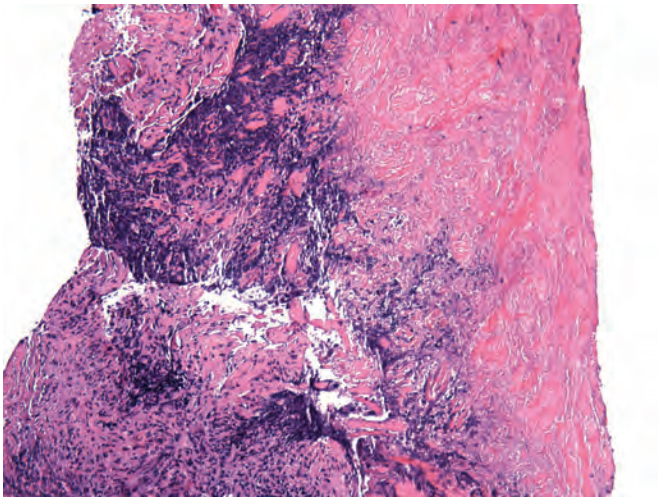
Mycobacteria can spread along the mucosal surfaces of the airways to produce ulcerated lesions in the larynx and tracheobronchial tree that can mimic Wegener granulomatosis (Fig. 8.64). However, as previously noted, the latter disorder rarely includes well-formed tuberculoid granulomas, and their presence favors the diagnosis of infection.

Reactivation Tuberculosis

Most clinically encountered cases of tuberculosis occurring in previously asymptomatic individuals with positive cutaneous responses to purified protein derivative (PPD) represent either



• **Figure 8.58** **A,** Polarized giant cells in early infection with *M. tuberculosis (arrow)*. These cells may reflect host difficulties in containing the infection. **B,** Mature infection shows Langhans giant cells and giant cells with central nuclei.



• **Figure 8.59** Necrotizing pneumonia due to *M. tuberculosis*.

• BOX 8.6 Classification of Mycobacteria

Tuberculosis Complex
M. tuberculosis
M. bovis

Runyon Group I
M. kansasii
M. marinum

Runyon Group II
M. gordonae
M. scrofulaceum

Runyon Group III
M. intracellulare
M. avium
M. xenopi

Runyon Group IV
M. fortuitum
M. chelonei
M. abscessus

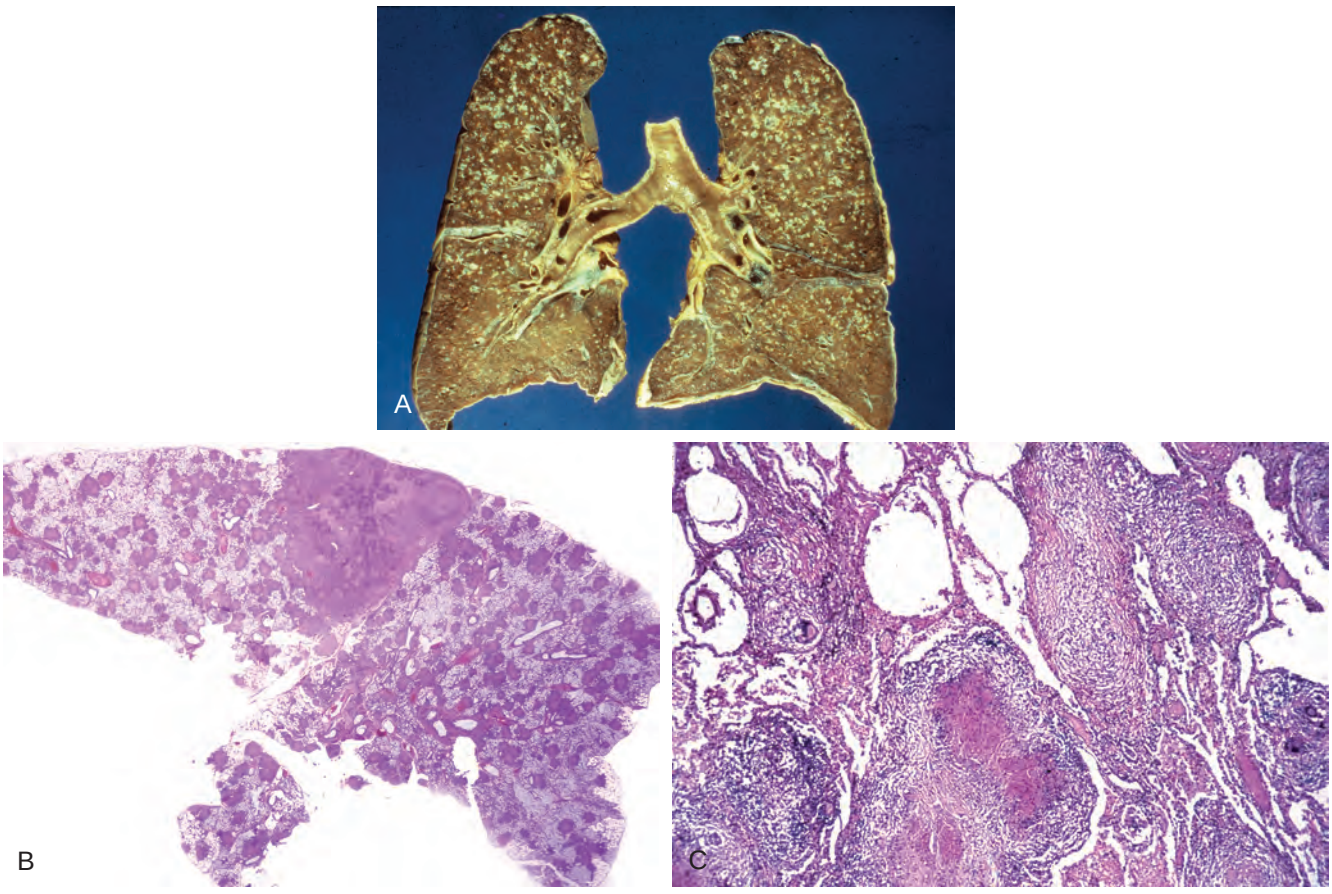
reactivation or reinfection. Reactivation indicates an acquired defect in cell-mediated immunity. Tuberculosis tends to reactivate in the upper lobes of the lung where ventilation/perfusion ratios are high. Histologically the lung shows necrotizing granulomas in areas of scarring and traction bronchiectasis (Fig. 8.65), due to the initial mycobacterial infection. In addition to reactivation of tuberculosis, the differential diagnosis of necrotizing granulomatous inflammation in this setting includes fungal infection and atypical mycobacterial infection, because both have a predilection to develop in areas of old pulmonary apical scarring. The distinction may be difficult in the case of atypical mycobacterial infection, as special stains cannot distinguish these possibilities, and culture or ancillary diagnostic tests are necessary (Fig. 8.66).

Atypical Mycobacteria

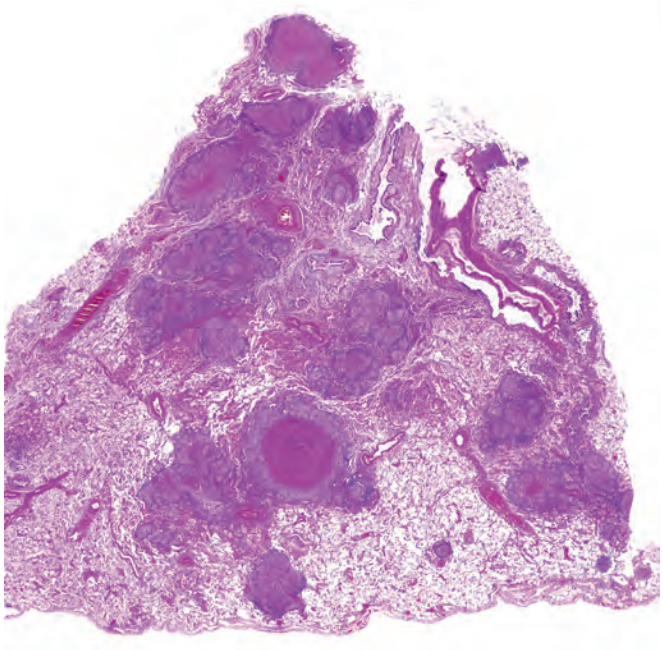
A number of mycobacteria that are genetically distinct from *M. tuberculosis* can produce pulmonary infection.[62] These organisms vary in virulence, and this is often reflected in the histologic appearance of the infection (Box 8.6). *Mycobacterium avium-intracellulare* or *Mycobacterium* avian complex (MAC) can attack the lung in a variety of clinical settings.[63] Patients immunosuppressed by HIV/AIDS can develop virulent infections with features that mimic tuberculosis. When adaptive T cell–mediated immunity is severely compromised, the host response may be limited to foamy histiocytes that have ingested large numbers of mycobacteria.[64] MAC can be demonstrated by both AFB and PAS stains, and clinical signs of both tuberculosis and MAC disease may only be recognized following treatment with antivirals, the so-called *immune reconstitution syndrome*.

In the immunocompetent host, MAC tends to affect older women (Lady Windermere disease) and patients with bronchiectasis or bullous emphysema. It is commonly seen in the right middle lobe syndrome due to bronchiectasis and chronic atelectasis. The lesions of MAC infection are detected radiographically as "tree-in-bud" opacities reflecting terminal bronchiolar infection (Fig. 8.67A), together with nodules that may be either solid or cavitary. Histologically the pathology often shows extensive areas of nonnecrotizing epithelioid histiocytes and is highly characteristic (see Fig. 8.67B). The causative mycobacteria are indistinguishable from *M. tuberculosis* and only culture or PCR can accurately establish the diagnosis.

A recently described variant of MAC infection is "hot tub" lung. In these cases the lung shows a microgranulomatous

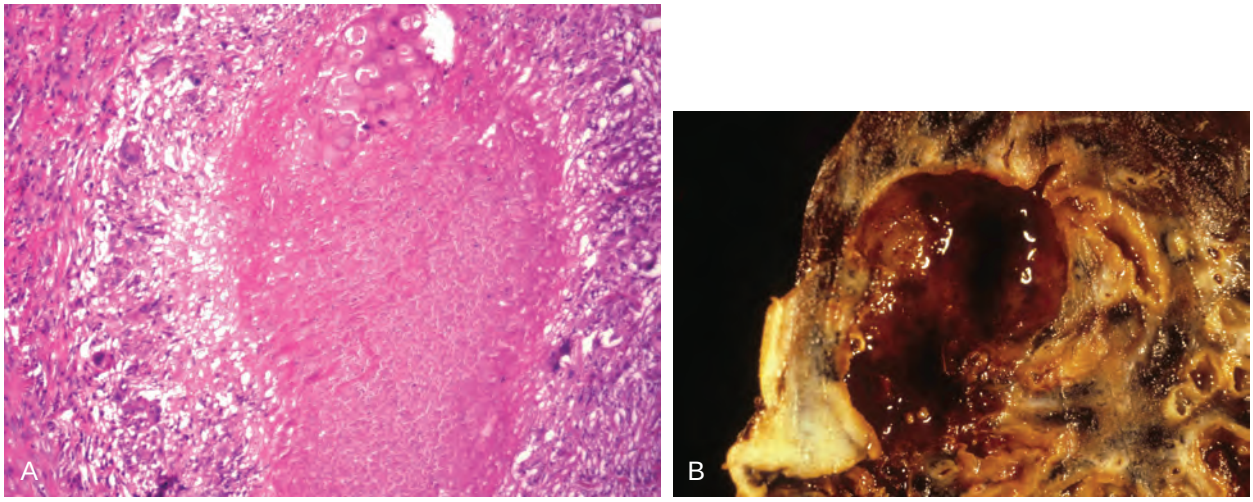


• **Figure 8.60** **A,** Miliary tuberculosis in lung reflects the failure to contain either primary or reactivation infection by the host. **B,** Innumerable millet-sized nodules are seen in lung with focus of necrotizing pneumonia. **C,** The response in miliary tuberculosis included poorly formed granulomas. With profound immunodeficiency, granulomatous changes may be absent.

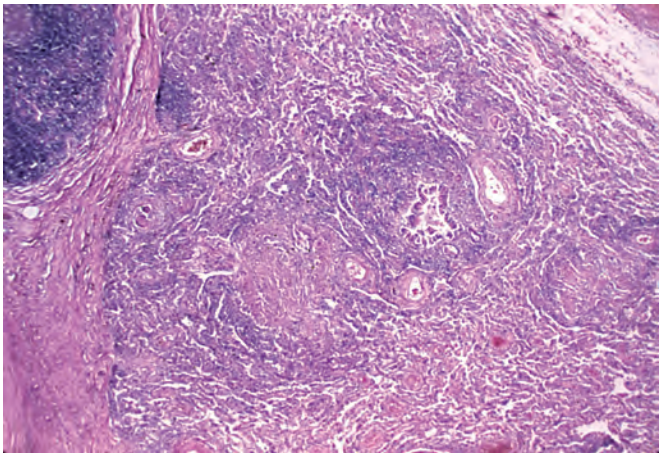


• **Figure 8.61** Geographic infiltrates of necrotizing acinar-nodose tuberculosis.

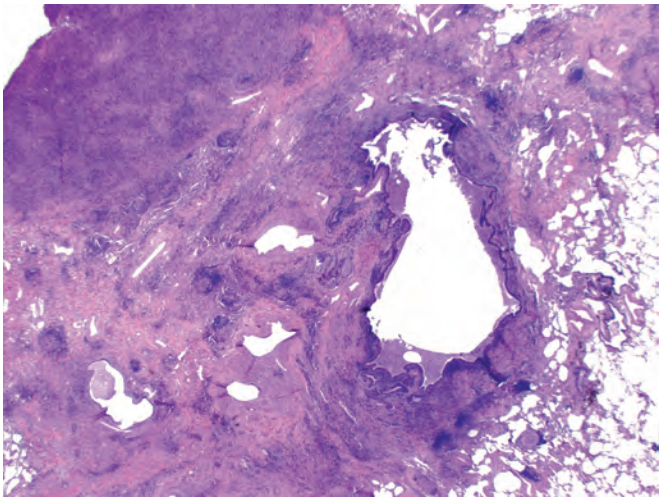
• **Figure 8.62** Localized tuberculoma.



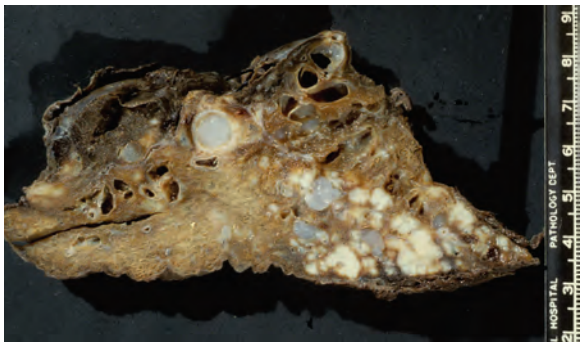
• **Figure 8.63** **A,** Cavitary tuberculosis. **B,** Erosion of a cavity into an adjacent pulmonary artery can produce a *Rasmussen aneurysm* and lead to fatal pulmonary hemorrhage.



• **Figure 8.64** Tracheobronchial tuberculosis. These lesions can mimic Wegener granulomatosis, but the presence of tuberculoid granulomas is a distinguishing diagnostic feature because they are rarely seen in Wegener granulomatosis.



• **Figure 8.66** Necrotizing granulomas in area of old calcified focus of bronchiectasis were due to atypical mycobacterial infection.

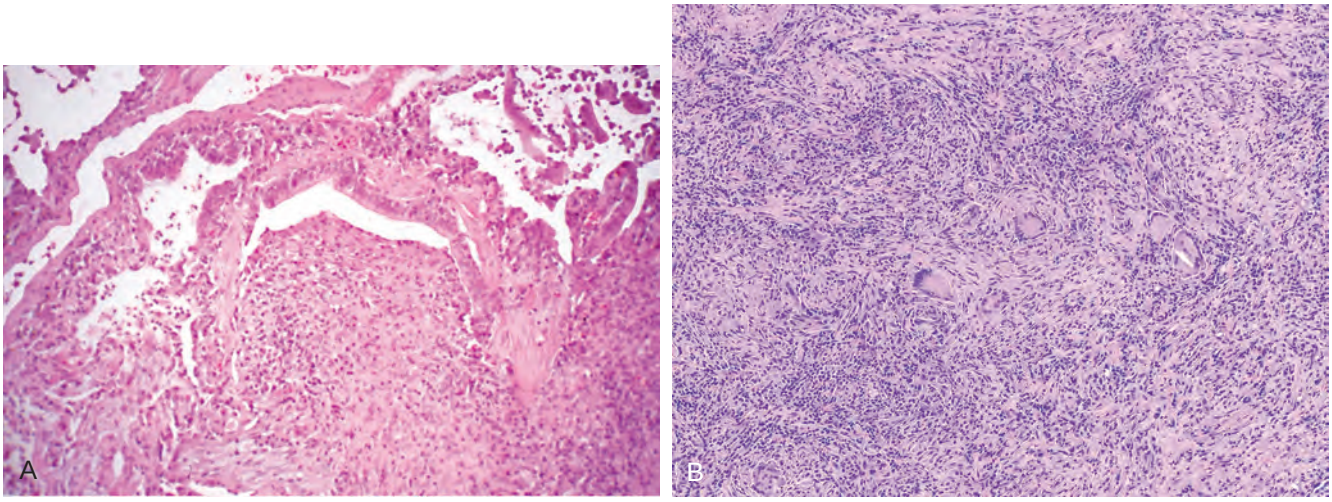


• **Figure 8.65** Bronchiectasis in tuberculosis.

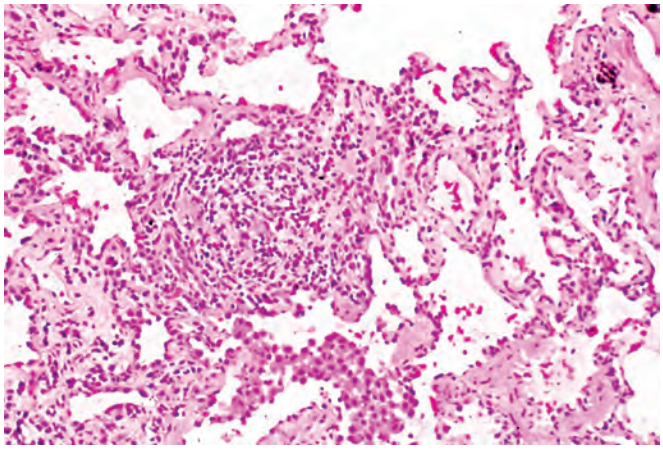
hypersensitivity pneumonitis that may be accompanied by necrotizing granulomatous inflammation. The pathology appears to represent primarily a cell-mediated hypersensitivity response to mycobacterial antigens, with a possible element of direct infection as well (Fig. 8.68).

An unusual presentation of MAC infection in the immunosuppressed host is the *pseudosarcomatous* nodule.[65] This can develop in the lung or in soft tissues and hematopoietic tissues. The nodules are composed of spindle cells and may be mistaken for a low-grade spindle-cell neoplasm (Fig. 8.69A). However, examination reveals the foamy appearance of the spindle cells, which prove to be $CD68^+$ macrophages containing large numbers of ingested mycobacteria, (see Fig. 8.69B), and the absence of mitotic activity. Within the spectrum of unusual mesenchymal reactions seen in the immunocompromised one must consider inflammatory myofibroblastic tumors that may include foamy histiocytes (Fig. 8.70A) associated with human herpesvirus 8 (HHV-8) infection (see Fig. 8.70B).

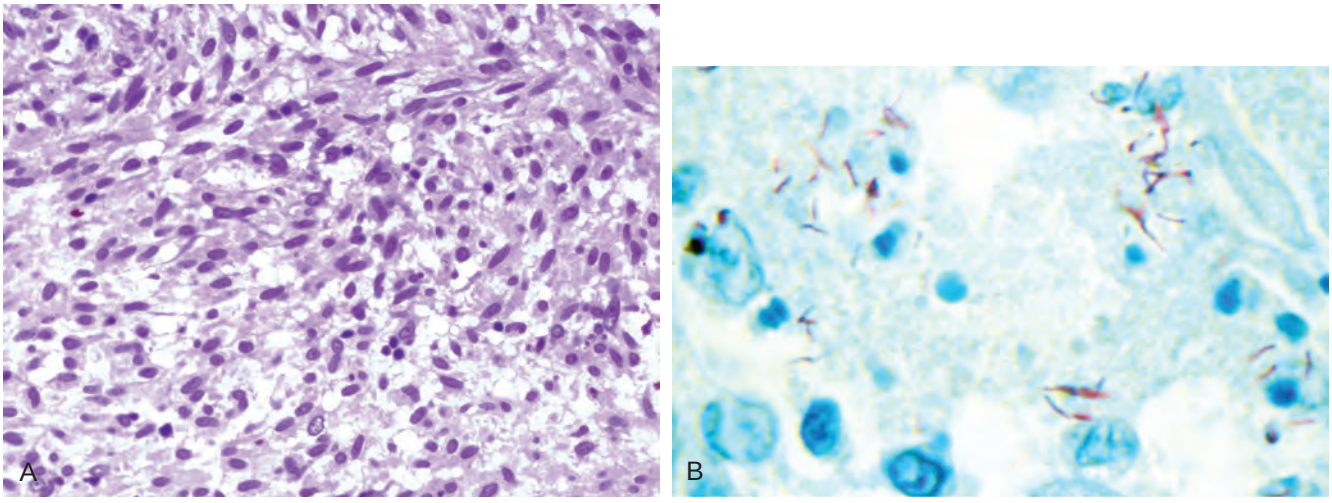
Other mycobacteria also cause pulmonary infection. *Mycobacterium kansasii* is a virulent species of atypical mycobacteria that produces necrotizing infection indistinguishable from tuberculosis. The organism often shows a prominent pattern of "cross-linking" on mycobacterial stains in situ that is characteristic but not diagnostic (Fig. 8.71). Other rapidly growing mycobacteria, including *Mycobacterium abscessus*, infect preexisting areas of



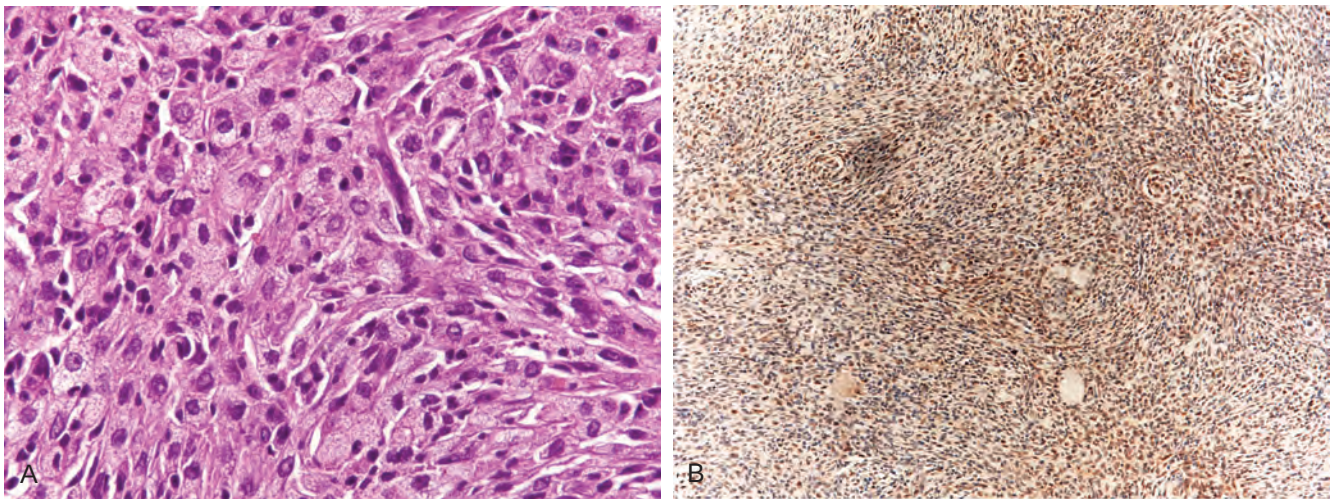
• **Figure 8.67** **A,** Nonnecrotizing granulomatous inflammation ulcerates a small airway in a patient with MAC. **B,** Sheets of epithelioid histiocytes are characteristically seen in MAC infection in nonimmunosuppressed patients with chronic airway disease.



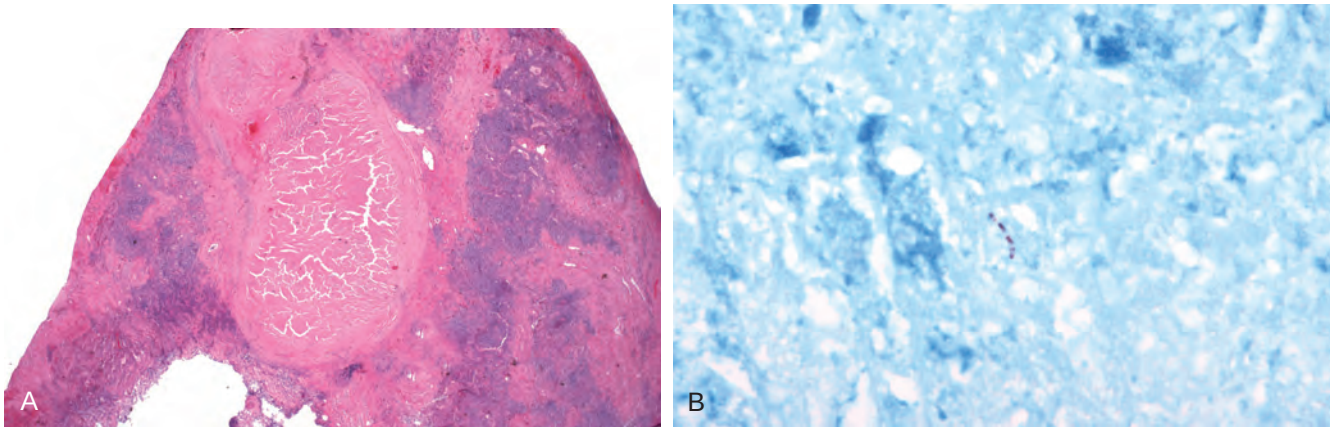
• **Figure 8.68** Micronodular granulomatous inflammation in "hot-tub" lung, a hypersensitivity reaction to MAC.



• **Figure 8.69** **A,** A pseudosarcomatous nodule in a patient with human immunodeficiency virus/acquired immune deficiency syndrome due to *Mycobacterium avian* complex. **B,** Large numbers of AFB/periodic acid–Schiff-positive mycobacteria are generally seen in this response.



• **Figure 8.70** **A,** Inflammatory myofibroblastic tumor with foamy histiocytes associated with **(B)** human herpesvirus 8 in patient with acquired immune deficiency syndrome can mimic *Mycobacterium avian* complex infection.



• **Figure 8.71** **A,** *Mycobacterium kansasii* is a virulent organism that produces necrotizing granulomatous inflammation comparable to *M. tuberculosis*. **B,** The elongate bacilli exhibit irregularity in their uptake of AFB stain, producing a characteristic pattern of "cross-linking."

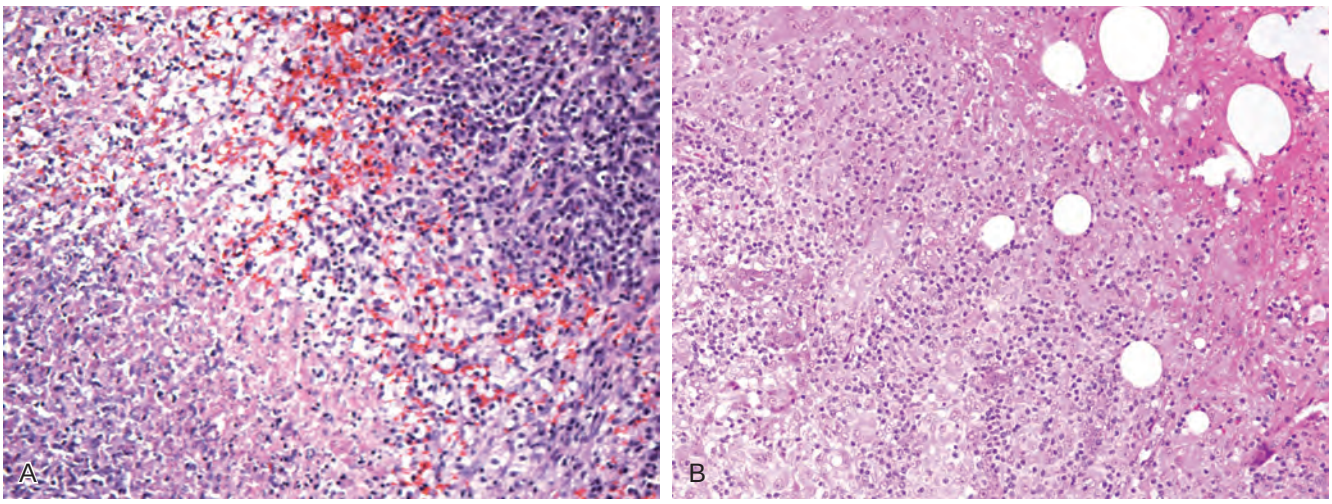
active bronchiectasis, particularly in cystic fibrosis (Fig. 8.72A), and rarely may involve the lung as miliary blood-borne disease (see Fig. 8.72B). *Mycobacterium fortuitum* produces limited infection in patients with diabetes, HIV/AIDS, and chronic upper gastrointestinal disease; all of the rapid growing nontuberculous mycobacteria, including *Mycobacterium smegmatis*, can complicate pneumonia due to aspiration of lipid-based substances like nose drops.[66]

Melioidosis

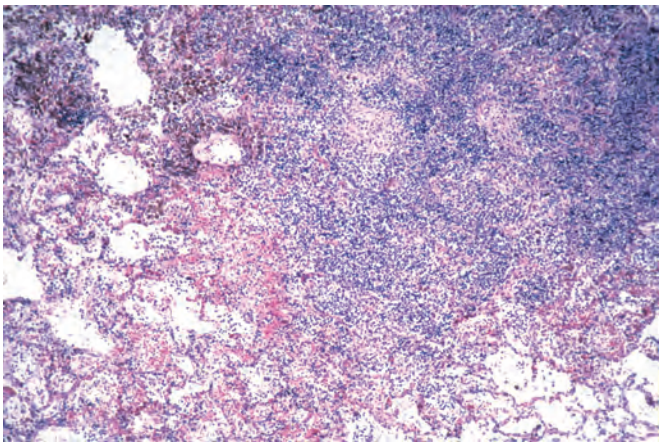
Rarely seen outside of Southeast Asia, where it is endemic, chronic infections due to *Pseudomonas pseudomallei* developed in veterans of the Vietnam War many years after they had left the region.[67] Acute melioidosis is a systemic infection that produces widespread coalescent microabscesses, but it may resolve without being recognized only to recur many years later in lung, lymph nodes, and bone. The lung shows necrotizing granulomatous lesions surrounded by a zone of fibrosis, (Fig. 8.73) and regional lymph nodes show stellate necrosis that can mimics "cat-scratch" disease due to *Bartonella henselae*. The offending organism is a gram-negative motile bacillus that shows bipolar staining that can be difficult to demonstrate in situ.

Fungal Infection Due to Yeasts

Fungi produce a spectrum of changes in the lung, ranging from benign colonization of airways to malignant angioinvasive infections. Some fungi grow as yeast at body temperatures, whereas others are hyphate molds (Table 8.2). Most fungal yeasts are soil organisms that are topographically distributed in the United States. Although *Histoplasma capsulatum* may be encountered virtually anywhere that soil and water coexist, most cases in the United States are endemic to the Mississippi and Ohio River valleys. *Blastomyces dermatitidis* predominantly affects individuals living in the Great Lakes regions and in the southeastern United States, whereas *Coccidioides immitis* is primarily encountered in the San Joaquin Valley of the Southwest. Despite their usual distribution, modern air travel and a highly mobile population has resulted in the possibility of these infections presenting virtually anywhere, and pathologists must be acquainted with their



• **Figure 8.72** **A,** *M. abscessus* complicates areas of bronchiectasis and may be exceedingly difficult to eradicate fully with antimycobacterial agents. **B,** Miliary *M. abscessus* in a patient with multisystem disease and mycobacteremia.



• **Figure 8.73** *P. pseudomallei*, the cause of melioidosis, may reactivate many years following initial exposure to produce a necrotizing granulomatous pneumonia that resembles tuberculosis.

characteristic histologic appearances. However, it is always prudent to inquire into possible travel prior to diagnosing an "exotic" fungal infection because substantial overlap can exist in fungal morphologies.

Histoplasmosis

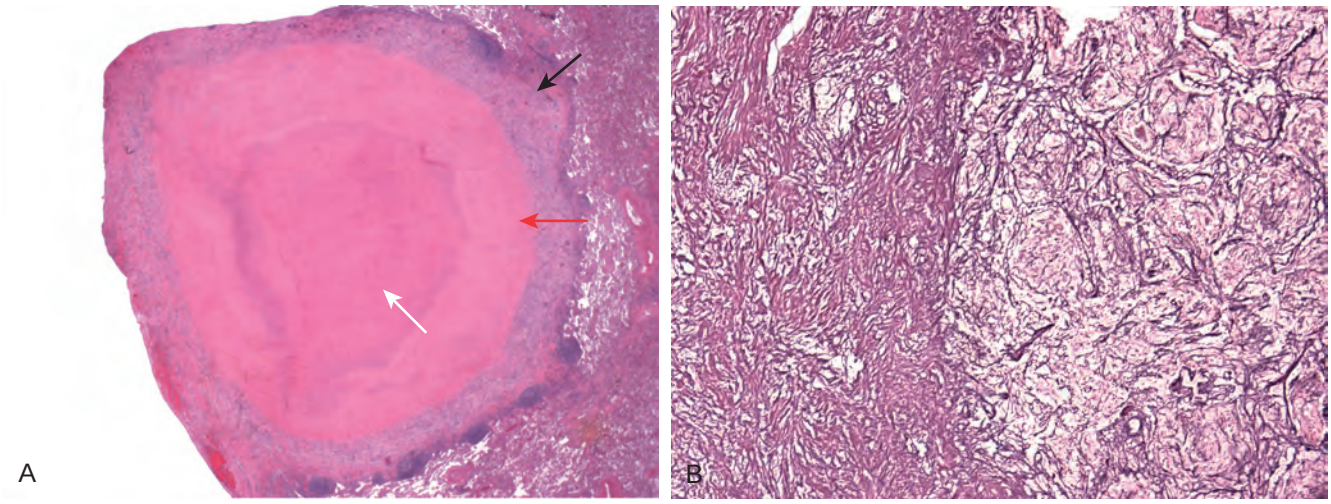
Primary *Histoplasma* infection produces a "viral-like" illness that generally resolves spontaneously.[68] However, if there is a defect in cell-mediated immunity or when the yeast burden is large, progression of infection may ensue.[69] Chronic histoplasmosis tends to develop in the lung apices in areas of bullous emphysema or bronchiectasis. Although most yeast can be identified in H&E-stained sections, *Histoplasma* require special histochemical staining due to their small size. GMS is the stain of choice because PAS at times fails to decorate the yeast.

Necrotizing granulomas due to *Histoplasma* spp. show central necrosis often surrounded by regions of mummefactive necrosis in which the "ghost outlines" of the underlying framework of the lung can still be distinguished in H&E- and reticulin-stained sections (Fig. 8.74A and B). The lining of the necrotic granuloma includes epithelioid histiocytes and giant cells but unlike tuberculosis does not show extensive tubercle granuloma formation, and the wall of the lesion characteristically exhibits paucicellular hyalinized fibrosis (Fig. 8.75).

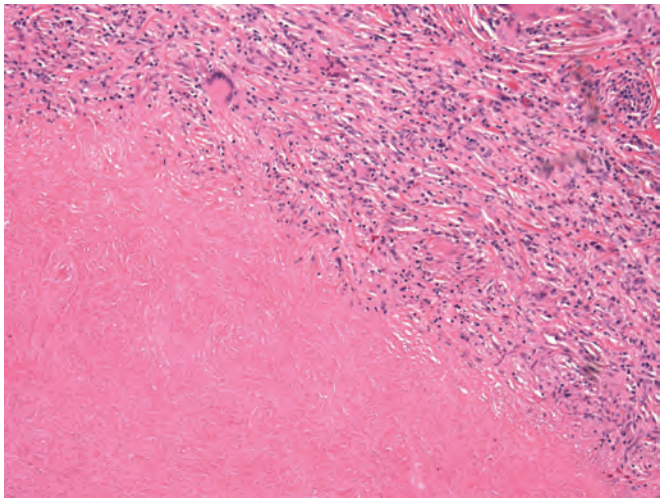
Although they are facultative intracellular pathogens, *Histoplasma* cluster in areas of necrosis and are often seen outside of histiocytes.[70] The 2- to 4-μM yeast show teardrop-shaped forms and reproduce by single, narrow-necked buds whose presence is diagnostic. Despite their name, no capsule is present (Fig. 8.76A). At times, pseudohyphae may be seen, and this should not dissuade the pathologist from making the correct diagnosis (see Fig. 8.76B) when all other diagnostic criteria are met.

TABLE 8.2 Fungal Identification in Tissue

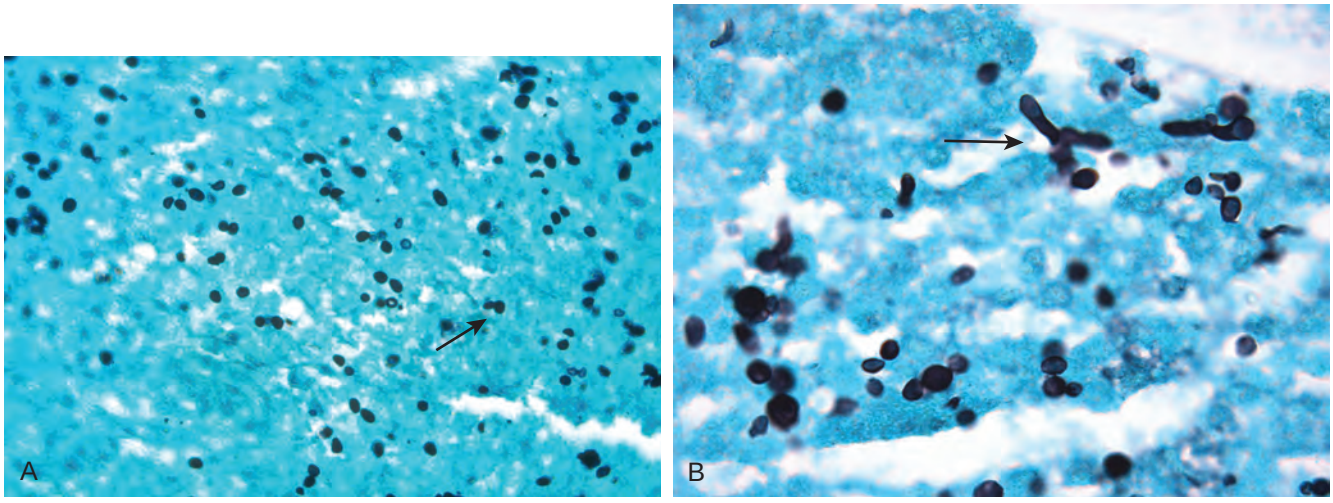
Organism	Size (Width μM)	Defining Morphology
H. capsulatum	2-5	Narrow-neck bud
C. neoformans	5-20	Narrow-neck bud
B. dermatitidis	15-30	Broad-based bud
C. glabrata	3-5	Budding, no pseudohyphae
Candida spp.	2-3	Yeast, pseudohyphae, hyphae
Aspergillus spp.	3-5	Acute-angle branching, septate, conidial head
Zygomyces spp.	5-8	Right-angle branching, ribbons, pauciseptate
Pseudallescheria spp.	3-4	Acute-angle branch, septate, terminal chlamydospore, pigmented conidia
Fusarium spp.	4-5	Acute- and right-angle branch, septate, narrowed branch points
C. immitis	20-200	Endosporulation



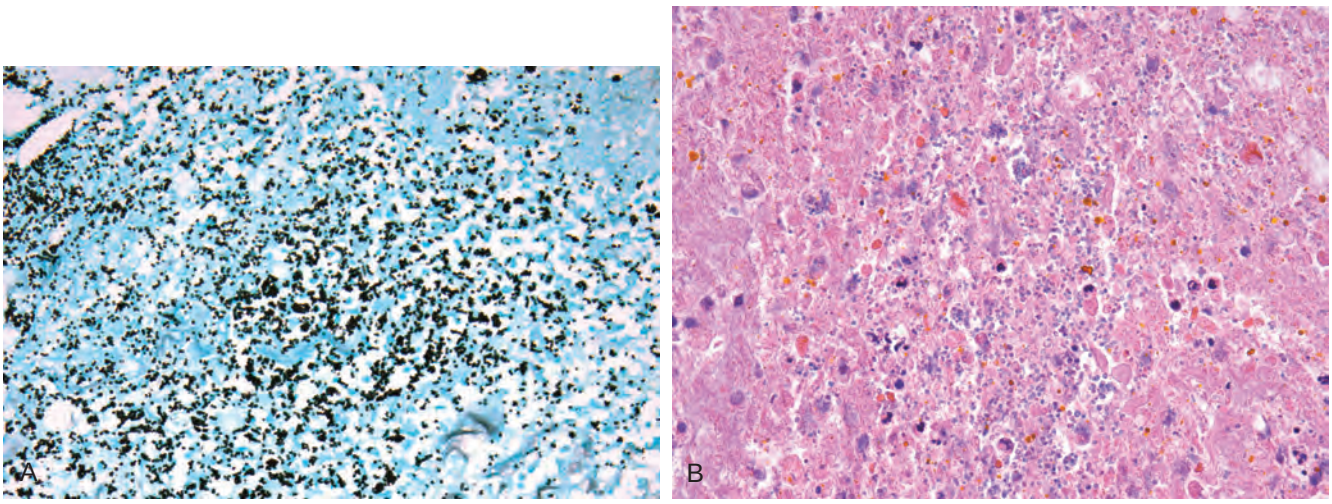
• **Figure 8.74 A,** Nodular pneumonia due to *H. capsulatum* often shows necrotizing granulomatous inflammation with three zones *(arrows)*. The outer capsule encloses an area of mummefactive necrosis that in turn surrounds an area of caseating necrosis findings confirmed by **(B)** reticulin stains.



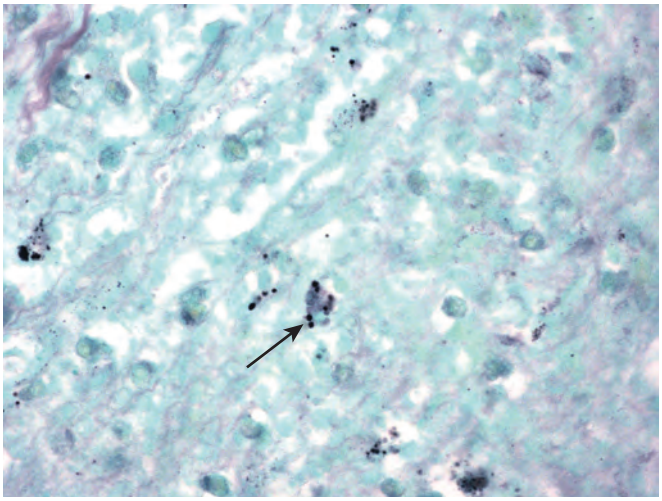
• **Figure 8.75** The wall of the necrotizing granuloma shows poorly formed granulomas and giant cells with a highly characteristic hyalinized basket-weave fibrosis.



• **Figure 8.76 A,** Narrow necked budding 2 to 4 μM yeast of *H. capsulatum* in Gomori methenamine silver stained section. Periodic acid–Schiff is not reliable for demonstrating this yeast. *Arrow* shows narrow-necked budding yeast. **B,** *H. capsulatum* infection with irregular yeast forms and pseudohyphae. *Arrow* points to pseudohyphal growth.



• **Figure 8.77** **A,** *C. glabrata* can easily be mistaken for *H. capsulatum* on Gomori methenamine silver stain, but **(B)** the yeast are amphophilic in hematoxylin and eosin–stained sections and easily differentiated on this basis.



• **Figure 8.78** Microcalcifications *(arrow)* can closely resemble *H. capsulatum*. At times ultrastructural examination may be required to exclude infection.

The differential diagnosis includes microforms of *Cryptococcus* or *Blastomyces*, *P. jiroveci*, and *Candida glabrata*. In the former, identifying the associated larger yeast forms eliminates the possibility of *H. capsulatum* infection. *P. jiroveci* shows irregularly shaped cysts with pericapsular accentuation on GMS stain and the organisms do not bud; *C. glabrata* can closely mimic the infection in GMS-stained sections, but unlike *Histoplasma*, they also stain amphophilic with H&E and are strongly gram positive (Fig. 8.77 A and B).

However, the most common and greatest difficulties in diagnosis can arise in distinguishing small, regular microcalcifications in GMS-stained sections because they can closely resemble degenerate yeast (Fig. 8.78). The presence of irregular calcifications is a clue to their actual nature, but at times, ultrastructural examination may be required to exclude infection. In the immunosuppressed host, disseminated infection is primarily distributed within interstitial and alveolar macrophages (Fig. 8.79).[71]

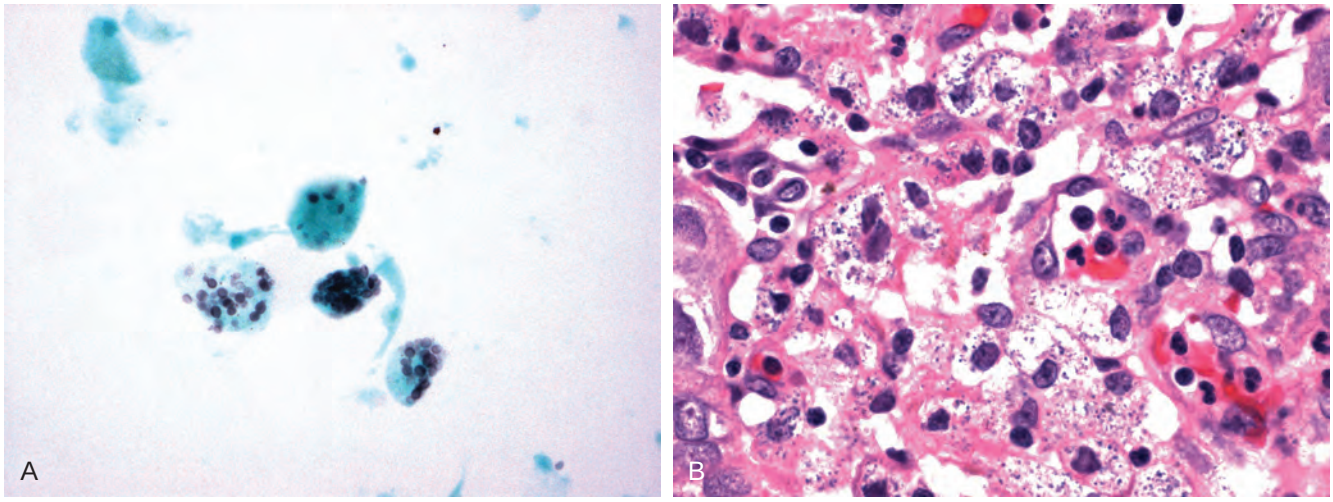
Extension of infection from a peribronchial lymph node can produce *mediastinal granuloma*, a lesion characterized by dense whorled hyaline fibrosis with aggregates of plasma cells (Fig. 8.80A). Organisms are rarely identified in the areas of paucicellular scarring, and this form of the disease appears to be immunologically mediated. At times, necrotizing granulomatous inflammation is concomitantly present and confirms the diagnosis (see Fig. 8.80B). The lesion can entrap the large vessels of the mediastinum, leading to the superior vena caval syndrome and death. Surgical excision is required but not always technically possible.

Remotely infected calcified peribronchial lymph nodes due to histoplasmosis can erode into adjacent airways to be either expectorated or aspirated as broncholiths. Surprisingly, these may show persistently viable yeast forms and colonization by aspirated oropharyngeal bacteria (Fig. 8.81).

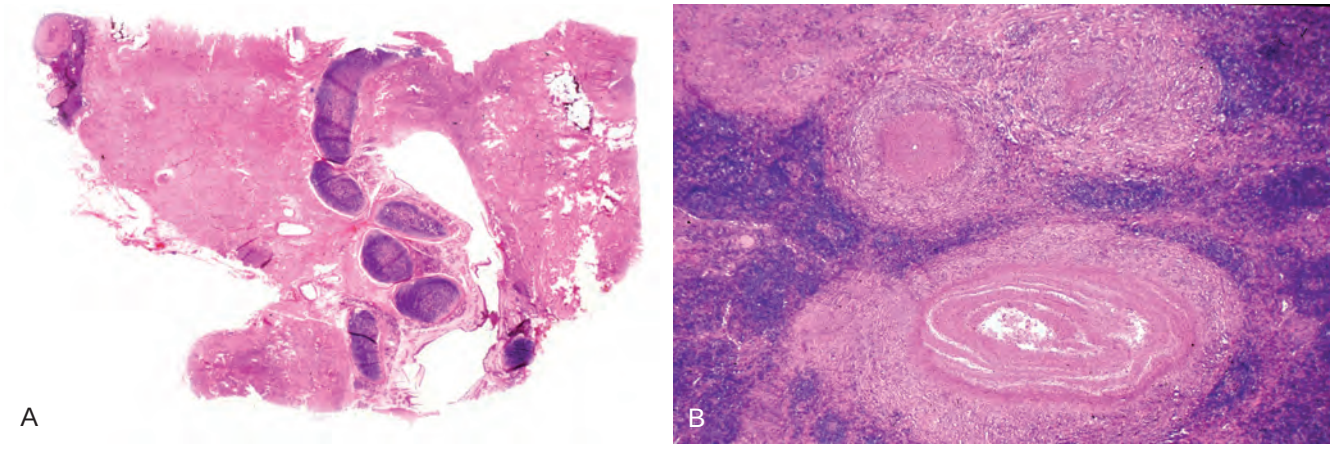
Old calcified granulomas are commonly encountered in surgical resection of lungs for neoplasia.[72] Most of these are due to healed tuberculosis or histoplasmosis, depending on exposure. In the vast majority of cases, no organism will be identified but occasionally nonviable degenerate histoplasma may be seen, although distinguishing them with confidence from microcalcifications can be difficult, and a high threshold for the diagnosis of histoplasmosis should be maintained in this setting.

Blastomyces

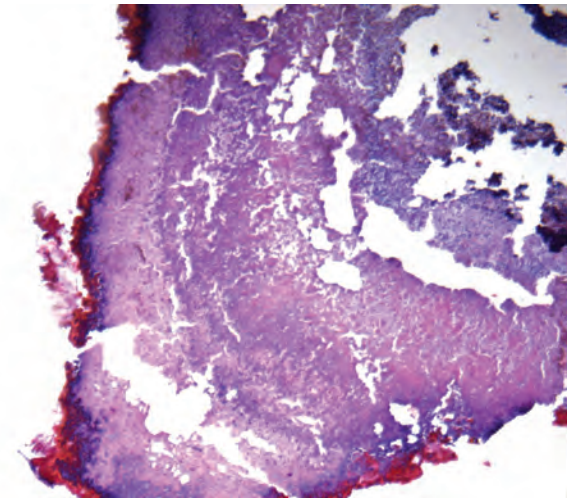
B. dermatitidis shows a propensity to infect lung, skin, and bone.[73] The spectrum of pulmonary presentations includes consolidative pneumonia and pulmonary nodules that radiographically may mimic pulmonary carcinoma (Fig. 8.82A). In rare cases, overwhelming infection may lead to acute respiratory failure and death (see Fig. 8.82B).[74] The pulmonary lesion characteristically shows a granulohistiocytic response (Fig. 8.83A). The yeast are large (15 to 30 μM) and easily identified in H&E sections, where they are distinguished by their thick refractile cell wall (see Fig. 8.83B). The organism is also multinucleate (see Fig. 8.83C) and proliferates via single, broad-based budding (see Fig. 8.83D). Microforms may be present and should not be confused with coinfection by *H. capsulatum*.[75] Giant yeast forms can also occur and may be confused with *C. immitis*.[76]



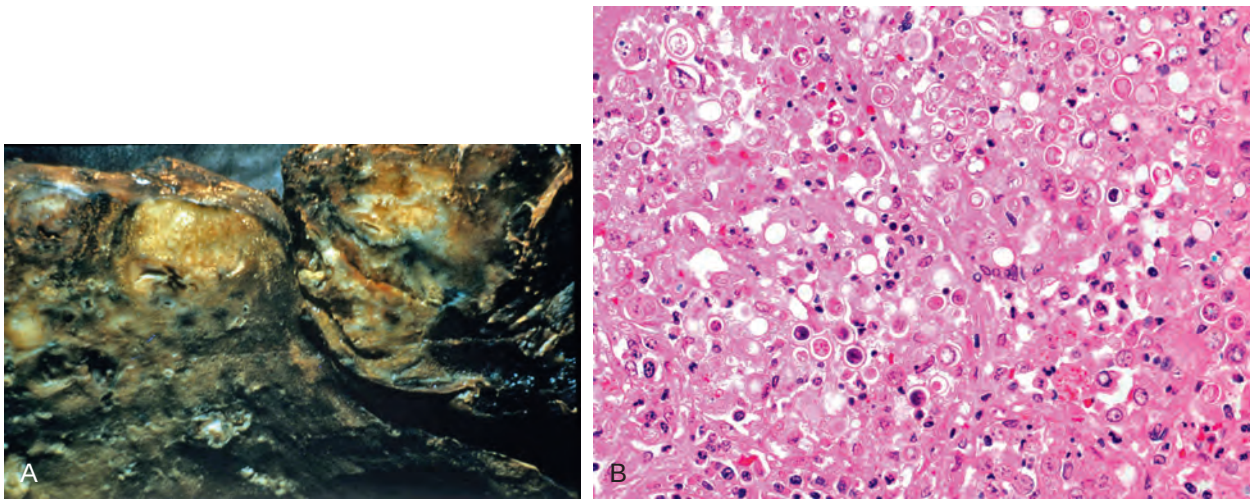
• **Figure 8.79 A,** Intracytoplasmic *H. capsulatum* in bronchoalveolar lavage macrophages in patient with disseminated infection and human immunodeficiency virus/acquired immune deficiency syndrome. **B,** Debris within macrophage phagolysosomes can resemble intracellular yeast.



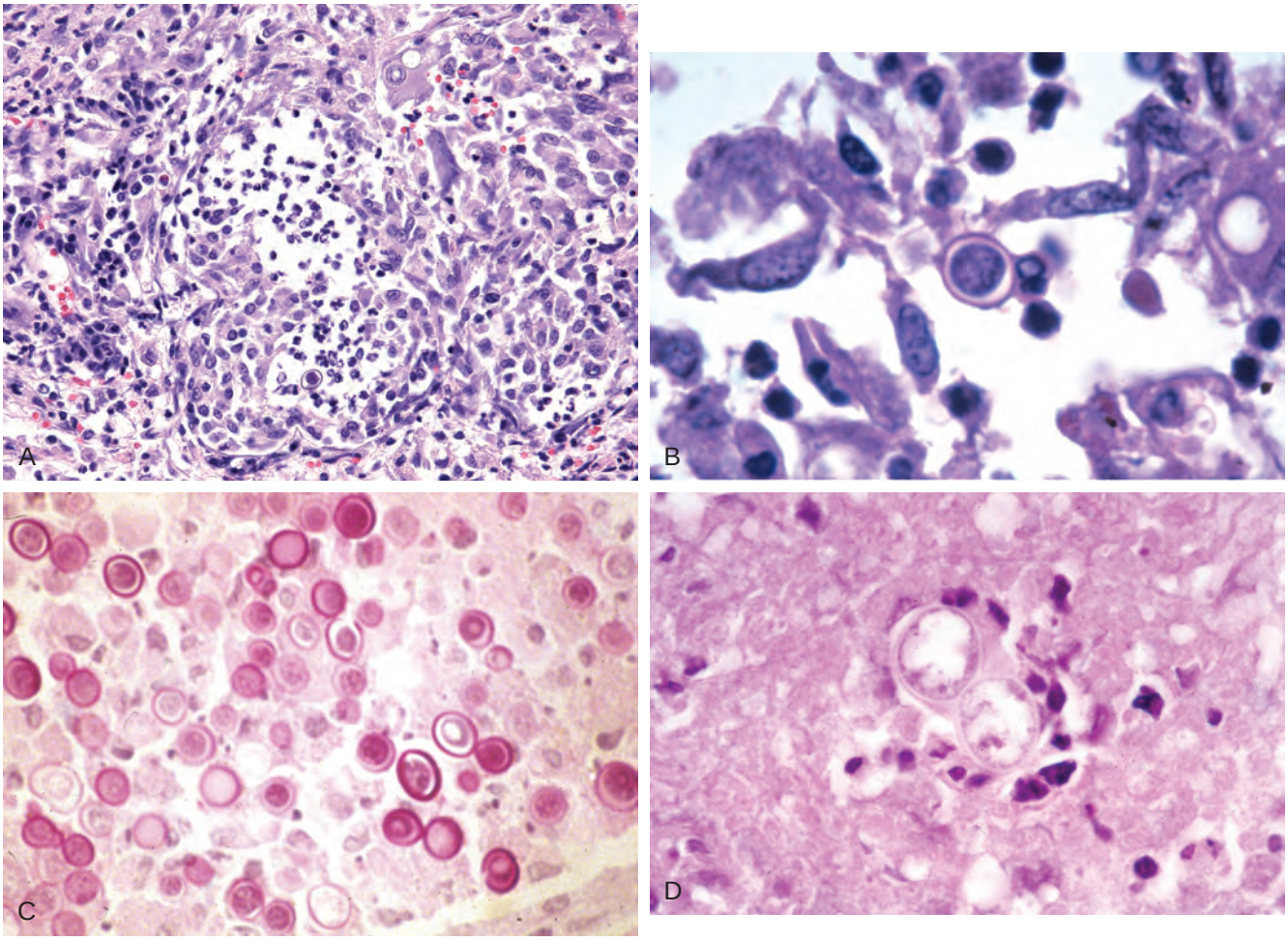
• **Figure 8.80 A,** Mediastinal granuloma is a serious complication of pulmonary histoplasmosis. The mediastinum shows paucicellular hyaline scarring and may include **(B)** foci of necrotizing granulomatous infection.



• **Figure 8.81** Expectorated broncholith due to histoplasmosis.



• **Figure 8.82** **A,** Pulmonary nodule excised as carcinoma due to blastomycosis. **B,** Blastomyces in patients who died from an overwhelming lung infection. Note presence of large numbers of yeast filling alveolar spaces.

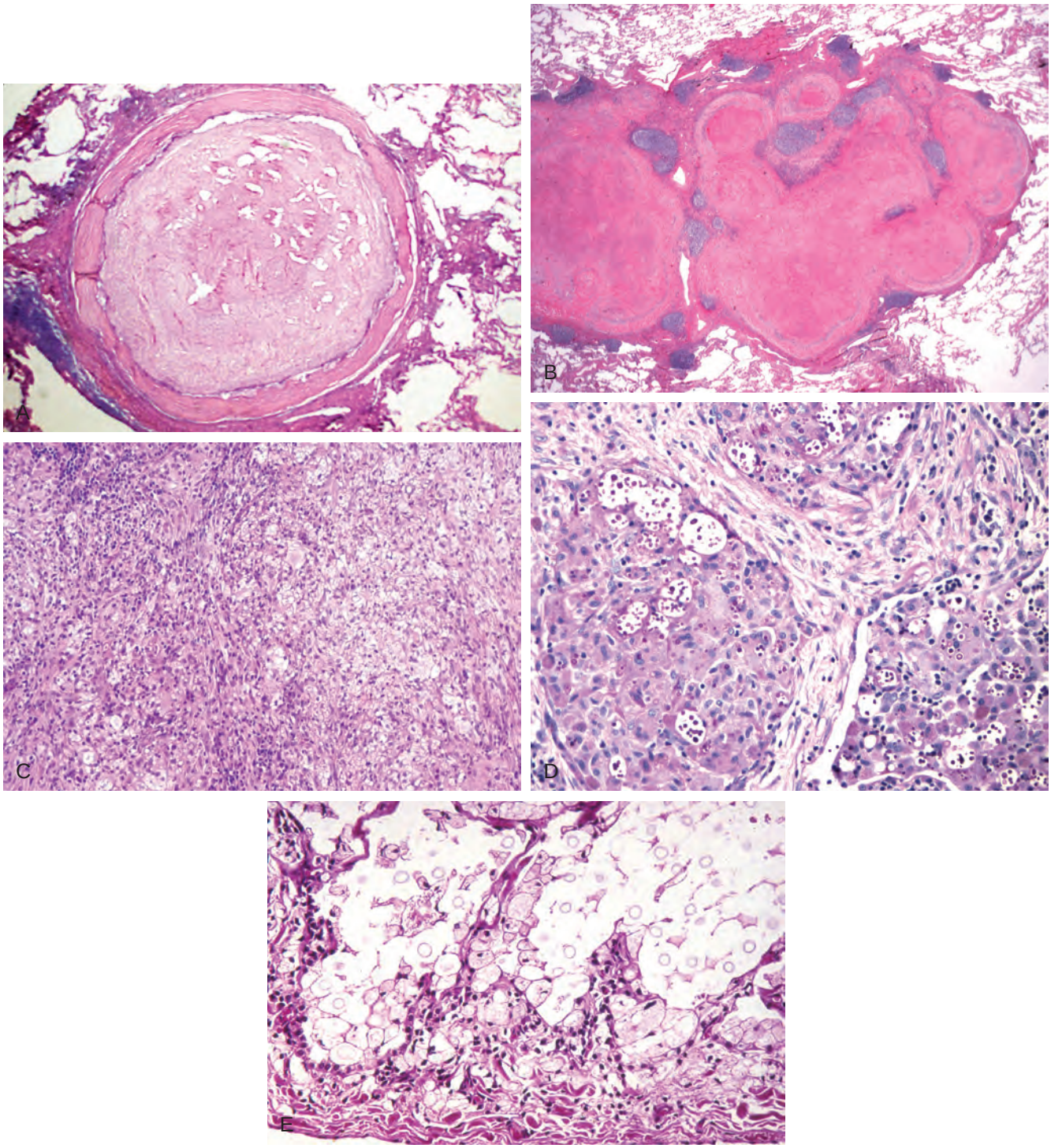


• **Figure 8.83** **A,** Granulohistiocytic response to *B. dermatitidis*. **B,** Yeast of *B. dermatitidis* with refractile cell wall. **C,** *B. dermatitidis* with multinucleation. **D,** *B. dermatitidis* showing broad-based budding.

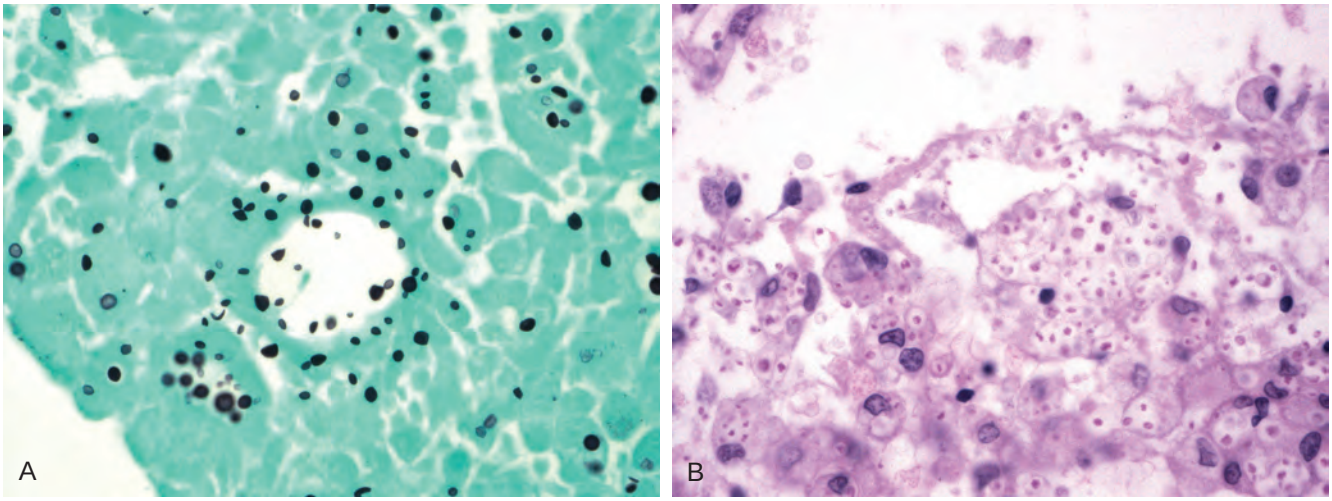
Cryptococcus

Cryptococcus neoformans infects immunocompromised patients but can also be seen in apparently normal hosts.[77] Meningoencephalitis is the most common clinical presentation, and it represents a complication of subclinical pulmonary infection. *C. neoformans* can produce localized necrotizing cryptococcomas (Fig. 8.84A), confluent bronchopneumonia (see Fig. 8.84B), granulomatous pneumonia (see Fig. 8.84C), or a null response characterized by "yeast lakes" with minimal inflammation (see Fig. 8.84D). Grossly, the infected lesions are glistening and "slimy."

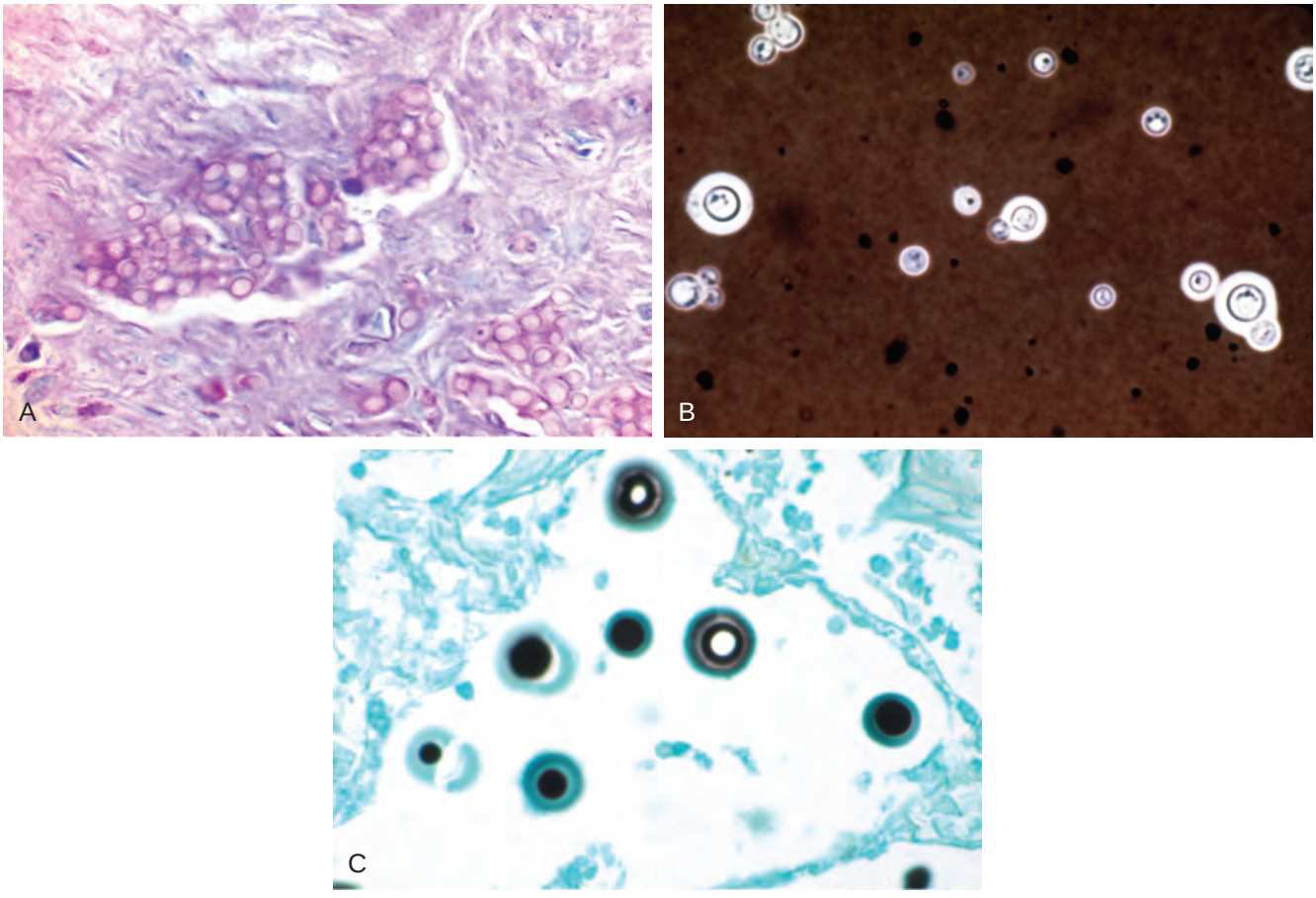
The organism show substantial variability in size (2 to 15 μM) and shape (Fig. 8.85A), and innumerable microforms can occasionally be seen that must be distinguished from histoplasmosis (see Fig. 8.85B). The yeast proliferate via single, narrow-necked



• **Figure 8.84** **A,** Nodular cryptococcoma. **B,** Confluent necrotizing bronchopneumonia. **C,** Histiocytic response. **D,** Granulomatous response to *C. neoformans.* **E,** Yeast lake.



• **Figure 8.85** **A,** Narrow-necked budding of *C. neoformans* showing variability in size and shapes. **B,** Microforms of *C. neoformans* within histiocytes must be distinguished from intracellular *H. capsulatum.*

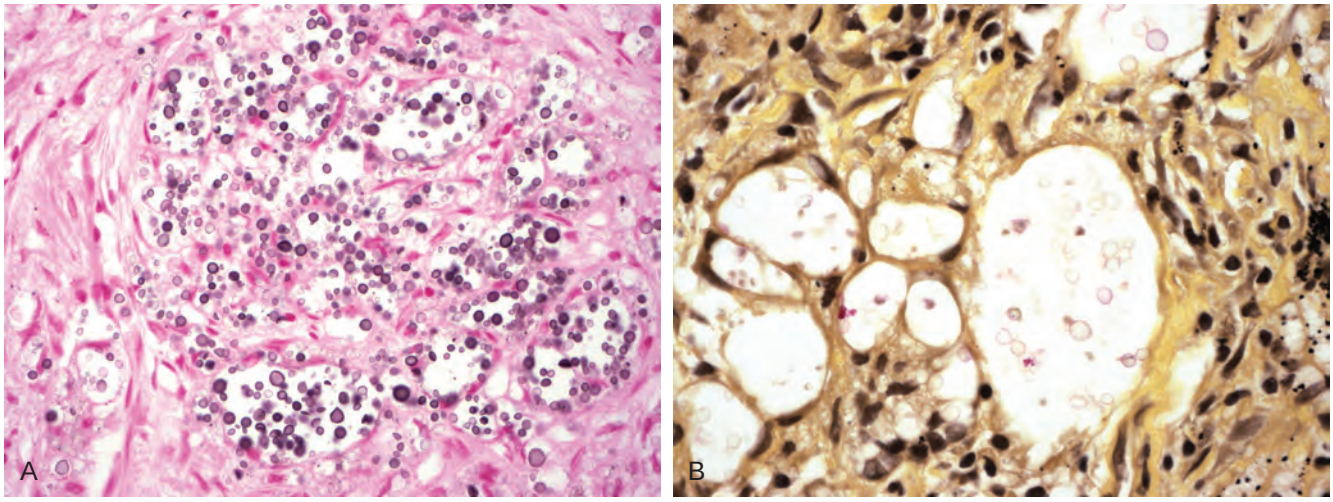


• **Figure 8.86** **A,** Mucicarmine stains capsule of *C. neoformans.* **B,** India ink preparation shows capsule of yeast. **C,** Both Gomori methenamine silver and periodic acid–Schiff stain body of yeast but not its capsule.

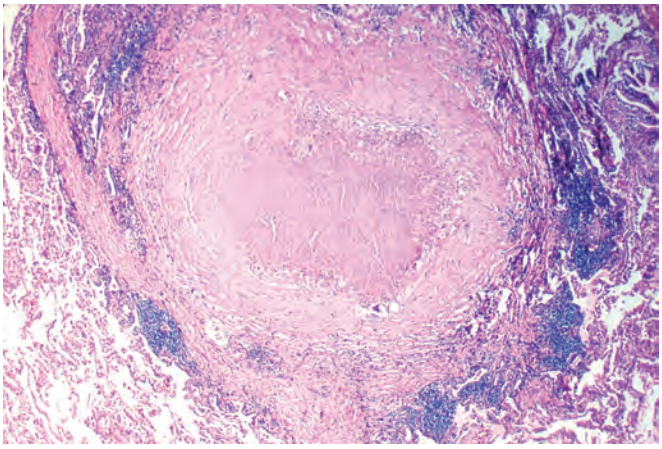
buds (secrete a capsule that is optimally visualized with mucicarmine in situ) (Fig. 8.86A). With GMS, the yeast body stains gray-black and the capsule is not decorated (see Fig. 8.86B). In capsular-deficient organisms the Fontana-Masson stain reacts with a melanin precursor in the yeast wall, highlighting the organisms (Fig. 8.87A), although a careful examination of the mucicarmine stain will invariably reveal a poorly developed rim of capsular staining (see Fig. 8.87B).[78]

Coccidioides immitis

Generally affecting patients from the southwestern United States, this organism is distinct from other yeast by virtue of its size (20



• **Figure 8.87** **A,** "Capsular-deficient" organisms stain with Fontana-Masson. **B,** "Capsular-deficient" yeast invariably show a faint rim of mucicarminophilic staining.



• **Figure 8.88** Fibrocaseous infection due to *C. immitis*.

to 200 μM) and its endosporulating mode of reproduction.[79] *C. immitis* produces a spectrum of changes that includes fibrocaseous granulomas, (Fig. 8.88) granulomatous pneumonia, and miliary disease, often accompanied by tissue eosinophilia. The endospores are contained within a spherular capsule and both stain well with GMS (Fig. 8.89A), whereas spherules are variably stained by PAS. The cysts of *C. immitis* have a characteristic tendency to collapse after having discharged their endospores in situ (see Fig. 8.89B).

In endemic areas, *C. immitis* can form fungus balls within preexisting pulmonary cavities (Fig. 8.90A). Because the organism is dimorphic, the presence of the infective hyphal arthroconidia should not be confused with a concomitant mold infection (see Fig. 8.90B).[80]

Paracoccidioides

Paracoccidioides braziliensis infection is endemic in South America, where it produces a range of pulmonary findings comparable to those of blastomycosis, but cases are rare in the United States. The organism is large (10 to 60 μM) and replicates by multiple narrow-necked buds that produce a "ship's wheel" appearance that is pathognomonic (Fig. 8.91).[81] However, when this feature is absent, the infection can be confused with other fungi.

Candida Spp.

Superficial colonization of the upper airways by *Candida* spp. is common in patients treated with inhaled corticosteroids or in chronically ill and diabetic patients (Fig. 8.92). Foci of aspiration pneumonia and abscess cavities may show colonization, but deep pulmonary infection is rarely seen in the absence of fungemia (Fig. 8.93A and B).[82]

Candida can have a pleomorphic morphology that includes yeast (blastoconidia), pseudohyphae, and true hyphae, although in some cases, only yeast forms may be present.[83] The organisms can be highlighted by either GMS or PAS and stain strongly gram positive (Fig. 8.94A). At times, blastoconidia of *Candida* spp. can be large and mimic other fungal infections (see Fig. 8.94B). *C. glabrata (torula glabrata)*[84] shows multiple, 2- to 5-μM budding yeast that are amphophilic in H&E-stained sections; they are distinct from other *Candida* species because pseudohyphae and hyphae are never present.

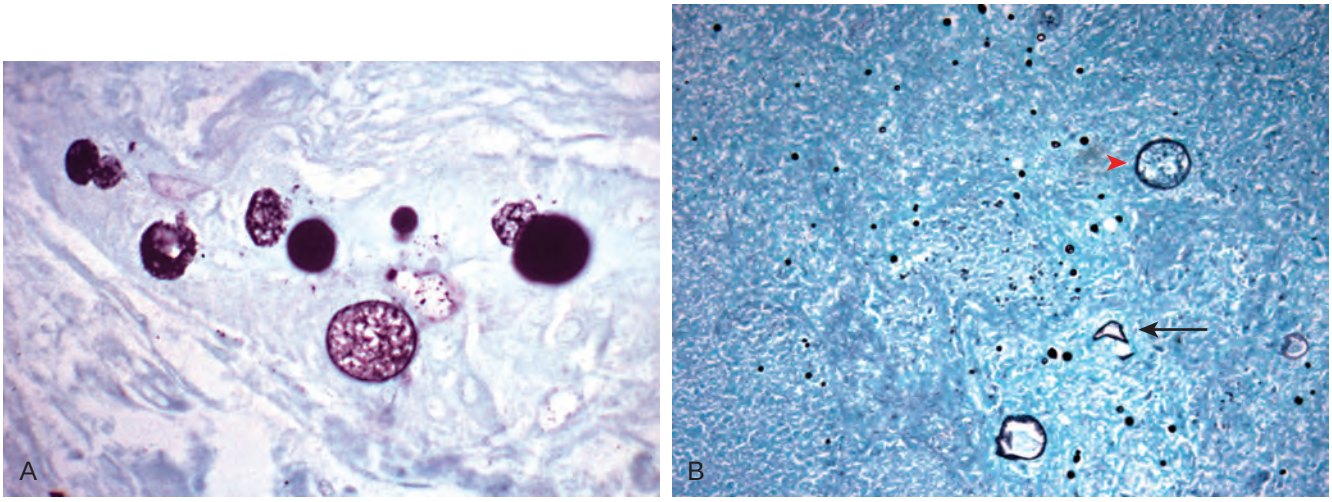
Hyphate Fungi

Hyphate fungi or "molds" are responsible for a range of pulmonary disorders ranging from colonization of pulmonary airways to angioinvasive, life-threatening infections. *Aspergillus* spp. account for the majority of pulmonary mold infections, but other organisms, including *Zygomyces*, *Pseudeallescheria*, and *Fusarium*, also produce pulmonary disease.

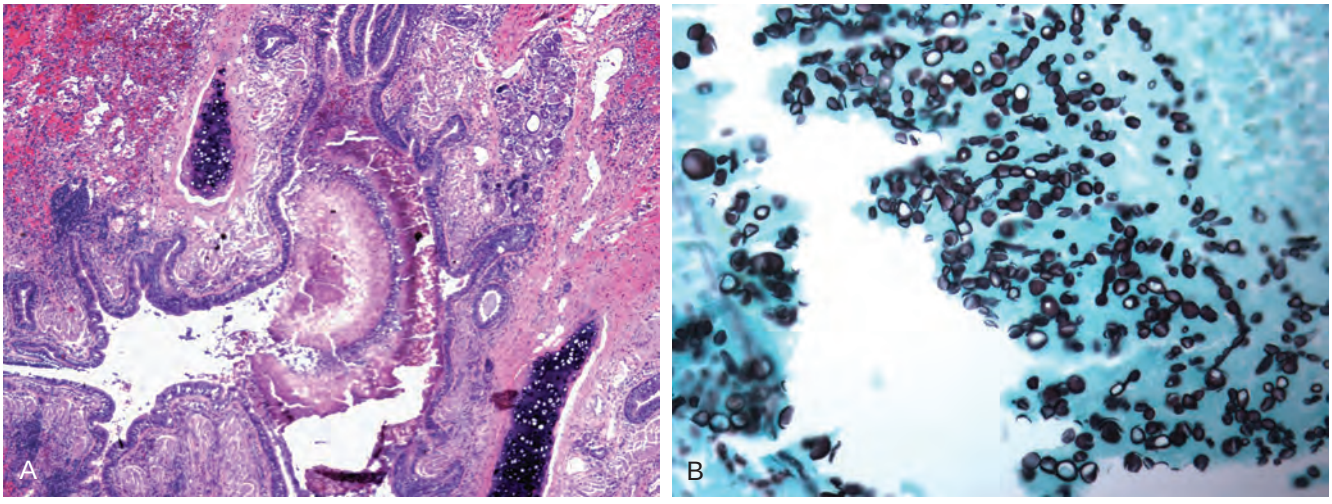
Aspergillus Spp.

The hyphae of *Aspergillus* spp. range in diameter from 2.5 to 4.5 μM and show frequent septation. *Aspergillus* spp. branch progressively, primarily at acute angles of approximately 45 degrees, mimicking an arborizing tree branch (Fig. 8.95A), but when cut in cross-section may be mistaken for yeast, although the absence of budding suggests the correct diagnosis. In areas of mycelial growth, organisms become tangled, bulbous, and distorted, and it may be impossible to confirm the diagnosis with accuracy, based on morphology (see Fig. 8.95B).

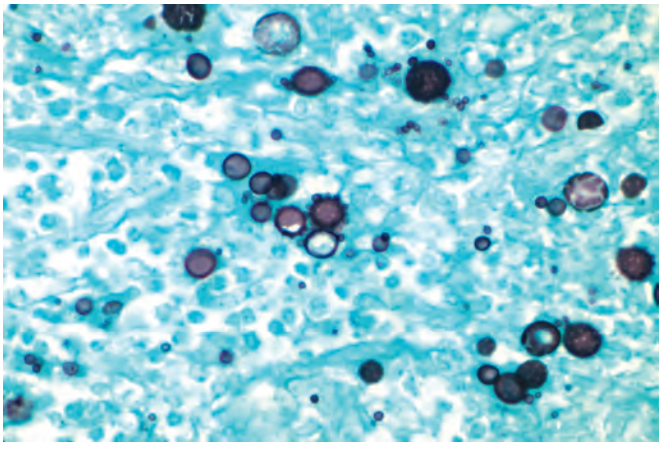
The aspergil, a ritual implement used in the Roman Catholic mass, resembles the fruiting body, and gives the fungus its name (Fig. 8.96A to C). Fruiting bodies develop from mycelia in areas



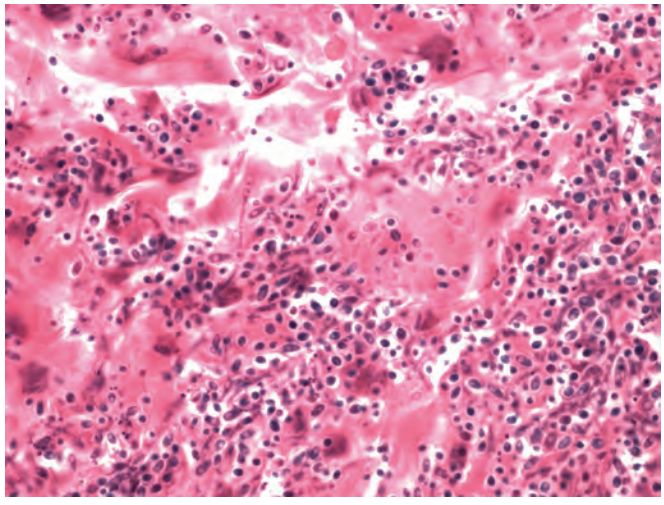
• **Figure 8.89** **A,** Endospores are variably present within periodic acid–Schiff-positive cysts. **B,** Cysts of *C. immitis* have a characteristic tendency to collapse after discharging their contents. *Red arrow* demonstrates intact fungal cyst. *Black arrow* shows a collapsed cyst.



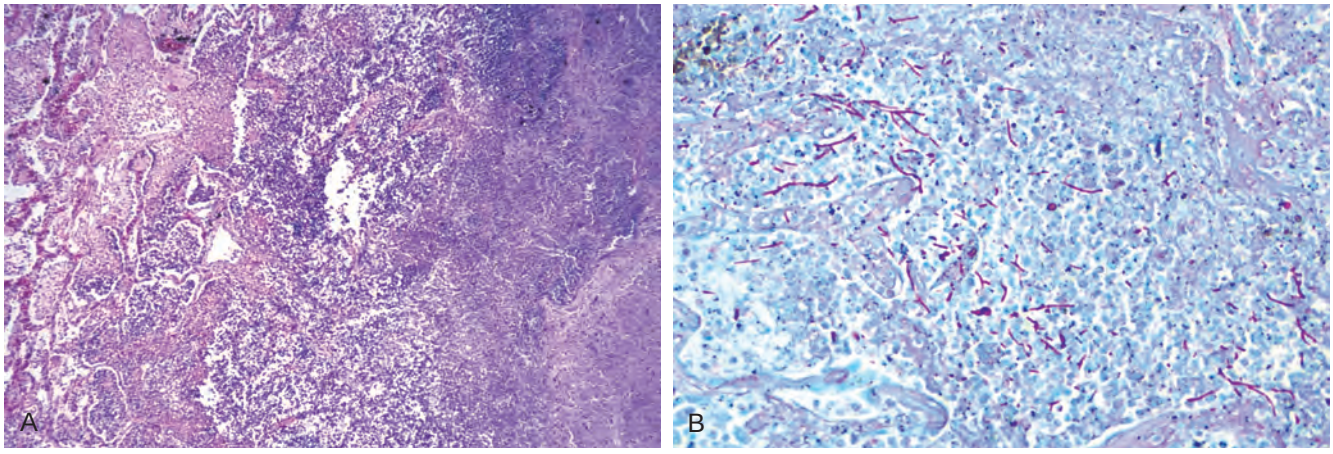
• **Figure 8.90** **A,** Fungus ball due to *C. immitis* with **(B)** yeast and arthroconidia that must not be confused with a concomitant mold infection.



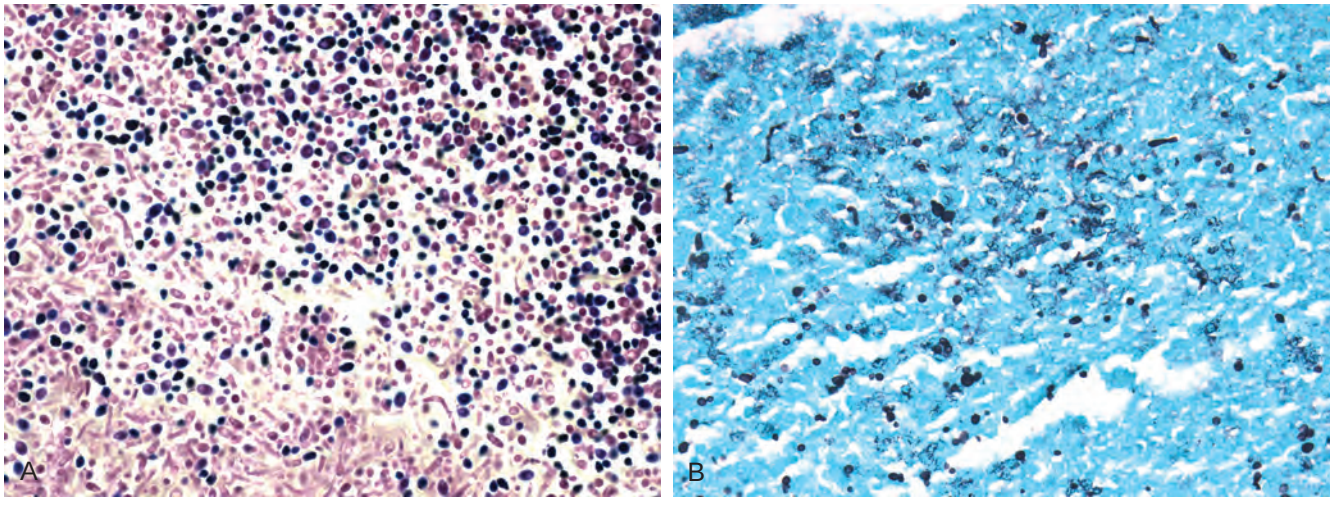
• **Figure 8.91** *Paracoccidioides brazilienses* shows multiple narrow buds that resemble a ship's wheel, but this diagnostic feature is not always present.



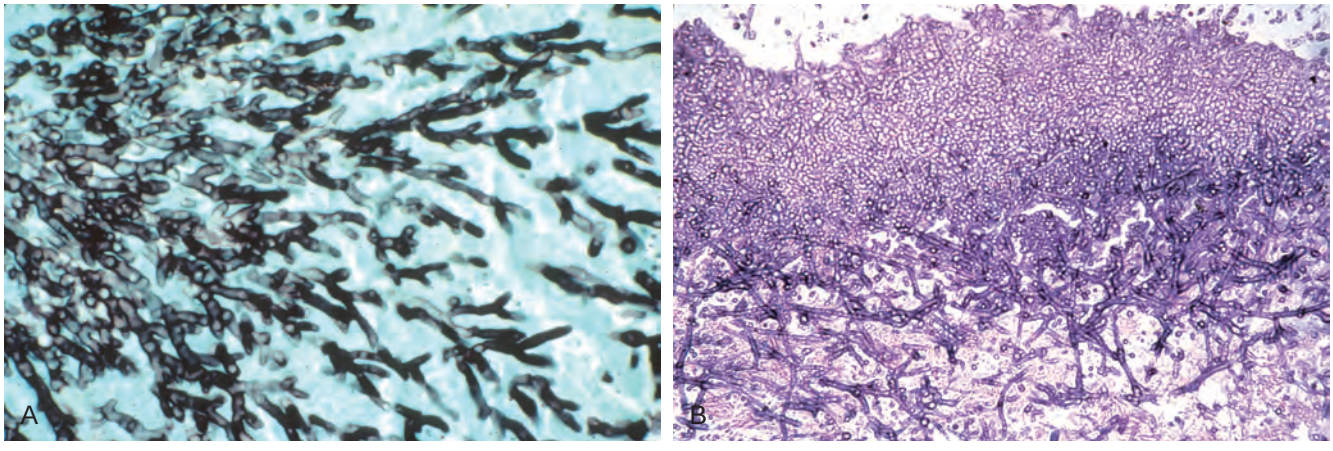
• **Figure 8.92** Dense colonization of airway with *C. albicans* in intubated patient.



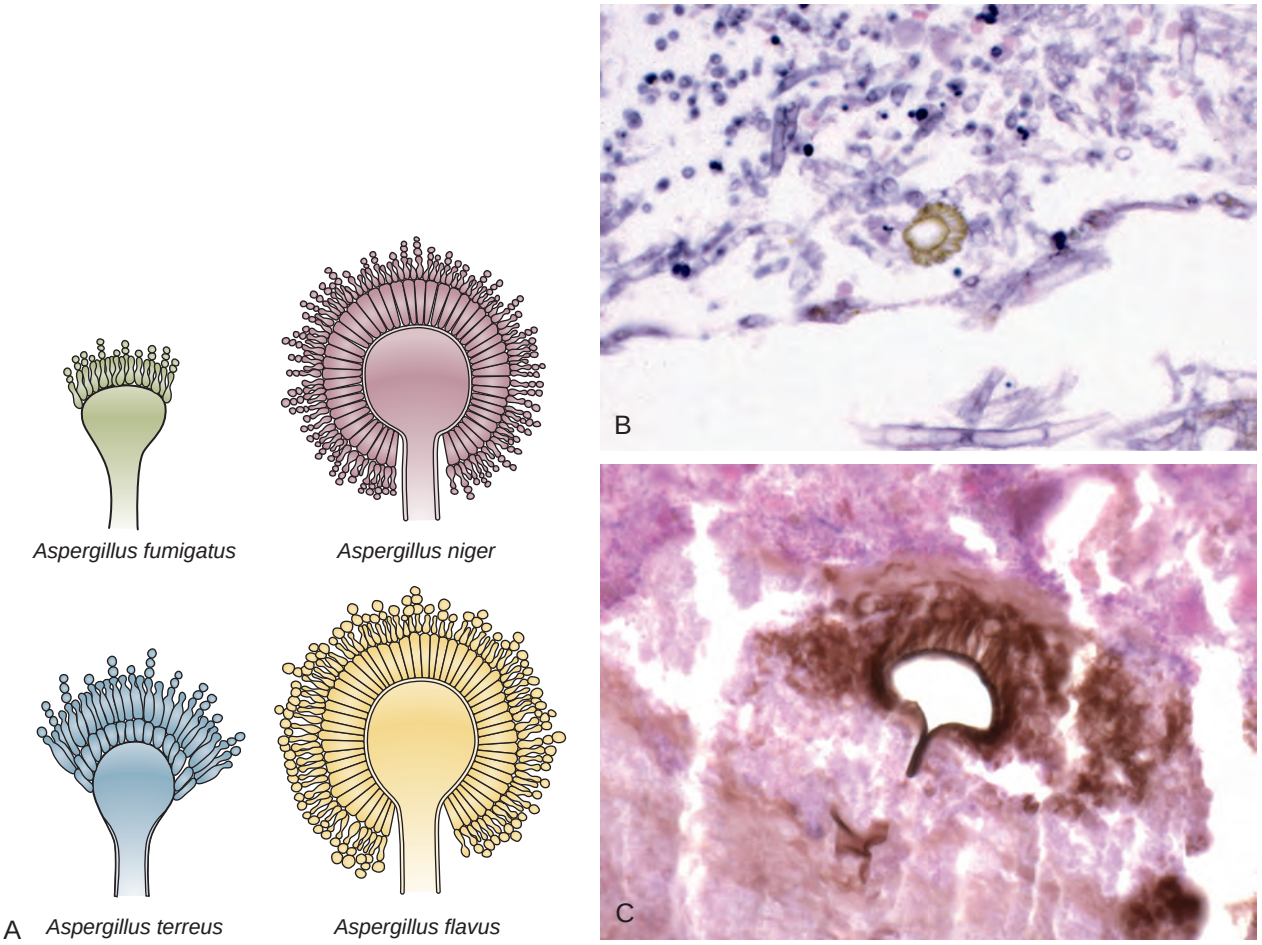
• **Figure 8.93** **A,** Necrotizing pneumonia due to *C. albicans* in fungemic patient. **B,** Periodic acid–Schiff stain highlights the organism.



• **Figure 8.94** **A,** *Candida* yeast are intensely gram-positive. **B,** Large yeast and pseudohyphae proved to be *Candida tropicalis*.



• **Figure 8.95** **A,** *Aspergillus* spp. characteristically branch dichotomously and progressively at acute angles. **B,** Tangled and distorted mycelial growth. It is impossible to speciate fungi based on this morphology.



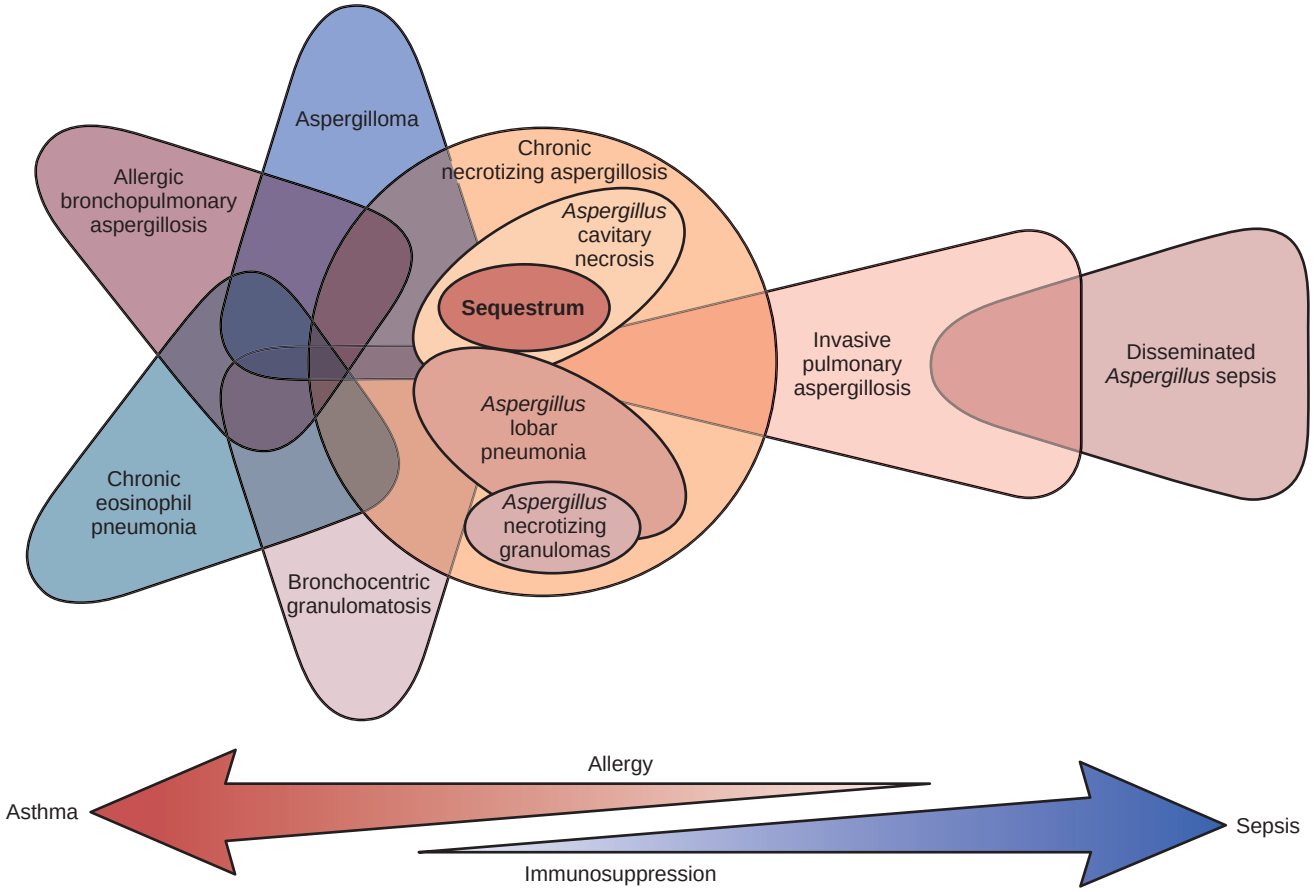
• **Figure 8.96** **A,** Morphology of aspergil fruiting body. **B,** Gram stain shows fruiting body of *Aspergillus fumigatus* with innumerable gram-positive conidia. **C,** *A. niger* with innumerable pigmented conidia.

of high oxygen tension, such as lung or sinus cavities but do not develop in tissues. They are composed of a vesicle with one or two layers of phialides that produce the infective conidial spores, and the morphology of the fruiting body allows for accurate speciation in situ. In general, the specific diagnosis of "aspergillosis" should be avoided unless the aspergil is identified because other fungi can be morphologically virtually indistinguishable in tissue. Diagnoses are therefore optimally phrased as "acute-angle branching hyphae consistent with aspergillus."

Immune Disorders Due to Aspergillus Infection. *Aspergillus* spp. give rise to a spectrum of disorders, some reflecting hypersensitivity responses to the organisms, whereas others are the consequence of invasive infection (Fig. 8.97). Distinguishing these is critical for the proper management of these disorders.

Allergic Bronchopulmonary Aspergillosis. *ABPA* shows a range of findings, including intractable asthma, proximal bronchiectasis,[85] and both peripheral blood eosinophilia. It is not certain whether the fungus plays an opportunistic role in exacerbating atopic responses or is primary in its pathogenesis.[86] Patients develop intractable bronchospasm with elevated serum IgE levels specific for *Aspergillus* spp. The pathology includes central cystic bronchiectasis with mucoid impaction (Fig. 8.98A). The impacted mucus is viscid and forms a cast of the airways, a disorder termed *plastic bronchitis*. Microscopically the mucus plugs show layers of degenerating eosinophils interspersed within the mucin (see Fig. 8.98B), and the surrounding lung may show patchy eosinophilic pneumonia. The fragmented fungal hyphae can at times be difficult to identify (see Fig. 8.98C) so that silver stains should be applied routinely to the evaluation of allergic mucus plugs. Whereas the clinical and histologic features of this disorder are most frequently caused by hypersensitivity to *Aspergillus* spp., other fungi (e.g., *Candida* spp.) can yield a comparable syndrome. A subset of patients with cystic fibrosis develops concomitant ABPA, and establishing the diagnosis in this setting can be difficult.[41]

Bronchocentric Granulomatosis. Bronchocentric granulomatosis (BCG) appears to reflect an abnormal cell-mediated response to *Aspergillus* spp. and less commonly other fungi (e.g., *Candida* spp. and *C. immitis*), in which small-caliber airways develop circumferential granulomatous inflammation, loss of the normal lining respiratory epithelium, and impaction of the airway lumen by granular basophilic mucin admixed with cellular debris (Fig. 8.99A to C). The disorder may be first noted radiographically as isolated or multiple airway-centered nodules.[87] BCG may be seen as part of the spectrum of findings in ABPA or as an isolated disorder. As in ABPA, fragmented hyphae may be difficult to identify and surrounding areas of eosinophilic pneumonitis are common. When the disease is suspected clinically, it can be treated noninvasively with corticosteroids; however, definitive resection may be undertaken to exclude neoplasia.



• **Figure 8.97** Spectrum of disease due to *Aspergillus* spp.

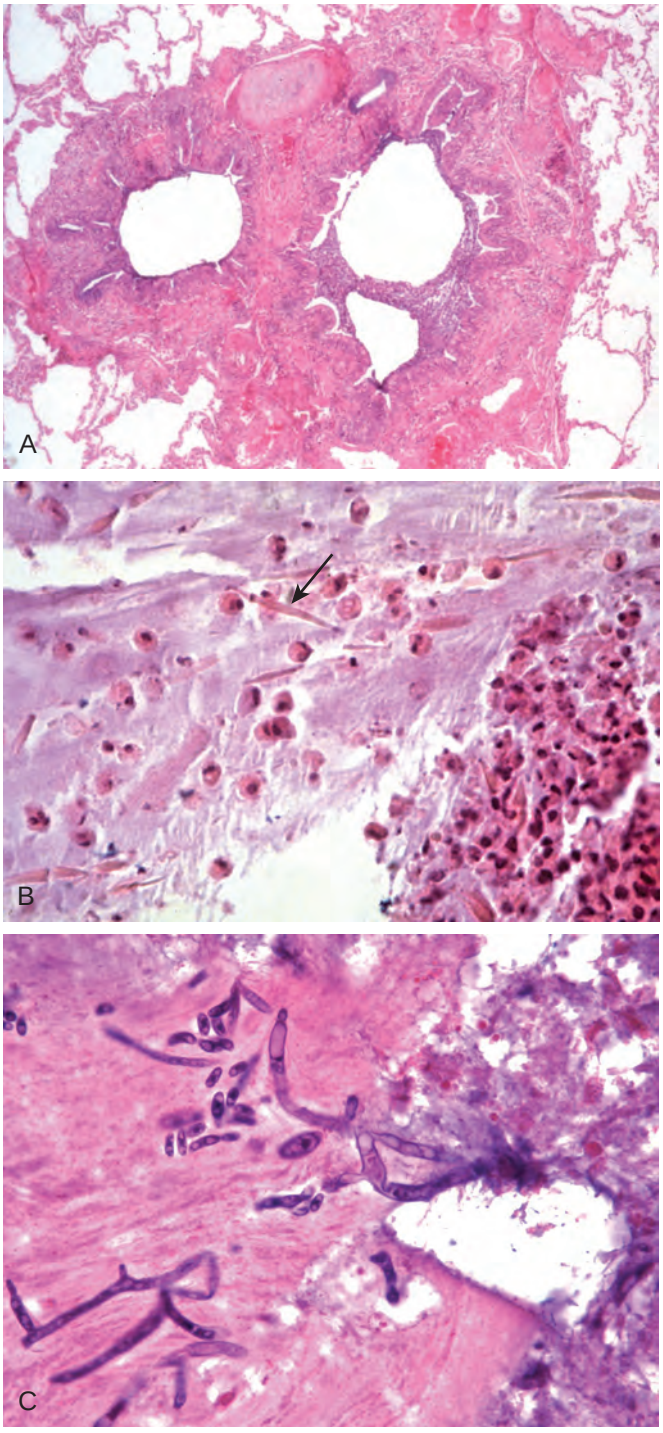
Hypersensitivity Pneumonitis. Hypersensitivity pneumonitis reflects a combined abnormality of humoral and cell-mediated immunologic responses to organic antigens. Most cases of hypersensitivity pneumonitis are caused by thermophilic actinomycetes, but hypersensitivity to *Aspergillus* spp. is well documented. Upper lobe predominance is the rule, and this can be a helpful feature in establishing the diagnosis.

The diagnosis of hypersensitivity pneumonitis is based primarily on establishing a historical link between antigen exposures and the clinical findings, but lung biopsies can establish the diagnosis. Microscopically the lung shows bronchiolocentric, lymphohistiocytic interstitial infiltrates with poorly formed microgranulomas (Fig. 8.100) and giant cells that may contain birefringent crystals and cholesterol crystals. Whereas $CD8^+$ lymphocytes characteristically predominate in BAL fluid specimens, immunostains will reveal dominance of either $CD4^+$ or $CD8^+$ lymphocytes in situ. In addition, other histopathologies, including nonspecific cellular interstitial pneumonitis (NSIP), organizing pneumonia, lymphoid interstitial pneumonitis (LIP), and nonnecrotizing granulomatous inflammation resembling sarcoidosis, can be caused by hypersensitivity pneumonitis. The presence of interstitial and alveolar eosinophils is characteristically seen in hypersensitivity pneumonitis due to *Aspergillus* antigens and is less common in response to other antigens.

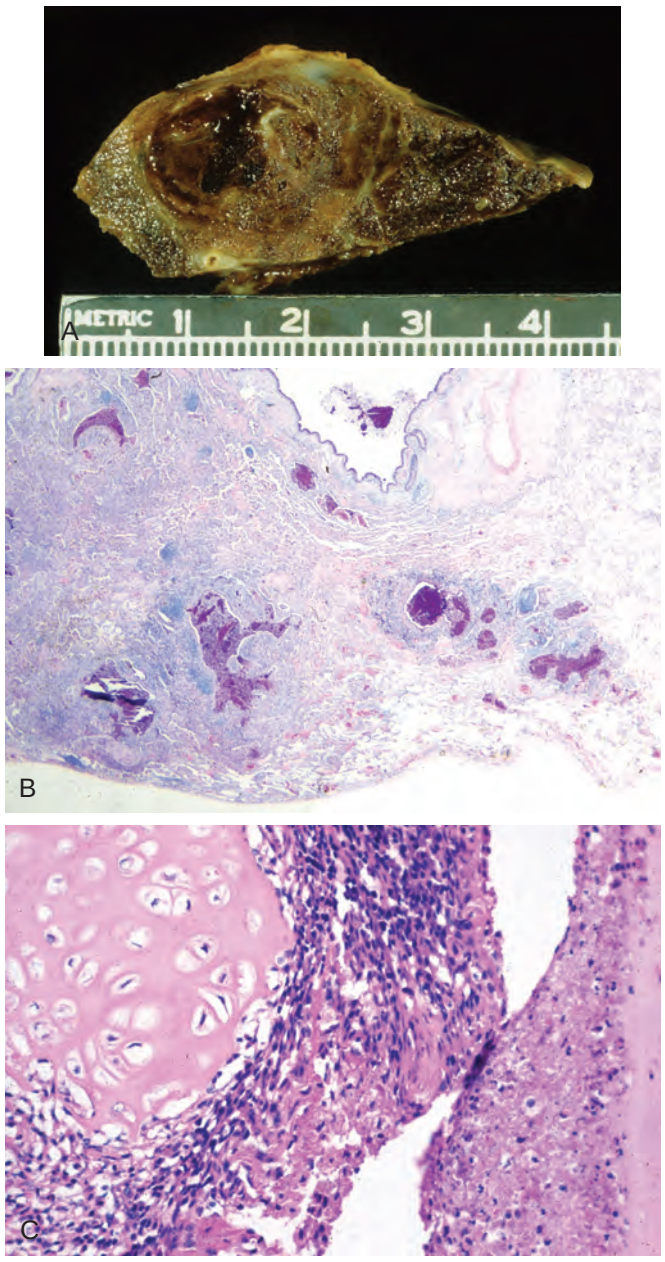
Aspergillus Bronchitis and Chronic Necrotizing Aspergillosis. The presence of primary airway infection by *Aspergillus* spp. is a poorly recognized entity. It is generally seen in the setting of modest immunosuppression accompanying such disorders as diabetes mellitus or the use of aerosolized steroids in asthmatics. In some cases, it is a precursor lesion for invasive disease. Fungal hyphae can be seen filling the lumen of airways (Fig. 8.101A) without evidence of frank tissue invasion. Elastic stains are helpful in determining whether organisms have begun to transgress normal tissue barriers (see Fig. 8.101B). Centrally necrotic lesions (Fig. 8.102A) of chronic necrotizing aspergillosis can resemble those due to mycobacteria and other fungi (see Fig. 8.102B).

Fungus Balls. The colonization of old fibrocavitary disease (e.g., areas of bronchiectasis due to healed tuberculosis and sarcoidosis, or emphysematous bullae by *Aspergillus* spp.) is the most common cause of a pulmonary fungus ball (Fig. 8.103A and B).[88] The term mycetoma should not be applied to intracavitary fungal mycelial growth because it accurately applies only to soft tissue infections. Patients may be asymptomatic or alternatively present with episodes of hemoptysis, at times massive and requiring emergent bronchial arterial embolization or definitive resection.

The morphology of the hyphae in a fungus ball is frequently distorted, and it can be impossible to identify diagnostic septate acute angle branching forms. *Aspergillus* fungus balls show heterogeneous staining intensity (see Fig. 8.103C), giving the impression of alternating zones of growth. The wall of the fungus ball frequently shows increased numbers of tissue eosinophils (see Fig. 8.103D). The *Splendore-Hoeppli* phenomenon is often present and is reassuring evidence that angioinvasion is unlikely, unless there has been a recent supervening cause of immunosuppression or neutropenia. The walls of the cavity are lined with granulation tissue, granulomatous inflammation, or metaplastic squamous



• **Figure 8.98** **A,** Central bronchiectasis with dense peribronchiolar scarring in a patient with allergic bronchopulmonary aspergillosis. **B,** Expectorated "allergic" mucus plug with eosinophils and Charcot-Leyden crystals. *Arrow* points to Charcot-Leyden crystal. **C,** Aspergillus hyphae in mucus plug.

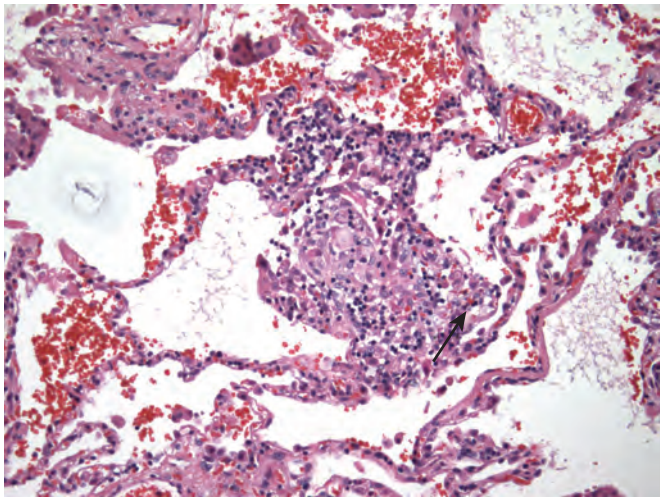


• **Figure 8.99** **A,** Gross appearance of bronchocentric granulomatosis showing **(B)** exquisite bronchocentric distribution in periodic acid–Schiff-stained section. **C,** The lining of a small airway is replaced by granulomatous inflammation, and the lumen is filled with a granular basophilic exudate.

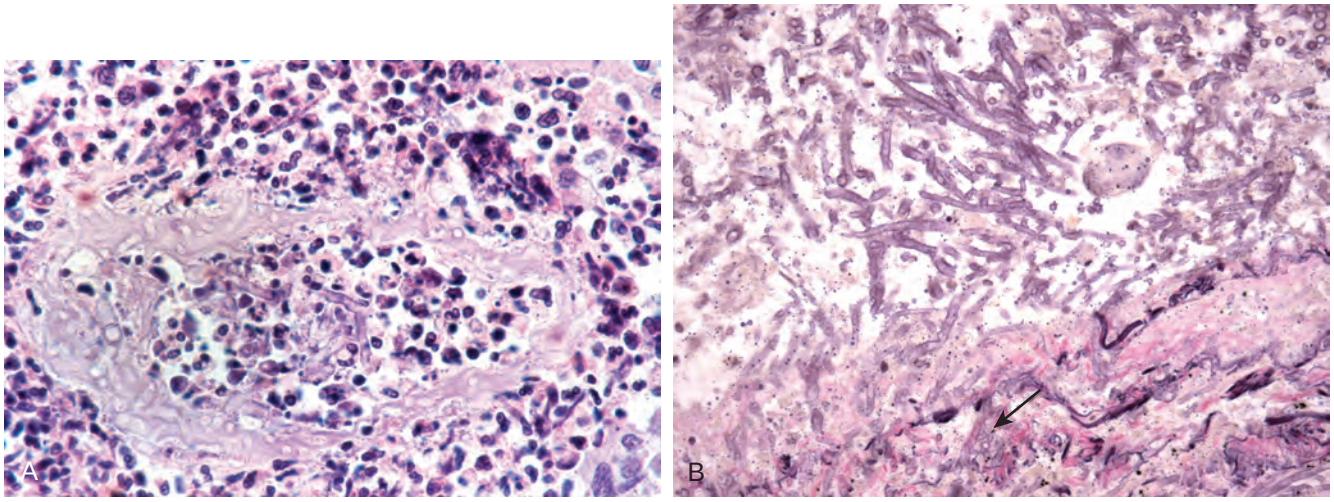
epithelium, depending on the activity of the disease. The occasional presence of germinative fruiting bodies of *Aspergillus* spp. with characteristic phialides and conidial forms allows definitive speciation.

One may rarely see rapid expansion of a cavity due to vascular thrombosis induced by calcium oxalate crystal deposition, a disorder termed *chronic pulmonary oxalosis*.[89] Oxalic acid is produced by a variety of *Aspergillus* spp. but is most commonly a feature of *Aspergillus niger* infection (Fig. 8.104A). Diffusion of oxalate into surrounding blood vessels is prothrombotic and can lead to extensive ischemic necrosis (see Fig. 8.104B and C). Oxalate crystal deposition in the renal tubules may also be seen. Emergency resection of the fungus ball is the only effective treatment.

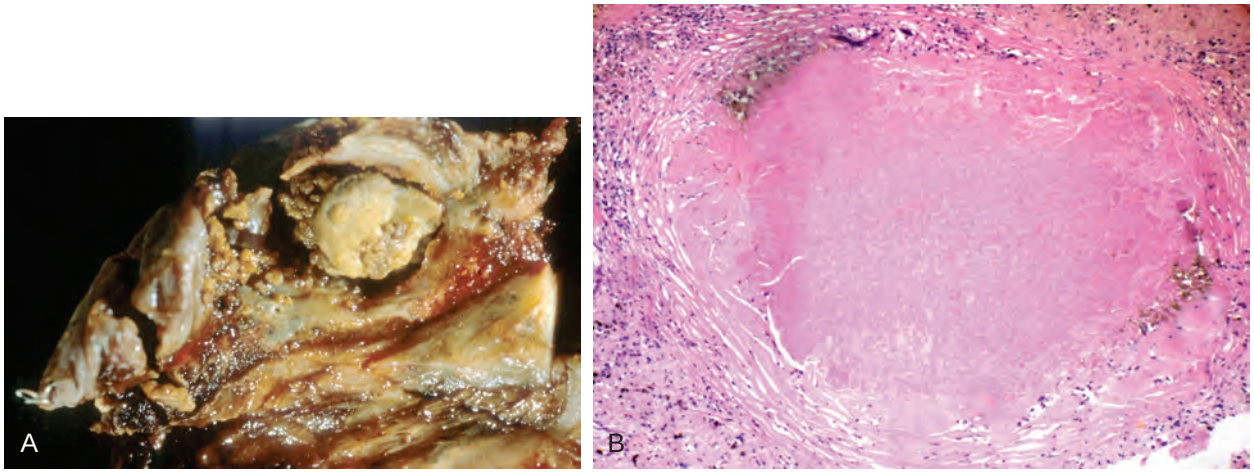
Angioinvasive Aspergillosis. This life-threatening infection is seen in patients who have been chronically immunosuppressed and/or neutropenic. It is a complication of bone marrow and solid organ transplantation, as well as of antileukemic chemotherapies. Angioinvasive aspergillosis is an uncommon complication of



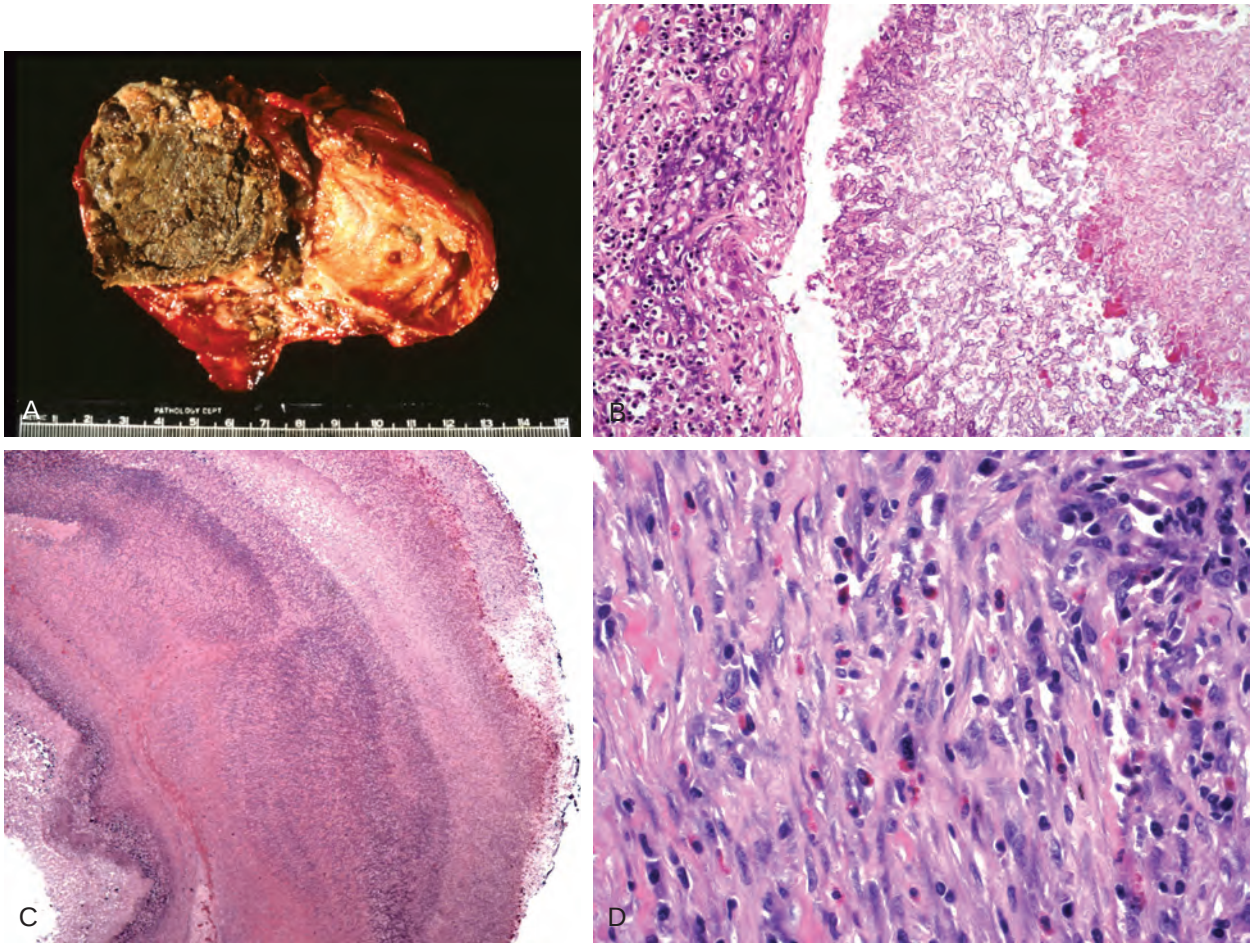
• **Figure 8.100** The microgranulomatous hypersensitivity response to aspergillus often includes tissue eosinophils.



• **Figure 8.101** **A,** Aspergillus bronchitis/bronchiolitis is a precursor to invasive disease. **B,** Elastic stains can determine whether tissue invasion has occurred *(arrow)*.



• **Figure 8.102** **A,** Necrotizing lesions due to aspergillus can **(B)** resemble mycobacterial or yeast infection.



• **Figure 8.103** **A,** Fungus ball growing in an old bronchiectatic cavity due to sarcoidosis. **B,** Histology shows tangled mass of hyphae and the Splendore-Hoeppli phenomenon. **C,** Fungus balls often show alternating "zones" of staining intensity with hematoxylin and eosin (and Gomori methenamine silver). **D,** Fungus balls frequently contain eosinophils in their wall.

HIV/AIDS, despite the associated profound immune deficiency. Grossly the lung shows "targetoid" lesions showing central thrombosed vessels secondary to angioinvasion, surrounded by a rim of consolidated lung, confluent bronchopneumonia, or lobar consolidation (Fig. 8.105A), and microscopically a necrotizing pneumonia (see Fig. 8.105B) at times featuring giant cells (see Fig. 8.105C) may be present.[90] Angioinvasion is identified microscopically and may be enhanced with silver and elastic stains (Fig. 8.106A and B). Rarely, foci of infarcted lung can produce an infected nonviable pulmonary sequestrum (Fig. 8.107).

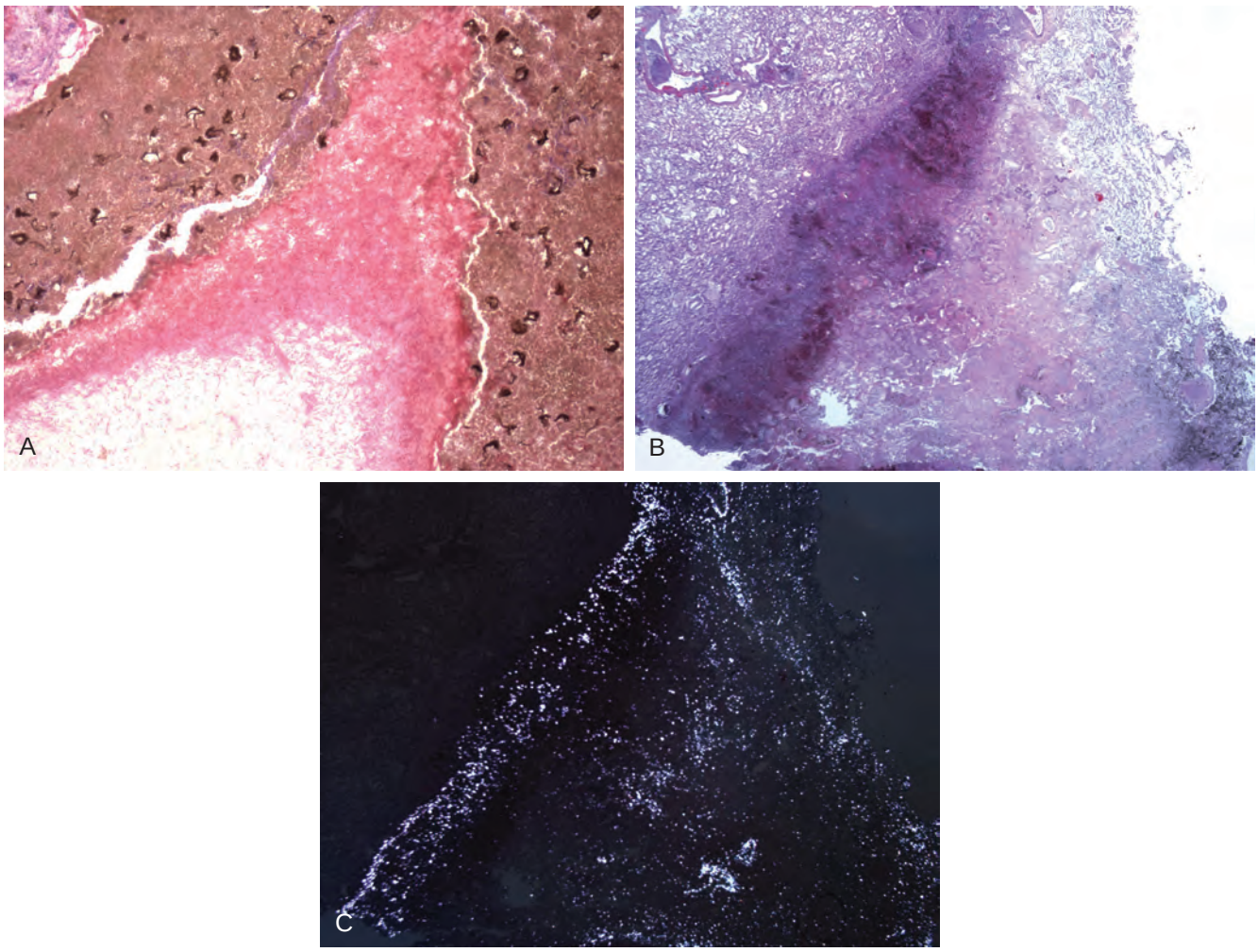
The fungal hyphae tend to invade blood vessels and to "metastasize" to other organs. The presence of sunburst vasculocentric hyphal growth is diagnostic of a "metastatic" focus of infection (Fig. 8.108), and virtually any organ may be secondarily involved. Circulating *Aspergillus* spp. can also seed abnormal cardiac valves to produce endocarditis with fatal embolic hemorrhagic infarctions to brain and other vital organs.

Other Aspergillus Species. *Aspergillus terreus* is an opportunistic fungus that infects patients with chronic granulomatous disease, as well as other immunocompromised hosts.[90] Its hyphae are pyriform with orthogonal branches (Fig. 8.109A and B). Fungus balls due to *Aspergillus nidulans* have a propensity to produce pale-staining, enlarged fungal forms or *Hulle cells* that show Maltese cross birefringence when examined under polarized light (Fig. 8.110A and B).[91]

Other Hyphate Fungi

Although the majority of acute-angle branching septate hyphae encountered in medical practice prove to be *Aspergillus* spp., exceptions do occur and can be difficult to distinguish in situ. Organisms that closely mimic *Aspergillus* include the *Zygogomyces*, *Pseudallescheria boydii (Scedosporium)*, and *Fusarium* spp. Pulmonary infection by *Zygomyces* is due to organisms of the order *Mucorales (Mucor)*, including *Mucor*, *Rhizopus*, *Absidia*, *Rhizomucor*, and *Apophysomyces*.[92] Infection generally occurs in immunosuppressed hosts and in patients with diabetic ketoacidosis or disorders of iron metabolism. As treatment invariably includes surgical resection, establishing an accurate diagnosis is critical.

The histologic responses to infection are comparable to those caused by angioinvasive aspergillosis. The hyphae of the *Zygomyces*



• **Figure 8.104 A,** Fungus ball due to *A. niger*. **B,** Extensive local ischemic infarction of lung and **(C)** deposition of calcium oxalate crystals are seen with polarized light.

are broad (5 to 25 μM), pauciseptate, tending to branch at right angles (Fig. 8.111A), although acute-angle branching does occur and its presence should not dissuade one from the correct diagnosis. The hyphae of the *Zygomyces* are well stained by hematoxylin, and they have a proclivity to produce ribbonlike structures (see Fig. 8.111B), but caution in diagnosis is required because treated aspergillus can assume this appearance. The fungus rapidly invades vessels, perineural lymphatics, and cartilage and tends not to respect tissue boundaries. *Zygomyces*, as well as all other hyphate fungi, can rarely form noninvasive fungus balls in the lung.

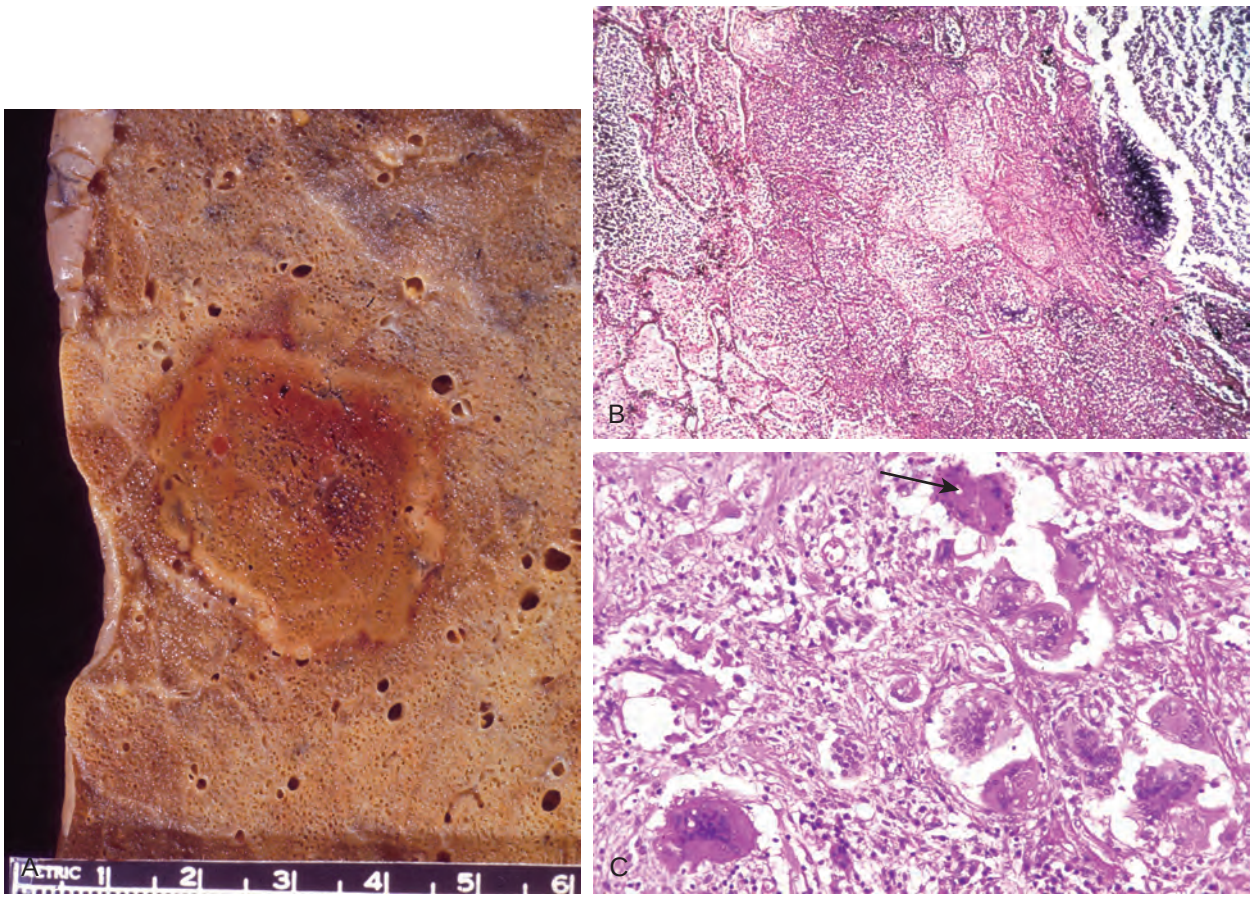
Pseudeallescheria (Scedosporium). The hyphae of *Pseudeallescheria* are smaller (2 to 4 μM) than those of *Aspergillus* spp.; the fungus branches predominantly at acute angles but, unlike *Aspergillus*, tends to branch haphazardly rather than progressively, and terminal chlamydospores mimicking a "tennis racket" may be apparent (Fig. 8.112). Although it is a hyaline fungus, the conidia produced by *Pseudeallescheria* in fungus balls are ovoid and pigmented.[11]

Fusarium. *Fusarium* is an opportunistic infection of lung that is seen in patients who are severely immunosuppressed. The septate hyphae of *Fusarium* spp. branch irregularly and at right angles, showing constrictions at branch points (Fig. 8.113).[11]

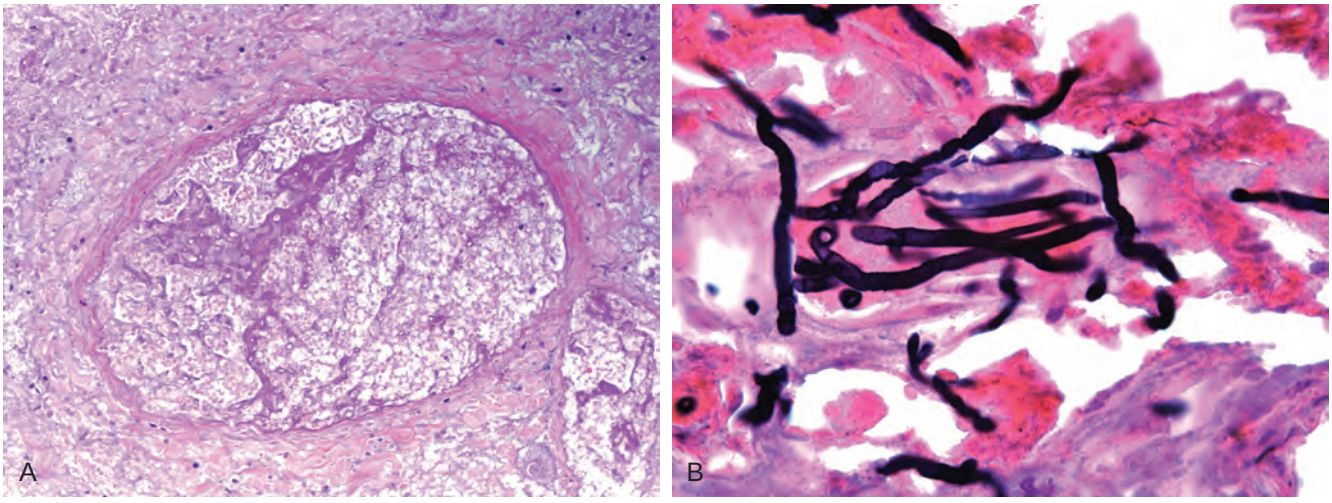
Differential Diagnosis

Whereas distinguishing hyphate fungal pathogens from one another in tissue can be exceedingly difficult, it is important because the efficacy of available fungal antibiotics depends on the diagnosis. The galactomannan assay is relatively specific for invasive aspergillosis and is rarely positive in mucormycosis, which can be helpful in distinguishing them noninvasively. The sensitivity is somewhat higher in the BAL than is serum.[93] However, this test should not be used to screen patients for aspergillus infection because its sensitivity in the nonimmunosuppressed and noninvasive disease is limited.

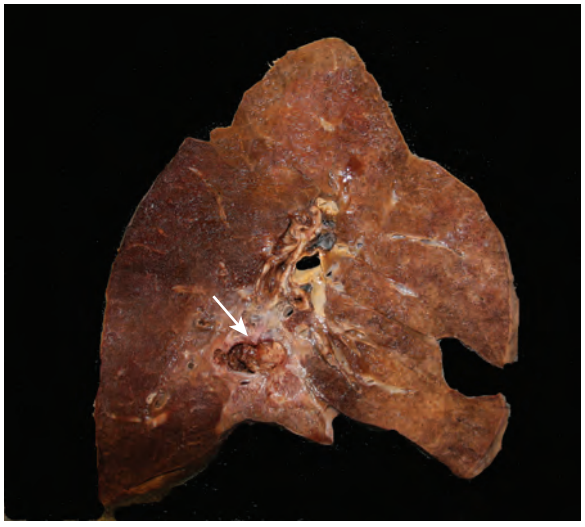
Whereas both *Aspergillus* spp. and *Pseudallescheria* are sensitive to voriconazole, *Pseudallescheria* is resistant to amphotericin. The sensitivity of *Fusarium* spp. to most antifungal antibiotics appears to be both unpredictable and limited. In all cases, culture remains the gold standard for diagnosis. Immunohistochemical reagents that can distinguish between hyphate fungi exist but are not



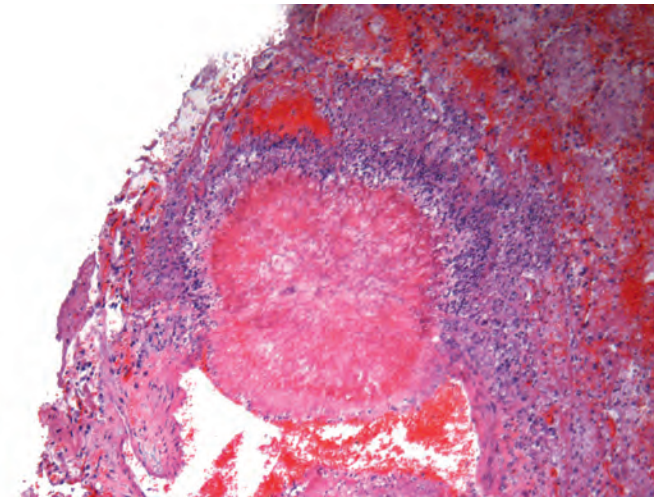
• **Figure 8.105** **A,** Angioinvasive aspergillosis with targetoid lesion due to vascular thrombosis, ischemic infarction, and fungal invasion into surrounding tissues. **B,** Necrotizing pneumonia due to angioinvasive aspergillosis with mycelial fungal growth developing at surface of cavitary lesion. **C,** Giant cells in pneumonia due to *A. fumigatus. Arrow* points to giant cells in inflammation.



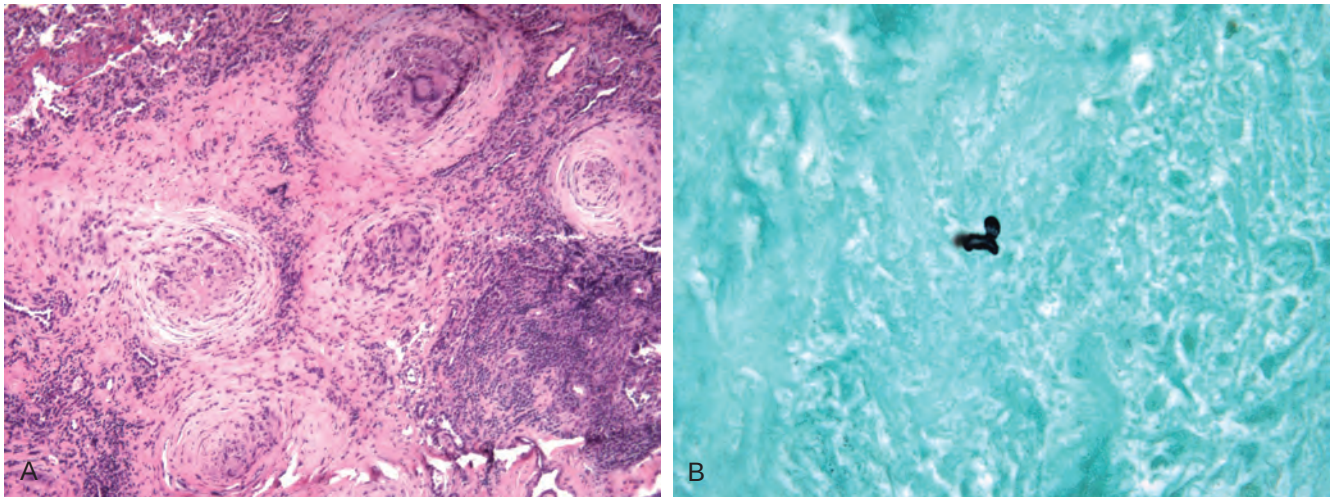
• **Figure 8.106** **A,** Blood vessel invasion may be seen with hematoxylin and eosin (H&E) and to better advantage in **(B)** section costained with Gomori methenamine silver and H&E.



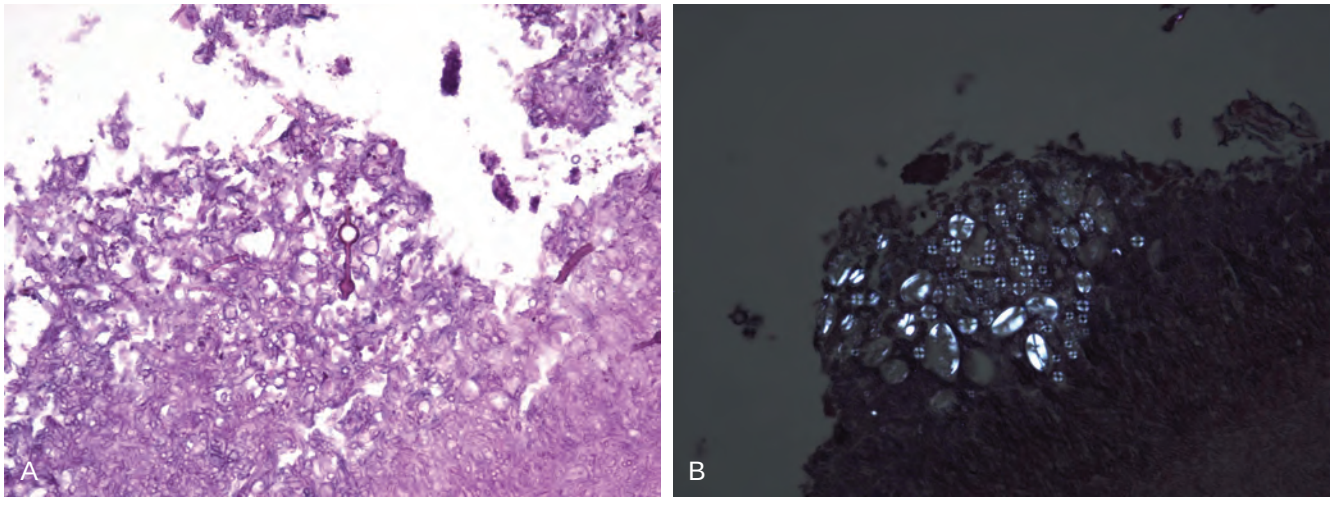
• **Figure 8.107** Area of nonviable lung (sequestrum) due to angioinvasive aspergillosis *(arrow)*.



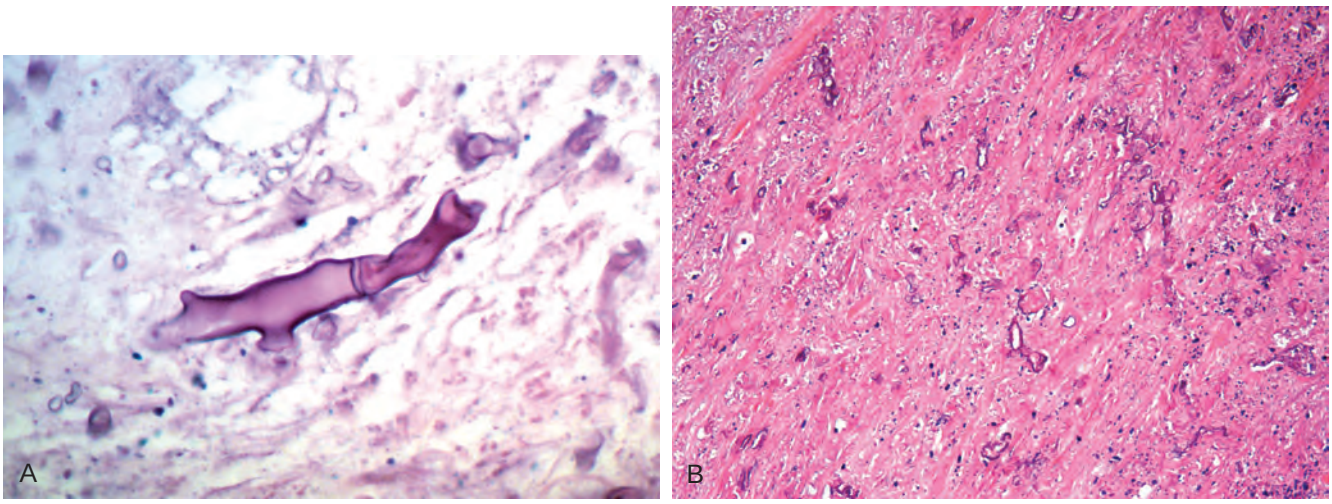
• **Figure 8.108** Focus of "metastatic" fungal infection showing sunburst pattern of growth out of blood vessel.



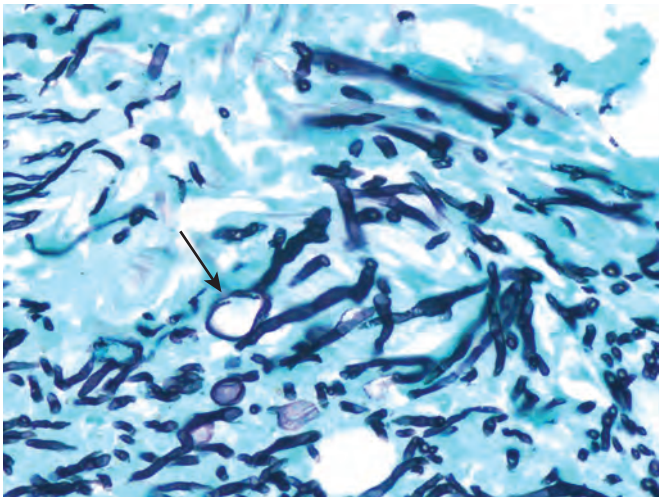
• **Figure 8.109 A,** *A. terreus* infection in patient with chronic granulomatous disease showing **(B)** fragmented pyriform hyphae branching at right angle.



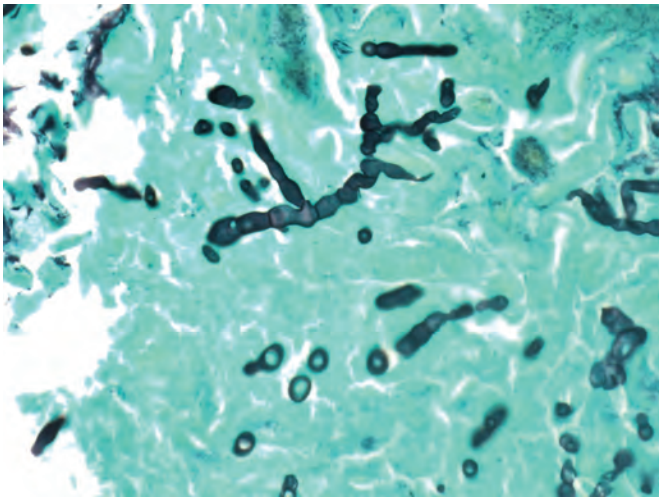
• **Figure 8.110 A,** Fungus ball due to *A. nidulans* with *Hulle* cells that **(B)** show Maltese cross configuration when examined with polarized light.



• **Figure 8.111** **A,** *Zygomyces* spp. hyphae are broad, pauciseptate, and tend to branch at right angles. **B,** Area of necrotizing angioinvasive disease with ribbonlike hyphae that stain well with hematoxylin.



• **Figure 8.112** *P. boydii* are slightly smaller than *Aspergillus* spp. and tend to grow randomly with prominent terminal chlamydospores *(arrow)*. Definitive diagnosis is generally not possible based solely on morphology in tissue sections.

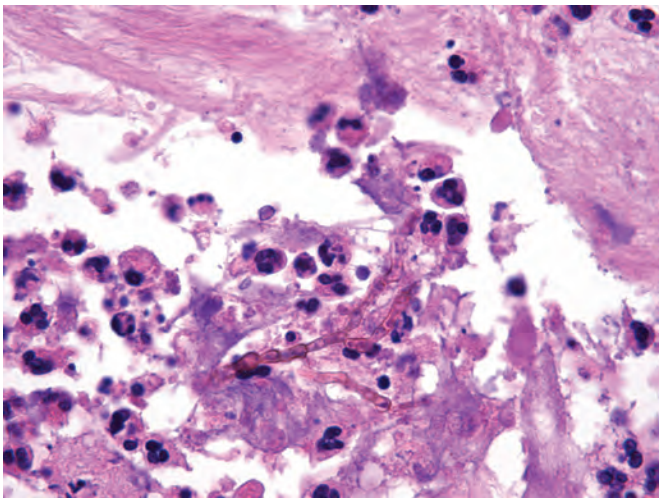


• **Figure 8.113** *Fusarium* tend to branch at right angles with narrow-branch points, but definitive classification is generally not achieved in tissue sections.

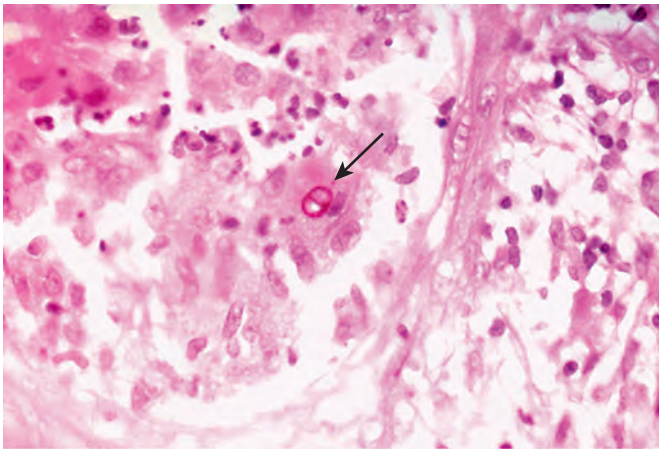
commercially widely available, and all reagents must be carefully tested with appropriate controls. The PCR assay is presently not an effective diagnostic approach.

Dematiaceous (Pigmented) Fungi

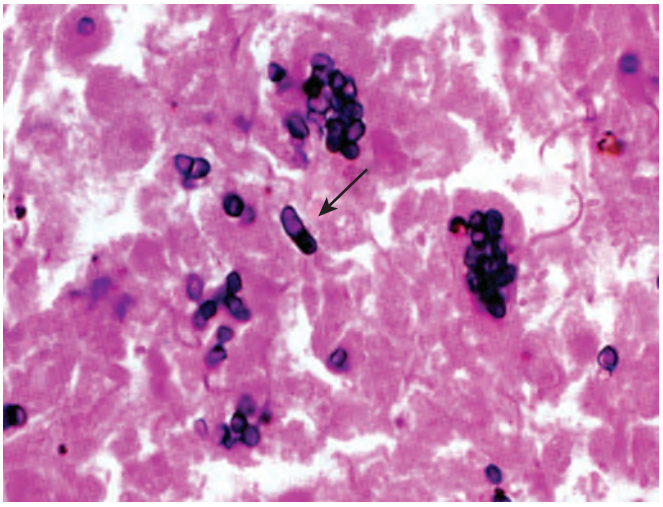
Pigmented fungi can be divided into those forming yeast (chromoblastomycosis) and those due to hyphate fungi (phaeohyphomycosis).[94] The specific organisms producing disease cannot be diagnosed morphologically, and isolation in culture is required. The Fontana-Masson stain can be applied when questions exist as to whether a fungus seen in H&E sections is pigmented. *Bipolaris*, *Curvularia*, *Alternaria*, *and others* are pigmented hyphate fungi that cause allergic pulmonary disorders mimicking those due to *Aspergillus* spp. (Fig. 8.114). The correct diagnosis is suggested by the presence of pigmented hyphae within allergic mucus or within fungus balls. *Chromoblastomycosis* is appreciated by the presence of sclerotic (Medlar) bodies. These are clusters of pigmented yeast that show characteristic multiaxial septa (Fig. 8.115).



• **Figure 8.114** Phaeohyphomycosis show pigmented fungal hyphae. Subclassification requires microbiologic culture.



• **Figure 8.115** Chromblastomycosis shows pigmented yeast forms with characteristic "sclerotic" Medlar bodies with multiaxial septation *(arrow)*.



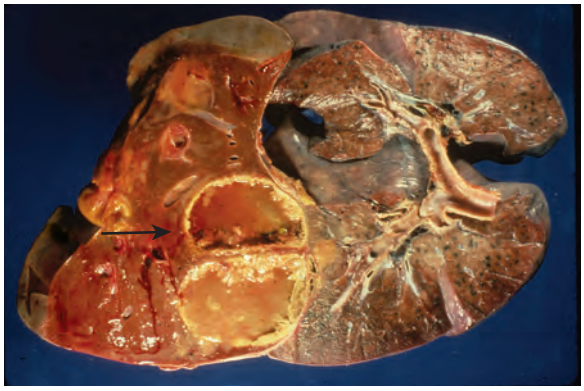
• **Figure 8.116** *P. marneffei* is a dimorphic fungus that is often seen within histiocytes and has a "sausage" shape with characteristic septation *(arrow)*.

Penicillium marneffei

Pulmonary infection due to *P. marneffei* is rarely seen outside of patients from Southeast Asia with HIV/AIDS, but it is a significant cause of necrotizing bronchopneumonia in this region. The organism is a small (2 to 5 μM) dimorphic fungus with an elongate sausage shape showing a characteristic septum. The organisms can be identified free within areas of necrosis or within foamy histiocytes (Fig. 8.116). The infection may also disseminate, commonly to involve the skin. Other fungi, including *Geotrichum* and *Sporothrix*, are rare causes of pulmonary infection.[95]

Parasites

The lung can be host to a variety of parasitic infections. Whereas many are seen primarily in tropical climates, others occur in temperate climes or as the result of immunosuppression. As previously noted, the current ease of global travel has greatly increased encounters with tropical diseases.



• **Figure 8.117** Amebic abscess with "anchovy paste" gross appearance. *Arrow* points to hematin pigment in cecum of schistosome.

Protozoa

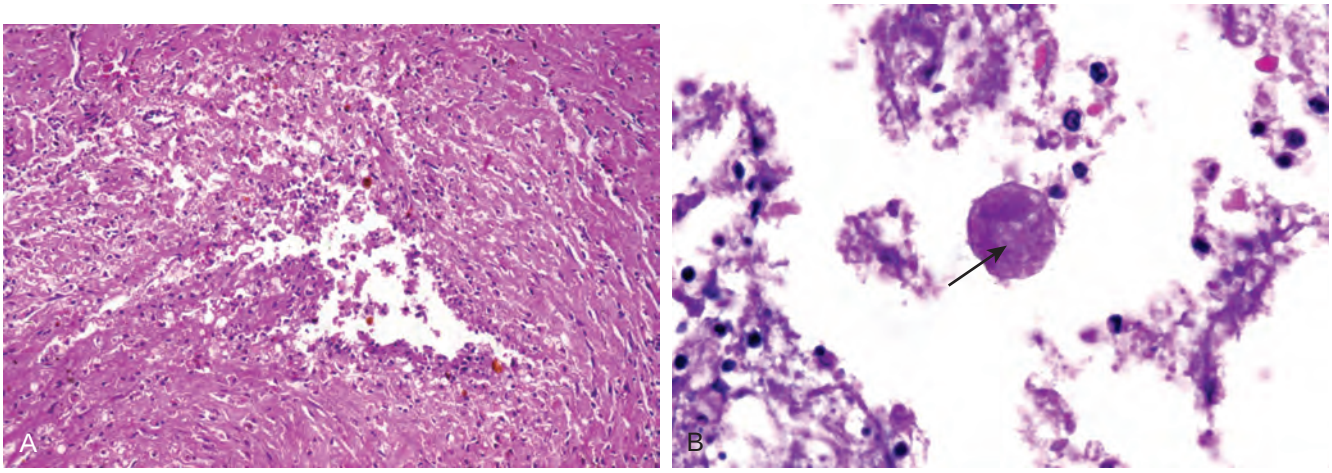
Ameba. Several species of protozoa can produce pulmonary infection. *Entamoeba histolytica* affects the lung secondary to the transdiaphragmatic extension of a hepatic amebic abscess or less commonly as the result of blood-borne spread from an intestinal source.[96] A pulmonary amebic abscess may extend to the pleura or rupture into an airway, leading to intrapulmonic dissemination. The amebic abscess shows liquefactive necrosis that grossly resembles "anchovy paste" (Fig. 8.117). The histologic response in areas of necrosis primarily includes neutrophils, but the margins of the lesion will show a polymorphous infiltrate of macrophages, lymphocytes, and plasma cells, and tissue eosinophilia is uncommon (Fig. 8.118A). Amebae can be distinguished from macrophages by their larger size, amphophilic bubbly cytoplasm, a sharply defined nuclear karyosome, (see Fig. 8.118B) and in the case of *E. histolytica*, by the ingestion of erythrocytes.

Free-living amebae, including *Acanthamoeba*, *Balamuthia*, *and Naegleria*, rarely affect the lung and primarily cause meningoencephalitis. However, in immunosuppressed patients, infection may spread beyond the confines of the nervous system to include the lung.

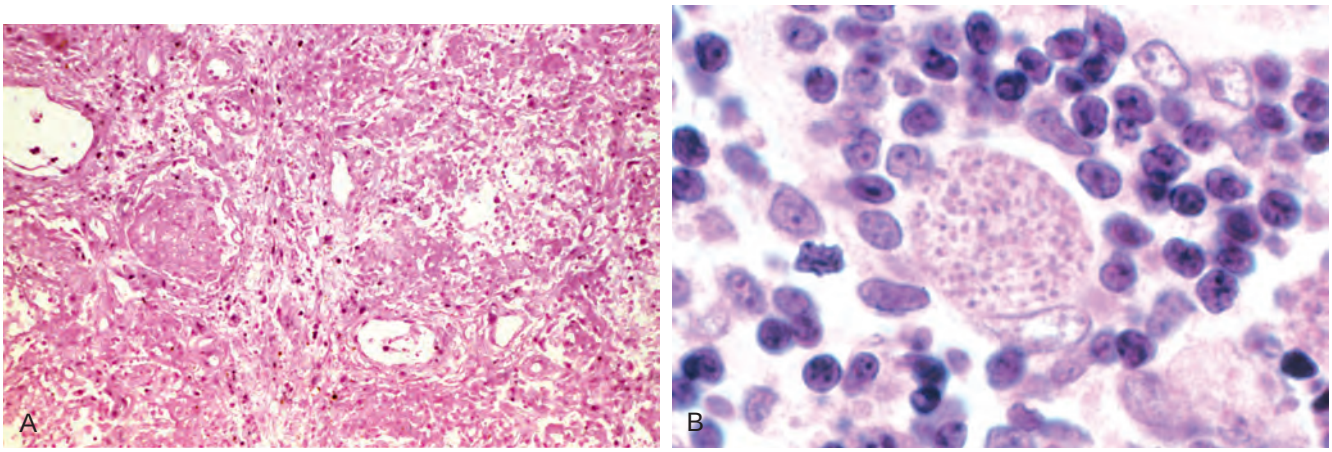
Toxoplasma. *Toxoplasma gondii* produces pneumonia in patients who are immunosuppressed, most often with HIV/AIDS.[97] Whereas Toxoplasma is a common infection of the central nervous system (CNS) and retina in HIV/AIDS, pulmonary infection is rare. When present, it includes a fibrinopurulent pneumonitis with areas of necrosis (Fig. 8.119A). The organisms are obligate intracellular parasites and are identified either as engorged pseudocysts containing crescent-shaped tachyzoites and/or GMS- and PAS-positive true cysts containing bradyzoites (see Fig. 8.119B).

Cryptosporidium. *Cryptosporidium parvum* is a water-borne opportunistic infection that affects patients with HIV/AIDS but has also been seen as outbreaks among children in daycare centers.[98] The disease primarily affects the small bowel, leading to cholera-like watery diarrhea. In patients with HIV/AIDS the infection can spread to the hepatobiliary tree, as well as to the pulmonary airways.

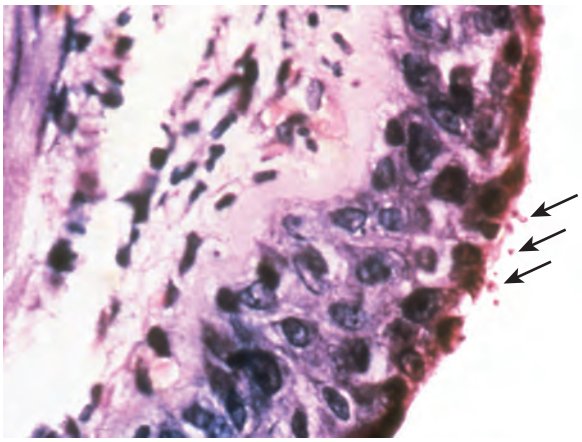
The diagnosis is made by identifying the amphophilic spores (3 to 5 μM) of *Cryptosporidia* along the surface of infected respiratory epithelium (Fig. 8.120). Although apparently extracellular by light microscopic determination, ultrastructural analysis demonstrates that the cysts are actually intracytoplasmic and invested by apical cytoplasm. The underlying mucosa shows a mild lymphocytic



• **Figure 8.118** **A,** Liquefactive necrosis in lung abscess due to **(B)** *E. histolytica* showing ingestion of erythrocyte *(arrow)*.



• **Figure 8.119** **A,** Necrotizing pneumonia in a patient with human immunodeficiency virus/acquired immune deficiency syndrome due to *T. gondii.* **B,** High power demonstrates bradyzoites within macrophage.



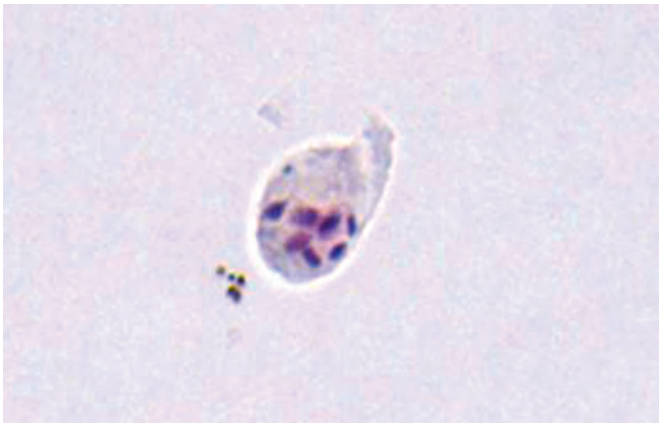
• **Figure 8.120** *C. parvum* seen along the surfaces of ciliated pulmonary epithelium *(arrows)*. The cysts are actually invested by apical cytoplasm.

infiltrate. Although well seen with H&E, the cysts are also stained by GMS, modified AFB, and Giemsa. The diagnosis is obvious once considered but can easily go unnoticed unless considered in the differential diagnosis.

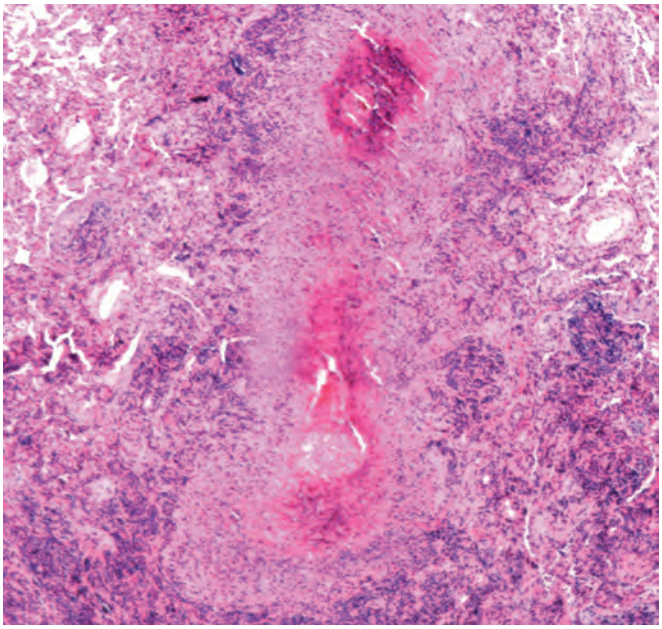
Microsporidia. Microsporidia are spore-forming protozoa that, like *Cryptosporidium*, infect immunosuppressed patients to produce a watery diarrheal illness. Extraintestinal disease, including bronchial involvement, is uncommon but has been observed. The organisms are intracytoplasmic and generally located within the apical cytoplasm.[99] Because of their small size (1 to 2 μM), they may be exceedingly difficult to detect, especially when the organism load is low. Special stains, including tissue Gram stain, trichrome, Warthin-Starry, and GMS, can assist in their identification (Fig. 8.121). Further speciation requires ultrastructural examination.

Nematodes (Round Worms)

Several nematodes (round worms) have a larval developmental phase in the lung (Box 8.7).[100] The larvae of *Ascaris*, *Necator*, *Ancylostoma*, *and Strongyloides* migrate through the airways towards the mouth, where they are swallowed or expectorated. This process can evoke wheezing, migratory pneumonia, and



• **Figure 8.121** Bronchoalveolar lavage macrophage contains *Microsporidium* spp. demonstrated by Brown-Hopps stain.



• **Figure 8.122** Necrotizing granulomatous and eosinophilic inflammation due to *S. mansoni.*

• BOX 8.7 Nematodes With Pulmonary Larval Phase

Ascaris lumbricoides
S. stercoralis
Necator americanes
Ancylostoma duodenale

blood eosinophilia, a complex termed Loeffler syndrome. The presence of track-like necrotizing granulomatous bronchitis and bronchopneumonia with prominent eosinophilic infiltrates should alert the pathologist to the presence of a migratory parasitic pulmonary infection (Fig. 8.122).

Strongyloides stercoralis is most often seen in the tropics; however, cases are endemic to the southeastern United States. Patients receiving high doses of corticosteroids are susceptible to infection and what is termed "hyperinfection." In hyperinfection, filariform organisms exit the gut and migrate to the lungs, where they may be found in airways, alveoli, and blood vessels, and produce a hemorrhagic and eosinophilic pneumonia. When normal pathways of maturation are inhibited, the filariform larvae mature to egg-laying adults that give rise to rhabditiform larvae, and the presence of expectorated ova indicates hyperinfection (Fig. 8.123A). Parasitemia predisposes to gram-negative sepsis that can lead to concomitant DAD (see Fig. 8.123B).

Dirofilaria. In temperate climates, *Dirofilaria immitis* is a zoonosis that causes canine "heartworm." Mosquitoes transfer microfilariae into subcutaneous tissues, where they mature silently and enter the systemic venous circulation. From there, they travel to the right heart, but in humans they do not mature further. Pulmonary disease reflects embolism of a nonviable helminth from the heart into the pulmonary circulation, where it generally lodges in a small muscular artery to produce an area of localized rounded infarction with a chronic immunologic reaction to the dead worm.[101] The embolic event itself may be clinically silent or associated with chest pain, fever, chills, hemoptysis, and peripheral blood eosinophilia. The disease is often first recognized as a solitary pulmonary nodule that is resected to exclude neoplasia.

Microscopically one sees a rounded area of pulmonary infarction (Fig. 8.124A). The diagnosis is facilitated by identifying the coiled nematode within an occluded muscular pulmonary artery. The area of surrounding necrosis is surrounded by a zone of granulomatous inflammation with lymphocytes, plasma cells, and variable degrees of tissue eosinophilia, all contained within a dense fibrous capsule. Trichrome, elastic, and reticulin stains highlight the features of the helminth and help to localize it in a vessel (see Fig. 8.124B and C). *Dirofilaria* are distinguished from other nematodes by their prominent muscular lateral cords and a striated cuticle that bulges inward in the region of the lateral cords, yielding a bat wings appearance.

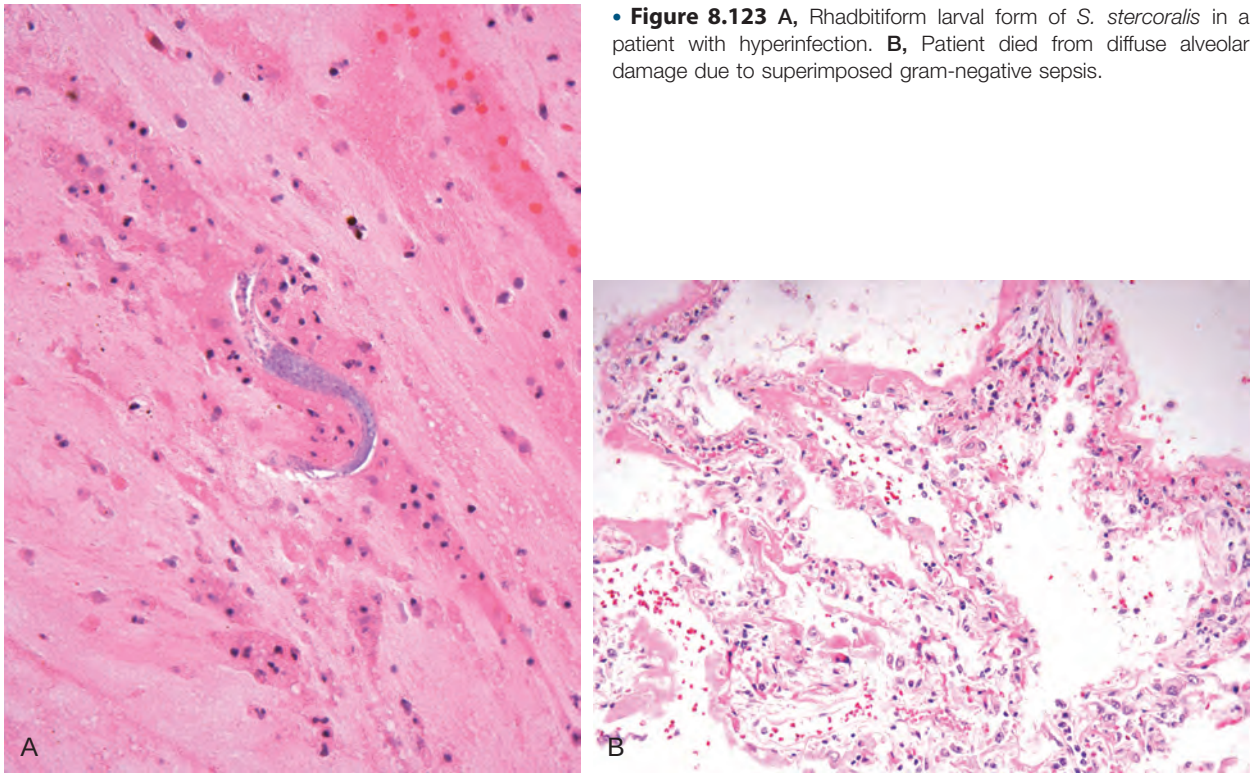
Trematodes (Flukes)

A number of trematodes produce pulmonary disease that can present as solitary pulmonary nodules, pulmonary infarctions, necrotizing granulomas, or eosinophilic pneumonia. The most frequent fluke infection in the United States is *Schistosomiasis*, due to it wide distribution in both the Eastern and Western hemispheres.

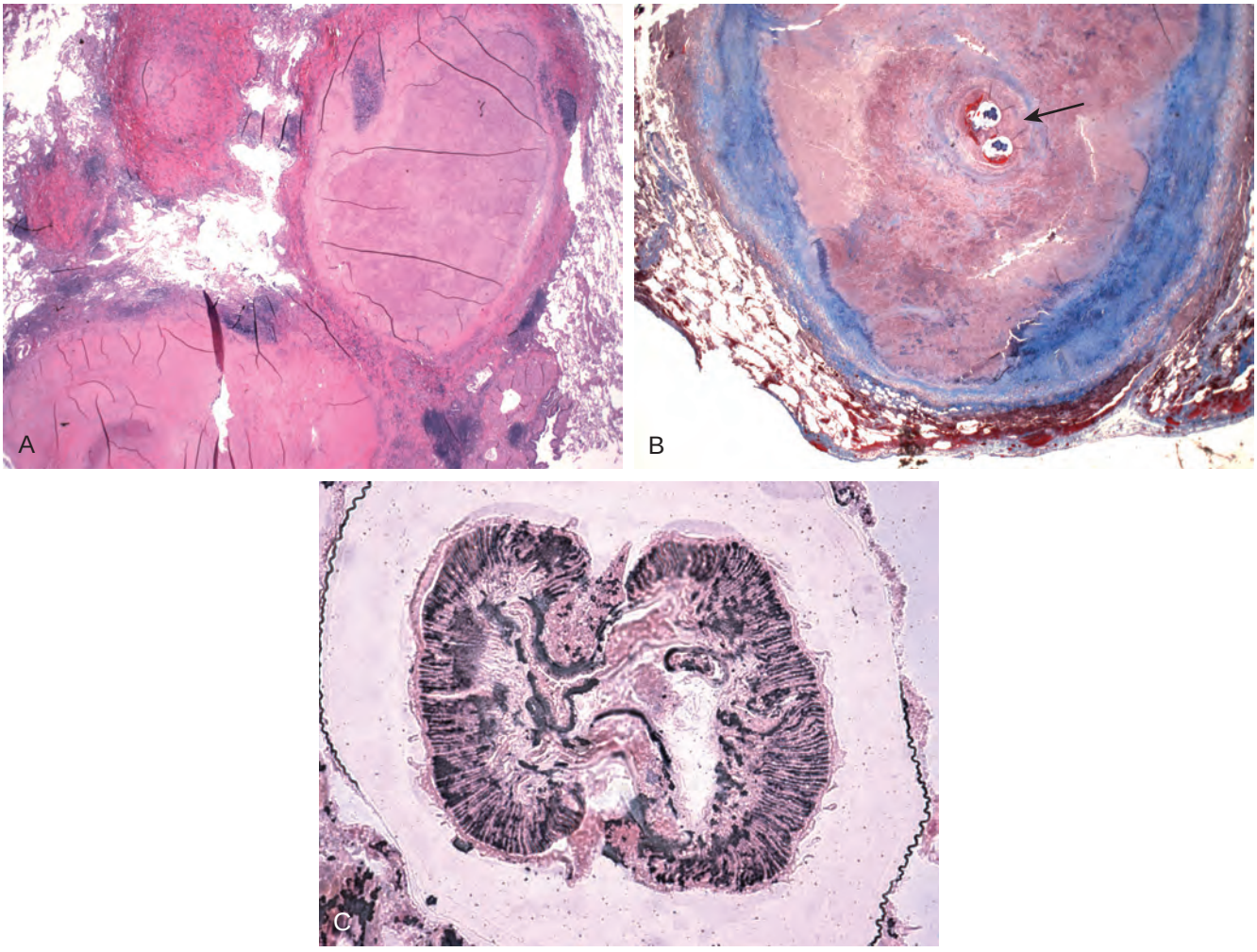
Pulmonary involvement reflects aberrant migration of adult worms into the lung, where they are found within pulmonary vessels (Fig. 8.125A). The helminth evokes an intense granulomatous and eosinophilic response, and extravascular refractile ova may be present and, like the helminth, can evoke granulomatous and eosinophilic reactions.

The ova are large (70 to 170 μM) depending on the species. The ova of *Schistosomiasis mansoni*, which produces most cases of pulmonary disease, show a prominent lateral spine that can be seen with H&E and that is highlighted by modified acid-fast stains, although the latter finding is inconstant (see Fig. 8.125B). Other species, including *Schistosomiasis hematobium* and *Schistosomiasis japonicum*, rarely cause pulmonary disease, and their ova show either a prominent or inconspicuous terminal spine, respectively.

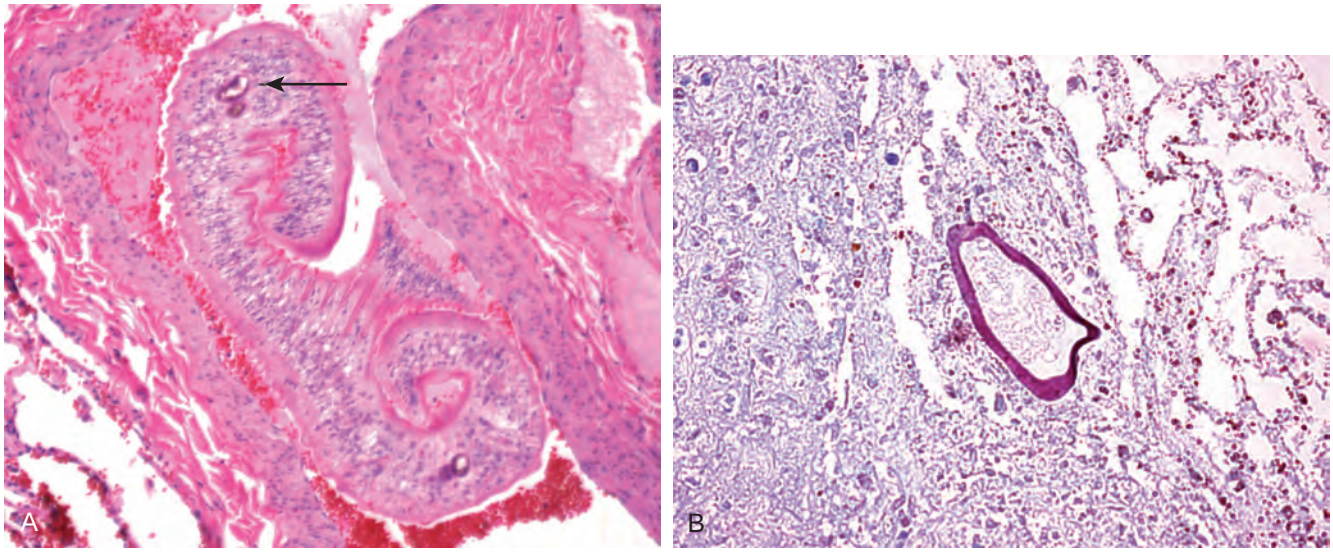
Schistosomiasis can also produce a granulomatous pulmonary hypertensive arteriopathy due to the presence of either ova or migrating schistosomules within small-caliber pulmonary arteries and arterioles (Fig. 8.126).[102] The thickened vascular walls show epithelioid histiocytes and giant cells with adventitial fibrosis and



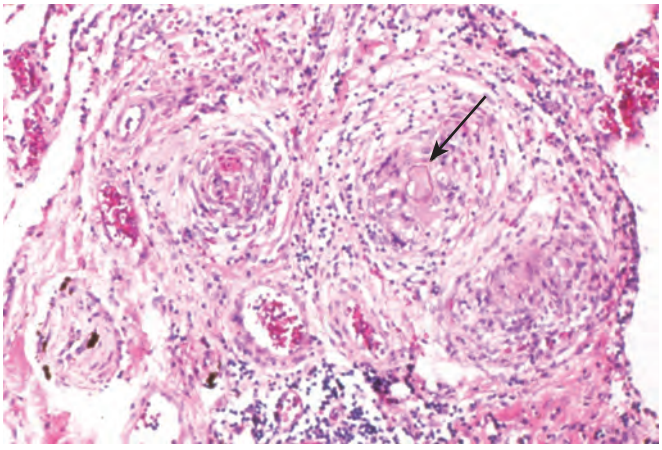
• **Figure 8.123 A,** Rhadbitiform larval form of *S. stercoralis* in a patient with hyperinfection. **B,** Patient died from diffuse alveolar damage due to superimposed gram-negative sepsis.



• **Figure 8.124 A,** Nodular pulmonary infarction due to *D. immitis* seen in **(B)** trichrome and **(C)** reticulin stains. Nematode shows muscular lateral chords with invagination of overlying cuticle.



• **Figure 8.125** **A,** Coiled tuberculated schistosome within pulmonary artery. **B,** Large lateral spine and cortex of *S. mansoni* ovum is decorated by modified AFB stain.



• **Figure 8.126** Granulomatous pulmonary arteritis due to *S. mansoni*. Both eggs *(arrow)* and schistosomules can be found in vessels, although their demonstration may require the examination of multiple sections.

eosinophils. This disorder usually arises from infection by *S. mansoni* or less commonly *S. japonicum* that has already produced pipestem hepatic fibrosis and presinusoidal portal hypertension, leading to shunting of portal blood into the systemic venous circulation, with ova swept into the pulmonary arterial bed, where they evoke the pathologic response.

Paragonimiasis. *Paragonimus*, often referred to as the "lung fluke," is a globally distributed trematode, with human disease limited to endemic regions.[103] Most cases in Asia are due to *Paragonimus westermani*, but other species are responsible for disease seen in Africa and Central and South America. Zoonotic forms of human infection rarely occur in North America secondary to *Paragonimus kellicotti* that affects rodents and cats.

The organism is a freshwater species that infects crabs and crayfish as intermediate hosts; the disease is transmitted to humans via ingestion of these crustaceans, although cases may be seen that are due to ingestion of contaminated seaweed or watercress. The larvae mature in the bowel and migrate through the diaphragm to infect the lung. Patients present with pulmonary infiltrates, fever, weight loss, and malaise. Hemoptysis is common. Pleural involvement is present in approximately half of patients. Peripherally located cystic lesions develop in the lung, together with areas of pneumonic consolidation, and concomitant bacterial and mycobacterial infections may be present.

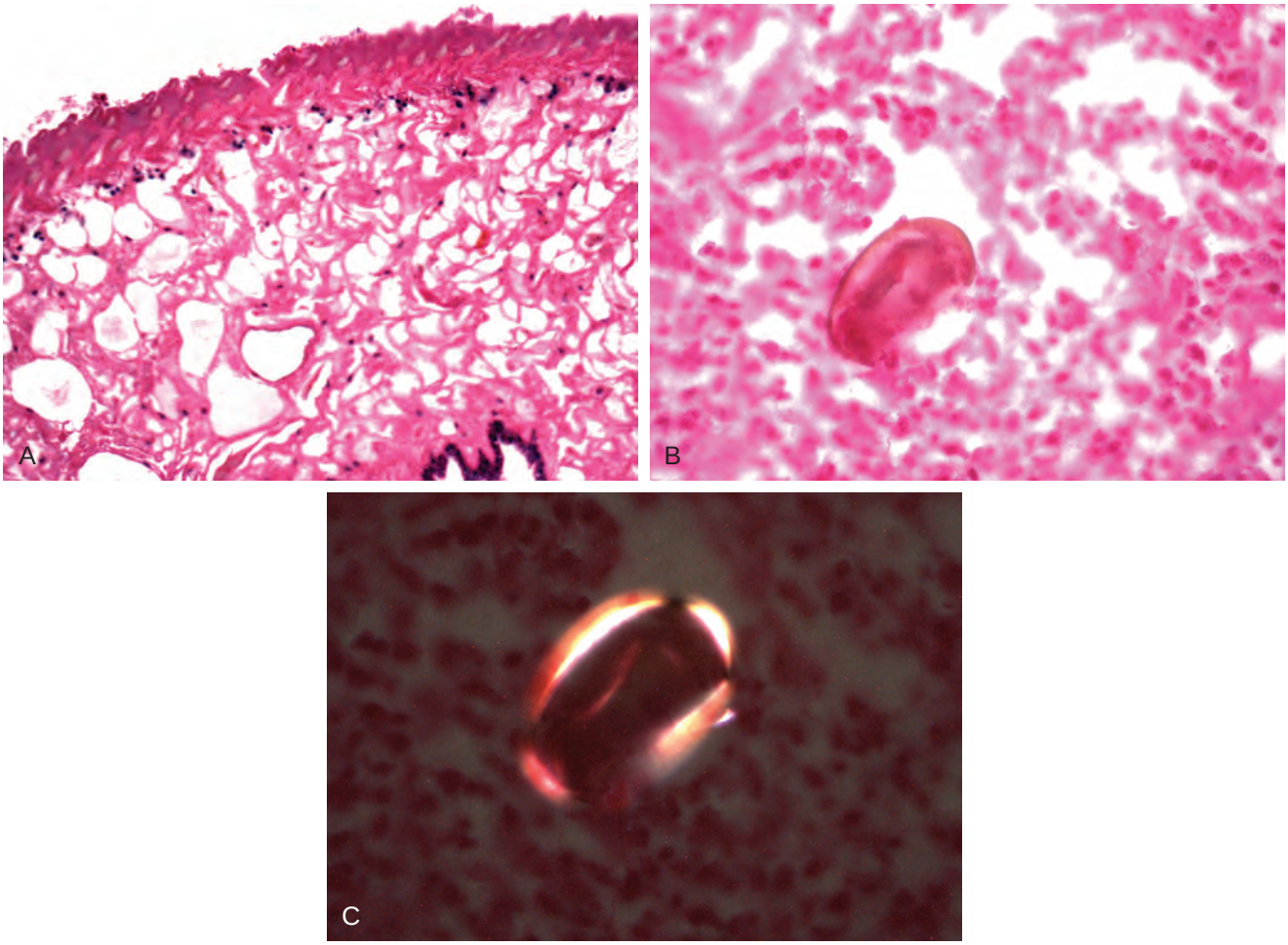
Grossly, *Paragonimus* is a large, 10-mm, reddish fluke. Its wall consists of a tegument with prominent spiny projections best seen on high-power examination of trichrome-stained sections (Fig. 8.127A). It exhibits prominent oral and ventral suckers and a loose internal stroma. The worm evokes a cystic necrotizing granulomatous and fibrotic response with tissue eosinophilia. The ova of *Paragonimus* are approximately 80 μM and show a flattened operculum (see Fig. 8.127B). They are also intensely birefringent under polarized light, and this is an important distinguishing factor with respect to schistosome ova (see Fig. 8.127C).

Cestodes (Tapeworms)

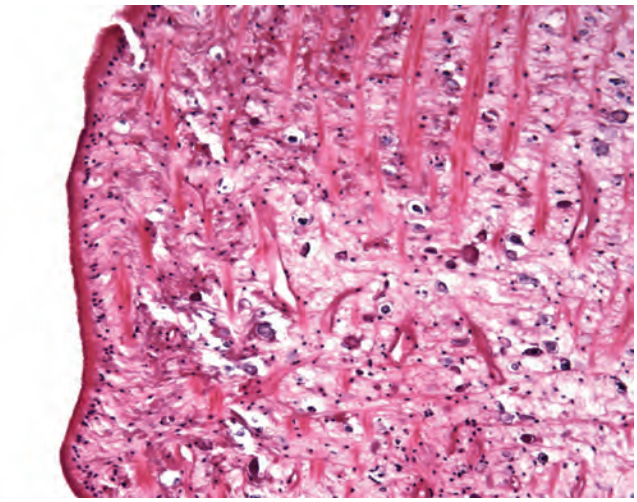
The most common cestode pathogen in the lung is *Echinococcus*, although other tapeworm larvae, including *Cystercerca* and *Sparganum* can rarely infect the lung (Fig. 8.128).[104] The adult tapeworm lives attached to the wall of the small intestine of carnivorous canids. Intermediate hosts are the grazing ungulates, including sheep, goats, deer, and bison. In the continental United States, the disease is endemic to midwestern and western states.

Pulmonary disease is generally the result of preexisting hepatic involvement by *Echinococcus granulosus*, although other species, including *Echinococcus vogeli* and *Echinococcus multilocularis*, are also pathogenic.[105] The appearance of the disease is characteristic on chest radiographs that show single or multiple large fluid-filled cysts (Fig. 8.129). These cysts can be asymptomatic or cause symptoms due to compression of the surrounding airways and lung. Rupture into an airway can lead to suppurative pneumonia, the proliferation of new cysts, and generate fatal anaphylactic reactions.

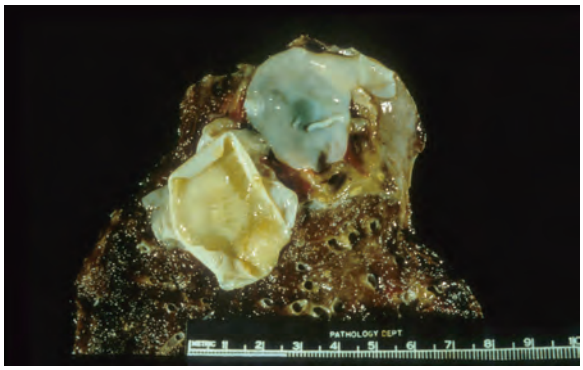
The cyst wall shows contributions from the cestode, as well as from the host. The inner germinative layer of the lamellar cyst wall is the matrix for protoscolices that detach to form secondary brood capsules (Fig. 8.130A). The rostellum of the attachment



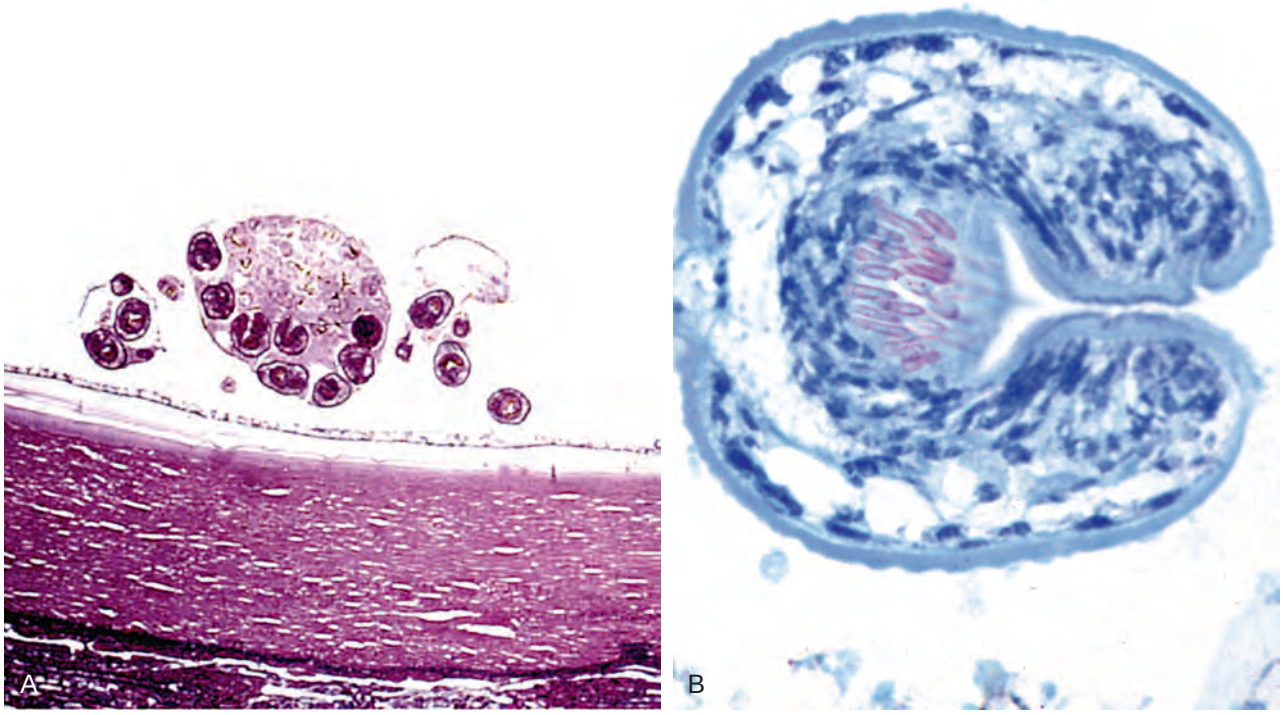
• **Figure 8.127** **A,** *Paragonimus* spp. are trematodes that show characteristic surface tegumental spikes. **B,** The ova of *Paragonimus* spp. are operculated, refractile, and **(C)** show diagnostic birefringence with polarized light.



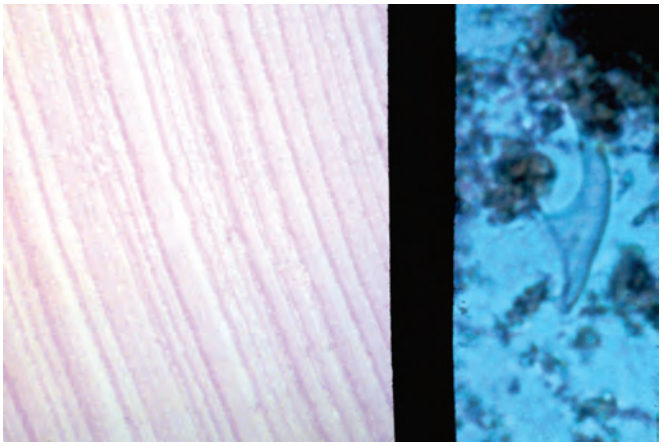
• **Figure 8.128** A cestode larval *sparganum* removed from lung.



• **Figure 8.129** Pulmonary cyst due to *E. granulosus.*



• **Figure 8.130** **A,** Germinative layer of echinococcal cyst giving rise to protoscolices. **B,** Rostellum and hooklets of a protoscolex stain with modified AFB.



• **Figure 8.131** Chitinous cyst wall *(left panel)* and refractile hooklet of *E. granulosus (right panel)*.

apparatus of the protoscolex contains rows of hooklets that are refractile and stain with modified AFB (see Fig. 8.130B). The cestode component is acellular chitinous lamellar wall that is apparent with H&E and further highlighted with GMS, and within the cyst wall, detritus and hooklets give rise to so-called hydatid sand (Fig. 8.131).

Microbes Associated With Bioterrorism

Recent world events have prompted interest in biological agents that can potentially be used as weapons of mass destruction. Several of these produce pneumonia and depend on dissemination via aerosolized secretions to achieve their ignominious goal. Whereas most of these infections are naturally virulent, they tend to occur under situations that are no longer commonly encountered in modern societies. Their adoption by bioterrorists may include the need to bioengineer the organisms to promote their attack rate.

Anthrax

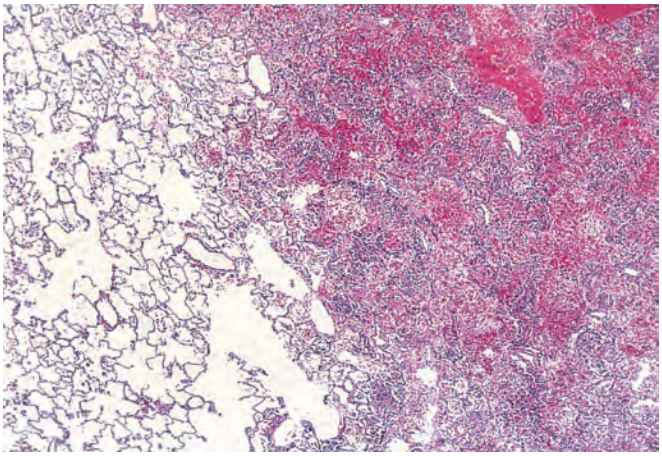
Bacillus anthracis, a toxin-producing gram-positive bacillus, was first isolated by Robert Koch as the cause of anthrax, a disease that primarily involved sheep and other farm animals. However, transmission to humans from infected animals has been recognized from antiquity as a complication of wool sorting (wool sorter's disease). An epidemic of inhalational anthrax occurred in 1979 at a biofacility in Sverdlovsk, in the former Union of Soviet Socialist Republics (USSR), and in 2001 there was a limited epidemic of anthrax due to contaminated letters sent through the US mail service by an unidentified terrorist.[106]

Cutaneous penetration by the bacillus produces a necrotic eschar with rapid extension to blood vessels, resulting in bacteremia, sepsis, meningitis, and death. However, the most deadly form of infection is pulmonary. In these cases the bacilli are inhaled, which evokes a localized hemorrhagic pneumonia and associated pleural effusion. Organisms proliferate rapidly in the lung, where they produce a localized hemorrhagic pneumonia with scant neutrophilic reaction, (Fig. 8.132) and spread via the pulmonary lymphatics to the regional lymph nodes to produce hemorrhagic mediastinitis, followed by bacteremia, toxic shock, and death in a high percentage of cases. The diagnosis must be suspected and treated early to be curable. However, it has most

often first been recognized at autopsy, where tissues prove to be teeming with bacteria.

Yersinia pestis (Plague Pneumonia)

Plague has played an important role in world history. The causative agent is *Y. pestis*, a gram-negative rod that is carried by animal fleas.[107] Infection occurs as the result of contact with infected animals via aerosol or direct contact with infected secretions. Throughout history, the black rat, *Rattus rattus*, has been most responsible worldwide for the persistence and spread of plague in urban epidemics, but any rodent can mechanically transmit infected fleas. Although *Y. pestis* has not yet been seen as a bioterrorist agent, it has received attention as a potential weapon of mass destruction because as few as 1 to 10 bacilli are sufficient to cause infection when introduced via the oral, intradermal, subcutaneous, or intravenous routes.



• **Figure 8.132** Hemorrhagic pneumonia due to anthrax.

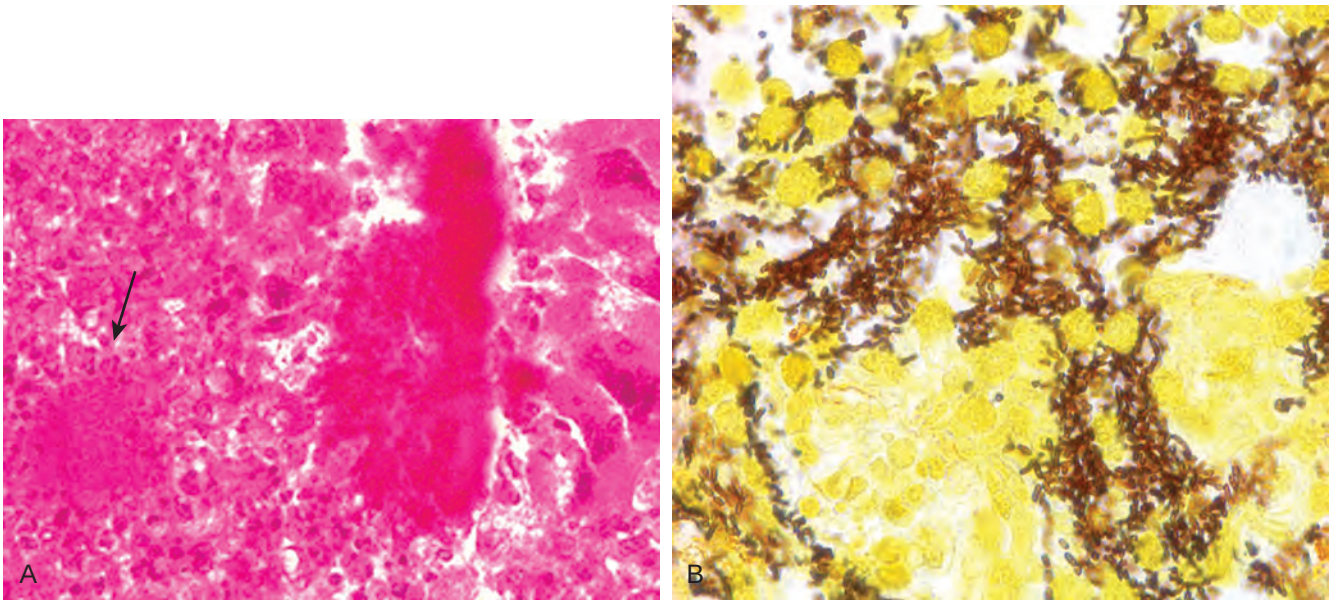
Yersinia produces a necrotizing hemorrhagic pneumonia and large numbers of extracellular organisms that can be seen with H&E (Fig. 8.133). Pulmonary infection leads rapidly to bacteremia and to death by sepsis.

Francisella tularensis (Tularemia Pneumonia)

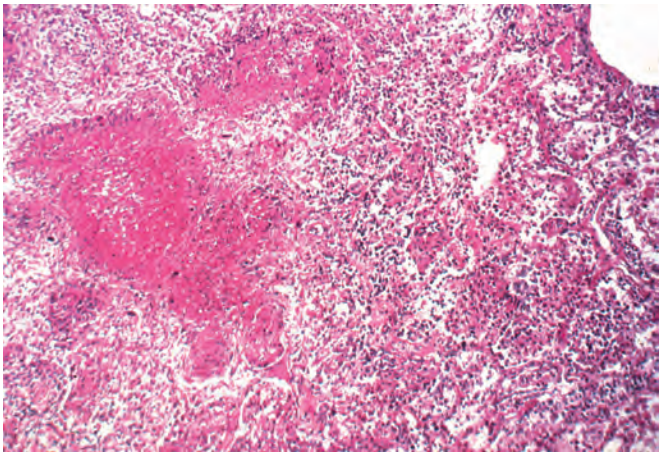
Tularemia causes a necrotizing bronchopneumonia that leads to sepsis and death. However, the gram-negative bacillus is less virulent than either anthrax or plague, and it produces a relatively slow progression of disease, a fact that limits its potential role as an agent of bioterrorism.[108] The histologic response in the lung is polymorphic and includes an early hemorrhagic granulohistiocytic response with microabscess formation (Fig. 8.134), followed by granulomatous inflammation. When these coexist, the appearance of this infection is characteristic. The short coccobacillary forms require silver impregnation to be visualized in tissues.

Pleural Infection

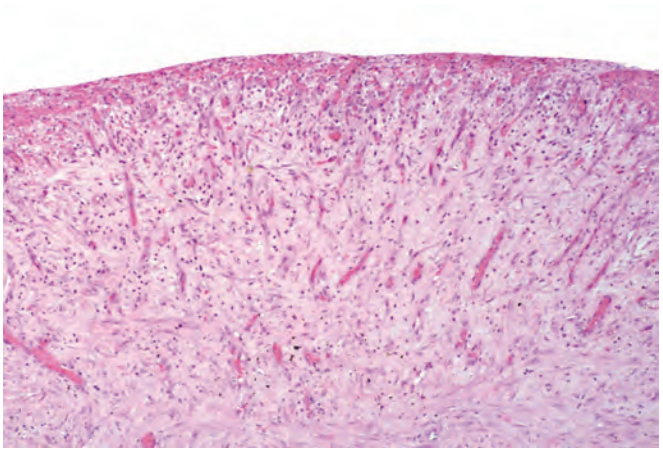
Parapneumonic effusions can complicate bacterial pneumonias, but they are rarely biopsied unless they lead to a restrictive rind around the lung requiring decortication (Fig. 8.135). Both gram-positive and gram-negative bacteria can produce empyema (i.e., abscess in the pleural space) (Fig. 8.136A and B). Mycobacteria (Fig. 8.137), actinomyces (Fig. 8.138), fungi (Fig. 8.139), and parasites can all produce pleural disease, and the nature of the inflammatory response will substantially assist in narrowing the differential diagnosis, even when organisms cannot be identified. Tuberculosis can result years later in a fibrothorax, often with dystrophic calcification, but with no residual granulomatous response. Pleural eosinophilia can be a clue to the presence of an underlying parasitic infection (Fig. 8.140) but can also be seen in fungal and mycobacterial infections, in response to drugs and pleural metastases, and following pneumothorax.



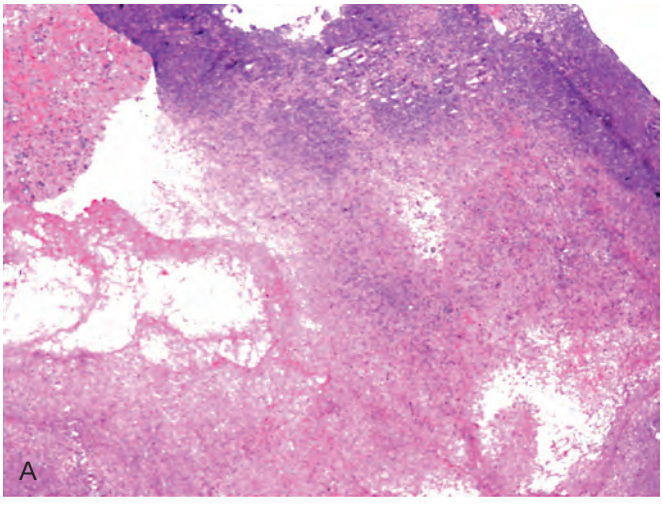
• **Figure 8.133** **A,** Necrotizing hemorrhagic pneumonia due to plague *(Y. pestis)*. Organisms are seen both in hematoxylin and eosin– *(arrow)* and **(B)** silver-stained sections.

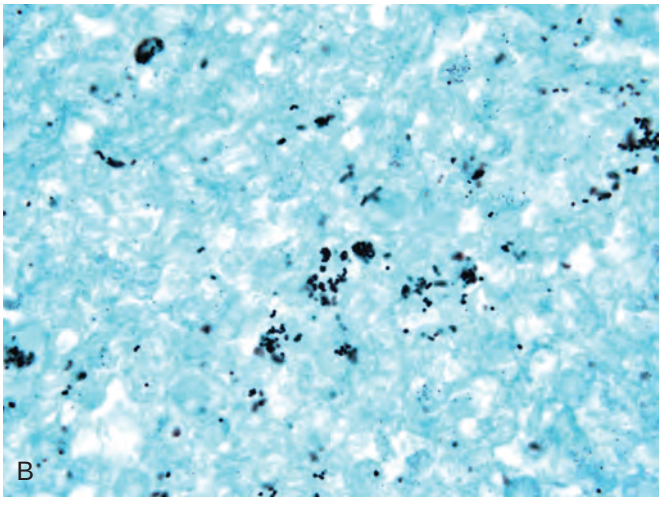


• **Figure 8.134** Granulomatous pneumonia due to tularemia.

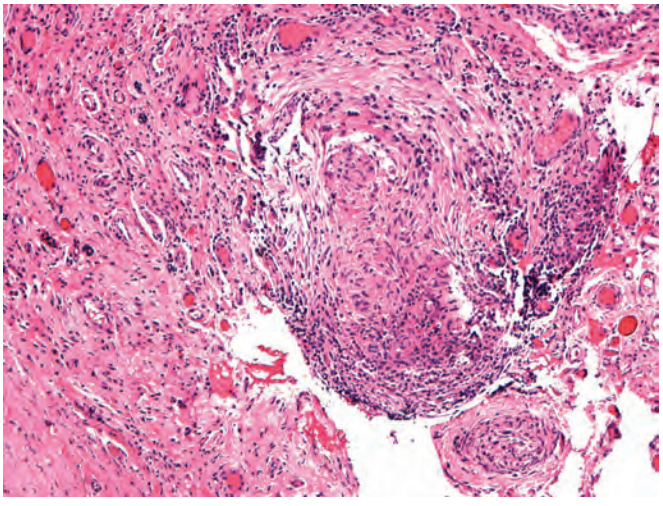


• **Figure 8.135** Organizing fibrinous pleuritis due to pneumococcal pneumonia. Patient had parapneumonic effusion.

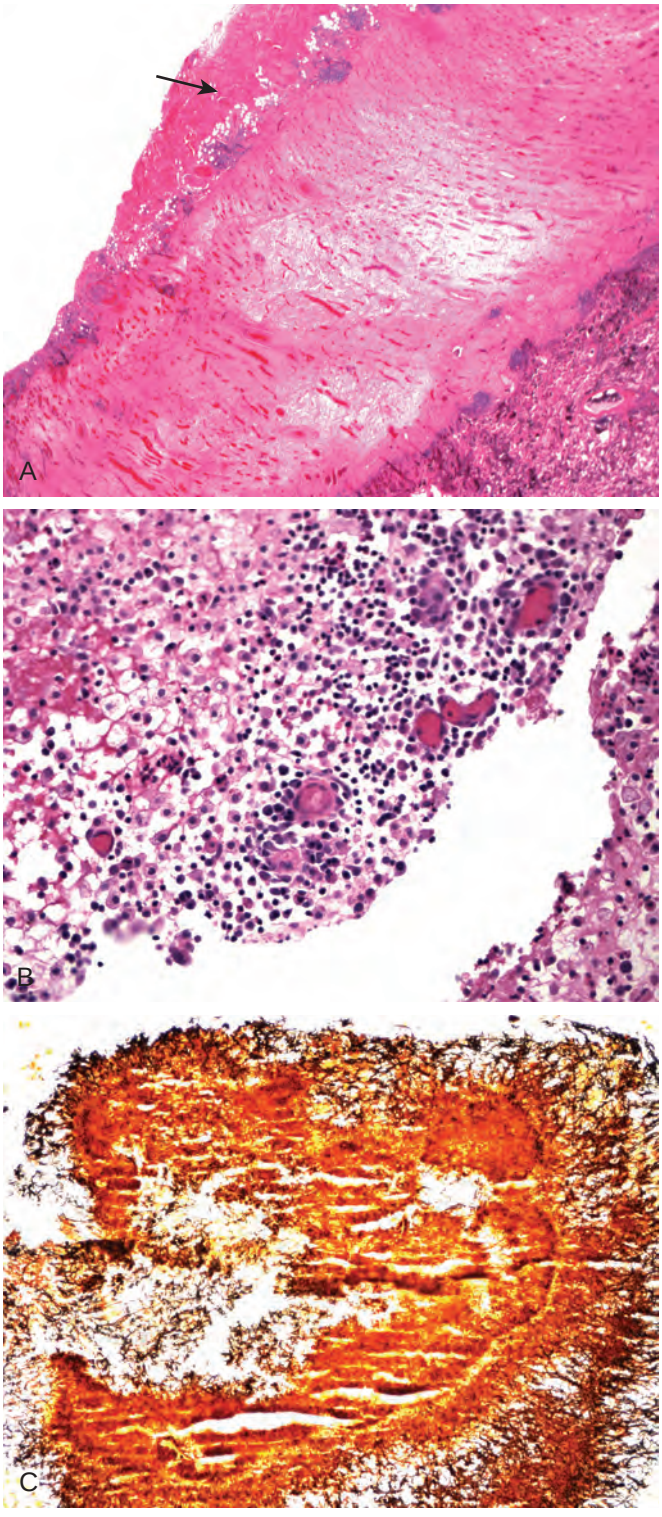




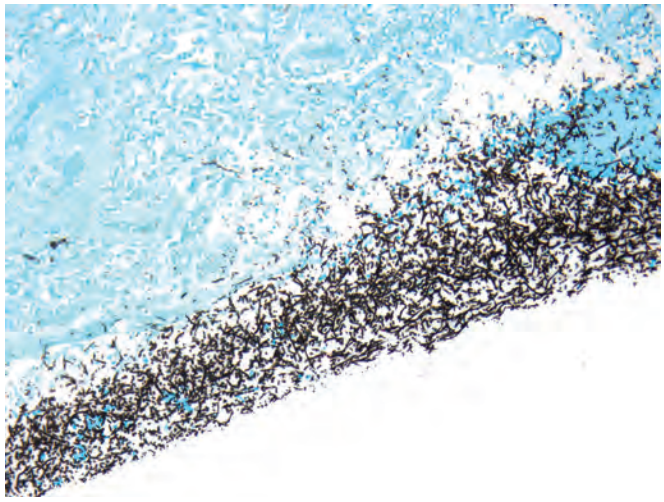
• **Figure 8.136** **A,** Empyema secondary to staphylococcal pneumonia. **B,** Clusters of cocci are stained by Gomori methenamine silver.



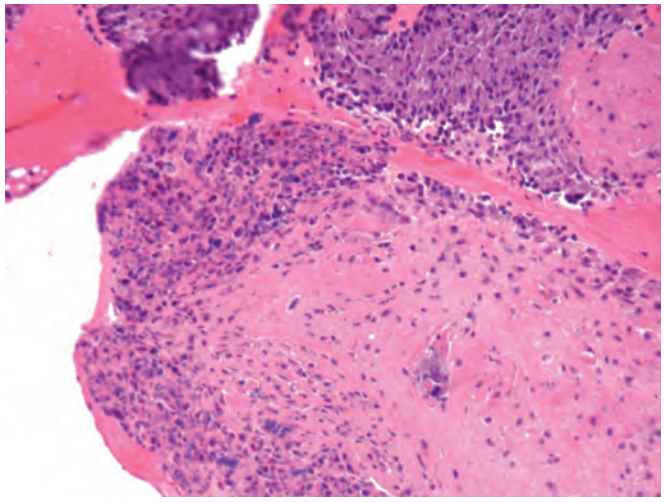
• **Figure 8.137** Granulomatous pleuritis in tuberculosis.



• **Figure 8.138** **A,** Dense pleural adhesion transgressing diaphragm *(arrow)*. **B,** Abscess cavity with granulohistiocytic inflammation. **C,** Steiner stain shows filamentous bacteria in sulfur granule. Organisms were also positive with tissue Gram stain and Gomori methenamine silver.



• **Figure 8.139** Aspergillus pleuritis.



• **Figure 8.140** Eosinophilic calcifying pleuritis in a patient with underlying *Trichuris* infection.

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9 Cardiac Infections

RICHARD L. KRADIN, H. THOMAS ARETZ

Introduction

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Pathology of Myocarditis
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Introduction

Infections can involve all three layers of the heart, and virtually all classes of organisms infect cardiac tissues. Because the entire cardiac output is delivered to the lungs and other vital organs by the cardiac ventricles with each cardiac cycle, the propensity for blood-borne infections to be carried to and from the heart is substantial.

This chapter is divided according to the layers involved, in the order of frequency seen by the surgical pathologist: *infective endocarditis* (IE) involving the valves (and, rarely, the endocardium lining the chambers); *myocarditis* involving the myocardium; and *pericarditis* involving the pericardium.

Pathology of Infective Endocarditis

The true incidence of IE is uncertain, but it has been estimated to account for 1 of every 1000 hospital admissions.[1] The morbidity of this disorder is substantial because it can cause cardiac valvular insufficiency, congestive heart failure, and cardiac conduction system abnormalities. Embolization of infected vegetations can damage vital organs, including brain, kidney, and lung. Circulating immune complexes that develop in response to endovascular infection can result in microvascular injury, arthritis, and renal failure.

Revised diagnostic criteria for IE have improved diagnosis,[2] although cases of IE continue to be discovered at the time of valve surgery and at autopsy. The emergence of fastidious microorganisms has led to increased recognition of the microbiologic spectrum of this disease. The widespread use of endovascular prosthetic devices and bioprosthetic grafts has expanded the scope of IE and complicated its management.[3-6] Extracorporeal membrane oxygenation is currently being used in major medical centers for the cardiorespiratory support of critically ill patients, and it has been associated with increased risk of endovascular infection, especially from *Enterobacteriaceae* and *Candida* spp. New left ventricular assist devices have also been associated with bloodstream infections.[4] Finally, research into bacterial interactions with vascular endothelium and recognition of the importance of bacterial biofilms promise to change how IE is treated in the future.[5]

The pathology of IE is complex; it reflects the virulence of the organism, host immunity, the biology of the endocardial surface, and the topography of infection. For example, infection of a cardiac valve by *Staphylococcus aureus* in a patient with acquired immunodeficiency syndrome (AIDS) is more likely to produce a rapidly progressive syndrome of valvular incompetence and acute heart failure than is endocarditis due to an organism of low virulence in a normal host.[6] Whereas IE can affect an ostensibly normal endocardial surface, it far more commonly targets anatomically distorted valves (Box 9.1).[7] The site of infection is critical with respect to the spectrum of possible complications; for example, IE on the left side of the heart is more likely to result in acutely life-threatening embolic events, including cerebral and myocardial emboli, than is right-heart endocarditis. Extension of aortic valve infection into the heart can produce myocardial abscess and complete heart block. Infected fistulous tracts forming between cardiac chambers can lead to intracardiac shunting of blood, depending on the anatomy of the involved valve. For these reasons, IE must always be approached nongenerically because the specific pathology determines both potential complications and the optimal therapeutic approach in each case.

Cardiac Valves

Most cases of IE involve the cardiac valves. The normal atrioventricular mitral and tricuspid valves of the cardiac inflow tracts and the semilunar aortic and pulmonary valves of the outflow tracts share a common structure. They consist of a dense, avascular, collagenous core, termed the valvular fibrosa, which is contiguous with the fibrous skeleton of the heart, surrounded by a spongiosa

• BOX 9.1 Modified Duke Infective Endocarditis Diagnostic Criteria

Pathologic

Histologic

Vegetation or intracardiac abscess confirmed histologically
OR

Bacteria

Demonstrated by culture or histology in a vegetation or in an embolic vegetation or intracranial abscess

Clinical

A: Supportive Laboratory Evidence

1. Positive blood culture for typical IE organisms from two separate blood cultures
2. Persistently positive for typical IE organisms blood culture drawn >12 hours apart
3. Persistently positive blood culture for typical IE organisms from all three or a majority of four blood cultures or a majority of four blood cultures
4. Single positive blood culture for *C. burnetii* or phase I antibody titer >1:800

B: Evidence of Endocardial Involvement

Echocardiogram supportive of endocarditis

1. Type of Study
   TEE recommended as first test in patients with (a) prosthetic valve, (b) possible endocarditis by clinical criteria, and (c) those suspected of paravalvular abscess
   Positive findings: oscillating intracardiac mass on valve or supporting structure, or in the path of regurgitant jets, or on implanted material in the absence of alternative explanation, abscess, or dehiscence of prosthetic valve or new valvular regurgitation

C: New Valvular Regurgitation

Minor Criteria

1. Predisposing heart condition or intravenous drug use
2. Temperature > 38.0°C
3. Vascular phenomena: arterial emboli, pulmonary infarcts, mycotic aneurysms, intracranial bleed, conjunctival hemorrhages, Janeway lesions
4. Glomerulonephritis, Osler nodes, Roth spots, positive rheumatoid factor
5. Positive blood culture that does not meet major criterion

Clinical Diagnosis of IE

Major criteria: 2
Minor criteria: 5
Major and minor one major + three minor

IE, *Infective endocarditis;* TEE, *transesophageal echocardiography.*
Modified from Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. *2000;30:633-638.*

that consists of a loose matrix of collagen, elastic fibers, and glycosaminoglycans. The valve surfaces are lined by endothelium. This topographic arrangement is critical to the normal function of the valves during the cardiac cycle. The cardiac ventricles fill with blood that passes through the open leaflets of atrioventricular valves during diastole, and the valves must remain tightly apposed during systole. Conversely, the semilunar valves must open during systole and remain competently closed during diastole. This is achieved by the elastic and deformable properties of the valves. Because the normal cardiac valve is normally avascular, stromal cells that contribute to the valvular fibrosa and spongiosa must dynamically maintain valvular structure.

With the mechanical wear and tear that accompanies age—valves must open and close almost 3 million times in the course of a 75-year lifetime—the normal configuration of the valve changes. Fibrosis is the most common complication of aging, whereas myxomatous change can result from altered hemodynamics (e.g., the functional insufficiency that follows dilatation of a valve ring) or genetically encoded defects (e.g., Marfan syndrome).

Mechanical degeneration leads to increased collagen deposition by valvular mesenchymal cells and to a relative decrease in the size of the spongiosa. In addition, shear forces produce focal endothelial denudation of the valvular surface, leading to the deposition of a platelet-fibrin coagulum that signals subendothelial fibrogenesis.

Valvular calcifications complicate both age-related fibrosis and postinflammatory valvulitis. The structural distortions produced by scarring are further enhanced by calcifications that grow by accretion to be nodular and bulky, leading to valvular stenosis and insufficiency.[8] In some cases, concomitant foci of ossification also develop.

Rheumatic fever, an immunologic complication of group A *Streptococcus* infection, is the most common cause of valvulitis. Rheumatic fever causes a pancarditis that affects the cardiac valves, myocardium, and pericardium. Histiocytic inflammation is accompanied by neovascularization and fibrosis. In the case of the atrioventricular valves the chordae tendineae become scarred and foreshortened, promoting the late hemodynamic consequences of valvular distortion. The end result is a scarred valve that is predisposed to further deformation by dystrophic calcification and functionally to both stenosis and insufficiency.[9]

Infective Endocarditis

IE results from the growth of microorganisms on the endocardial surfaces of the cardiac valves or vascular endothelium. Most infections are caused by bacteria that have been entrapped in a mesh of fibrin and platelets previously deposited along an injured endocardial surface. These deposits are termed vegetations. They are friable and grow by accretion to be potentially bulky, depending on the cause, location, and duration of the infection. In the absence of antimicrobial treatment, effective healing of vegetations does not occur, and the risk of infected platelet–thrombi dislodging and traveling into the circulation is substantial.

In the past, IE was termed "bacterial endocarditis," but with increased recognition that nonbacterial species, including fungi and rickettsia, can also cause endocarditis, the term IE is currently preferred. Modifiers, such as acute, subacute, or chronic, refer to the clinical course of the disease; however, they are imprecise and do not correlate well with the underlying pathology. Instead, IE should be considered a spectral disorder that can exhibit either an aggressive or an indolent course, depending on the circumstances of infection.

The once-universal mortality associated with IE has been substantially reduced by improved diagnosis, antimicrobial treatment, and aggressive surgical intervention. The current mortality rate ranges from 10% to 30%.[10] In the first half of the 20th century, most cases of IE were complications of rheumatic mitral valvular disease. However, with decreased prevalence of rheumatic valvular disease and increased aging of the population, the senile fibrocalcific aortic valve has become an increasingly common presentation.[11] New sources of infection include intravenous drug use and the widespread iatrogenic use of intravenous catheterization.[12] Host immunosuppression in patients receiving corticosteroids and other immunosuppressant agents, human immunodeficiency virus (HIV)-1 infection,[13] diabetes, renal failure, alcoholism,

and cirrhosis all substantially increase the risk for development of IE.[14]

Cardiac and vascular prostheses are potential nidi for infection and present a continued risk for IE after implantation.[15] Right-heart pacemaker implantation can lead to infection along the pacemaker leads and the tricuspid valve. *S. aureus* and *Staphylococcus epidermidis* are the most common pathogens in the early (first 60 days) and late periods, respectively, after implantation.[16] Left-ventricular assist devices are currently used in the treatment of intractable left ventricular failure and as a bridge to cardiac transplantation. The prevalence of IE associated with these devices ranges from 15% to 44%, and the diagnosis can be difficult to establish noninvasively. *Enterococcus* and *Staphylococcus* spp. are the most common infective agents, but fungi and low-virulence organisms also cause disease.[17]

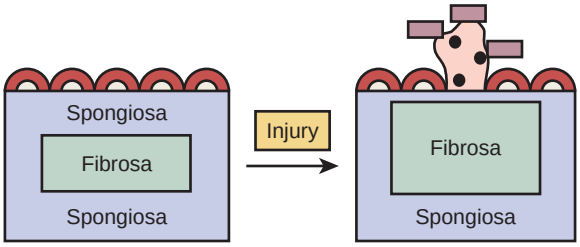
Pathogenesis of Infective Endocarditis

Experimental models have demonstrated that a catheter introduced into the right side of the heart of a rabbit or rat can cause endothelial injury to the tricuspid valve.[18] Endocardial injury greatly increases the risk of developing IE when coupled with subsequent exposure to circulating bacteria (Fig. 9.1). Endothelial injury exposes basement membrane proteins, including laminin, fibronectin, and vitronectin, which serve as adhesion molecules for bacteria. Activation of platelets and thrombus formation further promote bacterial adhesion. Bacterial binding to thrombus is followed by a lag period of several hours before bacterial proliferation is detected.[19]

The risk of developing experimental disease is a function of the virulence and size of the bacterial inoculum and whether the injurious catheter is left in place or removed. For bacterial inocula introduced after catheter removal, the risk of developing IE decreases progressively with time. The histopathology of the valve shows healing of the catheter-induced lesion by reendothelialization, which appears to protect against subsequent bacterial colonization.

Other animal models of IE that do not require an indwelling catheter have been developed. In the guinea pig, electrocoagulation of the aortic valve followed by inoculation with *S. aureus* or *Coxiella burnetii* yields IE. In addition, electrical stimulation of the cervical vagus nerve can result in injury of the mitral valve and predisposes to IE with subsequent bacterial challenge.

Specific microbial factors have been examined in the pathogenesis of IE.[20] Resistance by an *S. aureus* to a thrombin-induced microbicidal protein promotes IE. Fibronectin-binding proteins expressed by *S. aureus* facilitate binding to fibronectin and act as invasins. Organisms that do not bind fibronectin show decreased propensity to cause IE. Gelatinase/type IV collagenase enhances the virulence of *Streptococcus gordonii*, and an aggregation substance expressed by *Enterococcus* (formerly *Streptococcus*) *faecalis* increases virulence in the catheter injury model.



• **Figure 9.1** Pathogenesis of Infective Endocarditis. The diagram shows the sequence of events leading from the configuration of a normal cardiac valve to a scarred valve with increased valvular fibrosa, endothelial *(red)* denudation, and the deposition of fibrin *(pink)* with platelets *(black)* and bacteria *(purple)*.

Biofilm formation plays a critical role in the pathogenesis of IE and has important consequences with respect to its treatment.[21] Bacteria can survive as isolated, free-living planktonic organisms or as stationary colonies associated with a substratum. Four criteria have been proposed for the biofilm origin of infection: adherence of pathogenetic bacteria to a substratum; presence of bacteria in clusters or colonies associated with either an endogenous or a host-derived matrix; localized infection; and resistance to antibiotic therapy despite sensitivity of the planktonic organism. Most, if not all, cases of IE associated with prosthetic surfaces meet these criteria. Bacteria within biofilms produce extracellular polymeric substances (EPSs)—slime-producing glycocalices that limit the accessibility of host humoral and cellular defenses, as well as antibiotics, to the organisms that are embedded in the matrix. Biofilms confer other advantages to the bacterial colonies as well. If the availability of growth requirements is limited, biofilm bacteria can convert to a slow-growing, stationary state, so-called persisters. Water channels form within the biomass and serve as a circulatory system through which nutrients are shared and waste products released.

The activities of the biofilm are coordinated by redundant interbacterial genetic signals. The properties of the biofilm make it virtually impossible to eradicate infection as long as the stationary phase persists. Organisms can spread along surfaces by means of a ripple effect or, alternatively, as detached clumps of organisms that can break free of the substratum matrix and travel to distant sites in the circulating blood. These mechanisms explain the local and distant spread of infection in IE.

Hemodynamic Factors That Predispose to Development of Infectious Endocarditis

Structural deformation of a cardiac valve can lead to local shear stresses and injury.[19] Jet lesions produced by critically stenotic valves or shunts from the systemic to the pulmonary circulation increase the risk of IE. Bicuspid aortic valves are at risk because of their propensity to develop both stenosis and insufficiency.[22] Insufficiency of the aortic valve also increases the risk of mitral valve endocarditis because regurgitant flow through an incompetent aortic valve causes fluttering and superficial injury to the anterior mitral leaflet. A comparable mechanism accounts for mural endocarditis along the ventricular septum in aortic insufficiency. In mitral insufficiency, vegetations may develop along jet lesions formed in the left atrium.[23]

In the absence of primary involvement of the cardiac valve, other conditions, including mitral annular calcification and mural thrombi, become important sites of infection in IE. Left-to-right shunts due to congenital heart disease show a propensity to cause endocarditis at the maximum site of the jet stream. Ventricular septal defects are at high risk, whereas secundum atrial septal defects rarely develop IE. However, endocardial cushion or septum primum defects that involve the mitral apparatus and patent ductus lesions are at risk. Approximately 5% of patients with hypertrophic cardiomyopathy and asymmetric septal thickening develop IE.[24]

The Microbiology of Infective Endocarditis

Virtually all bacterial and fungal pathogens have been reported to cause IE (Box 9.2). The most common cause of IE continues to

• BOX 9.2 Conditions Associated With Infective Endocarditis

Rheumatic valvular disease
Bioprosthetic valve
Posttraumatic valvulitis
Annular calcifications
Atherosclerotic degeneration
Mural thrombi
Hypertrophic cardiomyopathy
Mechanical prosthetic valves
Swan-Ganz catheter
Pacemaker leads
Internal defibrillators
Left ventricular assist devices
Intravenous catheters
Intravenous hyperalimentation
Hemodialysis shunts

• Figure 9.2 Vegetations Along the Mitral Valve. The anterior leaflet of the mitral valve shows large polypoid vegetations caused by *S. aureus* infection. Note the predominant involvement of the atrial surface of the leaflet. The valve was distorted by rheumatic valvular disease, as evidenced by the thickened and shortened chordae tendineae *(yellow arrow)*. Calcification of the mitral annulus is present *(white arrow)*.

be *Streptococcus viridans*, a low-virulence organism that is part of the normal oral flora.[25] The classic clinical scenario for the development of IE is transient bacteremia caused by dental manipulation in a patient with rheumatic valvular disease. However, any site of active infection with access to the bloodstream can yield IE. These include cellulitis, puncture wounds, sinus disease, bronchiectasis, pneumonia, surgery within an unsterile field, and urogenital instrumentation.[26] Other oral flora β-hemolytic streptococci, including *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus mutans*, and *Streptococcus milleri*, account for approximately half of the cases of IE in the community. *Streptococcus pneumoniae* endocarditis is an unusual complication of a pyogenic pneumonia, otitis media, or meningitis.[27] Patients with defects of opsonization, splenic insufficiency, or diminished phagocytic capacity are at increased risk. IE due to group D streptococcal enterococci—*Streptococcus bovis*, *E. faecalis*, and *Enterococcus* (formerly *Streptococcus*) *faecium*—complicates bowel pathologies, including colon carcinoma and diverticulitis, as well as urogenital instrumentation. These organisms account for approximately 20% of cases of IE, and *S. aureus*, *S. epidermidis*, gram-negative bacilli,[28] culture-negative organisms, and fungi approximately account for the remainder.

IE of prosthetic valves is associated with a high rate of complications, including valvular incompetence, obstruction to blood flow, and embolic phenomena.[29] In bioprosthetic valves, IE usually affects the valve cusps, whereas mechanical prosthetic valves develop infections primarily along their sewing rings. Most cases of endocarditis occur within the first 6 months after implantation, but there is a steady annual rate of infection of 2% to 4%.[15] Early infection (≤60 days) is caused by *S. aureus*; by gram-negative bacteria, including *Klebsiella* spp. and coliforms; or by fungi, most often *Candida* spp. Coagulase-negative *S. aureus* accounts for approximately half of the late cases of IE, and nonvirulent organisms, including *S. epidermidis* and *Staphylococcus albus*, account for most of the remainder.

Intravenous drug users have a high incidence of IE.[29] The organisms isolated from blood in these patients tend to be virulent and include *S. aureus* and *Pseudomonas* spp. *Candida albicans* and other fungi account for 10% of cases. Involvement of the tricuspid valve is most common, but mutivalvular disease also occurs. Embolic complications may involve both the lungs and the left side of the circulation.

Blood culture–negative endocarditis accounts for approximately 10% of cases at most major medical centers.[30] Slow-growing gram-negative bacilli can require several weeks before they are detected in culture, and it is optimal laboratory practice to retain blood cultures for at least 3 weeks before discarding them as negative. This group of bacteria has collectively been referred to by the acronym HACEK (*Haemophilus* spp., *Actinobacillus* spp., *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingii*).

C. burnetii, the cause of Q fever endocarditis, often eludes diagnosis.[31] Blood cultures may be negative and vegetations difficult to detect. The noninvasive diagnosis rests on serologic and polymerase chain reaction (PCR) results. Other uncommon culture-negative organisms must also be considered in the differential diagnosis. *Tropheryma whipplei* (Whipple bacillus) can cause culture-negative endocarditis, and the noninvasive diagnosis centers on PCR because cross-reactivity with *C. burnetii* confounds serologic testing.[32] The diagnosis may require ultrastructural examination to detect the characteristic Whipple bodies. Cases of IE due to *Chlamydia* spp. are uncommon, and their clinical and pathologic features mimic those of *C. burnetii*.

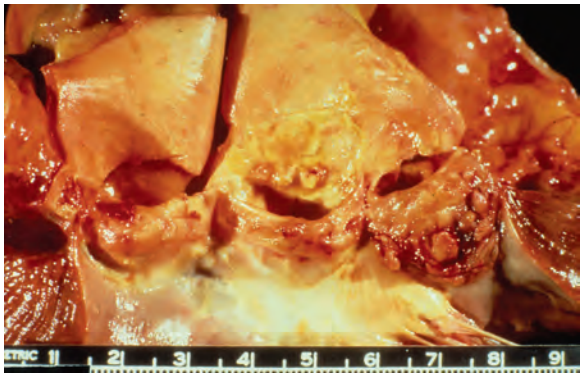
Gross Pathology of Infective Endocarditis

The sine qua non of IE is the vegetation. Vegetations are friable, fibrinous excrescences that develop along the endocardial surface. Most vegetations in IE are polypoid, but they can be sessile. Vegetations vary in size, in part depending on the causative organism, the duration of infection, and whether they affect the right or left side of the heart. In Q fever, vegetations may be inconspicuous, exhibiting only a thin coating of fibrin along the valve surface. Bulky vegetations occur on the tricuspid valve, reflecting the virulence of the organisms that tend to attack that valve in intravenous drug users and the lower systolic pressures of the right ventricle. They are white or yellow, as opposed to the usually dark red vegetations that occur in the left side of the heart. Tricuspid vegetations have a propensity to involve the anterior leaflet of the valve and to produce obstruction of the right ventricular outflow tract.

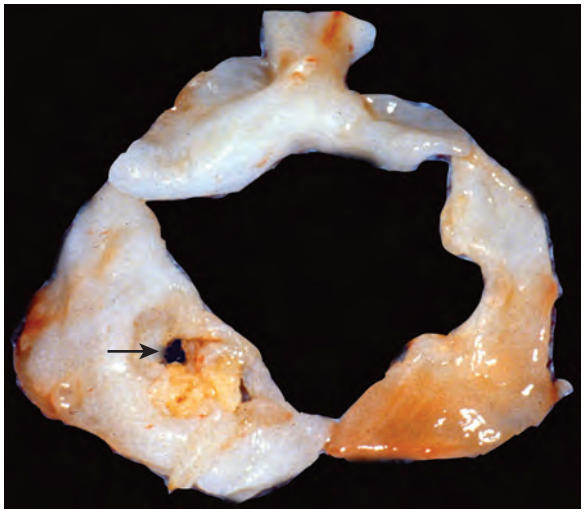
Vegetations along the mitral valve are dark tan or red (Fig. 9.2). They are distributed primarily along the atrial endocardial surfaces of the valve but do not respect lines of closure. They can extend from the valve surface to involve the adjacent atrial endocardium or inferiorly to infect the chordae tendineae. The continuity of the fibrous skeleton of the mitral and aortic valves allows

extension of infection by direct contiguity. Because the anterior leaflet of the mitral valve normally moves toward the aortic outflow tract during systole, infection can spread directly from one valve surface to the other by streaming of organisms at the surface of a biofilm. Infection may also extend directly into the adjacent valve annulus and from there into the subjacent myocardium to produce a myocardial abscess and fistulous tracts to other cardiac chambers.

Vegetations of the aortic valve are generally smaller than those seen along the atrioventricular valves (Fig. 9.3). Infection weakens the valve cusps, leading to aneurysmal dilatation of the sinuses of Valsalva or to tears and ruptures. Rupture of the chordal attachments of an atrioventricular valve can cause a sudden increase in valvular insufficiency and acute pulmonary edema or hepatic centrilobular necrosis and peripheral congestion. If the disease is treated and arrested, healing of the valve takes place, but deformations can result in late hemodynamic complications. Local fibrosis or calcification may mark an area of healed endocarditis, and foci of limited rupture may persist as reendothelialized fenestrations (Fig. 9.4).



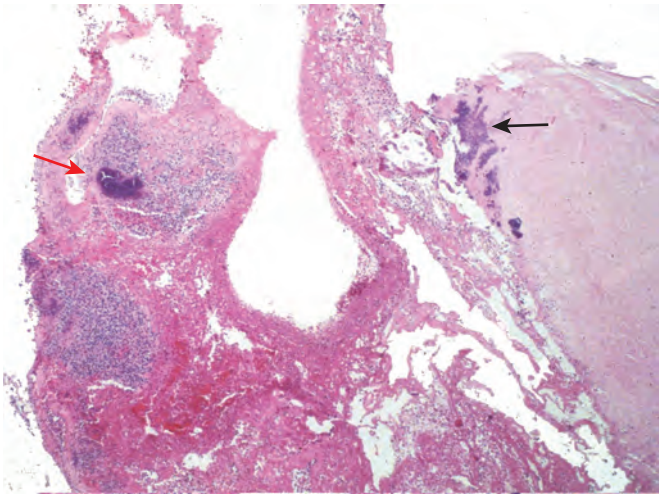
• **Figure 9.3** Aortic Valve Endocarditis. All three cusps of the aortic valve are involved by vegetations, with the left coronary cusp *(right)* showing the most severe disease. Note the relatively small size of the vegetations and the involvement of the ventricular surfaces of the cusps.



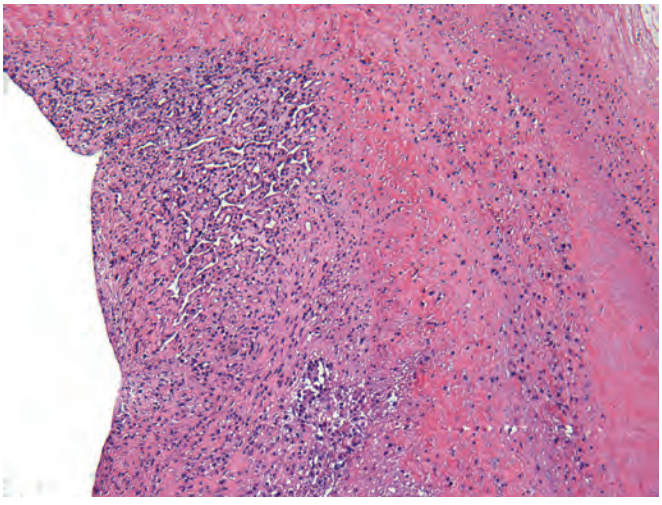
• **Figure 9.4** Healed Aortic Endocarditis. A healed vegetation is seen adjacent to a perforation of the noncoronary cusp *(arrow)*. The left coronary cusp is also eroded along its free margin. The valve was resected because of aortic insufficiency after antibiotic treatment.

Microscopic Pathology of Infective Endocarditis

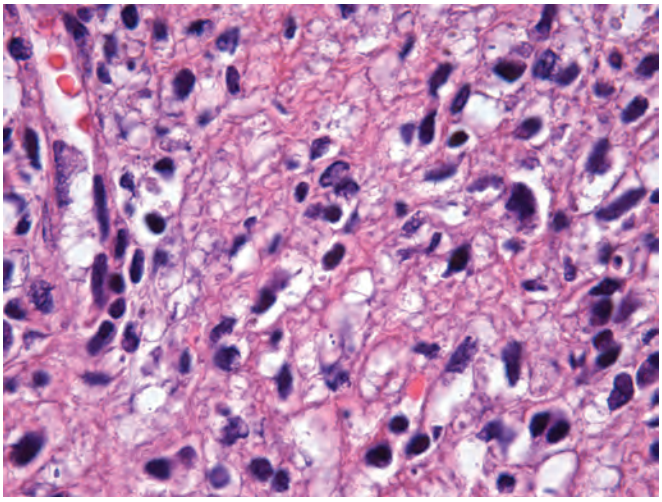
The microscopic appearance of IE can vary from subtle to exuberant. In all cases of active IE, it is possible to discern fibrin deposition along the disrupted endocardial surface. Large polypoid excrescences of fibrin and platelets containing colonies of bacteria or fungi may be evident (Fig. 9.5). The vegetations may be paucicellular or acutely inflamed with areas of valvular and leukocyte necrosis. The valve cusp is edematous and commonly shows a heterogeneous infiltration of neutrophils and histiocytes. The extent and type of inflammation vary with the offending organism. Virulent pyogenic infections, such as those caused by *S. aureus* or *C. albicans*, show substantial acute neutrophilic inflammation, whereas less virulent organisms may show subtle leukocyte infiltration. Early inflammation is rapidly followed by neoangiogenesis and active fibroplasia (Fig. 9.6). In IE caused by *C. burnetii*, *Chlamydia* spp., or *T. whipplei*, fibrinous vegetations are scant and the pathology primarily exhibits foamy histiocytes in areas of scarring (Fig. 9.7).



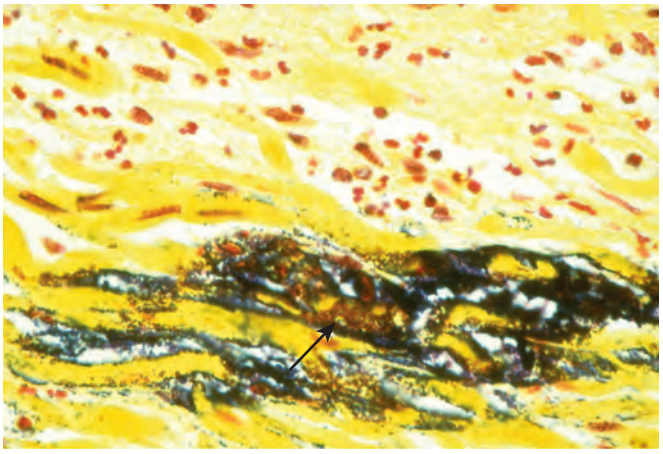
• **Figure 9.5** Histopathology of Vegetation. A fibrotic and calcified aortic valve cusp *(red arrow)* shows vegetation composed of fibrin, platelets, neutrophils, and a colony of *S. viridans (black arrow)* (×100).



• **Figure 9.6** Histopathology of Healing Vegetation. Mitral valve in *S. viridans* endocarditis shows prominent neoangiogenesis and fibroplasia (×200).



• **Figure 9.7** Histiocytic Inflammation in *Coxiella burnetii* Endocarditis. An area of scarring is seen adjacent to an infected prosthetic aortic valve sewing ring with foamy histiocytic inflammation (×400).

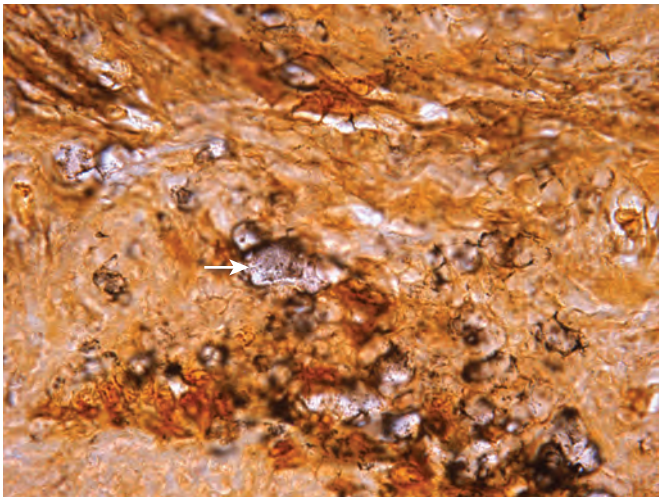


• **Figure 9.8** Tissue Gram Stain of Mitral Vegetation. Brown-Hopps stain shows a colony of gram-positive cocci consistent with *S. aureus*. Note the area of gram-variable staining *(arrow)* that could be mistaken for polymicrobial (mixed gram-positive and gram-negative) infection (×250).

Demonstrating Microorganisms In Situ. The demonstration of microorganisms in situ is an essential part of the pathologic evaluation of IE. In most cases of IE that come to surgery, the diagnosis has previously been established by blood culture and weeks of antibiotics have been administered before valve resection. It is incumbent on the surgical pathologist to be certain that the surgeon has properly cultured the infected tissue at the time of surgical excision. Rapid diagnosis may be enhanced by direct touch preparations of the vegetations and appropriate histochemical or immunohistochemical staining of organisms.

Microscopic examination of the valve must include an assessment of inflammatory activity and whether there are local structural complications, including tears, perforations, and myocardial extension. The minimal battery of histochemical stains includes a tissue Gram stain and a silver stain, such as Gomori methenamine silver (GMS). The tissue Gram stain demonstrates most gram-positive and gram-negative organisms, but some gram-negative organisms (e.g., *Legionella* spp.) require silver impregnation techniques. In addition, the phenomenon of variable staining of gram-positive organisms must not be mistakenly diagnosed as polymicrobial infection (Fig. 9.8). In cases that have been treated and where the underlying valvular pathology includes dystrophic calcifications, it may be difficult to distinguish microcalcifications from bacteria because both can stain positive on Gram and GMS stains. A positive von Kossa stain for calcium phosphate can help to exclude infection.

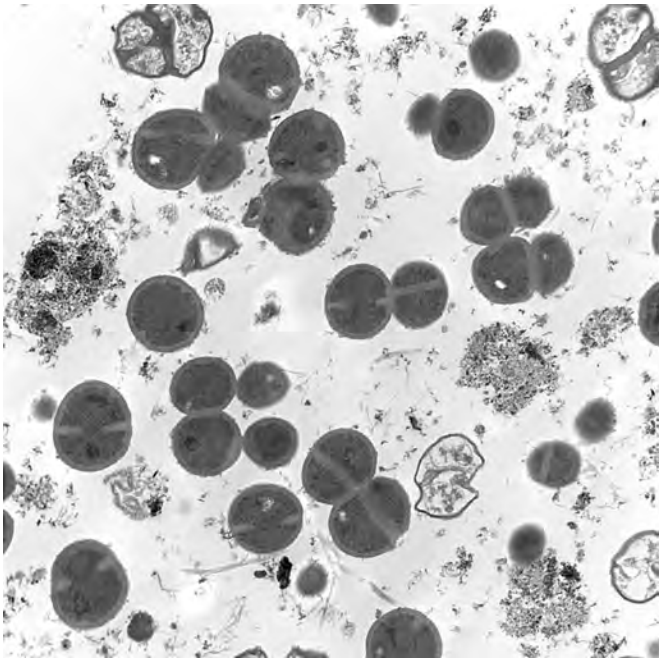
The GMS stain detects all gram-positive bacteria and some encapsulated gram-negatives (e.g., *Klebsiella*). In cases that have received antibiotic treatment, residual organisms may fail to stain with the tissue Gram stain but retain their GMS positivity. In those few cases in which it is impossible to decide whether the putative organisms are real or artifactual, the area of interest can be removed from the paraffin block and processed for ultrastructural examination. Most fungi are well visualized with hematoxylin and eosin (H&E) stain, but they are seen to better advantage with GMS. Screening for fungi should routinely include the GMS because some fungal organisms (e.g., *Histoplasma capsulatum*) are not optimally visualized with periodic acid–Schiff (PAS) stain.



• **Figure 9.9** Silver impregnation (Warthin-Starry) stain in a case of *C. burnetii* endocarditis. Warthin-Starry stain demonstrates small intracellular organisms *(arrow)* that proved to be *C. burnetii* by polymerase chain reaction assay (×250).

If a diagnosis of IE is clinically suspected but an organism has not been cultured, the pathologist must be prepared to demonstrate the presence of organisms by other methods. Mycobacteria can be demonstrated with routine or modified Ziehl-Neelsen stains. The modified acid-fast stain (Fite or Putts) also detects *Nocardia* spp., *Rhodococcus equi*, and *Legionella micdadei*.

Silver impregnation techniques, including the Warthin-Starry, Steiner, and Dieterle stains, decorate all eubacteria, including mycobacteria and actinomycetes. However, the high background in these stains can preclude a confident diagnosis. All culture-negative cases of endocarditis should be examined by silver impregnation (Fig. 9.9). Other special stains, such as the Gimenez stain, have been used to demonstrate *C. burnetii*, and the Macchiavello stain can assist in detection of *Rickettsia* and *Chlamydia* spp.



• **Figure 9.10** Ultrastructural appearance of gram-positive cocci from aortic valve endocarditis. The histologic appearance of a calcified infected valve was suspicious for bacteria, but a definitive diagnosis could not be established by light microscopy. Ultrastructural examination of a section of the paraffin block revealed diagnostic cocci (×7500).

• **Figure 9.11** Fistulous Tract Complicating Mitral Valve Endocarditis. Extension of infection from a vegetation of the mitral valve led to fistula formation between the left ventricle and right atrium. The patient died from acute right ventricular failure due to a large left-to-right shunt. A small thread illustrates the fistula.

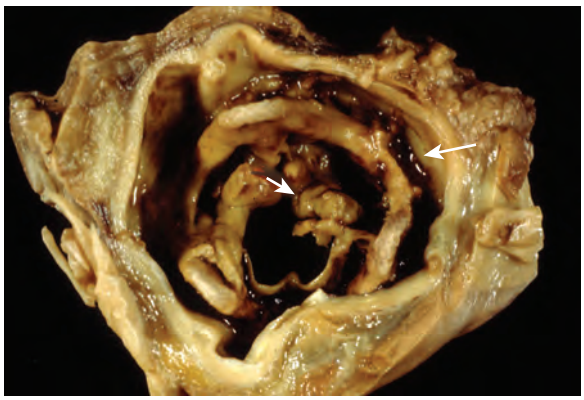
Ultrastructural examination is useful if there is morphologic suspicion of infection by light microscopic examination. Most microbes withstand processing for routine paraffin sections and can be identified with the electron microscope from the paraffin block (Fig. 9.10). Specific identification of microorganisms in situ can be achieved only by immunohistochemical or peptide nuclear agglutination techniques. Although antibodies to a wide variety of microbial agents have been developed, many are not commercially or widely available. In difficult cases, referrals to regional reference laboratories or to the Pathology Branch of the Centers for Disease Control and Prevention in Atlanta, Georgia, can help to establish the presence of an unusual pathogen.

Complications of Infective Endocarditis

Congestive heart failure is a major complication of IE. There are multiple pathways that lead to heart failure in IE, including valvular insufficiency, valvular stenosis, rupture of an infected fistulous tract, and conduction system abnormalities.

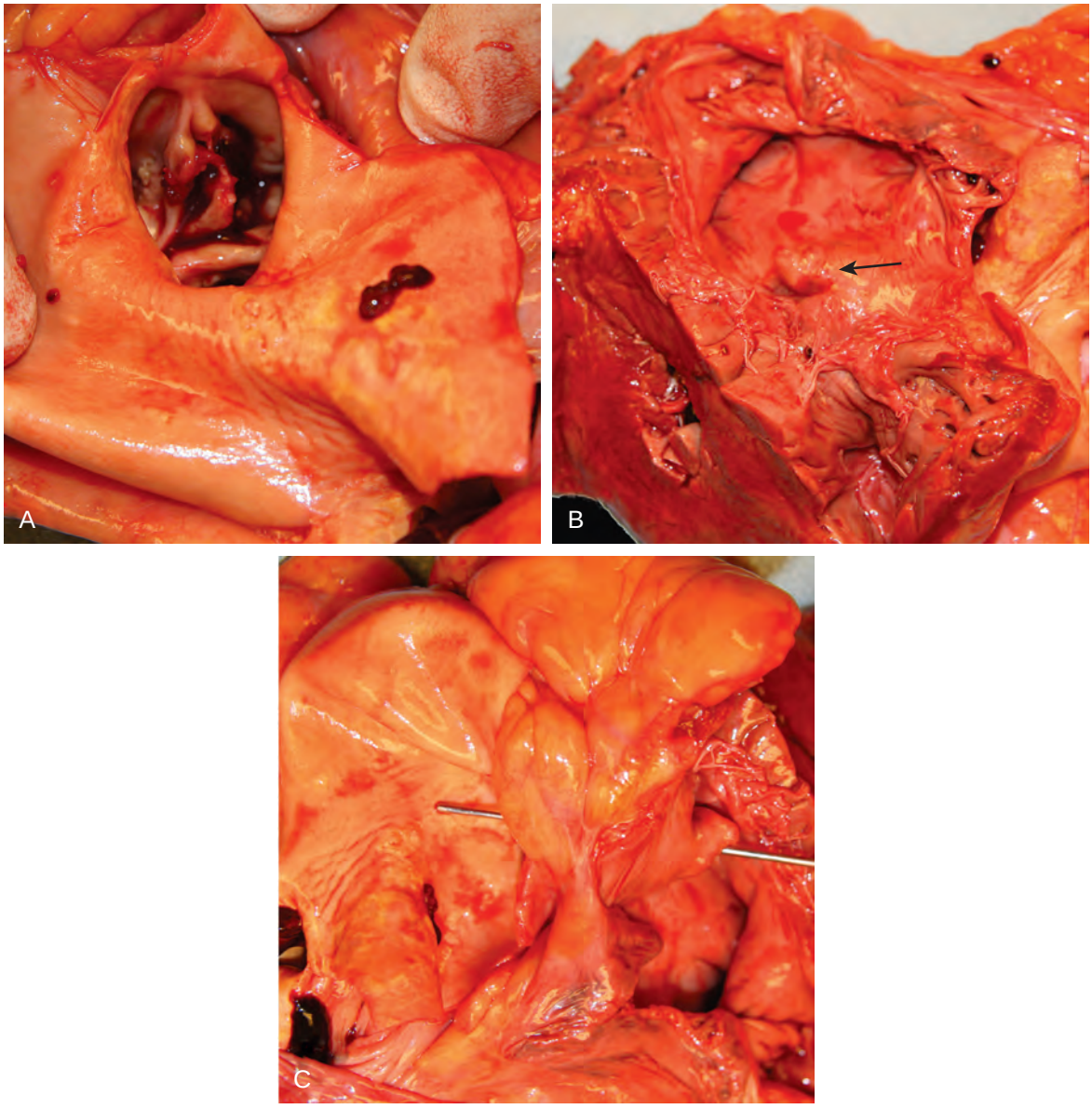
Valvular insufficiency is the most common complication of IE. It reflects either destruction of the valve by tears or penetrations or loss of structural support by tethering of the chordae or the valve ring. In mechanical prosthetic valves, direct involvement of Silastic balls or metal discs and rings is unusual, but paravalvular leaks secondary to dehiscence of the sewing ring are common (Fig. 9.11). Vegetations may interfere with the mechanics of the valve during the cardiac cycle, leading to both stenosis and regurgitation.

Abscesses of the mitral valve can form fistulous tracts between the left ventricle and right side of the heart (Fig. 9.12). Abscesses of the aortic sinuses of Valsalva can lead to fistulous tracts that communicate with the right atrium or right ventricle, leading to acute right heart failure due to massive left-to-right shunting (Fig. 9.13). Periaortic valvular abscesses can extend directly into the adjacent atrioventricular node, causing conduction abnormalities of the atrioventricular node and fascicular block (Fig. 9.14).



• **Figure 9.12** Infective Endocarditis of a Bioprosthetic Valve. An aortic bioprosthesis shows vegetations bridging the cusps *(small arrow)*, as well as involvement of the sewing ring that led to paravalvular insufficiency *(long arrow)*.

Embolic Complications. Embolic complications are common in IE. Involvement of the tricuspid valve by virulent organisms, such as *S. aureus* or *Candida* spp., is associated with bulky vegetations that fragment and travel to the lungs. Because vegetations are composed of both infected and bland fibrin–platelet excrescences, the result is potentially a mixture of bland pulmonary infarctions and septic pulmonary abscesses. Microthrombi can

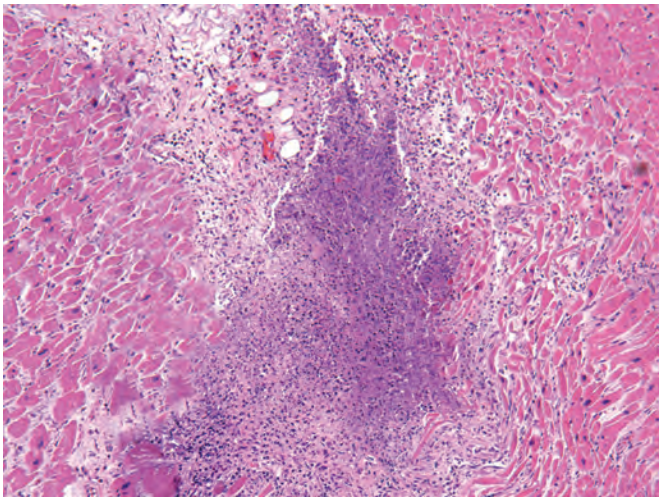


• **Figure 9.13** Rupture of Sinus of Valsalva Aneurysm (Posterior Noncoronary Cusp) in a Patient With Methicillin-Resistant *Staphylococcus aureus*. **A,** Aortic valve endocarditis led to an aneurysm of the noncoronary sinus of Valsalva, which ruptured acutely into the right atrium. **B,** The area of rupture appears as a small nubbin on the right atrial surface *(arrow)*. **C,** The path of the fistulous tract is demonstrated by a probe.

also travel through a patent foramen ovale to produce embolic complications on the left side of the circulation (Fig. 9.15).

Emboli on the left side can affect virtually any organ, but cerebral emboli are of greatest clinical concern (Fig. 9.16). Approximately 20% of patients with IE develop cerebral emboli, with an associated mortality rate of approximately 40%.[11] Hemorrhages and cotton-wool Roth spots reflect septic emboli to the microcirculation of the retina. Splenic emboli (Fig. 9.17) can cause flank pain or diaphragmatic irritation but may also be symptomatically silent. Renal microemboli produce a classic flea-bitten appearance of the cortex with focal segmental necrosis of the glomerular tuft. Janeway lesions are caused by microemboli to skin, whereas Osler nodes are caused by arteriolar injury due to immune complex deposition. Septic emboli to the skin must be distinguished from other immune-mediated dermatoses, such as Sweet syndrome (Fig. 9.18). Coronary artery emboli are uncommon and are most often a complication of prosthetic aortic valve endocarditis (Fig. 9.19). Microemboli to the myocardium can produce patchy areas of necrosis (Bracht-Wächter bodies), leading to myocardial dysfunction and congestive heart failure.

Immune Complex Disease. Circulating immune complexes are detected in the vast majority of patients with IE. Rheumatoid factor can be demonstrated in 50% of patients with endocarditis after 6 weeks of infection.[33] These circulating immune complexes include immunoglobulin, complement, and bacterial antigens.[34] Immune complexes cause arthritis, subungual splinter hemorrhages, and skin lesions.[35] In addition, the deposition of immune

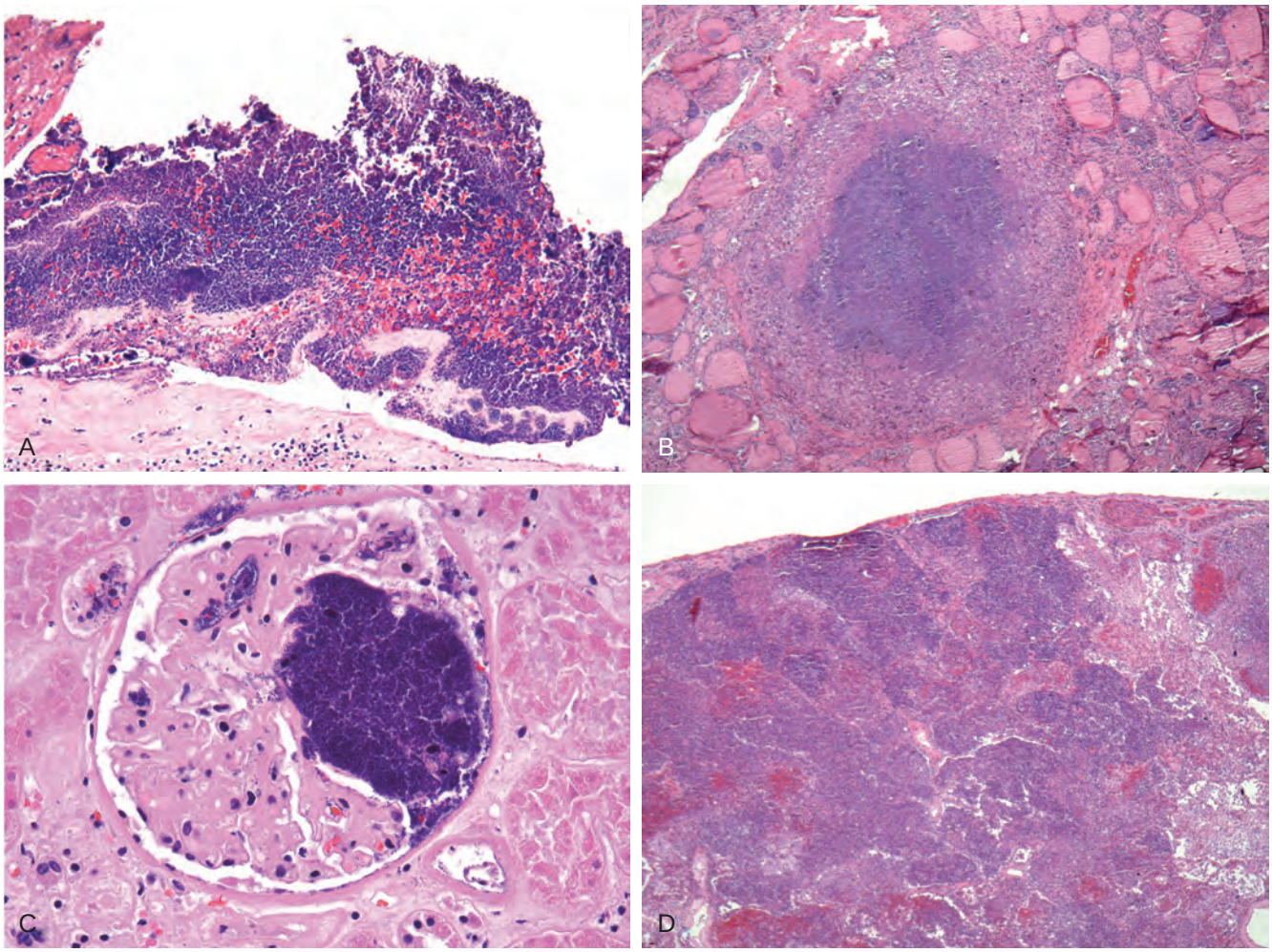


• **Figure 9.14** Myocardial Abscess. Aortic valve endocarditis due to *S. pneumoniae* extended into the adjacent myocardium, leading to complete blockade of the atrioventricular node. Disrupted myocardial muscle cells surround a central abscess (×150).

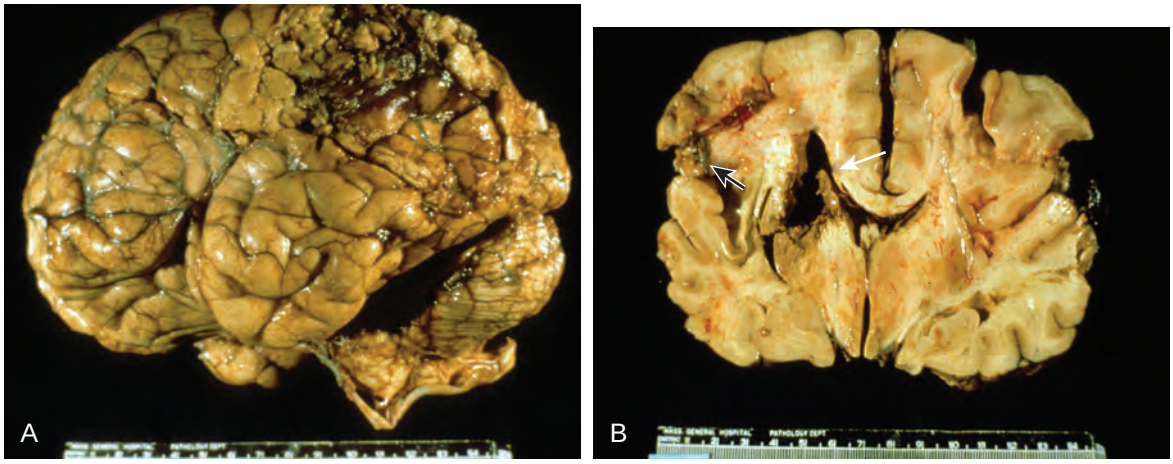
complexes leads to proliferative glomerulonephritis (Fig. 9.20) and renal failure. Mycotic aneurysms develop late in the course of IE and most likely reflect a combination of infective emboli and immune complex deposition that predisposes to aneurysmal dilatation and rupture of the affected vessel.

Lesions That Mimic Infective Endocarditis

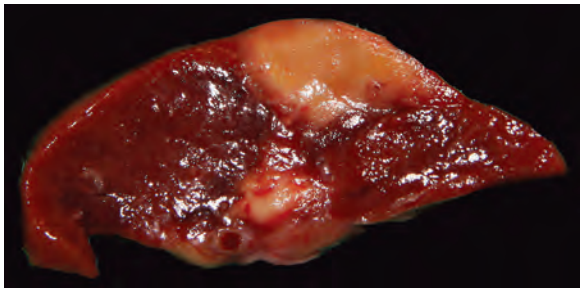
Other disorders can mimic both the clinical and the pathologic findings of IE. Noninfective (bacterial) thrombotic endocarditis (NBTE) is caused by the presence of bland fibrin thrombi localized along the aortic and mitral valves. As opposed to the vegetations of IE that tend to involve any aspect of the valve cusp or ring, the lesions of NBTE are limited to the valve's lines of closure. On average, the vegetations of IE are larger than those seen in NBTE, but these conditions cannot be distinguished based on size alone. NBTE represents a pathologic syndrome that is associated with carcinomatosis due to mucin-secreting malignancies, chronic infection, or the late stages of other wasting diseases (marantic endocarditis). The pathogenesis appears to reflect a hypercoagulable state in the absence of bacteremia.[36] The embolic



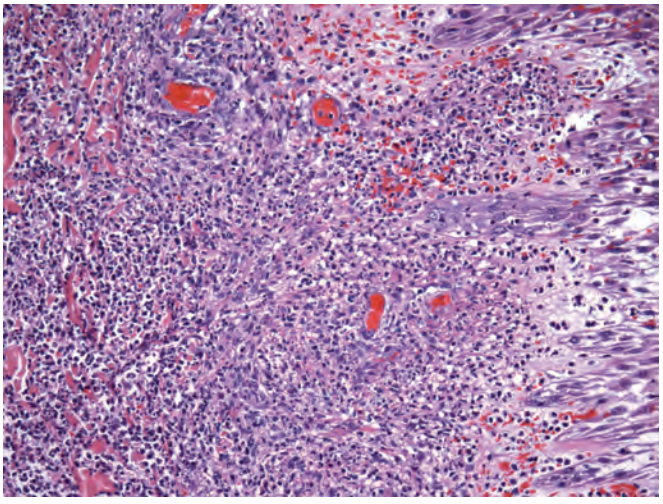
• **Figure 9.15** Tricuspid Endocarditis in a Patient With a Patent Foramen Ovale. An intravenous drug user with human immunodeficiency virus type 1 infection developed *S. aureus* endocarditis of the tricuspid valve (×250) **(A)**. At autopsy, infected lesions were found in thyroid (×200) **(B)**, kidney (×250) **(C)**, and lung (×150) **(D)**.



• **Figure 9.16** Bilateral Acute Cerebral Embolic Infarctions. A patient with *Staphylococcus aureus* aortic valve endocarditis developed acute hemiplegia and then rapidly lapsed into coma and died. **A,** The left cerebral hemisphere shows hemorrhagic infarction of the parietal, temporal, and occipital lobes. **B,** Coronal section shows hemorrhage in the left hemisphere *(black arrow)* with distortion of the lateral ventricle *(white arrow)* and diencephalic displacement.



• **Figure 9.17** Splenic Infarction. A pale subcapsular splenic infarction in a patient with *S. viridans* mitral endocarditis.



• **Figure 9.18** Neutrophilic Dermatosis in a Patient With Aortic Endocarditis. The patient was treated for *S. aureus* aortic valve endocarditis for several weeks with clearance of bacteria from the blood and decreasing size of valvular vegetations by echocardiography. Subsequently, raised purpuric lesions developed on the extremities, with hematuria and increased serum creatinine. Skin biopsy shows a neutrophilic infiltrate with no organisms, consistent with Sweet syndrome. A kidney biopsy showed immunoglobulin A nephropathy attributed to antibiotics (not shown) (×250).

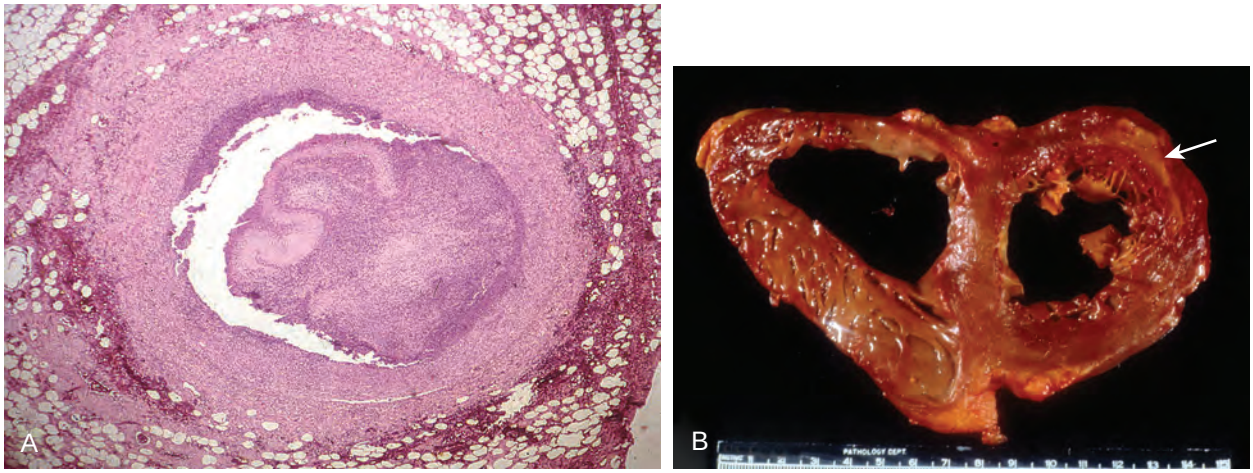
complications of NBTE are comparable to those seen in IE (Fig. 9.21).

Other disorders, including rheumatic fever, Libman-Sacks endocarditis, and carcinoid syndrome can produce lesions on the cardiac valves that mimic IE. Libman-Sacks endocarditis affects the mitral valve and is seen in 50% of fatal cases of systemic lupus erythematosus (SLE). The vegetations are flat, cover both surfaces of the valve cusp, and often extend to the adjacent atrial and ventricular myocardium. Histologically the valve shows fibrinoid necrosis (Fig. 9.22). The pulmonic and tricuspid valves may scar in response to a serotonin-secreting peripheral carcinoid. In the carcinoid syndrome, the pulmonic valve is either stenotic or incompetent, whereas insufficiency dominates with respect to the tricuspid valve. These findings are easily distinguished from vegetations but can be difficult to distinguish from previously treated IE.

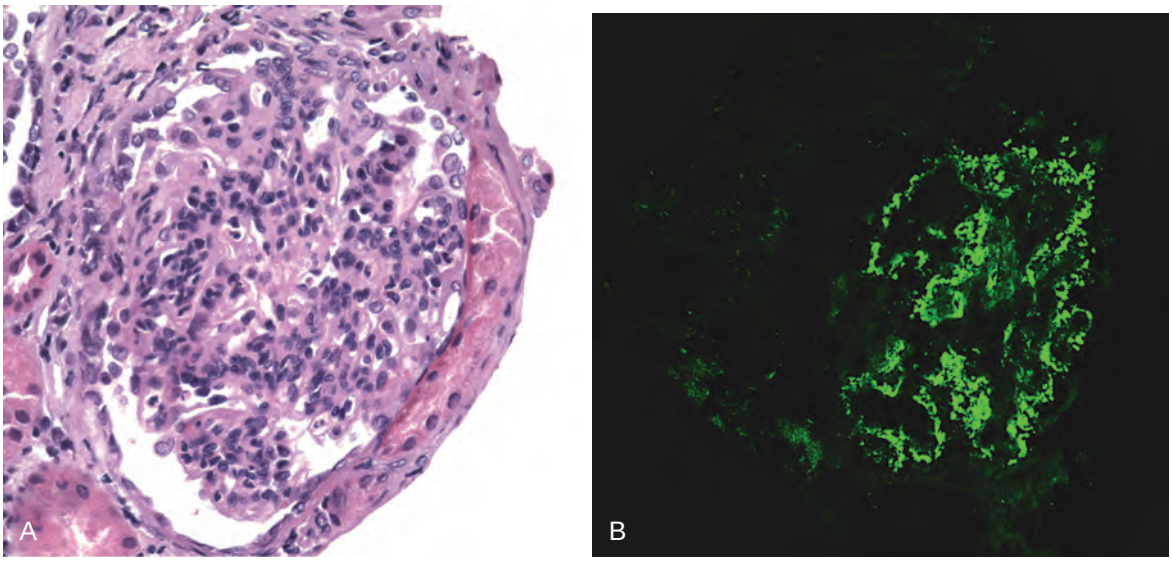
Infectious Aneurysms

IE can complicate atherosclerotic aneurysms, and the outcome in the absence of aggressive medical and surgical intervention is invariably fatal. Infrarenal abdominal aortic aneurysms are most often affected, but any site is susceptible. Patients can present with the signs and symptoms of IE, or the disease can be clinically silent. Pain in the region of the aneurysm is an ominous sign because it often heralds imminent rupture. Efforts should be made to sterilize the lesion with antibiotics, but the definitive treatment is surgical excision and must not be unduly delayed.

Pathologically the lesions show infection at the base of a mural thrombus, with bacteria and acute inflammation that extends through the wall of the aneurysm (Fig. 9.23A). *Salmonella typhimurium* has an increased propensity to infect atherosclerotic plaques and produces a characteristic lesion with aggregates of histiocytes that may include bacilli, best demonstrated by silver impregnation (see Fig. 9.23B and C). Pseudoaneurysms may form in response to blood leakage from the vessel wall. Involvement of vascular grafts by IE mimics the features of prosthetic devices, with infection primarily targeting the areas of graft anastomoses. Pseudoaneurysm and graft rupture are dreaded complications. Septic emboli may lodge at sites distal to the graft, leading to infarction and abscess formation.



• **Figure 9.19** Coronary Artery Embolus Resulting From Vegetation of Aortic Valve. **A,** An infected embolus is seen in the circumflex artery. **B,** The heart at autopsy shows an acute myocardial infarction of the lateral wall of the left ventricle *(arrow)*, caused by the embolus.



• **Figure 9.20** Immune Complex Glomerulonephritis. A patient with *Streptococcus viridans* mitral endocarditis developed acute renal failure (×150). **A,** Kidney biopsy showed diffuse proliferative glomerulonephritis (×250). Direct immunofluorescence revealed granular deposition of immunoglobulin G **(B)** and complement C3 (not shown) (×250).

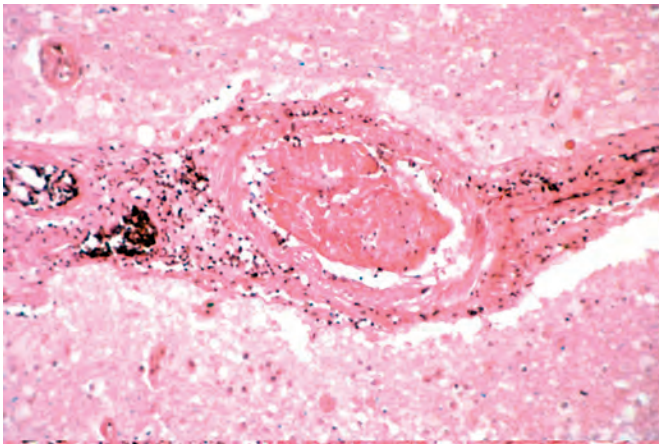
Treponema pallidum is the cause of syphilitic aortitis, which accounted for 1% of deaths in the United States between 1950 and 1965. Late syphilitic infections (tertiary syphilis) tend to involve the aortic valve and its ostia, as well as the ascending and transverse thoracic aortic arch. This results in aortic regurgitation and, rarely, aortic rupture.

Infection results from a characteristic spirochetal lymphoplasmacytic endarteritis that targets the vasa vasorum, yielding a thickened aortic wall with a gross "tree bark" appearance. Microgummas with focal areas of acute necrosis can include viable spirochetes, best demonstrated with the Warthin-Starry silver impregnation stain. Clinically the diagnosis is most often established by positive nontreponemal (Venereal Disease Research Laboratory [VDRL] test) or treponemal (fluorescent treponemal antibody [FTA] test) serology results; however, older patients may lack positive results on serologic studies.

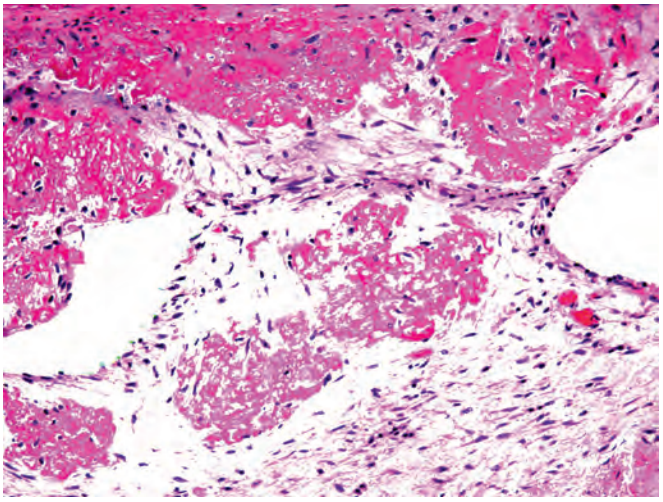
Other infectious causes of aortitis are rare. Tuberculosis can involve both the thoracic and abdominal aorta and usually results from contiguous spread of infection from the lung, a periaortic lymph node, or tuberculous osteomyelitis (Pott disease). The pathology is that of necrotizing granulomatous inflammation and must be distinguished from other noninfective causes of granulomatous aortitis, including giant cell aortitis, sarcoidosis, and Wegener granulomatosis.

Pathology of Myocarditis

Myocarditis can be simply defined as inflammation involving the myocardium. It is not surprising that there are numerous causes of myocarditis, both infectious and noninfectious. Although the definition seems rather clear and simple, the incidence, diagnostic criteria, pathogenesis, and treatment of myocarditis continue to



• **Figure 9.21** Cerebral embolus caused by noninfective thrombotic endocarditis of aortic valve. A patient with advanced carcinoma of the colon developed a lethal cerebral infarction. At autopsy, noninfective vegetations were seen along the lines of closure of the aortic valve, and a bland embolus was seen in the left carotid artery (×250).



• **Figure 9.22** Libman-Sacks Endocarditis. A 27-year-old woman died during an acute exacerbation of systemic lupus erythematosus. At autopsy, the mitral valve and surrounding endocardium showed small vegetations. Histologic examination showed the fibrinoid necrosis of the valve cusp that is characteristic of Libman-Sacks endocarditis (×250).

be debated. The clinical presentation of myocarditis is quite variable, ranging from nonspecific systemic symptoms to arrhythmias, restrictive cardiac physiology, and severe cardiac failure and sudden death, making a clear-cut clinical diagnosis difficult.[37]

The introduction of the endomyocardial biopsy should have made definitive pathologic diagnosis possible and reliable, but this promise was never realized because of a combination of sampling error and lack of agreement on diagnostic criteria (see later discussion). The variability of the clinical presentation, coupled with the difficulty of making the diagnosis, make the determination of the true incidence of myocarditis almost impossible. In selected populations studied prospectively, the incidence has been reported to be 8.6% to 12% in young adults with sudden death,[38,39] and it was identified as the cause of dilated cardiomyopathy in 9% to 16% of cases studied.[40-42] In an autopsy study of 12,000 people who died as a result of violence or accidents, the incidence of myocarditis was approximately 1%.[43] The true incidence in the general population is not known, but cardiac symptoms are frequently associated with viral infections; whether they represent evidence of myocardial inflammation is a matter of conjecture. With the use of molecular techniques, viral genomes have been identified in patients with left ventricular dysfunction[44,45] or other cardiac conditions,[46] lending further credence to the assumption that many cases of myocarditis are subclinical.

TABLE 9.1 Organisms That Cause Infective Endocarditis

Organism	% of Cases
Streptococci*	6-80
Enterococci	5-18
Staphylococci†	20-35
Gram-negative bacilli	2-13
Fungi	2-4
Culture negative	5-24

*Includes *S. viridans* and other *Streptococcus* spp.
†Includes coagulase-positive and coagulase-negative organisms.
From Fowler VG, Scheld WM, Bayer AS. Endocarditis and intravascular infections. In: Mandell GL, Bennett GE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 6th ed. Philadelphia: Elsevier; 2005.

• BOX 9.3 Etiologic Categories of Myocarditis

Infectious
Viral
Bacterial (including *Rickettsia* and *Chlamydia*)
Protozoal
Fungal
Parasitic

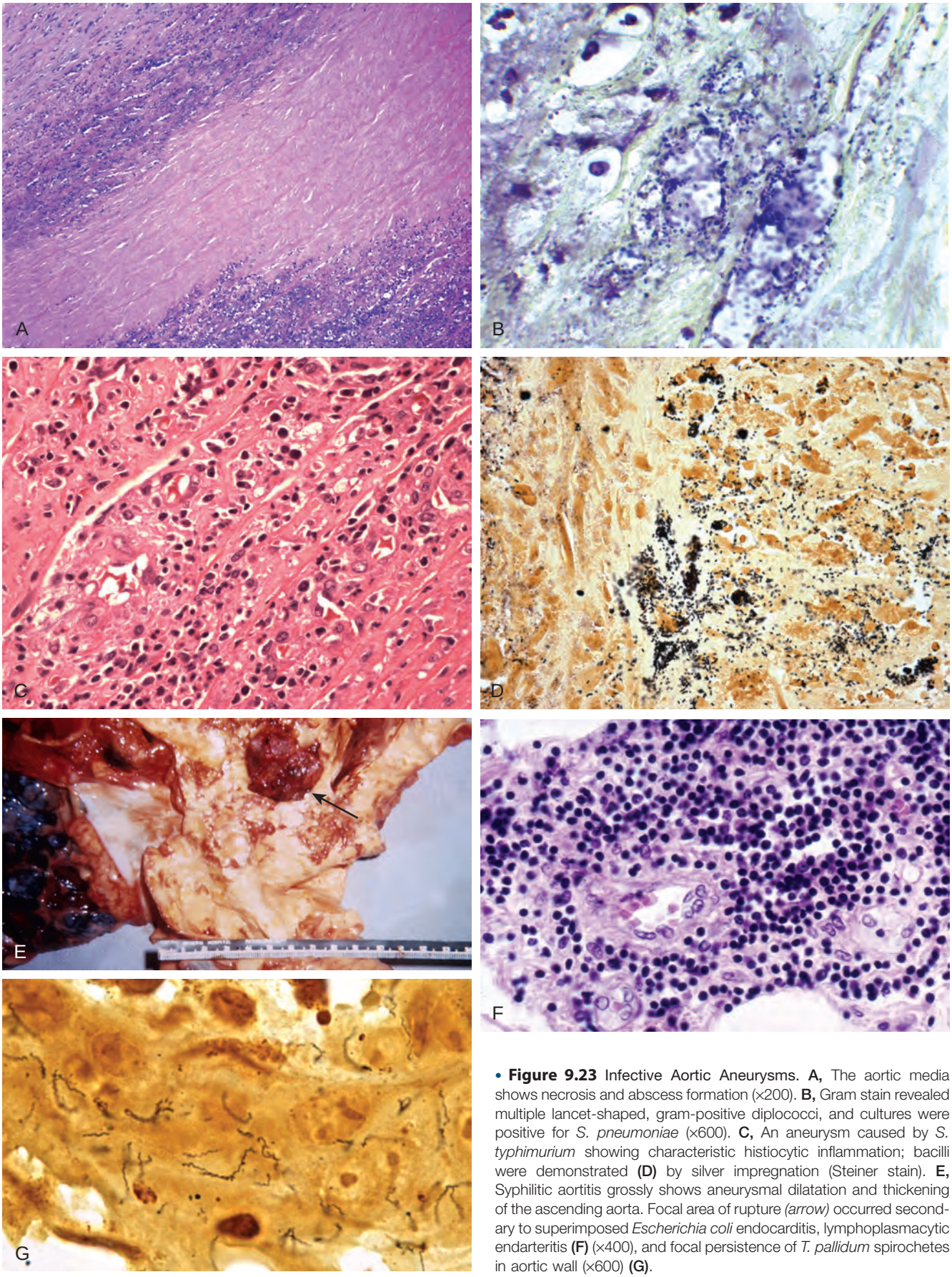
Noninfectious
Idiopathic
Giant cell myocarditis
Necrotizing eosinophilic myocarditis
Drug-induced myocarditis
Myocarditis associated with systemic disorders

Etiology of Myocarditis

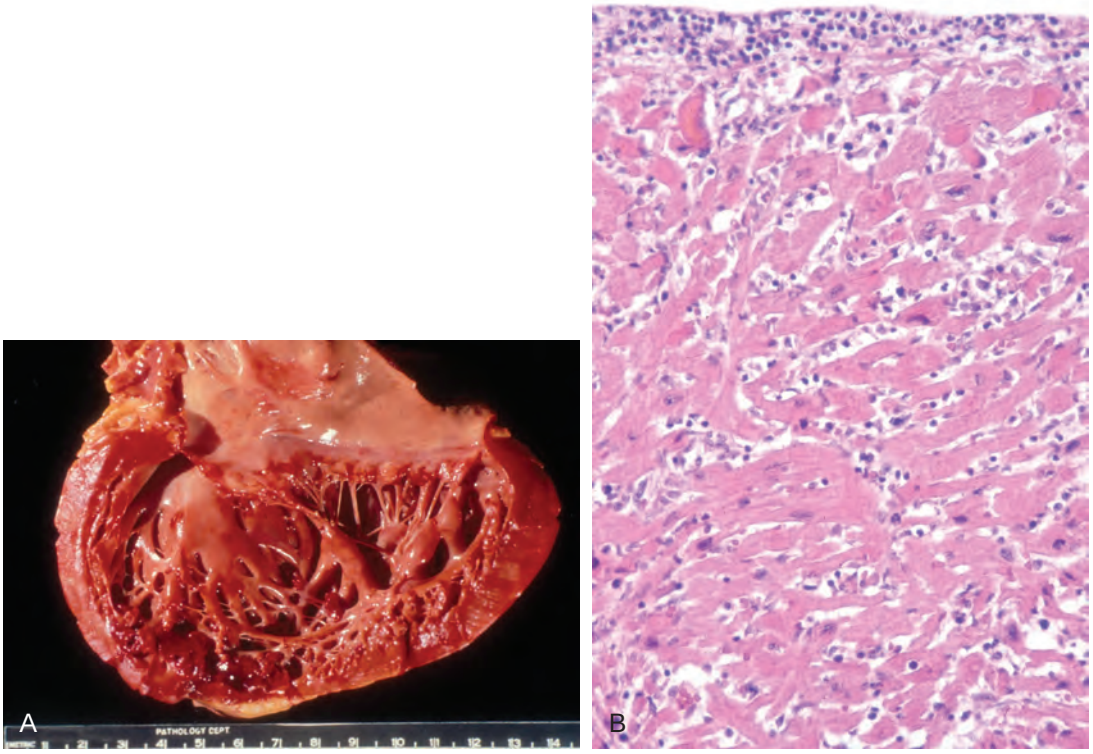
Table 9.1 and Box 9.3 summarize the major causes of myocarditis. The following sections discuss the infectious causes in greater detail but also touch on the noninfectious ones, especially as they need to be considered in the differential diagnosis of infectious myocarditis. Before proceeding to the pathology of the specific diseases, it is necessary to discuss general criteria for the diagnosis of myocarditis because they remain a source of controversy.

Diagnosis of Myocarditis

Until the advent of the endomyocardial bioptome, myocarditis could be diagnosed with certainty only at autopsy. The gross appearance can be quite variable, including completely



• **Figure 9.23** Infective Aortic Aneurysms. **A,** The aortic media shows necrosis and abscess formation (×200). **B,** Gram stain revealed multiple lancet-shaped, gram-positive diplococci, and cultures were positive for *S. pneumoniae* (×600). **C,** An aneurysm caused by *S. typhimurium* showing characteristic histiocytic inflammation; bacilli were demonstrated **(D)** by silver impregnation (Steiner stain). **E,** Syphilitic aortitis grossly shows aneurysmal dilatation and thickening of the ascending aorta. Focal area of rupture *(arrow)* occurred secondary to superimposed *Escherichia coli* endocarditis, lymphoplasmacytic endarteritis **(F)** (×400), and focal persistence of *T. pallidum* spirochetes in aortic wall (×600) **(G)**.



• **Figure 9.24** Lymphocytic (Viral) Myocarditis. This 11-year-old boy died suddenly at home while at rest after having developed a viral syndrome 2 weeks earlier. The heart **(A)** was dilated and contained interventricular thrombi in both ventricles. Microscopic examination **(B)** revealed a diffuse lymphocytic myocarditis. The photomicrograph shows the prominent endocardial involvement typical of myocarditis, allowing it to be distinguished from ischemic injury, in which the endocardium does not show an inflammatory infiltrate (×250).

normal-appearing heart tissue; the presence of fibrinous pericarditis as part of a myopericarditis syndrome; an edematous and "stiff" myocardium; and a "flabby," dilated heart with thin chamber walls and mural thrombi (Fig. 9.24).

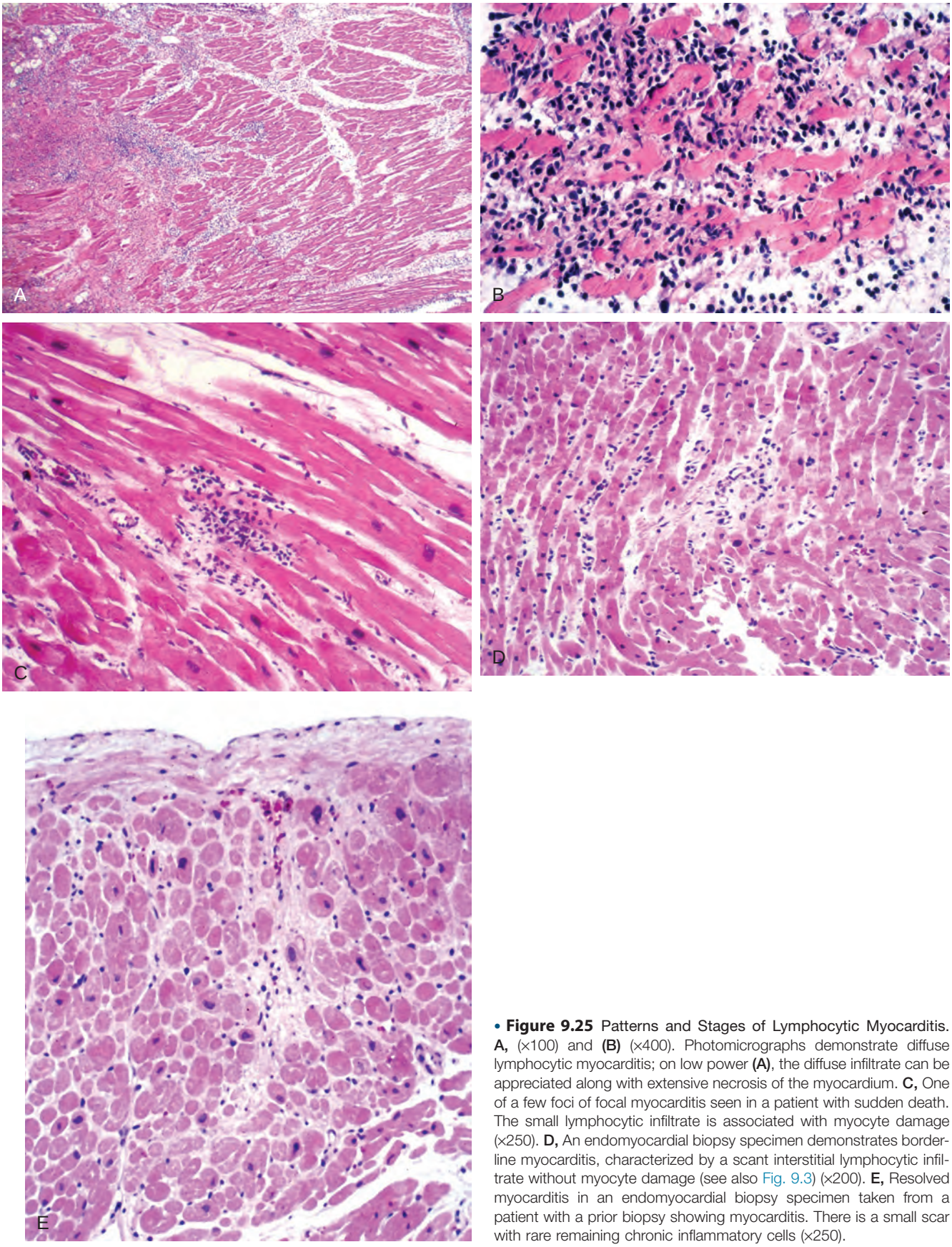
The microscopic criteria for diagnosis of myocarditis in endomyocardial biopsies were established in 1986 at a cardiology meeting in Dallas, and they have been known since then as the Dallas criteria.[47,48] The Dallas criteria essentially divided myocarditis into two forms. An "active" form is characterized by an inflammatory infiltrate associated with myocyte damage not characteristic of ischemic injury. In the absence of myocyte injury, the diagnosis of "borderline" myocarditis should be made. The classification further suggested that pathologists semiquantify the inflammatory infiltrate (e.g., diffuse, multifocal, focal), describe the nature of the inflammatory infiltrate (e.g., lymphocytic, giant cell, eosinophilic, mixed), and remark on the absence or presence of fibrosis and its location (e.g., perivascular, interstitial, replacement).

Fig. 9.25 illustrates examples of myocarditis in endomyocardial biopsy specimens. If repeat biopsies were performed, the Dallas classification called for the designation of the myocarditis as ongoing (persistent), resolving (healing), or resolved (healed). The classification served its purpose, to provide diagnostic consensus for a large multicenter myocarditis treatment trial,[49] but it has since been challenged by many authors as not being accurate enough based on sampling error,[50] interobserver variability,[51] or insufficient diagnostic criteria.[52,53] Suffice it to say that none of the proposed alternative classifications are in use, but molecular[46] and immunohistochemical techniques (Fig. 9.26) have clearly shown that the Dallas criteria may indeed underestimate the presence of myocarditis. Viral genome can be present in myocardial tissue in the absence of histopathologic myocarditis in accordance with the Dallas criteria.

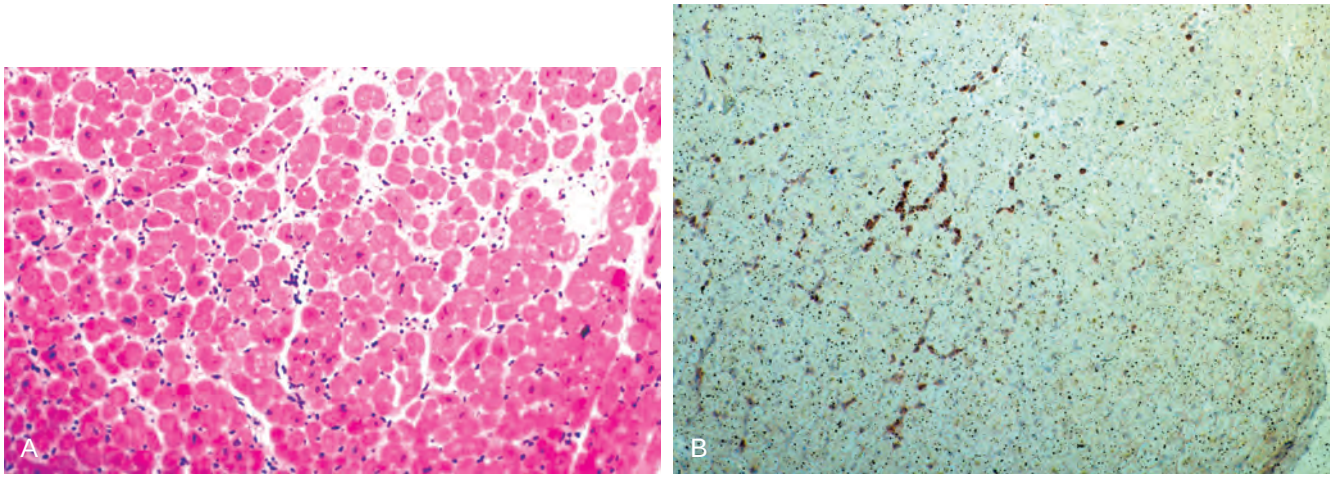
The recommended four to six biopsy specimens result in an unacceptable level of sampling error with standard histopathologic approaches[50]; probes directed at more generalized indicators of inflammatory myocardial disease have therefore been used in the diagnosis of myocarditis. Immunologic markers for the induction of intercellular adhesion molecule 1 (ICAM1) have been shown to be positive in endomyocardial biopsy specimens from patients with suspected myocarditis when no inflammatory infiltrates were found.[54] However, such findings are not accepted as standard diagnostic criteria, despite the declaration of the demise of the Dallas criteria by some.[55]

The inflammatory infiltrate itself can be quite variable. In one single-institution study of 112 consecutive myocardial biopsies, lymphocytic myocarditis was diagnosed in 55% of the cases, granulomatous myocarditis in 10%, giant cell myocarditis in 6%, and eosinophilic myocarditis in 6%, whereas 22% of the biopsies showed borderline myocarditis.[56] Although the correlation is not perfect, the nature of the infiltrate can serve as a general guide to the cause of myocarditis.[57] Although all forms of myocarditis can be idiopathic,

- Lymphocytic infiltrates suggest viral, rickettsial, spirochetal, toxic, or immunologic causes;
- Neutrophils are more often seen in myocardial injury associated with ischemia, bacteria, catecholamine toxicity, or the early stages of viral infections;



• **Figure 9.25** Patterns and Stages of Lymphocytic Myocarditis. **A,** (×100) and **(B)** (×400). Photomicrographs demonstrate diffuse lymphocytic myocarditis; on low power **(A)**, the diffuse infiltrate can be appreciated along with extensive necrosis of the myocardium. **C,** One of a few foci of focal myocarditis seen in a patient with sudden death. The small lymphocytic infiltrate is associated with myocyte damage (×250). **D,** An endomyocardial biopsy specimen demonstrates borderline myocarditis, characterized by a scant interstitial lymphocytic infiltrate without myocyte damage (see also Fig. 9.3) (×200). **E,** Resolved myocarditis in an endomyocardial biopsy specimen taken from a patient with a prior biopsy showing myocarditis. There is a small scar with rare remaining chronic inflammatory cells (×250).



• **Figure 9.26** Borderline Myocarditis. These photomicrographs show sections from the same endomyocardial biopsy specimen demonstrating borderline myocarditis (lymphocytic infiltrate without myocyte damage). Whereas the infiltrate can be appreciated in the hematoxylin and eosin–stained section (×100) **(A)**, the section stained by the immunoperoxidase method using a reagent against CD3-positive cells **(B)** shows this infiltrate much more clearly (×100).

- Eosinophils are most frequently associated with eosinophilic myocarditis, hypersensitivity reactions, parasitic diseases, and general states of hypereosinophilia;
- Giant cells are seen in giant cell myocarditis, sarcoidosis, hypersensitivity myocarditis, rheumatic fever, and rheumatoid diseases.

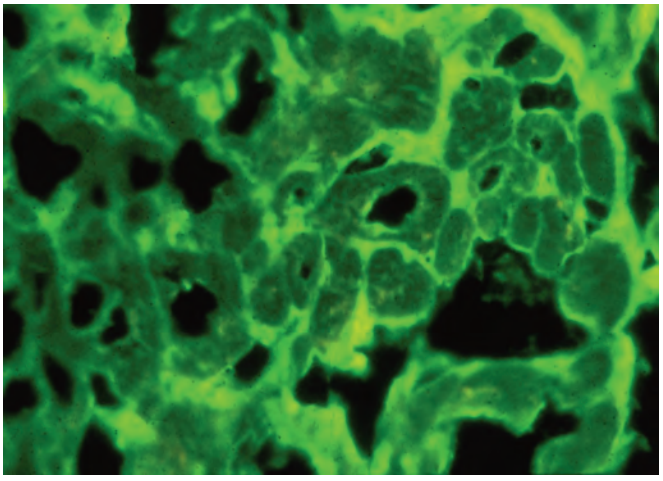
The following is a description of the pathologic findings in the various causes of myocarditis, along with short discussions of the pathogenesis, where appropriate.

Viral Myocarditis

Virtually all classes of microorganisms have been shown to cause myocarditis.[58] Most of what we know about viral myocarditis comes from numerous experimental studies, the detailed discussion of which is beyond the scope of this chapter. Viruses that have been implicated in myocarditis include adenovirus, enteroviruses (e.g., Coxsackie B virus), hepatitis C virus, cytomegalovirus, varicella, and HIV. Although varicella and cytomegalovirus infections can show the characteristic cytopathic effects, the inflammatory infiltrate in viral myocarditis is typically lymphocytic. On the other hand, with molecular techniques, a viral etiology cannot be demonstrated in most cases of lymphocytic myocarditis.[45]

Pathogenesis of Viral Myocarditis

Studies of the pathogenesis of viral myocarditis are ongoing, but experimental studies have shown several mechanisms ranging from direct cell damage to immunologically mediated effects. Numerous experimental studies have identified the role of CD4- and CD8-positive T cells, B cells, and cytokines (interleukin-1, interleukin-2, tumor necrosis factor, and interferon-γ) in initiation and continuation of the process of myocarditis. Studies have also shown the activation of inducible nitric oxide synthetase, which has mitigating effects in experimental viral myocarditis.[59] Summarizing these studies, there are at least three stages and five mechanisms that can explain myocardial damage and inflammation in viral myocarditis[37,60]:



• **Figure 9.27** Anticardiac Antibodies. In a frozen section of human myocardium stained by indirect immunofluorescence using the serum of a patient with biopsy-proved myocarditis, the presence of antibodies against sarcolemmal constituents is shown (×250).

1. Direct invasion of myocytes by cardiotropic viruses, such as Coxsackie B virus, leading to myocyte damage and dysfunction and persistence of the viral genome in the myocardium.
2. Cellular immune response directed at virus-associated antigens on the cell surface, resulting in a cellular infiltrate that includes natural killer cells and macrophages, followed by cytokine activation. $CD8^+$ cells directed at virus-associated antigens on the myocyte surface are an additional constituent of the inflammatory infiltrate. Subsequent activation of $CD4^+$ cells initiates the clonal expansion of B cells, leading to further cell damage, increased inflammation, and the production of anticardiac antibodies.
3. Autoimmunity (circulating antibodies and/or cellular mechanisms) directed at myocardial constituents has been shown to be present in many animal models and in human studies (Fig. 9.27).[61] The circulating antibodies that have been found are

directed against myocyte constituents responsible for signal transduction, ion transport (calcium), and energy metabolism. The autoimmune mechanisms can be explained by three possible mechanisms[60]:

a. Presence of self-antigens on infected cells
b. Molecular mimicry (i.e., presence of self-antigens that cross-react with antigens on infected or noninfected cells)
c. Epitope spreading (i.e., the expression of self-antigens in response to the infection on noninfected cells)

All those mechanisms could be present in one host, and they could in theory result in the development of chronic myocarditis. However, only mechanisms 1 through 3a require the persistence of virus in the cell; mechanisms 3b and 3c are independent of the presence of virus. These studies may explain why viral genome cannot be demonstrated in many cases of presumed viral myocarditis, and they may also explain why the response to viral and immunosuppressive treatments has been difficult to predict. Several studies have shown[62-64] that patients with anticardiac antibodies responded better to immunosuppressive treatment than those without antibodies, whereas patients with demonstrable viral genetic materials responded worse than those without.

Bacterial Myocarditis

The majority of direct infections of the myocardium by bacteria occur in the setting of sepsis or septic emboli secondary to bacterial endocarditis. A common form of myocarditis caused by a bacterium is acute rheumatic fever, which represents an immunologic response to an infection by *Streptococcus pyogenes*, rather than direct infection of the myocardium. Although rheumatic fever declined significantly in the United States before 1977, there has been an increase in the incidence of the disease since then. Grossly the presence of pericarditis and fibrinous vegetations on the line of closure of the valves may point one to the pancarditis of acute rheumatic fever, but the myocardium itself shows no gross features distinct from other forms of myocarditis. Microscopically there is a multifocal granulomatous interstitial inflammatory infiltrate consisting of lymphocytes and giant cells arranged in characteristic Aschoff bodies (Fig. 9.28). Rarely, this pathology can be seen in endomyocardial biopsy samples, but atrial appendages removed at cardiac surgery more frequently show these lesions. The pathogenetic mechanisms relate to the cross-reactivity (autoimmunity) with cardiac antigens, including myosin peptides, and the topic has been reviewed in detail in recent years.[65]

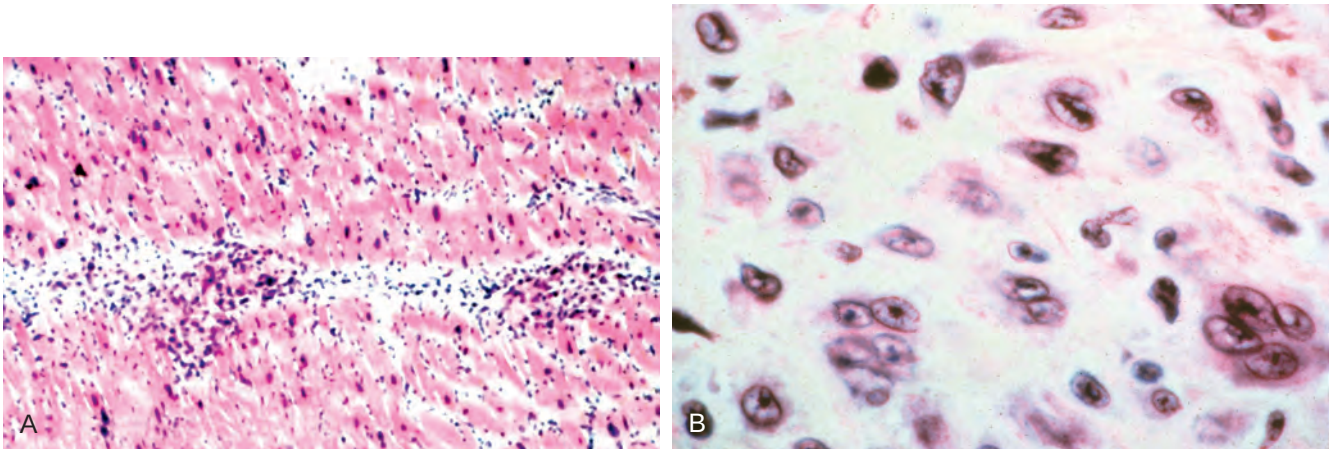
Borrelia burgdorferi, the agent responsible for Lyme disease in the United States, can cause a myocarditis characterized by a plasma cell infiltrate; the infectious agents can be demonstrated in the myocardium by silver stain.[66-68] Lymphocytic myocarditis is associated with rickettsial diseases, such as Rocky Mountain spotted fever,[69] and chlamydial diseases, such as psittacosis,[70] have also been associated with acute myocarditis. The production of bacterial toxins (e.g., in diphtheria) may lead to a toxic myocarditis.

Parasitic Infections

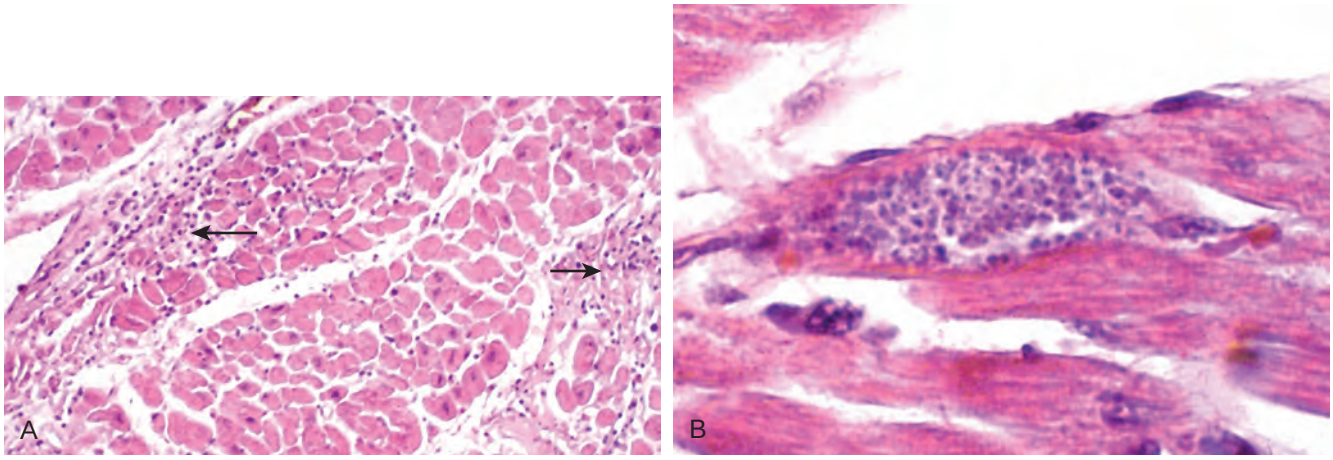
In endemic areas of the world, *Trypanosoma cruzi*, the etiologic agent of Chagas disease, is one of the major causes of cardiac dysfunction, and its true incidence in the United States may be underestimated.[71] Chagas disease is characterized grossly by dilated ventricles, sometimes associated with apical aneurysms, and, not surprisingly, intracavitary thrombi leading to embolic complications. Microscopically there is a diffuse lymphocytic myocarditis, associated with fibrosis and myocyte damage. In many patients the conduction system is involved. Intracellular parasites (Fig. 9.29) can be demonstrated in myocardial biopsy samples but are often absent.

The pathogenesis of Chagas disease, not unlike that of viral myocarditis, is quite complex. Although cellular destruction due to invasion by the parasite and the associated inflammation is clearly the first step in Chagas disease, occasionally leading to death during the acute phase, the development of the chronic form involves many more mechanisms,[72] including the following:

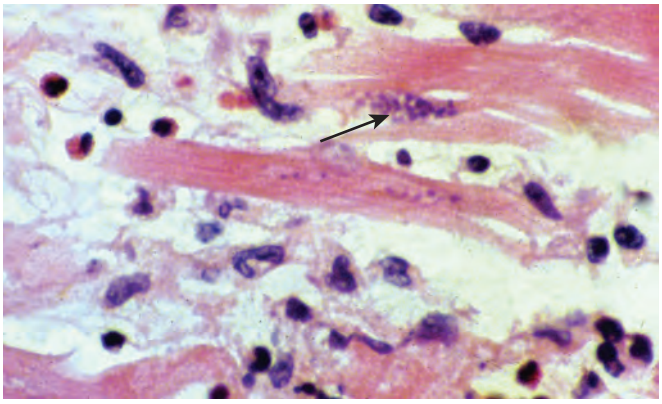
1. Myocytolysis caused by the parasite
2. Secretion of a toxin by the parasite
3. Microvascular damage induced by the parasite, which leads to small infarcts and myocyte damage
4. T-cell response to persisting parasite antigens, which results in delayed hypersensitivity reaction, causing further damage
5. Polyclonal B-cell activation, which leads to immunosuppression or autoimmune mechanisms. Autoantibodies against



• **Figure 9.28** Acute Rheumatic Fever. The low-power photomicrograph **(A)** (×200) shows an interstitial inflammatory infiltrate with two ill-formed granulomas typical of Aschoff bodies. On high power **(B)**, the typical Aschoff cells can be seen, characterized by ample basophilic cytoplasm and ragged cell borders with open, often multiple nuclei (×400).



• **Figure 9.29** Myocarditis of Chagas Disease. The low-power photomicrograph (×100) **(A)** shows multifocal myocarditis with areas of myocyte necrosis *(arrows)*. The intracellular *Trypanosoma cruzi* organisms can clearly be seen on high power **(B)**. Note that there is no inflammation surrounding the cell containing the organisms; the inflammatory infiltrate often does not correlate with foci of intracellular organisms (×400).



• **Figure 9.30** *Toxoplasma gondii* Myocarditis. The intracellular organisms *(arrow)* are present in this endomyocardial biopsy from an immunosuppressed patient. The inflammatory infiltrate shows a prominent eosinophilic component (×400).

myosin, actin, and laminin have been found in humans with Chagas disease.

As in viral myocarditis, a combination of these mechanisms may be active in myocardial damage and in the development of the chronic disease.

Other parasitic infections of the myocardium can be found in patients who are immunosuppressed, including those undergoing transplantation and patients with AIDS. *Toxoplasma gondii* is an intracellular organism (Fig. 9.30), whereas *Pneumocystis jiroveci* organisms are present in the interstitium and characterized by a typical foamy exudate.[73,74] In many parts of the world, parasitic infestations are common and cause a generalized hypereosinophilic state, which can cause eosinophil-associated cardiomyopathy.

Fungal Myocarditis

Fungal myocarditis is almost always secondary to septicemia and septic emboli, and *Aspergillus*, *Candida*, and *Cryptococcus* spp. (Fig. 9.31) have all been demonstrated in the myocardium by the usual staining techniques.

Noninfectious Causes of Myocarditis

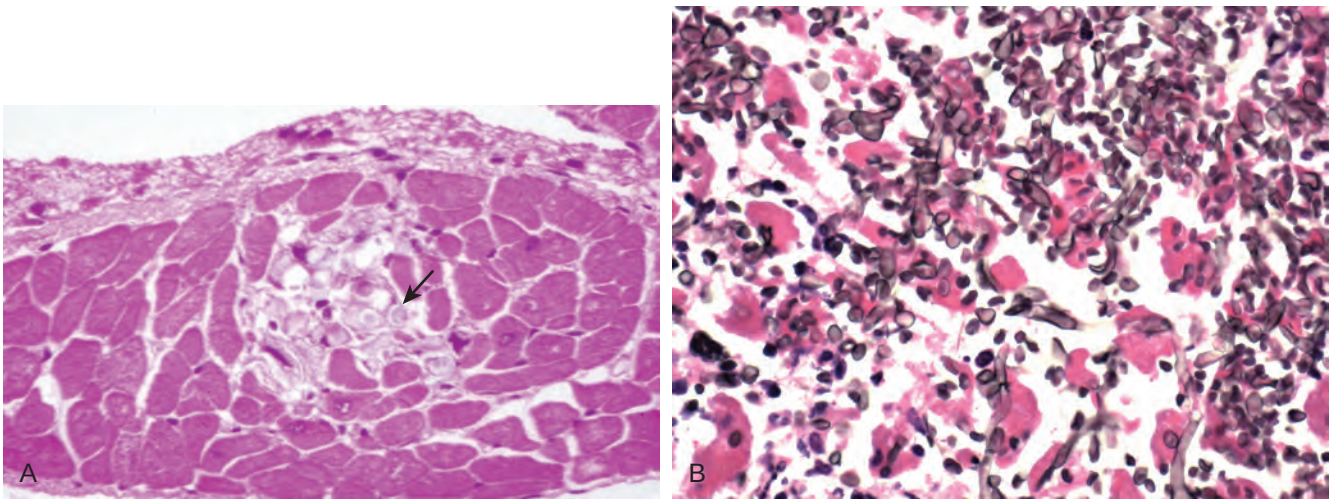
Idiopathic myocarditis is still the most common form of myocarditis, but there are other forms of noninfectious myocarditis that merit mention, including giant cell myocarditis, necrotizing eosinophilic myocarditis, drug-induced myocarditis, and myocarditis associated with systemic disorders.

Giant Cell Myocarditis

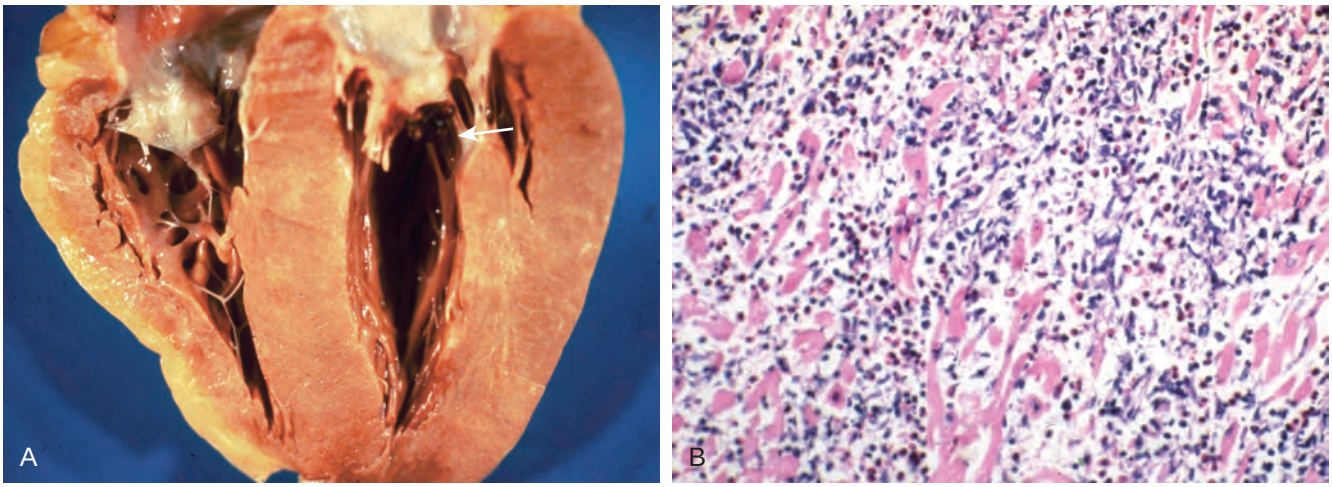
Giant cell myocarditis is a rare but fulminant and aggressive form of myocarditis, which occurs in young people and results in death or need for cardiac transplantation in 70% of cases.[75] Unlike in other forms of myocarditis, the diagnostic accuracy of endomyocardial biopsy in giant cell myocarditis is approximately 80%, presumably due to the extensive involvement of the heart. The infiltrate is composed of lymphocytes, eosinophils, and giant cells, and there is extensive myocyte destruction (see Fig. 9.34). It is most likely T cell mediated, and the lymphocytic infiltrate consists of mostly CD8-positive cells.[76,77] Studies have shown that immunosuppressive treatments aimed at T-cell function (e.g., muromonab-CD3, cyclosporine, steroids) prolong survival.[78] The disease recurs in transplanted donor hearts in about one quarter of the cases, but the 5-year survival rate is still approximately 70%.

Necrotizing Eosinophilic Myocarditis

Necrotizing eosinophilic myocarditis is another rare but aggressive, often lethal, form of acute myocarditis that frequently occurs in young adults. It is often preceded by a viral syndrome but may occur as an aggressive form of hypersensitivity myocarditis at any age (see later discussion). The presenting symptoms are nonspecific, and patients often die of sudden death or rapidly deteriorating cardiac function, but occasionally they present with cardiac tamponade.[79] Grossly the myocardium may be quite thickened due to extensive edema (Fig. 9.32A). Microscopically the myocardium is infiltrated extensively by a mixed inflammatory infiltrate with a prominent eosinophilic component, extensive myocyte necrosis, and interstitial edema (see Fig. 9.32B). If the condition is diagnosed in time by biopsy, successful treatment with steroids is possible.[80]



• **Figure 9.31** Fungal Myocarditis. **A,** Cryptococcal yeast forms are present *(arrow)* in histiocytes in myocardium of an immunosuppressed patient (×200). **B,** Gomori methenamine silver stain shows abundant yeast and pseudohyphae in a patient with *Candida tropicalis* fungemia (×400).



• **Figure 9.32** Necrotizing Eosinophilic Myocarditis. The gross photograph **(A)** shows the heart of a 26-year-old patient treated with antibiotics for endocarditis who died suddenly; residual vegetation is present on the mitral valve *(arrow)*. The ventricular walls were stiff and thickened, and on microscopic examination **(B)** there was a diffuse myocarditis characterized by a prominent eosinophilic component and extensive myocyte necrosis (×250).

Drug-Induced Myocarditis

Many drugs have been shown to cause various forms of myocarditis, and the list grows continuously. Many pathologic forms mimic infectious conditions, so a thorough drug history must be taken when determining the etiology of myocarditis. Box 9.4 summarizes the major cardiac effects, both inflammatory and noninflammatory, associated with drugs; a listing of the most common drugs can be found in recent publications.[37,57] The inflammatory forms are discussed in the following paragraphs.

Hypersensitivity Myocarditis. Many drugs have been implicated in the development of hypersensitivity myocarditis, the most common form of drug-induced myocarditis. It is characterized microscopically by a predominantly interstitial, mixed inflammatory infiltrate, with an extensive eosinophilic component, sometimes with giant cells and granulomas (Fig. 9.33). It was originally thought that myocyte necrosis needed to be absent to make the diagnosis of hypersensitivity myocarditis, but more

• BOX 9.4 Cardiac Effects Associated With Drugs

Inflammatory Forms

Inflammatory myocarditis
- Hypersensitivity myocarditis
- Toxic myocarditis

Vasculitis involving the myocardium
- Necrotizing
- Nonnecrotizing

Lupuslike reactions

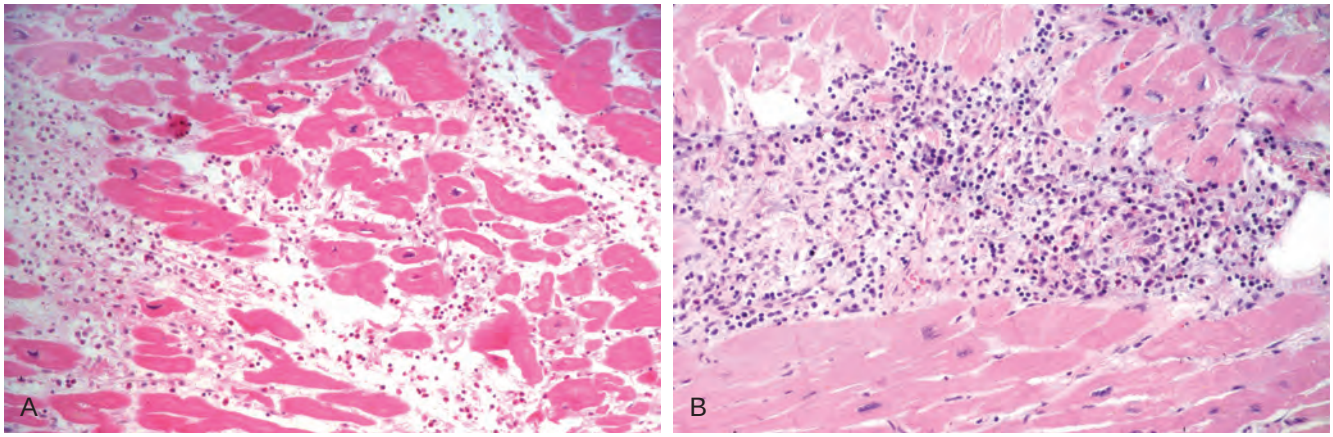
"Pressor lesions" (e.g., catecholamine effect; cocaine)

Noninflammatory Forms

Cardiomyopathy (e.g., Adriamycin)

Intracellular inclusions

Endocardial fibrosis



• **Figure 9.33** Hypersensitivity Myocarditis. **A,** A diffuse interstitial inflammatory infiltrate consisting of mostly eosinophils associated with extensive interstitial edema (×250). **B,** A photomicrograph (×200) taken from a myocardial apex specimen removed at the time of placement of a left ventricular assist device. The patient had been receiving intravenous dobutamine, and the photograph shows a mixed interstitial infiltrate with histiocytes surrounding degenerating collagen fibers. Myocyte necrosis is absent in both specimens.

recent studies and case reports[81] have shown that necrosis may be present and in some cases is quite extensive, resembling necrotizing eosinophilic myocarditis (see earlier discussion). Explanted recipient hearts have shown a high incidence of hypersensitivity myocarditis, and a particular preservative in intravenous dobutamine solutions was identified as the possible cause.[82] This lesion is associated with degenerating collagen bundles on microscopic examination.

Toxic Myocarditis. In contrast to hypersensitivity myocarditis, the lesions in toxic myocarditis are always associated with myocyte damage. The infiltrate is predominantly lymphocytic, admixed with neutrophils. With longer exposure to the toxic drug or toxin, new lesions are formed and older lesions heal. Therefore the lesions in toxic myocarditis are of varying stages of development, with active healing and healed (scarred) lesions being present in the same specimen.[83]

Other Inflammatory Forms of Drug-Related Myocarditis. Certain drugs can cause vasculitis syndromes, which can be either necrotizing or of the hypersensitivity type associated with a perivascular inflammatory infiltrate. These lesions may be found in the myocardium and may be confused with primary myocarditis.[84] Drugs that can cause lupuslike syndromes may also give rise to lupuslike lesions in the myocardium (see later discussion).

The cardiac effects of cocaine deserve special mention. Cocaine affects the heart in various ways. Its catecholamine-like effect can lead to microvascular spasm and platelet aggregation. Both mechanisms may lead to small foci of myocyte damage, associated with scant inflammatory infiltrates containing neutrophils and lymphocytes. Myocyte contraction bands are often seen in these lesions. Occasionally, cocaine can cause hypersensitivity myocarditis, with the inflammatory infiltrate containing eosinophils.[85,86]

Myocarditis Associated With Systemic Disorders

Sarcoidosis

Sarcoidosis is an idiopathic disorder that can mimic mycobacterial or fungal infection. Cardiac lesions are found in 25% to 50% of sarcoidosis patients at autopsy.[87,88] This may be an overestimation of cardiac involvement because congestive failure and sudden death caused by cardiac sarcoidosis may lead to bias in an autopsy population. The hearts of patients with long-standing sarcoidosis show extensive scarring, particularly in the apices of both ventricles, the papillary muscles, and the upper portion of the interventricular septum. The latter often leads to abnormalities in cardiac conduction, because the atrioventricular node and the major branches of the conduction system are located in that region. Dilation of both ventricles is also found because sarcoidosis can lead to congestive cardiomyopathy.

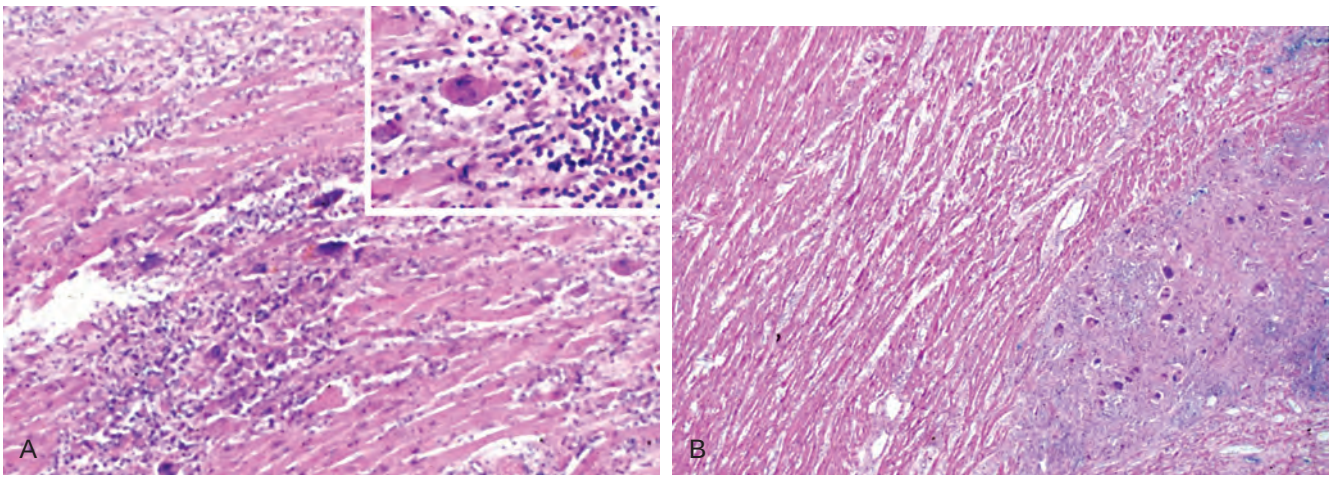
The hallmark of sarcoidosis on microscopic examination is the presence of noncaseating granulomas associated with a lymphocytic infiltrate and fibrosis. Early lesions of sarcoidosis may show very few giant cells and consist almost exclusively of lymphocytes with little fibrosis. As lesions mature, fibrosis increases and lymphocytes decrease. Sarcoidosis may be diagnosed by endomyocardial biopsy, which has been shown to be useful in the treatment of patients with the disease.[89]

Because the natural history and treatment of sarcoidosis and giant cell myocarditis are quite different, it is important to outline and contrast the histopathologic features of these two conditions (Fig. 9.34).[76,77]

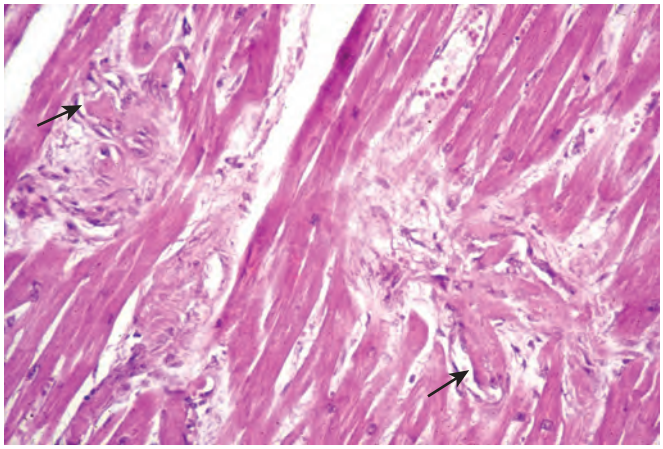
- The lesions of sarcoidosis are often rounded and have relatively sharp borders, whereas the inflammatory infiltrate of giant cell myocarditis is more infiltrative and quite diffuse.
- The predominant population of lymphocytes in sarcoidosis is made up of CD4-positive cells, whereas the lymphocyte population in giant cell myocarditis consists of mostly CD8-positive cells.
- Eosinophils are absent in sarcoidosis but are a prominent feature in giant cell myocarditis.
- Necrosis is extensive in giant cell myocarditis but is not prominent in sarcoidosis, whereas prominent fibrosis is a major feature of sarcoidosis.

Other Systemic Disorders

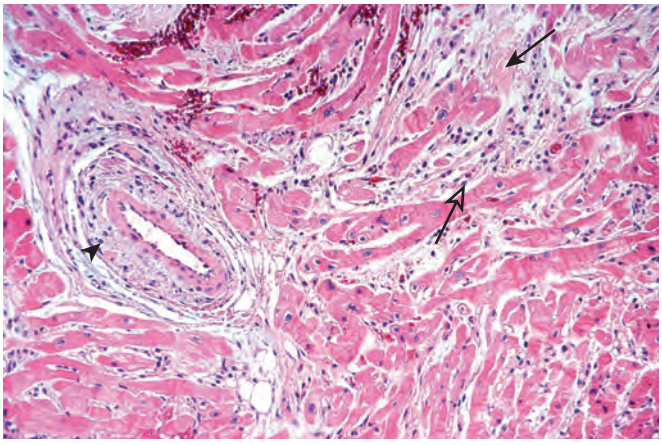
Many of the collagen vascular diseases can involve various parts of the cardiovascular system: pericardium, myocardium, valves, endocardium, and large and small vessels.[90] The discussion here highlights those that may manifest with myocarditis.



• **Figure 9.34** Giant Cell Myocarditis and Sarcoidosis. Giant cell myocarditis (×250) **(A)** and sarcoidosis **(B)** can be distinguished by the more ill-defined and diffuse infiltration in giant cell myocarditis, compared with the more circumscribed lesions in sarcoidosis (×200). Myocyte necrosis is also much more extensive in giant cell myocarditis. Both lesions contain giant cells, and the inflammatory infiltrate of giant cell myocarditis is shown at higher power in the inset.



• **Figure 9.35** Thrombotic Thrombocytopenic Purpura. Multiple fibrin-platelet thrombi *(arrows)* are seen in small myocardial vessels. Although absent in this field, myocyte necrosis with inflammation is often present in these specimens secondary to small vessel occlusions (×250).



• **Figure 9.36** Myocarditis Associated With Kawasaki Disease. This endomyocardial biopsy (×250) from a young patient with a history of Kawasaki disease shows an inflammatory infiltrate associated with interstitial *(open arrow)*, perivascular *(arrowhead)*, and replacement *(solid arrow)* fibrosis.

All layers of the heart may be involved in SLE. Fibrinous pericarditis is common, and valves may be involved by verrucous Libman-Sacks lesions. The myocardium may be involved in several ways. SLE causes a lymphocytic, presumably autoimmune, myocarditis; perivascular fibrinoid necrosis, when present, is characteristic of SLE myocarditis.[91,92] Because SLE can cause coronary arteritis, myocardial ischemia can result, which can be confused with myocarditis on pathologic examination. The development of anticardiolipin antibody is associated with SLE and causes microvascular thrombosis, which in turn can cause ischemic damage to the myocardium.[93]

Similar changes may be seen in thrombotic thrombocytopenic purpura (Fig. 9.35). Dermatomyositis/polymyositis can cause a lymphoplasmacytic myocarditis, and the inflammatory infiltrate of the myocarditis seen in Churg-Strauss syndrome contains CD83-positive cells.[94]

Kawasaki disease can involve all layers of the heart. Coronary arteritis is the best-known cardiac manifestation, occurring in 15% to 20% of untreated children. Although Kawasaki disease is most often not life threatening, it is often associated with myocarditis. Early in the disease, the lesions are characterized by edema and lymphocytic infiltrates, but with time there is increasing development of fibrosis, especially concentric perivascular fibrosis, and abnormal configurations of myocytes (Fig. 9.36).[95]

Complications and Natural History of Myocarditis

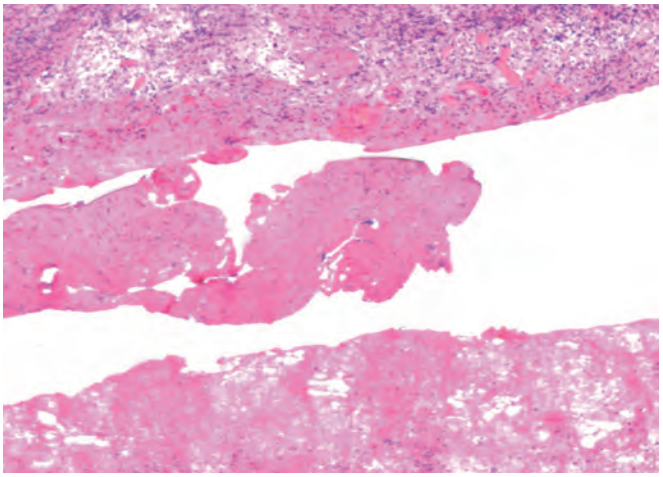
Acute complications of myocarditis are sudden death, arrhythmias, and acute dilated cardiomyopathy. Large pericardial effusions and pericarditis can be prominent features of the presentation.[96] Chronic sequelae relate to the development of

• BOX 9.5 Causes of Pericarditis

Infectious
Viral
Bacterial (including *Rickettsia* and *Chlamydia*)
Fungal

Noninfectious
Idiopathic
Uremia
Acute myocardial infarction (Dressler syndrome)
Postcardiac injury syndrome
Autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease, Sjögren syndrome, Whipple disease, scleroderma)
Irradiation

Modified from Aretz HT. The heart. In: Mills SE, Carter D, Greenson JK, et al., eds. Sternberg's Diagnostic Surgical Pathology. *4th ed. New York: Raven; 2003; and Bonfiglio T, Atwater E. Heart disease in patients with seropositive rheumatoid arthritis: a controlled autopsy study and review.* Arch Intern Med. *1969;124:714-719.*



• Figure 9.37 Exudative Pericarditis. Pericardium shows fibrinous exudate with chronic inflammation and mesothelial hyperplasia (×150).

congestive cardiomyopathy. Most patients recover completely or have mildly depressed cardiac function, but a small cohort of patients develop severe cardiac failure requiring transplantation. To date, there are no good clinical or histologic markers that allow reliable prediction of clinical outcome (except for giant cell myocarditis and necrotizing eosinophilic myocarditis). Five-year mortality rates in two series of patients presenting with cardiac dysfunction were approximately 50%[49,97]; the figure for giant cell myocarditis was less than 20%,[98] and the prognosis for HIV-associated myocarditis is equally dismal.[99] Despite multiple trials using a variety of immunosuppressive regimens, treatment remains mostly supportive.[37]

Pathology of Pericarditis

Normal Pericardium

The normal pericardium is a fibroelastic sac surrounding the heart that consists of the visceral and parietal pericardium. The visceral pericardium is a simple layer of mesothelial cells covering the pericardium, and the parietal pericardium is a sac made of fibrous and elastic tissue, typically no more than 2 mm thick. It is very well innervated. The potential space between the two layers contains no more than 50 mL of serous fluid under normal circumstances. The pericardium restrains acute dilation of the cardiac chambers, but it accommodates to chronic dilation and fluid accumulation. Pericardial specimens are typically received as part of pericardiectomies to treat cardiac constriction.

Etiology of Pericarditis

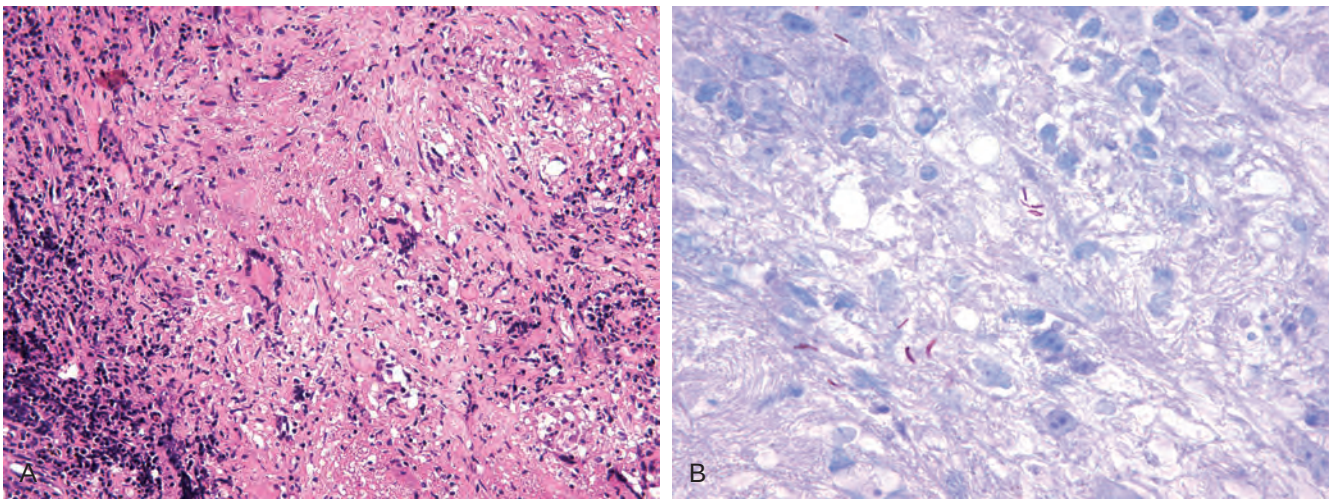
More than 90% of cases of acute pericarditis are idiopathic or viral in origin. The remainder may have many causes, the majority of which are summarized in Box 9.5,[57,100,101] that require clinical correlation.[102] Microscopically, viral pericarditis, uremic pericarditis, and the pericarditis associated with collagen vascular diseases are characterized by mesothelial hyperplasia, sometimes with extensive atypia, and a fibrinous exudate (Fig. 9.37). The cellular infiltrate consists of chronic inflammatory cells. Rheumatoid pericarditis rarely shows rheumatoid nodules.[103]

Bacterial pericarditis is caused by a variety of organisms, the most common of which are *Haemophilus influenzae*, meningococci, pneumococci, staphylococci, and streptococci. Microscopically a purulent exudate and severe acute inflammation are seen. Bacterial pericarditis can be a result of bacteremia but may also be caused by contiguous spread (e.g., pneumonia). The mortality rate is high, and pericardial drainage may be required in the acute phase.[104]

Tuberculous pericarditis accounts for fewer than 5% of cases of infectious pericarditis in the developed world, but it accounts for 50% to 70% of cases in sub-Saharan Africa.[105,106] It is much more common in HIV-infected patients in both settings. It can manifest as constrictive, constrictive-effusive, or effusive pericarditis.[107] The hallmarks of tuberculous pericarditis are the presence of caseating granulomas and demonstration of the tubercle bacillus (Fig. 9.38). If left untreated, it is fatal in more than half of patients and it leads to constrictive pericarditis in 30% to 50% of patients. Fungal pericarditis[108] occurs mostly in immunocompromised hosts or as part of endemic fungal infections (e.g., *Histoplasma*, *Coccidioides*); its lesions mimic those elsewhere.

Constrictive Chronic Pericarditis

The cause of constrictive pericarditis cannot be determined in most cases.[109] Except for the pericarditis associated with rheumatic fever and that associated with acute myocardial infarction, almost all forms of acute pericarditis can evolve into constrictive pericarditis. Fibrosis, often associated with calcifications and firm adhesions, can lead to significant constriction, as can loculated effusions.



• **Figure 9.38** Tuberculous Pericarditis. **A,** Pericardium showing granulomatous inflammation with multinucleated giant cells (×250). **B,** Mycobacteria are decorated by Ziehl-Neelsen stain (×400).

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10

Infections of the Gastrointestinal Tract

GREGORY Y. LAUWERS, MARI MINO-KENUDSON, RICHARD L. KRADIN

Infections of the Esophagus
- Bacteria
- Fungi
- Viruses

Infections of the Stomach
- Viruses
- Bacteria
- Fungi
- Parasites
- *Helicobacter pylori*–Associated Chronic Gastritis

Infections of the Small Bowel
- Bacteria
- Tropical Sprue
- Fungi
- Viruses
- Protozoa
- Helminths
- Trematodes
- Cestodes

Infectious Colitis
- Bacteria
- Viruses
- Protozoa
- Helminths
- Fungi
- Sexually Transmitted Diseases

The surgical pathologist plays a key role in the diagnosis of gastrointestinal infection. Virtually all types of pathogenic microbes can cause gastrointestinal infection, so the diagnostic pathologist must be cognizant of their varied histologic manifestations and acquainted with the complex morphologic features of viruses, bacteria, fungi, and parasites. Because certain noninfectious inflammatory conditions can mimic infection, recognizing their at times subtle distinctions is a critical aspect of the surgical pathologist's charge.

Infections of the Esophagus

Bacteria

Bacterial infection of the esophagus is uncommon; it is usually seen in neutropenic patients or as infection extending from the lung. Most cases occur after epithelial injury by severe acid reflux or previous viral infection. Neutropenia secondary to hematologic malignancy, intensive chemotherapy, or bone marrow transplantation predisposes to bacterial esophagitis.[1,2] Approximately 15% of infectious esophagitis is seen in immunocompromised patients, but it is less common in patients with acquired immune deficiency syndrome (AIDS) than in the oncology setting because of the relative sparing of granulocyte counts in AIDS.[3] Notably, immunomodulators, such as tumor necrosis factor (TNF)-α, have been reported with esophageal bacterial infection.[3a]

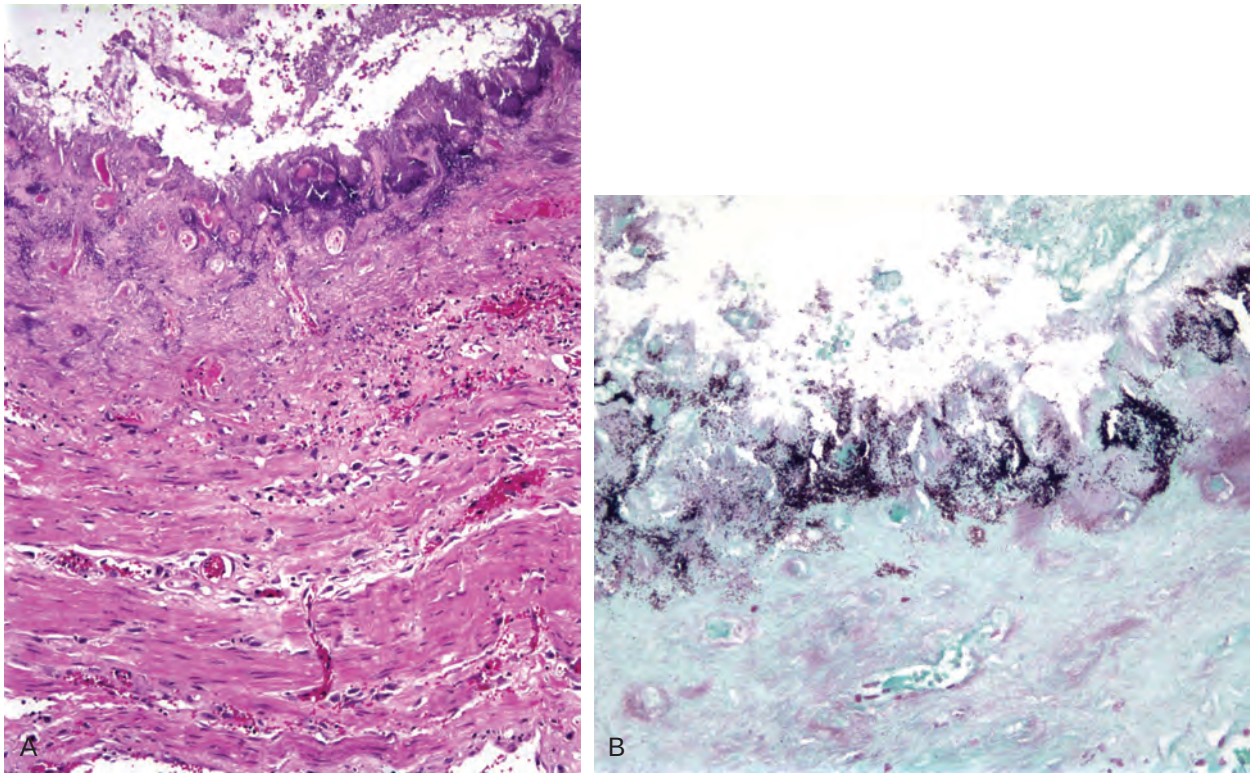
Clinical symptoms include odynophagia, dysphagia, and retrosternal pain. Fever is reported in a minority of cases.[2,4] Endoscopic findings are nonspecific and include mucosal friability, pseudomembranes, plaques, and ulcerations. Significant complications include perforation, fistula formation, and sepsis.[5]

The most common bacterial agents belong to the normal flora of the mouth and upper respiratory tract, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, and *Bacillus* spp. Polymicrobial infection is common.

Bacterial infection evokes a marked neutrophilic exudate, cellular necrosis, and degeneration (Fig. 10.1). In severely neutropenic patients, ulcers and pseudomembranes without substantial acute inflammation can be seen. Most bacteria can be identified in tissue sections by light microscopy with tissue Gram stain and oil immersion optics. The diagnosis of bacterial esophagitis is based on the presence of confluent bacteria invading subepithelial tissues (see Fig. 10.1B).[2] Bacterial cultures of endoscopic biopsy samples are usually of little value due to bacterial contamination by the endoscope.

Mycobacterium tuberculosis

Esophageal tuberculosis is usually caused by extension of infection from contiguous organs, particularly mediastinal lymph nodes, or from miliary spread.[6,7] Autopsy studies show esophageal involvement in 0.15% of patients dying with tuberculosis. Primary esophageal tuberculosis is extremely rare.[8-10] The most common symptoms of esophageal tuberculosis are dysphagia, weight loss, and retrosternal pain.[11] Imaging modalities may show extrinsic compression by involved lymph nodes in the mediastinum, traction diverticula, stricture, kinking of the esophagus, sinus or fistulous tract, and pseudotumoral masses.[12] Chest computed



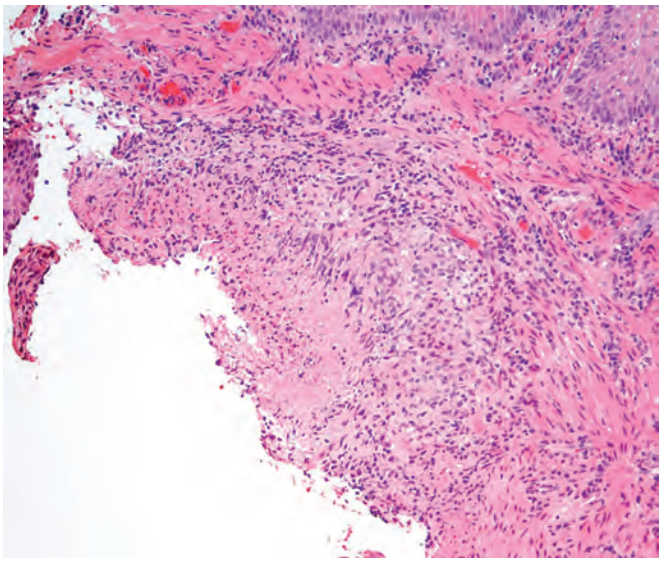
• **Figure 10.1** Nonspecific bacterial esophagitis. **A,** Esophageal mucosa is replaced by cellular necrosis and a fibrinopurulent exudate. **B,** Brown-Hopps stain highlights bacteria in subepithelial tissues. (Courtesy Dr. Laura Lamps.)

tomography (CT) and endoscopic ultrasonography reveal extrinsic nodular masses, consistent with mediastinal lymphadenopathy showing central hypodensity, rim enhancement, and calcification.[8,13] Endoscopic examination shows shallow ulcers with smooth border, a gray purulent base, and irregularly infiltrated edges.[14,15] Synchronous endoscopic ultrasound (EUS) commonly shows matted, heterogeneous lymph nodes with hypoechoic centers.[7]

The differential diagnosis includes carcinoma, fungal infection, syphilis, and Crohn disease.[16] In some cases, no preoperative diagnosis is established before esophagectomy[17,18]; however, even if a developed stricture has formed, antimycobacterial treatment can successfully save the patient from a complex surgical intervention.[19]

The characteristic histologic features of esophageal tuberculosis include necrotizing, often confluent granulomas, which can be present at any level of the esophagus (Fig. 10.2). A rim of lymphocytes may be present at the periphery. In some cases, granulomas are sparse, or hyalinized and calcified. Acid-fast stains may demonstrate the organisms, but culture confirmation is often required.[20] In a series of 32 cases, histology of the ulcers and fine-needle aspiration of lymph nodes yielded a diagnosis in 84% of the cases.[7] Polymerase chain reaction (PCR) for *M. tuberculosis* can be a rewarding diagnostic approach.[21-24]

Other granulomatous conditions, including mycobacterial infection due to *Mycobacterium kansasii* or *Mycobacterium bovis*, fungal infection, and Crohn disease, should be excluded. Features favoring Crohn disease include the presence of transmural lymphoid aggregates with deep fistulas and fissures.



• **Figure 10.2** *Mycobacterium tuberculosis*. Caseating granuloma involving esophageal wall.

Fungi

Fungal esophagitis is most frequently caused by *Candida* spp., but other pathogens, including *Histoplasma*, *Aspergillus*, *Cryptococcus*, *Coccidioides*, *Paracoccidioides*, *Trichosporon*, *Blastomyces*, and *Mucor*, can cause disease (Table 10.1). Fungal infection is often

TABLE 10.1 Comparative Morphology of *Candida*, *Aspergillus*, and *Histoplasma* Spp

Organisms	Morphologic Features
C. albicans *C. tropicalis*	Mixture of budding yeast and nonbranching pseudohyphae; occasional septate (true) hyphae
C. glabrata	Budding yeast No hyphae No halo effect
Aspergillus spp.	Septate hyphae in uniform width True dichotomous branching with regular, acute angles
H. capsulatum	Intracellular Oval budding yeast with narrow-based buds Halo effect around organisms on H&E stain

H&E, Hematoxylin and eosin stain.

superimposed on other infections, and efforts at identifying all possible pathogens are mandatory to design optimal treatment. Debilitated and immunocompromised individuals are susceptible to fungal infections, and irradiation, chemotherapy (including new targeted therapies), and chronic motility disorders are predisposing factors. Esophageal candidiasis is the most common cause of esophagitis in human immunodeficiency virus (HIV)-infected patients.[25,26]

Candidiasis

Candida albicans is a constituent of normal flora. Although it primarily affects patients with predisposing conditions, *Candida* esophagitis can occur in apparently normal hosts. A variety of *Candida* spp. are pathogenic, including *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida stellatoidea*, and *Candida krusei*. Of those, *C. tropicalis* tends to be more virulent, showing a propensity to invade submucosal blood vessels.[27] Patients with intact immunity usually develop inflammation at the infected site that limits penetration by the microorganisms.

Acute candidal esophagitis is the most common manifestation. Presenting symptoms are nonspecific and include odynophagia, dysphagia, and retrosternal chest pain. Esophageal bleeding, perforation, stricture, sinus formation (with secondary pulmonary abscess), extensive necrosis, and systemic invasion are rare.[28] Subacute candidal esophagitis is an uncommon disease that usually affects asymptomatic and immunocompetent patients, but it can cause esophageal strictures and pseudodiverticula. It typically follows an indolent course. Chronic candidal esophagitis is also rare and is usually seen as a feature of chronic mucocutaneous candidiasis that consists of several clinical syndromes, including autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. These patients usually have other gastrointestinal manifestations, including malabsorption and loss of parietal cell function.[29]

Candidal infections can result in significant morbidity and death, especially in high-risk patients. Patients who receive radiation therapy to the thorax for lung or esophageal cancer or inhaled corticosteroid for asthma or chronic obstructive pulmonary disease are predisposed to candidal esophagitis.

Candidal infections yield small, yellow-white, raised plaques with surrounding erythema in mild disease—and confluent linear and nodular plaques or membranes covering a friable, erythematous, ulcerated mucosa in extensive disease—particularly in the middle and distal esophagus.[28] Erosion, ulcers, and strictures can develop. Fungi are densely adherent to the inflamed mucosa. In advanced disease the esophagus may become stenotic and show mucosal irregularities. In chronic cases, umbilicated, wartlike lesions may develop. The gross features are easily confused with pseudodiverticulosis, varices, or carcinoma. Rarely, fungal esophagitis leads to a botryoid appearance resembling clusters of grapes projecting from the mucosa, and mucosal bridges may form. In severe candidiasis, necrosis of the entire esophageal mucosa may be seen.[30]

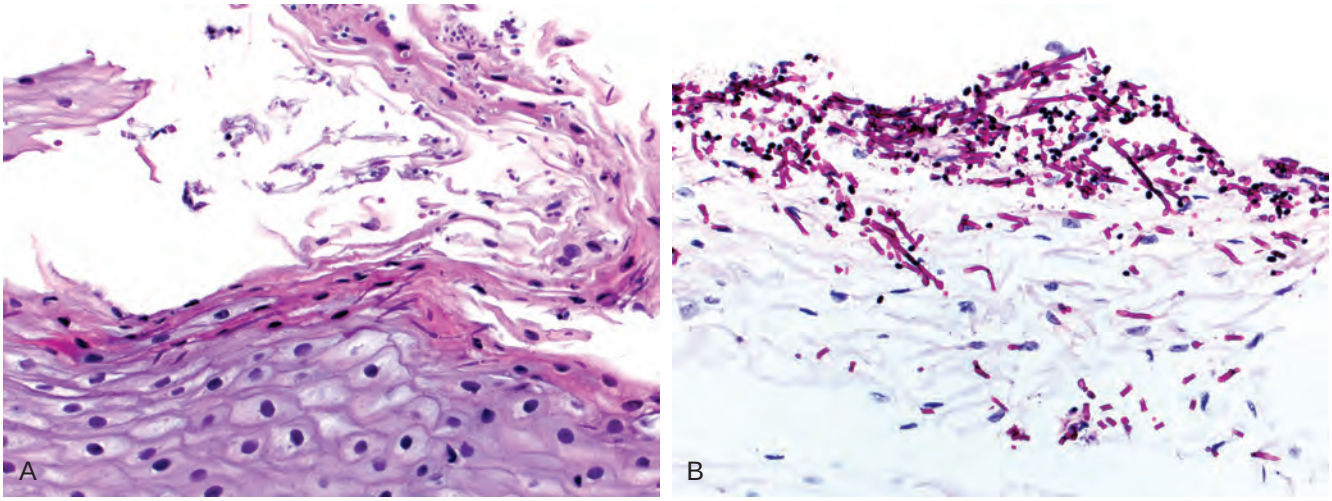
Candida spp. are characterized by a mixture of blastoconidial spores, 3 to 4 μm in diameter, and nonbranching pseudohyphae that may become quite large, up to 2 μm in diameter. True septate hyphae can also be seen. These structures are best seen with Grocott methenamine silver (GMS) or periodic acid–Schiff (PAS) stain, and the presence of all of these structures is diagnostic of *Candida* infection (Fig. 10.3). Isolated budding yeasts without pseudohyphae or evidence of tissue invasion are often seen in inflammatory exudates at the ulcer bed; however, pseudohyphae or true hyphae are required for accurate diagnosis. Superficial colonization, particularly of nonviable tissue, does not necessarily indicate clinically significant disease. Although cytologic brushings from the plaques are more sensitive for fungal detection, biopsies are required to ascertain whether the fungus has invaded tissue. For this reason, it is important to indicate in the pathology report the types of fungal forms that are present (yeast, pseudohyphae, or both) and whether they are seen only in exudates or actually invading tissue.

Candida infections also mimic other fungal infections, such as aspergillosis and histoplasmosis. *Candida* spp. can be differentiated from *Aspergillus* based on the width of hyphae, the presence of acute-angle dichotomous branching by *Aspergillus* spp., and the presence of blastoconidia in *Candida* infection. The presence of pseudohyphae in *Candida* generally differentiates it from *Histoplasma capsulatum*, but if only yeast forms are identified, the differentiation of *Candida* from *Histoplasma* may be difficult. However, tissue Gram stain almost always decorates the gram-positive yeast forms of *Candida*.

Aspergillosis

Aspergillus, which is a ubiquitous fungus, rarely causes esophagitis.[31-34] Several species can infect the esophagus, including *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*. *Aspergillus* commonly colonizes immunocompromised patients[35-37] but can invade tissues and disseminate via the bloodstream, posing a life-threatening condition. Isolated gastrointestinal aspergillosis is relatively rare but has been reported.[38] Patients with esophageal aspergillosis present with painful or difficult swallowing and weight loss. Concurrent mucosal candidiasis may be present.

Esophageal aspergillosis typically involves the mucosa and extends into the muscularis propria. When vascular invasion occurs, thrombosis and subsequent infarction may lead to perforation. *Aspergillus* is characterized cytologically by the presence of acute-angle branching septate hyphae with smooth, parallel walls, ranging in size from 2 to 4 μm in diameter. Characteristic conidiophores are rarely seen in this setting. *Aspergillus* can be differentiated from *Mucor* by its uniform thickness, mode of branching, and presence of frequent septation.



• **Figure 10.3 A,** Candidal esophagitis is characterized by a mixture of spores and nonbranching pseudohyphae that invade the superficial layer of squamous epithelium. **B,** Periodic acid–Schiff stain highlights the fungal elements.

Other Fungal Infections

Blastomyces, *Histoplasma*, *Mucor*, and *Cryptococcus* spp. rarely involve the esophagus[39] and usually only in patients with disseminated disease, but esophageal infection can arise from infections in contiguous structures, such as the lungs or mediastinum.[32] Patients with histoplasmosis can present with dysphagia resulting from esophageal compression by infected mediastinal lymph nodes or as a complication of mediastinal granuloma or sclerosing mediastinitis. Mediastinal granulomas can cause traction esophageal diverticuli.[39]

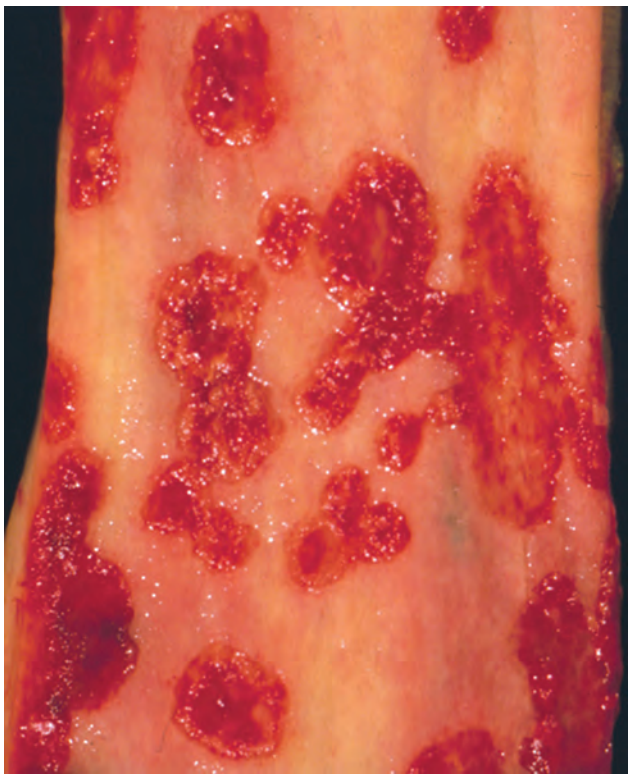
Viruses

Herpes Simplex Virus Types 1 and 2

Herpes infection may be seen throughout the gastrointestinal tract but is usually limited to squamous epithelium–lined structures (e.g., esophagus, anorectum). Herpes esophagitis has been reported with both herpes simplex virus type 1 (HSV-1) and HSV-2,[40,41] and it affects 0.5% to 6% of patients, primarily those who are immunocompromised due to AIDS, transplantation, or chemotherapy. However, immunocompetent adults and neonates also develop herpetic esophagitis.[42]

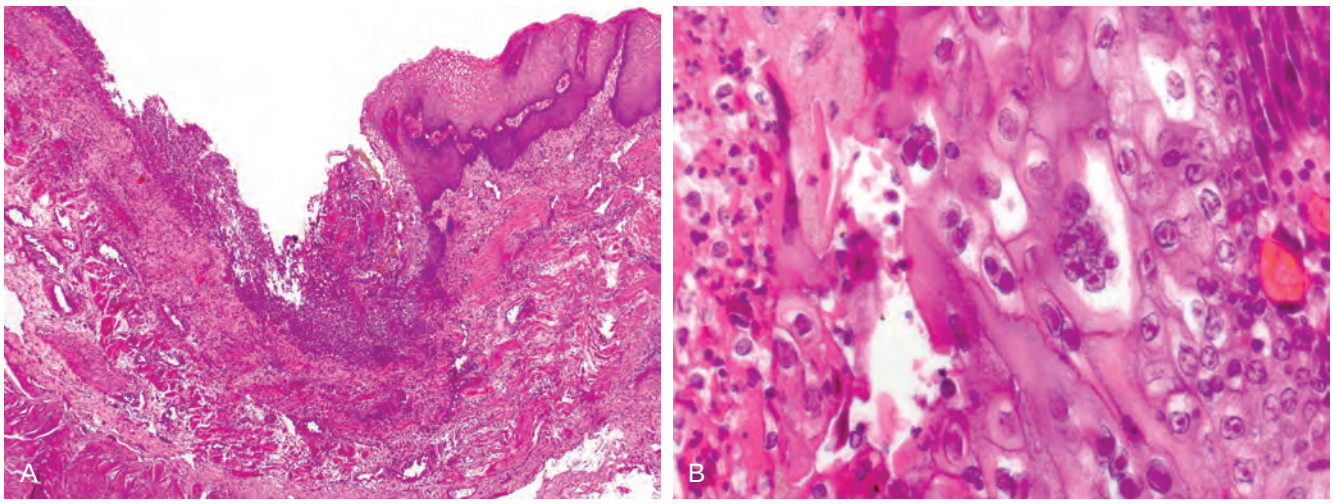
Primary infections are common in neonates with disseminated HSV.[43] In adults the disease typically manifests as a reactivation of latent disease. Usually, a previously healthy, immunocompetent patient with a history of recurrent "cold sores" (i.e., nasolabial herpetic lesions) who presents with concurrent esophageal symptoms most likely has HSV esophagitis. In this setting, infections are self-limited. However, immunocompromised individuals may experience severe prolonged infections, and they are at risk for serious complications, including mucosal necrosis, hemorrhage, strictures, tracheobronchial fistula, and disseminated infection.[44]

The endoscopic appearance of HSV esophagitis varies with the time of infection. Early lesions consist of rounded, 1- to 3-mm, clear vesicles seen predominantly in the middle to distal esophagus. Subsequently the epithelial roof is eroded to leave small, 1- to 3-mm ulcers. After most of the HSV-infected squamous cells have sloughed off, characteristic sharply demarcated ulcers with raised margins and a yellow-gray base are seen (Fig. 10.4). The uninvolved mucosa usually appears normal. If infection progresses, the ulcers may coalesce to produce inflammatory exudates that grossly resemble *Candida* esophagitis.[45-48]



• **Figure 10.4** Gross appearance of esophageal ulcers due to herpes esophagitis in a patient with human immunodeficiency virus infection. Numerous punched-out ulcers are present. (Courtesy Dr. Rhonda Yantiss.)

Typical histologic findings include focal ulceration, neutrophils in the lamina propria, and an inflammatory exudate with sloughed necrotic squamous cells. Aggregates of macrophages near the ulcer are characteristic of the inflammatory response.[49] Diagnostic cytopathic changes include nuclear molding, multinucleated giant cells, ballooning degeneration, and eosinophilic type A intranuclear inclusions showing margination of chromatin (Fig. 10.5). Cowdry



• **Figure 10.5** Herpes simplex virus esophagitis. **A,** Resection specimen shows a sharply demarcated ulcer with raised margins. The ulcer bed is partially covered by inflammatory exudate that contains sloughed squamous cells. **B,** Herpes simplex virus cytopathic changes consist of nuclear molding, multinucleated giant cells, and type A intranuclear inclusions with margination of chromatin.

type B inclusions are more frequently seen than type A inclusions. The edge of the ulcer and the sloughed squamous cells are the best places to sample for identification of the characteristic cytopathic changes. Viral culture is more sensitive than microscopic examination for diagnosis of HSV infection, and the combination of directed brushings and biopsy specimens submitted for culture and histology improves the sensitivity of endoscopic diagnosis compared with biopsy alone.[50,51] Accurate diagnosis can be confirmed in situ by immunostaining with commercially available specific monoclonal antibodies.

The differential diagnosis of HSV esophagitis includes other viral infections, such as cytomegalovirus (CMV) and varicella zoster virus (VZV) infection. Their histologic features overlap, so immunostains and in situ hybridization are required to accurately distinguish them. It is important to distinguish HSV from other infections because specific antimicrobial agents are required for effective treatment.

Multinucleated "reparative" squamous epithelial giant cells that are located predominantly in the proliferative basal and suprabasal zones of the preserved squamous epithelium have also been reported in the setting of reflux esophagitis, radiation therapy, and drug esophagitis. The epithelial giant cells may be confused with herpetic infection; however, the former typically lacks ground-glass nuclear inclusions and peripheral condensation of chromatin at the nuclear membrane. The multinucleated cells in herpes esophagitis are generally more prominent in the superficial aspects of the epithelium and in luminal inflammatory debris. An immunostain for herpes virus antigen can help to exclude HSV infection.[52]

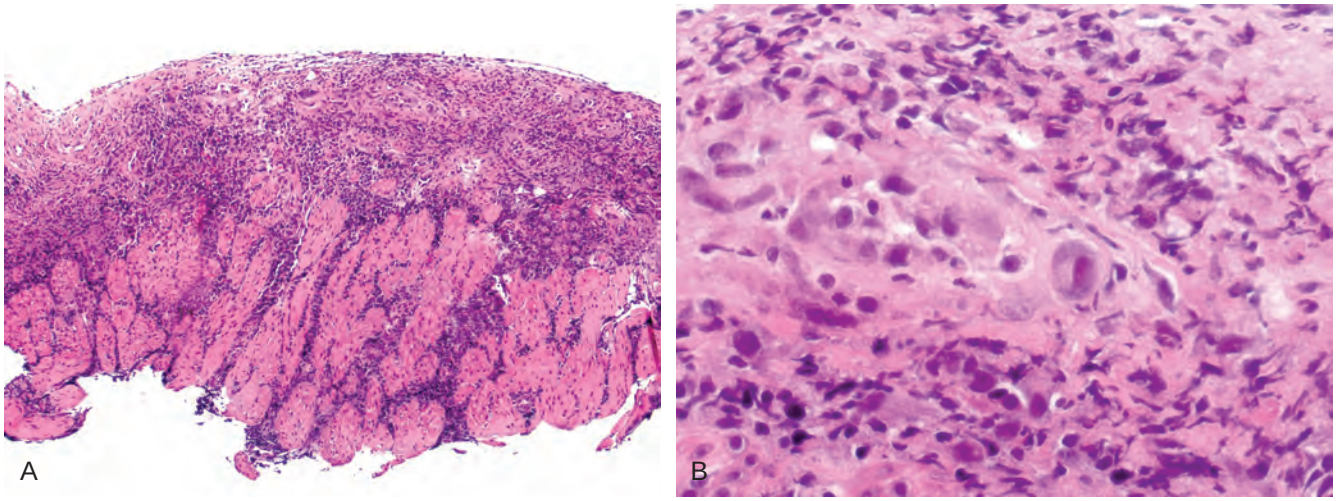
Cytomegalovirus

Esophagitis is the second most common gastrointestinal manifestation of CMV infection after colitis.[53] CMV esophagitis is reported in the setting of HIV infection, renal dialysis, or organ transplant. The onset of symptoms is more gradual than that of HSV esophagitis and includes fever, odynophagia, epigastric pain, nausea, vomiting, diarrhea, and weight loss, as opposed to the painful difficulties in swallowing and retrosternal pain commonly seen in HSV infection.[1,54,55] Endoscopically, CMV infection manifests as multiple, discrete, small, superficial ulcers in the middle or distal esophagus, but it may yield one or more large, flat, and elongated ulcers. Because HSV esophageal ulcers are rarely several centimeters in diameter, the presence of giant ulcers is suggestive of CMV esophagitis in patients with AIDS (Fig. 10.6A), although HIV-associated idiopathic ulcers may also become large.[56] Penetrating giant ulcers causing fistulas and pseudotumoral lesions may be seen in AIDS patients.[5] CMV esophagitis may coexist with both HSV and *Candida* infection in transplant recipients and AIDS patients.[1,57]

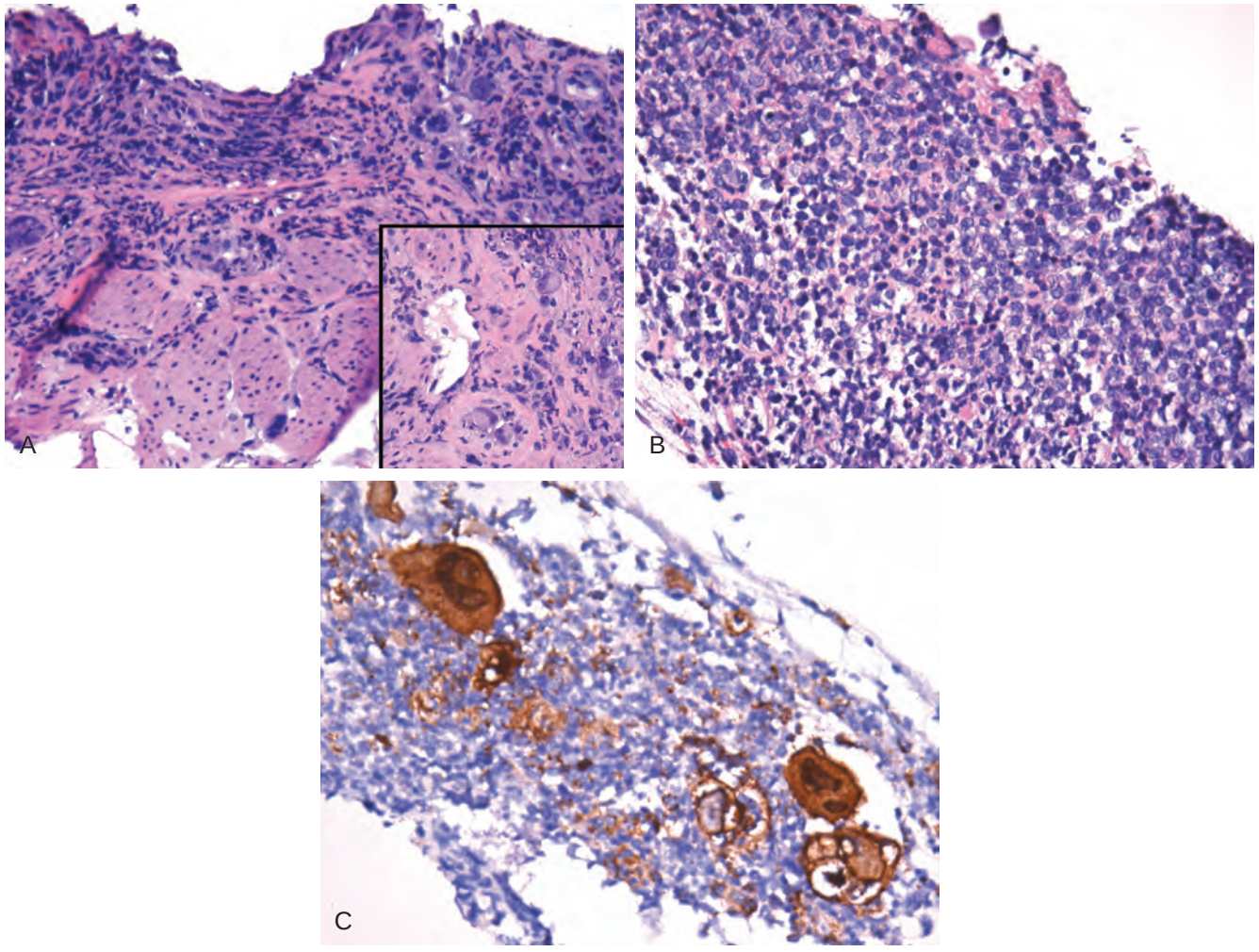
CMV cytopathic effects typically develop in endothelial cells and fibroblasts rather than in epithelial cells (see Fig. 10.6). Therefore superficial biopsy specimens that contain only squamous epithelium or brushings for cytology are usually insufficient for the evaluation of CMV infection, and it is recommended that biopsies include the granulation tissue of the ulcer bed. Classic cytopathic effects of CMV include intranuclear eosinophilic inclusions surrounded by a halo with margination of the chromatin to the nuclear membrane. In contrast to HSV and VZV, multiple small cytoplasmic inclusions may be seen with hematoxylin and eosin (H&E) stain and to advantage with PAS and GMS. CMV can superinfect the ulcerations caused by HSV, and mixed infections are common in severely immunocompromised individuals (Fig. 10.7). Immunohistochemistry with specific antibodies or genetic probing for virus may be necessary to establish the diagnosis, especially if the patient has received antiviral agents prophylactically, because such agents can inhibit nuclear cytopathic changes.

Other Viral Infections

Primary VZV infection (chickenpox) in immunocompromised children is often associated with severe visceral dissemination and has a mortality rate of 7% to 30%.[58,59] VZV esophagitis is rare and occurs in severely ill patients but not in AIDS patients.[3] VZV cytopathic changes are seen in epithelium, endothelium, and stroma cells and are indistinguishable from those of HSV.[60] Cytoplasmic rarefaction and vacuolization of the infected cells, as well as dissociation of the basal layer from the lamina propria, may be observed. To differentiate VZV infection from HSV



• **Figure 10.6** Cytomegalovirus infection of esophagus. **A,** Mucosal biopsy from a large ulcer in a patient with acquired immune deficiency syndrome demonstrates granulation tissue replacing lamina propria, as well as thickened and inflamed muscularis mucosae. **B,** High-power magnification reveals an endothelial cell with cytomegalovirus cytopathic changes.



• **Figure 10.7** Mixed infection in a severely immunocompromised patient. The esophageal biopsy consisted of granulation tissue **(A)**, with endothelial cells demonstrating characteristic cytomegalovirus cytopathic changes *(inset)*, and detached fragment of fibrinopurulent exudate **(B)**. **C,** Immunostain for herpes simplex virus (HSV) performed on deeper sections also revealed positive multinucleated cells consistent with HSV cytopathic changes.

infection, immunohistochemical staining with specific monoclonal antibodies is required.

Esophageal infections with human papillomavirus (HPV) may manifest as erythematous macules, white plaques, nodules, exuberant frondlike lesions, or even ulcerations.[61-64] Large lesions can be mistaken for malignancy on endoscopic examination, and small lesions may be confused with glycogen acanthosis.[65] Koilocytotic changes in squamous epithelium are characteristic. As in the cervix and anus, HPV infection is associated with well-defined papillomas (condylomas) and with mucosal hyperplasia.

Epstein-Barr virus (EBV) esophagitis is infrequently reported but can be seen in otherwise healthy patients with infectious mononucleosis. These patients present with odynophagia and hematemesis and develop denuded ulcers with erythema.[66,67] Histologically, EBV-infected esophageal mucosa is comparable to oral hairy leukoplakia seen in AIDS patients; it is characterized by epithelial hyperplasia, parakeratosis, and koilocytosis.[68,69] Deep biopsies show dense monocytic CD3-positive T cells.[66] EBV infections confirmed by in situ hybridization are also seen in AIDS.[69]

Human Immunodeficiency Virus

Esophageal ulceration is an important cause of morbidity in patients with HIV/AIDS and may result from a variety of factors. A prospective study reported that 50% of esophageal ulcers in HIV-infected patients were caused by CMV infection, and approximately 40% were classified as idiopathic ulcers. Other causes included HSV infection, gastroesophageal reflux disease, and *Candida* infection, noted in 24% of the cases in an African study.[70] In 10% of patients, more than one etiology was discovered during long-term follow-up.[71] Acute seroconversion to HIV can produce multiple small esophageal ulcers with an intense inflammatory infiltrate. Based on the ultrastructural detection of viral-like particles in biopsy samples, it was hypothesized that HIV was responsible for the ulcerations in these individuals.[72,73]

Large idiopathic esophageal ulcerations, after thorough evaluation for other infectious agents, have been documented in 4% to 12% of HIV-infected patients with esophageal symptoms, especially those in late stages of AIDS.[57,74,75] Some have suggested that HIV is causative, based on demonstration of HIV by in situ hybridization[76] and positive immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) for HIV p24 core protein in macrophages lining the ulcerations.[77,78] However, HIV PCR was also positive in the majority of the examined patients with CMV esophagitis, and HIV can be cultured in approximately 50% of esophageal specimens from otherwise asymptomatic HIV-infected patients,[79] raising doubt as to whether HIV is the cause of giant ulcerations. These patients may respond to systemic corticosteroid therapy,[76,78,80,81] but treatment also predisposes patients to infectious complications (Table 10.2).

Infections of the Stomach

Given the protective mechanism of low gastric pH, only a limited number of infectious agents have been demonstrated to thrive in the stomach, usually as a result of a high infectious burden or specific evolved protective mechanisms. Not surprisingly, immunodeficient patients are at greater risk for infectious gastritis. Although *Helicobacter pylori* is the most common cause of infectious gastritis, other agents also cause disease (Table 10.3).

Viruses

Cytomegalovirus

Although CMV infection may occur in immunocompetent patients,[82,83] gastrointestinal CMV usually occurs in immunocompromised individuals due to malignant disease, iatrogenic immunosuppression, or AIDS.[84] In these latter settings, CMV infection can be life-threatening, whereas CMV gastritis is generally asymptomatic in many patients with mononucleosis or like syndromes in immunocompetent patients.[83]

Clinical symptoms include epigastric pain, fever, and atypical lymphocytosis. Endoscopically the gastric mucosa is congested and edematous, with multiple erosions and ulcerations.[85] A pattern of hypertrophic gastritis resembling Ménétrier disease and associated with protein-losing gastropathy has been described (commonly in immunocompetent pediatric patients).[86]

TABLE 10.2 Viral Pathogens of Esophagus

Virus	Location	Macroscopic Features	Histology
HSV, VZV	Middle to distal esophagus not extending to stomach (usually involves squamous mucosa)	Multiple small, sharply demarcated, and shallow ulcers	Epithelial ballooning and inclusions at ulcer edge; only inflamed granulation tissue in ulcer bed
CMV	Part of multiple organ involvement (stomach, intestine > esophagus)	Resembles HSV; occasionally one or more giant ulcers	Cytopathic effects involving endothelium, stromal cells, and/or submucosal glands; infection of squamous cells rarely seen
HPV	Esophagus occasionally involved	Normal, elevated lesions, or papillomas	Koilocytosis in squamous epithelium; condyloma, epithelial hyperplasia, or normal-appearing mucosa
EBV	Middle esophagus	Multiple small, deep, and linear ulcers	Similar to oral hairy leukoplakia; epithelial hyperplasia, parakeratosis, and koilocytosis
HIV	Esophageal involvement hard to document	Large ulcers or small ulcers resembling HSV	No specific changes

CMV, Cytomegalovirus; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus; *HPV,* human papillomavirus; *HSV,* herpes simplex virus; *VSV,* varicella zoster virus.

TABLE 10.3 Pathogens Associated With Infectious Gastritis

Etiology	Clinical Setting	Histology
Viruses		
Cytomegalovirus	Immunocompromised patients	Erosive gastritis
Herpes simplex virus	• AIDS	—
Varicella zoster virus	• Cancer • Immunosuppressive therapies	— —
Bacteria		
Streptococcus	• Large intake of alcohol • Upper respiratory tract infection, AIDS, and other immunocompromised states	Necrotizing gastritis —
E. coli	—	—
Enterobacteriaceae		
S. aureus	—	—
M. tuberculosis	Endemic area	Erosive and fibrosing gastritis with necrotizing granulomas
H. pylori	General population	Chronic active gastritis
H. heilmannii	—	—
M. avium	AIDS	Ill-formed granulomas
Actinomyces	—	Suppurative and mass-forming gastritis
Syphilis	Sexually transmitted disease	Diffuse inflammatory gastritis
C. perfringens, *E. coli*, *Streptococcus*, *Enterobacter*, *P. aeruginosa*	• Gastroduodenal surgery • Corrosive material • Gastrointestinal infarction	Emphysematous gastritis — —
Fungi		
Candida	• Cancer • Immunocompromised patient • Severe alcoholism • Corrosive gastritis	Aphthous, or linear ulcerations or even larger ulcers — —
H. capsulatum	• Cancer	Inflammatory mass
Phycomycosis	• Immunocompromised patient • Corrosive gastritis	Ulceration and hemorrhagic necrosis
Parasites		
Cryptosporidiosis	AIDS	Minimal inflammation
S. stercoralis	Immunosuppressed patient (e.g., AIDS, diabetes)	Diffuse mucosal involvement
Anisakiasis	Consumption of raw shellfish	Eosinophilic and granulomatous gastritis
A. lumbricoides	Gastric outlet obstruction	

The biopsy specimens demonstrate mixed inflammatory infiltrates with characteristically enlarged endothelial, stromal, or epithelial cells showing diagnostic owl's-eye intranuclear inclusions. However, cytopathic changes can be difficult to identify, and multiple granular basophilic cytoplasmic inclusions may be seen instead.[87] It has been noted that inclusions are usually observed in the endothelial cells if the mucosa is not ulcerated, whereas they are commonly seen in endothelial and stromal cells when the mucosa is ulcerated.[88]

Gastric involvement with HSV or VZV is rare, particularly in immunocompetent children, but may be associated with severe abdominal pain.[89] Reactivation of infection acquired at an early age is often the result of radiation therapy, chemotherapy, or malignancies in immunocompromised patients.[90] In the latter the infection is particularly dangerous, with a 50% to 60% fatality rate despite the use of antiviral therapy. Symptoms include nausea, vomiting, fever, chills, and fatigue. Gastroscopic examination shows erosions and multiple small ulcers, at times yielding a

cobblestone appearance, with ulcerated plaques or linear superficial ulcers. Microscopic examination reveals epithelial cells with ground-glass nuclei and eosinophilic intranuclear inclusions surrounded by a clear halo.[91,92] Acute gastritis due to EBV has been reported but is rarely biopsied because it may arise in the setting of infectious mononucleosis. The pathology is characterized by dense and diffuse lymphoid infiltrate with atypical lymphocytes that can mimic diffuse lymphoma.[93] Rare cases of acute measles gastric infection with giant cells of Warthin-Finkeldey type have been described.[94]

Bacteria

Acute Suppurative Gastritis and Emphysematous Gastritis

Acute suppurative gastritis is a rare disorder caused by bacterial infection of the submucosa and muscularis propria. In severe cases, necrotizing gastritis may result.[95] It is associated with ethanol abuse, upper respiratory tract infection, AIDS and other immunocompromised states, and infected peritoneal-jugular venous shunts; it rarely occurs as a complication of surgical biopsy.[96,97]

Patients are acutely ill with acute upper abdominal pain, peritonitis, purulent ascitic fluid, fever, and hypotension. The outcome is frequently fatal. Ultrasonography and CT may establish the diagnosis.[98] Gastroscopic examination reveals a thick and edematous gastric wall. The mucosa may be granular with a dark green exudate. The diagnosis is commonly delayed, and the mortality rate is high. Gastric resection combined with broad antibiotic therapy directed against the most common organisms is the treatment of choice.

Microscopic examination reveals an acute suppurative inflammation and edema of the submucosa and necrosis of muscularis propria with numerous gram-positive and gram-negative organisms. Offending organisms include *Streptococci*, as well as *S. aureus*, *Escherichia coli*, *Enterobacteriaceae*, and other gram-negative bacilli.[99] Intravascular thrombosis is common, and extensive mucosal and mural necrosis can be seen.

Emphysematous gastritis is a rare variant resulting from infection by gas-forming organisms, most commonly *Clostridium perfringens*, *E. coli*, *Streptococcus*, *Enterobacter*, and *Pseudomonas aeruginosa*. Predisposing factors include gastroduodenal surgery, ingestion of corrosive material, gastroenteritis, and gastrointestinal infarction. In most cases, radiographic studies show gas bubbles conforming to the contours of the stomach.

Mycobacterium tuberculosis

Since the introduction of pasteurization, gastric infection due to *M. tuberculosis* has been rare; it usually represents a secondary spread from pulmonary tuberculosis. The patient commonly presents with abdominal pain, nausea and vomiting, fever, and weight loss.

Endoscopic examination may demonstrate multiple bleeding ulcers or erosions; a narrowed, deformed antrum; and possibly gastric outlet obstruction due to a thickened wall. Microscopic examination may show an ulcerated mucosa, but the presence of necrotizing granulomas is diagnostic. The presence of acid-fast bacilli (AFB) can be confirmed on acid-fast staining,[100] but PCR for *M. tuberculosis* can also be contributory. However, the mere demonstration of granuloma is not sufficient to establish the diagnosis, since gastroduodenal Crohn disease and sarcoidosis should be entered into the differential diagnosis.

Although *Mycobacterium avium* complex (MAC) infection is common in AIDS patients, the stomach is rarely involved. Gastric MAC may be associated with refractory chronic ulcers. Presenting symptoms include fever, night sweats, anorexia, weight loss, diarrhea, and abdominal pain. Abdominal CT scans may show mesenteric lymphadenopathy.[101] Microscopic examination shows a lamina propria expanded by numerous foamy histiocytes. These aggregate in ill-formed, nonnecrotizing granulomas. AFB, often numerous, can be highlighted by Ziehl-Neelsen or Fite stain.

Actinomycosis

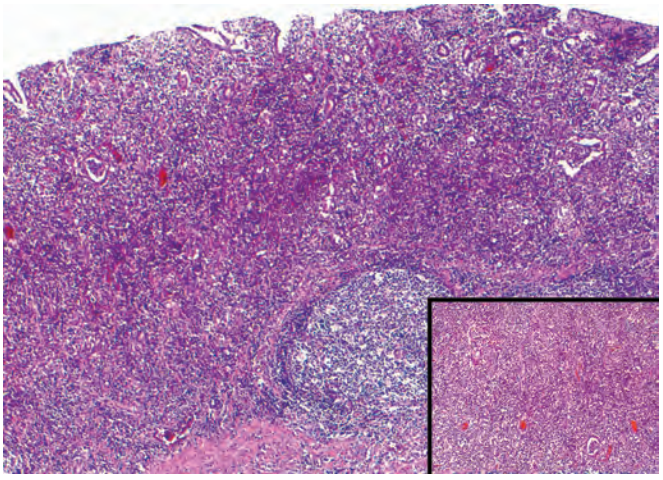
Primary gastric actinomycosis is a rare, chronic, suppurative disease. Symptoms include fever, epigastric pain, and bleeding. The stomach reveals a large, ill-defined ulcerative mass that mimics malignancy. Histologically, collections of neutrophils and sinuses are seen. A pattern of xanthogranulomatous gastritis also has been reported.[102] *Actinomyces*, a gram-positive, filamentous, anaerobic bacterium, is best observed with a silver stain. The finding of sulfur granules containing long, filamentous, gram-positive bacilli is diagnostic.[103]

Syphilis

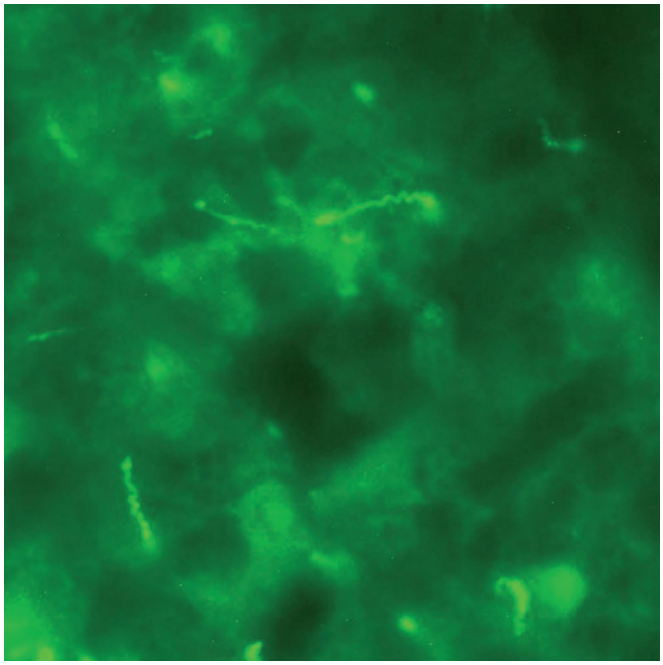
Syphilitic infection of the stomach is rare but on the rise. Given the protean manifestations of this disease, gastric involvement in secondary or tertiary syphilis remains a diagnostic challenge. Endoscopic examination may show numerous shallow ulcerations with surrounding erythema, or a nodular mucosa with enlarged, thickened folds, suggestive of adenocarcinoma or lymphoma. Microscopic examination reveals severe gastritis with dense plasma cell infiltrates and varying numbers of neutrophils and lymphocytes. Gland destruction, vasculitis with endothelialitis, and ill-formed granulomas are seen. Warthin-Starry silver stain or modified Steiner stain may reveal numerous spirochetes. However, in many cases, immunohistochemistry, immunofluorescence studies, and PCR are required to establish a definitive diagnosis (Figs. 10.8 and 10.9).[104]

Fungi

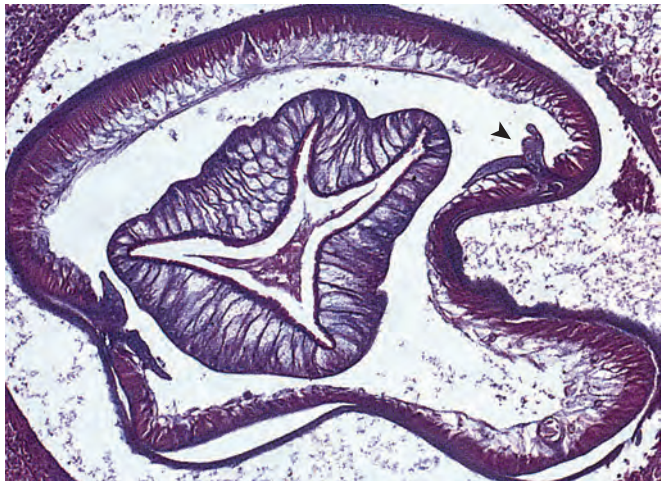
Fungal colonization of the stomach may be seen in patients with underlying malignancy or in immunocompromised patients



• **Figure 10.8** Syphilitic involvement of stomach. The mucosa is markedly thickened, and the normal architecture is effaced by a lymphoplasmacytic infiltrate *(inset)*.



• **Figure 10.9** Syphilitic involvement of stomach. Immunofluorescence demonstrates numerous *T. pallidum*.



• **Figure 10.10** Anisakiasis. The helminth displays characteristic Y-shaped lateral cords *(arrowhead)*.

treated with antibiotics or corticosteroids. *Candida* infection also occurs in alcoholic patients and in those who have ingested corrosive chemicals. The organisms can be found on H&E-stained sections but are more readily seen with PAS with diastase or silver stains. The mucosa shows multiple, aphthous, and linear ulcerations, or single or multiple larger ulcers. Microscopically the superficially necrotic, fibrinopurulent mucosa is infiltrated by yeast and pseudohyphae.

Fungal contamination of peptic gastric ulcer with *Candida* spp. is not uncommon and is likely of limited clinical significance, although it remains a matter of debate. These ulcers tend to be larger and are more often suspected of being malignant.[105]

Gastric histoplasmosis is a rare condition that may manifest with hypertrophic gastric folds, masses mimicking adenocarcinoma, or small ulcerations.[106] Biopsy shows intense infiltration by macrophages containing *H. capsulatum*. Fatal hemorrhage from gastric ulcer has been reported.[107]

Gastric phycomycosis is rare and highly lethal. It is classified as either invasive or noninvasive. The former type is characterized by deep invasion of the gastric wall and blood vessels. Ulceration and hemorrhagic necrosis of the mucosa and wall, with infiltrating pigmented nonseptate hyphae, are seen.[108,109]

Parasites

Gastric parasitic infections are rare and are reported in specific clinical settings. For example, cryptosporidiosis has been reported in AIDS patients.[110,111] The stomach is rarely affected by *Strongyloides stercoralis*, although colonization of peptic ulcers has been reported, and diffuse mucosal involvement may be seen in immunosuppressed patients. On biopsy, the parasite is easily identified infiltrating the mucosa.[112]

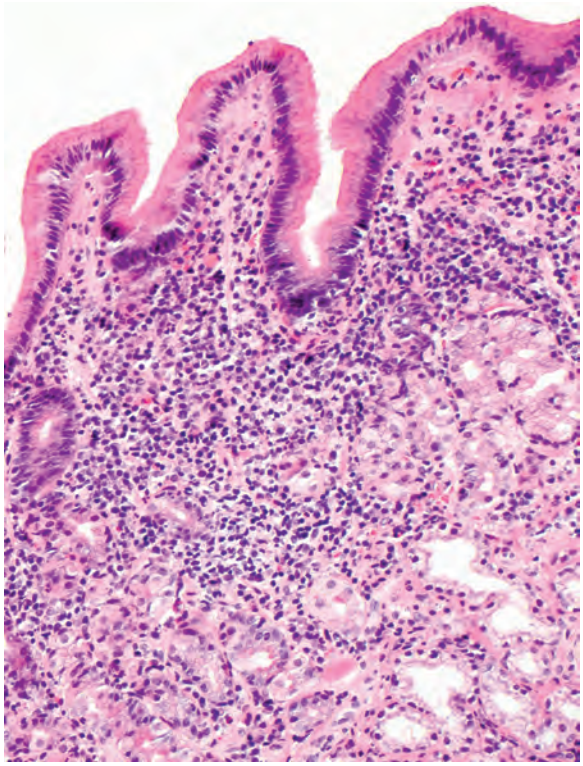
Gastric invasive anisakiasis is commonly acquired after ingestion of raw shellfish containing the nematode larvae of *Anisakis*, although cases caused by *Pseudoterranova* spp. have also been reported in the United States. Patients typically present with epigastric pain as the parasite migrates into the gastric wall. Peripheral blood eosinophilia is not universal. Small hemorrhagic erosions are detected endoscopically, and microscopic examination shows either a phlegmonous reaction or an eosinophilic abscess with granulomatous features. The offending nematode larvae of *Anisakis* can be identified based on the presence of characteristic Y-shaped lateral cords and a Y-shaped intestinal lumen at midsection (Fig. 10.10).[113] The lateral cords of *Pseudoterranova* are characteristically butterfly shaped. Both lack the lateral alae of *Ascaris* larvae.

Rare examples of gastric infection by *Ascaris lumbricoides* have been reported in association with outlet obstruction caused by adult worms.[114]

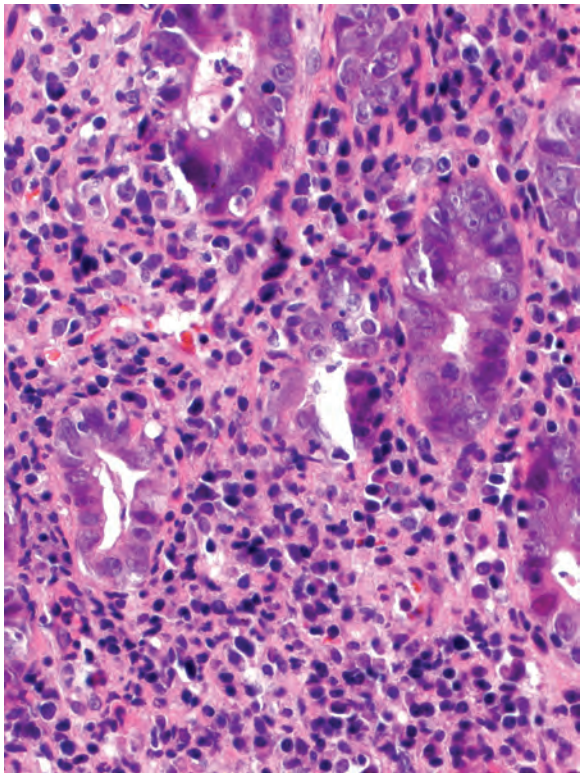
Helicobacter pylori—Associated Chronic Gastritis

H. pylori is a gram-negative rod that has a propensity to infect the gastric mucosa. *H. pylori* are 3.5 μm long and generally comma-shaped or slightly spiral in form.[115] The infection is usually acquired in childhood and causes chronic gastritis if the organisms are not eradicated. The majority of infected patients carry and transmit *H. pylori* without any symptoms. The prevalence of the infection varies worldwide, ranging from less than 15% in some cohorts to almost 100% in underdeveloped countries.[116,117] Currently, despite a decrease of infection worldwide, at least 50% of the world's population is actively infected. The infection is transmitted by close personal contact.

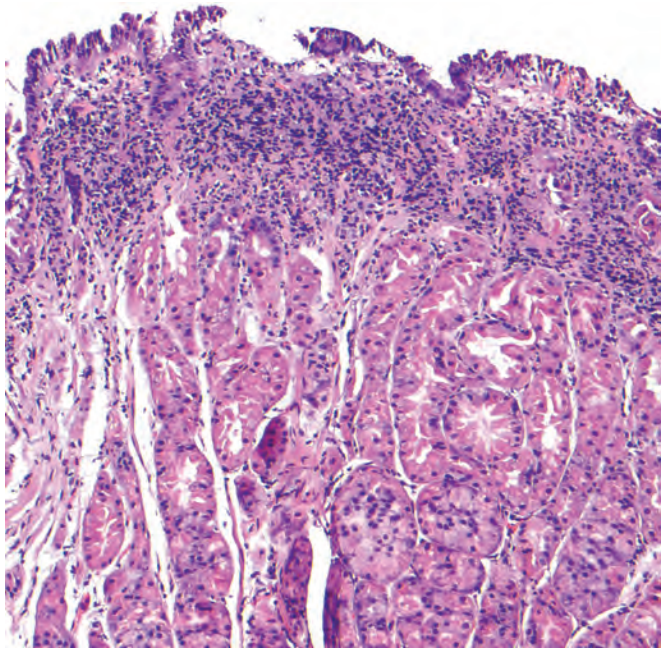
H. pylori produces active and chronic gastritis. The lamina propria is expanded by a mononuclear cellular infiltrate, lymphocytes, and plasma cells, and lymphoid aggregates and follicles can also be seen (Figs. 10.11 and 10.12). If lymphoid inflammation is prominent, the term *follicular gastritis* has been adopted.[118] Normally, lymphocytes rarely enter the epithelium. Various degrees of neutrophilic infiltrate, reported as evidence of activity, may be seen. There are significant variations among infected individuals, based on host factors and the strain of infection and its associated virulence factors.[119,120] Among the latter, the presence of cytokeratin-associated gene (cagA) is associated with increased inflammation and risk of developing intestinal metaplasia. *H. pylori* swim freely within the gastric mucus layer that



• **Figure 10.11** *Helicobacter pylori* infection. Low magnification of antral mucosa reveals a superficial band of dense chronic inflammation that is characteristic.



• **Figure 10.12** *Helicobacter pylori* gastritis. A dense lymphoplasmacytic infiltrate is seen around the foveolae, together with scattered neutrophils.



• **Figure 10.13** *Helicobacter pylori* infection of fundic mucosa. This is the characteristic appearance in a patient receiving proton pump inhibitor therapy without eradication of the bacteria.

overlays the apical side of the gastric surface cells, as well as in the foveolae.[120-122] In patients taking proton pump inhibitors, the organism translocates deep into the gastric glands (Fig. 10.13). Although *H. pylori*–induced damage is related to humoral and cell-mediated immune mechanisms, the morphologic activity varies significantly, from mild, with rare neutrophils seen, to severe, with glandular microabscesses.

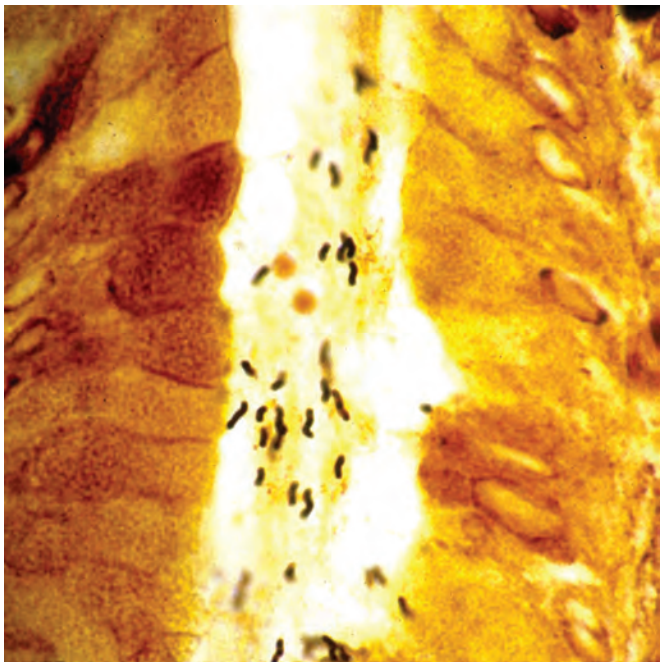
H. pylori–associated chronic gastritis includes a heterogeneous group of clinical and pathologic changes. The most common phenotype observed at our institution is a mild antral gastritis or pangastritis with limited disruption of gastric acid secretion and usually no symptoms. A second syndrome, the duodenal ulcer phenotype, shows a marked antral gastritis with high gastric secretion and preserved fundic mucosa. The patients commonly experience symptoms of duodenal ulceration.

Although approximately 90% of infected patients experience spontaneous regression,[123-125] some gastritis progresses with time. Overall the lifetime risk of ulcer disease is 5% to 10%, and these patients have a 3 to 6 times increased risk of developing gastric cancer.[126] When chronic gastritis develops, the mucosa undergoes changes that include glandular atrophy and intestinal metaplasia of the body fundus mucosa, accompanied by hypochlorhydria or achlorhydria. These changes increase the risk of gastric dysplasia and carcinoma. It has been calculated that once chronic atrophic gastritis develops, the patients have up to a 16-fold increased risk of developing gastric cancer. They are also at risk for development of mucosa-associated lymphoid tissue (MALT) lymphoma (extranodal marginal zone B-cell lymphoma).

In most cases, *H. pylori* can be identified in H&E-stained sections, although special stains (e.g., Giemsa, silver stains) and specific immunohistochemistry can aid in the detection of bacteria (Fig. 10.14).

Helicobacter heilmannii is a rare cause of diffuse, antral-predominant gastritis. The organism, in the past reported as

Gastrospirillum hominis, is larger than *H. pylori* and shows corkscrew-like spirals. The infection by the gram-negative rod may be acquired from contact with domestic pets (dogs, cats) and cattle. The patients present with epigastric pain or dyspepsia, and the endoscopy commonly shows patchy antral erythema, but in some cases pangastritis can be observed. Histologically a mild chronic, active gastritis is confined to the antrum with a 5- to 9-μm long, tightly coiled organism with corkscrew appearance present on the epithelial surface.[127] *H. heilmannii* infection has been associated with the development of MALT lymphoma but not with gastric cancer. Special stains used for *H. pylori* also highlight this organism (Fig. 10.15).[128-131]



• **Figure 10.14** Silver stain demonstrating numerous curved *H. pylori*.

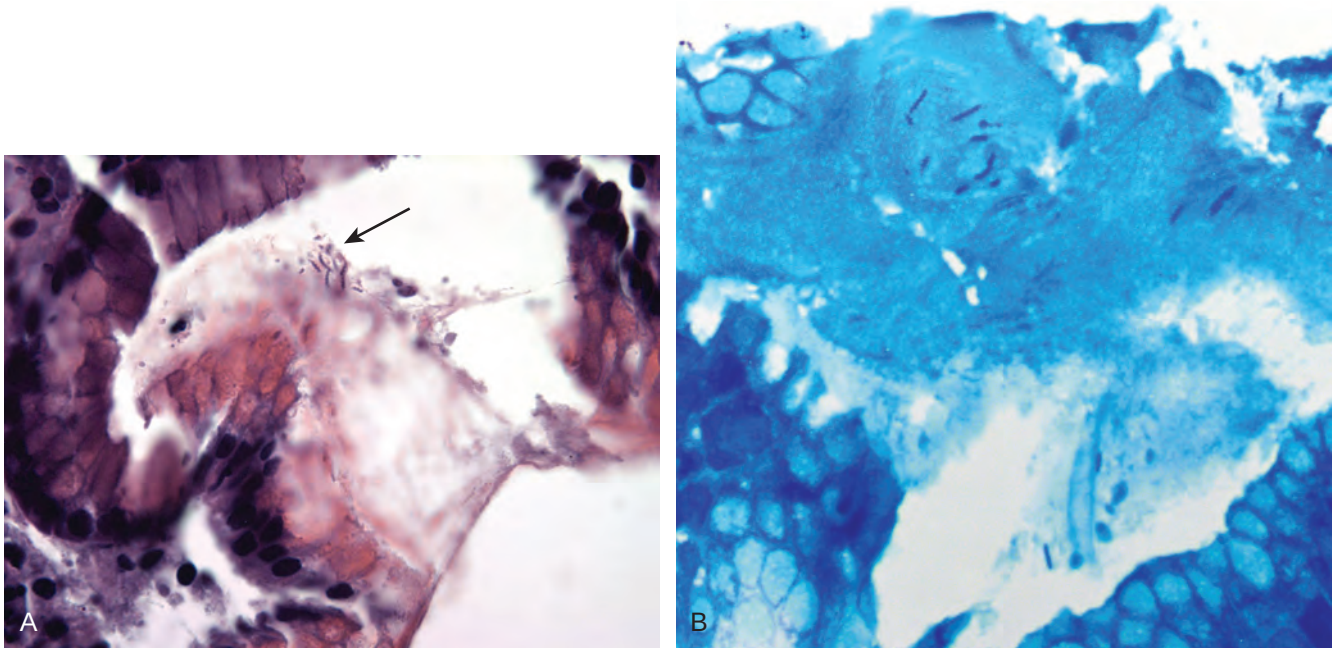
Infections of the Small Bowel

Bacteria

Escherichia coli

Although most *E. coli* strains are harmless, some can cause serious food poisoning. The harmless strains belong to the normal intestinal flora and benefit the host by producing vitamin K2[132] or by preventing the establishment of pathogenic bacteria within the intestine.[133,134] Virulent strains of *E. coli* that can cause gastroenteritis are classified on the basis of serologic characteristics and virulence properties. They are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), enteroadherent *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC). Of these, EPEC, EIEC, and EHEC efface or invade the enterocyte, whereas ETEC and EAggEC are noninvasive and induce diarrhea by producing enterotoxins or a hemolysin or both. EAEC are neither invasive nor toxigenic[135]; they avidly adhere to the epithelial brush border via specific receptors, and the histology of the intestine in infected individuals is often normal. Of the invasive forms, EPEC preferentially colonize the small intestine before causing diarrhea.

Enteropathogenic *Escherichia coli*. EPEC is a leading cause of infantile diarrhea in developing countries, and some types of EPEC are also an important cause of traveler's diarrhea. The mucosal biopsy reveals moderate-to-severe damage with irregular atrophy of surface epithelium and subnuclear vacuolization of crypt epithelium. Ultrastructural study reveals bacteria adherent to enterocytes with flattening of microvilli, loss of the cellular terminal web, and cupping of the plasma membrane around individual bacteria—characteristic attachment–effacement lesions.[136]



• **Figure 10.15** *Helicobacter heilmannii*. The thick, elongate, corkscrew bacteria are demonstrated in hematoxylin and eosin–stained section (**A,** *arrow*) and with thiazine stain **(B)**.

Salmonella

Salmonella typhi usually causes a mild, self-limited illness, but older adults, infants, and immunocompromised patients may develop a serious course with sepsis and death. A worrisome feature is that there is growing evidence showing that multidrug resistant *S. typhi* strains are increasing worldwide.[137] Nontyphoid species (*Salmonella paratyphi*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella muenchen*, *Salmonella anatum*, and *Salmonella give*) infections result in milder, self-limited gastroenteritis.

Based on a study that examined intestinal function and jejunal biopsy specimens obtained soon after the recovery phase from acute disease in 29 patients with *Salmonella* infection, normal or near-normal small-intestinal histology was seen in more than 90% of cases, despite defective absorption, which was transiently observed in 50%. The remaining patients developed partial villous blunting with increased inflammation in the lamina propria and surface epithelium associated with reactive epithelium.[138] If the disease manifests with severe gastrointestinal bleeding that requires surgical intervention, *Salmonella* spp. commonly involve the ileum and cecum. In these cases the bowel wall is thickened, and the mucosa displays erosions and ulcers that can be aphthous, linear, discoid, or full thickness in nature (Fig. 10.16A). Perforation with or without fistula formation may be seen.[139]

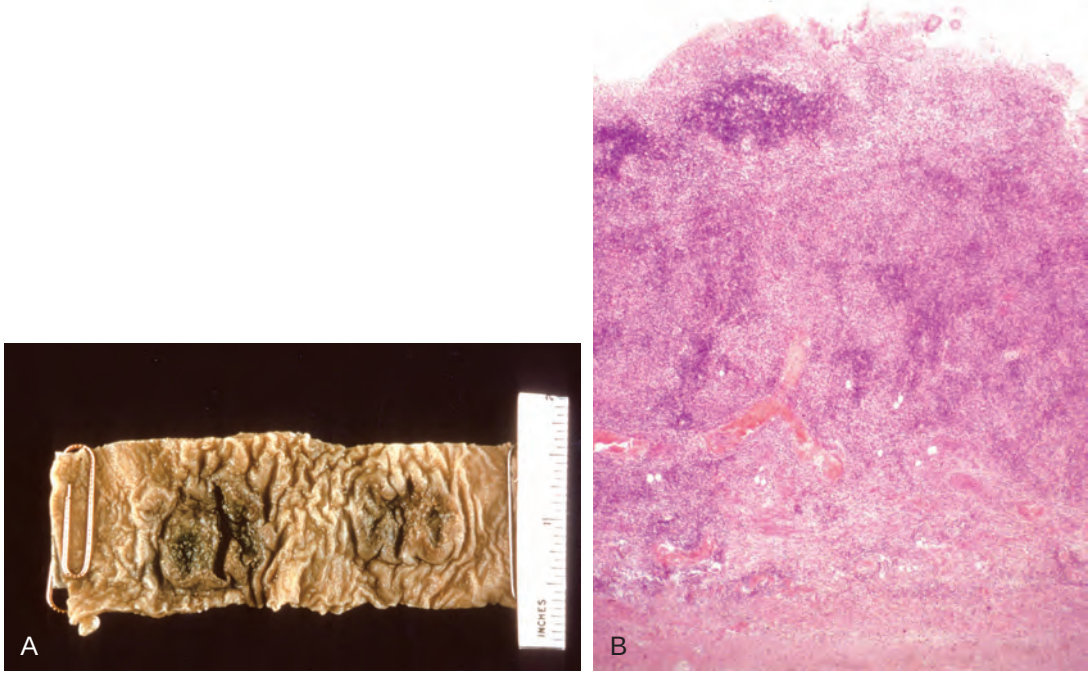
Microscopically there is ileal and cecal mucosal thickening resulting from a predominantly monocytic infiltrate. Low-power examination shows nodular and diffuse areas of infiltration, with the latter present predominantly at the center of the lesion (see Fig. 10.16B). The nodules are of two types. The first is a germinal center rimmed by a ragged and compressed mantle zone. The second and predominant type of nodule consists of uniform sheets of monocyte-macrophages with numerous apoptotic bodies and cellular debris, surrounded by small lymphocytes. The centers of these monocytic-rich foci consist of amorphous eosinophilic debris and degenerating cells.

The interfollicular and diffuse areas are replaced by phagocytic monocytes showing round to irregularly shaped nuclei and intermingled with small, mature lymphocytes. Neutrophils are rare, even in the areas of mucosal ulceration. The inflammatory process breaches the muscularis propria and may extend into the serosa. Small mucosal erosions in uninvolved portions of the ileum reveal a predominantly lymphoid infiltrate with germinal center formation and clusters of monocyte-macrophages, most likely representing early lesions.[139]

The regional lymph nodes show a necrotizing lymphadenitis and marked sinusoidal and paracortical expansion due to a proliferation of monocyte-macrophages identical to those seen in the intestine. Subcapsular sinuses are distended by monocytes but may be compressed or obliterated. Macrophages may be actively phagocytic with many containing intracellular apoptotic bodies and cell fragments. The necrotic foci tend to be well circumscribed with a rim of foamy macrophages that blend smoothly with the reminder of the node.[139]

The closest mimic of *S. typhi* enterocolitis is *Yersinia* infection.[140-142] In both lesions the terminal ileal mucosa and mesenteric lymph nodes are distorted by lymphoid and histiocytic hyperplasia; however, deep, penetrating ulcers and abundant epithelioid granulomas are characteristic features of *Yersinia* infections and not of *S. typhi* enterocolitis.

Also in the differential diagnosis, especially of the nodal involvement, are histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease) and sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). However, these entities rarely involve the intestine as the primary site.[139]



• **Figure 10.16** **A,** Opened, formalin-fixed specimen of terminal ileum from a patient with typhoid fever, showing ulceration of the Peyer patches. **B,** Low-power magnification of the ileum reveals diffuse inflammatory infiltrate transmurally involving the intestinal wall with surface ulceration, as well as nodular lymphoid aggregates.

Vibrio

Eleven *Vibrio* spp. are recognized to cause human infection.[143-148] Infection with *Vibrio cholerae* results in cholera, an important cause of watery diarrhea and dysentery that may lead to significant dehydration and death. Although the disease is widespread, the role of intestinal biopsy is limited because *V. cholerae* is a noninvasive, toxin-producing organism that causes minimal or no histologic changes. The intestinal mucosa appears to be intact, with mucin-depleted, dilated crypts in a background of edematous lamina propria with vascular dilatation and no significant inflammation. Ultrastructural examination reveals widening of the intercellular spaces and alteration of apical junctional complexes prominently in the villus epithelium, whereas blebbing of the microvillus border and mitochondrial changes occur in the crypt epithelium. The extent of the changes correlates with clinical severity.[149]

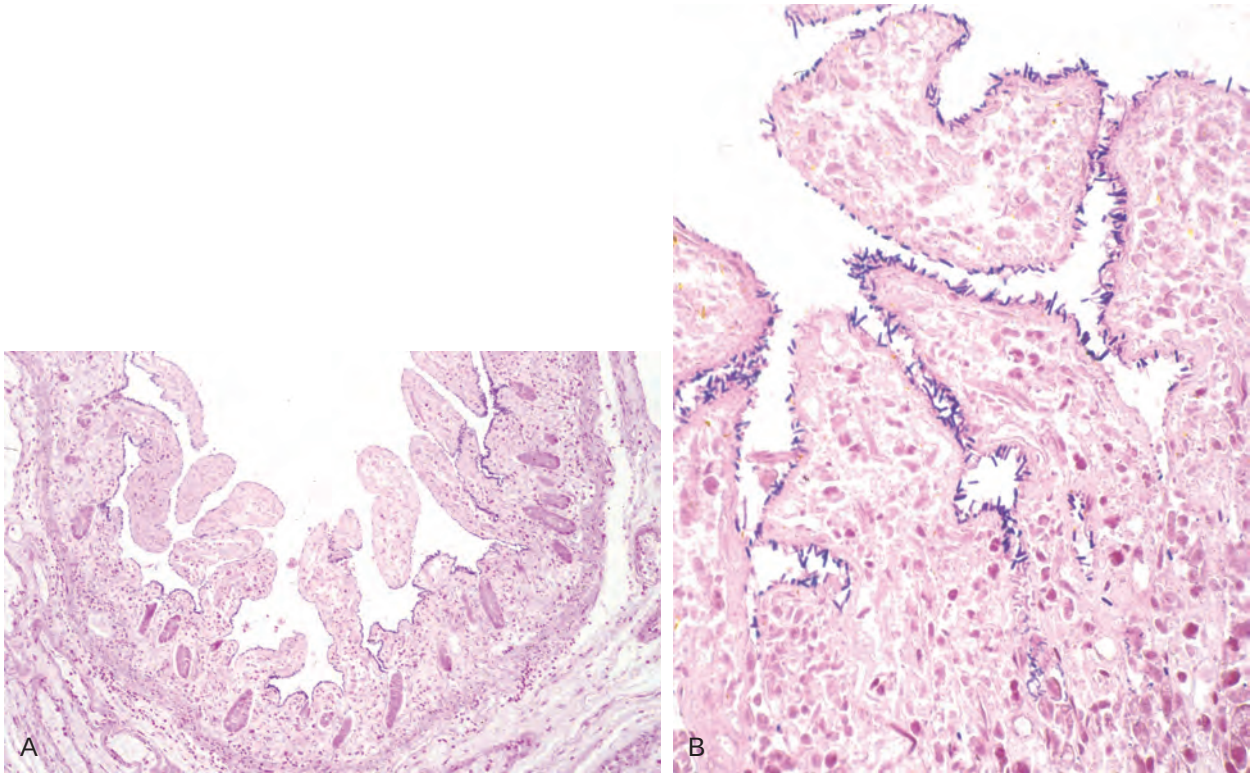
Clostridium

Of several forms of Clostridial infections, enteritis nectoticans is unique because it primarily involves the small intestine, whereas the others are typically seen in the colon.

Enteritis necroticans is a life-threatening infectious disease caused by the commensal organism *C. perfringens (welchii)*, type C, a β toxin–producing strain. It is characterized by segmental necrosis of the proximal jejunum and a high mortality rate if not diagnosed early and appropriately treated. It can be related to food poisoning but is not associated with hospitalization or antibiotic therapy. In addition to antibiotics, advanced cases require surgical resection of necrotic bowel. The disease was first reported as Darmbrand (burnt bowels) in northern Germany after World War II, where starved children and adults developed it after ingestion of large meals of meats and vegetables. It was prevalent between 1944 and 1948 but then disappeared in Europe as the nutritional status of its population improved. In 1963 a similar disease was reported from the Highlands of Papua New Guinea, where predominantly male children and young adults presented with severe abdominal pain after ceremonial feasting on large amounts of sweet potatoes and inadequately cooked pork contaminated with pig intestine. It was termed "pigbel," a pidgin English term for abdominal pain following a pig feast. Nowadays, enteritis necroticans is rarely seen in developed countries, and the majority of affected subjects are diabetics.[150,151] An impaired ability to degrade exogenous toxins due to decreased secretion of pancreatic protease in diabetics and individuals on low-protein diets, or the presence of natural protease inhibitors in sweet potato or soybean flour, together with delayed transit through the small intestine, may contribute to this unique form of clostridial infection.[150]

Histopathologically the lesion starts abruptly in the proximal jejunum and usually extends distally to the ileum and cecum. Affected bowel segments are dilated, edematous, markedly congested, and thickened. Unaffected skip segments may be seen. Sloughing of the necrotic mucosa leaves severely ulcerated lesions, and transmural inflammation may be seen in severe cases.

The pathology varies depending on the severity and duration of disease, but common findings include mucosal necrosis with or without pseudomembrane formation, marked submucosal edema and hemorrhage, and fibrinous or fibrous serosal exudate (Fig. 10.17). Severe cases are accompanied by transmural necrosis and perforation. Pneumatosis may be observed. The necrosis tends



• **Figure 10.17** *Enteritis necroticans.* **A,** Severe mucosal necrosis. **B,** The necrotic mucosa is covered by numerous club-shaped, gram-positive rods. (Courtesy Dr. Elena Brachtel.)

to be more prominent at the crown of plicae circulares, whereas the mucosa may appear normal at the vale of plicae circulares.[151] The necrotic mucosa is often covered by large numbers of club-shaped, gram-positive rods, ranging in length from 0.5 to 1.2 μm. Immunohistochemistry or PCR for the α and β toxins of *C. perfringens*, type C (designated *cpa* and *cpb*, respectively) confirms the diagnosis.[150,151]

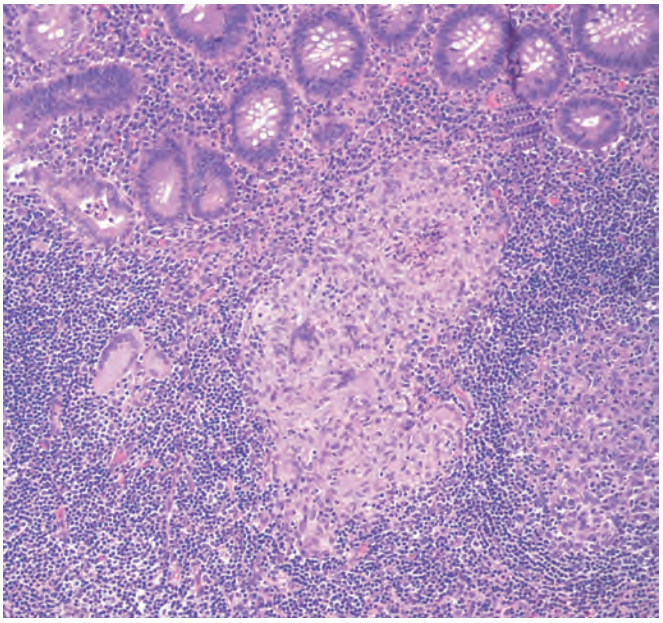
Yersinia

The genus of *Yersinia* includes three species that are pathogenic for humans and rodents: *Yersinia pestis* (the causative agent of plague), *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis.* These gram-negative coccobacilli most often cause self-limited enterocolitis and mesenteric lymphadenitis in humans.[152] An acute abdomen presentation due to acute gastroenteritis, colitis, or pseudoappendicitis resulting from acute terminal ileitis is the most characteristic manifestation of the disease. Patents with iron overload,[153-155] those receiving desferrioxamine,[156-158] those with liver disease,[154] and those who are immunocompromised or debilitated[159] are at an increased risk of severe disease. *Yersinia* can occasionally cause extraintestinal manifestations, such as arthritis and erythema nodosum,[156-158] as well as fulminate septicemia and peritonitis.[153-155,160]

Enteropathogenic *Yersinia* strains have a tropism for lymphoid tissue.[161] Bacteria bind to and invade M cells within the epithelium overlying the lymphoid follicles of Peyer patches.[162,163] Following their entry into the Peyer patches, the bacteria induce a host immune response characterized by infiltration of neutrophils and macrophages.[164] Because of its lymphoid tropism, *Yersinia* infections preferentially involve the ileocecal and appendiceal regions, although any segment of the small or large intestine can be affected.[165] The intestinal wall is congested and edematous. Diffuse or focal aphthous mucosal ulcers can develop. The serosa appears dull and hyperemic. Enlarged lymph nodes contain yellowish microabscesses that may become matted.

Microscopically, severe active enteritis with cryptitis, crypt abscesses, and ulceration can be seen in both *Y. enterocolitica* and *Y. pseudotuberculosis* infections. Crypt hyperplasia occurs throughout the small intestine, with villous atrophy.[141] Sharply demarcated areas of lymphoid hyperplasia contain prominent germinal centers. The follicular ileitis may persist for months. Notably, the mucosa overlying the follicles develops small, punctate, aphthoid ulcers resembling the early mucosal lesions of Crohn disease.[141] The ulcers are covered by fibrinopurulent exudates and large numbers of gram-positive coccobacilli. Epithelioid granulomas with central necrosis and prominent lymphoid cuffing are usually present in the mucosa, submucosa, and lymph nodes but can also be seen on the serosa and lymph nodes (Fig. 10.18). The muscularis propria and serosa may exhibit mixed inflammatory infiltrates including eosinophils. Acute vasculitis and intussusception have been reported to cause segmental bowel ischemia.

The differential diagnosis of *Yersinia* infections includes Crohn disease, and this can be difficult to distinguish on histologic grounds alone. Cultures requiring special media,[165] serologic titers, and PCR assays must be considered in the evaluation of a patient for inflammatory bowel disease, in particular Crohn disease, or a patient with suspected Crohn disease for whom steroid therapy has failed, before more aggressive immunosuppressive therapy is considered.[165] Features favoring a diagnosis of Crohn disease include crypt distortion, muscularis mucosae thickening, and prominent neural hyperplasia; these features are evidence of chronicity.



• **Figure 10.18** *Yersinia* infection. Epithelioid granulomas surrounded by prominent lymphoid infiltrate are seen in the deep mucosa and submucosa. (Courtesy Dr. Laura Lamps.)

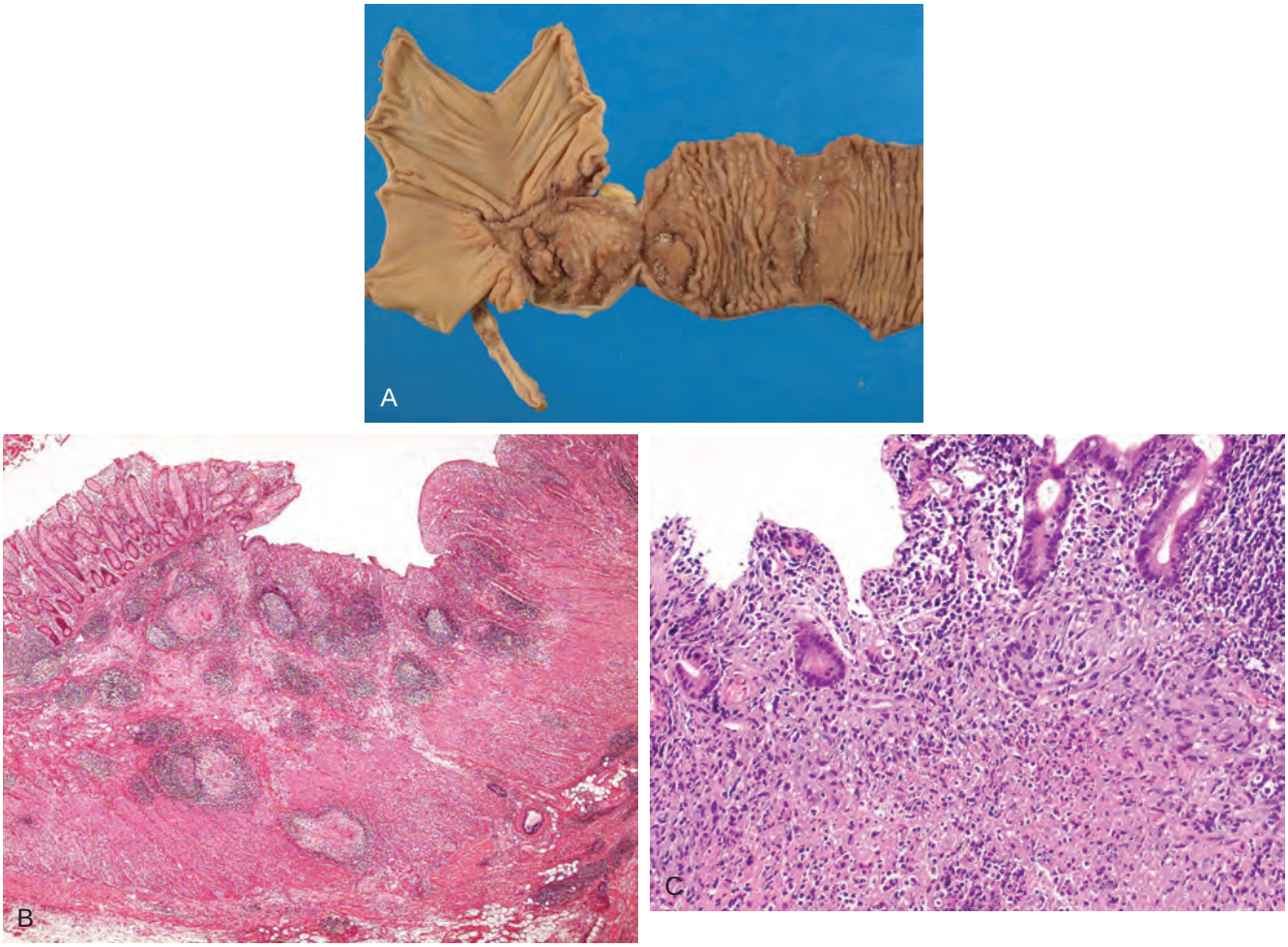
Mycobacterium tuberculosis

In accordance with the distribution of lymphoid tissue, the ileocecal region is affected in 90% of patients with *M. tuberculosis* infection involving the gastrointestinal tract. Grossly, intestinal tuberculosis displays an ulcerative, hypertrophic, or mixed pattern. The ulcerative form, characterized by multiple superficial ulcers, is most common and has been associated with a virulent clinical course and high mortality. The hypertrophic form is least common and mimics Crohn disease because of its scarring, fibrosis, and heaped-up mass lesions.[166] In the ulcerohypertrophic form, the intestinal wall becomes thickened and ulcerated by an inflammatory mass that consists of mesenteric fat, fibrotic tissue, and inflamed lymph nodes. It is centered on and frequently obscures the ileocecal valve.[167]

Tubercles begin in the Peyer patches or lymphoid follicles, giving the mucosa a cobblestone appearance. As disease progresses, they involve the entire intestinal wall with multiple nodules that may also produce serosal and mesenteric "studs." The ulcerative form of the disease begins as ragged ulcers that vary in number and size. In contrast to Crohn disease, tuberculous ulcers are circumferential, with a long axis perpendicular to the lumen; fissure formation that extends into the muscularis propria may be seen.[166] The ulcers may contain AFB, even in the absence of granulomas. The mucosa appears edematous and hemorrhagic.

Hypertrophic lesions are caused by pronounced mural thickening with ulceration and obstruction. Fibrosis, strictures, and stenosis that developed in the healing process of ulcers may be broad, at times several centimeters in length. Epithelioid granulomas with obvious caseation (Fig. 10.19) occur more frequently in ulcerative than in hypertrophic lesions and are distributed throughout the entire thickness of the intestinal wall. Regional mesenteric lymph nodes become enlarged and also contain areas of caseous necrosis.

Isolated organisms can be detected in the granulomas and lymph nodes with the use of special stains and are recoverable in tissue culture. However, tissue samples are positive for AFB in



• **Figure 10.19** **A,** *M. tuberculosis* of terminal ileum and ileocecal valve demonstrating ulcers that are circumferential, with their long axis perpendicular to the lumen. **B,** The intestinal wall is thickened by epithelioid granulomas, lymphoid aggregates, and fibrosis. **C,** High power reveals caseous necrosis and epithelioid histiocytes. (Courtesy Dr. Sachiko Minmiguchi.)

only approximately one-third of cases.[168] In this setting the detection of mycobacterial DNA in formalin-fixed paraffin-embedded tissue by duplex PCR can confirm the diagnosis. Other mycobacteria (e.g., *M. kansasii*, *M. bovis*) can produce similar pathologic features.

Although granulomas are characteristic of tuberculosis, they can be seen in other causes, including fungal infections and Crohn disease. The latter may be difficult to distinguish from tuberculosis; transmural lymphoid aggregates, deep fistulas, and fissures favor Crohn disease (Box 10.1).

Mycobacterium avium-*intracellulare* Complex

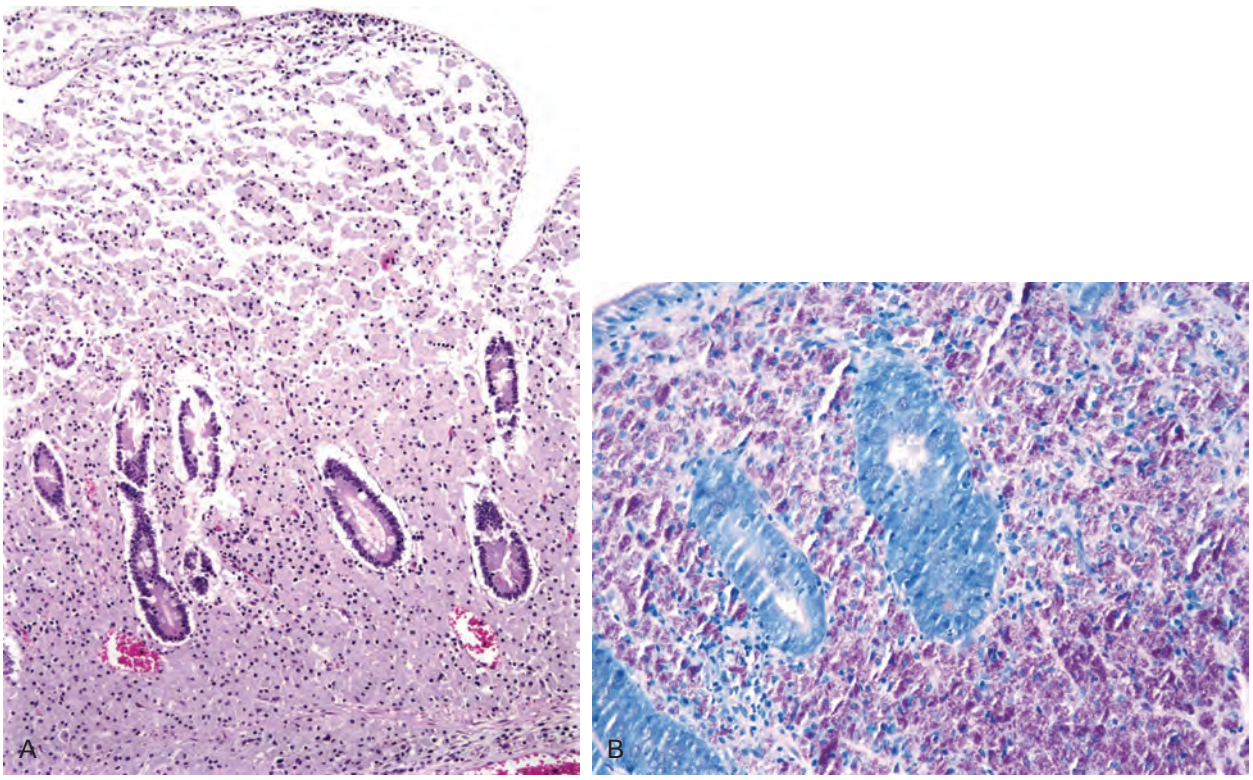
MAC is an AIDS-defining opportunistic infection. Disseminated MAC infection is usually seen in patients with advanced HIV infection, generally those with CD4-positive T-lymphocyte counts lower than 50 cells/μL, and is associated with significant morbidity and mortality.[169,170] Since the induction of antimicrobial prophylaxis and highly active antiretroviral therapy (HAART), the incidence of disseminated MAC infection has dramatically declined, leading to improved survival.[171]

Disseminated MAC infection results from a primary infection, and its common portal of entry in AIDS patients appears to be

• BOX 10.1 Causes of Granulomas in Small Intestine

Crohn disease
Sarcoidosis
Infections
- *M. tuberculosis, M. avium-intracellulare, M. bovis*
- *Yersinia*
- *Histoplasma*
- *Schistosoma*
- *Strongyloides*
- *Actinomyces*
- *Salmonella*
- *Campylobacter*
- *Brucella*

Foreign material: sutures, barium, talc, feces due to fistula or perforation
Pneumatosis intestinalis
Malacoplakia
Langerhans cell histiocytosis



• **Figure 10.20** *Mycobacterium avium-intracellulare* complex infection. **A,** Intestinal villi are obliterated by infiltration of numerous plump macrophages with foamy cytoplasm in the lamina propria. **B,** The lamina propria macrophages are filled with acid fast stain–positive mycobacteria.

the gastrointestinal tract.[172] The mycobacteria penetrate the gastrointestinal mucosa by yet undetermined mechanisms. This occurs rapidly, and solitary organisms can be seen in the lamina propria without apparent mucosal abnormality. Mycobacteria within the lamina propria are phagocytosed by macrophages, but intracellular killing does not occur. Instead, the macrophages become stuffed with organisms as they multiply intracellularly. With continued bacterial replication, the host cell ruptures, leading to the presence of sheets of AFB-laden macrophages. Unimpaired replication of mycobacteria results in massive thickening of the intestinal wall,[173] and infection spreads via mural lymphatics to involve lymph nodes. Mycobacteria replicate in the lymph nodes as well, and eventually the normal histology of the lymph nodes is effaced. Hematogenous dissemination occurs concurrently. Reticuloendothelial organs, such as the liver, spleen, and bone marrow, are the most frequent distant sites.[172]

The common presenting symptoms include diarrhea, fever, weight loss, and abdominal pain. Gastric ulcer, enterocolitis, enteric fistulas, intraabdominal abscesses, and hemorrhage can also be seen as gastrointestinal manifestations.[174] Intussusception secondary to hyperplasia of Peyer patches can occur, and clinical and radiologic pictures may resemble ileal Crohn disease. The duodenum is most frequently involved, followed by the rectum, ileum, colon, esophagus, jejunum, and stomach, for unknown reasons. On endoscopic examination, multiple raised, yellow-white nodules are seen in the duodenum, but the mucosa may appear normal. Other endoscopic findings include, in descending order, ulceration, erythema, edema, friability, confluent nodules, and strictures.[174]

Histologically the nodular lesions show atrophic mucosa with villous blunting, as well as widening of lamina propria by an infiltrate of plump macrophages exhibiting granular foamy cytoplasm (Fig. 10.20). However, the overall architecture of the intestine is usually preserved. Other inflammatory cells, such as lymphocytes, plasma cells, and neutrophils, are sparse, if present. Regional lymph nodes display comparable infiltrates. Epithelioid granulomas are rarely seen[173]; if present they are poorly formed and rarely contain multinucleated giant cells. Small areas of necrosis are present in up to 30% of cases.[175] If necrosis is marked, the infiltrates are easily recognized on H&E stain even on low-power magnification, but detection of focal involvement may require special stains. In addition to Ziehl-Neelsen and Fite stains, the PAS stain highlights mycobacteria. In contrast to *M. tuberculosis* infection, mycobacteria are usually present within macrophages and not in giant cells or areas of caseous necrosis.

Whipple Disease (Tropheryma whipplei)

Whipple disease is a multisystem disease that results from infection with a gram-positive rod-shaped bacterium, *T. whipplei*. It is a rare disorder, and only approximately 1000 cases have been reported to date. Although it occurs in people of all ages, it typically affects middle-aged white men.[176] *T. whipplei* appears to be present in the general environment, although neither its source nor its mode of transmission is well established. Because an association between Whipple disease and *Giardia lamblia* infection has been reported, it is plausible that both microorganisms occupy the same ecologic niche.[177] Considering that many people may be exposed to *T. whipplei* but the disease develops in only a fraction of them, it is likely that undefined predisposing immune factors exist.[178]

Several studies have demonstrated defective function of macrophages, with inability to degrade bacterial antigens efficiently,

most likely due to inadequate production of interleukin-12 (IL-12),[179] which may lead to diminished production of interferon-γ by T cells and defective macrophage activation. A decrease in IL-12 production may prevent the development of an effective type 1 helper T-cell immune response and favor a shift toward a type 2 helper T-cell response. Replication of *T. whipplei* in macrophages is associated with apoptosis of the host cell that correlates with the expression and release of IL-16.[180]

Whipple disease is characterized by two stages: a prodromal stage and steady-state stage. The prodromal stage is marked by protean symptoms, along with chronic nonspecific findings, mainly arthralgia and arthritis. The steady-state stage is characterized by weight loss, diarrhea, or both, and occasionally by other manifestations secondary to other organ involvement.[181] The average time between the prodromal and the steady-state stage is 6 years. Patients on immunosuppressive therapy, such as corticosteroids or tumor necrosis factor antagonists, may experience more rapid clinical progression.[182,183]

Whipple disease involves the small intestine, and jejunum and ileum more frequently than the duodenum.[184] On endoscopic examination, pale yellow, shaggy mucosal changes, attributed to lipid deposits, alternating with eroded, erythematous, or mildly friable mucosal patches, are often seen in the distal aspect of the duodenum and jejunum.[185,186]

The histologic hallmark of Whipple disease is the presence of macrophages with cytoplasmic PAS-positive, diastase-resistant granules or sickle-form particles; these are known as sickle-form particle-containing cells, or SPC cells. Bacilli may also be seen within and between epithelial cells, especially before the initiation of antimicrobial therapy.[186] Intestinal villi are blunted and distorted by the collections of macrophages. Subtotal or total villous atrophy can be seen in severe cases, whereas the villous architecture is relatively normal in about one-fifth of the cases.[186]

The majority of macrophages accumulate in the lamina propria of the villi, just beneath the luminal epithelial basement membrane (Fig. 10.21). A small number of macrophages may also be seen in the pericryptal lamina propria or submucosa or both.[186] Lymphocytes, neutrophils, eosinophils, and plasma cells can be seen amidst the macrophage collections. Although rare, nonnecrotizing granulomas composed of epithelioid cells, which are PAS-negative in 40%, may be present in the mucosa, lymph nodes, and other organs, resembling sarcoidosis.[185,187,188] Lymphatic obstruction can cause dilatation of lacteals leading to lipid deposits in the mucosa.

Antimicrobial treatment may affect the histology of the disease. With a decrease in the number of PAS-positive macrophages, a change occurs in the pattern of mucosal involvement. It may go from diffuse to patchy; the distribution of macrophages may shift from the villous interstitium to the pericryptal interstitium; and the intensity of PAS-positive staining may diminish.[185]

PAS-positive macrophage collections in the intestinal lamina propria can be seen in other entities. These include *M. avium-intracellulare* infection, histoplasmosis, chronic granulomatous disease, xanthomas, storage disease, and common variable immunodeficiency. Although the macrophages may appear similar in these conditions, lipid deposits are seen only in Whipple disease. Whipple disease can be differentiated from MAC infection with negative acid-fast staining. Importantly, given that 10% of the patients do not show characteristic histologic features,[185] PCR assay is required to confirm the diagnosis in clinically suspicious cases with nondiagnostic histology (Table 10.4).

TABLE 10.4 Comparison of *Mycobacterium avium-intracellulare* Infection and Whipple Disease

	M. avium-intracellulare	Whipple Disease
Distribution in small intestine	Duodenum > ileum > jejunum	Jejunum and ileum > duodenum
Macroscopic features	Yellow-white nodules or normal	Erythema alternating with pale yellow, shaggy mucosal changes
Villous architecture	Blunted or normal	Blunted
Lacteals	Not dilated	Dilated
Lipid deposits	–	+
PAS stain	+	+
AFB stain	+	–

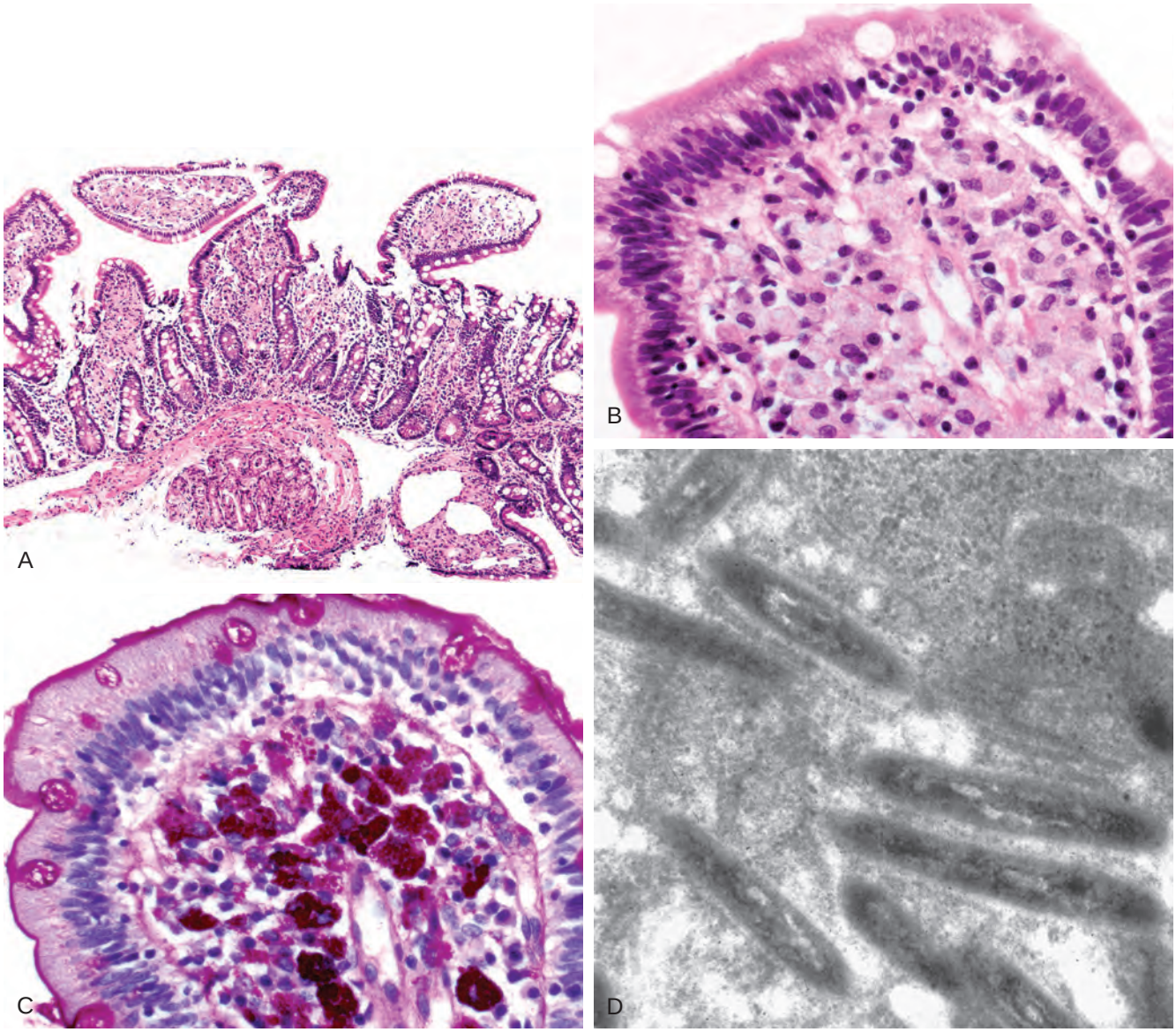
AFB, Acid-fast bacilli; *PAS,* periodic acid–Schiff.

Tropical Sprue

Tropical sprue is an intestinal malabsorption syndrome that affects residents and visitors to the tropics, including Puerto Rico, the Caribbean, northern South America, West Africa, India, and Southeast Asia.[189] Although its etiology and pathogenesis remain unclear, an infectious cause is strongly suspected because of the occurrence of tropical sprue particularly in rural areas with poor hygiene, susceptibility of visitors from developed countries in endemic regions,[190] and a favorable response to antibiotic therapy.[191] Patients also have been shown to have aerobic bacterial contamination of the small bowel, although no specific microorganisms are common to all patients.[192] In addition, preexisting prolonged orocecal transit time may contribute to the disease process in some patients.[192] Diagnostic evaluation of tropical sprue requires its differentiation from infectious etiologies, especially parasitic diarrheal diseases and Whipple disease, and from other malabsorption syndromes, such as celiac disease. Jejunal biopsies are necessary to establish the presence of characteristic histology and to exclude other diseases.

Histologic features of tropical sprue consist of incomplete villous blunting of the duodenum, chronic inflammatory cell infiltration (plasma cells, lymphocytes, histiocytes, and particularly eosinophils), and increased numbers of intraepithelial lymphocytes.[193] Nuclear-cytoplasmic maturational dissociation (i.e., nuclear enlargement and decreased mitotic figures) may be observed in the enterocytes.[194,195] The histologic findings, although similar to those of celiac disease, are not identical, and total villous atrophy is rare (<10%) in tropical sprue.[196-198] Of note, the ileum displays more marked inflammation and villous blunting than the proximal small bowel.[193]

The disease first involves the proximal small intestine, then spreads distally to the ileum. In the early stage the mucosa may appear normal or may show only increased numbers of intraepithelial lymphocytes. In well-established disease the pathologic changes become at least equally prominent in the ileum.[198] Conversely, celiac disease commonly shows prominent mucosal changes in the proximal small intestine. This difference in disease distribution explains the more common association of tropical



• **Figure 10.21** Whipple disease involving duodenum. **A,** Mucosal biopsy demonstrates partial villous blunting by collections of macrophages in the lamina propria. **B,** Large number of macrophages have accumulated in the lamina propria beneath the luminal epithelial basement membrane. **C,** Periodic acid–Schiff stain highlights intracytoplasmic granular particles that are resistant to diastase. **D,** Electron micrograph shows intact bacilli with characteristic thick walls and intracytoplasmic vacuoles.

sprue with megaloblastic anemia caused by deficiencies of vitamin B_{12} and folate.

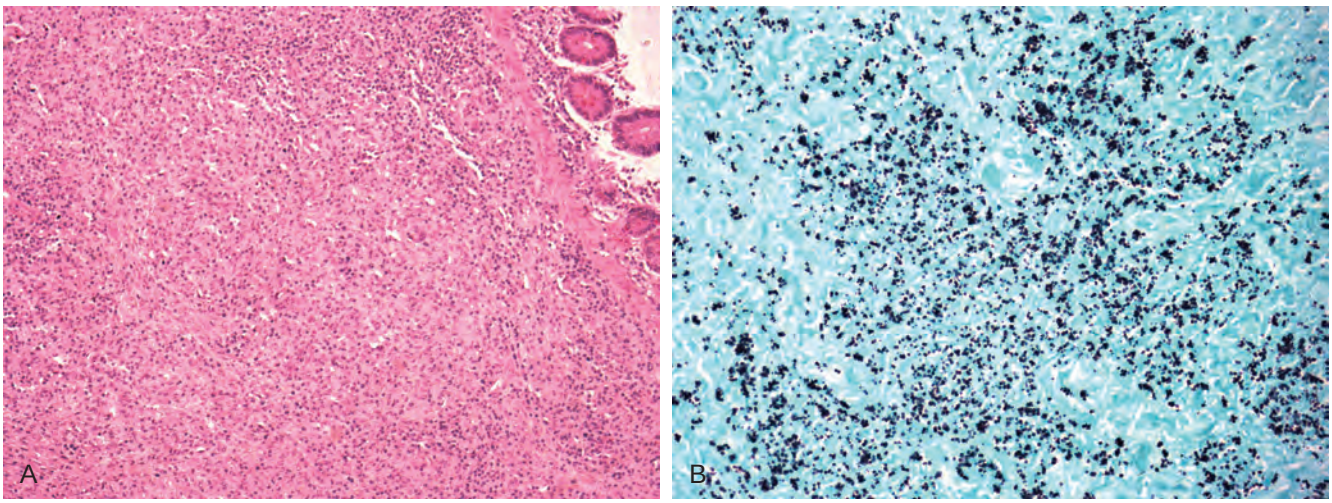
Fungi

Candida and *Aspergillus* spp. account for the majority of fungal infections of the small bowel. In comparison to *Candida*, *Aspergillus* hyphae are often seen in submucosal vessels spreading within the bowel wall in a radiating pattern.[199] Other fungi that can involve the small bowel include *Histoplasma*, *Mucor*, *Paracoccidioides*, and *Penicillium marneffei*.

Of these, *H. capsulatum* is the most common endemic systemic mycosis in the United States; it occurs primarily, but not exclusively, in the central regions of the country. Gastrointestinal involvement occurs in 40% of patients with disseminated disease, and involvement of the small intestine is seen in 27%[200]; however, in contrast to other forms of disseminated histoplasmosis, fever and pulmonary involvement are unusual in gastrointestinal histoplasmosis.[201] Terminal ileal involvement is commonly seen. Deep ulcerative lesions with undercut margins are characteristic of *H. capsulatum* involvement,[201] but masses mimicking carcinoma, pseudopolyps, and lesions resembling xanthomas may also be seen.[202] Histologically, yeast forms ranging from 2 to 4 µm in size lie within well-formed granulomas or macrophages scattered in the lamina propria in a pattern resembling Whipple disease or early enteritis caused by *M. avium-intracellulare* (Fig. 10.22).

Candida

Small intestinal candidal infections are rare in immunocompetent individuals with intact mucosal integrity, but they can be seen in



• **Figure 10.22** **A,** *H. capsulatum* infection of the small intestine shows ulceration of the mucosa with nonnecrotizing granulomas in the bowel wall. **B,** Grocott methenamine silver stain reveals innumerable budding yeast forms.

up to 20% of patients with disseminated infection in autopsy.[203] All *Candida* spp. can infect immunocompromised patients, but there is a higher frequency of disseminated *C. tropicalis*.[27] The gross appearance is variable; it includes mucosal flecks, sloughed membranes, ulcers of varying configuration, and nodular masses. Most invasive *Candida* infections are superficial[199] and must be carefully differentiated from benign colonization. In colonization, *Candida* spp. may be identified embedded in the fibrinopurulent exudate that covers blind loops or in devitalized tissues without invasion of viable tissue. In immunocompromised or chronically debilitated patients, *Candida* can also invade deep into the intestinal wall and gain entry to the microvasculature, leading to ischemia and sepsis.

Emmonsia spp.

The genus *Emmonsia* contains three species that are associated with human disease: *Emmonsia crescens* and *Emmonsia parva* that cause adiarpiromycosis and *Emmonsia pasteuriana*. Infection caused by a new *Emmonsia* spp. (that is most closely related to *E. pasteuriana*) is a newly described HIV-associated dimorphic fungal infection. A report of the *New England Journal of Medicine*[204] presented a series of 13 cases, all in HIV patients and identified in Cape Town, South Africa. Most patients present with fever, loss of weight, anemia, and skin lesions. The extent of the gastrointestinal involvement has not been well described, but we have seen a case with gastric involvement (courtesy Dr. Tomas Slavik).

Viruses

Enteric Virus Infections

Numerous viruses, including rotavirus, enteric adenovirus, Norwalk virus (norovirus), coronavirus, echovirus, enterovirus, calicivirus, and astrovirus,[205-209] can cause gastroenteritis. Diagnosis is made based on viral culture, electron microscopy, ELISA of stool specimens, or genetic probes. Pathologists rarely obtain biopsies from patients with viral enteritis, but if they do, the bowel displays nonspecific findings, such as reactive and degenerative changes of the epithelium and a mononuclear cell infiltrate in the lamina propria. Architectural changes of microvilli may also be seen.

Norovirus

Approximately 20 million Americans develop acute gastroenteritis related to norovirus every year. Most affected patients do not seek care, but 1.6 million do. Approximately 14% of those seeking urgent care need hospitalization, and up to 0.2% of them may die of associated complications.[210] In a large study of 1099 cases, it is shown that the overall incidence of norovirus-associated gastroenteritis (among those who sought medical attention) was 5.6 per 1000 person-years. However, the rate was higher among children younger than 5 years (25.6 per 1000 person-years), followed by that of adults 85 years and older (7.8 per 1000 person-years).[209]

Acute infection with norovirus leads to reversible histopathologic changes in the jejunum, with apparent sparing of stomach and rectum.[211-215] They are rather nonspecific, characterized by villous blunting with mononuclear and polymorpholeukocytic infiltrations in the lamina propria. These changes are generally clear within 2 weeks after the onset of illness, but some changes may last as late as 6 weeks after the onset.

Cytomegalovirus

CMV infection of the small intestine accounts for only 4% of all CMV infections, and CMV infection of the duodenum accounts for 22% of all CMV infections of the gastrointestinal tract.[216] Immunosuppressed patients, including not only those with HIV infection but also those who undergo small bowel transplantation for short bowel syndrome, are prone to CMV infection. The latter group of patients show a high incidence of recurrence due to the heavy immunosuppression required to manage the graft.[217,218] In this setting, PCR analysis of small-intestinal biopsy specimens may be a sensitive method for early detection of CMV and can be useful for preemptive therapy.[219] The clinical manifestations of CMV infection include a wide spectrum of symptoms, such as malaise, anorexia, fever, nausea, diarrhea, abdominal pain, ileus, gastrointestinal bleeding, and perforation. Severe complications, such as hemorrhage and perforation, are often preceded by

vague symptoms for up to 2 weeks.[220,221] The mortality associated with CMV enteritis is related to older age (>65 years) and delay in instituting therapy but not to the anatomic site of the infection.[222]

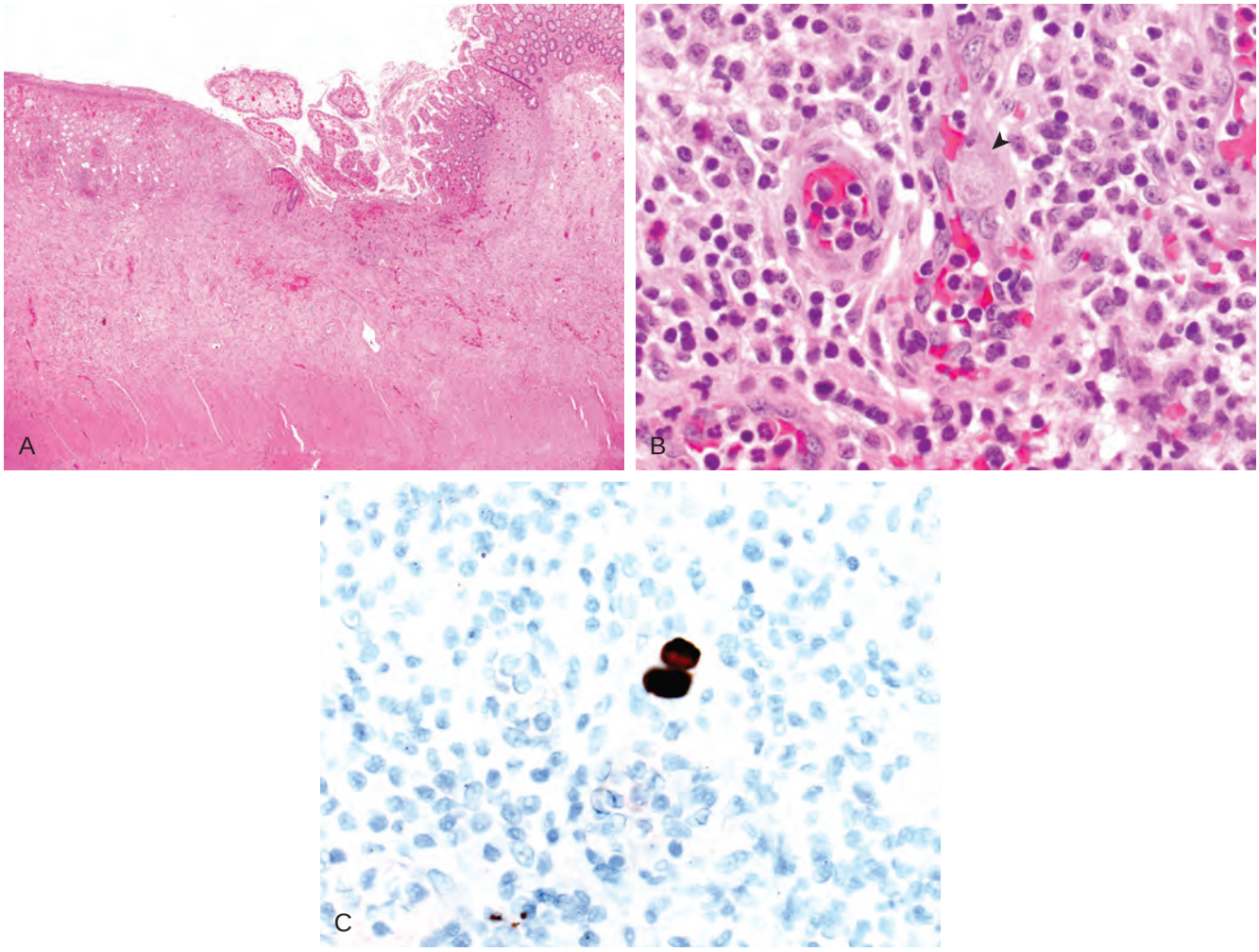
CMV enteritis tends to involve only a single region, rather than causing a panenteric infection.[223] The pathologic changes range from few stromal or endothelial cells exhibiting CMV viral inclusions with no tissue reaction to frank ulceration and perforation. Almost any cell type may be infected, although CMV most frequently involves vascular endothelial or stromal cells (Fig. 10.23). The infected and swollen endothelial cells lead to luminal compromise, with fibrin thrombi formation that results in ischemic mucosal injury and, subsequently, ulceration.[220]

Human Immunodeficiency Virus—Related Enteropathy

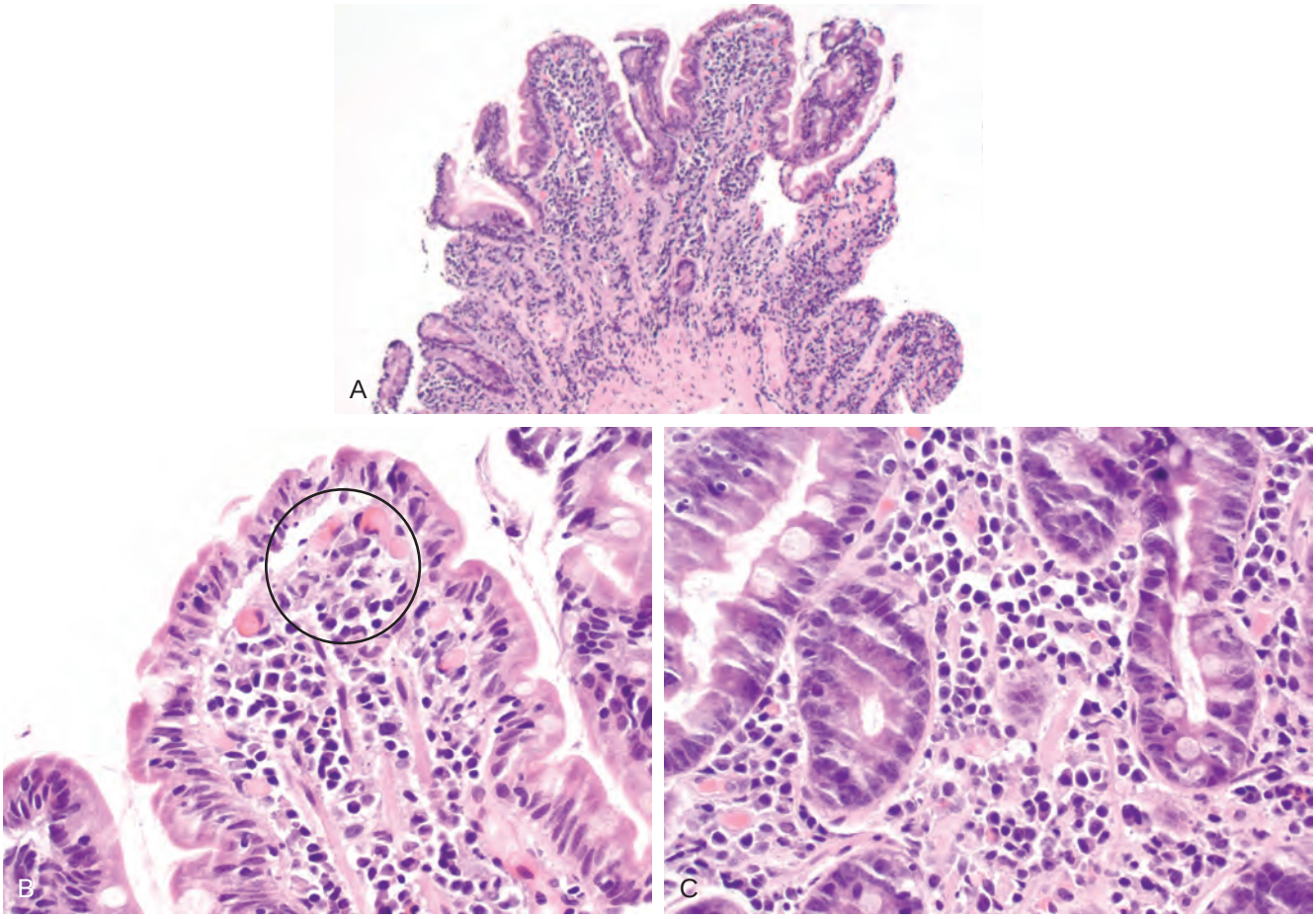
HIV enteropathy is a poorly defined clinical entity that represents pathogen-negative diarrhea in an HIV-infected individual. The possibility that it is related directly to local HIV infection is supported by the detection of HIV proteins and nuclear acids in various cell types of the intestine (i.e., epithelial cells, lymphocytes, and macrophages). Furthermore, combination antiretroviral therapy has led to improvement of gastrointestinal symptoms in these individuals.[224]

Two competing hypotheses have been advanced to explain the diarrheal disease of mucosal HIV infection. It has been suggested that HIV enteropathy is a pathophysiologic consequence of exposure to gp120, which arises from mononuclear cells of the lamina propria, and therefore is independent of epithelial HIV infection.[225-227] Others have argued that the diarrhea is driven by cytokines (tumor necrosis factor and related moieties) released from infected lymphoid cells that can inhibit mucosal ion flux,[228,229] resulting in malabsorption, diarrhea, and weight loss.[230-232]

Vacuolated enterocytes may show conversion from columnar to cuboidal morphology, with cellular apoptosis, seen in a background of either villus and crypt atrophy or hyperplasia (Fig. 10.24). These epithelial changes are associated with mild mucosal inflammation, intraepithelial lymphocytosis, and dilated lacteals. The histologic changes are often mild and disproportionate to the symptoms.[233,234]



• **Figure 10.23** CMV infection superimposed on chronic ischemia of small intestine. The resection specimen of small intestine from a patient with human immunodeficiency virus infection who presented with small-bowel obstruction reveals chronic enteritis with ulceration and mural fibrosis **(A)**. Proliferating capillaries in the submucosa contain endothelial cells exhibiting atypical CMV cytopathic changes, that is, cytomegaly with multiple small eosinophilic granules in the cytoplasm *(arrowhead)* **(B)** that react with CMV immunostain **(C)**.



• **Figure 10.24** Human immunodeficiency virus enteropathy. **A,** Small-intestinal mucosa with partial villous blunting demonstrates apoptosis within the epithelium, as well as in the lamina propria mononuclear cells. **B,** Region of apoptosis is seen *(within circle)*. **C,** The lamina propria shows increased plasma cells.

Protozoa

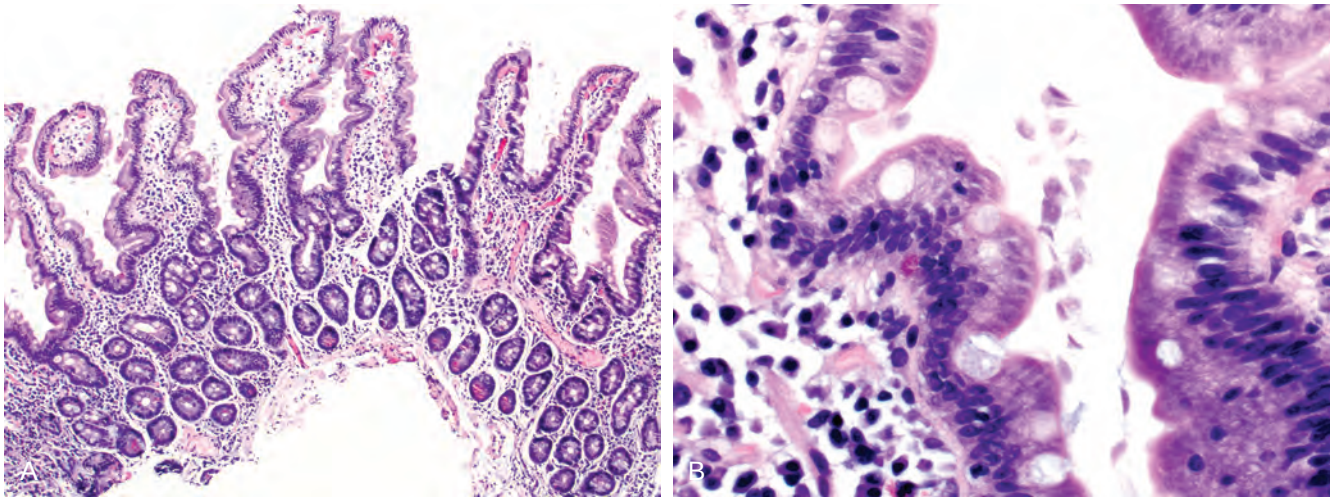
Flagellates

Giardia intestinalis (*G. lamblia*) is the most prevalent human protozoan pathogen and the leading cause of water-borne diarrheal outbreaks in North America. *G. intestinalis* infection is associated with a broad spectrum of manifestations. Serious diarrhea illness with intestinal malabsorption and marked weight loss can be seen in young children and in previously unexposed adults, particularly travelers from low- to high-prevalence parts of the world. Infants and young children may suffer impairment of growth and development. However, in the vast majority of individuals worldwide, the parasite is apparently carried without significant morbidity.[235]

The life cycle of *Giardia* includes two stages: cyst and trophozoite. The former is the infectious form; it is both relatively inert and environmentally resistant. After ingestion, excystation occurs in the duodenum as a result of exposure to gastric juice and pancreatic enzymes, yielding two trophozoites from each cyst.[236,237] These trophozoites replicate in duodenal crypts and in the upper jejunum, reproducing asexually by binary fission.[235] The cysts may be round or oval and measure 11 × 10 μm^2. They each have four nuclei, axonemes, and two median bodies. The trophozoites are 10 to 20 μm in length and 5 to 15 μm in width and have the shape of a teardrop when viewed from the dorsal or ventral aspect. They exhibit two identical nuclei and a ventral concave sucking disk with four pairs of flagella, two axonemes, and two median bodies.[235] Disruption and distortion of the intestinal microvilli occurs at the site where the ventral disk interfaces with the microvillus membrane.[238]

The duodenal and/or jejunal biopsies from infected patients with normal absorption demonstrate preserved villous architecture with or without signs of epithelial damage, and intraepithelial lymphocytes are not increased (Fig. 10.25). Conversely, infected patients with proven intestinal malabsorption are likely to show mild to marked villous blunting, inflammatory expansion of the lamina propria, and increased intraepithelial lymphocytes.[239-242] However, a flattened mucosa is rarely seen. Even without apparent histologic abnormalities, trophozoites are usually found if at least two biopsy specimens are obtained from the duodenum.[239] The organisms can be detected in gastric or ileal biopsies and rarely in the colonic biopsies as well.[240] Nodular lymphoid hyperplasia of the small intestine may also be seen.[239] Patients whose biopsies lack lamina propria plasma cells usually have coexisting hypogammaglobulinemia.

If normal or relatively preserved villous architecture is present, the differential diagnosis includes celiac sprue (Marsh I classification), other infectious agents (e.g., postviral enteritis, cryptosporidia, bacterial overgrowth), nongluten food sensitivity (especially in children), use of nonsteroidal antiinflammatory



• **Figure 10.25** *Giardia* infection of duodenum in a patient who presented with acute onset of diarrhea after a camping trip. **A,** At low power the duodenal mucosa is unremarkable and without significant inflammation or villous architectural distortion. **B,** High power reveals characteristic teardrop-shaped trophozoites between villi.

drugs, autoimmune disorders, immunodeficiency, and inflammatory bowel disease. If villous blunting is present, the most important differential diagnosis is celiac sprue (Marsh III), given that both conditions are often associated with intestinal malabsorption. Other diseases to consider would include tropical sprue, autoimmune enteropathy, immunodeficiency, nongluten food sensitivity, and infectious enteritis or protracted infection of the small intestine. However, with careful review of the small-intestinal biopsies, the offending trophozoites can usually be identified.

Coccidians

Cryptosporidium, *Microsporidium*, *Isospora*, and *Cyclospora* have all been identified in the small intestine of AIDS patients but are also important pathogens in otherwise healthy persons, including infants and children in developing countries (Table 10.5).[243,244] Of these, microsporidia are single-celled, obligate intracellular parasites that have been reclassified from protozoa to fungi but will nevertheless be discussed in this section.[245] Transmission is via the fecal-oral route, either by direct contact or through contaminated water or food.[244]

In immunocompetent individuals, infection is usually self-limited and typically lasts for a few days but may persist for up to 4 weeks. Symptoms often consist of diarrhea, abdominal pain, and malaise. Nausea, vomiting, and fever may be reported. Conversely, immunocompromised patients are at risk for severe and chronic diarrhea that may be life threatening. Weight loss and cramping abdominal pain are proportional to the severity of the diarrhea.

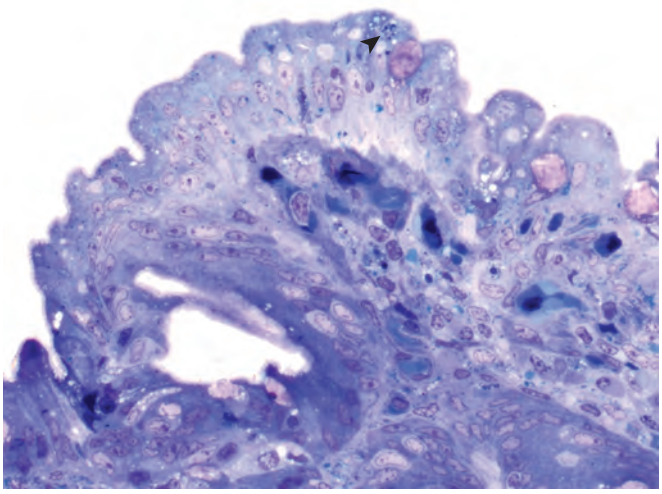
Other enteric opportunistic pathogens may infect profoundly immunocompromised patients.[244] Patients with isosporiasis are likely to have peripheral eosinophilia. Endoscopic examination often shows normal intestinal mucosa or mild erythema, mucosal granularity, atrophy, and superficial erosions. Although all four coccidians primarily involve the mucosa of the small intestine, especially in the distal segment, the colonic mucosa and biliary tract can also be involved in the setting of heavy infection.[244]

Diagnosis is usually based on the examination of stool specimens. Less frequently, examination of duodenal/jejunal aspirates with special stains can establish the diagnosis. Evaluation of small bowel biopsy specimens by electron microscopy may be more sensitive than stool examination for the diagnosis of intestinal microsporidiosis (Figs. 10.26 and 10.27).[246,247] With the development of sensitive diagnostic tests (e.g., ELISA), immunohistochemistry, and PCR,[63] it is likely that low-intensity infections will be more frequently identified, with some probably of little clinical significance.[244]

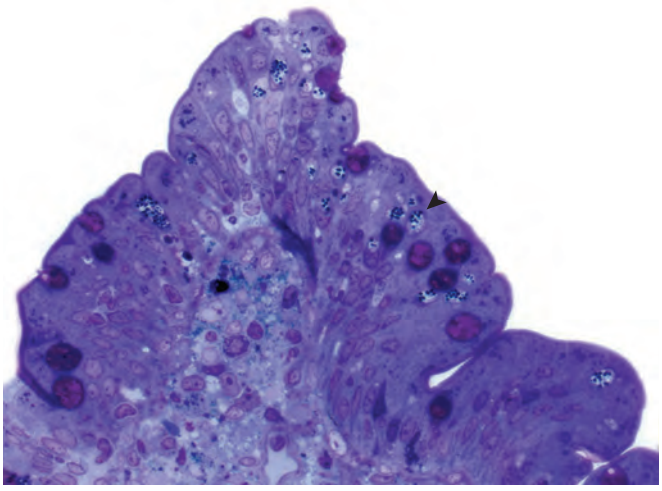
TABLE 10.5 Comparison of Coccidians

Coccidians	Morphology
C. parvum	2-5 μm basophilic spherical bodies protruding from the apex of the enterocytes GMS negative
Cyclospora cayetanensis	8-10 μm crescent or ovoid microorganisms normally located in enterocytes but can be present at the cell surface At some stages of infection, surrounded by a parasitophorous vacuole (EM) GMS negative
Isospora belli	20-μm oval, blue enterocyte inclusions, both perinuclear and subnuclear Rarely present in the lamina propria or in macrophages At some stages of infection, surrounded by a parasitophorous vacuole (EM)
Microsporidia (*Enterocytozoon bieneusi* and *Septata intestinalis*)	Difficult to detect in H&E-stained sections 2-3 μm apical inclusions in the enterocytes

EM, Electron microscopy; *GMS,* Grocott methenamine silver stain; *H&E,* hematoxylin and eosin stain.



• **Figure 10.26** *Enterocytozoon bieneusi* infection of duodenum. A mucosal biopsy shows partial villous blunting, with the enterocytes infected with microsporidia spores *(arrowhead)* (toluidine blue stain).



• **Figure 10.27** *Septata intestinalis* infection of duodenum. Spores are grouped in supranuclear vacuoles of enterocytes *(arrowhead)* (toluidine blue stain).

In all coccidial infections, the small-intestinal mucosa shows nonspecific findings, including normal intestinal mucosa in low-density infections or intraepithelial lymphocytosis, various degrees of villous atrophy, crypt hyperplasia, and mixed inflammation in the lamina propria if the organism burden is high.[248,249] The diagnosis is based on organism morphology by light microscopy and/or ultrastructural examination of biopsy specimens. Microsporidia are difficult to detect in H&E-stained sections, but special stains, such as GMS, PAS, modified acid-fast, modified trichrome, auramine, Warthin-Starry, and Brown-Brenn stains, can aid greatly in the diagnosis (see Table 10.5).

Helminths

The conventional method used to diagnose gastrointestinal helminth infections is examination of stool for ova and parasites, but pathologists may at times encounter these organisms in endoscopic biopsies or in resection specimens. Thus helminths that can affect the small intestine are briefly described in this section.

Enterobius vermicularis *(Pinworm)*

Pinworms are one of the most common human parasites and tend to infest young children. They have a worldwide distribution, but they are more commonly seen in cold or temperate climates and in developed countries. The worms live and reproduce in the ileum, cecum, proximal colon, and appendix; however, pathologists are most likely to encounter them in colonic specimens, usually as an incidental finding in the appendix.

Ascaris lumbricoides

The distribution of ascariasis is worldwide, with the greatest frequency being in moist tropical climates. The mode of infection is fecal contamination of toys, soil, and fingers; contaminated raw vegetables; and drinking water. Usually children are more vulnerable than adults to *Ascaris* infection. Patients may be asymptomatic, or they may complain of vague abdominal pain. However, massive infection with obstruction, perforation with peritonitis, appendicitis, and pancreatobiliary obstruction does occur.[250-252]

Tissue damage may be seen primarily at the sites of attachment, but adult *A. lumbricoides* in the small intestine usually produces no diagnostic lesions.[250-252] This nematode (roundworm) is characterized by its large size, even in its larval form. The female worms reach lengths of greater than 30 cm; males are somewhat smaller. The helminth displays an annulate, multilayered cuticle with prominent hypodermis and conspicuous lateral cords. The female pseudocoelem includes paired genital tubes that contain innumerable ova which, when mature, exhibit prominent mamillations (Fig. 10.28). Even if the organism is no longer viable, it can be readily identified by virtue of its size.

Ancylostomiasis (Hookworm)

The distribution of hookworm (*Necator americanus* and *Ancylostoma duodenale*) is worldwide, with particular prevalence in rural areas of the moist tropics where there is inadequate sanitation and people walk barefoot. The filariform larvae penetrate the skin of the feet or hands, enter venules, and are carried to the capillaries of the lung, where they break through the capillaries into alveolar sacs. They subsequently migrate through proximal airways to the gastrointestinal tract. Adult hookworms are creamy white, cylindrical nematodes with a large buccal capsule that includes teeth or cutting plates.[251,253,254]

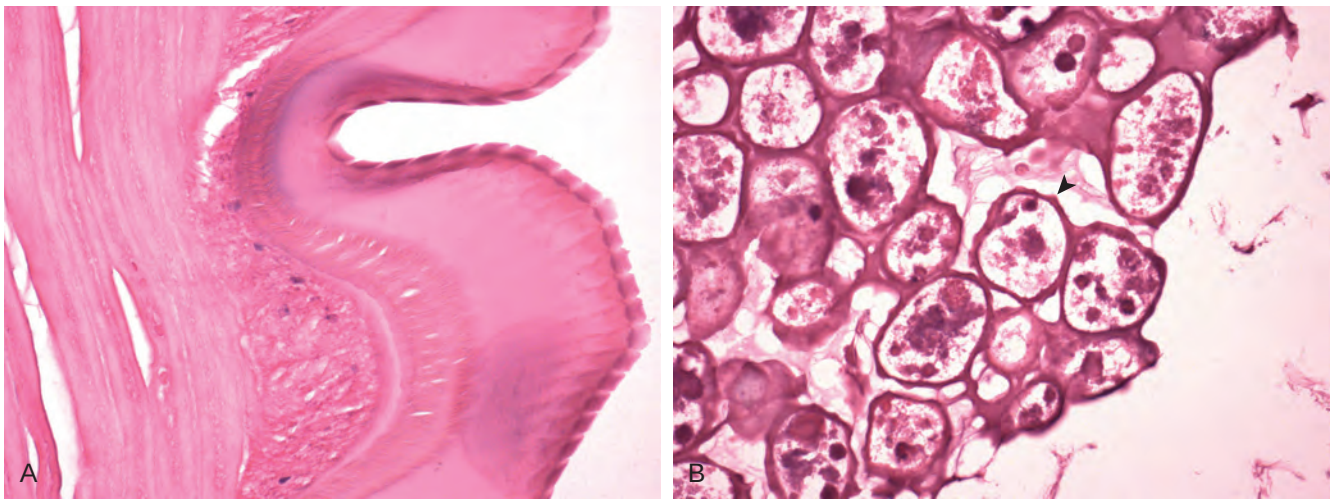
The patients are usually asymptomatic, but dyspepsia, nausea, and epigastric discomfort may occur. In addition, because the worms attach to the intestinal mucosa and withdraw blood from villous capillaries, the patient develops anemia if the infestation is left untreated.

Mucosal damage is usually minor, but dense eosinophilic infiltrate extending into the submucosa or villous blunting may be seen. Biopsy specimens may occasionally reveal intestinal mucosa in the buccal cavity of the worm.[251,253,254]

Strongyloides stercoralis

The distribution of *S. stercoralis* is worldwide, with the greatest prevalence in warm and wet regions. Adults, especially old, hospitalized, institutionalized, or immunocompromised individuals, are more prone than others to be infected by *Strongyloides*.

Filariform larvae penetrate the skin, enter the venous system, travel to the lung, and migrate up the proximal airways and down into the esophagus, eventually reaching the small intestine. Adult female worms parasitizing human small intestine lay eggs in the intestinal mucosa that hatch into rhabditiform larvae, which are



• **Figure 10.28** **A,** *Ascaris lumbricoides* with thick annulate cuticle and hypodermis. **B,** The mature ova of *Ascaris* exhibit prominent mamillations *(arrowhead)*.

shed in the stool. In an unfavorable environment, rhabditiform larvae transform into filariform larvae (parasitic phase). In the autoinfection cycle, rhabditiform larvae change into infective filiform larvae in the intestine or on the perianal skin and directly invade the host. This autoinfective capability leads to prolonged illness. Disseminated infection (i.e., migration of larvae to organs beyond the range of the autoinfective cycle) can occur in immunocompromised hosts and results in severe, life-threatening illness.[250,251,255-262] Systemic parasitic infection predisposes to gram-negative sepsis.

Adult female worms are 2 to 3 mm in length, with 1- to 2-µm thick cuticles and fine transverse striations. Eggs are 50 to 60 µm, oval, thin shelled, and embryonated when they exit the female. Rhabditiform larvae are 200 to 300 µm, and filariform larvae are 300 to 600 µm.[250,251,255-262]

Many patients are asymptomatic carriers, but abdominal pain, diarrhea, nausea, vomiting, anorexia, weight loss, and gastrointestinal bleeding, in any order or combination, have been reported. Ileus, small-bowel obstruction, and malnutrition also occur. CT scans occasionally reveal intra-abdominal lymphadenopathy. Gastrointestinal manifestations can be accompanied by pruritus, rash, eosinophilia, and pulmonary symptoms.[250,251,255-262]

Mucosal lesions may lead to esophagitis and gastritis, in addition to duodenitis, jejunitis, ileitis, and colitis with pseudomembranous colitis. Mucosal ulceration is most commonly seen in the small intestine, but it can occur at any level of the gastrointestinal tract. Histologically the crypts are often distended by numerous larvae and adult worms (Fig. 10.29), but the organisms may be difficult to detect due to their patchy distribution. Other histologic features include villous blunting, ulcers and necrosis, edema, and a dense eosinophilic infiltrate of the lamina propria. Granulomas may also be seen.[250,251,255-262]

Capillaria philippinensis (Intestinal Capillariasis)

Although endemic in the Philippines and Thailand, cases of *C. philippinensis* have been reported in nonendemic areas. Transmission to humans is through ingestion of freshwater fish infected by larvae. The worms inhabit both the small and large intestine. At autopsy, as many as 200,000 worms can be recovered from a liter of intestinal contents. Autoinfection is attributed to the huge numbers of the organisms.[251,253,263,264]

The female worms range in size from 2.5 to 4.3 mm, and the male worms are slightly smaller. *C. philippinensis* eggs, 36 to 45 µm long, are barrel shaped with flattened bipolar plugs. *C. philippinensis* resembles *Trichuris* and *Trichinella* spp. but is distinguished from them by its characteristic ova. The symptoms of infection are nonspecific and include dull gastric pain or generalized abdominal pain, as well as watery and voluminous diarrhea with an increased amount of fat. Intractable diarrhea can lead to ascites and weight loss, followed by cachexia and death. The natural course of the disease is relatively rapid, and the period between onset of symptoms and death is as short as 2 to 3 months.[251,253,263,264]

Grossly the small intestine is thickened, congested, and distended with fluid. An extremely large number of adult worms, larvae, and ova may be seen within the jejunum, the proximal portion of the ileum, and occasionally the duodenum. Worms are most commonly found in the crypts of the small intestine, but they may also invade the lamina propria. The intestinal villi tend to exhibit secondary changes, such as villous atrophy and epithelial sloughing (Fig. 10.30).[251,253,263,264]

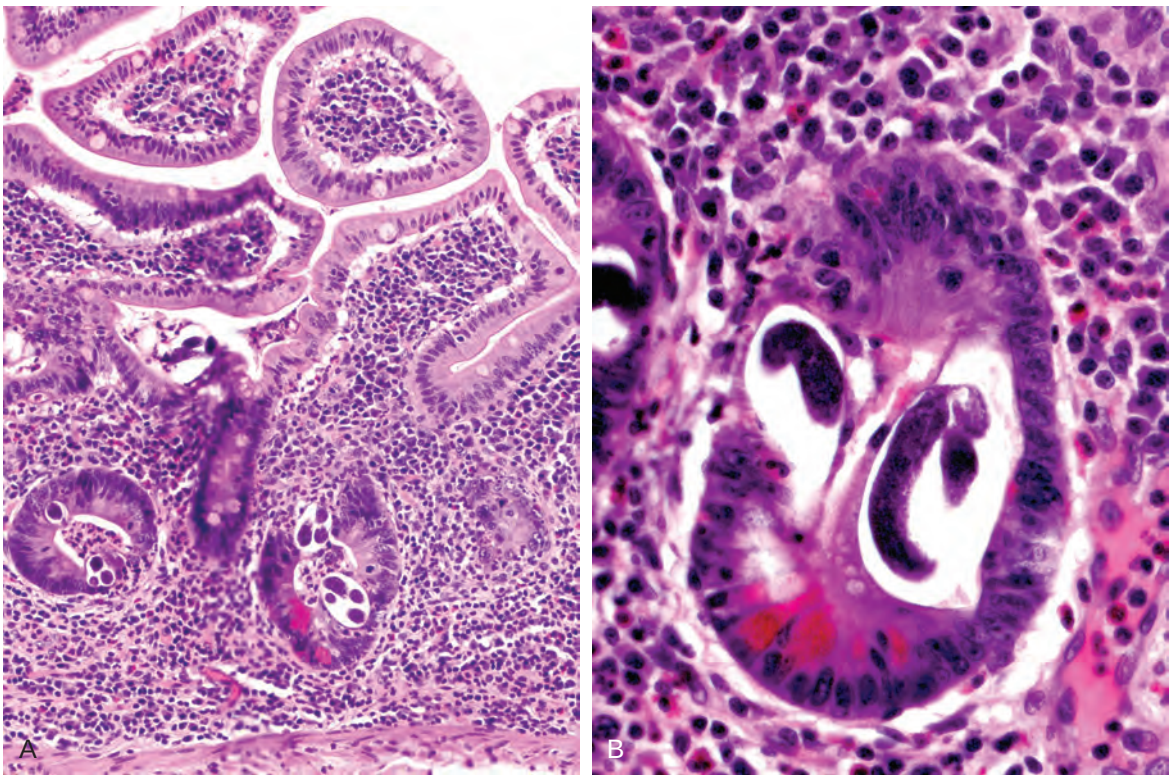
Trematodes

Schistosomiasis is the most common disease caused by trematodes worldwide. All *Schistosoma* spp. cause disease, and any level of the gastrointestinal tract may be affected. However, pathologists are most likely to encounter the organisms in colonic specimens.

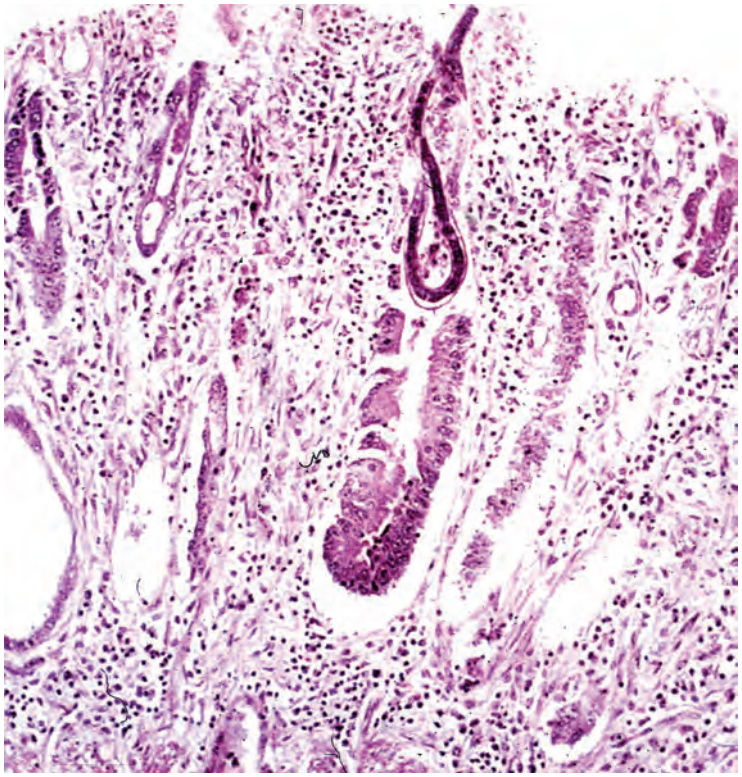
Fasciolopsis buski (Intestinal Fluke)

F. buski infection is prevalent in countries of eastern Asia and the southwest Pacific, and the highest incidence is reported in eastern China. Transmission of the fluke to humans is via ingestion of aquatic plants, such as water chestnuts, containing infective metacercariae. These encyst in the duodenum, attach to the mucosa, and mature into adult worms in approximately 3 months.[250,265-269]

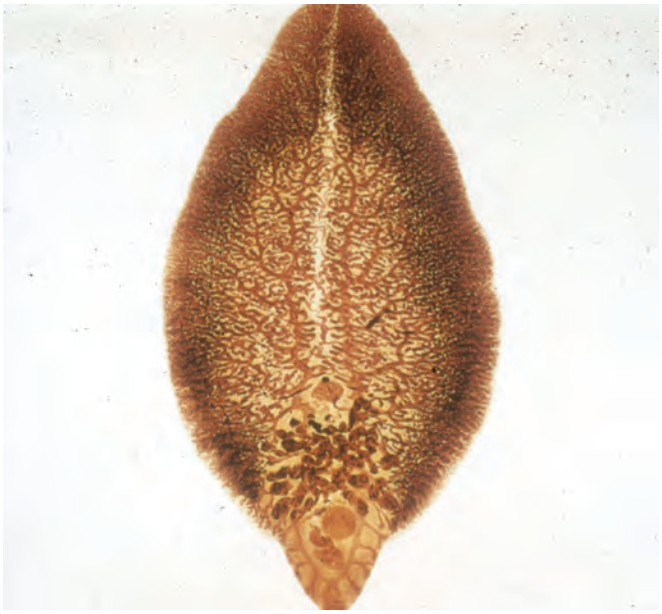
The adult is a flat, fleshy, ovate trematode that is 2 to 7.5 cm in length (Fig. 10.31). Eggs measure 130 to 140 µm in length and are ellipsoid, with a small operculum at one end.[250,265-269] The majority of infections remain asymptomatic; however, in cases of heavy infection, patients may develop diarrhea, often alternating



• **Figure 10.29** Ileum of a patient infected with the adult *S. stercoralis* **(A)** and rhabditiform larvae **(B)** in crypts.



• **Figure 10.30** *Capillaria philippinensis*. The involved ileum exhibits villous atrophy, epithelial sloughing, and a helminth in a crypt.



• **Figure 10.31** *Fasciolopsis buski* (130 to 140 µm in length).

with constipation, epigastric pain, nausea and vomiting, and hemorrhage, secondary to intestinal obstruction and mucosal injury. In addition, the absorption of parasitic metabolites can cause generalized edema, which is most striking in the face. The duodenum and jejunum are most severely affected, with the large adult worms attaching to the mucosa and inducing an intense inflammation with possible ulceration and abscess formation. Leukocytosis and peripheral eosinophilia are also frequently associated with fasciolopsiasis.[250,265-269]

Cestodes

Adult *Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), and *Diphyllobothrium latum* (fish tapeworm) are among the largest parasites that infect humans, and they occasionally cause gastrointestinal disease. Approximately 40% of patients with *Hymenolepis nana* (dwarf tapeworm) infection have low vitamin B_{12} levels because the tapeworm competes with the host for the vitamin.[250,270,271]

Infectious Colitis

Although there is worldwide variation in the most common intestinal infectious agents, diseases that were once considered tropical are no longer diagnosed only in distant exotic places but may be encountered anywhere, as a result of international travel and migratory populations. In the West, breakdown in sanitary handling of food and contamination of poultry flocks, as well as the use of raw or partially cooked foods, have led to epidemics of infectious colitis.[272]

Infectious colitis manifests as an acute diarrheal disease frequently associated with hematochezia or microscopic evidence of blood loss. In North America the diarrheas, commonly of bacterial etiology, are usually self-limited and are commonly referred to as *acute self-limited colitis*. However, in immunodeficient patients, presentations may be severe and a benign clinical outcome less certain.

Bacteria

Common Histologic Features of Bacterial (Invasive) Colitis

In response to infection the colonic mucosa shows remarkable histologic variability. In practice, the dilemma is to distinguish between infectious colitis and the early manifestations of chronic inflammatory bowel disease. Early in the course of infectious colitis, edema of the lamina propria with clusters of neutrophils and ectatic capillaries are the dominant findings. Established crypt abscesses are rare. The changes are commonly patchy, and the overall mucosal architecture is preserved. Minimal degenerative changes, limited to the upper half of the mucosa, can be seen. These include mucin depletion and epithelial damage with flattened cytoplasm associated with cryptic dilatation. Concurrently, the inflammatory infiltrate of the lamina propria is mixed, characteristically with more neutrophils than lymphocytes and plasma cells. Microthrombi can plug dilated capillaries and can account for the focal hemorrhage of the lamina propria.

Later in the course of the infection, biopsies may show scattered neutrophils, inflammation with slightly decreased goblet cells, mucin depletion, and cellular degeneration, sometimes with vacuolization. A mild increase in plasma cells is commonly noted in the lamina propria, sometimes in association with lymphoid aggregates. However, a well-established dense basal lymphoplasmacytic infiltrate is uncommon. These changes habitually resolve in 2 to 3 weeks or certainly within a trimester.[273-275]

Histologic Features Associated With Bacterial Infection

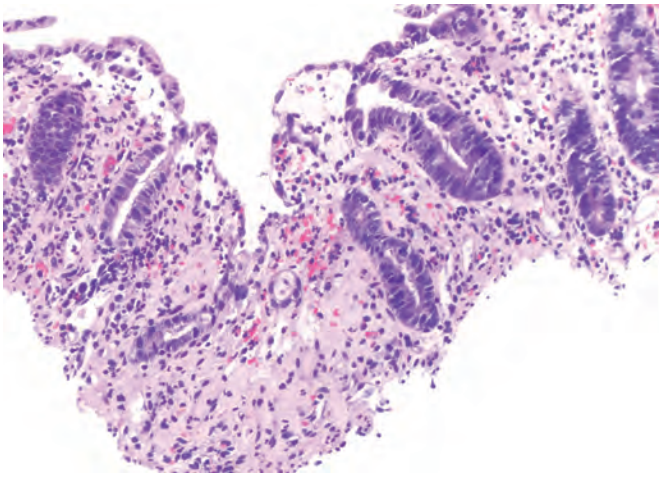
Escherichia coli. *E. coli* is the most prevalent aerobic bacterium of the gut. Five variants are responsible for most common diarrheal illnesses (ETEC, EPEC, EIEC, EHEC, and EAEC species).[276] The ETEC species include O157:H7, which produces a verotoxin and results in a hemorrhagic colitis with features similar to those of ischemic colitis. The diarrheogenic *E. coli* (ETEC, EAggEC, and EIEC *E. coli*) account for more than 50% of traveler's diarrhea.[277]

In North America, *E. coli* O157:H7 is the strain most commonly associated with the hemolytic and uremic syndrome. Its pathogenicity is attributable to two Shiga-like toxins (I and II). These toxins interact with a mucosal receptor in the Peyer patches. The absorbed toxins eventually cause epithelial and endothelial damage of not only the colon but also the kidneys.[278-280] Incubation takes approximately 4 days, and the illness lasts approximately 1 week. In many cases the disease is self-limited, with minimal or even no diarrhea.

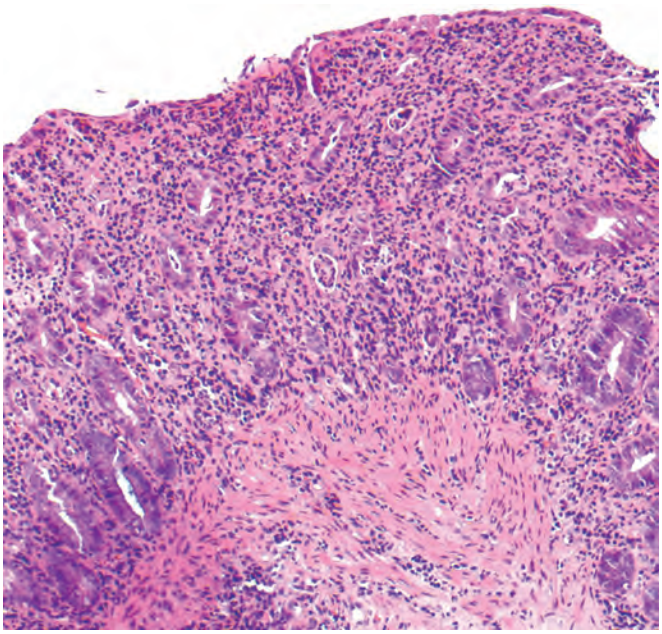
Those who develop a colitis may show various endoscopic appearances, ranging from normal mucosa to edema, erosion, and pseudomembranes. The histologic findings include overlapping features of both ischemic colitis and infectious colitis. Submucosal edema, hemorrhage, pseudomembranes, and withering crypts are seen, along with marked inflammatory infiltrate and cryptitis (Fig. 10.32).[281,282]

Aeromonas. Members of the bacterial genus *Aeromonas* produce a wide area of virulence factors and have been associated with cases of gastroenteritis, particularly in young children. Chronic symptoms also have been reported.[283]

Campylobacter. *Campylobacter* bacteria rank among the most common causes of infectious diarrhea. Most infections are self-limited cases of simple diarrhea, occasionally associated with systemic symptoms, but severe infections can occur. Domestic animals, particularly poultry, are reservoirs of infection, and the



• **Figure 10.32** Colitis associated with *E. coli* O157:H7 infection. The histology shows evidence of ischemic colitis with hyalinized stroma and withering crypts.



• **Figure 10.33** Colitis associated with *Salmonella typhi* infection. The lamina propria is moderately expanded by mixed lymphoplasmacytic infiltrate, as well as scattered neutrophils. Cryptitis is present as well. The morphologic features are not specific.

organism is primarily transmitted by the fecal-oral route. Infections peak in summer and early fall and generally result from ingestion of contaminated food or water.

Campylobacter jejuni, the most important species, produces typical infectious colitis that lasts from 1 to 7 days. The symptoms usually begin with a prodrome of fever, headache, and myalgias 12 to 24 hours before the onset of gastrointestinal symptoms. Colitis is reported in up to 80% of infections. Colonoscopic findings are nonspecific and range from segmental ulceration to diffuse colitis.[284] Organisms may be seen in the lamina propria by ultrastructural analysis, and granulomatous changes with giant cells may also occur. On biopsy, a focal active colitis is observed.[285] Rare cases produce changes mimicking ulcerative colitis.[284] Complications include toxic megacolon and the development of Guillain-Barré syndrome.[286,287]

Shigella. The enteroinvasive *Shigella* bacteria cause mucosal changes resembling those of chronic inflammatory bowel disease.

Salmonella. *Salmonella* spp. are associated with a common form of infectious diarrhea. Classic typhoid fever due to *S. typhi* is primarily an ileal disease (see earlier discussion). However, nontyphoid salmonellosis may involve the colon as well.

At endoscopy, a wide range of nonspecific changes are seen. These include mild edema with petechial hemorrhage and, in severe cases, friability and ulceration. The histologic changes are indistinguishable from other types of bacterial diarrhea and some inflammatory bowel diseases, especially if the specimens were obtained early in the course of inflammatory bowel disease. Complications include the occasional development of toxic megacolon (Fig. 10.33).

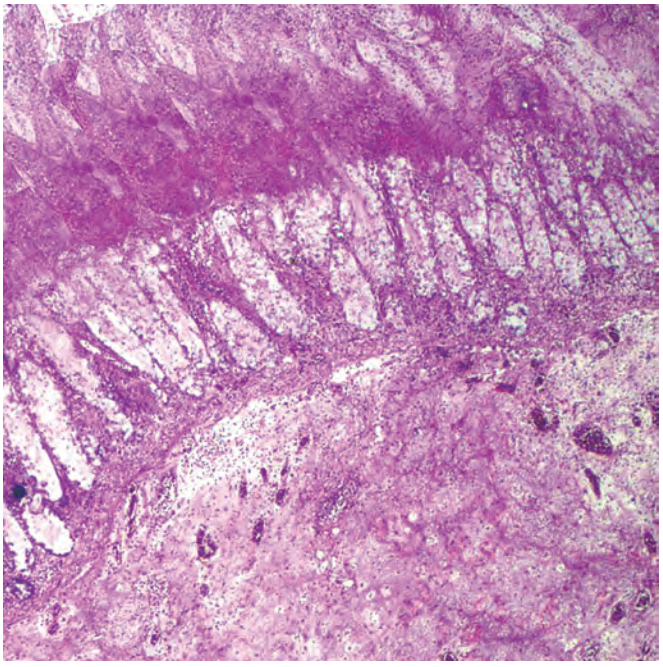
Clostridium difficile. *C. difficile* is a gram-positive bacillus that produces two principal toxins that play a role in pathogenesis of disease. Toxin A is an enterotoxin responsible for food accumulation in the gut and for damage to the enterocolonic mucosa; toxin B is a labile motility-altering cytotoxin that leads to food accumulation.

The spectrum of *C. difficile* colitis ranges from asymptomatic carriage to fulminant colitis. The presence of *C. difficile* infection has been steadily increasing in recent years, particularly among institutionalized patients, and it currently represents a major cause of hospital-acquired diarrhea. *C. difficile* infection is also prevalent in patients with inflammatory bowel disease (particularly ulcerative colitis) who are receiving maintenance immunomodulation therapy, and it is associated with exacerbation of the disease. It appears to play a minor role in sporadic diarrhea. It is well established that *C. difficile* infection follows exposures to antibiotics targeting gut anaerobes, and transmission occurs nosocomially from one host to another.

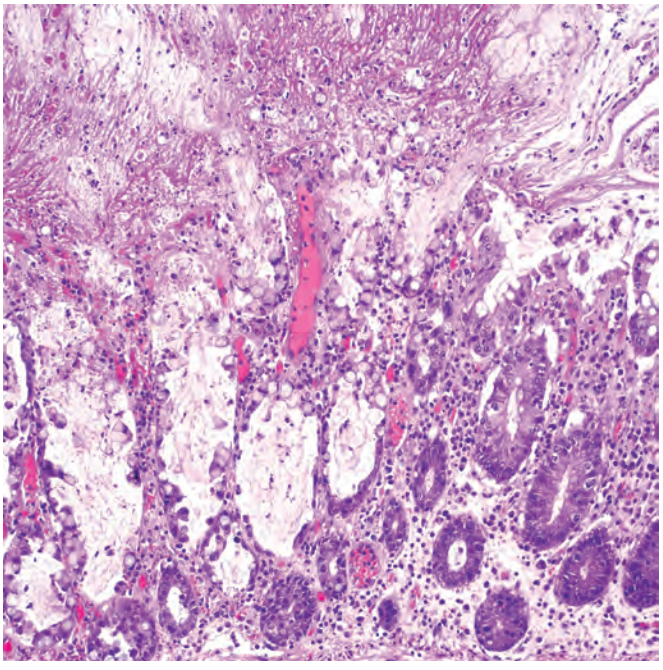
Some researchers have created a risk score to identify patients at risk for *C. difficile* infection after hospitalization. A model based on age, past hospitalizations, and use of third/fourth-generation cephalosporin, clindamycin, or fluoroquinolone antibiotics resulted in a predictive risk score with patients within the low-risk group developing *C. difficile* colitis with 28 days or more of hospitalization at a much lower rate than those within the high-risk group (0.3% vs 1.6%).[288]

Toxigenic *C. difficile* is responsible for more than 90% of cases of pseudomembranous colitis and 30% to 40% of cases of antibiotic-associated colitis. The diarrhea usually begins within a few days after antibiotics are started. Some patients develop pseudomembranous colitis, whereas others suffer only mild diarrhea.

Pseudomembranous colitis due to *C. difficile* shows a spectrum of histologic changes, beginning with mild lesions that exhibit a luminal spray of mucus and neutrophils above a background mucosa with minimal inflammation. More advanced disease shows marked inflammatory erosion of the lamina propria covered by a fibrinopurulent cap and cystification of crypts. Finally, mucosal inflammation and erosion extends to form confluent pseudomembranes. In some patients, toxic megacolon ensues.[289] A signet ring cell-like morphology can be see due to mucosal degeneration, but in this setting the finding of signet ring cells should not be mistaken as indicating a carcinoma (Figs. 10.34 to 10.36).[290] The standard for diagnosis of *C. difficile* is a toxin test that is positive in more than 90% of patients with classic histologic changes of pseudomembranous colitis.

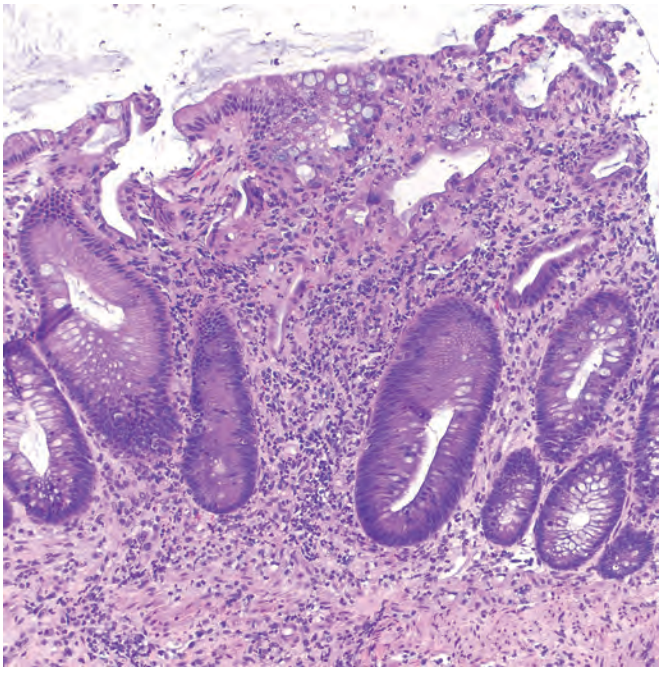


• **Figure 10.34** Low-power view of specimen from a patient with *C. difficile*–associated pseudomembranous colitis characterized by edema of the submucosa and destructive changes of the mucosa.



• **Figure 10.35** At higher power, *C. difficile* pseudomembranous colitis is characterized by sloughing off of superficial epithelial cells. The coating of fibrinopurulent material creates a pseudomembrane.

Yersinia. *Y. enterocolitica* and *Y. pseudotuberculosis* are gram-negative aerobic coccobacilli. The latter species causes colitis that usually involves the terminal ileum and mesenteric lymph nodes (see earlier discussion). In *Y. enterocolitica*–associated acute colitis, the mucosa is erythematous and friable, with tiny superficial ulcerations. Granulomas are absent, but aggregates of histiocytes are frequently seen.



• **Figure 10.36** Colitis in a patient with a positive toxin test for *C. difficile* colitis but no evidence of pseudomembrane formation. Nonspecific degenerative changes of the surface epithelium, neutrophils, and mild expansion of the lamina propria are present.

Mycobacterium tuberculosis. Tuberculosis of the colon, which is increasing in prevalence due to global travel, is essentially limited to the ileocecal region. Most patients have concomitant pulmonary tuberculosis. The tubercle bacillus can reach the gastrointestinal tract by several routes, including swallowed sputum, infected food, adjacent tissues, lymphatic spread, and via the bloodstream. Colonoscopy can be challenging, given the presence of ulceration and hypertrophic polypoid masses. Ulcerative, hyperplastic, and sclerotic variants may be seen. The differential diagnosis includes ileocecal Crohn disease and carcinoma.[291]

Biopsies reveal mucosal ulceration, granulation tissue, and microabscesses. Characteristic necrotizing granulomas are usually seen in the ulcer bed. The granulomas in tuberculosis are much larger than those seen in Crohn disease. They are confluent, and caseation is a common feature. AFB are demonstrated in 35% to 60% of cases. If *M. tuberculosis* is suspected, acid-fast staining of tissues and cultures is required to establish the diagnosis, and the development of PCR technology can increase the diagnostic yield.

Intestinal Spirochetosis. The prevalence of intestinal spirochetosis in rectal biopsies is between 2% and 7% in the West but between 11% and 34% in developing countries. Homosexual men and HIV-infected patients are reported to have the highest prevalence (up to 54%).

Whether the organism is of clinical significance or is a commensal bacterium has been debated. Colonoscopy reveals mucosal erosions and hyperemia. Histologically the irregular luminal aspect of the surface epithelium seen on H&E-stained sections is emphasized by PAS or Warthin-Starry stains. Ultrastructural analysis also identifies the spirochetes. The disorder is most frequently seen in the right colon.[292] The spirochetes or brachyspira, *Brachyspira aalborgi* and *Brachyspira pilosicoli*, are found between and parallel to the microvilli and do not penetrate the cytoplasm

in most cases (Fig. 10.37). Mild inflammation may be seen in colonic biopsies. Improvement of symptoms and decrease in immunoglobulin E plasma cells within the lamina propria has been observed after treatment with metronidazole. Cases of severe colitis are rare but have been reported.[293,294]

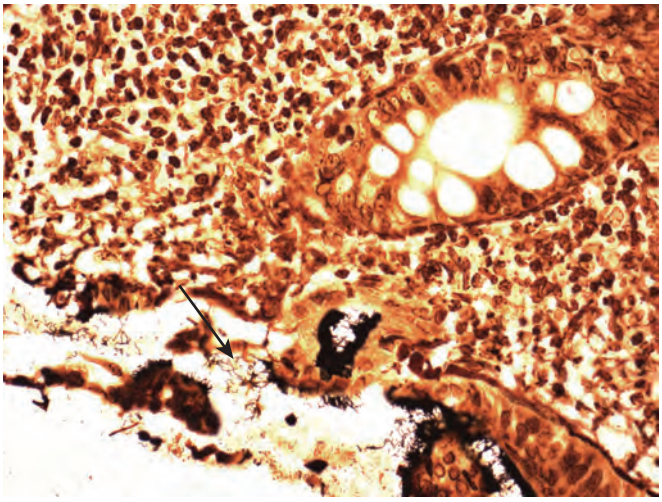
Viruses

Many viruses, including rotavirus, enteric adenovirus, calicivirus, astrovirus, CMV, herpesvirus, and adenovirus, can produce colitis. CMV, HSV, and adenovirus are considered here because they are the only ones likely to be identified on endoscopic biopsies. A wide array of changes, from normal or edematous mucosa to ulcerative colitis, may be seen (Fig. 10.38).

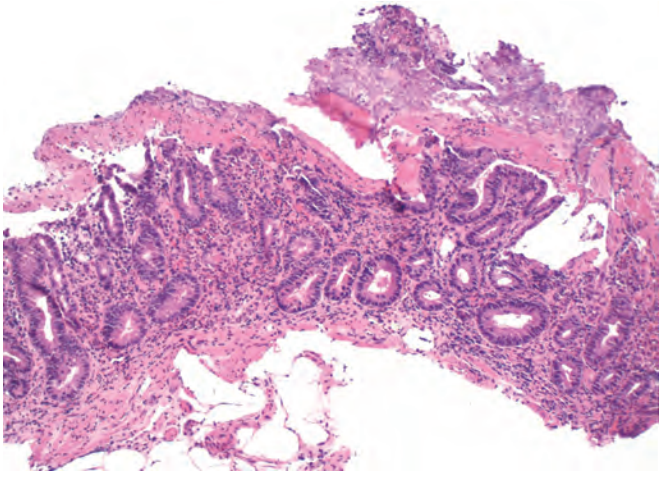
Although CMV infection is usually subclinical, symptoms are common in immunologically suppressed patients, such as patients with AIDS, cancer patients undergoing chemotherapy, transplant recipients, and patients with inflammatory bowel disease.[295,296] CMV colitis can be recognized by the presence of ulceration with or without colitis. The diagnosis of CMV infection depends on the identification of characteristic intranuclear inclusions. Infected cells typically show endothelial, stromal, or epithelial nuclear and cytoplasmic enlargement with a single, dark red, amphophilic nuclear "bull's-eye" inclusion. However, in the gastrointestinal tract, it is not uncommon for infected cells to show indistinct, smudged, hematophilic nuclei. The cytoplasm of the infected cells can show either granular inclusion or a foamy appearance. The diagnosis can be rendered difficult by granulation tissue arising secondary to the ulceration and the reactive stromal cells. Immunohistochemistry allows a diagnosis to be made in atypical cases, especially in inflammatory bowel disease patients. CMV cytopathic changes are often atypical and are seen in a small number of stromal cells; thus conducting immunohistochemistry with a low threshold is advised.[297]

HSV is a common cause of proctitis in male homosexual patients, in AIDS patients, and in other immunosuppressed individuals. The ulcerations commonly demonstrate peripheral giant cells. Nonspecific cases of severe acute colitis with widespread ulceration have been reported.[298]

Adenovirus is believed to be a common cause of diarrhea in children and patients with HIV infection. Infected vacuolated epithelial cells are found close to the mucosal surface, with amphophilic nuclei that can be either enlarged or crescent shaped, and the presence of virus can be confirmed by immunocytochemistry. Small intranuclear inclusions are also visible.



• **Figure 10.37** Intestinal spirochetosis. With Warthin-Starry stain, numerous tangled spirochetes are seen distributed along the apical surfaces of the mucosa *(arrow)*.



• **Figure 10.38** Amebiasis of the rectum. The colonic mucosa is moderately inflamed and shows a surface erosion with fibrinopurulent material.

Protozoa

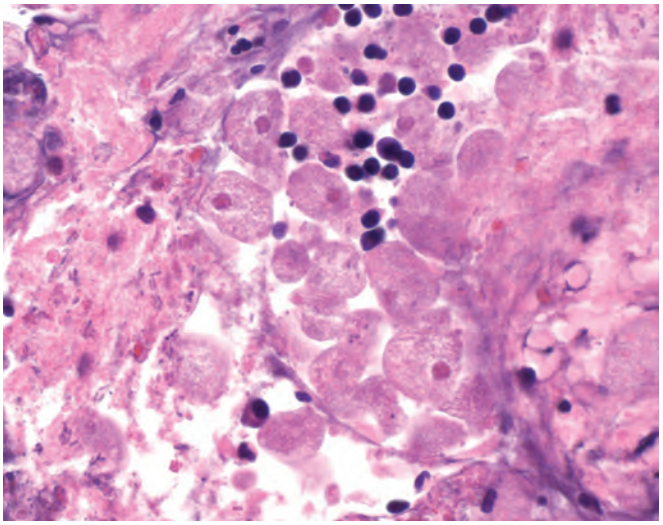
The diagnostic identification of protozoa is best done by examination of fresh stool. There is no characteristic histologic pattern on biopsy, and the organisms are usually overlooked on sections unless a careful inspection is made.

Amebiasis

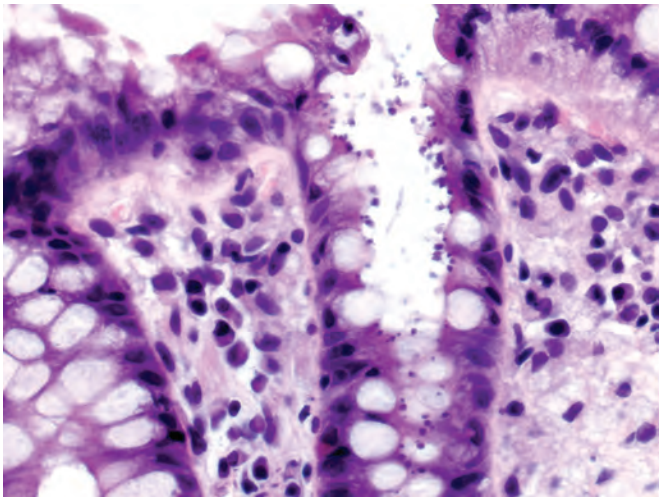
Entamoeba histolytica is found in all climates. Infection is acquired via fecal contamination and ingestion of cysts. In the West a history of foreign travel to areas of poor sanitation is common, although sexually transmitted cases have been recognized. Infected individuals may present with various symptoms, including toxic megacolon. The range of symptoms is explained in part by the existence of 22 different zymodemes.

Early in infection, edema and nonspecific inflammation with clusters of neutrophils are seen in the lamina propria and surface epithelium. The amebas may be present on the surface, usually in the overlying inflammatory exudate. With time, a large, flask-shaped ulceration may develop. The epithelium shows goblet cell depletion and microulceration with organisms in the exudate overlying the denuded lamina propria (see Fig. 10.38). Although there are numerous neutrophils in the lamina propria, crypt abscesses are not conspicuous, and tissue eosinophilia is not a feature.

Deep necrosis with ulceration extending to the submucosa can be seen in severe cases. The mucosa is replaced by a thick, amorphous inflammatory fibrinoid material with abundant organisms and scattered inflammatory cells. The infective trophozoites are at times substantially larger (10 to 60 µm) than macrophages, display foamy amphophilic cytoplasm with ingested erythrocytes, and sport a punctate central karyosome (Fig. 10.39). The PAS stain aids in recognition of the amebas but obscures the presence of the diagnostic karyosome and ingested erythrocytes, and a trichrome stain aids in detection of erythrocytes in the cytoplasm.



• **Figure 10.39** Amebiasis of the rectum. Higher magnification shows numerous *E. histolytica* distributed along the surface epithelium.



• **Figure 10.40** *Cryptosporidia parvum* infection in an immunosuppressed patient. The organisms coat the surface epithelium and should not be mistaken for extruded mucin globules.

Of note, antidiarrheal preparations can destroy the protozoa and should be avoided before biopsy. Current diagnostic tools include antigen detection in stool and PCR (see Figs. 10.38 and 10.39).[299]

Coccidia

Coccidia is a collective name for the suborder that includes other human pathogens, such as *Isospora*, *Sarcocystis*, and *Toxoplasma*. The diagnosis of these intracellular pathogens is difficult to evaluate on colorectal biopsy. *Cryptosporidium parvum* can be seen in immunocompromised and normal subjects, but with a markedly different clinical presentation. The former may present with severe diarrhea, sometimes with toxic megacolon. In the setting of AIDS, several other microorganisms are frequently associated. In immunocompetent individuals, *C. parvum* infection usually manifests as a self-limited, flulike gastroenteritis; it has also been noted as a cause of traveler's diarrhea.

On biopsy, a nonspecific inflammatory infiltrate and rare ulceration are seen. Cryptosporidia are observed as clusters of tiny hematoxylin dots on the epithelial surface or within the crypt. PAS, silver, or Giemsa stain is also helpful in highlighting the organisms (Fig. 10.40).

Other Protozoan Infections

Giardiasis is more commonly recognized in the small bowel, but rare cases of colonic infection have been documented.

Balantidium coli is a ciliated protozoan. Patients are infected through contact with pigs or rats under conditions of poor personal hygiene. After ingestion of cysts, the trophozoite resides predominantly in the colon. The mucosal lesions are similar to those of amebiasis. Symptoms may include acute diarrhea, fulminant colitis, and perforation. Asymptomatic carriers are recognized.[300]

Helminths

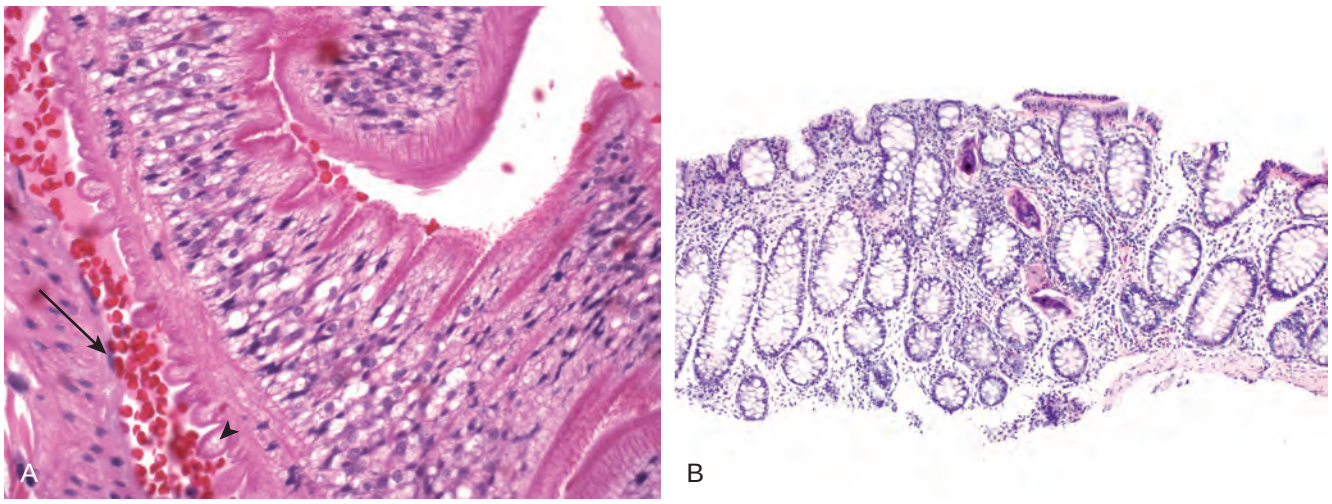
Three classes of helminths can lead to human infection: trematodes (flukes), nematodes (roundworms), and cestodes (tapeworms). Only schistosomiasis leads to colorectal infestation.

Schistosoma mansoni and *Schistosoma japonicum* are the two common trematode worms affecting the colon. The adults reside in the major mesenteric veins, and the disease is caused primarily by an inflammatory reaction to the eggs. The rectal mucosa and submucosa are a good location for identification of *S. mansoni* eggs, whereas those of *S. japonicum* are generally seen in the right colon. The presence of miracidial nuclei indicates that organisms are viable. The ova of *S. mansoni* are characterized by subterminal spines, whereas the eggs of *S. japonicum* have ill-defined subterminal knobs. The eggs are usually surrounded by inflammatory infiltrate in which eosinophils predominate. A granulomatous reaction may develop. In some cases the eggs are surrounded by an eosinophilic zone of fibrin material, representing an antigen-antibody complex. With time, fibrosis develops around the calcified ova. The lateral spine of *S. mansoni* and the cortical shell stain variably with modified AFB stain; unlike paragonimiasis, the ova are nonrefractile. In countries with endemic infection the entire colon may become fibrotic, studded with polyps and ulcers. There is an increased incidence of adenocarcinoma in these patients (Figs. 10.41 and 10.42).[301,302]

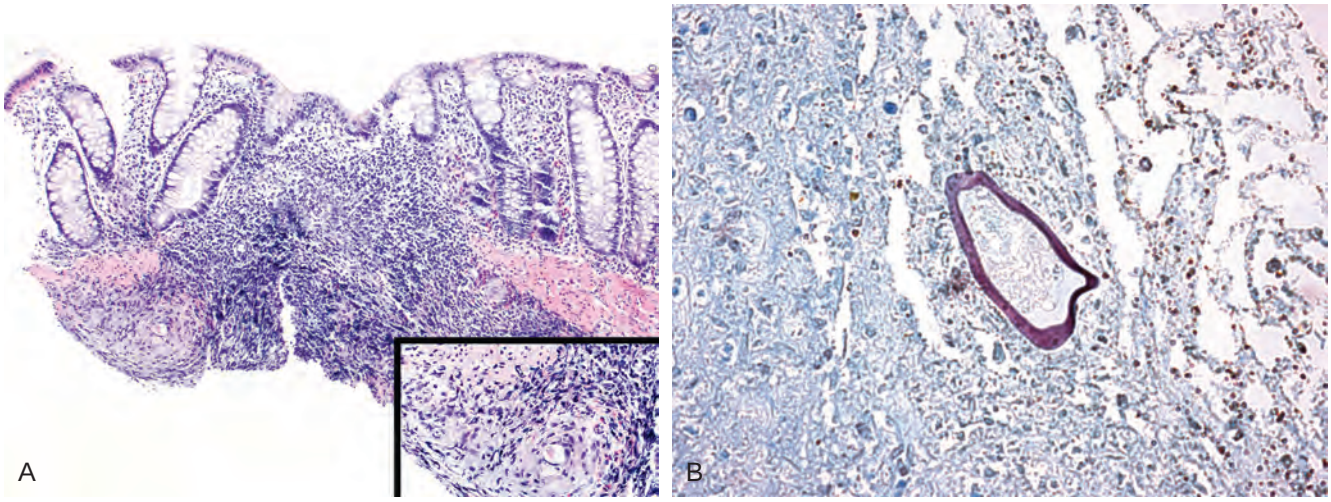
Strongyloidiasis, trichuriasis, and oxyuriasis are common nematode infestations of the lower gastrointestinal tract. Although *S. stercoralis* is usually restricted to the small intestine, migratory larvae can implant in the colonic mucosa, leading to autoinfection, particularly in the setting of massive infestation. The parasites are found in crypts and superficial mucosae, usually surrounded by transmural eosinophilic inflammation.[303]

Trichuris trichiura (whipworm) preferentially infests the cecum. The body structure of the worm is unique. The anterior three-fifths of the worm is threadlike and embeds into the mucosa, whereas the remainder of the organism floats within the lumen. The esophagus shows a characteristic stichosome, and the anterior wall shows characteristic bacillary bands. The posterior part of the worm exhibits the reproductive organs (Fig. 10.43). *Trichuris* causes chronic dysentery. On biopsy, the worm is usually surrounded by an intense focal eosinophilic infiltrate.

Oxyuriasis, or *Enterobius vermicularis*, is the most prevalent parasitic helminth recognized in the West. The adult pinworm attaches to the cecal mucosa, and the worm is commonly observed in the lumen of the appendix. Symptoms result from the nocturnal migration of the adult female; she lays eggs on the perianal skin, causing intense pruritus. Diagnosis is classically established by

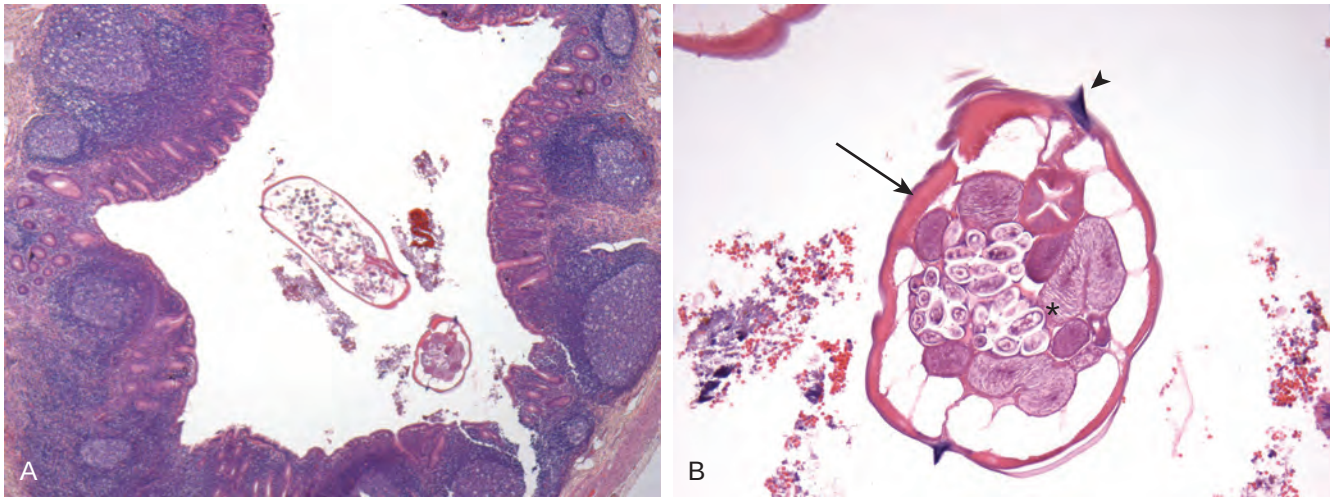


• **Figure 10.41** **A,** The tuberculated tegument *(arrowhead)* of an adult male *S. mansoni* lodged within a mesenteric vein *(arrow)*. **B,** Low-power magnification demonstrates degenerating ova within the rectal mucosa.



• **Figure 10.42** **A,** Colonic schistosomiasis characterized by an ill-formed submucosal granuloma. The inset demonstrates the terminal spine with surrounding ill-formed granulomatous reaction. **B,** A Fite stain highlights the cortex and lateral spine of *S. mansoni*.

• **Figure 10.43** *Trichuris trichiura*. The anterior portion of the nematode with stichosome *(long arrow)* and bacillary band *(arrowhead)* is embedded in the superficial mucosa, while the posterior two-thirds of the helminth containing the sexual organs projects into the bowel lumen *(short arrow)*.



• **Figure 10.44** **A,** *E. vermicularis* within the appendiceal lumen. **B,** The helminth shows prominent lateral alae *(arrowhead)*, platymyarian muscle *(arrow)*, and flattened refractile ova *(asterisk)*.

adhering the deposited ova to cellophane tape and examining them under the microscope. Refractile ova are characteristically flattened on one side. The helminth is small, shows a muscular wall, and exhibits prominent lateral alae projecting from the cuticle throughout its length. The central oviducts contain the characteristic flattened eggs. The surrounding mucosa is generally inflamed, and tissue eosinophilia may be present (Fig. 10.44). Inflammatory reaction may produce a presenting polyp or even a mass, sometimes mistaken for a carcinoma.[304] Aberrant migration can lead to similar lesions in the vagina and bladder.

Fungi

Fungal infections of the large bowel are rare. They are commonly opportunistic infections of immunocompromised hosts, although in some regions, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis are endemic. *Histoplasma* is the organism most likely to involve the gut, and the diagnosis depends on identification of the fungus on biopsy. This is best achieved with silver stains. The dimorphic fungus is most often represented in its yeast form, but mycelial growth may be present. The mass mimics carcinoma, from which it must be distinguished.

Sexually Transmitted Diseases

Proctitis is common in homosexual men and is frequently related to infection by *Neisseria gonorrhoeae*, *Treponema pallidum*, chlamydia, or HSV.[305] The symptoms of gonorrhea, caused by the gram-negative diplococcus *N. gonorrhoeae*, vary from pruritus to severe proctitis and diarrhea. Biopsies frequently are not diagnostic and show either normal mucosa or nonspecific inflammatory change. Fewer than 5% of patients show infectious proctitis.[306]

Secondary syphilis can manifest as proctitis. The changes include marked inflammation, in which small granulomas with giant cells are usually prominent.[305] *Chlamydia trachomatis* is an obligate intracellular bacterium that ranks among the most common causes of sexually transmitted clinical proctitis.[305]

Lymphogranuloma Venereum

In acute cases of lymphogranuloma venereum, the mucosa shows a mixed inflammatory infiltrate. Giant cell granuloma can be seen in association with disrupted crypts. A common differential diagnosis is ulcerative colitis, and the diagnosis is confirmed by immunofluorescent methods. The inflammatory infiltrate may be transmural or may give a pattern of follicular proctitis. Strictures and adenocarcinoma have been reported in chronic infection.

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11

Liver and Bile Duct Infections

RICARD MASIA, JOSEPH MISDRAJI

Viral Infections of the Liver and Bile Duct
- Hepatitis A
- Hepatitis B
- Hepatitis C
- Hepatitis D
- Hepatitis E
- George Barker Virus Type C (Hepatitis G)
- Epstein-Barr Virus
- Cytomegalovirus
- Herpes Simplex Virus
- Varicella Zoster Virus
- Adenovirus
- Parvovirus B19
- Rubella (German Measles)
- Rubeola (Measles)
- Severe Acute Respiratory Syndrome Coronavirus
- Human Immunodeficiency Virus
- Yellow Fever
- Dengue
- Rift Valley Fever
- Lassa Fever
- Ebola Virus
- Marburg Virus

Mycobacterial Infections of the Liver and Bile Duct
- Tuberculosis
- *Mycobacterium avium-intracellulare* Complex
- Leprosy
- Bacillus Calmette-Guérin

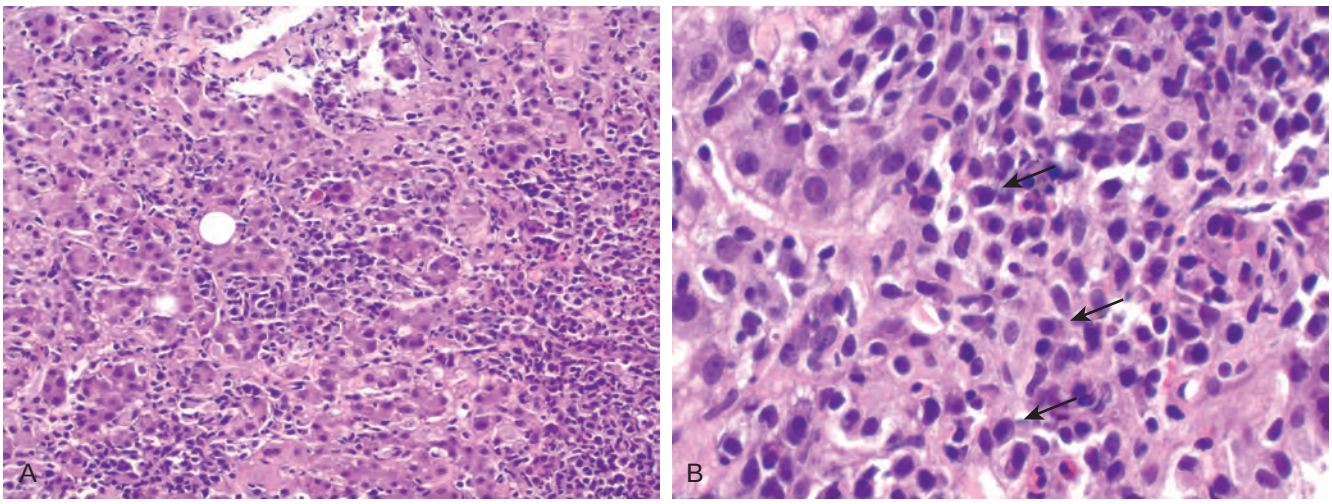
Other Bacterial Infections of the Liver and Bile Duct
- Pyogenic Liver Abscess
- Acute Cholangitis
- Recurrent Pyogenic Cholangitis (Oriental Cholangiohepatitis)
- Brucellosis
- *Bartonella* Infection (Cat-Scratch Disease)
- Enteric Fever (Typhoid and Paratyphoid Fever)
- Tularemia
- Melioidosis
- Listeriosis
- Spirochetes
- Rickettsia

Fungal Infections of the Liver and Bile Duct
- Histoplasma
- Candida
- *Pneumocystis jiroveci*
- *Aspergillus*
- Mucormycetes/Zygomycetes
- Penicilliosis
- Cryptococcosis
- Coccidioidomycosis
- Blastomycosis
- Paracoccidioidomycosis (South American Blastomycosis)
- Microsporidiosis

Helminth Infections of the Liver and Bile Duct
- Schistosomiasis
- Strongyloides
- Enterobiasis (Pinworm)
- Echinococcosis
- Toxocara
- Capillariasis
- Fascioliasis
- Clonorchiasis
- Ascariasis

Protozoal Infections of the Liver and Bile Duct
- Amebiasis
- Malaria
- *Leishmania*
- Toxoplasmosis
- Cryptosporidiosis

The liver is the target of many infectious agents, most notably hepatotropic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV). In addition, many infectious organisms can involve the liver in the setting of disseminated infection, in immunosuppressed patients, or as a medical curiosity. The major challenge facing the pathologist in diagnosing these conditions is that many of these diseases have overlapping histopathologic characteristics. Hepatitis, necrosis, or granulomas are characteristic of many liver infections, and distinguishing the exact cause often requires a meticulous search for organisms, attention to subtle morphologic clues, or, not uncommonly, clinical, epidemiologic, or serologic data. Although some organisms can be readily detected in tissue, many are not, even with the use of ancillary techniques. Furthermore, many of the



• **Figure 11.1** Liver biopsy in hepatitis A. **A,** Panlobular inflammation and lobular disarray. **B,** High power shows many plasma cells within the infiltrate *(arrows)*.

ancillary techniques used to detect organisms in tissue are not widely available. Therefore, although the pattern of injury can provide a differential diagnosis, in some cases the final diagnosis relies on culture or serologic studies.

Viral Infections of the Liver and Bile Duct

Hepatitis A

Hepatitis A virus (HAV) is an RNA virus in the Picornaviridae family. Although the incidence of HAV infection has fallen dramatically since the introduction of vaccines, it still causes approximately 60,000 infections per year and occasionally causes dramatic outbreaks with fulminant hepatitis and death.[1] Fecal-oral transmission is facilitated by extensive viral shedding in feces during the 3- to 6-week incubation period, which reaches a maximum just before the onset of hepatocellular injury.[1] An increasing incidence has been noted among urban homosexual men. An effective vaccine has been developed.

The signs and symptoms of hepatitis A are related to patient age. In children younger than 3 years, more than 80% of infections are clinically silent, whereas in adolescents and adults, more than 75% of cases are symptomatic.[2] Symptoms include fever, malaise, abdominal pain, and jaundice. Marked transaminase elevations are characteristic. Approximately 100 cases of HAV-related fulminant liver failure are reported each year, predominantly in adults.[2] Chronic infection does not occur. However, HAV infection can precipitate autoimmune hepatitis, which can progress to chronic hepatitis with fibrosis or cirrhosis.[3-6]

Liver injury in HAV infection is the result of an immunopathologic response to infected hepatocytes rather than a direct cytopathic effect of the virus.[1] The adaptive immune response is highly effective in eliminating the virus. The earliest antibody response is largely that of immunoglobulin M (IgM), with IgG production beginning shortly thereafter; therefore the diagnosis is established by the detection of anti-HAV IgM (with or without IgG). Anti-HAV IgG persists for life and confers protection against reinfection.[1]

Acute HAV infection may be indistinguishable from other acute viral hepatitides (see discussion of hepatitis B). However, portal plasma cell infiltrates and periportal necrosis may be prominent, causing confusion with autoimmune hepatitis (Fig. 11.1).[7] In rare cases, perivenular cholestasis with relatively little inflammation mimics cholestatic drug reactions. Fibrin ring granulomas have been reported.[8,9]

Hepatitis B

Chronic hepatitis B affects an estimated 400 million persons worldwide, of whom 1 million die annually.[10,11] Three-quarters of patients with chronic hepatitis B in the world are Chinese, and sub-Saharan Africa also has high prevalence.[11] In the United States the incidence of newly acquired HBV infection has been declining due to screening of pregnant women, vaccination, and safer injection practices.[10]

Virology of Hepatitis B Virus

HBV is a DNA-containing virus with four overlapping open reading frames.[12] Its four genes are core, surface, X, and polymerase genes. The core gene encodes the core nucleocapsid protein, which is important in viral packaging, and hepatitis B e antigen (HBeAg). The surface gene encodes pre-S1, pre-S2, and S protein, which are large, middle, and small surface proteins, respectively. The X gene encodes the X protein, which may be important in carcinogenesis. The polymerase gene encodes a large protein that has a role in packaging and DNA replication.[12]

There are eight major HBV genotypes: A is pandemic, B and C are found in Asia, D in southern Europe, E in Africa, F in the United States and South America, G in the United States and France, and H in South America.[11] To some extent, genotype influences the severity of hepatitis and its outcome. The severity of chronic hepatitis is greater with genotype C than with B, and there is a higher frequency of cirrhosis and hepatocellular carcinoma (HCC) in patients infected with HBV genotype C. A higher rate of virologic response is achieved among patients infected with genotype B, compared with genotype C.[13]

Pathophysiology of Hepatitis B Virus

Hepatitis B is not directly cytotoxic to hepatocytes. Instead, the pathogenesis of HBV infection is related to the host immune response to viral infection. More vigorous immune responses cause more severe liver injury. Patients with a vigorous immune

response may suffer fulminant infection with severe liver injury followed by rapid viral clearance, whereas hosts with less vigorous immune responses may become asymptomatic carriers.[10]

Natural History of Hepatitis B Virus

Transmission of HBV is parenteral. In developed countries, sexual contact, intravenous drug use, acupuncture, and transfusion constitute the most common modes of transmission. In developing countries, vertical transmission is more significant.

Acute hepatitis B manifests as an icteric illness after an incubation period of 6 weeks to 6 months in up to 50% of infected persons.[10] A subset of patients experience a prodromal phase characterized by arthralgias and urticarial skin rash.[14] Acute infection is diagnosed by the detection of hepatitis B surface antigen (HBsAg), IgM antibodies to hepatitis B core antigen (anti-HBcAg), and HBeAg. The outcome of acute hepatitis depends on the immune status and age of the host. Chronic HBV infection develops in as many as 90% of neonates and infants but in only 1% to 5% of immunocompetent adults.[10,11] Patients with chronic HBV infection rarely have extrahepatic manifestations, such as polyarteritis nodosa or glomerulonephritis.[14] Many remain asymptomatic until they present with cirrhosis, HCC, or both.

The presence of HBsAg in serum for 6 months or longer is indicative of chronic HBV infection. Chronic HBV infection manifests in one of several well-defined stages. The immune tolerance phase is seen largely in patients who acquire infection at birth or in early childhood. These patients have high levels of HBV replication but little to no liver inflammation and normal serum aminotransferase levels. Serum HBeAg is detectable, and HBV DNA is markedly elevated.[10,11,15,16] As the host immune system matures, the patient enters the immune clearance phase, which is characterized by immune-mediated liver injury. Patients who acquire infection as children come to clinical attention in this stage, and those who acquire infection as adolescents or adults have a very short or no immune tolerance phase and rapidly move into this second phase of the infection. Viral levels decrease, but HBV DNA is still elevated, and HBeAg is detectable. Serum aminotransferases increase, and liver histology shows active chronic hepatitis with evolving fibrosis.[10] Although most patients remain asymptomatic, some present with flares that mimic acute hepatitis, and this may precede the development of antibodies to HBeAg and remission of hepatitis activity.[10] Spontaneous seroconversion to anti-HBeAg antibody (HBeAb)-positive status occurs in up to 90% of white adults with chronic hepatitis B within 10 years of follow-up, and it is more likely in those with high transaminase levels, which indicate a vigorous immune response to HBV.[10,16]

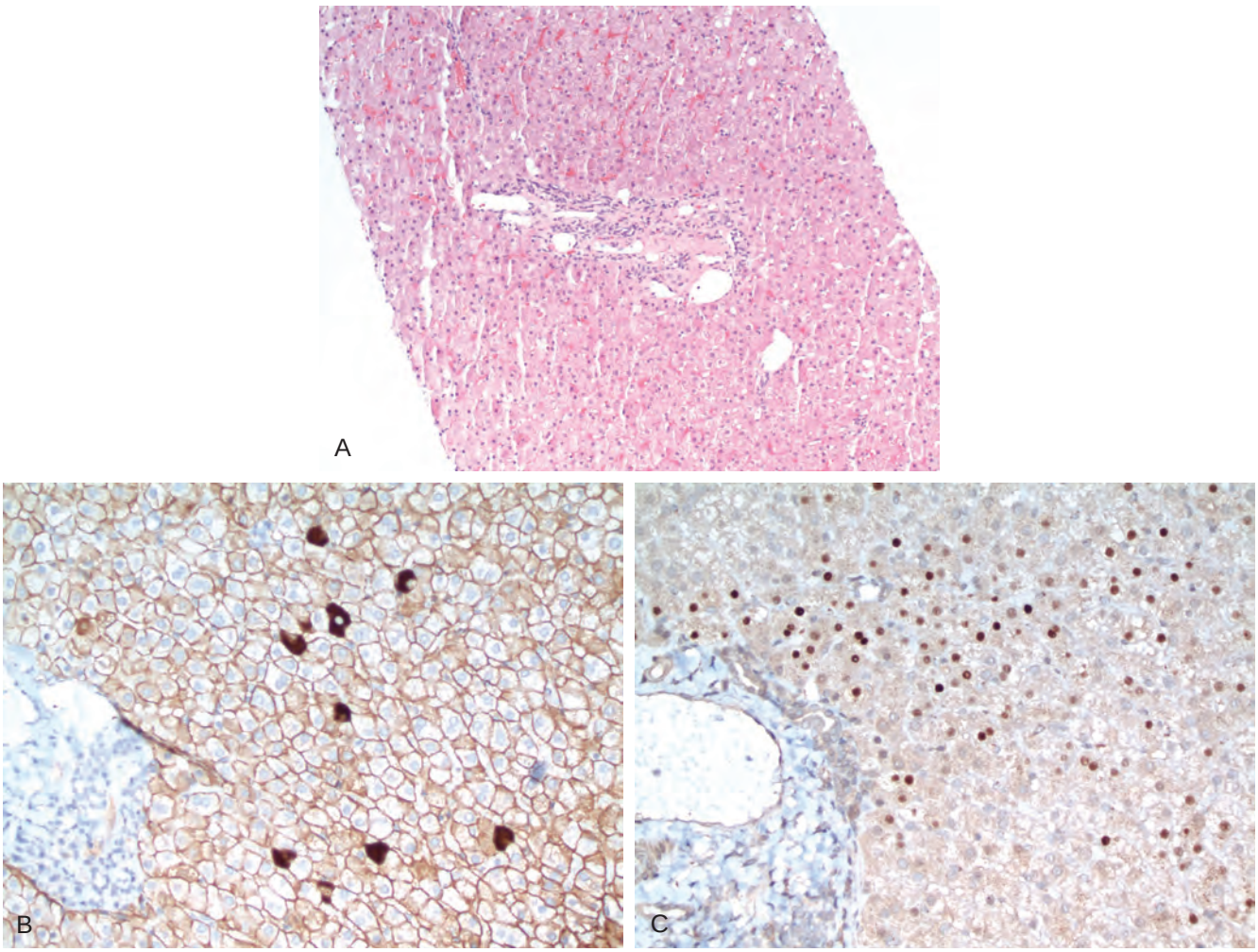
Seroconversion is followed by the low or nonreplicative HBsAg carrier stage, characterized by normalization of aminotransferases and low or undetectable HBV DNA levels.[10,16] Histologically, minimal to mild hepatitis with variable fibrosis is seen.[10] Most patients remain in this stage, particularly if they acquired the infection as adults; viral clearance may occur, but in patients with established cirrhosis, monitoring for HCC must continue.[10,17] Up to 20% of patients serorevert to HBeAg-positive status, with a flare of activity.[10,15,17] In approximately 20% of patients, chronic hepatitis recurs without seroreversion; this is known as HBeAg-negative chronic hepatitis, due to mutations in the precore or core-promoter regions of the HBV genome.[10,15,17] In this phase, despite the presence of HBeAb and the absence of HBeAg, HBV DNA is detectable, serum aminotransferases rise, and histologic examination of the liver shows chronic hepatitis.[10] However, patients with HBeAg-negative chronic hepatitis tend to have lower HBV DNA levels than patients with HBeAg-positive chronic hepatitis and to have a more fluctuating course.[15] In addition, they are generally older and have more advanced liver fibrosis.[10] Resolved chronic hepatitis B is defined by the loss of HBsAg and acquisition of antibody to HBsAg. Approximately 0.5% of persons with inactive chronic hepatitis clear HBsAg yearly, and most of these will develop antibodies to HBsAg.[15] However, covalently closed circular DNA remains in the nucleus of hepatocytes, even in patients with serologic evidence of resolved infection, and poses a livelong risk for reactivation of infection.[15]

Cirrhosis develops at an annual incidence of 8% to 10% in patients with HBeAg-negative chronic hepatitis and 2% to 5% in patients with HBeAg-positive chronic hepatitis.[10] Cirrhosis is the major risk factor for the development of HCC; the annual incidence of HCC is 1% for HBV carriers without cirrhosis and 2% to 3% for those with cirrhosis.[10] The risk factors for cirrhosis and HCC are similar and include high HBV DNA levels, HBeAg positivity, older age, and male gender.[10,16] Additional risk factors for HCC include abnormal alanine aminotransferase (ALT) levels, long duration of infection, coinfection with HCV or hepatitis D virus (HDV), a family history of HCC, excessive alcohol intake, cigarette smoking, HBV genotype C, and core-promoter mutations.[10]

Histopathology of Hepatitis B Virus

Acute HBV infection is indistinguishable from other forms of acute viral hepatitis. Portal tracts exhibit a moderate to marked lymphocytic infiltrate. Lobular mononuclear inflammation is associated with widespread lobular injury in the form of hepatocyte ballooning, although in the early stages the injury may be confined to centrilobular regions. Numerous acidophil bodies, canalicular cholestasis, and Kupffer cell hyperplasia may be seen. In more severe cases, bridging necrosis may span between portal tracts and central veins. Panacinar necrosis or multiacinar necrosis may also be a feature. Fulminant cases are characterized by submassive or massive necrosis with marked ductular reaction. Numerous macrophages laden with lipofuscin and hemosiderin may be seen in necrotic areas. Hepatic lobular regeneration may also be evident, with mitotic figures and lobular disarray. The latter can be highlighted by reticulin stains, which serve to delineate the loss of normal hepatic plate architecture.

The histology of chronic hepatitis B varies according to the phase of the disease and host immunity. The immune tolerance phase may show no or minimal portal and lobular inflammation and no fibrosis, despite rapid viral replication (Fig. 11.2). In the immune clearance phase, chronic hepatitis B shows portal mononuclear infiltrates with interface hepatitis and variable fibrosis (Fig. 11.3). Varying degrees of lobular necroinflammatory activity are present, but typically not to the extent seen in acute viral hepatitis. Hepatocyte anisonucleosis may be conspicuous. A characteristic feature of chronic HBV is the presence of ground-glass hepatocytes (Fig. 11.4), which contain HBsAg. These hepatocytes show a finely granular cytoplasmic inclusion that displaces the nucleus and is surrounded by a pale halo. Ground-glass hepatocytes can be demonstrated by various histochemical stains, such as Victoria blue, orcein, or aldehyde fuchsin,[18] and by immunohistochemical stains for HBsAg. In some cases the hepatocyte nuclei have a "sanded" appearance due to the accumulation of HBcAg, although these are difficult to recognize and also are seen in delta hepatitis.[18]



• **Figure 11.2** Liver biopsy in the immune tolerance phase of hepatitis B. **A,** Portal tract without inflammation or fibrosis. **B,** Immunohistochemical staining for hepatitis B surface antigen shows strong membranous staining of hepatocytes, as well as individual hepatocytes with strong cytoplasmic staining, consistent with high viremia. **C,** Immunohistochemical stain for the hepatitis B core antigen shows nuclear staining of many hepatocytes, consistent with high viral replication.

Immunohistochemistry of Hepatitis B Virus

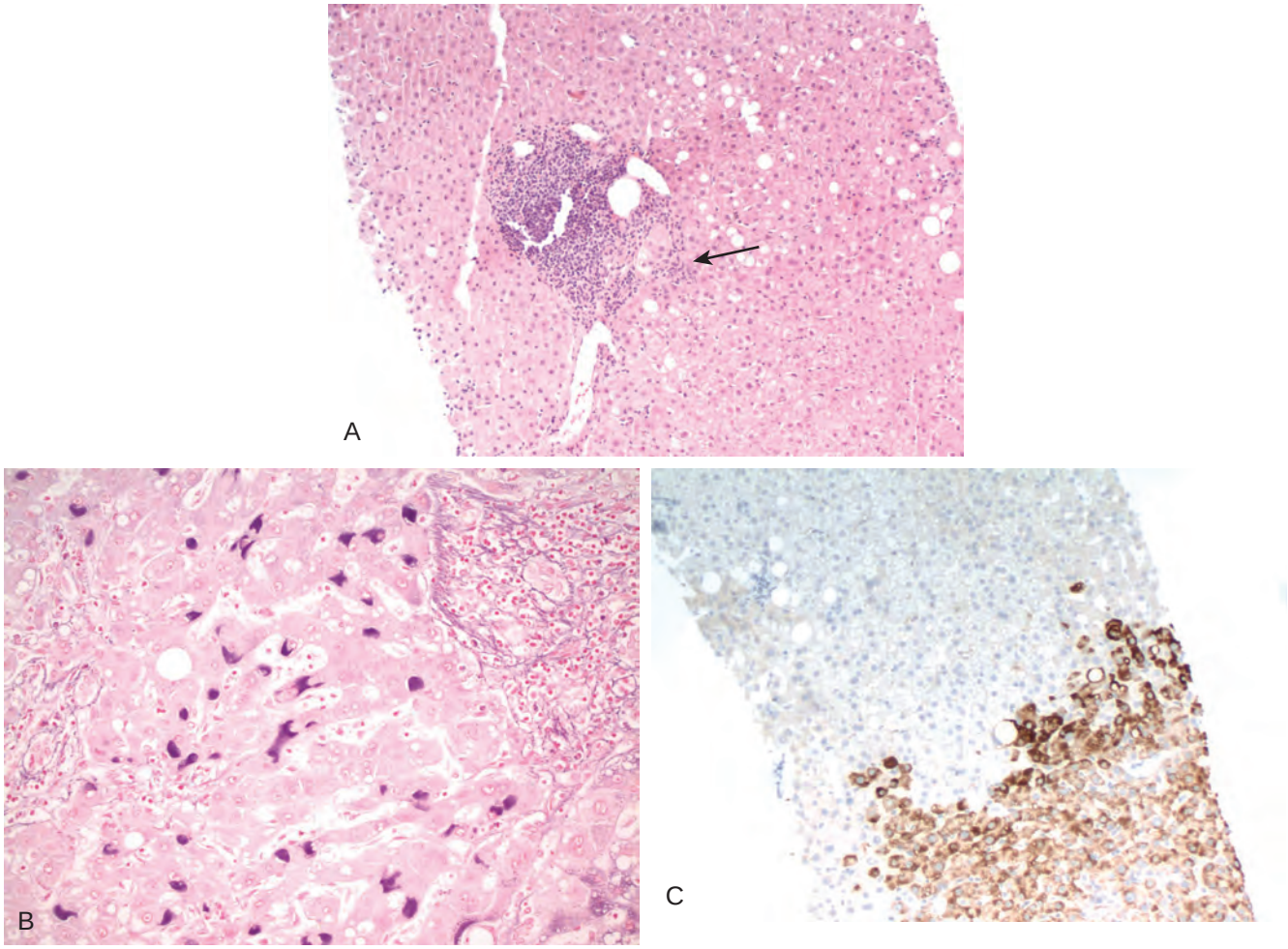
Immunohistochemistry for HBV antigens can be used to evaluate the pattern of antigen expression, which correlates with viral replication and disease activity (Table 11.1). Membranous expression of HBsAg in hepatocytes with strong cytoplasmic expression in individual hepatocytes indicates high viremia and is seen in the immune tolerance phase.[19,20] In contrast, cytoplasmic expression of HBsAg in clusters of hepatocytes is more often seen in patients with low or absent viremia and without active viral replication; these cells contain integrated HBV DNA, and clonal expansion of such cells may explain their clustering.[19,20]

Expression of HBcAg can be cytoplasmic, nuclear, or a mixed pattern. Nuclear expression correlates with the degree of viral replication and the level of HBV DNA[21]; biopsy specimens from patients in the immune tolerance phase or from immunosuppressed patients often show widespread nuclear staining, whereas those from patients with chronic hepatitis and low-replicative states have rare positive nuclei. Cytoplasmic HBcAg expression is associated with active liver damage and higher ALT level,[22] suggesting that HBcAg is the likely target for immune-mediated cytolysis.[19] Coinfection with delta virus can suppress HBcAg production and is one possible cause of a negative HBcAg stain in the setting of active hepatitis.

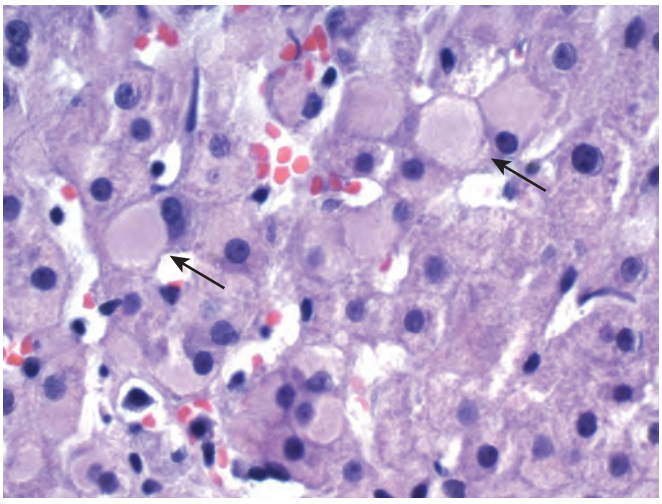
In general, immunohistochemistry findings for HBV antigens are negative in acute or fulminant hepatitis. Presumably, the inability to detect HBV antigen expression is a result of the short time interval of infection and insufficient accumulation of the proteins in hepatocytes to permit detection by immunohistochemistry.

Management of Hepatitis B Virus

The management of chronic HBV infection has improved significantly in the past decade with the introduction of nucleoside and nucleotide analogues. These agents are orally administered, well tolerated, and very effective at suppressing HBV DNA replication.[23] However, HBsAg clearance is rarely observed, and lifelong therapy is often required. Because lifelong treatment exposes patients to adverse reactions and high costs and may induce viral resistance,[23] the question of when to begin treatment remains an important one in the management of these patients. In general, patients with active disease benefit most from therapy. Although



• **Figure 11.3** Liver biopsy in the immune clearance phase of hepatitis B. **A,** Low-power view of a portal tract with a dense mononuclear infiltrate and focal interface hepatitis *(arrow)*. **B,** Aldehyde fuchsin stain confirms the accumulation of hepatitis B surface antigen within hepatocytes. **C,** Immunohistochemical stain for HBsAg shows cytoplasmic staining of clusters of hepatocytes, indicative of low viremia.



• **Figure 11.4** Ground-glass hepatocytes in chronic hepatitis B *(arrows)*. Ground-glass hepatocytes show finely granular cytoplasmic inclusions that displace the nucleus to the edge of the cell and are surrounded by a clear halo.

ALT can be an indicator of active disease, it has been shown that approximately 20% of patients with normal ALT levels have significant inflammation and/or fibrosis on biopsy, particularly among patients who are older than 35 years of age, who are HBeAg negative, or who have fluctuating ALT levels.[24] Therefore a liver biopsy is frequently used in questionable cases to determine whether there is sufficient necroinflammatory activity to warrant therapy.

Current American Association for the Study of Liver Diseases (AASLD) guidelines recommend antiviral therapy for adults with immune-active chronic hepatitis B, whether HBeAg negative or positive.[15] Immune-active disease is defined as elevation of ALT greater than 2 times the upper limit of normal or evidence of significant histologic disease plus elevated HBV DNA above 2000 IU/mL (HBeAg negative) or above 20,000 IU/mL (HBeAg positive).[15] Additional factors, including gender, age, family history of HCC, and presence of extrahepatic manifestations, may be taken into consideration for patients whose ALT or HBV DNA levels do not meet these criteria. The AASLD recommends against treating adults with immune-tolerant hepatitis B, but ALT levels should be tested periodically to monitor for the transition to immune-active stage.[15] Moderate to severe necroinflammatory

TABLE 11.1 Immunohistochemistry for Hepatitis B

	HBsAg		HBcAg	
	Membranous	Cytoplasmic	Nuclear	Cytoplasmic
Immune tolerance phase	++	++ (individual cells)	++	–
Immune clearance phase	–/+	+ (individual cells)	+ (few cells)	+/–
Low-replication states (chronic carriers)	–	++ (clustered cells)	–/+ (rare cells)	–
HDV/HCV coinfection	+	+/–	–	–
HBV in immunodeficiency states	++	+/–	++	+

–, Absent; +, present; *HBcAg,* hepatitis B core antigen; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HDV,* hepatitis D virus.

activity or fibrosis on liver biopsy warrants consideration to initiate antiviral therapy.[15]

All guidelines recommend continuing therapy in HBeAg-positive individuals at least until they achieve HBeAg seroconversion and have undetectable HBV DNA levels followed by 12 months of consolidation therapy. However, many experts feel that HBeAg seroconversion is not an acceptable endpoint and recommend therapy until HBsAg is lost, particularly in patients with advanced fibrosis or cirrhosis. Because HBsAg clearance is rare, most patients will be treated for life.[23]

Viral Mutants of Hepatitis B Virus

Precore and Core Gene Mutations. Precore and core gene mutations are associated with decreased production of HBeAg despite continued production of infectious virions. The most common mutation results in a stop codon that prematurely terminates the synthesis of HBeAg.[16] Other mutations in the basic core promoter downregulate HBeAg synthesis at the transcriptional level.[16] Patients infected with these mutants exhibit absent HBeAg but positive HBsAg, elevated serum HBV DNA, and elevated transaminases.

Surface Gene Mutations. Surface gene mutations are responsible for vaccine escape. Although patients infected with these mutants are infectious, HBsAg is not detectable.[12]

Polymerase Gene Mutations. Mutations in the YMDD catalytic site of the polymerase significantly reduce the effectiveness of lamivudine and famciclovir.[12,25] Despite developing this mutation, patients continue to receive some benefit from lamivudine therapy because the YMDD variant HBV exhibits reduced replication competence and reduced virulence.[12] Discontinuation of lamivudine may cause mutant virus to revert to wild-type, with renewed efficacy of lamivudine.[25]

Posttransplantation Hepatitis B

HBV frequently infects liver allografts after transplantation for HBV and may lead to deterioration of graft function, although some patients enjoy excellent graft function for many years despite active viral replication.[26] The risk of reinfection is greater after transplantation for chronic HBV infection with cirrhosis, compared with acute HBV.[27] The pattern of hepatitis in this setting ranges from purely immunohistochemical evidence of viral antigen expression without histologic features of HBV infection, to acute hepatitis, and to chronic hepatitis with cirrhosis.[26] Acute HBV hepatitis after transplantation may show marked ballooning and degenerative changes in the hepatocytes, scattered acidophil bodies, and extensive immunohistochemical evidence of viral antigen expression but with remarkably scant inflammation.[26,27]

A distinctive pattern of hepatitis in the transplantation population is known as fibrosing cholestatic hepatitis, initially described by Davies et al. in 1991.[26-29] This pattern is characterized by canalicular and cellular cholestasis, ballooning of hepatocytes, and scattered acidophil bodies with relatively scant parenchymal inflammation. Portal tracts are mildly to moderately inflamed and show periportal fibrosis, with immature fibrous tissue extending as thin perisinusoidal strands into the acinus. At the interface, a proliferation of ductal-type cells lends a hypercellularity to the portal areas. Immunohistochemical stains show extensive cytoplasmic and membranous expression with HBsAg and extensive nuclear and cytoplasmic HBcAg.[26,28,29] The combination of high HBV antigen expression, marked hepatocyte injury, and relatively little inflammation suggests that the virus itself may be cytopathic in this setting.[26,27,29] Fibrosing cholestatic hepatitis is associated with a high rate of viral replication, with high serum HBV DNA, high serum HBsAg titers, and rapid deterioration.[27-29] It has also been described in HBV infection in other settings involving immunosuppression, including human immunodeficiency virus (HIV) infection[30] and bone marrow transplantation.[31]

Coinfection With Hepatitis B Virus and Human Immunodeficiency Virus

Approximately 90% of HIV-infected persons show evidence of prior HBV infection, and 5% to 15% have chronic HBV infection.[32] In patients coinfected with HIV and HBV, the rate of clearance of HBsAg and HBeAg is reduced, compared with non–HIV-infected individuals.[33] This reduced clearance rate is most likely due to a weakened immune system. However, the reduced immune reaction is potentially responsible for the relatively reduced inflammation in these patients despite their higher HBV viral replication rates.[33] Even though there is reduced inflammation, HBV infection is more progressive in HIV-positive patients, including development of cirrhosis and its complications.[33] A pattern of hepatitis similar to fibrosing cholestatic hepatitis has been described in HIV patients with concurrent HBV infection.[30] The HBV-related mortality in HIV patients has increased since the introduction of highly active antiretroviral therapy (HAART), possibly because of increased immunologic injury to the liver with immune reconstitution, toxicity of the antiviral drugs, or longer life spans in HIV patients.[33] Conversely, the use of antiretroviral agents that also have activity against HBV may slow the progression of chronic HBV and even result in

seroconversion, whereas their discontinuation can cause significant liver disease due to reemergence of HBV replication.[32]

Hepatitis C

Hepatitis C affects between more than 170 million people worldwide.[34]

Virology of Hepatitis C Virus

HCV is an RNA flavivirus that was characterized in the late 1980s.[35] Its genome is a positive, single-stranded RNA with a large open reading frame that encodes a 3011- to 3030-amino-acid polyprotein.[36] This polyprotein is processed into an array of structural and nonstructural proteins. The structural proteins include the core protein and two envelope proteins, E1 and E2.[36] The nonstructural proteins are p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (RNA polymerase).[34,36]

There are six major genotypes and more than 50 subtypes. However, the genome of HCV is highly mutagenic, and a given host carries a mixture of viral particles with closely related sequences known as quasispecies.[36,37] The high mutation rate may allow the virus to escape the immune system; patients with chronic infection harbor highly diverse quasispecies, whereas those who clear the infection have low virus diversity and patients with fulminant hepatitis have the lowest level of viral diversity.[37] Genotypes 1, 2, and 3 have worldwide distribution, but their relative prevalence varies geographically.[38] Genotype 1a is the predominant genotype in North America (70%).[39] In Japan, subtype 1b is responsible for up to 73% of infections. Subtypes 2a and 2b are common in North America, Europe, and Japan, whereas subtype 2c is common in northern Italy. Genotype 3 is endemic in Southeast Asia, and it is also prevalent among intravenous drug users in Europe and the United States.[39] Genotype 4 is prevalent in North Africa and the Middle East; genotype 5 is largely confined to South Africa; and genotype 6 is found in Hong Kong, Macao, and Vietnam.[38,39]

The genotype affects the rate of evolution to chronic hepatitis, the severity of liver disease, and the response to interferon (IFN) therapy.[38] For example, genotype 1 is associated with a poor response to IFN therapy, whereas genotypes 2 and 3 respond more favorably.[39] An association between genotype 1b and an increased risk of developing severe liver disease and HCC has been reported.[39,40]

Natural History of Hepatitis C Virus

HCV is primarily transmitted parenterally, such as by recreational drug use, injection with contaminated syringes or needles, or blood transfusion.[35,41] Although sexual and vertical transmission occur, they are less important with HCV than with HBV. The incidence of HCV in the United States has fallen since the introduction of widespread blood donor screening and needle exchange programs.[37] However, since 2000, there has been an increase in the number of cases of acute HCV occurring in HIV-positive men who have sex with men (MSM).[34]

Acute infection can be diagnosed in a variety of ways, including documentation of anti-HCV seroconversion and detection of HCV RNA in the absence of HCV antibodies.[42-44] The mean incubation time for HCV is 6 to 8 weeks.[35] Although the majority (60% to 75%) of affected patients do not experience symptoms when acutely infected,[35] acute HCV still accounts for approximately 20% of cases of acute hepatitis.[41,45] The symptoms of acute HCV are malaise, fatigue, lethargy, anorexia, abdominal pain, jaundice, mild hepatosplenomegaly, maculopapular rash, and arthralgia.[35] Fulminant hepatitis is rare.

A minority of patients (approximately 15% to 50%) clear the infection, but most develop chronic viral hepatitis. Symptomatic onset of disease and female gender are associated with a higher chance of viral clearance after acute infection.[45] The serologic diagnosis of chronic HCV infection is made by detection of HCV antibodies, usually by enzyme immunoassay.[35] These assays have a 95% to 99% sensitivity and can detect antibodies 6 to 8 weeks after exposure. Polymerase chain reaction (PCR)-based methods can detect HCV RNA 1 to 3 weeks after exposure.[35] Patients with chronic HCV infection may present with normal transaminases. These patients are often identified during blood donation or screening. The rate of progression to fibrosis or cirrhosis is very low in this group.[41] Patients with elevated transaminases may suffer from fatigue or from nonspecific symptoms.[41]

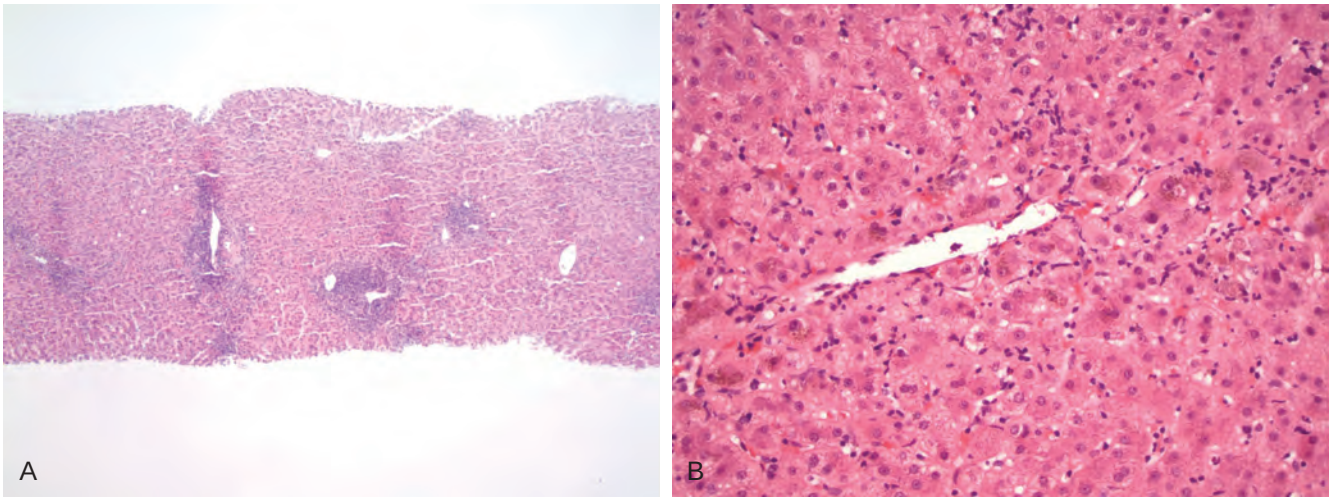
Extrahepatic manifestations may include mixed essential cryoglobulinemia, membranous or membranoproliferative glomerulonephritis, non-Hodgkin lymphoma, Sjögren syndrome, lichen planus, autoimmune thyroid disease, and porphyria cutanea tarda.[35,41,46] A subset of patients with HCV demonstrate autoantibodies similar to those seen in autoimmune hepatitis, namely antinuclear antibodies (ANAs), smooth muscle antibody (SMA), perinuclear antineutrophilic cytoplasmic antibody (p-ANCA), and anti-asialoglycoprotein receptor, although often at lower titer than is typically seen in autoimmune hepatitis.[46] Less often, liver-kidney microsomal (LKM1) autoantibodies are detected, although the epitopes recognized by these antibodies in HCV differ from those in autoimmune hepatitis type 2.[46] Patients with autoantibodies tend to be females and to have higher transaminase levels.[47] Some of these patients experience exacerbation during IFN-α therapy that may respond to steroid therapy, suggesting either preexisting autoimmune hepatitis or induction of autoimmune hepatitis in these patients.

The rate at which chronic hepatitis C progresses to cirrhosis depends on several factors. Factors that increase the rate of progression include male gender, older age at infection acquisition, longer duration of infection, immune suppression (e.g., HIV coinfection), HBV coinfection, alcohol use, and obesity.[35] The risk of developing cirrhosis is approximately 20% to 30% after 10 to 20 years of infection.[41] After cirrhosis has developed, the risk of liver disease–related mortality is 2% to 5% per year and the risk of developing HCC is 3% to 5% each year.[41]

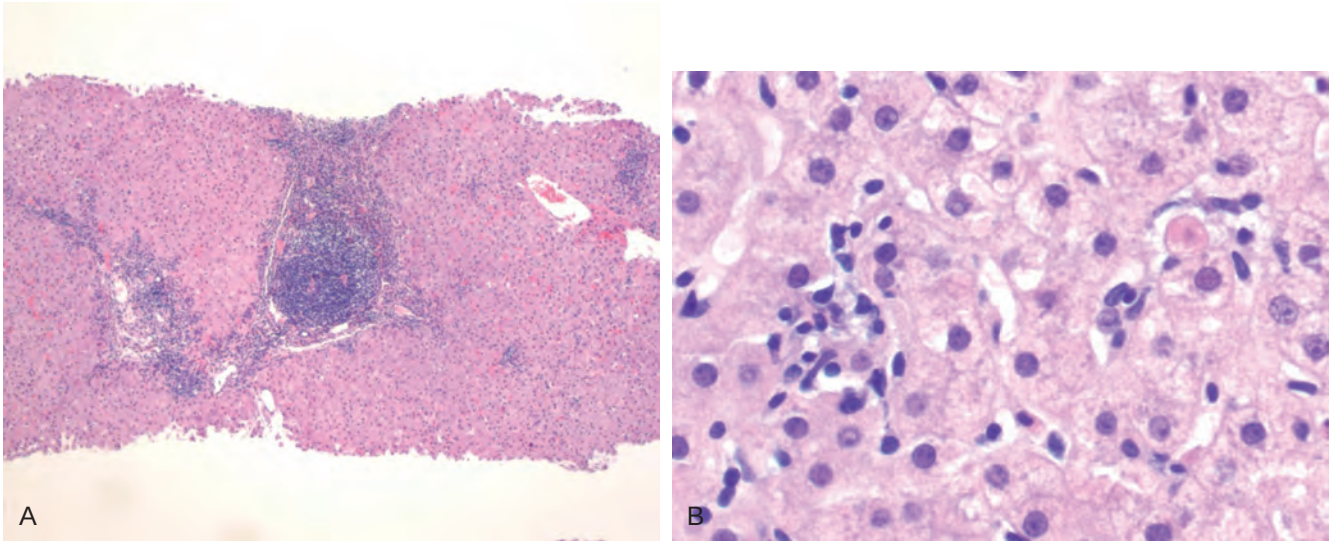
Histopathology of Hepatitis C Virus

Acute hepatitis C is characterized by panlobular inflammation, numerous acidophil bodies, and lobular disarray similar to that seen in other acute hepatitides. A sinusoidal pattern of inflammation can mimic Epstein-Barr virus (EBV) hepatitis. More severe patterns of acute hepatitis, such as bridging necrosis or panacinar necrosis, are typically absent. Portal tracts harbor dense mononuclear infiltrates, resembling chronic HCV infection (Fig. 11.5). Bile duct injury may be present. Cholestatic forms of acute HCV infection occur rarely, predominantly in the immunosuppressed population.

Chronic HCV infection is characterized by dense mononuclear cell aggregates or follicles in portal tracts, with mild to moderate interface hepatitis.[48] Bile duct injury may be prominent, although typically it is mild. The lobules show scattered acidophil bodies (Councilman bodies) or foci of lytic necrosis marked by a small cluster of mononuclear cells (Fig. 11.6).[48] Kupffer cell prominence and lymphocytic infiltration of sinusoids may be seen.[18,48] Variable



• **Figure 11.5** Liver biopsy in acute hepatitis C. **A,** Low-power view shows mononuclear cell infiltrates within portal tracts, similar in appearance to chronic hepatitis C virus infection. **B,** High-power view of the centrilobular region shows sinusoidal lymphocytic infiltrates surrounding a central vein.



• **Figure 11.6** Liver biopsy in chronic hepatitis C. **A,** Low-power view shows a dense lymphoid aggregate within a portal tract. Bridging fibrosis is also evident, with mononuclear infiltrates in the septum. **B,** Lobular inflammation shows lytic necrosis on the left with a cluster of mononuclear cells indicating a focus of hepatocyte necrosis and a Councilman body (apoptotic hepatocyte) on the right; the latter is characteristic of hepatitis C virusinfection.

fibrosis is present, and its extent often drives the decision of whether to treat the infection. Demonstration of virus by immunohistochemistry[49] or in situ hybridization[50] has been described but does not play a significant role in the routine evaluation of liver biopsies in hepatitis C.

Mild to moderate steatosis is characteristic of chronic HCV infection.[48] Steatosis may be related to direct viral cytopathic effects in patients with genotype 3 but to underlying metabolic status in patients with other genotypes.[51,52] HCV core protein expression produces steatosis in mice through mitochondrial toxicity and production of reactive oxygen species.[51] The severity of steatosis correlates with fibrosis.

Sarcoid-like granulomas are occasionally seen in liver biopsy specimens from patients with HCV. In one series, 9.5% of hepatic granulomas were attributed to HCV.[53] In another series, 5 (10%) of 52 liver explants for HCV-related cirrhosis had granulomas for which no other cause could be identified.[54] In a biopsy series, 14 of 155 biopsies for HCV had granulomas, but half of them could be ascribed to another cause (sarcoidosis, schistosomiasis, primary biliary cirrhosis [PBC], or mycobacterial infection).[55] In a large series of 542 biopsies for HCV, only 2% had granulomas. In that series, the presence of granulomas predicted a better response to IFN-α therapy.[56] Others have described granulomas in HCV after treatment with IFN-α in patients who did not respond well to IFN-α.[57-59] In short, when a granulomatous process is encountered in a patient with HCV, other causes of granulomas in the liver must be rigorously excluded before they can be attributed to HCV. A history of IFN-α therapy should be sought.

The granulomas in HCV may occur in portal tracts or in the lobules. It is well known that HCV can be associated with mild bile

duct injury. If the granulomas are in the portal area, their presence in conjunction with injured bile ducts may mimic PBC. Other clinical information (e.g., antimitochondrial antibody [AMA], alkaline phosphatase level) may be needed to distinguish the two.

After successful treatment of hepatitis C, most patients experience either stable or reduced fibrosis and have a reduced risk for hepatic decompensation and HCC. However, curiously, a small percentage of patients who appear to have been successfully treated for HCV actually continue to progress and may still have inflammation in liver biopsies.[60,61] One factor that might explain this variable response is that patients with early cirrhosis are more likely to regress than those with advanced cirrhosis. In advanced cirrhosis, the extensive deposition of elastin and increased cross-linking of the extracellular matrix may prove more resistant to degradation.[61] Another theory that has been proposed as a possible explanation for this phenomenon is the concept of "occult HCV" in which very sensitive assays are able to detect the presence of virus in plasma, lymphocytes, and macrophages in patients with a confirmed sustained viral response (SVR).[61] Finally, genetic variants may be responsible because the rate of fibrosis regression differs among different ethnic groups.[61] Patients who did not enjoy a good response to therapy tend to progress similarly to those who were not treated.[60]

Management of Hepatitis C Virus

The management of HCV has changed dramatically in the past several years. From the mid-1980s until 2014, the mainstay of treatment for chronic HCV included IFN. Standard IFN therapy was administered 3 times weekly and resulted in a fluctuating HCV RNA level.[62] By attaching a polyethylene glycol moiety to IFN (pegylated interferon [peg-IFN]), a single weekly injection became possible. The addition of ribavirin (RBV) to peg-IFN improved SVR rates and became the mainstay of therapy.[62] This combination achieved an SVR in approximately 40% to 50% of patients with genotype 1 and in approximately 80% of patients with genotypes 2, 3, 5, and 6.[63] An important discovery during this era was that polymorphisms of the *interleukin-28B (IL-28B)* gene predicted response to therapy in patients with genotype 1.[62] Among persons with genotype 1, the non-C/C genotype (CT or TT) was associated with reduced rates of SVR.

After the HCV genome had been mapped out, it became possible to target structural components of the virus itself. Direct-acting antivirals (DAAs) target several steps in the life cycle of HCV, including NS3-NS4A, NS5A, and NS5B RNA-dependent RNA polymerase.[63] In 2011 the first DAAs, boceprevir and telaprevir, protease inhibitors (PIs) of NS3-NS4, were approved for the treatment of chronic HCV, in combination with peg-IFN and RBV.[63] However, the initial enthusiasm for these agents was dampened by their significant toxicity. Second-generation PIs, such as simeprevir, have fewer drug-drug interactions and less severe side effects, with increased efficacy against genotype 1.[63]

NS5B inhibitors include nucleotide/nucleoside polymerase inhibitors (NPIs) and nonnucleotide/nucleoside polymerase inhibitors (NNPIs). NPIs are incorporated into the nascent RNA chain and cause chain termination, whereas NNPIs act as allosteric inhibitors of the polymerase.[63] Sofosbuvir is the first NS5B NPI available in the United States and has become the backbone of HCV therapy given its high barrier to resistance, pangenotypic activity, and favorable pharmacologic profile. In 2014 IFN-free combinations became available that proved to have extremely high efficacy in achieving SVR in patients with chronic HCV, including patients considered difficult to treat, such as cirrhotics and those with genotype 1. Still, genotype may continue to be useful information in the decision of which combination to use because some, not all, DAAs have consistent activity across all genotypes and some genotypes are more likely to harbor resistance mutations. Despite the excitement IFN-free treatment of HCV has produced, the high cost of these drugs and other barriers to access remain as challenges to the eradication of HCV.

Posttransplantation Hepatitis C

Historically, after transplantation for chronic HCV, reinfection of the graft was almost universal, although with the advent of highly successful therapy, patients will increasingly be treated before transplantation to prevent reinfection of the allograft. Although most patients do well in the long term, recurrent HCV can be progressive and can lead to graft dysfunction. The histologic features of recurrent HCV hepatitis progress from acute lobular hepatitis with scattered acidophil bodies and sinusoidal lymphocytic infiltration in the early stage to portal-based hepatitis with portal lymphoid aggregates typical of HCV infection in the chronic phase.[64,65] Progressive fibrosis and cirrhosis of the graft may result. A rapidly progressive cholestatic form of HCV infection has been described in the transplantation population, similar to the fibrosing cholestatic hepatitis described with HBV.[66] Fibrosing cholestatic hepatitis secondary to HCV has also been described in other immunosuppressed patient populations, such as in HIV patients[67] and after heart or kidney transplantation.[68-70]

Coinfection With Hepatitis C Virus and Human Immunodeficiency Virus

In the United States and Europe 33% of HIV-infected persons are coinfected with HCV.[32] The clearance rate of HCV after acute infection is reduced in HIV-infected patients.[32] In chronic infection, HCV RNA levels are higher in HIV-coinfected patients, the efficacy of anti-HCV therapy is reduced, and the incidence of cirrhosis and HCC is higher.[25,32,33] In hemophiliac patients infected with HCV, coinfection with HIV has been associated with increased severity of hepatitis and increased risk of developing cirrhosis and liver failure.[71,72] Fibrosing cholestatic hepatitis related to HCV has been described in patients with HIV coinfection.[67] With the introduction of HAART, HCV-related liver disease has become an important factor in hospitalizations and mortality of HIV patients.[33] Patients with HCV are more likely to suffer from hepatotoxicity related to HAART and to have impaired immune reconstitution.[32]

Hepatitis D

HDV (delta virus) is a defective, single-stranded, circular RNA virus that requires the lipoprotein coat of HBV for its replication; therefore infection from HDV alone does not occur. Eight genotypes are known; genotype 1 is the most common worldwide. There are two modes of infection: coinfection with HBV and superinfection of prior HBV infection. The severity of hepatitis in coinfection depends on the virulence of the concomitant HBV infection and ranges from mild hepatitis to severe acute hepatitis with high mortality.[73] The usual outcome is complete recovery, and only 2% of patients develop chronic disease; resolution produces immunity to both viruses. Superinfection presents as exacerbation in a patient with preexisting chronic hepatitis or as a new hepatitis in a previously asymptomatic HBV carrier. In superinfection, chronic infection develops in more than 90% of cases.[73] HDV infection inhibits HBV replication, resulting in

little to no evidence of HBV replication, with antibodies to HBeAg and low HBV DNA.[73] Treatment with IFN-α inhibits HDV replication, but relapse is common.

HDV infection may be associated with autoimmune manifestations in a subset of patients. Anti-liver kidney microsomal 3 (LKM3) autoantibodies are detected in 13% of patients with HDV.[46] However, one study found liver-specific autoantibodies (anti-asialoglycoprotein receptor) and non–organ-specific autoantibodies (e.g., ANA, anti-SMA) in 60.3% and 22.1% of HBV patients, respectively, regardless of whether HDV was present.[74] The pathology of HBV and delta virus is indistinguishable from that of HBV alone,[75] except that the degree of inflammation and hepatocyte necrosis is often more prominent than with HBV alone. Liver specimens from an outbreak among Venezuelan Indians showed a range of histologic severity from diffuse small-droplet steatosis with focal necrosis in the earliest stages to confluent necrosis and, in the late stage, postnecrotic cirrhosis with massive collapse.[76] Immunohistochemistry in cases of hepatitis D demonstrates delta antigen in nuclei of hepatocytes, reduced or absent expression of HBcAg, but continued expression of HBsAg.[75-77]

Hepatitis E

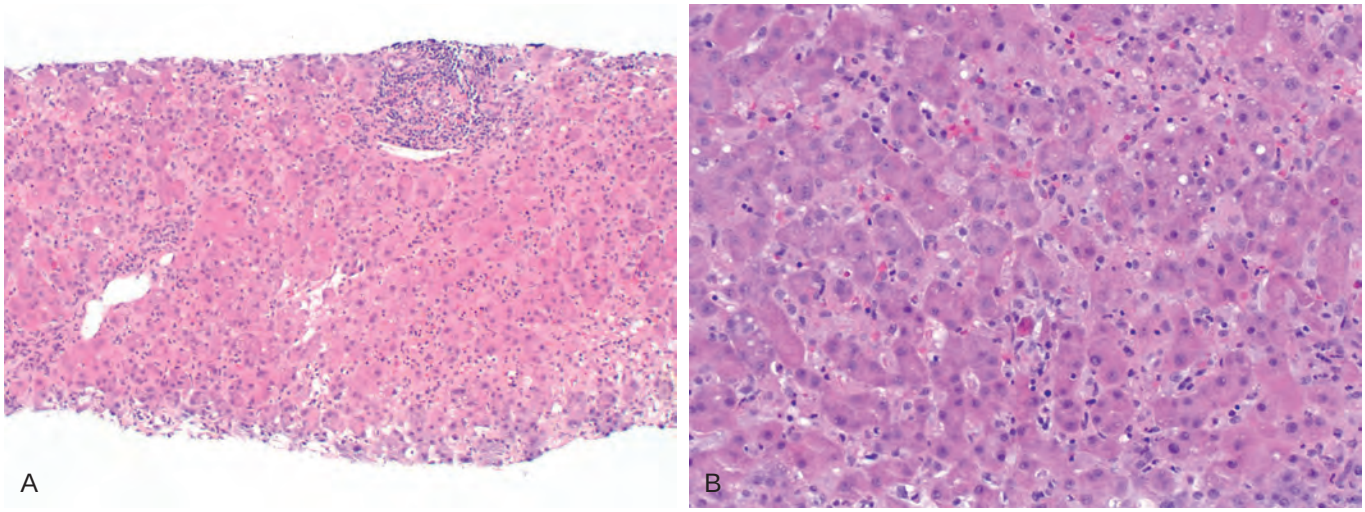
Hepatitis E virus (HEV) is a nonenveloped, positive-sense, single-stranded RNA virus of the Hepeviridae family. There are four genotypes: genotypes 1 and 2 cause human disease in endemic areas, predominantly in developing countries, whereas genotypes 3 and 4 infect animals and cause zoonotic infections in humans in nonendemic areas. The virus is spread through fecally contaminated water or food, and epidemics have occurred in central and Southeast Asia, the Middle East, and North Africa.[78,79] In contrast, genotype 3 has been detected in domesticated pigs, wild boars, deer, and rodents and can be transmitted by porcine meat.

Most often, hepatitis E presents as acute hepatitis that is clinically indistinguishable from hepatitis A, ranging from asymptomatic infection to fulminant hepatitis. Mortality rate is 1% to 4% overall, but it is approximately 20% among pregnant women.[78,79] In immunocompetent individuals, there is no progression to chronic hepatitis. Although hepatitis E has been regarded as a disease largely restricted to underdeveloped countries with warm climates, it is being increasingly recognized in industrialized countries; indeed, a small percentage of presumed cases of drug hepatitis may be acute hepatitis E.[80] Among 400 patients with acute hepatitis in Montenegro, 6% were demonstrated to have HEV by the presence of IgM anti-HEV.[81]

In immunosuppressed patients, chronic HEV infection with prolonged viremia has been described. Chronic HEV infection occurs largely in the solid organ transplant population,[82,83] but rare cases in HIV-positive individuals are described.[84] Chronic HEV might explain some cases of otherwise unexplained chronic hepatitis in liver allografts that, in some patients, leads to cirrhosis, sometimes requiring retransplantation.[85] Factors that predispose to chronic HEV include liver transplantation (relative to kidney transplantation) and tacrolimus immunosuppression rather than cyclosporin A.[82] Most patients eventually clear the virus, usually because of reduced immunosuppression, although treatment with RBV and peg-IFN may be beneficial in some patients.

Serologic diagnosis of HEV infection is hampered by the high prevalence of anti-HEV in the general population and a high false negative rate in immunocompromised individuals. The prevalence of anti-HEV ranges from 6% to 53% of the population, depending on the region surveyed. For example, in a nationwide survey of blood donors in France, 22.4% of the population had IgG to HEV and 1% of the population had IgM to HEV.[86] Areas of highest prevalence of IgG corresponded to areas of higher consumption of pork meat, raw pork liver sausage, game meat, offal, and oysters. In the United States the seroprevalence rate between 1988 and 1994 was 21%, with the likelihood of exposure increasing with age.[87] The presence of anti-HEV IgM is required to make the diagnosis of acute infection, but specific testing may not be available in all geographic locations. A rising IgG anti-HEV titer can provide evidence of infection, and PCR for HEV RNA provides additional supportive evidence.

The histopathology of acute HEV is similar to HAV in that it usually produces classic acute hepatitis (Fig. 11.7). Cases from endemic regions have been described as having ballooning degeneration of hepatocytes, apoptotic bodies, pseudorosette formation of hepatocytes, steatosis, and portal plasma cells.[88] Cases of



• **Figure 11.7** Liver biopsy in acute hepatitis E. **A,** Medium-power view showing lymphohistiocytic infiltrate in sinusoids, mild lobular disarray, and mononuclear cells in portal tracts. **B,** High-power view shows occasional acidophil bodies *(center)*, mild lobular disarray, small droplet fat in some hepatocytes, and lymphohistiocystic inflammation.

autochthonous HEV hepatitis due to genotype 3 show acinar and portal inflammation with lymphocytes, plasma cells, and polymorphs, with equal numbers of eosinophils and neutrophils. Neutrophilic or lymphocytic cholangitis, cholestasis, lobular disarray with ballooning degeneration, hepatocyte rosettes, perivenular edema, confluent necrosis, anisonucleosis, and Kupffer cell aggregates are also described.[89,90] In transplant patients, biopsies may show mild portal and lobular hepatitis with acidophil bodies, progression to portal-based chronic hepatitis, and variable fibrosis including cirrhosis.[83,85]

George Barker Virus Type C (Hepatitis G)

During the investigation for the cause of non-A, non-B hepatitis, a virus was discovered that was initially classified as hepatitis G but is now considered nonpathogenic. In 1966 a surgeon, George Barker (GB), became ill with acute hepatitis, and his blood was used to infect marmosets, which developed hepatitis. Serum from these animals was used to infect other primates. Eventually, two viruses were discovered in this primate group and called GB virus A (GBV-A) and GB virus B (GBV-B), after the surgeon.[91,92] Attempts to isolate these viruses from humans failed, but a related virus, GB virus C (GBV-C), was isolated from patient material.[91] Meanwhile, independent investigators discovered a novel virus that they classified as hepatitis G, and soon it was discovered that GBV-C and hepatitis G were genetically identical.[91] Like HCV, GBV is classified as members of the family Flaviviridae.[92]

Most immunocompetent people clear the virus in approximately 2 years, although some patients have persistent viremia. Clearance of the virus is associated with the production of antibodies to the envelope glycoprotein E2. Infection with GBV-C is worldwide and common. One percent to 4% of blood donors are viremic at the time of donation, and another 13% have antibodies.[93] Among HIV-positive homosexual men, approximately 85% are viremic or have antibodies indicating prior infection,[93] evidence of sexual transmission. Prostitutes also have high prevalence. Other methods of transmission are transfusion and maternal transmission.[91]

Because GBV-C was found in patients with hepatitis and at risk of developing parenteral hepatitis, it was initially believed to be a hepatotropic virus responsible for some cases of transfusion-related hepatitis, acute hepatitis, or chronic hepatitis. However, the virus is actually lymphotropic, not hepatotropic, and no convincing association between the virus and human disease has been identified.[92] Therefore the virus is currently believed to be nonpathogenic.

Epstein-Barr Virus

The liver is involved in more than 90% of cases of infectious mononucleosis, which is caused by EBV infection; hepatomegaly is present in 10% to 15% of cases, splenomegaly in 50%, and jaundice in only 5%. Most often, the hepatic manifestations of EBV infection consist of self-limited elevations of hepatic transaminases. Rarely, EBV hepatitis has more serious consequences, such as the induction of autoimmune hepatitis,[94] severe hepatitis with jaundice,[95,96] or liver failure.[97-99]

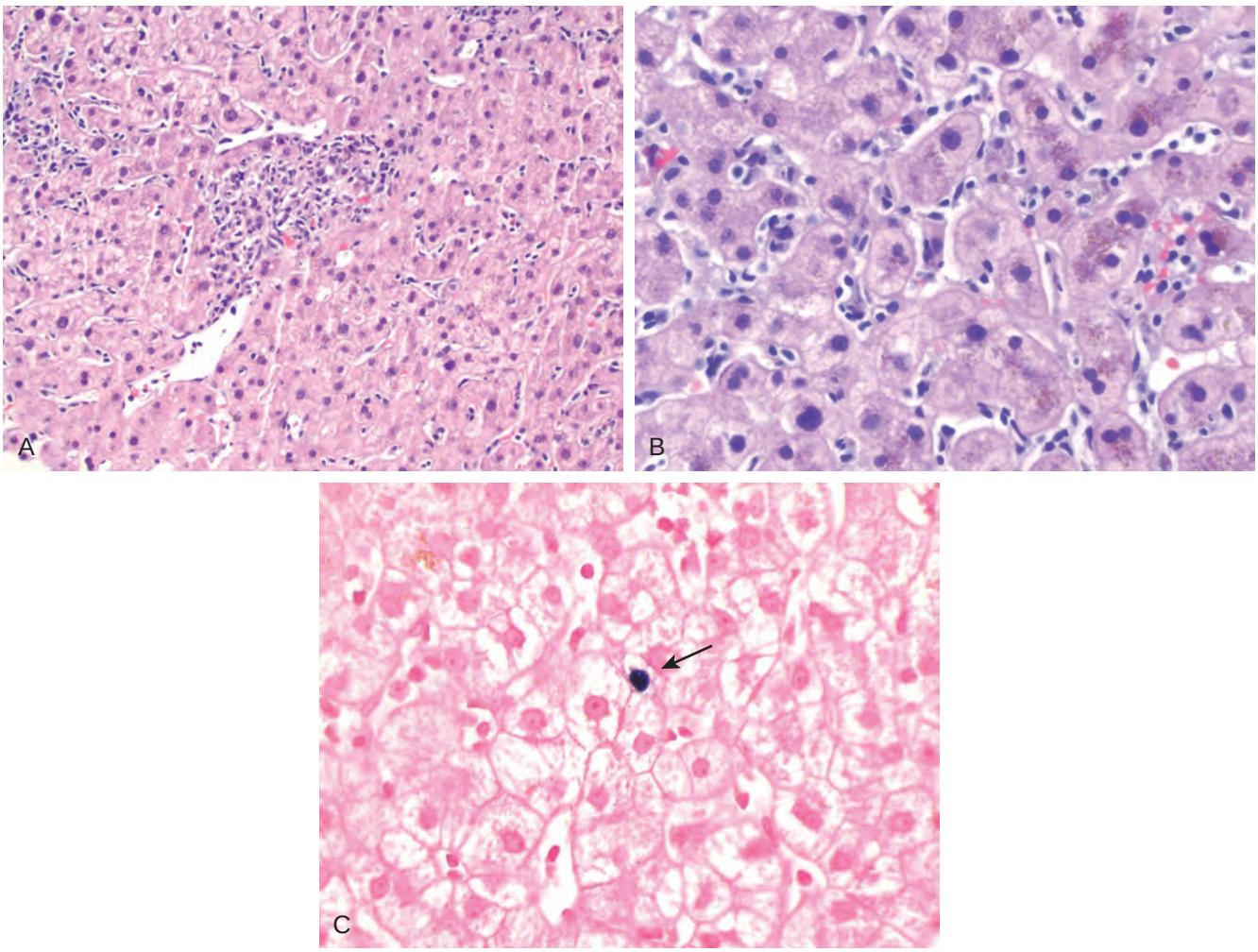
Liver biopsy shows portal and periportal infiltrates of small and large lymphocytes, with occasional larger immunoblastic cells resembling the Reed-Sternberg cells of Hodgkin disease. A characteristic feature is sinusoidal infiltration by these same lymphocytes, which creates a beaded appearance (Fig. 11.8).[100] Bile duct injury may be subtle.[95] Liver cell ballooning is not prominent, although hepatocyte regeneration, canalicular cholestasis, and Kupffer cell hyperplasia are variably seen. Areas of necrosis may be infiltrated by collections of mononuclear cells, creating a granulomatous appearance. Although these histiocytes usually do not form true epithelioid granulomas, well-developed nonnecrotizing granulomas[101] and fibrin ring granulomas have been reported in patients with EBV hepatitis.[102] In fatal cases, submassive lobular necrosis has been seen.[97]

Detection of EBV is done by in situ hybridization for EBV-encoded RNA (EBER) or by PCR for EBV DNA.[103] Immunohistochemistry for EBV latent membrane protein has not proved to be a reliable method for detecting EBV in EBV hepatitis.

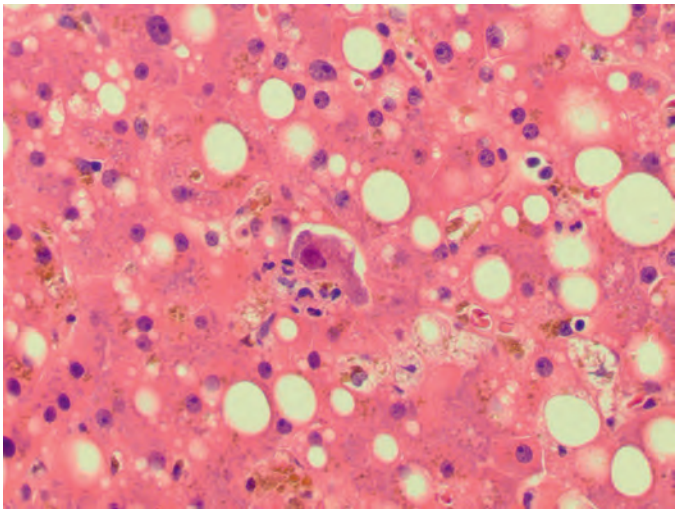
Cytomegalovirus

In neonates, cytomegalovirus (CMV) infection has been associated with neonatal hepatitis or neonatal cholestasis, although establishing a serologic diagnosis in these cases is challenging due to maternal transfer of antibodies and poor IgM production in neonates.[104] Several studies have found CMV DNA in the serum or liver tissue of neonates with liver dysfunction or cholestasis.[104,105] The histology in these cases may resemble extrahepatic biliary atresia, with portal expansion, bile ductular proliferation, and bile stasis. Fatal cases may show widespread hepatocyte necrosis, lobular disarray with pseudorosettes, giant cell transformation of hepatocytes, and ductular metaplasia of hepatocytes.[106] Cytomegalic inclusion bodies have been described in hepatocytes, endothelial cells, and bile duct epithelium.[106] However, inclusions may be absent by routine histologic assessment, and immunohistochemistry or in situ hybridization may be useful, although their utility in this population has not been formally studied.[105] CMV has also been implicated in extrahepatic biliary atresia, based on studies demonstrating CMV DNA in liver tissue, serologic evidence of CMV infection, or positive urine cultures in a subset of infants with biliary atresia.[107-109] Inclusions have not been described by histologic assessment.[108] Although intriguing, the possible association between biliary atresia and CMV may lead to delay in diagnosis of biliary atresia when a child presenting with cholestasis is found to have clinical evidence of CMV infection.[107,108] Clinical evidence of CMV infection should not preclude evaluation for other causes of cholestasis, particularly biliary atresia, which requires early surgical intervention.

In adults, CMV infection is clinically mild and self-limited; CMV accounts for 8% of cases of infectious mononucleosis–like syndrome with hepatic involvement. Rare cases of massive hepatic necrosis have been reported, usually in immunocompromised hosts. In immunocompetent hosts, CMV infection can result in a histologic picture virtually identical to that of EBV hepatitis, with sinusoidal beading, atypical lymphocytes within sinusoids, lymphocytic infiltrates in portal tracts, and areas of necrosis with aggregates of Kupffer cells resulting in a granulomatous appearance.[110,111] CMV infection is rarely associated with well-formed nonnecrotizing granulomas[112] or fibrin ring granulomas.[113] In immunocompromised patients, viral inclusions may be found in hepatocytes, endothelial cells, Kupffer cells, and duct epithelium (Fig. 11.9). Virally infected cells show a large amphophilic nuclear inclusion surrounded by a halo, known as an "owl's-eye" nuclear inclusion, and coarsely granular cytoplasmic inclusions. The inclusions may be isolated, or they may elicit an inflammatory reaction that can be granulomatous or neutrophilic, the latter being especially common in transplantation patients.[110] In fact, a



• **Figure 11.8** Liver biopsy in Epstein-Barr virus infection. **A,** A portal tract shows a mild mononuclear infiltrate, and the lobules show infiltration of sinusoids by mononuclear cells. **B,** High magnification shows prominent sinusoidal lymphocytic infiltration without hepatocyte necrosis. **C,** In situ hybridization for Epstein-Barr virus–encoded RNA shows nuclear staining of a single infected lymphocyte *(arrow)*.

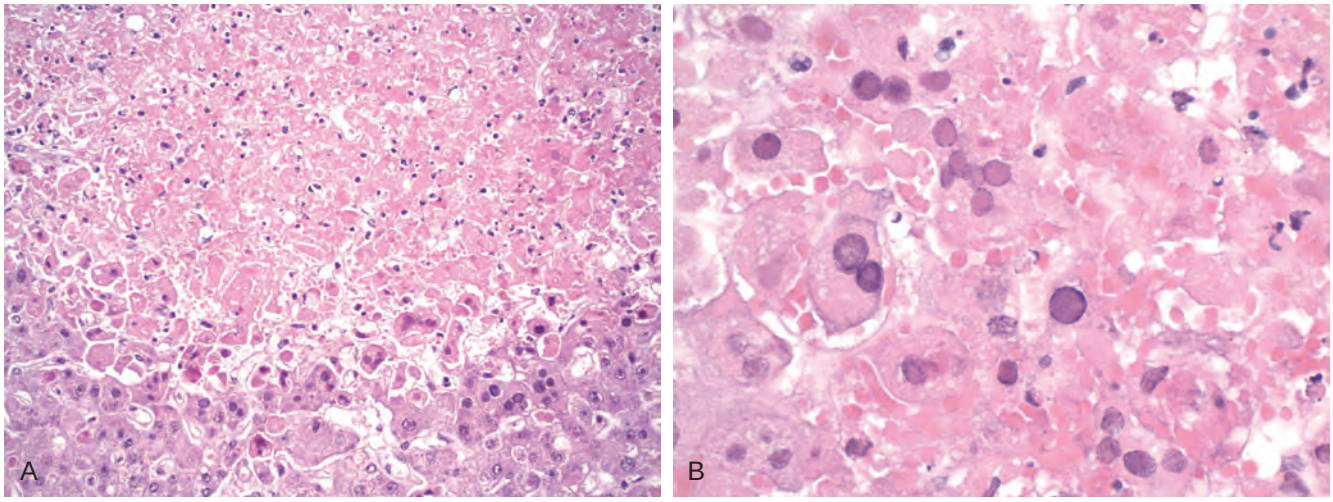


• **Figure 11.9** Cytomegalovirus (CMV) hepatitis, demonstrating a CMV inclusion in a hepatocyte associated with a few mononuclear inflammatory cells.

pattern of "disseminated focal hepatitis" with small inflammatory foci in the lobules composed of either neutrophils or mononuclear cells should prompt exclusion of CMV in transplant patients by searching for inclusions or immunohistochemistry.[114] In HIV-positive patients, bile duct involvement may result in sclerosing cholangitis (HIV-associated cholangiopathy). Immunohistochemical stains for CMV can highlight cells with inclusions, including atypical inclusions, and may stain infected hepatocytes that are not recognizable as infected cells on routine stains, increasing sensitivity for the diagnosis of CMV hepatitis.

Herpes Simplex Virus

Herpes simplex virus (HSV) viremia can result in visceral involvement, affecting mainly the esophagus, lungs, and liver. Liver involvement occurs primarily in neonates, pregnant patients, and immunocompromised patients, although immunocompetent adults are rarely affected as well.[115,116] Dissemination usually occurs in primary infection (particularly if the patient is immunocompetent) but has also been described in reactivation.[117] In children, severe protein-calorie malnutrition and the postmeasles



• **Figure 11.10** Herpes simplex virus infection. **A,** The liver shows necrotic regions without an inflammatory response. **B,** Hepatocytes at the edge of the necrotic regions show glassy nuclear inclusions and multinucleation, consistent with herpes simplex virus infection.

state predispose to disseminated HSV infection.[117] Oral ulcers and skin lesions may or may not be present. HSV hepatitis is rapidly lethal and requires early recognition and institution of antiviral therapy to improve outcome.

HSV hepatitis is characterized by patchy, nonzonal coagulative necrosis with minimal to absent inflammatory response (Fig. 11.10). Mild to severe fatty change may be present.[117] Intranuclear inclusions of two types can be found in hepatocytes at the edge of the necrotic foci, and virally infected cells are often multinucleated.[115,116] Cowdry type A inclusions are large, eosinophilic intranuclear inclusions surrounded by a halo, whereas type B inclusions replace the entire nucleus with a basophilic ground-glass appearance. Immunohistochemistry for herpes type I and type II antigens highlights the nuclear inclusions, and overlap between the two antibodies is frequent.[115,116]

Treatment with antiviral drugs before the biopsy is obtained may result in a biopsy specimen with extensive necrosis but without diagnostic inclusions.[116] The main differential is varicella zoster virus (VZV) infection and adenovirus infection, both of which have a similar histologic appearance; immunohistochemical staining or PCR may be necessary to distinguish these infections. HSV serologic studies are not helpful in establishing the diagnosis but virologic cultures may be positive.

Varicella Zoster Virus

Rarely, the rash of VZV can be accompanied by potentially life-threatening noncutaneous manifestations, including encephalitis, pneumonitis, myocarditis, and hepatitis, especially in immunocompromised patients, neonates, and adults. Dissemination is virtually always seen in primary infection, although dissemination in reactivation can occur in the immunocompromised patient.[117] Primary infection in immunocompetent adults can cause severe acute hepatitis and, rarely, fulminant hepatic failure. Transplantation patients and immunocompromised patients are at higher risk for a fatal fulminant hepatitis. In children, the convalescent phase can be associated with Reye syndrome (microvesicular steatosis, hyperammonemia, coagulopathy, and cerebral edema), particularly if aspirin has been administered.

Serology is of little use, especially in the immunocompromised patient. The appearance on liver biopsy resembles HSV. Immunohistochemistry can confirm the presence of a herpes virus, but, depending on the antibody, may not distinguish which one. PCR may be necessary to distinguish HSV from VZV infection.

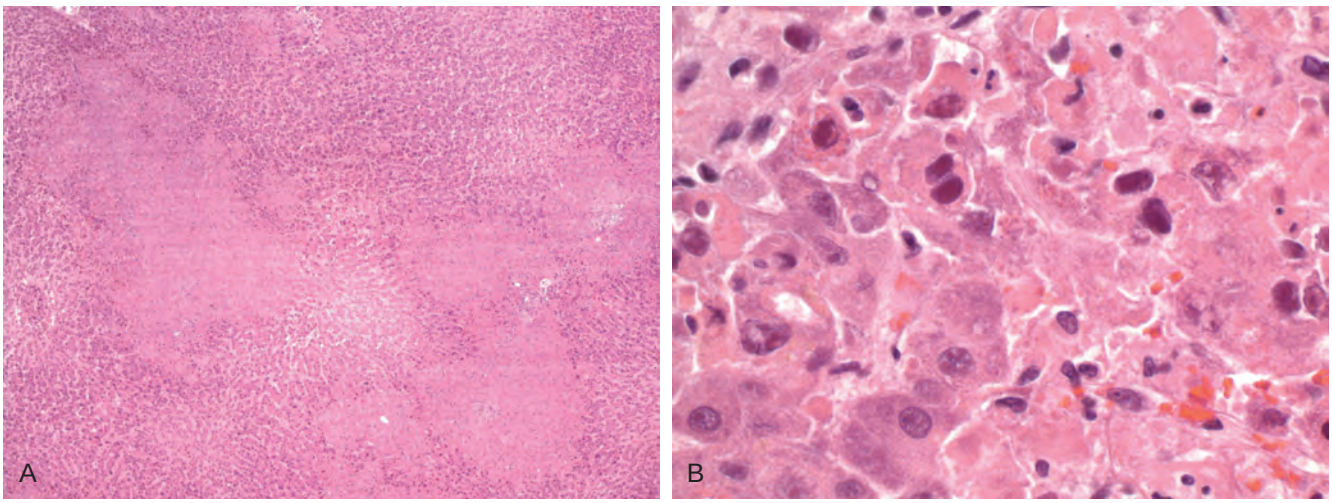
Adenovirus

Although infection by adenoviruses is generally restricted to the upper respiratory tract and conjunctivae in the normal host, disseminated infection can occur in immunodeficient patients, particularly liver transplant recipients, bone marrow transplant recipients, and patients receiving chemotherapy.[118] Sixty-four percent of cases in the literature occurred in patients younger than 18 years of age.[118] Liver biopsies or postmortem livers in patients with adenovirus hepatitis have shown widespread necrosis with little inflammation, mild steatosis, and viral inclusions with a deep blue-purple smudged appearance (Fig. 11.11).[118-120] Immunohistochemistry, in situ hybridization, electron microscopy, PCR, or viral culture can be helpful in confirming the diagnosis.[118,120]

Parvovirus B19

Parvovirus B19 produces several clinical manifestations, including erythema infectiosum (fifth disease) in children; hydrops fetalis; arthritis associated with acute infection in adults; various hematologic disorders (e.g., leukopenia, thrombocytopenia, transient aplastic crisis); and, rarely, involvement of other organs, including neurologic, cardiac, hepatic, and vascular disease.

The role of parvovirus B19 infection in acute or fulminant hepatitis is controversial. Several reports raise the possibility that parvovirus B19 may cause acute hepatitis, fulminant hepatitis, or fulminant hepatic failure. These cases describe several lines of evidence that implicate parvovirus B19, including the presence of parvovirus B19 DNA in the liver of patients with fulminant hepatic failure, the presence of parvovirus B19 DNA in liver tissue or serum of patients with acute or fulminant hepatitis, and the presence of IgM to parvovirus B19 in serum in patients with acute hepatitis.[121-126] In patients with fulminant hepatic failure,[123]



• **Figure 11.11** Adenovirus hepatitis. **A,** Low-power view shows several regions of necrosis without an inflammatory reaction. **B,** High-power view shows hepatocytes with smudgy nuclear inclusions similar to the Cowdry type A inclusions of herpes simplex virus.

parvovirus B19 DNA was found in liver tissue in four of six patients with associated aplastic anemia and in two of four patients without aplastic anemia but not in six patients with known causes of acute liver failure. Histology studies in these and other[125] cases showed a range of injury, from mild lobular hepatitis to massive or submassive hepatic necrosis and collapse with either no inflammatory infiltrates or small aggregates of lymphocytes. Viral inclusions are not seen on routinely stained slides, but parvovirus VP1 antigen (a structural protein produced by the virus) was detected by immunohistochemistry in one case report.[125] In contrast, other investigators found no parvovirus B19 DNA by PCR in 33 cases of cryptogenic acute liver failure but detected parvovirus B19 DNA in several patients with known causes of acute liver failure.[127] In that study, several patients had low-titer IgM positivity without confirmatory PCR positivity. In another study, parvovirus B19 DNA was found with similar frequency in patients with fulminant hepatitis and with hepatitis B or C.[128] Furthermore, RNA transcripts could not be detected in any of the liver tissue samples, arguing against active viral replication.[120] These studies suggest that low levels of PCR positivity may reflect remote infection, and they raise questions about the role of parvovirus B19 in acute hepatitis and acute liver failure.

The role for parvovirus B19 in chronic hepatitis is equally controversial. Although there are reported cases of chronic hepatitis secondary to parvovirus B19,[129] studies have shown that parvovirus B19 DNA can persist in liver tissue for prolonged periods.[130,131] Whether coinfection with parvovirus B19 worsens the prognosis of patients with hepatitis B or hepatitis C is uncertain. Some studies found that coinfected patients have more severe disease, but most studies do not corroborate that finding.[131-133]

Rubella (German Measles)

Acute hepatitis has been reported in adults who acquire rubella. The morphology shows ballooning degeneration of hepatocytes, focal hepatocyte necrosis, and infiltrating mononuclear cells, similar to classic acute hepatitis.[134,135] In addition, a case of fatal fulminant hepatic failure with giant cell transformation of hepatocytes in a child has been reported.[136]

Rubeola (Measles)

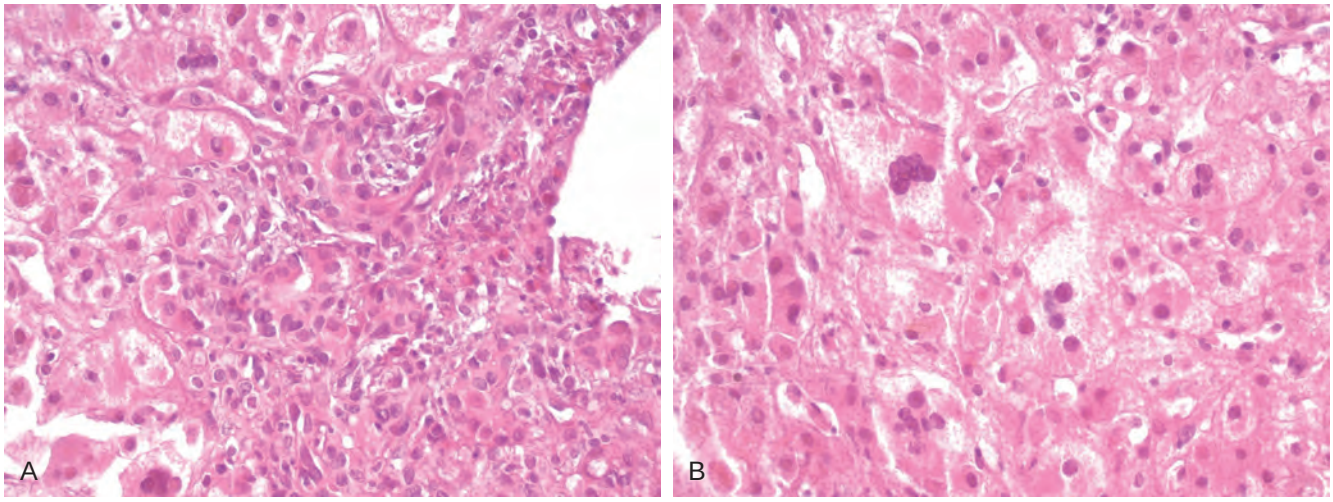
Measles is a contagious, acute, febrile illness that predominantly affects children and causes a maculopapular rash, Koplik spots, cough, conjunctivitis, fever, and lymphadenopathy. The virus enters the body through the lungs or conjunctivae, replicates at these sites, and then is transported to the reticuloendothelial system, where further replication and lymphoid proliferation occur. Transient transaminase elevation is not uncommon in measles.[137] In one reported case, liver biopsy demonstrated acute cholangitis, periportal inflammation with lymphocytes, eosinophils and neutrophils, periportal hepatocyte necrosis, ballooning degeneration and cholestasis, and multinucleated giant cells (Fig. 11.12). At autopsy, viral inclusions were noted in hepatocytes.[138]

Severe Acute Respiratory Syndrome Coronavirus

In 2003 a novel coronavirus was found to be the causative agent of severe acute respiratory syndrome (SARS), an atypical pneumonia that can progress rapidly to acute respiratory distress syndrome. Hepatic impairment is common in these patients, with elevated transaminases seen in approximately 60% of cases. Liver histology shows lobular hepatitis with occasional acidophil bodies, prominent Kupffer cells, increased numbers of hepatocyte mitoses, focal mild ballooning degeneration, and mild portal tract inflammation.[139]

Human Immunodeficiency Virus

HIV is associated with liver disease in several ways. During seroconversion, a small proportion of patients may experience hepatitis with transaminase elevations, although the histology of this hepatitis has not been described.[140,141] Opportunistic infections such as CMV, *Histoplasma capsulatum*, *Mycobacterium avium-intracellulare* complex (MAC), and *Pneumocystis jiroveci* can infect the liver. HIV can also increase the risk of progression of nonopportunistic pathogens, such as *Mycobacterium tuberculosis*, HBV, or HCV (see earlier discussions). Patients with acquired



• **Figure 11.12** Liver biopsy in a patient with measles hepatitis. **A,** Portal tracts show inflammation, cholangitis with bile duct injury, periportal hepatocyte loss, and ballooning degeneration. **B,** The lobules show widespread hepatocyte ballooning degeneration, cholestasis, and multinucleated hepatocytes.

immune deficiency syndrome (AIDS) may develop unusual reactions to certain pathogens, such as bacillary epithelioid angiomatosis secondary to *Bartonella henselae* or *Bartonella quintana* infection (discussed later).[142,143]

Granulomas are a common finding in liver biopsy of patients with HIV infection. The more common causes are *M. tuberculosis*, MAC, *Histoplasma*, *Candida*, CMV, and *Cryptococcus*.[144] In some of these patients the granulomatous response is poorly developed and composed of loose aggregates of histiocytes, but silver or acid-fast stains show numerous organisms. Therefore acid-fast and silver stains should be done routinely on biopsy specimens from patients with HIV/AIDS.

AIDS-related cholangiopathy is a syndrome that manifests with right upper quadrant abdominal pain, fever, and marked elevations of serum alkaline phosphatase. On cholangiography, the bile duct shows strictures and irregularities indistinguishable from those of primary sclerosing cholangitis (PSC). Several opportunistic pathogens have been implicated in this disorder, including *Cryptosporidium*, CMV, and microsporidia *(Enterocytozoon bieneusi)*.[145-150] In a significant minority of cases, there is no identifiable pathogen; it is unclear whether these cases are due to unknown pathogens, occult infection, altered immunity, direct infiltration of the bile duct mucosa by HIV, or PSC coincidentally occurring in patients with AIDS.[149,150]

The liver can be involved by HIV-associated neoplasms, such as lymphoma or Kaposi sarcoma.[151] The latter is caused by another virus, Kaposi sarcoma–associated herpes virus (KSHV), also known as *human herpesvirus 8* (HHV-8). Finally, immune reconstitution with HAART can precipitate autoimmune hepatitis.[152]

Yellow Fever

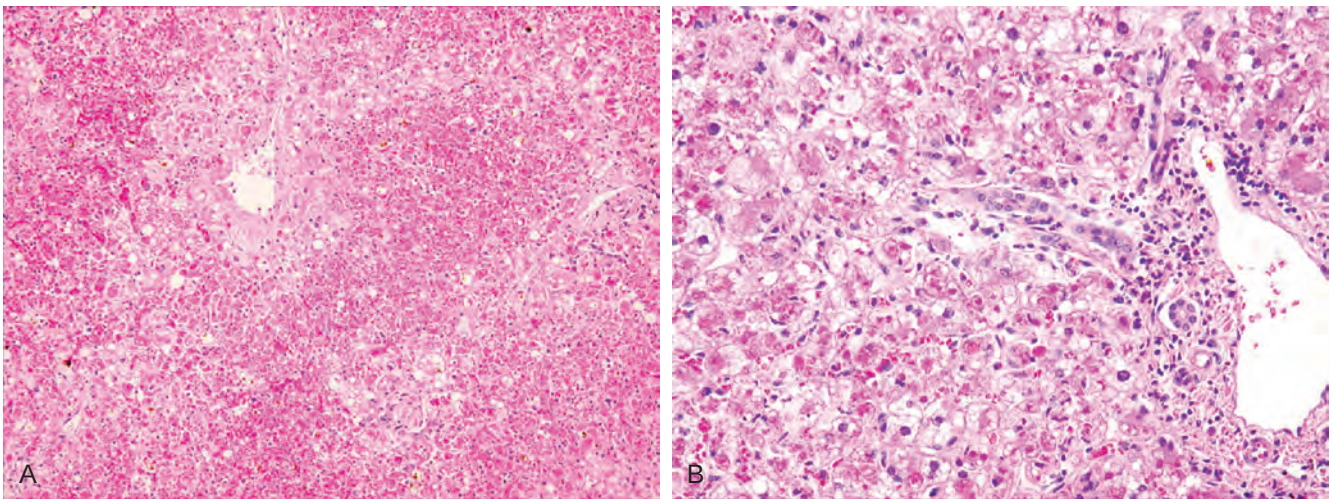
Yellow fever is a viral hemorrhagic fever that can vary from subclinical to rapidly fatal. It is transmitted by the *Aedes* mosquito. Classic symptoms include sudden onset of fever, rigors, and headache. Jaundice and hemorrhagic manifestations portend a poor prognosis. The pattern of injury is frequently described as midzonal and sometimes centrilobular.[153-156] Others describe panlobular injury with only a collar (one or two cells thick) of preserved hepatocytes around central veins and portal tracts.[157] The key findings are necrosis with numerous apoptotic hepatocytes (Councilman bodies), microvesicular steatosis, and absence of inflammation (Fig. 11.13).[153-156] Eosinophilic intranuclear inclusions, Torres bodies, are rarely seen in humans.[157] Immunoperoxidase assays are not widely available, but the diagnosis can be confirmed serologically. The differential diagnosis includes other viral hemorrhagic fevers, such as dengue and Rift Valley fever.

Dengue

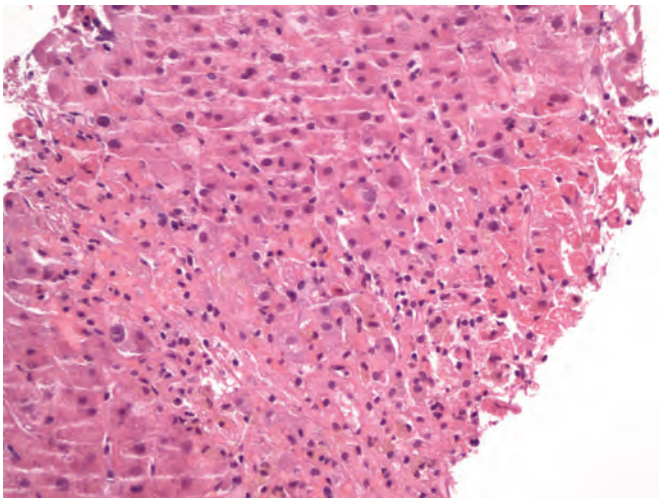
The dengue virus, a member of the Flaviviridae family, is also transmitted by the *Aedes* mosquito. Dengue virus infection is one of the most important mosquito-borne diseases in the world, and the resurgence of this disease in the last few decades may be related to human population growth, inadequate wastewater management, lack of effective mosquito eradication programs, and emergence of more virulent strains.[158] There are four serotypes of dengue virus, and infection with one serotype confers future protective immunity against that serotype only. Infection with a second serotype may result in antibody-dependent enhancement of the illness with immune complex formation.[158]

The clinical manifestations range from asymptomatic infection to severe infections known as dengue fever, dengue hemorrhagic fever, or dengue shock syndrome. Dengue fever manifests as high fever, severe headache, arthralgias, myalgias, and sometimes a rash. Patients with dengue hemorrhagic fever suffer from bleeding phenomena and circulatory failure. Dengue shock is caused by severe plasma leakage and manifests as cyanosis, hypotension, and encephalopathy.[158] The severity of hepatic involvement parallels the severity of the dengue infection. Serotypes 3 and 4 are associated with more severe liver disease.[158] Acute liver failure may develop at a median of 7.5 days after the onset of fever.[159] The prognosis varies; children may have higher mortality than adults.[159-161]

The histology is characterized by necrosis, with Councilman bodies involving the centrilobular region or, as in yellow fever, zone 2, although in most cases the degree of necrosis is not as severe as in yellow fever (Fig. 11.14).[156,157] Other features include hemorrhage, edema, macrophages in sinusoids, and platelets in



• **Figure 11.13** Yellow fever. **A,** Low-power view shows panlobular necrosis with numerous necrotic hepatocytes primarily in zones 2 and 3. **B,** High-power view shows relative preservation of periportal hepatocytes with steatosis, ballooning degeneration, and scattered necrotic hepatocytes. (Courtesy Dr. Shu-Yuan Xiao.)



• **Figure 11.14** Liver biopsy specimen from a patient who died of dengue shock demonstrates centrilobular necrosis with numerous acidophil bodies *(right)* and no inflammatory reaction.

the sinusoids with loss of endothelium.[162] Microvesicular steatosis is frequent, although relatively mild, and often localizes to the periportal space.[162] As with other arboviruses, little inflammation is present.[163,164] The distinction from yellow fever may require geographic data, clinical features, and virologic studies. The contributions of virus, the immune response, or shock to the pathologic changes remain uncertain.[157,165,166] However, immunohistochemical demonstration of the virus within hepatocytes has been described.[162-164] Viruslike particles have also been detected in hepatocytes using transmission electron microscopy, and in the same case, dengue virus polypeptides were found by electron immunomicroscopy.[167]

Rift Valley Fever

Rift Valley fever primarily affects sheep and cattle in Africa, although humans who have direct contact with animals or carcasses may become infected. Fever, severe headache, and myalgia may be followed by facial inflammation, encephalitis, and macular degeneration.[153] Hemorrhage and jaundice are poor prognostic features. Autopsies of cases with hemorrhage show hepatic necrosis, either widespread or predominantly in the periportal and midzonal areas, without prominent inflammatory infiltrates; numerous acidophil bodies; and hemorrhage.[153,157,168]

Lassa Fever

Lassa virus is associated with severe febrile illness among missionaries and travelers returning from West Africa. Symptoms include chills, malaise, headache, and myalgia. Petechiae, ulcerative tonsillitis, and lymphadenopathy are common.[153] The liver shows haphazardly distributed areas of hepatocyte necrosis, both as foci of contiguous cells and as individual cells, either acidophilic or coagulative in type.[157,169] There is little inflammatory reaction apart from histiocytes phagocytosing necrotic debris.[169] The nonzonal distribution of the necrosis distinguishes this disease from yellow fever.[153,169]

Ebola Virus

Ebola virus causes a severe and frequently fatal viral hemorrhagic fever. Outbreaks occur intermittently in Sub-Saharan Africa; the largest outbreak to date occurred in 2014-2016 and primarily affected Guinea, Liberia, and Sierra Leone. The disease is similar to Marburg virus infection, with disseminated intravascular coagulopathy and bleeding occurring in the majority of patients.[153] The histology is characterized by foci of hepatocellular necrosis randomly distributed throughout the parenchyma and eosinophilic inclusions within hepatocytes.[153,157] Mild to moderate steatosis, mild mononuclear infiltrates in the periportal area, and Kupffer cell activation are additional findings.[157]

Marburg Virus

Marburg virus has been associated with African green monkeys. Fever, malaise, headache, and myalgia may be accompanied by a

maculopapular rash and conjunctivitis.[153] The histology is similar to that of Ebola virus infection. On electron microscopy, many hepatocytes contain inclusions composed of uniformly packed filaments arranged in parallel arrays.[153]

Mycobacterial Infections of the Liver and Bile Duct

Tuberculosis

Worldwide, *M. tuberculosis* is one of the most common causes of hepatic granulomas. The organisms may reach the liver hematogenously from the lungs (or less commonly an extrapulmonary site), through the portal circulation in the setting of gastrointestinal tuberculosis (TB), or via lymphatics.[170] The prevalence of hepatic involvement in patients with active TB has been estimated at approximately 1%.[171] TB can affect the liver in several forms. Most often (approximately 80% of cases of hepatic TB), the liver is affected in the setting of generalized miliary TB.[172,173] Less often (approximately 20%), hepatic TB manifests as a localized mass lesion that mimics a neoplasm, causes obstructive jaundice from extrinsic compression of the hepatic duct, or causes portal hypertension from compression of the portal vein.[170,174] Localized hepatic involvement can occur in primary infection, in which case there is no evidence of antecedent infection, or as reactivation TB.[175] Tuberculous cholangitis, in which the bacillus primarily infects the biliary tree, is extremely rare.[176] Hepatic TB is common among HIV-positive individuals: it was identified in 26% of untreated HIV-positive patients undergoing liver biopsy in one series from Brazil[177] and may be found in the liver at autopsy in approximately a third of HIV-positive patients.[171] HIV-positive patients tend to have more severe manifestations of hepatic TB.

The most common presenting symptoms of hepatic TB are hepatomegaly, fever, respiratory symptoms, abdominal pain, and weight loss; splenomegaly, ascites, and jaundice may also be seen but are less common.[171] Diffuse abdominal pain is more common in localized hepatic TB, whereas respiratory symptoms (such as cough with or without sputum production) are more common in miliary hepatic TB. The relatively nonspecific presentation makes the diagnosis difficult in the absence of a high index of suspicion. Alkaline phosphatase elevations and hyponatremia usually are prominent features.[175,178,179] An inverted albumin to globulin ratio *(A/G)*, in which serum globulin is 1.25 to 1.86 times higher than serum albumin, is common.[171] In 65% to 78% of patients with hepatic TB, respiratory symptoms or chest radiographs suggest pulmonary TB as well.[170,175,178,179] Abdominal computed tomography imaging shows multiple hypodense micronodules throughout the liver or hepatomegaly without nodules, whereas in localized TB it shows one large solitary nodule or 2 to 3 hypodense nodules.[171] Liver calcifications are seen in approximately half of patients.[170,173,180,181] Presentation with acute liver failure is rare but has been reported.[182]

Liver biopsy with mycobacterial culture is considered the most specific diagnostic test for hepatic TB. Caseating granulomas are a hallmark of hepatic TB (seen in 51% to 83% of cases), particularly miliary TB in the setting of primary infection; in reactivation TB, noncaseating granulomas may be present instead (Fig. 11.15).[171] The granulomas may reside in the lobules or in portal tracts; the former location may be more common in miliary TB, whereas the latter may be more common in localized TB.[171] In TB-endemic countries, such as Iran, India, and Saudi Arabia, TB is the most common cause of hepatic granulomas (accounting for approximately 50%), as opposed to non–TB-endemic countries where histoplasmosis, sarcoidosis, or PBC are more common etiologies.[171] Sinusoidal congestion and peliosis hepatis may also be seen.[183] Acid-fast bacilli (AFB) smears are positive in 0% to 59% of cases (median 25%), and organisms are more likely to be found with caseating necrosis, but smears may be positive due to infection from nontuberculous mycobacteria.[171,175,176,178] Cultures provide the strongest evidence of hepatic TB, but the sensitivity may be less than 10%. Histologic evidence of caseating granulomas has a median sensitivity of 68% (range: 14% to 100%) and is generally sufficient to establish the diagnosis in TB-endemic areas.[171] PCR for *M. tuberculosis* DNA has a 53% to 88% sensitivity and a 96% to 100% specificity for detecting hepatic TB.[170,184,185] Immunocompromised patients may present with a wasting syndrome in which multiple organs, including the liver, contain necrotic miliary nodules surrounded by histiocytes that do not aggregate into well-formed granulomas. AFB stain shows numerous organisms in these lesions.[186] Prompt initiation of four-drug anti-TB therapy should lead to clinical improvement in most patients with hepatic TB.

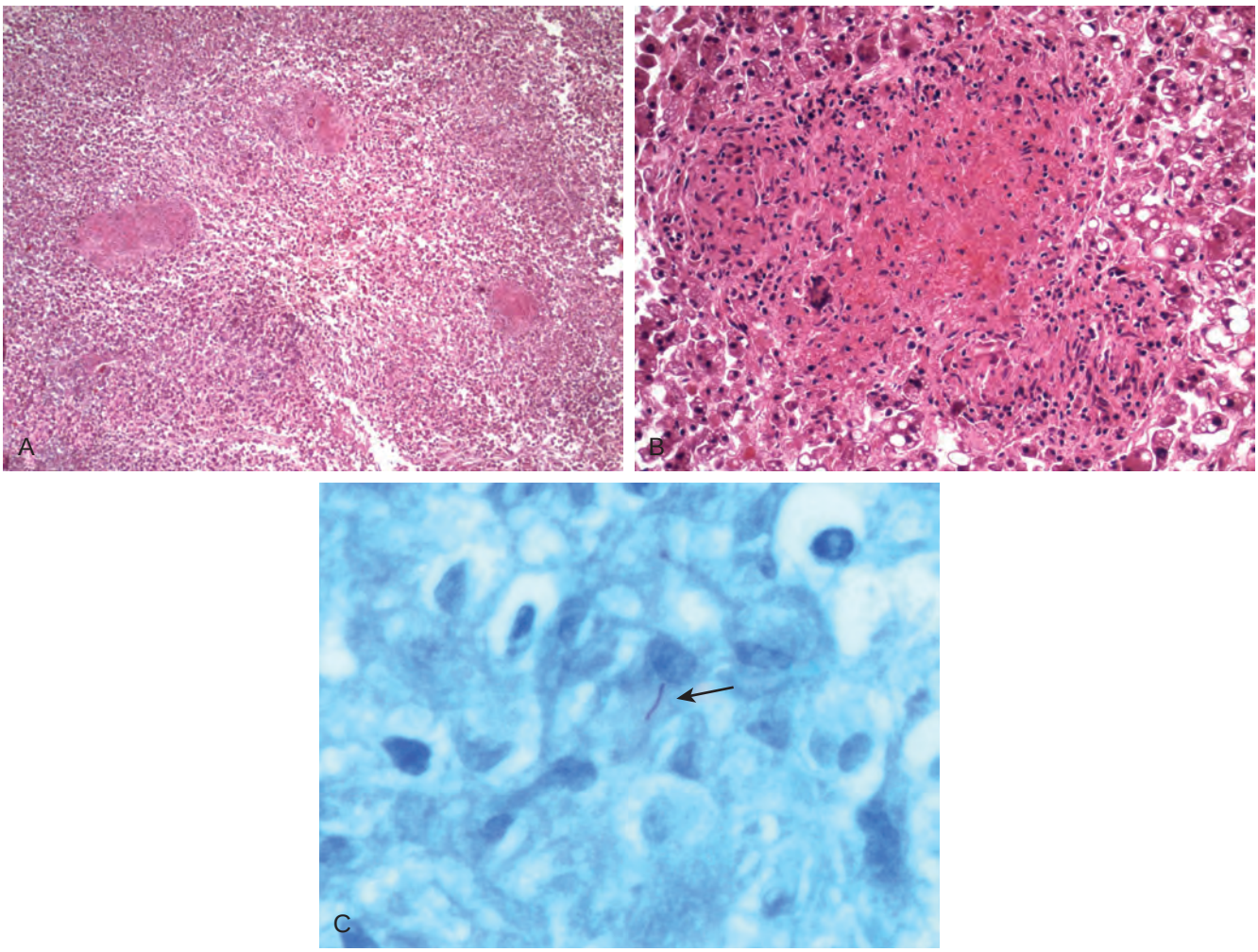
Localized hepatic TB includes tuberculoma and tuberculous abscess. Radiologically, it mimics tumors except that calcification may be prominent and may assume a bull's-eye configuration.[174,187-189] The diagnosis in these cases is more elusive because liver biopsy, unlike in the setting of diffuse hepatic involvement, often shows nonspecific findings. Combined with the clinical and radiologic suspicion of malignancy, this may lead to surgical resection of localized hepatic TB.[190,191] Grossly, localized hepatic TB appears as cheesy or chalky white, irregular nodules. Histologically, tuberculomas are composed of confluent granulomas, contain few organisms, and are encountered in immunocompetent patients, whereas tuberculous abscesses are centrally suppurative, contain numerous organisms, and are encountered mainly in immunodeficient patients.

Biliary involvement manifests as obstructive jaundice, which might have as its cause compression of the bile duct by a hepatic tuberculoma, small duct involvement by granulomas, or isolated biliary tree involvement.[173] The latter may result in bile duct strictures that can be mistaken for cholangiocarcinoma or PSC.[173,180,192] Biliary involvement mimicking cholangiocarcioma has also been reported with the atypical *Mycobacterium abscessus*.[193]

In HIV patients initiating HAART, coinfection with TB may precipitate an inflammatory reaction to TB antigens known as the immune reconstitution inflammatory syndrome (IRIS). IRIS is characterized clinically by hepatomegaly, abdominal pain, elevated liver enzymes, jaundice, and constitutional symptoms, such as fever. The syndrome, which generally occurs within 8 weeks of initiation of HAART, may be difficult to distinguish from drug-induced liver injury. Liver biopsy shows necrotizing and nonnecrotizing granulomas, but the granulomas may exhibit a more "inflamed" or "activated" appearance than is typical in TB (with numerous admixed neutrophils, lymphocytes, and plasma cells).[194]

Mycobacterium avium-intracellulare Complex

Of the atypical mycobacteria, *M. avium* and *M. intracellulare* are significant hepatic pathogens. MAC is commonly encountered in immunocompromised patients, particularly those with AIDS. These organisms cause disseminated infection that commonly affects lung, liver, spleen, lymph nodes, and bone marrow. Patients with hepatic involvement present with fever, elevated alkaline



• **Figure 11.15** Miliary tuberculosis involving the liver. **A,** Low-power view shows several necrotizing granulomas. **B,** High-power view of a necrotizing granuloma. Note the central region of necrosis surrounded by histiocytes and a giant cell. **C,** An acid-fast stain highlights a single bacillus *(arrow)* within the granuloma.

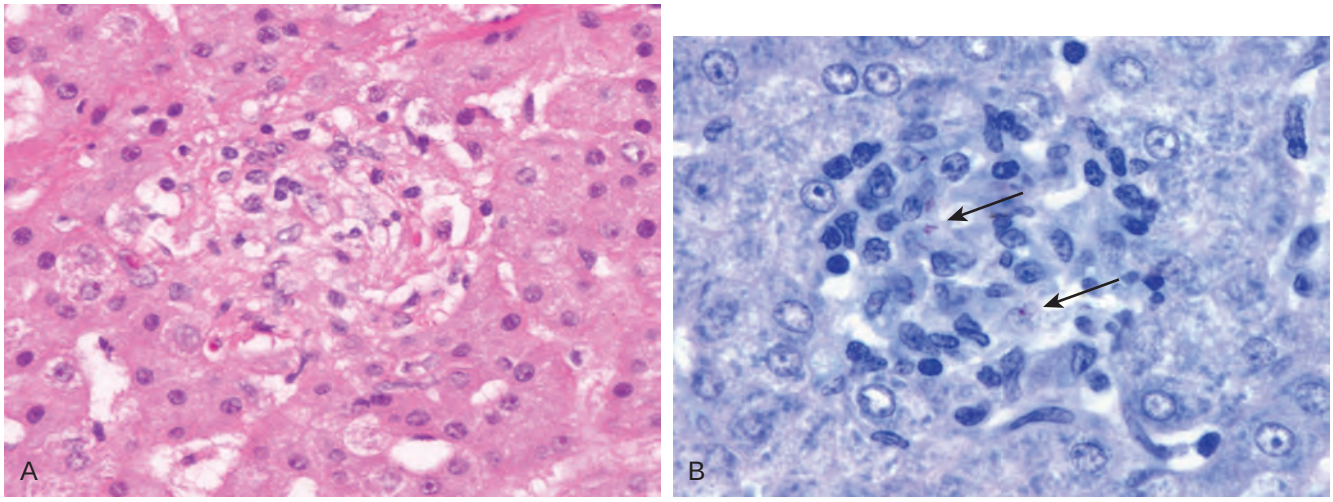
phosphatase, and hepatomegaly.[195] The histopathologic findings range from numerous well-formed, nonnecrotizing granulomas with rare acid-fast organisms on Ziehl-Neelsen stain to less well-formed granulomas with large numbers of acid-fast organisms spilling out of the loose granulomas into nearby Kupffer cells (Fig. 11.16).[186,196] Although the latter pattern differs from the usual case of hepatic TB, the degree of immune compromise, rather than the species of mycobacteria, may be the more important factor in determining the number of organisms and type of granulomas seen, because T-cell function is required for the formation of well-formed granulomas, and hepatic TB in patients with AIDS also may show large numbers of organisms and few well-formed granulomas.[186,195] Granulomas in hepatic MAC may exhibit a portal or periportal distribution.[197] IRIS in HIV patients has been described with hepatic MAC.[198]

Leprosy

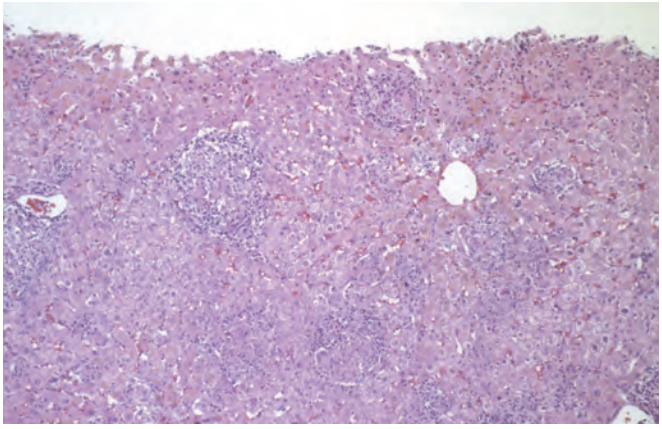
Infection by *Mycobacterium leprae* commonly involves the liver, although clinical manifestations are often mild or absent. Hepatic granulomas are found in 90% of patients with lepromatous leprosy but in fewer than 20% of patients with tuberculoid leprosy.[199] The appearance of the granulomas depends on the type of leprosy. In lepromatous leprosy, collections of foam cells within the lobules or portal tracts contain numerous AFB.[199,200] In tuberculoid leprosy, epithelioid granulomas with rare or no acid-fast organisms are seen (Fig. 11.17).[199] An individual patient may have both lepromatous and tuberculoid granulomas.

Bacillus Calmette-Guérin

Bacillus Calmette-Guérin (BCG) is an attenuated form of *Mycobacterium bovis*, immunologically related to *M. tuberculosis*, that has been used as a vaccine against TB, as intralesional treatment of dermal malignancies, and as intravesical therapy for superficial bladder cancer. Hepatic granulomas have been described in patients after intradermal injections of BCG.[201,202] Pneumonitis or granulomatous hepatitis, or both, affect 0.7% of patients who receive multiple intravesical instillations of BCG and are more likely after traumatic catheterization, extensive tumor resection, or bladder perforation.[203] Rarely, acute hepatic failure with encephalopathy may develop.[204] The liver shows noncaseating epithelioid granulomas, epithelioid giant cells, mild steatosis, hepatocyte necrosis, and portal inflammation.[205-207] Often, AFB



• **Figure 11.16** *Mycobacterium avium-intracellulare* complex infection in an immunocompromised patient. **A,** The liver shows loose collections of histiocytes, suggestive of poorly formed granulomas. **B,** On acid-fast staining, several bacilli are present *(arrows)*.



• **Figure 11.17** Liver biopsy sample from a patient with leprosy. Several noncaseating granulomas are present.

are not identified, and cultures are negative. This has led to the suggestion that the granulomatous hepatitis represents a hypersensitivity reaction to antigens present in the BCG preparation,[201] particularly because some cases show additional features of extrahepatic granulomas, leukocytoclastic vasculitis, or bile duct injury and eosinophils (Fig. 11.18).[208,209] However, in some reported cases, AFB have been identified in the granulomas, blood cultures have been positive for *M. bovis*, or PCR for mycobacterial DNA has been positive, indicating disseminated BCG infection.[209-212] Some have suggested that two types of adverse reactions may occur, one a disseminated infection that responds to anti-TB therapy and the other a sterile hypersensitivity reaction that is delayed in appearance and responds to steroids.[209]

Other Bacterial Infections of the Liver and Bile Duct

Bacterial infections of the liver can produce a wide range of pathology, including hepatitis, parenchymal necrosis, microabscesses, pyogenic liver abscess, and granulomatous hepatitis. Mixed patterns are frequent, and many organisms can produce several patterns. Microabscesses or pyogenic liver abscesses can be caused by a multitude of aerobic and anaerobic organisms, fungi, and parasites. Granulomatous hepatitis with or without a necrotizing component can be seen in a number of infections (Box 11.1).[213-215] However, in some entities the granulomas are not true epithelioid granulomas but rather aggregates of histiocytes in foci of parenchymal necrosis. Because these "microgranulomas" do not generate the same differential diagnosis as true epithelioid

• BOX 11.1 Infections Associated With Granulomatous Inflammation in the Liver

Viral
- HCV
- CMV
- EBV

Mycobacterial
- TB
- Leprosy
- Atypical tuberculosis

Bacterial
- Brucellosis
- Cat-scratch disease
- Enteric fever; typhoid fever
- Listeriosis
- Melioidosis
- Tularemia
- Whipple disease[213]
- *Pasteurella multocida*[214]
- *Yersinia enterocolitica*[215]
- Actinomycosis
- Nocardiosis

Rickettsial
- Q fever

Chlamydial

Lymphogranuloma venereum
- Psittacosis

Spirochetal
- Syphilis
- Lyme disease

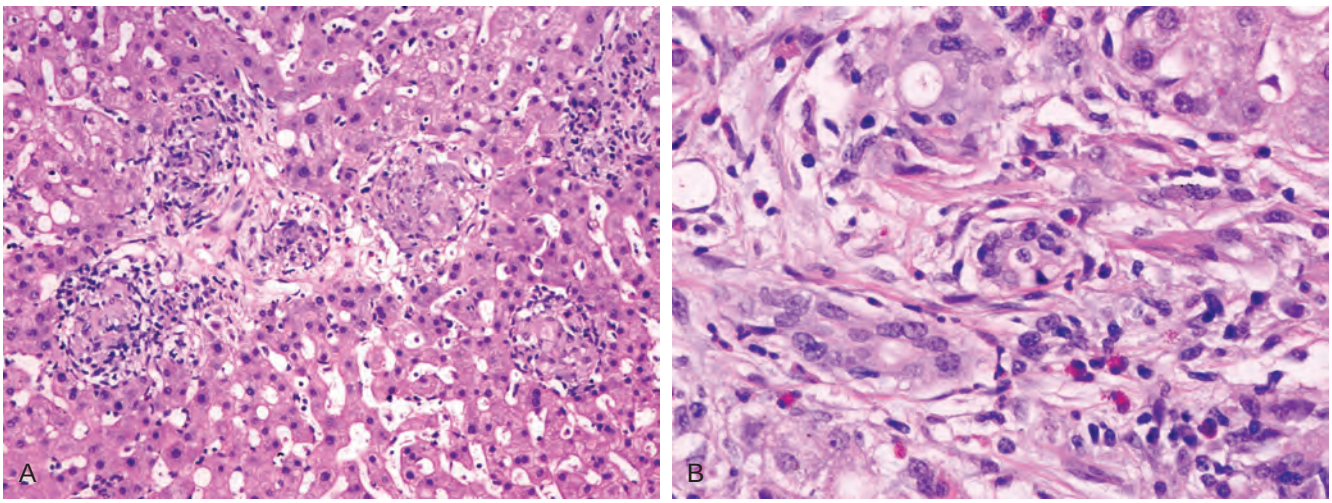
Fungal
- Aspergillosis
- Blastomycosis
- Candidiasis
- Coccidioidomycosis
- Cryptococcosis
- Histoplasmosis
- Mucormycosis
- Paracoccidioidomycosis

Pneumocystis

Helminthic
- Ascariasis
- Capillariasis
- Fascioliasis
- Opisthorchiasis
- Schistosomiasis
- Strongyloidiasis
- Visceral larva migrans

Protozoal
- Leishmaniasis
- Toxoplasmosis

CMV, *Cytomegalovirus;* EBV, *Epstein-Barr virus;* HCV, *hepatitis C virus;* TB, *tuberculosis.*



• **Figure 11.18** Liver biopsy in a patient with bacillus Calmette-Guérin infection. **A,** Several small granulomas are present in this view. **B,** In some portal tracts, bile duct injury and scattered eosinophils are present, suggesting a hypersensitivity reaction.

granulomas, the diagnosis of "granulomatous hepatitis" in these cases is best avoided. Biliary involvement can produce acute cholangitis or cholecystitis.

Pyogenic Liver Abscess

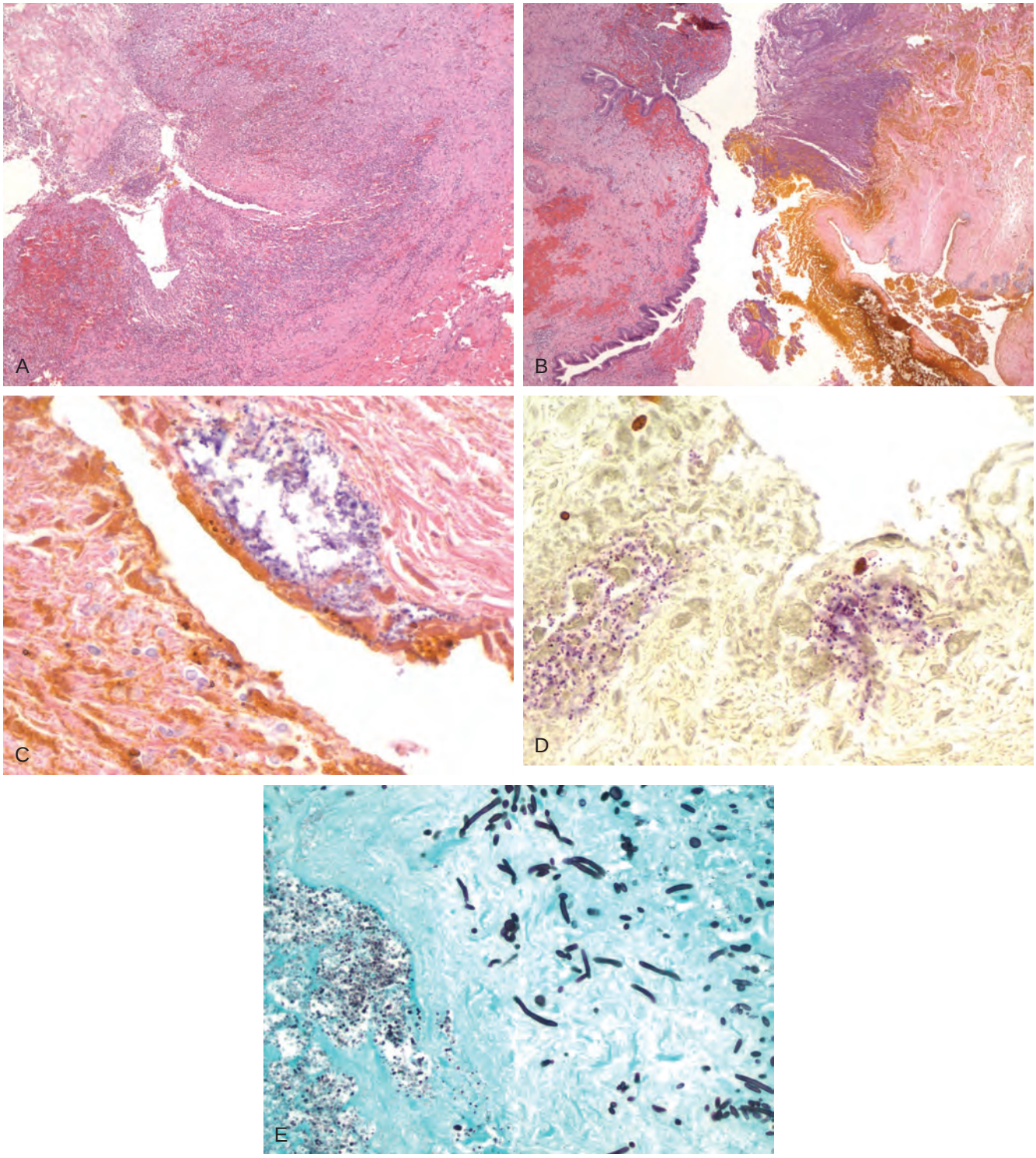
Historically, pyogenic liver abscess was associated with acute appendicitis or intra-abdominal infection, and in those settings it was seen more often in children.[216] More recently, biliary disease has emerged as a common etiology, and with that shift, the age of the patients has also increased, to 55 to 60 years of age.[217-219] Diabetes mellitus is a strong risk factor for the development of pyogenic liver abscess.[219,220] Other frequent comorbidities are malignancy, alcohol abuse, cirrhosis, hypertension, recent surgery, and immunosuppression.[217,218,221] A significant minority of cases remain cryptogenic. Although the disease carries a high mortality, improved diagnosis, abscess drainage, and antibiotic therapy have reduced the mortality rate to 5% to 31%.[217,221] Patients typically present with fever, chills, right upper quadrant pain, and elevated alkaline phosphatase.[217,218] Men are affected more often than women in most series.[217-219] Most abscesses are solitary; multiple abscesses occur in 25% to 45% of cases.[217-219,221] Most abscesses are right sided (55% to 70%), with left-sided and bilateral disease occurring less often.[216,217,219] Liver abscesses are rare in neonates but may be seen in the setting of sepsis, particularly if there is a history of umbilical catheterization.[222]

Culture of aspirated purulent material from the abscess or of blood may yield the offending organisms, although both of these are occasionally negative. Sometimes blood culture results do not correlate with pus culture results; therefore blood culture alone is not sufficient to determine the etiology.[217] Depending on the organism, serology may be useful. The most commonly isolated organisms are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus*, *Streptococcus*, and *Pseudomonas* spp.[217,219,221] A third of infections are polymicrobial (Fig. 11.19). Organisms that produce formic hydrogenlyase, such as *Klebsiella* spp. and *E. coli*, can convert acids that accumulate within the abscess to carbon dioxide and hydrogen gas; infection with these organisms can result in gas-forming pyogenic abscess, which carries a higher risk of septic shock, bacteremia, and mortality.[223] The incidence of *K. pneumoniae* abscesses has increased in recent years, and this organism is now the leading cause of pyogenic liver abscesses in Asia. *K. pneumoniae* abscess may be suspected on the basis of the following features on abdominal CT: a thin abscess wall, presence of necrotic debris within the abscess cavity, presence of metastatic infection, and absence of underlying biliary disease.[224] Abscesses secondary to *Yersinia enterocolitica* or *Yersinia pseudotuberculosis* are often associated with underlying hemochromatosis.[225-228] Anaerobes are isolated in up to 25% of cases, sometimes together with aerobes.[218,229,230] Microaerophilic streptococci, *Bacteroides fragilis*, *Fusobacterium necrophorum*, and *Clostridium* spp. are the most commonly implicated anaerobes. *Actinomyces* spp. are infrequently found by either anaerobic culture of aspirated pus or identification of filamentous bacteria and sulfur granules on histologic examination of resection material (Fig. 11.20); these organisms may be associated with the formation of sinus tracts.[231-234] Rarely, *Francisella tularensis*,[235] *Burkholderia pseudomallei* (the agent of melioidosis),[236,237] or *Listeria monocytogenes*[238] causes liver abscesses. Fungi, such as *Candida* and *Aspergillus*, are found in approximately 15% of cases.[229,239]

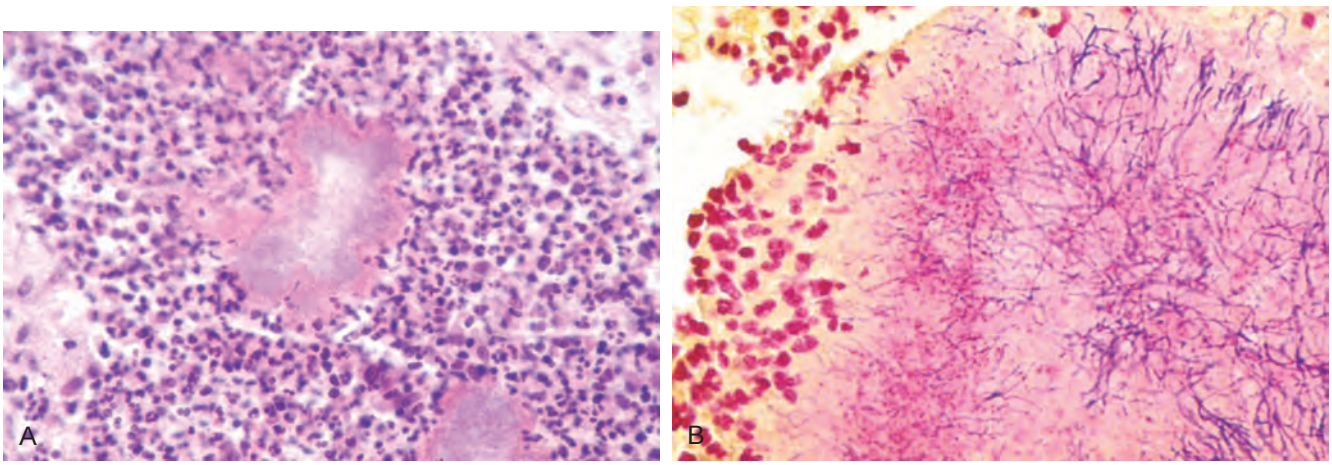
Complications of pyogenic liver abscess include metastatic infections, such as endophthalmitis, meningitis, osteomyelitis, pyelonephritis, and pneumonia. The two main risk factors for metastatic infection are infection with *K. pneumoniae* and underlying diabetes mellitus.[221,240,241] Although surgical management was once the mainstay of therapy, percutaneous drainage and antibiotics have become the first line approach.[217]

Acute Cholangitis

The term *acute cholangitis* refers to an infection of the bile ducts characterized by jaundice, abdominal pain, and sepsis, in combination with biliary obstruction. Bacterial colonization of the biliary tree in the absence of obstruction produces no symptoms; conversely, sterile obstruction of the bile duct produces a picture of aseptic obstructive jaundice.[242] The route by which bacteria colonize the bile ducts is uncertain. Possible sources include reflux of intestinal contents through the choledochal sphincter; passage from an infected gallbladder; and lymphatic, hepatic arterial, or portal venous bacteremia.[242] Obstruction is most often due to



• **Figure 11.19** Hepatic abscesses arising from polymicrobial cholangitis with *Enterococcus* and *Candida* in a liver transplant recipient. **A,** The abscess contains a center of necrotic debris, fibrin, and neutrophils surrounded by granulation tissue and inflammation, with a fibrotic rim. **B,** In some areas, residual bile duct epithelium at the abscess edge betrays origin from cholangitis. **C,** Yeast forms *(lower left)* are present adjacent to bacterial cocci *(upper right)* in the bile duct lumen. **D,** Gram-positive cocci are revealed by Brown-Hopps stain. **E,** Yeast forms and pseudohyphae consistent with *Candida (upper right)* and bacterial cocci *(lower left)* are present (Gomori methenamine silver stain).



• **Figure 11.20** *Actinomyces*. **A,** Sulfur granule with aggregate of filamentous bacteria and neutrophils. **B,** Brown-Hopps stain shows gram-positive filamentous bacteria in the sulfur granule. (Courtesy Dr. Laura Lamps.)

gallstones. Other causes include strictures, malignancy, and congenital anomalies of the ampulla of Vater. How bactibilia and obstruction leads to biliary septicemia is uncertain. Cholangiovenous reflux with increased intrabiliary pressure can be demonstrated using contrast, and higher biliary pressures are associated with an increased incidence of purulent bile, sepsis, and bacteremia.[242]

Manifestations of acute cholangitis range from mild, recurrent illness to overwhelming sepsis. Most patients are middle aged, and men and women are affected equally. Fever and jaundice are the most common symptoms. In 1877 Charcot proposed a clinical triad: right upper quadrant abdominal pain, fever, and jaundice.[243] Approximately 70% of patients present with the full triad. Reynolds arrived at a pentad in 1959 by adding hypotension and delirium.[244] Although rare, the pentad describes patients with high mortality risk.[245] Alkaline phosphatase and bilirubin are elevated in most patients, but blood cultures are positive in fewer than half of patients.

The bacteria that infect the biliary tree usually derive from the gut. The bacteria most commonly isolated from bile are *E. coli*, *Enterococcus*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*.[242,246] Improved culture techniques have shown a significant incidence of infection by anaerobes, such as *B. fragilis* and clostridial species, frequently in association with previous biliary-intestinal anastomosis, an older adult patient, and more severe illness.[242,247] Overall, 56% of cases are polymicrobial. Mycotic infection of the bile duct, principally by *Candida albicans*, is rare and affects older patients, patients with malignancy, immunosuppressed patients, and diabetics.[242]

The histology of acute cholangitis is similar to that of large duct obstruction, with portal edema, neutrophilic portal and periductal inflammation, and neutrophils within duct epithelium.[248,249] Ductular reaction and canalicular and ductular cholestasis may also be seen.[250] In some cases, neutrophils accumulate within the duct lumina; although this is suggestive of infection, the association is not sufficiently reliable to be predictive.[248,249] Microabscesses and macroscopic abscesses may be seen. Thrombophlebitis may result in portal vein thrombi.[249] Patients suffering from shock may show necrosis of the liver.[249] Chronic changes include strictures, dilatations, portal fibrosis, and potentially ductopenia. Histologic findings frequently do not correlate with clinical status, in that most cases of acute cholangitis show relatively minor histologic changes, and patients with mild symptoms can have frank abscesses.[249] Histology is also not predictive of survival.[245] Therefore clinical and microbiologic correlation is frequently required to make the diagnosis of acute bacterial cholangitis.

Most patients respond well to antibiotics. The efficacy of a specific antibiotic depends on its biliary secretion, which is affected by the size of the compound. The inflammatory process also can cause impairment of secretion. Treatment of the obstruction is necessary to prevent recurrence. Some patients require emergent biliary drainage in addition to antibiotics, and there may be a survival advantage to surgical management of the obstruction.[245] Complications of acute cholangitis include renal failure and the development of hepatic abscesses.[242]

Recurrent Pyogenic Cholangitis (Oriental Cholangiohepatitis)

Recurrent pyogenic cholangitis is a disease that historically was seen largely in the Far East but is reported in increasing numbers in the West, largely among Asian immigrants. The disease is characterized by abdominal pain, fever, chills, and jaundice, resulting from recurrent attacks of suppurative cholangitis associated with intrahepatic biliary stones.[251] The cause is not fully known, but bacterial infection of the biliary tree is perhaps the inciting event. Enteric organisms, particularly *E. coli*, are cultured from bile in most cases.[251] Bacteria deconjugate bilirubin glucuronide, which then precipitates with calcium in the bile as soft, brown, friable calcium bilirubinate stones.[251,252] Exactly what predisposes patients to biliary tract infection is unknown, but the geographic distribution of this disease mirrors that of *Clonorchis sinensis* and *Ascaris lumbricoides*, and biliary parasites are detected in a significant minority of patients.[251] Dead flukes and ova within the bile ducts, accompanied by bile stasis, may lead to bacterial infection. However, large numbers of people in endemic areas are infested with liver flukes without recurrent pyogenic cholangitis. In any case, the recurrent cholangitis results in intense periductal inflammation and fibrosis, strictures, and dilated ducts filled with sludge, predominantly affecting the left lobe.[252]

Morphologically, large ducts show chronic and acute cholangitis, fibrosis, and peribiliary gland hyperplasia ("chronic proliferative cholangitis"). Pigmented stones and pus are seen in ducts;

liver abscesses may be present.[251] During acute infection, there is acute cholangitis with portal edema, erosion/ulceration of large ducts, and thrombophlebitis of portal vein branches.[253] Bile ductular proliferation with cholangiolitis and periductal fibrosis develops in small interlobular portal tracts. Small portal vein branches are reduced in number and may show fibrous obliteration.[253] Atrophy of liver parenchyma may also be seen. Biliary dysplasia and cholangiocarcinoma are complications and were seen in 13% and 23% of patients undergoing hepatic resection in one study, respectively.[254]

The changes in small interlobular tracts may lead to consideration of PSC, particularly in the West; however, ductopenia in recurrent pyogenic cholangitis is not as significant as PSC, and ductular reaction is only rarely observed.[253] In addition, chronic cholestatic changes (such as copper deposition in periportal hepatocytes) are typically not seen, and progression to biliary cirrhosis is rare. These differences, as well as the different clinical presentations and epidemiologic associations, should allow distinction of recurrent pyogenic cholangitis from PSC and other biliary diseases in which secondary stone formation may occur.[253]

Brucellosis

Brucellosis is the most common zoonotic disease in the world, with 500,000 cases per year, and is particularly common in the Mediterranean basin, the Middle East, India, and Central and South America. It largely affects handlers of livestock, particularly those in the meatpacking industry, due to direct contact with animals and animal parts. It may also be acquired by ingestion of unpasteurized dairy products or infected meat, or by occupational exposure in veterinarians and clinical laboratory workers. The organism escapes destruction by replicating intracellularly within phagocytic cells, and thus involvement of the liver is common given its large population of reticuloendothelial cells. Although mildly elevated transaminases or hepatomegaly are seen in almost all patients, a true hepatitis (defined as a five-fold or greater increase in transaminases) is seen in approximately 25% of cases.[255] A cholestatic presentation may also be seen. *Brucella melitensis* accounts for most cases of hepatic involvement, whereas *Brucella abortus* and *Brucella suis* are seen in a minority of cases. Most (approximately 90%) patients present with an acute illness (0 to 2 months in duration) characterized by malaise, fever, chills, sweats, arthralgia, weight loss, and headache, but some patients become symptomatic after many years of dormancy or are episodically symptomatic.[255-257] Fever in brucellosis patients can be intermittent and has been described as "undulant fever." Subacute and chronic presentations are less common but do occur.

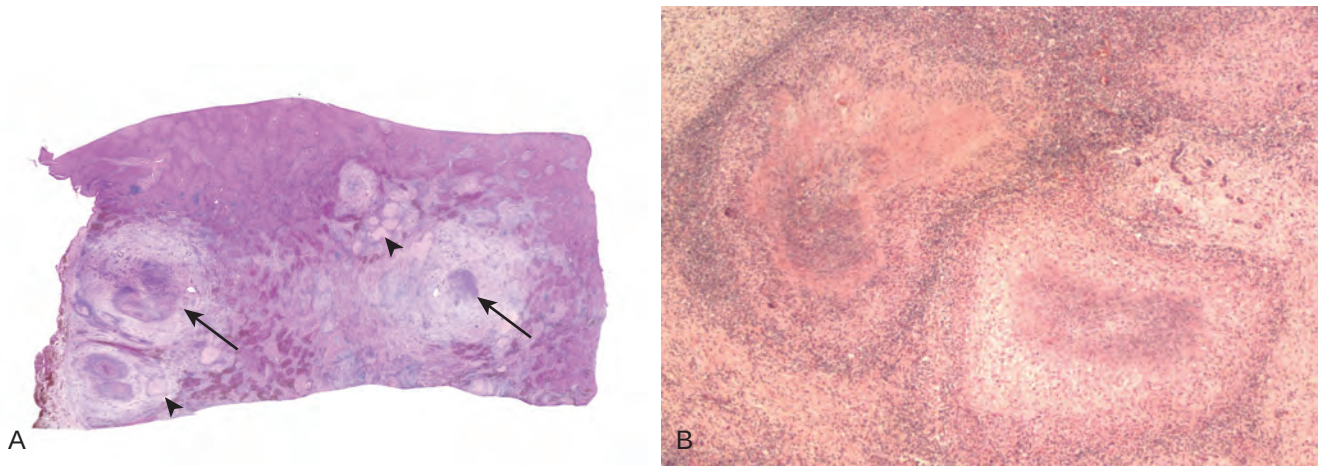
The characteristic histopathologic pattern of hepatic brucellosis is a granulomatous hepatitis.[258-260] In a series of 14 patients with brucellosis, all had hepatic granulomas in both portal tracts and lobules[261]; in another, 28 of 40 patients (70%) had granulomas.[258] However, in a prospective study in which patients presenting with acute brucellosis underwent liver biopsy, the most common finding (15 of 20 cases, 75%) was a nonspecific hepatitis characterized by portal and/or lobular mononuclear inflammation. Of these cases, only four (20%) also showed granulomas.[262] The remainder of the cases showed mixed inflammation (one case, 5%) or no significant inflammation (four cases, 20%). Thus granulomas may correlate with more severe liver disease because in other studies liver biopsies were usually done because of biochemical evidence of liver dysfunction. Granulomas in brucellosis may be indistinguishable from TB or sarcoidosis, and brucellosis should be considered in any patient with hepatic granulomas.[260] Some authors consider granulomas in brucellosis to be smaller and less clearly defined than in TB, sarcoidosis, or histoplasmosis[258]; the term "microgranuloma" has also been used to describe them.[262] The presence of granulomas does not reliably distinguish among the various species of *Brucella*,[263-266] despite a report that *B. melitensis* is not associated with granulomas.[267] Other findings may include nonspecific acute hepatitis with hepatocyte necrosis, portal inflammation, and Kupffer cell hyperplasia.[256-258,261,266] Liver involvement by brucellosis generally follows a benign course, and a full recovery in greater than 95% patients is expected after treatment with doxycycline in combination with an aminoglycoside and/or rifampin.[256] Relapse of chronic brucellosis may be precipitated by viral illness or trauma.[257]

The diagnosis is established by rose bengal test or *Brucella* agglutination titer (Wright serum agglutination test or standard tube agglutination [STA]), which are positive in greater than 90% of cases.[255,256] Indirect Coombs test is almost invariably positive and may be used to make the diagnosis in patients with negative STA tests. Culturing the organism from blood, body fluids, or tissue is difficult; cultures are positive in only approximately 40% of patients, although the yield has increased in recent years due to improvements in technique. PCR testing for *Brucella* spp. DNA may also be performed.

Rarely, patients present with hepatosplenic abscesses or mass-like lesions (brucelloma) mimicking a tumor.[257,268,269] In one series of 15 patients with brucelloma in the liver or spleen, half had suffered from brucellosis many years before, suggesting reactivation of latent disease.[270] Radiologically, brucellomas have central calcification with peripheral necrotic areas.[257,268-272] Two general presentations of hepatosplenic *Brucella* abscesses are seen. The most common is a solitary liver abscess, often large and located in the right lobe, with a central calcification, which occurs only in adults and should immediately prompt suspicion of brucellosis in an endemic setting. A less common presentation is multiple small abscesses in the liver and spleen, known as "miliary" pattern, which occurs in children and adults and mimics disseminated TB.[268] Histologically, a brucelloma shows necrotic areas surrounded by a palisaded granulomatous reaction.[269,270] Some cases may show only fibrosis with dense chronic inflammation.[272] Organisms are isolated from cultures of abscess material in only 18% of cases. When an organism is identified in patients with liver abscesses (combining cultures of blood and abscess material), the majority (80%) are due to *B. melitensis*, and the remainder are due to *B. abortus* (12%) and *B. suis* (8%).[268] In addition to antibiotics, management of brucellomas requires surgery in approximately half of cases, and resolution of the lesions is expected in the overwhelming majority of cases.

Bartonella Infection (Cat-Scratch Disease)

Cat-scratch disease is a self-limited infection caused by infection with *Bartonella* spp. after inoculation by a cat, which usually manifests as a local skin reaction and lymphadenopathy. Most cases are attributed to *B. henselae*, but *B. quintana* has been implicated in some.[273] Approximately 1% to 2% of patients with *Bartonella* infection develop severe systemic disease with involvement of the liver, spleen, bone, central nervous system, or lung.[274] Most patients with hepatosplenic presentation are children 5 to 10 years of age.[273,275,276] Patients often have nonspecific symptoms, including fever, abdominal pain, chills, headache, malaise, and weight loss. Approximately 25% of patients have



• **Figure 11.21** Cat-scratch disease involving the liver. **A,** Low-power view of a section of liver shows multiple stellate abscesses *(arrows)* and several older, hyalinized scars *(arrowheads)*. **B,** Medium-power view of stellate abscesses shows central necrosis surrounded by palisaded histiocytes, chronic inflammation, and fibrotic tissue.

lymphadenopathy, but often the classic skin papule of cat-scratch disease is absent.[273-275] Hepatic lesions are typically multiple and associated with abdominal lymphadenopathy and, in some cases, splenic lesions; many patients come to biopsy to exclude neoplasia.[274,276,277] On laparotomy, the liver may be found to be studded with hard nodules of varying sizes.[277]

The hallmark lesion in hepatic cat-scratch disease is an irregular, stellate microabscess surrounded by a layer of palisading histiocytes, lymphocytes, and a rim of fibrous tissue (Fig. 11.21).[274,277,278] Younger lesions may show more necrosis with less organization of the inflammatory granulomatous response, whereas older lesions may show confluent granulomas with scarring and scant residual necrosis.[274] These hepatic lesions are similar to the ones seen in lymph nodes in patients with cat-scratch disease, but they have also been noted in infections with *Y. enterocolitica*, *F. tularensis*, lymphogranuloma venereum, mycobacterial species, *Candida*, and *Actinomyces*.[274] Other lesions may appear as small, rounded granulomas with giant cells and small foci of central necrosis, similar to caseating granulomas in mycobacterial or fungal infections. The background liver parenchyma shows sinusoidal dilatation, portal mixed but predominantly lymphocytic inflammatory infiltrates, portal fibrosis, periductal concentric fibrosis, and focal bile ductular proliferation.[274] These changes are attributed to mass effect and are also seen in other infections that result in space-occupying lesions, such as pyogenic abscesses.[274]

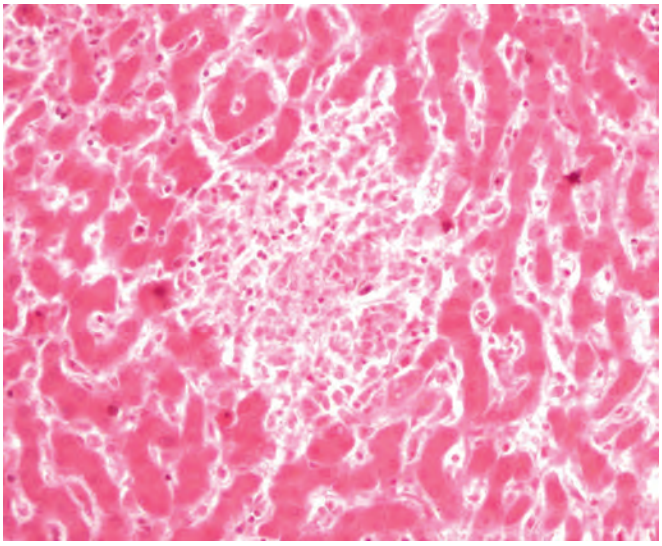
Warthin-Starry stains identify bacilli in some cases, and the organisms often cluster around vessels or along collagen fibers; the stain is not specific for the organism.[275] Culture is difficult. The diagnosis can be confirmed by PCR and Southern blot for *Bartonella* DNA on tissue, skin testing, or serology.[273,274,278,279] The disease is self-limited without long-term hepatic dysfunction. The infection responds dramatically to rifampin, erythromycin, or doxycycline, but antibiotics are unnecessary in most cases.

Bartonella also may cause vascular proliferative lesions in the liver. Bacillary epithelioid angiomatosis is a vasoproliferative tissue reaction to *B. henselae* or *B. quintana* that usually occurs in immunocompromised hosts. Hepatic involvement shows sharply demarcated periportal areas in which the normal parenchyma is replaced by vascular tissue with extravasated erythrocytes, delicate spindle cells, neutrophils, and karyorrhexic debris, mimicking Kaposi sarcoma.[142] Similar lesions have been designated as bacillary peliosis hepatis; these are characterized by the presence of multiple blood-filled cystic spaces, foci of necrosis, fibromyxoid stroma, and clumps of granular purple material that correspond to organisms on Warthin-Starry stain and electron microscopy.[142] The latter lesions can be mistaken for nonbacillary peliosis hepatis. Although these infections can be progressive and fatal, they respond to antibiotics.

Enteric Fever (Typhoid and Paratyphoid Fever)

Enteric fever (the inclusive term for typhoid and paratyphoid fever) is caused by infection with *Salmonella enterica* serotype Typhi *(Salmonella typhi)* or serotype Paratyphi *(Salmonella paratyphi)*. The disease is transmitted via the fecal-oral route from food or water contaminated by an acutely ill person or a chronic carrier.[280] Human beings are the only known reservoir. Most infections nowadays occur in countries where sanitary conditions are poor; the Indian subcontinent has a particularly high incidence.[280] Travelers to these areas can also be affected. Although *S. typhi* causes 80% of infections, *S. paratyphi* may be more important among travelers, possibly due to a vaccine effect that protects only against *S. typhi*.[280] In indigenous populations, enteric fever is a disease of young children and adolescents, whereas among travelers, the age of the patients reflects the age of travelers.[280]

Ingestion of the organism is followed by an asymptomatic period of approximately 7 days, during which the organism multiplies within mononuclear phagocytic cells in Peyer patches, mesenteric lymph nodes, liver, and spleen.[281] This is followed by a bacteremic phase, with fever, chills, headache, and rose spots. During this phase, invasion of the gallbladder occurs, either directly or from infected bile. Chronic biliary carriage occurs in 2% to 5% of cases, even after treatment, particularly among women, older adults, and patients with cholelithiasis.[280,281] In these cases, shedding of virus continues for more than a year and is a public health risk. The majority of patients experience minor degrees of hepatomegaly and elevated transaminases. However, the presentation can resemble acute viral hepatitis, with jaundice and very high transaminases or even fulminant hepatic failure, although coinfection with HAV or HEV may be responsible for



• **Figure 11.22** Typhoid nodule in typhoid fever. An aggregate of mononuclear cells, including histiocytes and lymphocytes, is present in the lobule. The surrounding liver shows prominent Kupffer cells in the sinusoids.

some of these cases.[280] Intestinal perforation, hemorrhage, and encephalopathy are the most important complications. The diagnosis is made by blood culture or Widal serology assay.

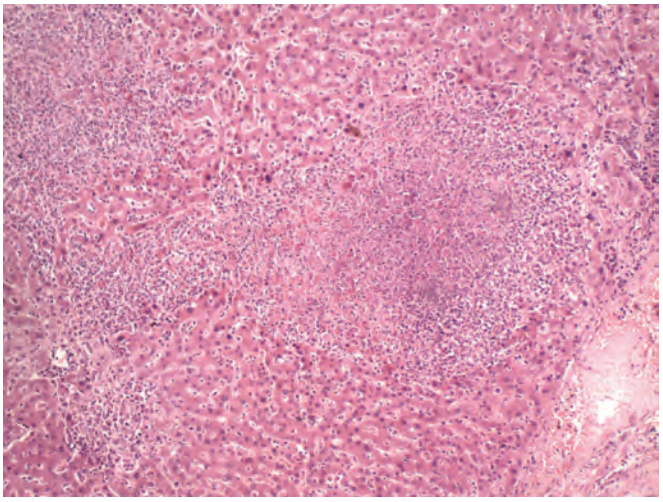
In the liver, hyperplasia of the reticuloendothelial system results in prominence of mononuclear cells in the sinusoids and typhoid nodules (Fig. 11.22).[282,283] Typhoid nodules are lobular collections of macrophages that ingest bacteria, erythrocytes, and degenerated lymphocytes.[283] These nodules may resemble granulomas but are not true epithelioid granulomas, although granulomatous hepatitis has also been described.[284,285] Abscesses are rare.[286]

Tularemia

Tularemia is caused by the coccobacillus *F. tularensis.* The disease is most often transmitted to humans by ticks or by animal contact, especially with rabbits. Inhalation outbreaks have been associated with lawn mowing and brush cutting in contaminated areas.[235] Most patients have pulmonary involvement. Other features include fever, cutaneous ulcers, lymphadenopathy, headache, and malaise. Hepatic involvement occurs in up to 75% of patients and includes hepatosplenomegaly and mild to moderate elevations of transaminases.[287] Severe cases may show jaundice. Liver histology shows suppurative microabscesses and areas of necrosis (Fig. 11.23). As the lesions age, they become more granulomatous.[288] The diagnosis is often established serologically because the organism is difficult to culture and is rarely seen on tissue Gram stain. PCR assays for *F. tularensis* in fresh tissue and on formalin-fixed tissue have been described.[235]

Melioidosis

Melioidosis is a glanderslike disease caused by *B. pseudomallei*, a gram-negative aerobic bacillus that survives within phagocytic cells. The organism is a soil saprophyte that prefers wet soils and is an important community-acquired pathogen in Southeast Asia and northern Australia, particularly during rainy seasons.[237]

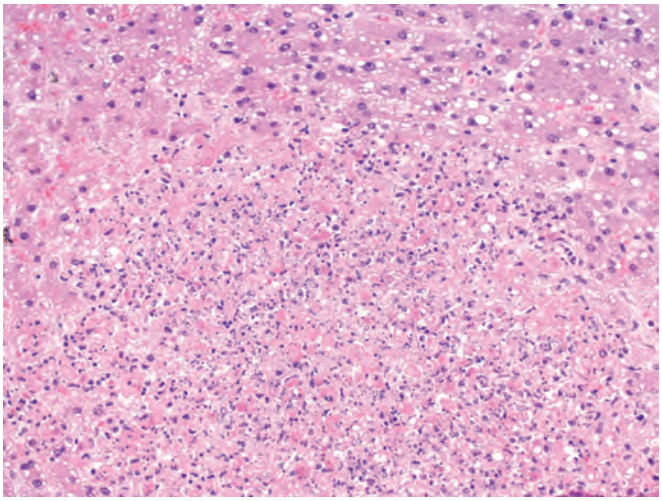


• **Figure 11.23** Tularemia involving the liver. Several small abscesses are present throughout the lobules.

Percutaneous inoculation and inhalation are modes of transmission. Travelers can be exposed when they come in contact with wet soil, such as during adventure tours.[289] Occasionally, patients have chronic disease or reactivation of a latent focus years after leaving an endemic area, a possibility that caused concern for veterans returning from Vietnam.[289] Predisposing factors include diabetes mellitus, renal disease, alcoholism, cirrhosis, and immunosuppression.[236,237] The acute form of the disease is characterized by abscess formation, predominantly in the lung, but seeding and abscess formation also occur in the liver, spleen, skeletal muscle, and lymph nodes; often, multiple sites are involved.[236,237,290] Large abscesses tend to be multiseptate and multiloculated, resulting in a "honeycomb" appearance on radiology.[235] Chronic melioidosis shows granulomatous inflammation with central irregular abscesses, indistinguishable from the stellate abscesses of cat-scratch disease, tularemia, or lymphogranuloma venereum.[290] The granulomas may show caseous-type necrosis, mimicking TB.[290] The lesions in chronic melioidosis are usually confined to a single organ. Serology is not completely reliable, but *B. pseudomallei* can be readily cultured from abscess material.[236] The organism can also be detected in Gram stains as a gram-negative bacillus with a characteristic "safety pin" appearance.

Listeriosis

L. monocytogenes is a gram-positive, facultative intracellular bacillus that is an occasional contaminant of food, even despite proper refrigeration. Infection is associated with pregnancy, extremes of age, diabetes mellitus, and immunosuppression. Transplacental transmission causes granulomatosis infantisepticum, which is characterized by abscesses and granulomas in various fetal organs.[291] After ingestion, the organisms invade the Peyer patches and are transported to the liver, where they are cleared from the bloodstream by Kupffer cells. Some organisms escape from Kupffer cells and invade hepatocytes, where, protected from the host immune response, they replicate. Neutrophils are recruited to the area, forming abscesses, and after a few days, granulomas form in an attempt to impede further spread. After the organisms escape the granulomas and are released into the blood, they have a predilection for the central nervous system and placenta.[291]



• **Figure 11.24** Liver from a patient who died of systemic listeriosis, showing microabscess and steatosis in the surrounding hepatocytes.

Hepatic involvement can manifest as solitary liver abscess, multiple liver abscesses, or diffuse hepatitis (Fig. 11.24).[238,291-293] Solitary liver abscess is associated with diabetes mellitus, absence of bacteremia or extrahepatic manifestations, and a relatively good prognosis.[238,291] Multiple liver abscesses are more often associated with bacteremia, extrahepatic sites of involvement, and meningitis and carry a worse prognosis.[238,291] Diffuse hepatitis is associated with underlying conditions, such as viral hepatitis, pregnancy, or alcohol abuse,[291,294] although it may also occur in healthy individuals.[295] Bacteremia is highly likely in cases with diffuse hepatitis. The histology is that of an active hepatitis with extensive necroinflammatory activity, or sometimes a granulomatous hepatitis.[291,294] Cholestasis and sinusoidal infiltration by mononuclear inflammatory cells may also be seen.[296] Diffuse hepatitis due to *Listeria* often mimics acute viral hepatitis clinically and pathologically (fever, jaundice, substantially elevated transaminases, active hepatitis on biopsy) and may lead to misdiagnosis and delayed treatment.[293-297] The diagnosis is made by culture of the organism from blood or abscess material.

Spirochetes

Syphilis

Treponema pallidum infection can affect the liver in secondary syphilis, in tertiary syphilis, or congenitally. Involvement by the liver in secondary syphilis has been reported in men and women. It is common in HIV-positive individuals. Among HIV-positive patients with syphilis, the rate of liver involvement (based on abnormal liver function tests [LFTs]) is between 19% and 38%.[298-301] However, others have argued that the true rate of syphilitic hepatitis in this patient population is substantially lower, given that many other etiologies may cause liver dysfunction in HIV-positive patients, including viral hepatitis, drug hepatotoxicity, and fatty liver disease.[302] Liver involvement by syphilis is also common among MSM,[303-309] possibly from portal transport of the organism after anal intercourse,[309] although this may be confounded by high rates of MSM among HIV-positive patients.[299-304] A characteristic clinical picture is cholestatic jaundice, hepatomegaly, and a disproportionately elevated alkaline phosphatase level.[305,307,308,310-313] The trademark palmar rash of syphilis may be the key to diagnosis.[298,311,312]

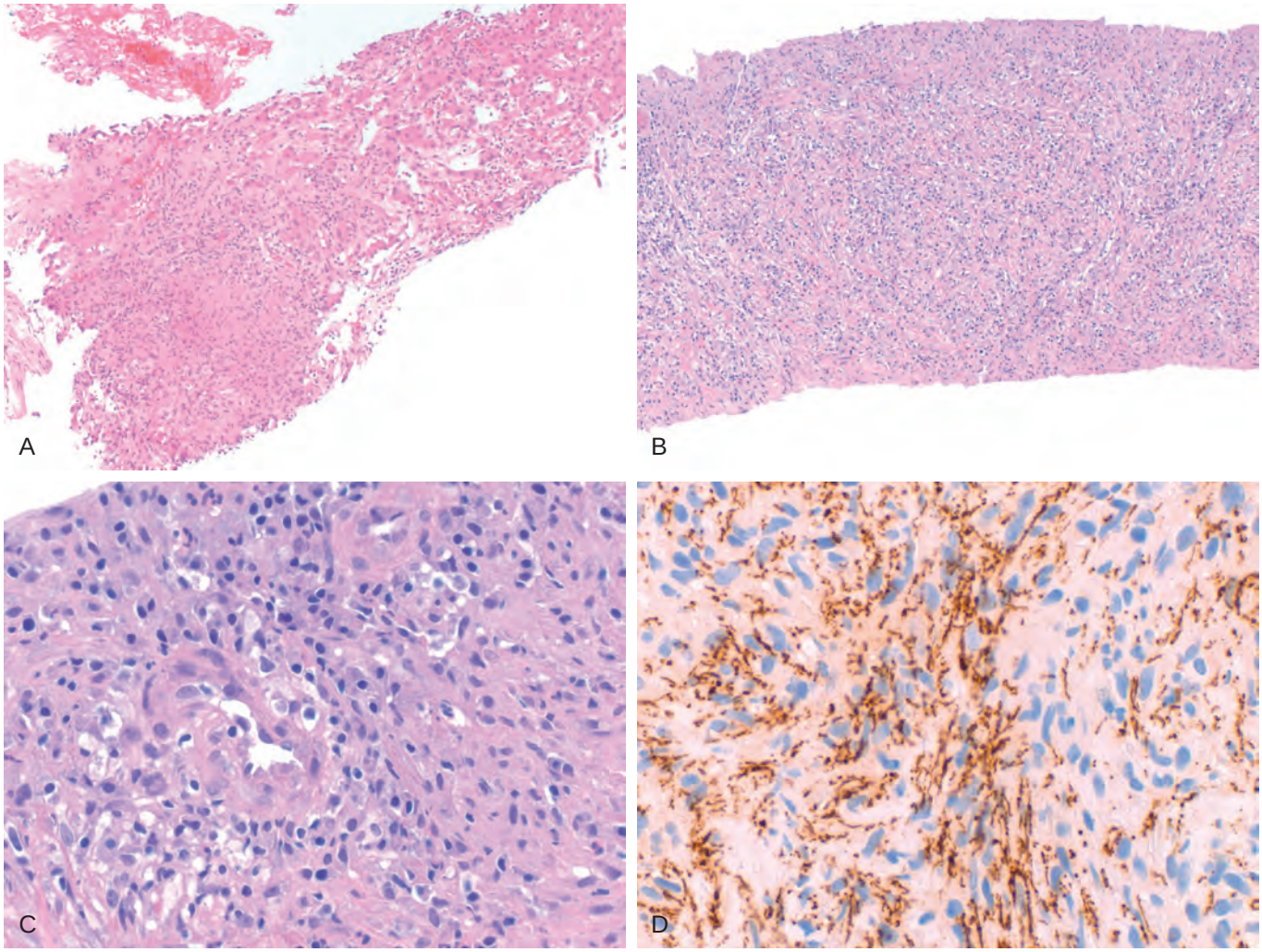
Histologic examination of liver may show a variety of pathologic patterns. In most cases, portal inflammation with mononuclear cells or neutrophils surrounding damaged bile ducts (pericholangitis) is the main finding.[306,307,313-316] Scattered foci of necrosis may be present in the lobules, sometimes accompanied by inflammation around the central veins and Glisson capsule.[304,307,309,316,317] Granulomatous hepatitis is described in some cases (Fig. 11.25).[310,317,318] Rarely, other patterns can be seen, such as diffuse sinusoidal histiocytic infiltration or postinfantile giant cell hepatitis.[319] Spirochetes are only rarely identified in histologic sections with silver stains, such as Warthin-Starry or Steiner.[305,311,316] Organisms can be demonstrated with immunohistochemical stains.[303,320-322]

In tertiary syphilis, multiple gummas in the liver may mimic metastatic carcinoma.[323,324] Histologically, gummas resemble TB, in that they are composed of masses of granulomas with caseating necrosis, surrounding fibrosis, chronic inflammation, and histiocytic inflammation.[324] Organizing abscesses with abundant eosinophils have also been reported.[323] Healed gummas may be replaced by fibrous scars (Fig. 11.26). Retraction of these scars can distort the liver by producing pseudolobules or so-called hepar lobatum.[324] Scars near the hilum may result in portal hypertension. Less commonly, secondary syphilis may also present as mass-forming lesions in the liver, leading to a radiologic diagnosis of malignancy.[303,321,322,325] Lesions are typically multiple in number, ranging from two to innumerable, and may be up to 5.7 cm in size. Histologically the lesions consist of a variably cellular storiform spindle cell proliferation with mixed inflammation, sometimes with abscesses.[303,323,325] The lesions contain numerous spirochetes, particularly within the walls of small blood vessels.[303,321,322] The background liver may show mixed inflammation with portal edema, pericholangitis/cholangitis with duct injury, and granulomas. Although pathologically the lesions mimic a spindle cell neoplasm, awareness of the clinical scenario, as well as the background inflamed liver, should enable the pathologist to suspect syphilis. Treatment with intramuscular benzathine penicillin or, less commonly, oral ceftriaxone or doxycycline, should result in resolution of mass lesions and LFT abnormalities.

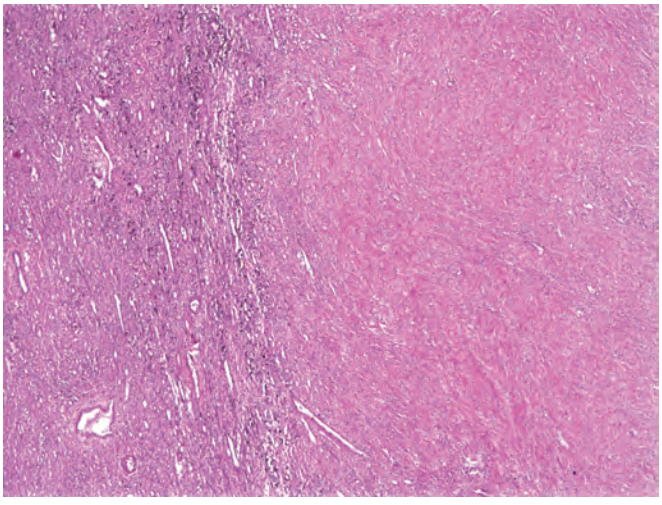
Congenital syphilis is frequently fatal, but surviving infants may have hepatomegaly and jaundice, with diffuse fibrosis. Spirochetes can be seen within connective tissue septa, parenchymal cells, and the walls of small vessels.[326]

Leptospirosis

Leptospirosis is an acute febrile illness caused by *Leptospira interrogans* that affects humans and animals in all parts of the world.[327] The disease is biphasic, with a septicemic phase followed by an immune phase with antibody production and urinary excretion of the organism.[327] Most patients experience a mild anicteric illness, with fever, headaches, and myalgias. Severe cases, characterized by hepatic, renal, and pulmonary involvement, are known as Weil syndrome.[327] Liver pathology is predominantly a cholestatic hepatitis with reactive hepatocellular changes and Kupffer cell hyperplasia.[283,327-329] Scattered Councilman bodies, bile within canaliculi, and mild portal inflammation may be seen.[283,330] Very rarely, spirochetes can be demonstrated on silver stain. Immunohistochemical staining for *Leptospira* is also available from the Centers for Disease Control and Prevention,[328] and PCR assays can be performed from formalin-fixed, paraffin-embedded tissue.[331] However, the diagnosis is usually based on serologic assays.



• **Figure 11.25** Syphilis in the liver. **A,** Granulomatous inflammation is seen adjacent to inflamed hepatic parenchyma. **B,** Medium-power view of an inflammatory tumor in syphilis composed of spindle cells with a storiform appearance and mixed inflammation. **C,** High-power view shows a mononuclear infiltrate with plasma cells surrounding small vessels. **D,** Immunohistochemical stain for *T. pallidum* showing numerous organisms in the inflammatory mass.



• **Figure 11.26** Old gumma in the liver, showing a hyalinized nodule with mild chronic inflammation at the periphery. (Courtesy Dr. Laura Lamps.)

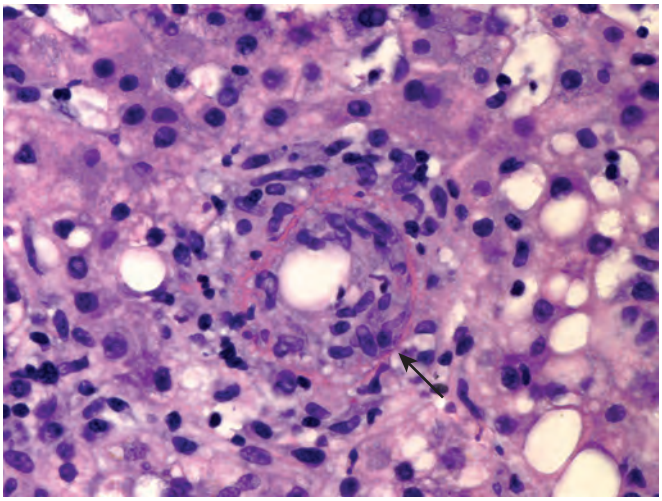
Lyme Disease

Lyme disease is caused by *Borrelia burgdorferi* and is transmitted by *Ixodid* ticks, usually during the months of May through October. Hepatomegaly and symptoms of hepatitis are uncommon. Elevated transaminases indicate mild hepatocellular injury, although a nonhepatic source is possible in the case of Lyme disease–associated myositis.[287] In the liver, mononuclear cells, plasma cells, and granulocytes are seen in sinusoids. Kupffer cell hyperplasia, steatosis, and hepatocyte ballooning are also described.[287,332] Granulomatous hepatitis is uncommon; necrotizing granulomas have been reported.[333] Organisms are only rarely seen in hepatic sinusoids and parenchyma with silver stain, such as Dieterle.[332]

Rickettsia

Q Fever (Coxiella burnetii)

Q fever is a zoonotic rickettsial disease that is caused by the aerosol spread of *Coxiella burnetii* from infected sheep and cattle. The disease is endemic in the southwestern United States and in



• **Figure 11.27** Fibrin ring granuloma. A collection of histiocytes contains a central fat droplet surrounded by a rim of histiocytes, a ring of fibrin *(arrow)*, and another rim of histiocytes.

southwestern Ontario. Most patients suffer an acute, self-limited infection that typically manifests as pneumonitis, although chronic infection with endocarditis is well known. Unlike other rickettsial diseases, Q fever does not produce a rash.[334] Hepatic involvement is common, and the disease may even manifest as hepatic disease without pulmonary symptoms.[335] Hepatic abscess formation detected radiologically has been reported.[336] Some cases may be associated with the hemophagocytic syndrome.[337] The diagnosis is established by complement-fixing antibodies to phase II *C. burnetii* antigen in serum.

The classic lesion of Q fever is the fibrin ring granuloma, which can be found in liver or bone marrow (Fig. 11.27).[334,335,337-344] Also known as doughnut or ring granulomas, these lesions contain a central fat vacuole that can lead to their being mistaken for lipogranulomas, but on closer inspection a ring of fibrin surrounds the fat vacuole. The fibrin ring is highlighted on trichrome stain. Q fever is the most commonly reported etiology, but fibrin ring granulomas have also been reported in other infections and in noninfectious conditions, including hepatitis A,[8,9] *Staphylococcus epidermidis* infection,[345] allopurinol hypersensitivity,[346-349] visceral leishmaniasis,[348,349] giant cell arteritis,[350] CMV,[113] EBV,[102,351] Hodgkin disease,[349,352] toxoplasmosis,[348] and boutonneuse fever.[348]

In addition to fibrin ring granulomas, epithelioid granulomas with or without central necrosis may be seen and can be mistaken for TB.[334,340,343,348,353,354] Other findings in Q fever hepatitis include moderate steatosis, focal liver cell necrosis, Kupffer cell hyperplasia, hemosiderin deposits, acute cholangitis, and, rarely, microabscesses.[340,343]

Ehrlichiosis

Ehrlichiosis is caused by gram-negative obligate intracellular organisms that are transmitted by a tick vector, predominantly in the southeastern United States. Human monocytic ehrlichiosis is caused by *Ehrlichia chaffeensis* and *Ehrlichia canis*, whereas human granulocytic ehrlichiosis is caused by *Ehrlichia phagocytophila* and *Ehrlichia equi*.[287] Symptoms include fever, headache, anorexia, and myalgia. The relatively low incidence of rash (20%) contrasts with the frequent presence of a rash in Lyme disease and Rocky Mountain spotted fever.[355,356] Transaminases are often mildly elevated, although they may be in the range of viral hepatitis. Alkaline phosphatase and bilirubin are less likely to be elevated but may be so with severe cholestasis.[355,356] Liver biopsy may show lobular lymphohistiocytic aggregates and sinusoidal infiltration by lymphohistiocytic cells associated with erythroleukophagocytosis.[357] In cholestatic cases, bile stasis, duct epithelial injury, and neutrophilic infiltration of medium-sized ducts may suggest extrahepatic obstruction.[355,357] Other reported findings include focal hepatic necrosis, steatosis, granulomas, and foamy Kupffer cells with scattered apoptotic bodies.[356,357]

Rocky Mountain Spotted Fever

Rickettsia rickettsii is transmitted by the wood tick, *Dermacentor andersoni*, in the Rocky Mountain region and by the dog tick, *Dermacentor variabilis*, in the eastern United States. Mild to moderate transaminase elevations and occasionally jaundice may be seen. The main pathologic lesion is vasculitis, and rickettsiae are capable of infecting the endothelial lining cells of portal blood vessels, especially arteries and arterioles, and sinusoidal lining cells.[287,358] Reports of autopsy livers describe portal inflammation, portal vasculitis with subendothelial and intramural mononuclear leukocytes, portal vascular thrombi, portal tract hemorrhage, sinusoidal leukocytosis, and erythrophagocytosis by Kupffer cells.[358,359] Immunofluorescence techniques can be used to identify the organisms in tissue.

Boutonneuse Fever/Mediterranean Spotted Fever

Boutonneuse fever, also known as Mediterranean spotted fever, is caused by *Rickettsia conorii* and is transmitted by the dog tick *Rhipicephalus sanguineus*. The disease is found in Mediterranean countries between the months of June and September and classically presents with fever, maculopapular rash, and a black eschar at the bite site (known as "tache noire," or black spot in French).[360] As is the case with Q fever, boutonneuse fever may be associated with the hemophagocytic syndrome.[337] Hepatic involvement shows Kupffer cell swelling and increased sinusoidal cellularity. Small foci of hepatocyte necrosis are seen, associated with collections of histiocytes, a few neutrophils, and lymphocytes.[361,362] The mononuclear cell aggregates in the areas of necrosis may resemble poorly formed granulomas, but they are not true epithelioid granulomas. The diagnosis is typically made by serology.

Fungal Infections of the Liver and Bile Duct

Histoplasma

Histoplasma is considered to be the most common fungal cause of hepatic granulomas, and it is a common cause of granulomatous hepatitis in areas where *H. capsulatum* is endemic, such as in the Ohio River valley.[363] In one series from that region, 15 (65%) of 23 children with hepatic granulomas had histoplasmosis demonstrated by PCR.[364] In another series, 50% of liver biopsy specimens with granulomas cultured positively for *Histoplasma*. Although histoplasmosis is usually self-limited in infants or immunocompromised patients, it can manifest with progressive disseminated disease requiring treatment.[364] Progressive disseminated histoplasmosis often involves the liver, with hepatomegaly in 62% of patients and abnormal liver function studies in 84%.[365]

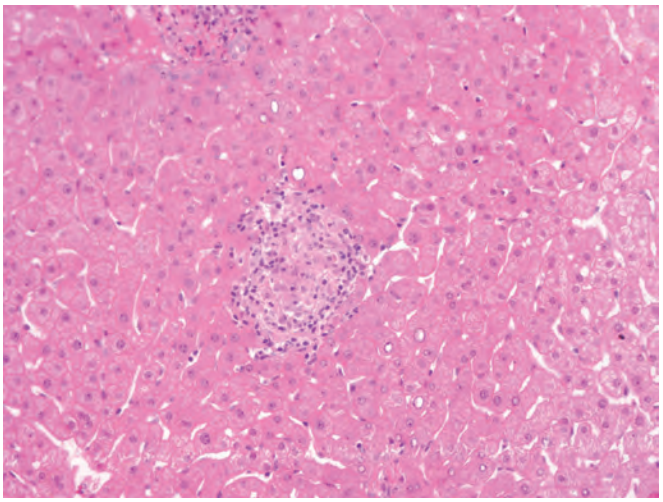
Histologically the appearance is identical to sarcoidosis, with numerous well-formed epithelioid granulomas (Fig. 11.28).[176,366] In immunocompromised hosts, loose collections of histiocytes

with numerous intracellular organisms can mimic leishmaniasis (Fig. 11.29). The absence of a kinetoplast on Giemsa stain helps to distinguish *Histoplasma* from *Leishmania*.[367] The diagnosis can be established with methenamine silver staining of involved tissue, fungal culture, or complement fixation tests.[365]

Candida

Candidiasis is especially problematic in immunocompromised patients, particularly those with leukemia or cytotoxic chemotherapy-related neutropenia.[368] *Candida* accounts for 62% to 91% of fungal infections in the liver transplantation population, affecting 5% to 42% of all liver transplant recipients.[110] Patients present with fever, abdominal pain, and elevated alkaline phosphatase; blood cultures are negative in at least half of the cases.[369] Usually, patients are in the recovery phase from the neutropenic episode, suggesting a role for the host inflammatory response in defining the lesions.[368] The diagnosis is often not made until postmortem examination. *Candida* is an uncommon but important cause of hepatic abscesses in preterm neonates.[370-372]

Radiologically the lesions appear targetoid and, in the case of older lesions, calcified.[368,369] Grossly the liver and spleen are studded with white to yellow nodules. In the early stages, the nodule consists of a necrotic center and surrounding neutrophilic infiltrate. Periodic acid–Schiff (PAS) or Gomori methenamine silver (GMS) stains demonstrate yeast or pseudohyphae in the center of the abscess. As the lesion matures, a palisaded histiocytic reaction may develop at the periphery of the lesion, surrounded by a fibrous wall (Fig. 11.30).[369] Eventually, well-defined granulomas with giant cells may be seen.[368] As the lesions progress with increasing fibrosis, organisms become more difficult to detect, so their absence does not exclude the diagnosis. The adjacent liver parenchyma frequently shows sinusoidal dilatation, portal edema, neutrophilic infiltrate, and ductular reaction, presumably due to mass effect.[369] Candidal infection of the biliary tree and gallbladder occurs in disseminated candidiasis, with bacterial cholangitis, or as isolated candidal cholangitis or cholecystitis.[373,374] *Candida* species can be identified from liver biopsies using PCR techniques, which may be useful in cases in which patients are on antifungal treatment and organisms are not identified by pathologic examination.[375]



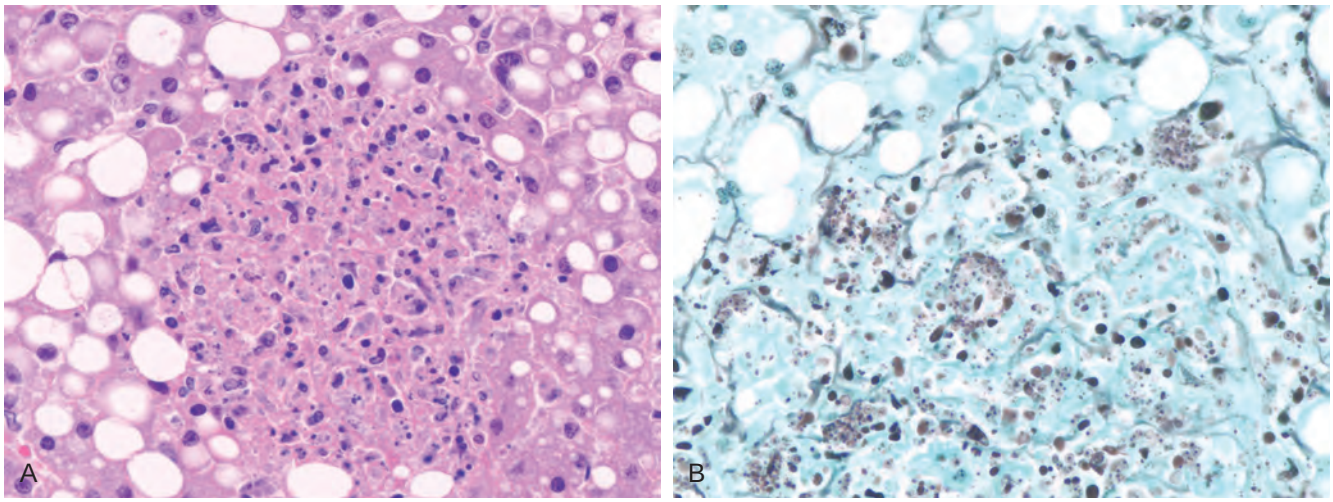
• **Figure 11.28** Liver biopsy sample in an immunocompetent patient with hepatic and splenic histoplasmosis. A solitary, well-formed granuloma is present in the liver.

Pneumocystis jiroveci

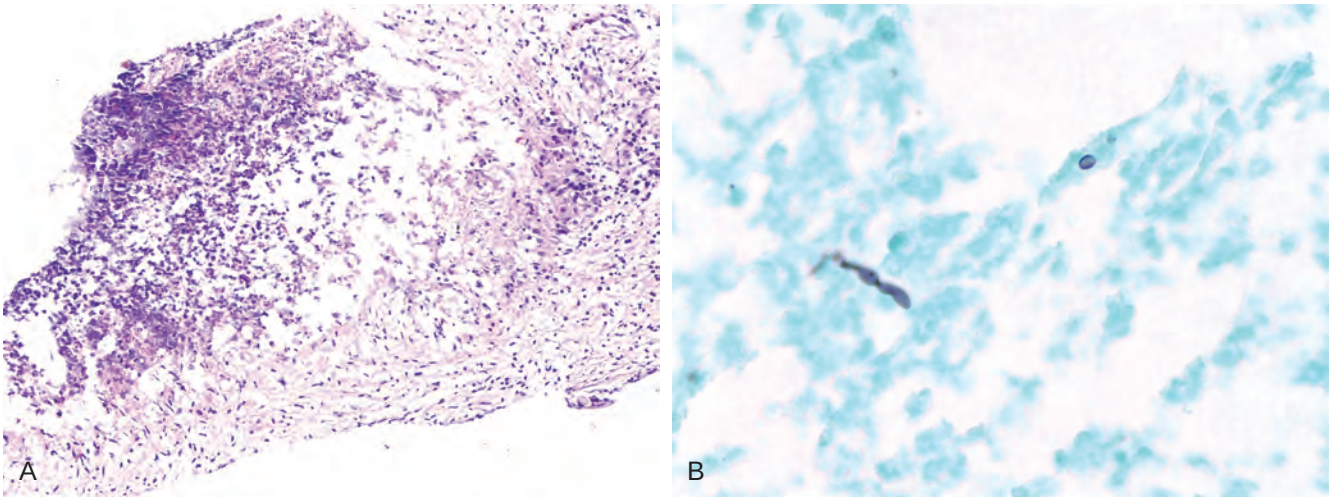
P. jiroveci (formerly *Pneumocystis carinii*) is a fungal organism that typically causes lung infections in immunocompromised patients. Extrapulmonary infection is rare but has been associated with the use of prophylactic aerosolized pentamidine, which suppresses pulmonary disease but might allow for dissemination of the organism.[376,377] Grossly the liver is studded with yellow-white nodules. The pathology ranges from caseating granulomas to massive infiltration of sinusoids and vessels by the organism with little inflammatory reaction (Fig. 11.31).[376,377] The organism appears as indented, helmet-shaped "cysts" on silver stains.

Aspergillus

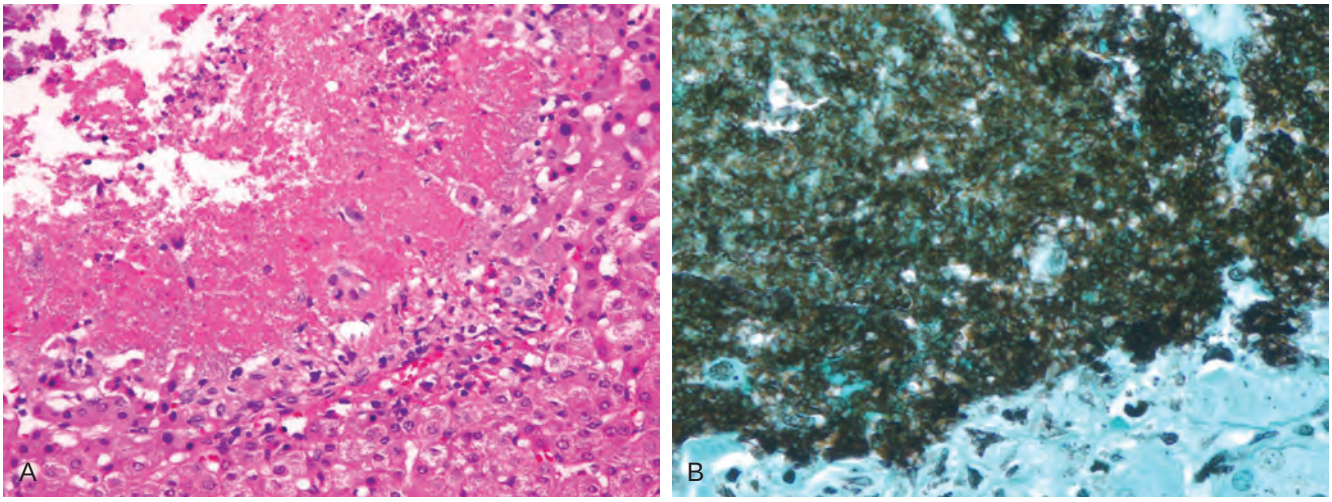
Aspergillus spp. (*Aspergillus fumigatus* or *Aspergillus flavus*) involving the liver is seen in cases of disseminated aspergillosis and



• **Figure 11.29** Histoplasmosis in an acquired immune deficiency syndrome patient. **A,** Necrotic lesions are composed of histiocytes and debris. **B,** Gomori methenamine silver stain shows yeast in macrophages in the necrotic lesion.



• **Figure 11.30** Candidal abscess in a patient with leukemia. **A,** Abscess with necrosis and neutrophils *(upper left)* surrounded by a palisaded histiocytic reaction. **B,** Rare yeast and pseudohyphae on Gomori methenamine silver staining.



• **Figure 11.31** *Pneumocystis jiroveci* in the liver. **A,** Necrotic lesion composed of granular eosinophilic material with mild histiocytic reaction at the periphery. **B,** Gomori methenamine silver stain demonstrates that the granular material is composed of masses of *Pneumocystis* organisms.

in immunosuppressed patients, particularly organ transplant recipients. Liver abscess and cholangitis secondary to *Aspergillus* infection have been described.[239,378-381] The organism is easily demonstrated on methenamine silver or PAS stains and shows characteristic septate hyphae with acute-angle branching. Speciation can be accomplished by culture (with examination of fruiting bodies) or PCR-based techniques.

Mucormycetes/Zygomycetes

Fungi of the class *Mucormycetes* (formerly *Zygomycetes*) are ubiquitous in nature, occurring in decaying material and soil. The members of this class most often implicated in human disease are *Rhizopus*, *Rhizomucor*, *Mucor*, and *Absidia.*[382] Hepatic zygomycosis has been reported in immunocompromised patients, in patients receiving chemotherapy, in stem cell transplant recipients, and in solid organ transplant recipients.[382-386] Involvement of liver allografts leading to retransplantation has been described.[387,388] Less commonly, it may affect immunocompetent patients.[389] Hepatic infection in the absence of pulmonary disease may be caused by spread from a gastrointestinal source, and thus many patients have concurrent involvement of a site in the tubular gastrointestinal tract.[382,390,391] Patients may present with single or multiple hypodense lesions on abdominal CT.[392,393] Mass effect may lead to stenosis and dilatation of the extrahepatic bile ducts, mimicking cholangiocarcinoma.[389] Peripheral eosinophilia may be present.

Pathologic specimens commonly show organizing abscesses with necrosis, with or without granulomatous inflammation. Some cases contain abundant eosinophilic infiltration and may be described as eosinophilic granulomas; this is common in zygomycosis due to *Basidiobolus ranarum*.[390,394-396] These fungi have broad (10 to 20 μm), aseptate or hyposeptate, ribbonlike hyphae with right-angle branching, and they have a propensity

to invade blood vessels, leading to extensive necrosis, infarctions, and dissemination.[382-385,393,397] When eosinophilic inflammation is prominent, degenerated fungal organisms may be associated with dense, hyalinized, eosinophilic material (Splendore-Hoeppli phenomenon). Diagnosis requires identification of the fungus in tissue or culture. However, tissue culture sensitivity is poor, possibly because the grinding of tissue in preparation for plating destroys the delicate hyphae, and this technique is no longer recommended.[382] PCR-based techniques for diagnosis are also available. Blood cultures are always negative. In tissue identification, erroneous classification as *Aspergillus* may occur if the hyphae are hyposeptate.[382] Some evidence suggests that iron is required for growth of zygomycetes, and thus there is interest in using iron chelators in combination with antifungal agents to treat zygomycosis; however, it is not established whether this approach is more effective.

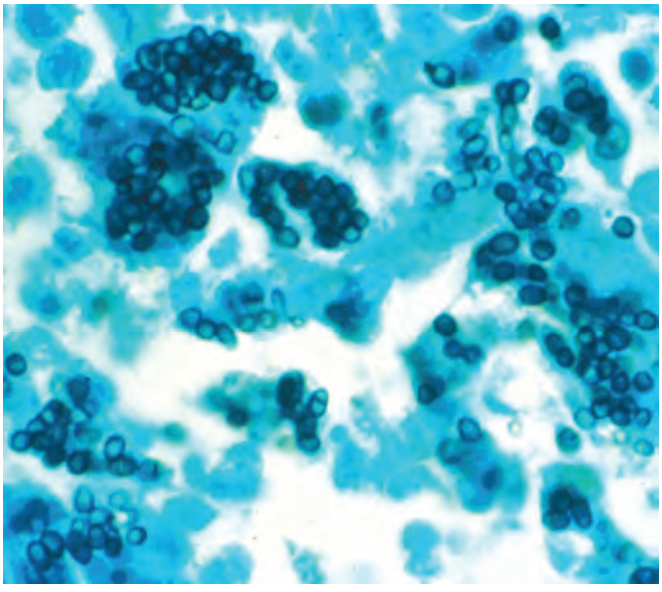
Penicilliosis

Penicilliosis is an infection caused by the dimorphic fungus, *Penicillium marneffei*, which is endemic in Southeast Asia.[398] This pathogen has emerged as the third most common opportunistic infection among AIDS patients in Southeast Asia and southern China, although it affects people with apparently normal immunity as well.[399] Disseminated infection commonly presents with fever and skin lesions, and hepatic abscess formation may occur.[400] Pathologic findings include a granulomatous hepatitis with relatively few organisms, diffuse sinusoidal infiltration by foamy macrophages containing numerous organisms, and suppurative abscesses or necrotic lesions consisting of histiocytes filled with organisms, the latter being typical in immunocompromised patients.[398,399,401] The organisms appear as small spherules, 2 to 4 μm in diameter, along with some elongated and septate forms; they are highlighted with methenamine silver stain or by PAS stain with diastase digestion (Fig. 11.32).[398,399,401] Isolated organisms grow as mold at 25°C on Sabouraud glucose agar and produce a characteristic soluble red pigment that diffuses into the agar.[399,400] The main differential diagnosis is *H. capsulatum*.

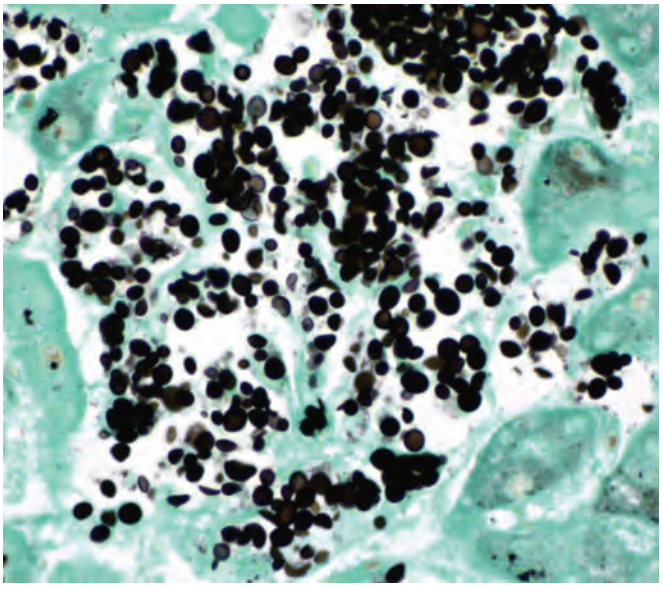
Cryptococcosis

Cryptococcosis is caused by the encapsulated yeast, *Cryptococcus neoformans*. Lung and central nervous system are the two most commonly affected organs, but hepatic infection has been reported in both immunocompromised and immunocompetent patients.[402,403] In immunocompromised patients, hepatic involvement commonly occurs in the setting of disseminated infection or multiorgan involvement.[404-406] In contrast, immunocompetent patients tend to present with an isolated biliary infection that clinically and radiologically mimics sclerosing cholangitis or cholangiocarcinoma.[407-412] These patients exhibit obstructive jaundice, and radiologic studies may show dilatation of intrahepatic bile duct dilatation, stricturing and wall thickening of extrahepatic bile ducts, and masslike lesions in the hilum. Most reported immunocompetent patients with biliary cryptococcosis are children or young adults.[407-409,412]

Histopathologically, immunocompetent cases are characterized by granulomas, whereas in AIDS patients, an inflammatory reaction is poorly developed or absent. Autopsies in cases of disseminated infection have shown areas of necrosis with innumerable yeast cells, yeast cells diffusely within tissue admixed with cellular debris and inflammation, or yeast cells within Kupffer cells (Fig. 11.33).[406,411] The organism can be demonstrated on special stains (silver impregnation, PAS), and mucicarmine stain can be used to highlight the capsule.[404-406,409,410] The capsule is a virulence factor and is helpful in the diagnosis, but in rare instances, capsule-deficient strains can cause infection, particularly in immunocompromised patients (see Fig. 11.33). Capsule-deficient forms can be confused with other yeasts. The diagnosis can be made by serology but may be elusive in cases with atypical presentations



• **Figure 11.32** Penicilliosis. A Gomori methenamine silver stain shows numerous yeast forms in macrophages. The organisms are round to oval, with a few slightly elongate and septate forms. (Courtesy Drs. David Walker and Laura Lamps.)



• **Figure 11.33** Capsule-deficient *Cryptococcus.* A Gomori methenamine silver stain shows yeast cells of varying sizes within a collection of histiocytes in the liver. (Courtesy Dr. Laura Lamps.)

(e.g., without prominent pulmonary symptoms), leading to delayed treatment and poor outcomes.

Coccidioidomycosis

Coccidioides immitis is endemic to the southwestern United States and northern Mexico. Disseminated infection can show granulomatous hepatitis or microabscesses containing the thick-walled spherules of *C. immitis*, which can be highlighted with PAS and methenamine silver stains.[413-415] The spherules may or may not contain endospores; immature spherules without endospores can be mistaken for *Blastomyces dermatitidis* or *C. neoformans*.[415] Serology and culture results can be used to establish the diagnosis.

Blastomycosis

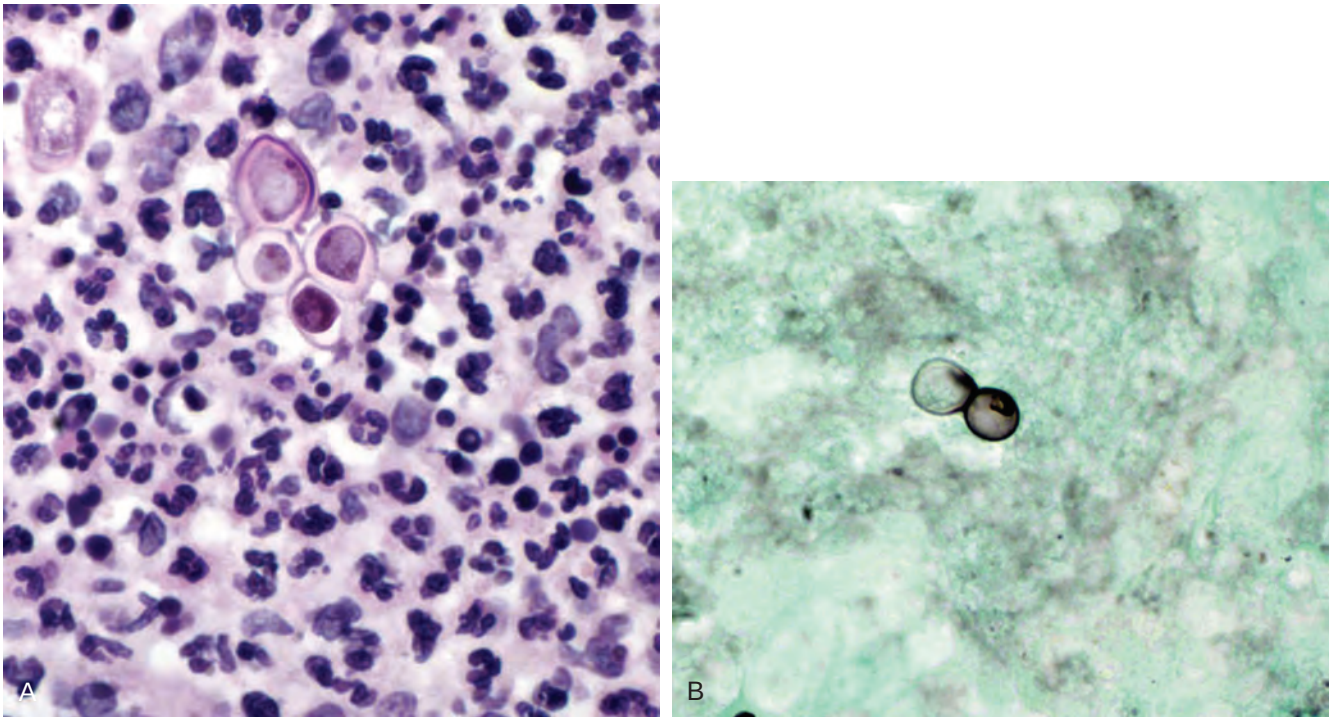
B. dermatitidis is a dimorphic fungus that usually involves skin and lungs. The fungus is endemic to the southeastern and south central United States, the areas bordering the Great Lakes, and the Ohio and Mississippi River basins. Hepatic granulomas and cholangitis have been described.[416] Thick-walled yeast forms with broad-based budding are seen on PAS or silver stains (Fig. 11.34).

Paracoccidioidomycosis (South American Blastomycosis)

Paracoccidioides brasiliensis causes a chronic granulomatous disease known as South American blastomycosis. The disease is endemic in rural South America and affects males more often than females. Although pulmonary lesions are typical, extrapulmonary disease can occur. In the liver the fungus usually causes a granulomatous reaction. Less often, it causes focal necrosis with neutrophilic exudates containing numerous organisms.[417] The inflammatory reaction may involve the portal tracts and be associated with bile duct injury, ductular reaction, and portal fibrosis, which may progress to bridging.[418] Yeast forms can be demonstrated by silver impregnation stains. Intrahepatic ductal dilatation may result from biliary involvement and may mimic cholangiocarcinoma radiologically.[419]

Microsporidiosis

Microsporidia are obligate intracellular organisms that were originally believed to be primitive eukaryotes but have been reclassified as fungi, based on DNA sequence data. Infection of bile duct epithelium has been described as a cause of AIDS-related cholangitis.[146,148] Several species have been implicated in AIDS-related cholangitis: *E. bieneusi* (the most common), *Encephalitozoon cuniculi*, and *Septata intestinalis* (previously *Encephalitozoon intestinalis*).[420] The presentation is similar to AIDS cholangitis due to other organisms, including elevation of alkaline phosphatase and PSC-like changes on endoscopic retrograde cholangiopancreatography (ERCP). *S. intestinalis* may also cause radiologically detected hypodense parenchymal lesions, which on biopsy show necrotizing cholangitis consisting of periportal mixed inflammation coalescing into granulomatous microabscesses with foamy macrophages that contain organisms.[421] *S. intestinalis*, unlike *E. bieneusi*, may infect macrophages, fibroblasts, and endothelial cells, in addition to epithelial cells; thus the presence of foamy macrophages suggests *S. intestinalis* infection.[422] The organisms may be seen on Brown-Hopps or Giemsa stains and appear as ovoid to ellipsoid spores measuring 2.2 to 2.4 µm in greatest dimension, with characteristic cross-banding and polar vacuoles.[421] A case of hepatitis has also been reported, with



• **Figure 11.34** Blastomycosis. **A,** Broad-based, budding yeast forms are present in an area of suppurative inflammation. **B,** Gomori methenamine silver stain shows broad-based, thick-walled yeast cells. (Courtesy Dr. Laura Lamps.)

sinusoidal congestion and microgranulomas on light microscopy.[145] Electron microscopy is necessary to identify the organisms in most cases, but often the organism has already been identified in intestinal biopsy specimens by the time cholangitis develops. Treatment with albendazole may eradicate the organism, but microsporidial cholangitis in HIV patients has a poor prognosis (as does AIDS cholangitis in general) and often follows a progressive course.

Helminth Infections of the Liver and Bile Duct

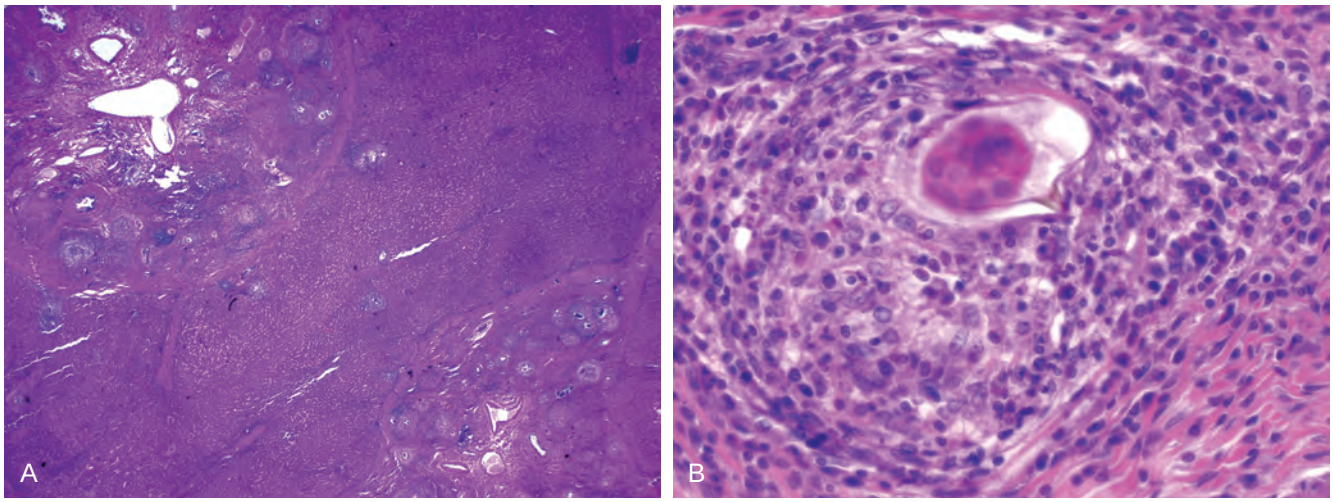
Schistosomiasis

Schistosomiasis is the second most common parasitic infection of humans after malaria. Several species of schistosomes affect humans. *Schistosoma japonicum* and *Schistosoma mansoni* are the two major species associated with hepatic and intestinal schistosomiasis and account for the disease burden in endemic areas.[423] Uncommonly, hepatic involvement has also been documented with *Schistosoma hematobium*, *Schistosoma intercalatum*, and *Schistosoma mekongi*. The intermediate host is a population of snails that is not found in North America or Europe.[424] Infection occurs when the cercariae exit the snail and penetrate the skin of a vertebrate host. In the human host the cercariae migrate to the lungs, where they mature, reach the left side of the heart, and then are carried to the portal hepatic circulation. In the mesenteric venous plexus, the male occupies the gynecophoric canal of the female, and the worms remain in a state of continuous copulation for their life span of 3 to 5 years.[424] Eggs released in the mesenteric venous plexus can migrate to the intestine (intestinal form) or into the liver (hepatosplenic form), where they lodge in the small portal vein tributaries and release antigens that elicit a granulomatous immunologic reaction. Granuloma formation around the eggs leads to pyelophlebitis and periportal fibrosis. *S. mansoni* eggs are ovoid, measure 140 × 60 μm, and have a prominent lateral spine, whereas *S. japonicum* eggs are smaller and more rounded (100 × 60 μm) and have a smaller lateral spine that is often obscured.[423] Degenerated eggs may become calcified, which is more commonly seen with *S. japonicum* than with *S. mansoni*.

Histology shows portal fibrosis with partial or complete destruction of the main branches of the portal vein but sparing of arteries and ducts.[424] Adult worms metabolize large amounts of hemoglobin and regurgitate hemozoin pigment, which is engulfed by macrophages in the sinusoids and portal tracts and can be accentuated by Prussian blue stain. Despite the scarring, lobular architecture is maintained, so the fibrosis does not represent true cirrhosis, and there is evidence that it may undergo regression with clearance of the organisms.[425] This pattern of fibrosis is known as Symmers pipestem fibrosis because of the clay pipestem appearance on gross pathology (Fig. 11.35).[424] The dense periportal fibrosis can also be seen on radiology as characteristic low-attenuation bands or rings around large portal vein branches, which may be associated with calcified eggs.[426]

Eventually, portal hypertension supervenes with splenomegaly and esophagogastric varices. However, compensatory increase in hepatic arterial blood flow maintains hepatic perfusion and hepatic function.[424] Often, patients lack other stigmata of chronic liver disease. The diagnosis is based on the demonstration of ova in stool specimens. Serology to detect anti-schistosomal antibodies does not distinguish active from resolved infection. Enzyme-linked immunosorbent assays (ELISAs) have been developed that detect schistosomal antigens in the serum and urine of actively infected patients. Antigen levels correlate with worm burden.[424] Coinfection with HBV or HCV is associated with a more aggressive course of hepatitis B or C and accelerated progression of fibrosis.[423]

In endemic areas, recently infected patients may present with an acute form of the disease (sometimes referred to as Katayama fever) caused by a systemic hypersensitivity reaction to migrating cercariae and eggs.[423] Clinical symptoms include fever, headache, myalgias, pneumonia, diarrhea, hepatosplenomegaly, and peripheral eosinophilia. Pathologic examination shows granulomas with extensive central necrosis and dense eosinophilic infiltrates surrounding eggs in the liver, intestines, and other organs.[427]



• **Figure 11.35** Schistosomiasis. **A,** The liver shows marked expansion of two portal areas with fibrosis and numerous granulomas, consistent with Symmers pipestem fibrosis. Note the absence of cirrhosis in the background liver. **B,** A high-power view of a granuloma surrounding a schistosome ovum with the characteristic lateral spine.

Strongyloides

Strongyloides stercoralis exist as rhabditiform larvae in soil and as filariform larvae in humans. The organisms reside in the intestine, where they produce eggs that develop into rhabditiform larvae, which are shed in feces. In certain settings, rhabditiform larvae may develop into the infectious filariform larvae within the intestine; this form is capable of invading the intestinal mucosa and traveling throughout the body, typically the central nervous system, liver, and lungs. This unique cycle of autoinfection is accelerated in immunosuppressed patients, particularly those receiving corticosteroids, and is referred to as hyperinfection syndrome or disseminated strongyloidiasis; untreated, its mortality rate approaches 100%.[428] The diagnosis is based on detection of larvae in stool in conjunction with serologic testing. In the liver the larvae can cause granulomatous hepatitis with or without larval remnants. The larvae measure between 6 and 13 μm, and they may be seen in sinusoids, portal tract lymphatics, or branches of the portal vein (Fig. 11.36).[429] The larvae occasionally do not provoke an inflammatory reaction.[429] Steatosis and cholestasis are additional features.

Enterobiasis (Pinworm)

Rarely, *Enterobius vermicularis* travels from the anus to unusual locations, such as the urethra or vagina. Rupture of the appendix or other intestinal disease may also provide the nematode with a mode of reaching the liver.[430] Subcapsular nodules in the liver may be found at the time of surgery or may be radiologically detected during staging of patients with carcinoma, leading to misdiagnosis as metastases.[431,432] Histologic examination shows large necrotic nodules with surrounding histiocytic reaction and fibrosis, hyalinizing granulomas, or calcifying granulomas; the lesions contain remnants of pinworms with characteristic lateral alae and multiple ova.[430-433]

Echinococcosis

Echinococcosis or hydatid cyst disease is caused by species of the tapeworm, *Echinococcus*. *Echinococcus granulosus* is endemic in Mediterranean countries, Iran, India, China, Chile, and Argentina and causes cystic disease, whereas *Echinococcus multilocularis* is endemic in central Europe, the Near East, Russia, China, northern Japan, and Alaska and causes alveolar disease.[434,435] The adult tapeworms live in the jejunum of dogs and other carnivores, the final hosts; the larvae infect herbivores, the intermediate hosts, when they ingest the eggs released in the feces of the carnivore host. Humans are incidental intermediate hosts because further development of the cestode requires ingestion of the larvae by a carnivore.[436]

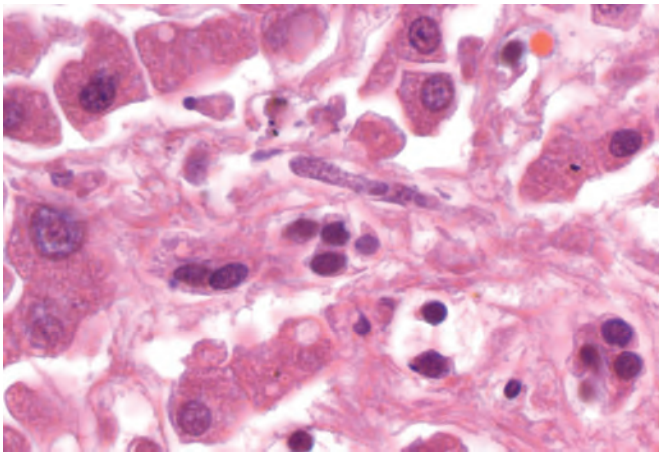
After being ingested, the egg develops into a larval oncosphere, which penetrates the intestinal mucosa, enters the portal circulation or lymphatics, and, on reaching the target organ (usually the liver, in 70% of cases, or the lung, in 20%), forms a rounded, multinucleated mass that becomes cystic and progressively enlarges.[435] The hydatid cyst or metacestode consists of an external, acellular, laminated cuticle and an inner germinal membrane that gives rise to brood capsules containing new larvae called protoscolices (Fig. 11.37).[435,437] The fully developed protoscolex stage contains suckers, hooks, a cup-shaped foramen, and calcareous corpuscles, which are irregularly spherical or ovoid in shape and have a diameter of 2 to 16 μm.[438] The central region of the calcareous corpuscles is composed of an electron-lucent matrix containing granular deposits of inorganic salts (chiefly calcium and magnesium carbonate) and paired membrane lamellae.[439]

Hydatid cysts are initially uniloculated but develop internal septations as they form daughter cysts, either from the germinal membrane or from the protoscolices or brood capsules. Cysts cause a granulomatous host reaction that leads to development of a fibrous tissue layer or pericyst.[436] In the case of *E. granulosus* the cysts are slowly enlarging masses that remain asymptomatic for a long period. They may eventually cause dull abdominal pain and a palpable mass.[374] Abdominal ultrasound is key for the diagnosis of cystic echinoccocosis and further classifies the cysts as active, transitional, or inactive based on a classification scheme developed by the World Health Organization (WHO) which helps to guide management.[440] Serologic testing is available but has low sensitivity and is used primarily as a confirmatory test. Approximately 40% of cases are associated with complications, including erosion into bile ducts, through the hepatic capsule into the peritoneal cavity, into adherent organs, or through the diaphragm.[374] Therapeutic options include medical therapy, percutaneous aspiration injection and reaspiration (PAIR), and surgical resection.[441] In alveolar hydatid disease due to *E. multilocularis*, the hepatic parenchyma is invaded and replaced by fibrous tissue with numerous embedded vesicles (each with a germinal layer and laminated cuticle) that mimics carcinoma.[434] Dense granulomatous inflammation, microcalcifications, and necrotic cavitation may be present.[437]

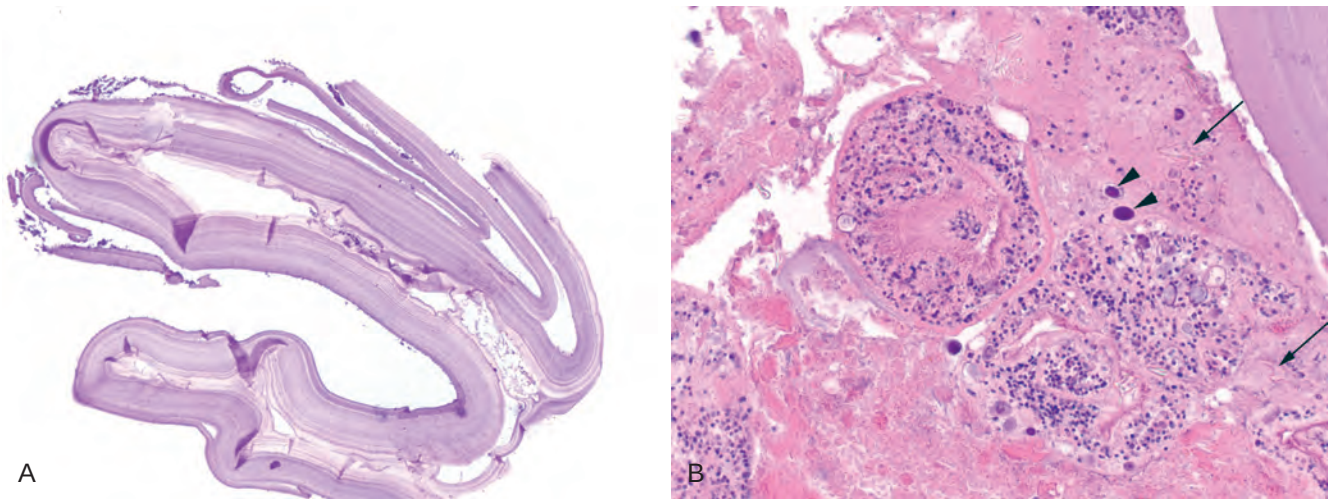
Alveolar hydatid disease carries a high mortality without surgery, due to liver failure, invasion of contiguous structures, or, less often, metastases to the lung and brain.[434,437] Transplantation has been attempted, but there is a high risk of recurrence in the donor liver.[437] As with cystic echinococcosis, the diagnosis can be made radiologically and confirmed serologically, although some patients do not develop an immune response.[434,437]

Toxocara

Toxocariasis refers to infection by larvae of the dog ascarid, *Toxocara canis*, or, less commonly, the cat ascarid, *Toxocara cati.* In humans, these larvae do not develop into adult worms but travel throughout host tissue, primarily liver and lungs; therefore the



• **Figure 11.36** *Strongyloides* in an AIDS patient with disseminated *Strongyloides* infestation. A larval form is present in a small portal lymphatic channel.



• **Figure 11.37** Echinococcal cyst. **A,** Collapsed cyst wall is composed of an acellular laminated cuticle. **B,** Closer inspection of the cyst debris shows protoscolices, calcareous corpuscles *(arrowheads)*, and refractile hooklets *(arrows)*.

disease is known as visceral larva migrans.[442] Humans acquire the infection by ingesting the eggs from contaminated soil or by eating undercooked meat or other animal tissues (e.g., cow liver) that are infected by the encapsulated larvae. The larvae are released in the intestines, burrow through the intestinal wall, and reach the liver via the portal vein. From there, they travel to various other organs, causing eosinophilic inflammation, granulomas, and abscesses.[442] Most patients are asymptomatic but demonstrate peripheral eosinophilia and hypergammaglobulinemia. Vague abdominal pain, fever, cough, and dyspnea are occasional complaints.[442] In the United States, visceral toxocariasis affects primarily children between 2 and 7 years old.[443] Radiologic studies show multiple, ill-defined, oval or angular hypoechoic lesions that improve or resolve spontaneously, similar to *Fasciola hepatica* and *Capillaria hepatica*. The nonspecific radiologic appearance may lead to consideration of other etiologies, such as microabscesses, sarcoidosis, and malignancies.[444] Serologic testing for IgG to *Toxocara* antigens has high sensitivity and specificity and is used to make the diagnosis.

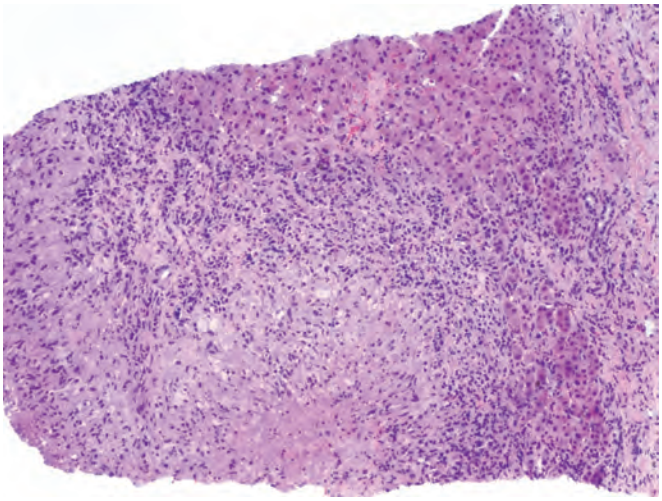
In liver biopsy specimens, multiple granulomas and confluent granulomas may be the main finding.[445,446] Grossly the liver may show 0.3- to 1-cm, white-gray lesions, primarily in the subcapsular region of the right lobe.[447,448] These granulomatous lesions show central necrosis surrounded by a mixed inflammatory infiltrate that includes numerous eosinophils and palisading epithelioid histiocytes and may be referred to as eosinophilic granulomas.[444] The central necrotic zone shows granular debris or eosinophilic material. Charcot-Leyden crystals may be seen in the areas of necrosis. Remnants of parasites are found in a minority of cases and measure 15 to 21 µm in diameter.[447] The differential diagnosis includes other causes of visceral larva migrans, including *C. hepatica* and *Ascaris.*

Capillariasis

C. hepatica is a nematode that mainly affects rodents but is also found in carnivores. The organism requires only a single host, with the liver containing both the adult parasite and its ova.[449] Eggs are deposited in soil, where, under favorable conditions, they embryonate. Ingestion of the unembryonated eggs does not result in hepatic disease but only in abdominal discomfort; in these spurious cases, eggs are passed in the stool. Ingestion of embryonated eggs results in hepatic disease; the larvae bore through the intestinal wall and reach the liver via the portal circulation where they mature into adults. Eggs are not passed into stool.[449] Infection of humans is rare; children are more commonly affected, probably because of frequent soil-to-mouth contact.[449] Patients present with chronic fever, hepatomegaly, and peripheral eosinophilia.[450] Imaging may show portal lymphadenopathy and multiple ill-defined, subcentimeter hypodense nodules throughout the liver.[451] Liver biopsy specimens may show granulomas and eosinophils. Granulomatous lesions with central eosinophilic necrosis rimmed by palisaded histiocytes and numerous eosinophils similar to toxocariasis may be seen.[447,451-454] Fibrosis may develop over time. Remnants of the parasite and its eggs may be identified.[449] The parasite measures 50 µm or larger in diameter[447] and can be identified by the presence of its internal organs (bacillary bands, esophagus, and associated stichosome).[450] The eggs are elliptical, measure 54 to 64 µm by 29 to 33 µm, and have a double-layered envelope with an outer striated wall and bipolar plugs ("bioperculated eggs").[449,450,453-455] Serology for *Capillaria* is not available. Thus, unlike other helminthic diseases in which the diagnosis is made primarily by serology, a definitive diagnosis of hepatic capillariasis requires pathologic identification of the adult or eggs in biopsy material. Serology should still be performed to exclude other infections in the differential diagnosis, particularly toxocariasis.

Fascioliasis

F. hepatica and *Fasciola gigantica* are trematode bile duct flukes found primarily in sheep and cattle, with a broad geographic distribution.[434,456] The leaf-shaped worms reach a size of approximately 2 cm and may remain viable in the bile ducts for more than a decade. The eggs pass in feces, hatch in water, and infect lymnaeid snails.[434] Snails release a cercarial stage of the parasite that contaminates aquatic plants that are ingested by sheep, cattle, or humans. Watercress is often associated with human



• **Figure 11.38** Fascioliasis. Necrotic tract is surrounded by a palisaded histiocytic reaction in a patient with *Fasciola* infestation.

infection.[457] After being ingested, metacercariae penetrate the intestine, traverse the peritoneal cavity, and penetrate the liver capsule.[458] They burrow through the liver parenchyma for 1 to 3 months, while maturing, and finally enter the bile ducts to complete the life cycle.

Acute (invasive) fascioliasis manifests with fever, right upper quadrant discomfort, hepatomegaly, and eosinophilia.[456] Infrequently, patients experience respiratory, cardiac, or neurologic symptoms that are believed to be immunoallergic in origin because the organism only rarely appears at these sites.[456] As the flukes course through the liver, they create tracks of necrosis infiltrated by eosinophils, sometimes surrounded by a reaction of palisaded histiocytes (Fig. 11.38).[457,458] These tracks appear as yellow-white subcapsular nodules or cords at laparoscopy.[457,458] Common findings on CT or magnetic resonance imaging (MRI) include the classic picture of multiple lesions which change in position, attenuation, and shape over time; tracklike subcapsular hypodense lesions; and subcapsular hematomas.[459] As the lesions age, they become cavities bound by granulation tissue, fibrous tissue, and calcifications.[458] Immature flukes that fail to reach the liver may produce ectopic abscesses, most often in the skin.[456]

After the flukes penetrate ducts, egg production initiates chronic (obstructive) fascioliasis, characterized by local inflammation, fibrous thickening of the duct wall, ascending cholangitis, episodic biliary pain, and obstructive jaundice.[456] Peripheral eosinophilia is mild or absent in this stage. The flukes attach to the bile duct epithelium using suckers and cause ductal epithelial proliferation, possibly due to their production of proline.[458] Large necrotic granulomas can develop around entrapped eggs, and tissue eosinophilia is typical in these lesions. Adult flukes can be found in the bile ducts or gallbladder, along with stones, hypertrophied muscle coat, and mucosal hyperplasia. Cholangiocarcinoma is not associated with fascioliasis, but development of cirrhosis has been described. Radiologic findings in the chronic phase are nonspecific and may include hepatosplenomegaly, periportal fibrosis, cystic lesions, and calcifications, as well as dilated common bile duct and thickened gallbladder.[459] Diagnosis is made with serologic and antigen-identification tests or stool examination for ova, although the latter is useful only in chronic disease and may be negative if ova production is intermittent.[456]

Clonorchiasis

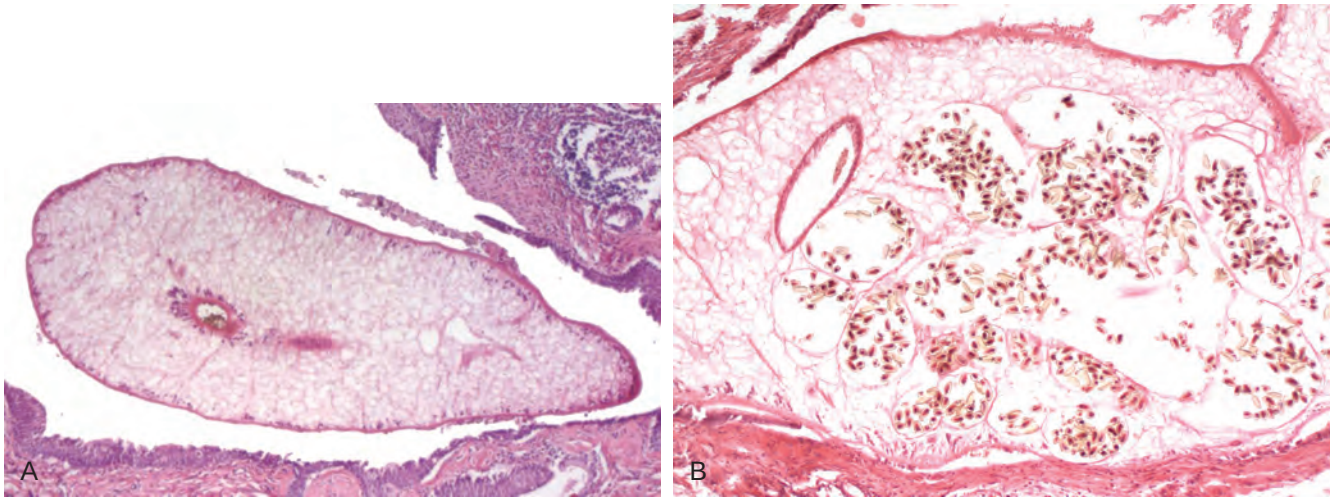
The trematode liver flukes *C. sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus* are similar and are usually considered together. *C. sinensis* infects persons in China and east Asia and is common among immigrants to New York City. *O. viverrini* is limited to Thailand, Laos, Vietnam, and Cambodia, and *O. felineus* infects cats and humans in parts of Russia and eastern Europe.[459,460] These bile duct flukes are acquired through ingestion of raw fish containing the metacercariae.[459,460]

In contradistinction to *Fasciola*, The flukes migrate into the ducts via the ampulla of Vater, where they mature in approximately 25 days.[459,460] The adults reside in medium-sized and small intrahepatic ducts and occasionally in the extrahepatic ducts, gallbladder, or pancreatic duct, where they can live for a decade or longer.[459] Human hosts excrete eggs that hatch in water, pass through snails and fish, and infect other humans or animals. Patients are usually asymptomatic but may present with abdominal pain, hepatomegaly, and eosinophilia or with the syndrome of recurrent pyogenic cholangitis.

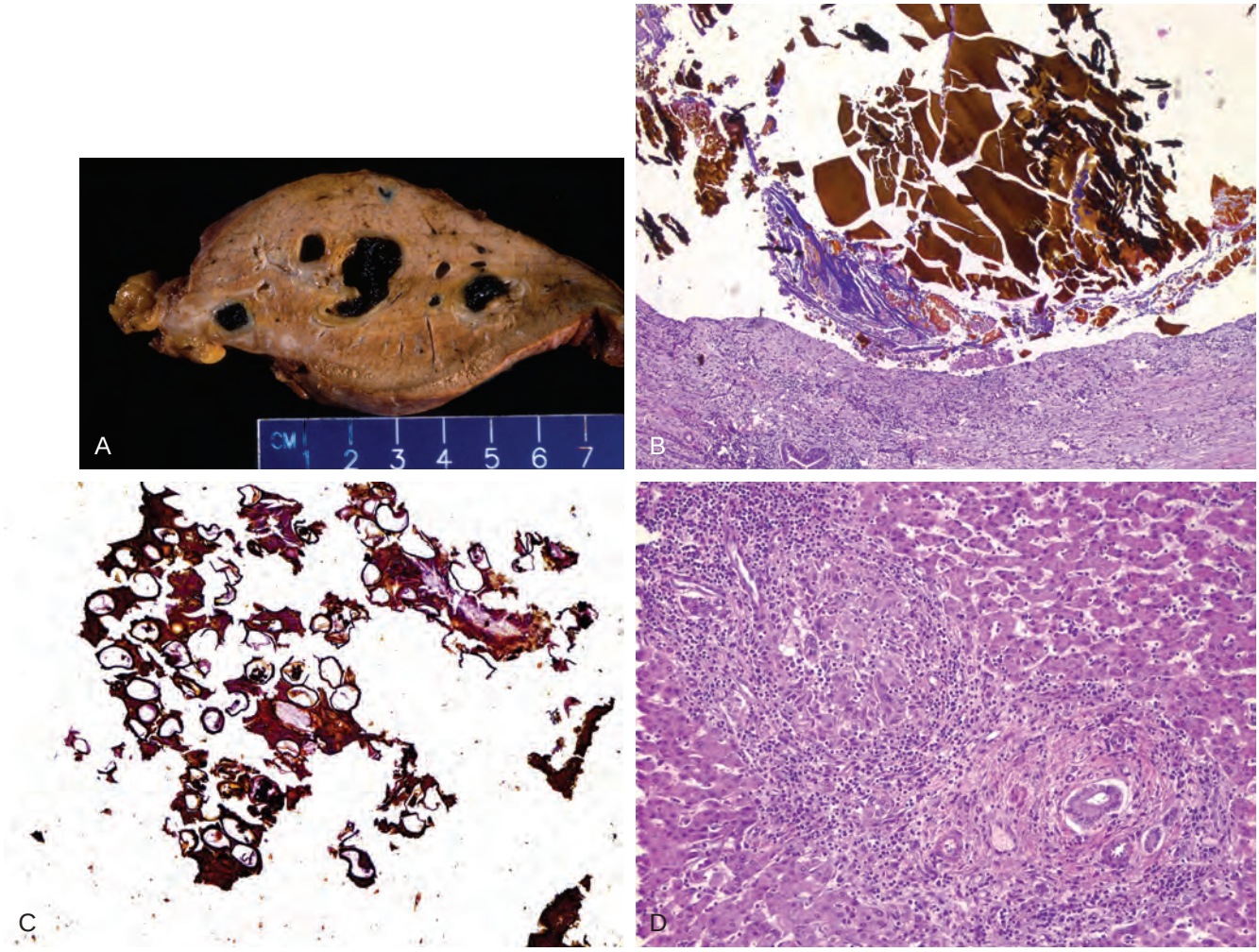
Grossly, focal dilatation of segments of smaller ducts may be seen, with thickened walls and possibly worms in the lumen. The left lobe is affected more often because the left intrahepatic bile duct is straighter and wider, allowing easier access for the worms. Bile ducts that harbor these flukes show dilatation, irregular thickening, adenomatous epithelial hyperplasia, and variable eosinophilia (Fig. 11.39).[459] *Opisthorchis* flukes contain an egg-filled branched uterus flanked by paired ceca.[461] Eggs are not usually seen in tissues. Ascending cholangitis, usually from *E. coli*, can lead to purulent exudates in dilated ducts. Bile duct obstruction can result from the worms, strictures, or calculus formation.[459] Stones often have dead worms as their nidus. Imaging may show intrahepatic duct dilatation, increased periductal echogenicity, and gallbladder sludge. Diagnosis is made by identifying the ova in stool or by ultrasonography. Infection with *O. viverrini* or *C. sinensis* is strongly associated with development of cholangiocarcinoma, which is often intrahepatic, multicentric, and mucin secreting. In fact, *O. viverrini* is considered a group 1 carcinogen. The mechanisms of carcinogenesis in chlonorchiasis are not well understood but may be multifactorial. Serology does not distinguish between present and past infection. Therefore the diagnosis of active infection may require the detection of ova in stool.[459]

Ascariasis

A. lumbricoides rarely causes biliary ascariasis when the roundworms, which usually reside in the lumen of the small intestine, ascend the bile duct; this occurs usually in patients who live in endemic areas, have biliary abnormalities, or have undergone sphincterotomy. Although children are infected with *Ascaris* commonly, biliary involvement is rare in children, probably because of the narrow ampulla of Vater and bile duct lumen.[462] Bile duct infestation manifests as biliary colic, obstructive jaundice, or pancreatitis. Dying worms, in conjunction with *E. coli* and other bacteria that they transport into the liver, can cause suppurative cholangitis, liver abscesses, or the syndrome of recurrent pyogenic cholangitis (Fig. 11.40).[248,463,464] Hepatolithiasis in association with ascariasis has also been described.[465] The worms can also penetrate liver tissue (visceral larva migrans), perforate the Glisson capsule, and exit into the subdiaphragmatic space.[248] Adult worms or ova may be present in tissue sections. The ova can cause granulomas and pseudotumors.[466] Bile ducts may show



• **Figure 11.39** *Clonorchis* infestation. **A,** A fluke is present within a large duct that shows squamous metaplasia and periductal chronic inflammation. **B,** Higher magnification shows cross-sections of the uterus filled with ova.



• **Figure 11.40** *Ascaris* infection in a patient with the clinical diagnosis of recurrent pyogenic cholangitis. **A,** Gross photograph of a section of liver shows dilated bile ducts inspissated with darkly stained sludge. **B,** On microscopic examination, the inspissated sludge is found to contain laminated, bile-stained structures suggestive of dead *Ascaris.* **C,** Focally, a collection of *Ascaris* ova is present in the sludge. **D,** Elsewhere in the liver, granulomatous inflammation was seen in the lobules adjacent to portal tracts.

mucosal ulceration and a mixed inflammatory infiltrate associated with embedded ova.[467] Fine-needle aspiration cytology from a liver abscess may reveal numerous fertilized, decorticated *Ascaris* eggs in a background of necrosis, inflammation, and Charcot-Leyden crystals.[468] Ultrasonography can identify worms within the gallbladder or extrahepatic ducts or the presence of a dilated common bile duct.[462] Ova or adult worms can be found in stool in 91% of patients.[462] In some patients the worms exit the ducts spontaneously; in others, antihelminthic therapy or surgery is required, particularly if abscess formation has occurred.[462-464]

Protozoal Infections of the Liver and Bile Duct

Amebiasis

Liver abscess is the most common extraintestinal manifestation of amebiasis, which is endemic in several tropical regions of India, Southeast Asia, Central America, and Africa. Amebic liver abscesses most likely arise from hematogenous spread of the trophozoites, probably via the portal circulation.[469] The classic presentation is fever, right upper quadrant pain, and hepatic tenderness.[470] Often, patients with liver abscess have no bowel symptoms, and stool microscopy is negative for *Entamoeba histolytica* trophozoites and cysts.[469] Amebic abscesses are approximately 10 times more common in men; proposed explanations for this disparity include inhibition of amebic growth by iron deficiency anemia (which is common in women in affected areas), hormonal factors, and higher rates of alcohol consumption in men. In North America, patients may present years after travel to an endemic area.[471] Compared with pyogenic liver abscesses, amebic abscesses are more likely to be solitary, are more common in men, and tend to occur in younger patients without underlying liver disease or other comorbidities.[471,472] In the United States the disease commonly affects young Hispanic men from southwestern states.

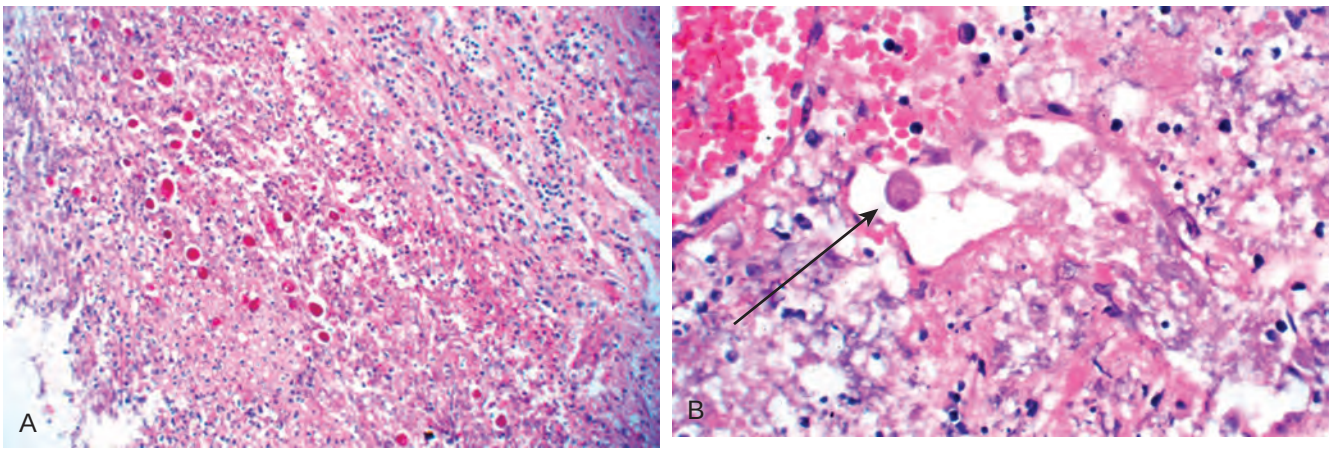
The histopathologic findings in hepatic amebiasis consist of well-circumscribed regions of dead hepatocytes, liquefied cells, and cellular debris. A rim of connective tissue with few inflammatory cells and amebic trophozoites surrounds the necrotic lesion (Fig. 11.41). The adjacent hepatic parenchyma is often completely unaffected.[469] The number of trophozoites may be surprisingly small relative to the size of the abscess. The diagnosis is often made by identification of a lesion in the liver in the setting of positive amebic serology.

Malaria

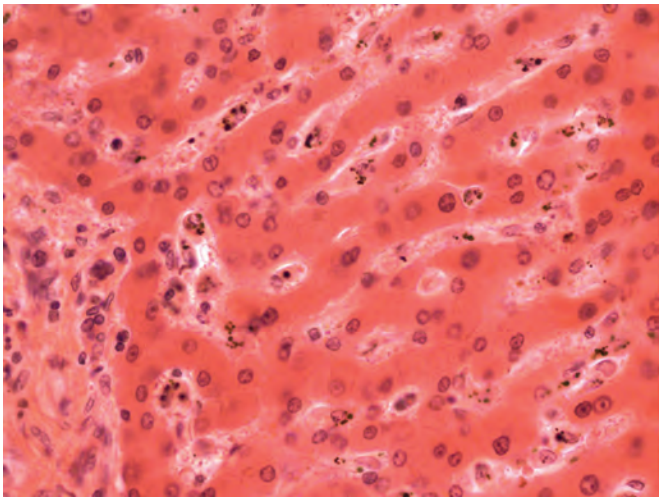
The *Plasmodium* parasite has a life cycle that involves both mosquitoes and humans. When the parasite is first transmitted to a human host, it is incapable of infecting red blood cells and must first pass through the liver. The parasite grows rapidly within hepatocytes, producing merozoites that can infect red blood cells. Through unknown mechanisms, the parasites render hepatocytes resistant to apoptotic signals.[473]

Hepatic dysfunction is well described in malaria, particularly with infection by *Plasmodium falciparum* or mixed infection with both *P. falciparum* and *Plasmodium vivax*, and usually involves mild abnormalities of liver function studies or jaundice, although in some instances there may be a more severe acute hepatitis.[474-476] Hepatic dysfunction has also been reported with *Plasmodium malariae* and *Plasmodium knowlesi*.[477,478] Hepatomegaly and abdominal pain may be seen. The combination of fever and jaundice may lead to a misdiagnosis of acute viral hepatitis. Hepatic encephalopathy is rare but does occur.[479] Hepatic dysfunction and jaundice may be multifactorial in etiology, including hemolysis, sequestration of infected red blood cells in sinusoids, or coexistent viral hepatitis.[476]

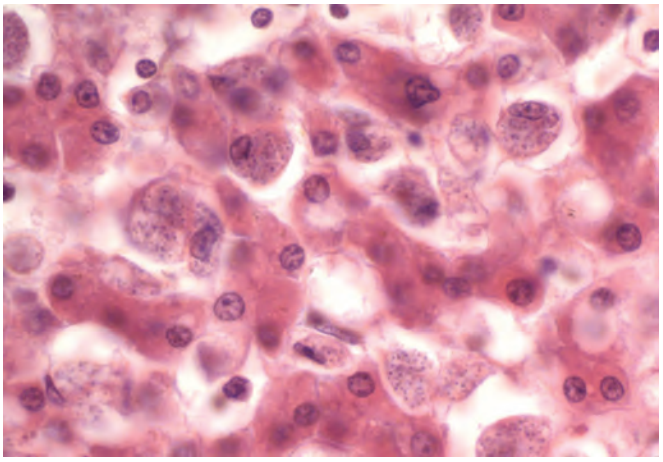
The histologic hallmarks of malarial hepatitis are Kupffer cell hyperplasia and deposition of malarial pigment or hemozoin in Kupffer cells and macrophages (Fig. 11.42).[480-483] Hemozoin pigment is coarse and birefringent when examined with polarized light. Pigment is seen throughout the lobule in parasitemic cases but is concentrated in portal areas in nonparasitemic cases, suggesting movement towards portal areas with resolution of infection.[481] The degree of inflammation and hepatocyte injury is generally mild. Sinusoidal congestion and centrilobular necrosis may be caused by adherence of red blood cells in sinusoids, resulting in ischemia. Kupffer cell activation, fatty change, and cholestasis are other common findings.[476,482] Exoerythrocytic forms of the parasite in liver biopsy material can be demonstrated by immunofluorescence.[484] In cases of fatal malaria, examination of the liver at autopsy may also show submassive coagulative-type



• **Figure 11.41** Amebic abscess in the liver. **A,** Low-power view shows the edge of an abscess cavity with inflammation and debris. **B,** High-power view of an amebic trophozoite *(arrow)* within the necrotic debris. (Courtesy Drs. David Walker and Laura Lamps.)



• **Figure 11.42** Malarial pigment within Kupffer Cells.



• **Figure 11.43** Visceral leishmaniasis (kala-azar) in the liver. In the sinusoids, Kupffer cells are filled with amastigotes.

necrosis, likely as a result of shock and hypoperfusion in the setting of multiorgan system failure.[482]

Leishmania

Visceral leishmaniasis, or kala-azar, is caused by an obligate intracellular protozoan, *Leishmania donovani*, that is transmitted to humans by the sandfly, *Phlebotomus argentipes*. Worldwide, India has the highest incidence of kala-azar. Children comprise the majority of patients. In southwest Europe, visceral leishmaniasis affects adults, many of whom are coinfected with HIV.[485] In this population, the majority of patients are intravenous drug users, and the organism is transmitted by the sharing of syringes.[485] In addition, reactivation of latent disease may occur in immunocompromised patients years after travel to endemic areas, making the infection difficult to suspect.[486] The organisms exist as flagellated promastigotes in the gut of female sandflies and as amastigotes in animal or human hosts. In visceral leishmaniasis, an incubation period of 10 days to 1 year is followed by low-grade recurrent fevers, malaise, wasting, anemia, and hepatosplenomegaly.[487] Death follows in 2 to 3 years, commonly from secondary infections.

The accumulation of mononuclear phagocytic cells in infected tissues leads to reticuloendothelial hyperplasia affecting the liver, spleen, intestinal mucosa, bone marrow, and lymph nodes.[487] In the liver, the typical pattern consists of marked hyperplasia of Kupffer cells, many of them parasitized by numerous amastigotes (Fig. 11.43).[488] Well-formed granulomas may be seen alongside Kupffer cells with intracellular amastigotes.[487,489] Other patterns include a nodular pattern, with collections of macrophages, lymphocytes, and plasma cells within the lobules and portal tracts harboring few parasites, and a fibrogenic pattern, with perisinusoidal fibrosis that isolates small groups of liver cells with regenerative changes.[488,490] The differential diagnosis includes *H. capsulatum*, *P. marneffei*, and *Toxoplasma gondii*.[486] Fibrin ring granulomas have been reported.[349,491] The diagnosis depends on demonstration of the organism in tissue, typically bone marrow smears or spleen aspirates.[487] In liver tissue, demonstration of the parasites is best done with Giemsa staining, preferably of touch preparations on glass slides.[484] The presence of an eccentrically located rodlike kinetoplast on Giemsa stain is characteristic of *Leishmania* and distinguishes this parasite from *Histoplasma* and trophozoites of *T. gondii*.[484] In addition, *Leishmania*, unlike *Histoplasma*, lacks a cell wall and is negative on PAS and silver impregnation stains. Immunohistochemical stains and serologic assays are available.

Toxoplasmosis

T. gondii is an obligate intracellular parasite usually acquired from ingestion of undercooked pork or lamb, or transplacentally. Cats are a major reservoir. Disseminated infection occurs in bone marrow and solid organ transplant recipients and in AIDS patients. Hepatitis has been reported in apparently healthy persons during acute infection; these patients present with jaundice, rash, and elevated levels of transaminases and alkaline phosphatase.[492-495]

Histologically, *Toxoplasma* hepatitis is characterized as a diffuse hepatitis with infiltration of portal tracts and sinusoids by mononuclear cells associated with focal hepatic necrosis, similar to EBV or CMV hepatitis.[492-494] Granulomatous hepatitis has also been described.[495] In some cases, bradyzoite-containing *Toxoplasma* cysts have been identified in histiocytes and granulomas or within the hepatic parenchyma.[493-496] Free tachyzoites have been described within degenerating or necrotic hepatocytes, although they can be difficult to distinguish from detritus.[492,495] Giemsa stain, PAS, immunohistochemical stains, or immunofluorescent stains may assist in the detection of organisms in tissue. Serologic assay for IgM antibodies to *T. gondii* can be used to establish the diagnosis.

Cryptosporidiosis

Cryptosporidium is a protozoan that causes an opportunistic infection of the gastrointestinal tract, resulting in diarrhea. In patients with AIDS or other immunocompromised states, including primary immunodeficiency syndromes and solid organ transplantation, the organism has been known to infect the biliary tree, producing a syndrome similar to PSC.[147,497-501] The incidence of *Cryptosporidium* biliary infection is particularly high in patients with X-linked hyper-IgM syndrome; these patients lack the CD40 receptor CD40L, which suggests an essential role of the CD40-CD40L interaction in the immune response to *Cryptosporidium*.[500-503] Signs and symptoms include right upper quadrant abdominal pain, nausea, vomiting, fever, and

biochemical evidence of anicteric cholestasis.[147] Dilatation of the common bile duct is often seen on ultrasonography or CT scanning, and cholangiography reveals attenuation and pruning of duct branches, stricturing and beading of the proximal intrahepatic bile ducts, and dilatation or stricturing of the extrahepatic duct.[147,503,504] The histologic picture reflects the intense periductal inflammatory response elicited by the organism, with portal edema, inflammation, and hyperplastic periductal glands. The biliary epithelium may be reactive or frankly necrotic. Some cases may show prominent periductal "onion-skinning" fibrosis with minimal inflammation, indistinguishable from PSC.[499] Portal fibrosis and ductular reaction may develop, and progression to biliary-type cirrhosis has been reported.[497,502] Organisms can be detected on the luminal surface of the epithelial lining of small biliary radicles, peribiliary glands, the pancreatic duct, and the gallbladder.[147,498,501] Concomitant CMV infection may be found. *Cryptosporidium*-associated sclerosing cholangitis may lead to biliary dysplasia and cholangiocarcinoma in patients with X-linked hyper-IgM syndrome.[500,502,504] Although the organism may be cleared in non-AIDS patients, leading to resolution of liver disease, the infection appears difficult or impossible to cure in AIDS patients.

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12

Infectious Lymphadenitis

JUDITH A. FERRY

Lymphadenitis of Viral or Possible Viral Etiology
- Epstein-Barr Virus/Infectious Mononucleosis
- Cytomegaloviral Lymphadenitis
- Herpes Simplex Viral Lymphadenitis
- Human Immunodeficiency Virus–Associated Lymphadenopathy
- Measles Lymphadenitis
- Human Herpesvirus 6–Associated Lymphadenitis
- Kawasaki Disease
- Kikuchi Disease

Bacterial Lymphadenitis
- Pyogenic Bacterial Lymphadenitis
- Cat-Scratch Disease
- Syphilitic Lymphadenitis
- Brucellosis
- Mycobacterial Lymphadenitis
- Whipple Disease
- Chronic Granulomatous Disease

Fungal Lymphadenitis

Protozoal Lymphadenitis
- Toxoplasmosis
- Leishmania Lymphadenitis
- *Pneumocystis jiroveci* Lymphadenitis
- Filarial Infections

Lymph nodes play an important role in reaction to infectious processes. In many cases the histologic changes are nonspecific and a particular etiology cannot be assigned; in other cases the findings are characteristic of specific entities. This chapter focuses on histologically distinctive types of infectious lymphadenitides and on their differential diagnosis.

Lymphadenitis of Viral or Possible Viral Etiology

Epstein-Barr Virus/Infectious Mononucleosis

Infectious mononucleosis is a symptomatic primary infection by Epstein-Barr virus (EBV), characterized by the classic triad of fever, pharyngitis, and cervical lymphadenopathy. Infectious mononucleosis was first described as a distinct entity in 1920, when six college students with pharyngitis, fever, and lymphadenopathy (a syndrome referred to previously as glandular fever) were all found to have an absolute lymphocytosis in the peripheral blood with mononuclear cells with unusually abundant cytoplasm. Subsequently, Paul and Bunnell found that the serum of patients with infectious mononucleosis caused sheep red blood cells to agglutinate due to the presence of what they called a heterophile antibody, providing a laboratory test for the diagnosis of infectious mononucleosis. The definition of infectious mononucleosis was further refined when a laboratory worker exposed to EBV, which had only recently been discovered and identified as being associated with endemic Burkitt lymphoma,[1] developed heterophile-positive infectious mononucleosis, establishing EBV as the causative agent.[2]

Currently the diagnosis of infectious mononucleosis is usually established clinically. When enlarged lymph nodes or tonsils are biopsied, it is often because the presentation is not typical for infectious mononucleosis and the possibility of infectious mononucleosis was not considered. On pathologic evaluation, problems in differential diagnosis are not uncommon.

Clinical Features

Infectious mononucleosis is a fairly common disease of adolescents and young adults. Cases of infectious mononucleosis in young children have been reported, although EBV infection in this age group is usually subclinical. Rare cases are also described in older adults. Males and females are affected equally. Development of symptomatic infectious mononucleosis is most likely related to a combination of socioeconomic and genetic factors.[3] Infectious mononucleosis is more commonly encountered among individuals in higher socioeconomic groups, who are less likely to be exposed to EBV during early childhood. Transmission is through close contact with another individual who is EBV infected, with an incubation period of 4 to 7 weeks.[4]

The classic findings at presentation include fever, pharyngitis, and cervical lymphadenopathy, accompanied by an atypical peripheral blood lymphocytosis and a positive heterophile antibody (Monospot) test. Lymphadenopathy is usually symmetrical and tender.[2] Both anterior and posterior triangle lymphadenopathy may be prominent, in contrast to bacterial tonsillitis, in which lymphadenopathy typically involves only the upper anterior cervical lymph nodes.[4] Splenomegaly is also common, occurring in approximately 50% of cases.[5] A maculopapular or urticarial rash

may develop, mainly among patients who are treated with a β-lactam antibiotic.[2]

Supportive therapy is usually sufficient, and spontaneous recovery after an illness of up to 4 weeks is typical, except for the small minority of patients with serious complications. In more severe cases, there may be generalized lymphadenopathy, hepatomegaly with hepatic dysfunction, splenic rupture, peripheral cytopenias, and development of a hemophagocytic lymphohistiocytosis. In most cases the illness is self-limited, but, rarely, intercurrent infection, Guillain-Barré syndrome, or splenic rupture with hemorrhage results in death. Rare patients develop chronic active EBV infection (CAEBV).[6]

Problems in establishing a diagnosis on clinical and laboratory grounds sometimes arise because the heterophile antibody test may not be positive, especially early in the illness and among children, sometimes requiring repeated Monospot tests. In some individuals, particularly those at the extremes of age, the heterophile antibody is never detected.[2] In such cases, serologic studies for EBV-associated antigens help to establish a diagnosis. Another scenario creating diagnostic difficulties is when patients have clinical features diverging from the classic presenting symptoms (e.g., presentation with diffuse lymphadenopathy without prominent pharyngitis). Patients with unusual clinical or laboratory features are more likely to undergo lymph node excision to establish a diagnosis than those patients with classic infectious mononucleosis.

Pathologic Features

The lymph nodal architecture is typically distorted but not effaced by an expanded paracortex containing a polymorphous population of lymphoid cells, including small lymphocytes, intermediate-sized lymphoid cells, immunoblasts, tingible body macrophages, and mature and immature plasma cells. The immunoblasts may occur in clusters or sheets and may be atypical, with pleomorphic or lobated nuclei. Binucleated cells resembling Reed-Sternberg cells may be identified.[7,8] Mitotic figures may be numerous. Apoptosis is common, and zonal necrosis may be present. Primary follicles or reactive follicles are often present at the periphery of the lymph node, but follicular hyperplasia is not usually a prominent feature. Sinuses are patent in at least some areas, and frequently they are dilated. Sinuses contain histiocytes and a polymorphous population of lymphoid cells similar to that found in the paracortex, including immunoblasts. Lymphoid cells sometimes infiltrate the capsule and extend into perinodal fat. Similar histologic features are found in the tonsils of patients with infectious mononucleosis (Fig. 12.1). In tonsils, crypts are usually present, although the epithelium lining them may be necrotic.[9-13] Rare patients with acute infectious mononucleosis or with a recent history of infectious mononucleosis have florid follicular hyperplasia, sometimes accompanied by monocytoid B-cell hyperplasia. EBV-infected cells may be found in follicles and the interfollicular areas in such cases.[14,15] Thus an uncommon pattern of acute or resolving infectious mononucleosis is florid follicular hyperplasia rather than a paracortical immunoblastic reaction.

The small and intermediate-sized cells in the paracortex are predominantly T cells, including many that are activated in response to the presence of the virus. The CD4 to CD8 ratio is decreased. The paracortical EBV-infected immunoblasts are B cells that are typically $CD20^+$, MUM1/$IRF4^+$, $CD10^-$, $BCL6^{-/dim}$, BCL2 variable, $OCT2^+$, $BOB1^+$. Thus the EBV-positive immunoblasts have a postgerminal center immunophenotype.[7,8] The B-immunoblasts may coexpress CD43.[15] Using immunostains or in situ hybridization for kappa and lambda immunoglobulin light chains, plasma cells are polytypic. Polytypic light chain expression can often also be demonstrated in the immunoblasts.[7] The immunoblasts often show staining for the activation antigen CD30; they are CD15 negative.[8,10,13,16-18] Using in situ hybridization, EBV-encoded RNA (EBER) can be detected in the paracortical immunoblasts and scattered smaller cells.[16]

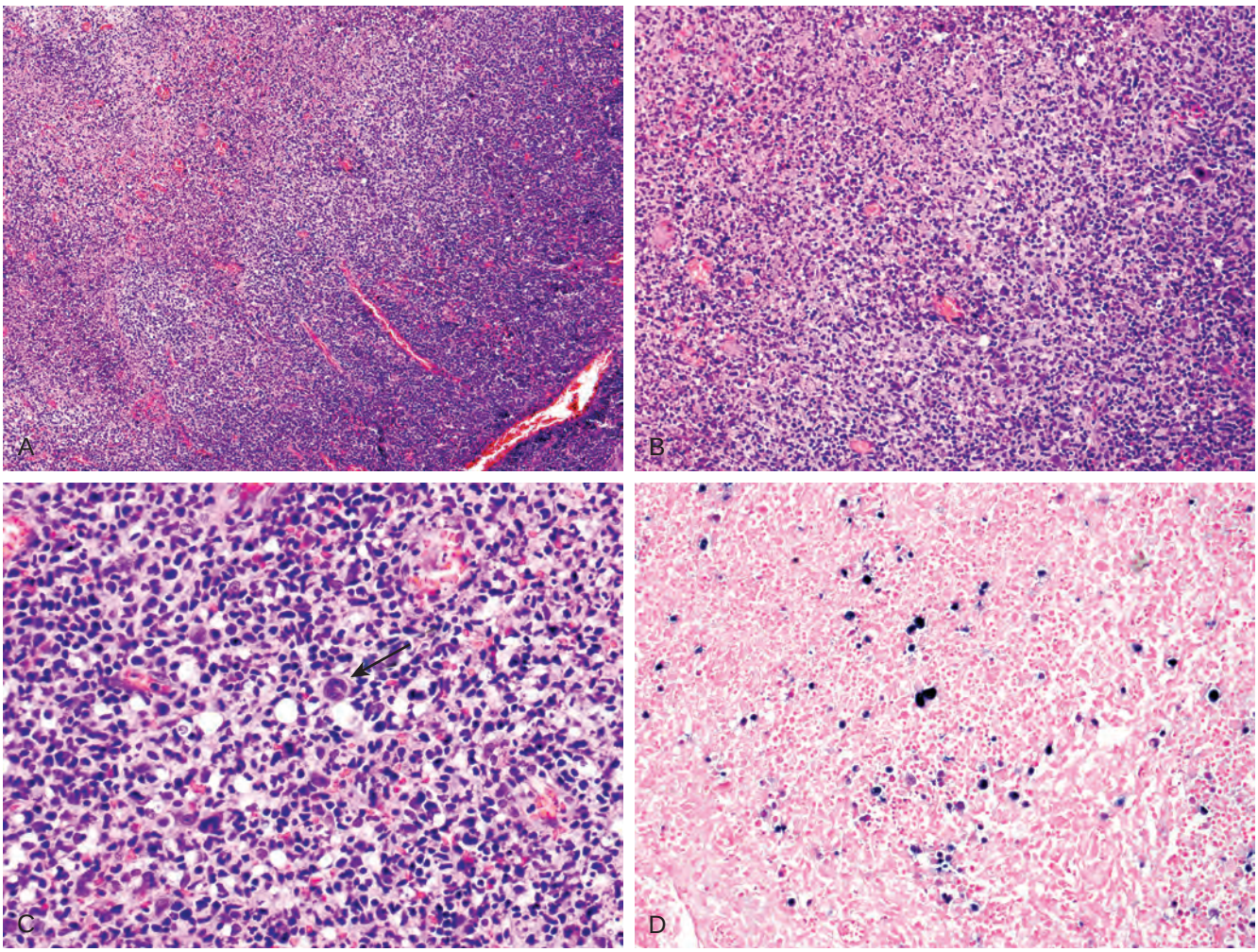
Clonal rearrangement of the TCR gamma gene has been described in association with infectious mononucleosis and in an otherwise typical case of infectious mononucleosis should not be considered to support a diagnosis of T-cell lymphoma.[19] The clonal T cells likely represent a focused immune response to EBV.

Differential Diagnosis

The differential diagnosis of infectious mononucleosis is broad. A variety of infectious processes can be associated with an illness with clinical features resembling infectious mononucleosis, including cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), herpes simplex virus type 1 (HSV-1), human immunodeficiency virus (HIV), group A β-hemolytic *Streptococcus pyogenes* (strep throat), and *Toxoplasma gondii*.[2] Careful clinical evaluation, augmented by laboratory studies, is required to establish a diagnosis.

The differential based on pathologic features includes a variety of reactive and neoplastic conditions. When immunoblasts are numerous, the possibility of diffuse large B-cell lymphoma is often a consideration. In favor of infectious mononucleosis are lack of architectural effacement, presence of areas readily recognizable as reactive hyperplasia, a polymorphous background of lymphoid cells, patent sinuses containing lymphoid cells (including immunoblasts), and lack of monotypic immunoglobulin expression on immunophenotyping.[12,13] The typical immunophenotype of the immunoblasts ($CD10^-$, $BCL6^{-/dim}$, $BCL2^{+/-}$, $MUM1^+$) may also be helpful because the majority of diffuse large B-cell lymphomas express BCL6. Diffuse large B-cell lymphomas are usually EBV-negative unless the patient is immunocompromised, so the presence of EBV should raise the possibility of infectious mononucleosis. In EBV-positive diffuse large B-cell lymphoma, EBV-positive cells will virtually all be large, whereas in infectious mononucleosis, they are of a range of sizes.[19]

Classical Hodgkin lymphoma is often included in the differential diagnosis because Reed-Sternberg–like cells are often seen in infectious mononucleosis (see Fig. 12.1C); however, most large cells in infectious mononucleosis have the appearance of immunoblasts rather than Reed-Sternberg cells or variants. Hodgkin lymphoma is more often associated with obliteration of the lymph nodal architecture. The polymorphous background of lymphoid cells ranging from small to intermediate and large in the paracortex and sinuses seen in infectious mononucleosis is very helpful in excluding Hodgkin lymphoma, in which background lymphocytes are all typically small. Granulocytes, especially eosinophils, and a background of fibrosis are more common in Hodgkin lymphoma. Immunoblasts in infectious mononucleosis are typically $CD20^+$, $CD15^-$, in contrast to the $CD20^-$, $CD15^+$ typically found Reed-Sternberg cells. However, in some cases, CD20 is absent or only weakly expressed and Pax5 may be dim or negative in infectious mononucleosis,[8] overlapping with findings in Hodgkin lymphoma, so that immunophenotype must be relied upon with caution. The CD4 to CD8 ratio is usually normal or elevated in Hodgkin lymphoma, in contrast to the CD8 predominance typically seen in infectious mononucleosis. Reed-Sternberg



• **Figure 12.1** Infectious mononucleosis, tonsil. **A,** Low power shows a dense polymorphous lymphoid proliferation with extensive necrosis *(upper left)*. **B,** Higher power shows numerous small- to medium-sized cells and scattered large cells, as well as numerous apoptotic cells *(upper left)*. **C,** High power shows a Reed-Sternberg–like trinucleate cell *(arrow)* with prominent nucleoli in a background of lymphoid cells of various sizes. **D,** Scattered small and large cells are positive for Epstein-Barr virus (EBV) (in situ hybridization using EBV-encoded RNA probe).

cells in approximately 40% of cases of classical Hodgkin lymphoma are EBV positive, so the presence of EBV does not exclude Hodgkin lymphoma. Similar to EBV-positive diffuse large B-cell lymphoma, the EBV-positive cells will all, or nearly all, be the large neoplastic cells in Hodgkin lymphoma, in contrast to infectious mononucleosis. Obtaining adequate clinical information is important to make the correct diagnosis. If the patient is known to have clinical or laboratory evidence of infectious mononucleosis, a diagnosis of Hodgkin or non-Hodgkin lymphoma should be made with caution.

Other viral infections, vaccination, and acute reaction to severe necrotizing processes can produce lymphadenopathy with histologic features similar to or indistinguishable from those of infectious mononucleosis. Reaction to certain drugs, including phenytoin and carbamazepine, can mimic the histologic features of infectious mononucleosis. Clinical information can be helpful in investigating the etiology of the lymphadenopathy. In addition, in infectious mononucleosis, EBV-positive immunoblasts are typically present in large numbers, whereas in other conditions, EBV-positive cells are usually absent.[16,20-23] The Monospot test and serologic testing for EBV-specific antibodies can be helpful in establishing a diagnosis.

Cytomegaloviral Lymphadenitis

Clinical Features

CMV is an uncommon cause of lymph nodal enlargement; lymphadenopathy may be localized or generalized.[24,25] CMV lymphadenitis may occur in the setting of an infectious mononucleosis-like illness in which patients have sore throat, fatigue, and malaise; it may also be found in patients who are otherwise asymptomatic.[2,26-28]

CMV lymphadenitis can occur in individuals of any age; males and females are equally affected. The risk of CMV lymphadenitis is increased in the setting of congenital or acquired immunodeficiency, and CMV infection is described in patients who have or have had Hodgkin lymphoma and non-Hodgkin lymphoma,[29] but CMV lymphadenitis also occurs in immunologically normal individuals. CMV infection is common in patients with common variable immunodeficiency.[25,29,30] Most patients have a self-limited

illness and require no specific therapy. Patients with an associated lymphoproliferative disorder or immunodeficiency may have a more severe course.

Pathologic Findings

On microscopic examination, involved lymph nodes most often show follicular hyperplasia and monocytoid B cell hyperplasia; this may be sufficiently florid to distort the nodal architecture. Paracortical hyperplasia may also be prominent. Infected cells contain a large eosinophilic intranuclear inclusion (mean size: 9 μm) and often also contain multiple tiny eosinophilic to amphophilic cytoplasmic inclusions.[29] Cells with inclusions are usually present focally in relatively small numbers, but occasionally they are numerous.[24,29] Neutrophils and histiocytes are often scattered around the infected cells. Inclusions are typically found among monocytoid B cells, but the infected cells are most likely histiocytes or endothelial cells rather than lymphocytes; finding inclusions in the paracortex has also been described.[25,29,31]

Immunoperoxidase stains for CMV-associated antigens can be used to confirm the diagnosis when inclusions are found on routine sections and can help to identify CMV-infected cells when inclusions are difficult to find.[24,29] Cells containing inclusions express CD15, usually with a Golgi region or diffuse cytoplasmic pattern of staining, but membrane staining is less common.[29]

Differential Diagnosis

The differential diagnosis of CMV lymphadenitis includes reactive hyperplasia due to causes other than CMV and lymphoma, including both Hodgkin and non-Hodgkin lymphoma. If the viral inclusions are overlooked, the changes may be interpreted as nonspecific reactive lymphoid hyperplasia. The histologic features of CMV lymphadenitis overlap with those of toxoplasma lymphadenitis and early cat-scratch disease in that all three are characterized by follicular and monocytoid B-cell hyperplasia. Toxoplasma lymphadenitis is characterized in addition by clusters of epithelioid histiocytes, and cat-scratch disease, even at an early stage, typically also shows small foci of necrosis with fibrin and neutrophils.

Cells with nuclear viral inclusions may resemble Reed-Sternberg cells and variants on routinely stained sections, raising the question of classical Hodgkin lymphoma. CD15 expression by the virally infected cells enhances the resemblance to Hodgkin lymphoma. However, CMV lymphadenitis is much less likely to cause obliteration of the nodal architecture, and Hodgkin lymphoma is unlikely to be found in a lymph node occupied by florid follicular hyperplasia. Cells harboring virus may contain numerous granular cytoplasmic inclusions, in contrast to the agranular cytoplasm of Reed-Sternberg cells. Membrane staining by CD15 is more common in Reed-Sternberg cells than in CMV-infected cells.[29]

Occasionally the follicular and monocytoid B-cell hyperplasia can be so florid as to distort the nodal architecture, potentially suggesting follicular lymphoma or nodal marginal zone lymphoma. Identification of CMV-infected cells, confirmed by immunostaining for CMV in a background of a follicular and monocytoid B-cell proliferation, establishes a diagnosis of CMV lymphadenitis.

Herpes Simplex Viral Lymphadenitis

Clinical Features

HSV-1 or HSV-2 infection is rarely associated with lymphadenitis. In some cases, primary infection with HSV-1 occurs in the setting of an infectious mononucleosis-like syndrome, with pharyngitis, fever, and cervical lymphadenopathy.[2] This type of lymphadenitis has been described in individuals over a wide age range. Males and females are equally affected. The risk of HSV lymphadenitis appears to be increased among immunodeficient individuals; some patients have also had a hematologic malignancy. The most common associated disorder is chronic lymphocytic leukemia,[32,33] but other B-cell lymphomas and myeloid leukemias are also occasionally associated with HSV lymphadenitis. HSV can cause localized or generalized lymphadenitis or can involve lymph nodes in the setting of widespread visceral infection. When localized, the lymphadenopathy most often affects inguinal nodes, with cervical nodes next most often affected. The enlarged lymph nodes are characteristically tender or even painful. The presence of concurrent ulcerated cutaneous or mucosal lesions provides a clue to the diagnosis, but such lesions are not always present, and, when present, they may be inconspicuous and overlooked. Rarely, HSV-1 is responsible for Parinaud oculoglandular syndrome; manifestations include conjunctivitis, periorbital swelling and tenderness, and enlarged, painful preauricular lymph nodes.[34]

Some patients have had no specific therapy and some have received acyclovir or valacyclovir. In those with an associated hematologic disorder, therapy may be directed at that rather than directly at the HSV infection. The prognosis depends on the extent of infection and on the prognosis of any underlying hematologic disorder or immunodeficiency. Disseminated HSV infection has a poor prognosis, but isolated HSV lymphadenitis is self-limited in most cases.[32,33,35-44]

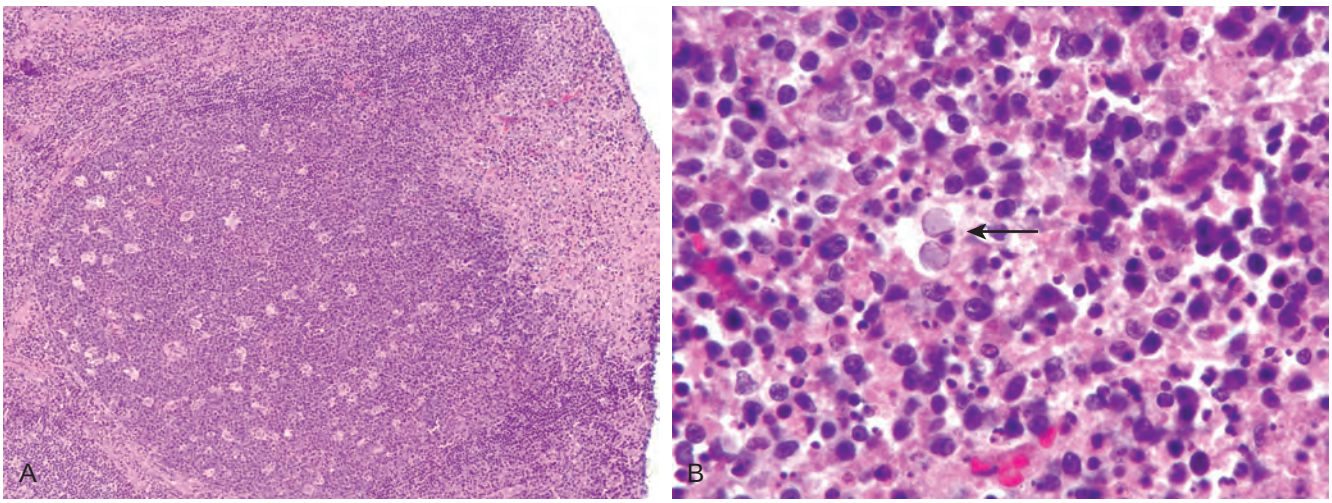
Pathologic Features

HSV infection results in a necrotizing lymphadenitis. Viable areas show prominent paracortical hyperplasia, often with numerous immunoblasts. Necrosis appears to begin in the paracortex at one pole of the lymph node and often shows prominent extension into perinodal soft tissue. The necrotic areas contain neutrophils, karyorrhectic or amorphous eosinophilic debris, and a variable number of viral inclusions, ranging from rare to abundant. Most infected cells are uninucleate, although a few multinucleated cells are seen (Fig. 12.2). This is in contrast to HSV infection of squamous epithelial cells, in which multinucleated cells with nuclear molding are characteristic. Intact neutrophils are most abundant in early lesions and may be absent in long-standing lymphadenitis. Histiocytes often surround the necrotic areas, but granulomas are not found.[35-41] Similar changes are found in the tonsils in herpetic tonsillitis.[43] Varicella zoster virus rarely causes lymphadenitis; the histologic features are similar to those of HSV lymphadenitis.[45,46]

The diagnosis can be confirmed readily using immunohistochemical stains for HSV-1– and HSV-2–associated antigens; polymerase chain reaction (PCR), in situ hybridization, electron microscopy, or viral culture can also be used.

Differential Diagnosis

The differential diagnosis includes other types of necrotizing lymphadenitis as well as lymphoma. The lack of granulomatous inflammation with epithelioid or palisading histiocytes provides evidence against infections caused by mycobacteria, fungi, yersinia, cat-scratch bacilli, and lymphogranuloma venereum.[39] Infectious mononucleosis typically shows paracortical expansion with immunoblasts and some necrosis, but EBV does not produce viral inclusions. In those cases with marked paracortical expansion with numerous immunoblasts, a high-grade lymphoma with



• **Figure 12.2** Herpes simplex lymphadenitis. **A,** A discrete focus of necrosis lies adjacent to a floridly hyperplastic lymphoid follicle. **B,** Within the necrotic focus there are small and large lymphoid cells, necrotic debris, and rare cells with nuclei replaced by homogeneous, glassy viral inclusions *(arrow)*.

necrosis may enter the differential diagnosis. In HSV lymphadenitis the nodal architecture may be distorted, although it is not obliterated, in contrast to most nodes involved by lymphoma. Viral inclusions are absent in lymphoma, except in cases in which HSV and lymphoma concurrently involve the same lymph node.[38,42,44]

Human Immunodeficiency Virus–Associated Lymphadenopathy

Reactive changes in lymph nodes directly related to HIV infection are discussed below. Lymphadenitis or lymphadenopathy in HIV-positive patients related to infectious agents other than HIV is discussed separately in those sections.

Clinical Features

Most patients are young to middle-aged adult males, but occasional women and children of either gender may also be affected. There are a number of different clinical settings in which HIV-associated lymphoid hyperplasia can be found: lymphadenopathy, occurring in the setting of an infectious mononucleosis-like illness, in association with primary infection by HIV; persistent generalized or isolated lymphadenopathy, which may be accompanied by constitutional symptoms; cystic lymphoid hyperplasia, involving periparotid lymph nodes and causing multicystic, often bilateral, masses in the area of the parotid glands; and hyperplasia of extranodal lymphoid tissue, with symptoms related to mass effect.

Upon acute infection by HIV, some patients develop an infectious mononucleosis-like illness with fever, pharyngitis, and cervical lymphadenopathy.[47] This has been referred to as the acute retroviral syndrome.[2] Persistent generalized lymphadenopathy, defined as extrainguinal lymphadenopathy persisting for at least 3 months, involving at least two noncontiguous node groups, is common among HIV-positive patients. Persistent generalized lymphadenopathy mainly affects adult males and is often accompanied by fever, weight loss, headaches, and malaise. When periparotid nodes are affected, the hyperplastic lymphoid tissue can obstruct intranodal ductal structures, resulting in the formation of cysts within the nodes. This results in multicystic, often bilateral lesions in the area of the parotid. The process has been called cystic lymphoid hyperplasia and also lymphoepithelial cysts.[48,49] When the lymphoid tissue of Waldeyer ring is affected, patients may present with a large, obstructive nasopharyngeal mass, nasal stuffiness, or nasal bleeding. The hyperplastic lymphoid tissue may obstruct the eustachian tubes and cause hearing loss.[50]

Therapy is directed against the HIV infection itself. Outcome is related to the underlying HIV infection, but with antiretroviral therapy (ART) the prognosis has significantly improved.

Pathologic Features

A range of changes is found in lymphadenopathy associated with HIV infection. Similar changes may be found in organized extranodal lymphoid tissue. In early stages of HIV infection, lymph nodes show florid follicular hyperplasia. Follicle centers are often large and irregularly shaped and have an expanded dark zone with numerous blast cells, numerous mitoses, many tingible body macrophages, and attenuated or absent follicle mantles. Follicle lysis, in which the follicle center appears disrupted and fragmented by irregularly infiltrating small lymphocytes, sometimes accompanied by tiny foci of hemorrhage, is common. The interfollicular region contains a mixture of immunoblasts, plasma cells, lymphocytes, and histiocytes. Monocytoid B cells are often prominent. Sinuses are patent and may show sinus histiocytosis, sometimes with erythrophagocytosis. Scattered epithelioid histiocytes and polykaryocytes/Warthin-Finkeldey–type giant cells may be seen.

In advanced stages of immunodeficiency, there is lymphoid depletion. Follicles are decreased in number. Those follicles present are small and may be ill-defined and difficult to identify. Follicle centers are "burnt out" or regressively transformed and contain a decreased number of B cells. The paracortex appears hypocellular. It is occupied by scattered lymphocytes and immunoblasts, often with prominent vascularity. As in early stages of infection, plasma cells are often abundant. Amorphous eosinophilic interstitial material may be present, or there may be fibrosis, and the node may have a pale, depleted appearance. In some cases, lymph nodes show changes intermediate between florid follicular hyperplasia and lymphoid depletion.[51-54]

Cutaneous rashes are common among HIV-positive patients; these individuals often have a component of dermatopathic lymphadenopathy.

In both early and late stages, B cells in follicles are polytypic. T cells show a decreased, usually reversed CD4 to CD8 ratio.[53,55] Follicles with follicle lysis show a fragmented follicular dendritic cell (FDC) network; this may be seen with antibodies to CD21 or CD23. Few to many scattered cells infected by EBV may be found using in situ hybridization for EBER, reflecting poor immunologic control of EBV-infected B cells.

The pathologic changes are not completely specific, but if an individual has lymphadenopathy with the features described previously and the patient is not known to be HIV positive, performing a test for HIV is warranted. HIV-positive patients, particularly those with histologic changes consistent with advanced immunodeficiency, are at increased risk for opportunistic infections, Kaposi sarcoma, or lymphoma, and lymph nodes should be examined carefully to be certain one of these serious complications of HIV infection is not present. If follicles show changes of the type that may be seen in Castleman disease, a stain for HHV-8/Kaposi sarcoma–associated herpesvirus (KSHV) should be performed to investigate the possibility of HHV-8–associated multicentric Castleman disease (MCD) (see Castleman disease, later).

Most patients who have been treated with ART show improvement in lymphoid architecture, as well as an increase in $CD4^+$ T cells and a decrease in $CD8^+$ T cells within lymphoid tissue. However, HIV often is detectable in FDCs in lymphoid tissue, even after a prolonged course of ART, and even in the absence of detectable virus in the peripheral blood.[56]

Measles Lymphadenitis

Measles is an infectious disease caused by a paramyxovirus of the genus *Morbillivirus*. Measles is uncommon in the United States because of widespread inoculation of children; however, it continues to be a significant health problem in multiple countries.[57] Patients typically present with fever, rash, malaise, cough, sore throat, and headache.[57] Lymphadenitis in the setting of measles occurs in a minority of cases. Lymph nodes show follicular hyperplasia; the most distinctive feature is the presence of Warthin-Finkeldey giant cells within reactive germinal centers. Warthin-Finkeldey giant cells are found in the prodromal stage of infection and generally disappear as a rash develops.

Human Herpesvirus 6–Associated Lymphadenitis

HHV-6 is the etiologic agent of a common febrile illness among young children that is known as exanthem subitum, roseola infantum, or sixth disease. Adults infected by HHV-6 may develop a heterophile-negative mononucleosis-like illness with cervical lymphadenopathy.[2] Rare cases of lymphadenitis in the setting of a severe acute febrile illness due to HHV-6 in adults have been described.[58] Lymph nodes in these systemically ill patients showed marked paracortical expansion by a polymorphous population of cells of varying sizes, including many atypical-appearing cells containing large eosinophilic nuclear and/or cytoplasmic viral inclusions. Virally infected cells appear to be $CD4^+$ T cells. The differential diagnosis in such cases may include cytomegaloviral lymphadenitis because the inclusions bear some resemblance to those of CMV. The paracortical expansion in conjunction with many immunoblasts or virally infected cells resembling immunoblasts could raise the possibility of lymphoma.[58] A small proportion of cases of lymphadenitis with the morphology of Kikuchi disease may be due to HHV-6 infection (see Kikuchi disease, later). Immunostains for HHV-6–associated antigens, electron microscopy, PCR for HHV-6, and serologic studies may help to establish a diagnosis.[2]

Kawasaki Disease

Kawasaki disease, also known as mucocutaneous lymph node syndrome and infantile polyarteritis, is a systemic vasculitis of uncertain etiology, but evidence strongly suggests that infection triggers the development of Kawasaki disease in genetically susceptible children.[59] For this reason, it is included in this chapter.

Clinical Features

Kawasaki disease is an acute febrile disease of young children, with 85% of patients under the age of 5 years. Boys are more often affected than girls, with a male to female ratio of approximately 2:1. In the United States the incidence is approximately 17 cases per 100,000 children younger than 5 years per year. The incidence is up to 20-fold higher among Northeast Asians than among whites.[59] There is also a higher risk among family members of patients with Kawasaki disease.

The Centers for Disease Control and Prevention requirements for the diagnosis of Kawasaki disease include finding fever of 5 or more days, not responding to antibiotics, and a minimum of four of the following five findings:

(1) bilateral conjunctival congestion,
(2) abnormalities of lips and oral cavity, including diffuse erythema, dry fissured lips, and prominent lingual papillae (strawberry tongue),
(3) abnormalities of the skin of the distal extremities, including erythema of palms and soles with edema early on and desquamation of fingertips later in the course of the disease,
(4) polymorphous, nonvesicular, primarily truncal, rash, and
(5) acute, nonsuppurative cervical lymphadenopathy, not due to any other identifiable cause. The lymphadenopathy is typically in the anterior cervical triangle.[59] Although cervical lymphadenopathy is most characteristic, lymphadenopathy in a variety of other sites can develop.[60]

In addition to the diagnostic criteria noted previously, patients may have cardiac abnormalities (electrocardiogram changes, cardiomegaly, murmurs), diarrhea, arthritis or arthralgia, peripheral blood abnormalities (neutrophilic leukocytosis with a leftward shift, anemia and/or thrombocytosis), elevated sedimentation rate, aseptic meningitis, mild jaundice, proteinuria, sterile pyuria, elevated transaminases, or a combination of these. Most patients recover after an illness of 3 to 4 weeks' duration, but without therapy 20% to 25% develop complications of coronary arteritis (aneurysm, thrombosis), which may be fatal, sometimes years later. If coronary artery dilatation is demonstrated on echocardiography, a diagnosis of Kawasaki disease is confirmed, even if four of the five previously mentioned criteria are not met. A subset of patients present initially with fever and cervical lymphadenopathy alone; these patients are slightly older on average than patients with more classical presentation of Kawasaki disease. They may be thought to have some form of infectious lymphadenitis, potentially leading to a delay in diagnosis and delay in instituting appropriate therapy.[61]

Kawasaki disease can often be treated successfully with intravenous immunoglobulin (IVIg) and aspirin; steroids and other antiinflammatory agents can be used if symptoms persist.

Prompt administration of IVIg reduces the risk of coronary arterial changes, and thus of serious or fatal complications of this disease.

Pathologic Features

Only a limited amount of information is available on histologic findings in lymph nodes in Kawasaki disease. Lymph nodes are reported to show paracortical expansion by lymphocytes, plasma cells, and monocytes/macrophages, as well as small to large areas of necrosis containing karyorrhectic debris, sometimes with admixed neutrophils. Some reports also describe an admixture of immunoblasts. Increased numbers of blood vessels lined by swollen endothelial cells and containing fibrin thrombi may surround the necrotic areas. The capsule is inflamed, and in some instances there is inflammation and vasculitis in perinodal soft tissue.[60] Some lymph nodes show nonspecific changes without necrosis.[60] Activation of the immune response is believed to underlie the pathogenesis of the disease; important components are proposed to be immunoglobulin A (IgA), cytotoxic T cells, and monocytes/macrophages.[62-66]

Kikuchi Disease

Kikuchi disease, also known as histiocytic necrotizing lymphadenitis, subacute necrotizing lymphadenitis, Kikuchi lymphadenitis, and Kikuchi-Fujimoto disease, is an uncommon cause of lymphadenopathy. This disease is of unknown etiology, but an infectious etiology has been implicated in the pathogenesis of at least a subset of cases of Kikuchi disease.

Clinical Features

Most patients with Kikuchi disease are between adolescence and middle age, but it is mainly a disease of young adults, with a mean and median age in the third decade. There is a female preponderance that ranges from modest to pronounced in different series. Kikuchi disease appears to be more prevalent among Asians than in Western populations.[67-74] Most patients present with unilateral, often painful, cervical lymphadenopathy. Other lymph nodes are affected much less often.[74] Infrequently, there is generalized lymphadenopathy.[73] Approximately one-half of patients have fever at presentation. Some patients have anemia or neutropenia; one-quarter of patients have an atypical lymphocytosis. Leukocytosis is uncommon. Occasionally, patients have a rash.

The minority of patients who are in the pediatric age range show a slight male preponderance, in contrast to adolescents and adults.[75] Children are also more likely to have fever and to have a rash than older patients.[75]

Kikuchi disease is typically self-limited, and most patients recover without therapy. A small number develop recurrent lymphadenopathy. Rarely, Kikuchi disease occurring in immunocompromised patients results in a severe illness. A few patients thought to have Kikuchi disease later develop systemic lupus erythematosus; this may be coincidental or the patient may have had systemic lupus erythematosus mimicking Kikuchi disease from the outset.[67-71,74]

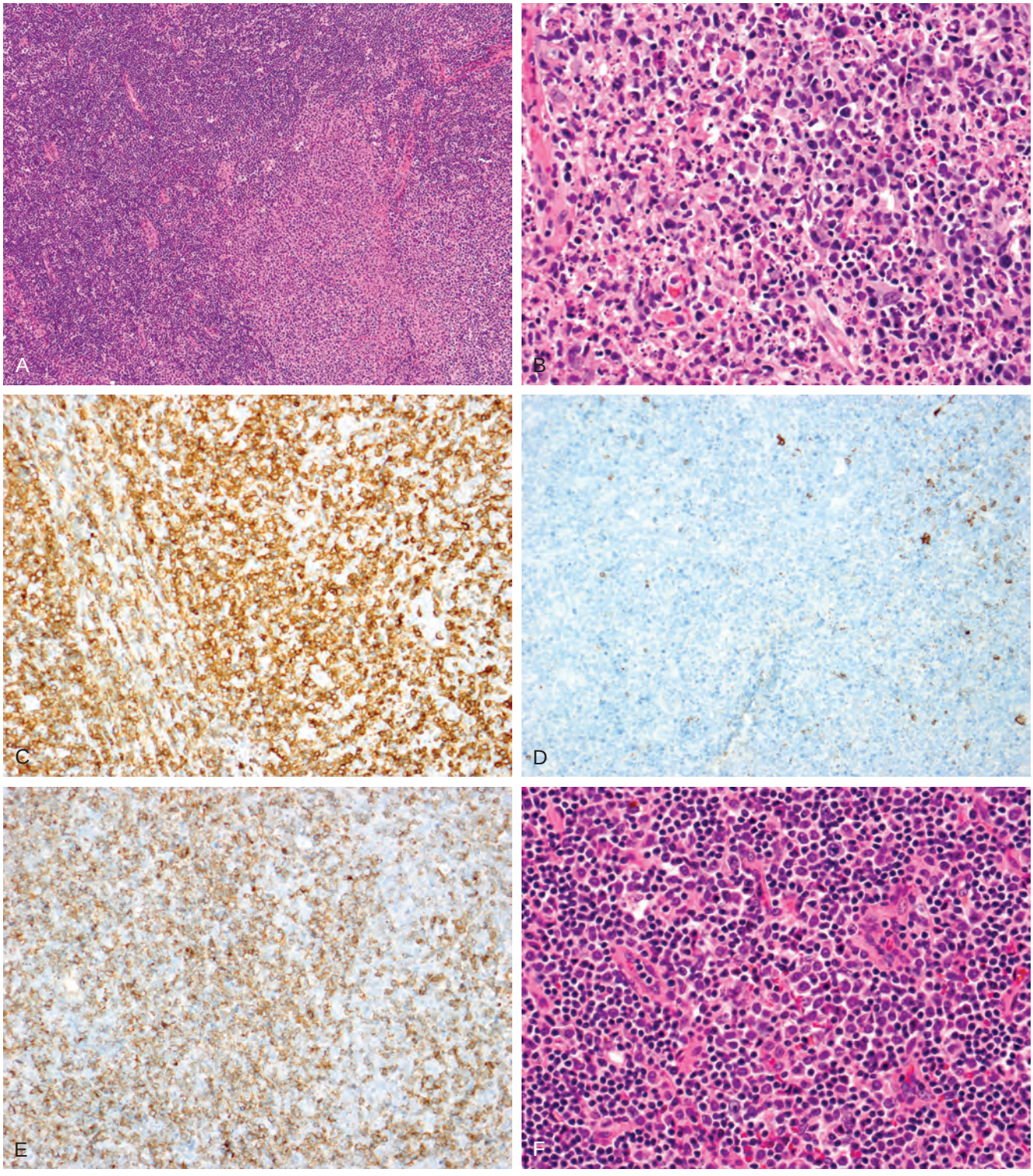
A variety of viral and other types of infectious etiologies have been suggested as potential causes of Kikuchi disease, including EBV, parvovirus B19,[76] HTLV-1,[77] HHV-6,[74] and HIV.[78] However, no one infectious agent stands out as the principle cause of Kikuchi disease, and when large series of Kikuchi disease are studied, the majority of cases have no associated specific, identifiable infectious agent.[73,74,79] The apparent evolution to systemic lupus erythematosus in a small number of cases raises the question of Kikuchi disease being an autoimmune disease that is self-limited in the vast majority of cases. Alternatively, it may be that these cases represented unrecognized cases of systemic lupus erythematosus that were misdiagnosed as Kikuchi disease. Some patients have developed lymphadenitis with features of Kikuchi disease as a reaction to foreign material or other types of noninfectious conditions. Kikuchi disease appears to be more common among individuals with certain human leukocyte antigen (HLA) class II genes.[67,68,71,80] It is possible that the histologic and immunohistologic features of Kikuchi disease can be produced by a variety of stimuli, depending on the susceptibility of the individual affected.

Pathologic Features

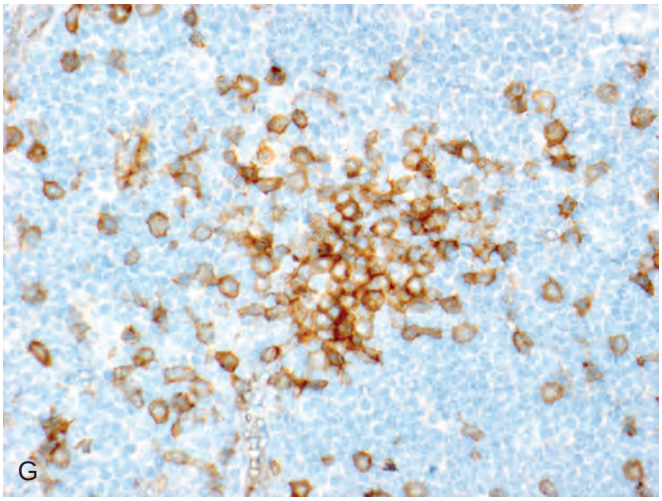
Lymph nodes show prominent paracortical hyperplasia with one or more round or irregular, discrete, or confluent eosinophilic areas in the cortex or paracortex containing histiocytes, lymphocytes, immunoblasts, plasmacytoid dendritic cells, and karyorrhectic and eosinophilic granular debris. The necrotic debris is most abundant in the centers of these areas, whereas immunoblasts are most numerous at the periphery. The cytologically bland histiocytes have oval, indented, or twisted nuclei and typically include a mixture of phagocytic and nonphagocytic forms. Vacuolated or xanthomatous histiocytes may be present. Histiocytes with eccentric sickle-shaped nuclei have been called "crescentic histiocytes." Plasmacytoid dendritic cells are medium-sized cells with round nuclei, dispersed chromatin, and a moderate amount of faintly amphophilic cytoplasm. In Kikuchi lymphadenitis, aggregates of plasmacytoid dendritic cells are frequently seen in viable paracortex; necrosis appears to begin within clusters of plasmacytoid dendritic cells. The necrosis is predominantly apoptotic. Thrombosed vessels may be seen around necrotic foci. Epithelioid histiocytes, plasma cells, eosinophils, and neutrophils are virtually absent (Fig. 12.3A, B, and F). The characteristic foci may be relatively small and confined to the paracortex, or may occupy the majority of the lymph node. The infiltrate may extend beyond the capsule into perinodal soft tissue. Reactive lymphoid follicles may be present, but they are not important as a diagnostic feature of Kikuchi disease.[67-71,73,74]

Kikuchi disease can been classified into three histologic types: proliferative, necrotizing, and xanthomatous. Proliferative and necrotizing patterns are more common than the xanthomatous pattern.[74] These may represent different stages in the evolution of the disease. In the proliferative type, microscopic examination reveals the mixture of cells described previously, with apoptosis but without coagulative necrosis. In the necrotizing type, lymph nodes show large areas of necrosis in addition to the changes characteristic of the proliferative type. In the xanthomatous type, there is a predominance of foamy histiocytes, and necrosis can be present or absent.[69-71]

Immunohistochemical analysis confirms the presence of a mixture of lymphoid cells, histiocytes, and plasmacytoid dendritic cells. The lymphoid cells, including the immunoblasts, are virtually all T cells in necrotic areas. In most cases the majority of T cells are $CD8^+$ (see Fig. 12.3C and E), often with coexpression of cytotoxic granule proteins, such as TIA-1, granzyme B, and perforin. The histiocytes are lysozyme positive and $CD68^+$, and surprisingly they are reported to coexpress myeloperoxidase.[69,71,80,81] The plasmacytoid dendritic cells express CD123 (see Fig. 12.3G) and CD68. They also express CD2, CD4, and CD43, markers that may be expressed by both T cells and monocytes, and lack



• **Figure 12.3** Kikuchi disease. **A,** Low power shows lymph node with a floridly hyperplastic paracortex *(upper left)* and an eosinophilic zone rich in histiocytes *(lower right)*. **B,** Higher power of the eosinophilic zone shows histiocytes, necrotic debris, lymphocytes, and occasional immunoblasts. **C** and **D,** The histiocyte-rich zone contains numerous T cells (**C,** $CD3^+$) and rare to absent B cells (**D,** $CD20^+$). **E,** Nearly all T cells are $CD8^+$. **F,** Plasmacytoid dendritic cells with nuclei slightly larger and paler than small lymphocytes, and with slightly more cytoplasm, form a loose cluster in the center of the field.



• **Figure 12.3, cont'd** **G,** An immunostain for CD123 highlights the plasmacytoid dendritic cells (**C, D, E, and G:** immunoperoxidase technique on paraffin sections).

T-cell specific antigens, such as CD3.[81-83] Although B cells are found if reactive follicles are present, B cells are sparse to absent in the areas of the lymph node with histiocytes and cellular debris (see Fig. 12.3D).

Differential Diagnosis

A variety of reactive and neoplastic disorders can enter the differential diagnosis of Kikuchi disease. The most important entity in the differential diagnosis is non-Hodgkin lymphoma. The distinction may be difficult when immunoblasts are abundant. Familiarity with the spectrum of changes found in Kikuchi disease, and identification of areas with the characteristic polymorphous infiltrate, are essential to rendering the correct diagnosis. In addition, Kikuchi disease more often shows partial nodal involvement than lymphoma. The vast majority of lymphomas in Western countries are B cell lymphomas. Only a minority of lymphomas in the United States are T-cell lymphomas, and most lymph node–based T-cell lymphomas are $CD4^+$. The predominance of T cells in Kikuchi disease, usually with a predominance of $CD8^+$ T cells, is an important clue in avoiding a misdiagnosis of lymphoma.

Because necrosis is such a prominent component of Kikuchi disease, infectious lymphadenitis is often a consideration. However, neutrophils, suppurative necrosis, and granulomas, features characteristic of many types of infectious lymphadenitis, are not found in Kikuchi disease. Herpes simplex lymphadenitis can resemble Kikuchi disease, particularly in older lesions with few intact neutrophils. Although histiocytes may be prominent in herpetic lymphadenitis, the histiocytic infiltrate is generally even more pronounced in Kikuchi disease, and viral inclusions are absent.

When crescentic histiocytes are prominent, metastatic carcinoma of the signet ring cell–type may enter the differential diagnosis. However, histiocytes in Kikuchi disease are cytologically bland, may contain cellular debris but do not contain mucin, and express histiocyte-related antigens rather than cytokeratin.[70] In addition, the classic clinical setting for Kikuchi disease—a young woman with isolated cervical lymphadenopathy—differs greatly from the usual clinical setting expected in a patient with signet ring cell carcinoma.

Lymphadenitis in patients with systemic lupus erythematosus can closely resemble Kikuchi disease.[69] Finding hematoxylin bodies, plasma cells, neutrophils, or deposition of nuclear material on the basement membranes of blood vessels (Azzopardi phenomenon) supports a diagnosis of lupus lymphadenitis over Kikuchi disease, but these features may not be found in every case of lupus lymphadenitis. Clinical features are sometimes helpful in making a definitive distinction. Because of the difficulty in definitively excluding lupus on microscopic examination and because a minority of patients diagnosed with Kikuchi disease have developed lupus later, clinicians may consider performing a workup for autoimmune disease in cases showing histologic features of Kikuchi disease.[71,79]

Bacterial Lymphadenitis

Pyogenic Bacterial Lymphadenitis

Lymphadenitis due to pyogenic bacteria, mainly *S. pyogenes* or *Staphylococcus aureus*, although not often biopsied, is likely the most common cause of acute unilateral cervical lymphadenopathy in children in the United States. When streptococcal lymphadenitis develops, it is often in the setting of pharyngitis. Adults may also be affected. Microscopic examination usually shows follicular hyperplasia and a variably prominent paracortical infiltrate of neutrophils.[84-87]

Cat-Scratch Disease

Clinical Features

Cat-scratch disease is among the most common causes of subacute or chronic, benign lymphadenopathy in the United States, with approximately 24,000 cases occurring per year.[86,88] For reasons that may be related to the breeding patterns of cats, it is more common between July and December than between January and June.[89-92] *Bartonella henselae* is the cause of nearly all cases of cat-scratch disease.[89,91-94] A few cases are due to *Bartonella quintana*, the louse-borne agent of the infamous trench fever of World War I,[95,96] or rarely *Bartonella alsatica*.[95] Although cat-scratch disease can affect patients of any age, most are younger than 30.[90,97-99] A history of exposure to a cat can be found in nearly all cases, if sought.[90,97] Dog exposure has rarely been implicated in cat-scratch disease.[100] *B. henselae* bacteremia is common among cats, although it is not associated with a serious illness. Kittens are more likely to be bacteremic than adult cats and thus could be more likely to transmit the disease. The infectious agent has been identified within the gastrointestinal tract of fleas; flea feces contaminating a cat scratch or bite could possibly initiate cat-scratch disease.[101] Three to 10 days after exposure, a red papule or papules develop in the skin at the inoculation site (usually the site of a cat scratch); the papules typically become vesiculated and then crusted over the next several days. Regional lymphadenopathy is usually found 1 to 2 weeks after the papule appears.[90,94]

The frequently tender lymphadenopathy usually involves only one node or one group of nodes.[90,97] Surrounding soft tissue may be edematous or inflamed.[102] Noncontiguous lymphadenopathy is occasionally found, probably because of more than one inoculation site or because the inoculation site is midline, allowing organisms to drain to lymph nodes bilaterally. The axilla is the most common site for lymphadenopathy in most series. Also commonly involved are cervical, supraclavicular, and epitrochlear lymph nodes. Femoral or inguinal nodes may be involved.[90,98,99,102,103]

In approximately 5% of cases the inoculation site is the eye, and patients may develop the oculoglandular syndrome of Parinaud (granulomatous conjunctivitis and preauricular or submandibular lymphadenopathy). Cat-scratch disease is the most common cause of this syndrome.[34] Rarely *B. quintana* rather than *B. henselae* is the cause of oculoglandular syndrome of Parinaud.[96] Up to half of patients have fever, which is usually low grade.[90,97] Patients may also experience malaise, anorexia, or, rarely, nausea or abdominal pain. The disease is usually mild and self-limited and may have resolved by the time a diagnosis is established. Some patients receive antibiotics,[102] although it is not clear that antibiotics shorten the course of the illness or are required in an immunocompetent individual without severe manifestations of *B. henselae* infection.[95,101]

Severe complications, occurring in up to 2%, include involvement of the nervous system, skeleton, lung, liver, spleen, or heart. Neurologic manifestations include seizures, encephalitis, meningitis, and involvement of cranial or peripheral nerves. Ocular disease can result in retinitis or uveitis. Splenic or hepatic abscesses may develop. The skeleton is rarely involved by a necrotizing granulomatous osteomyelitis, sometimes in the vicinity of an affected lymph node. Pulmonary involvement may take the form of pneumonia or pleural effusions.[88,94,97,102,104] Rare patients have died of complications of their infection.[98,103] In some patients with severe manifestations of cat-scratch disease, there is an underlying immunodeficiency,[102] including solid organ transplantation[103] or HIV infection. Older patients (>60 years) are infrequently affected by cat-scratch disease, but when it does occur, these individuals are slightly less likely to have lymphadenopathy and more likely to have malaise, endocarditis, encephalitis, and fever of unknown origin than younger patients. The uncommon occurrence of cat-scratch disease in older patients and its atypical clinical presentation may lead to a delay in diagnosis.[98]

Pathologic Features

The appearance of the infected lymph nodes changes over time. The earliest changes are follicular hyperplasia with a prominent proliferation of monocytoid B cells. Foci of necrosis with a few neutrophils, scant fibrin, and cellular debris appear within aggregates of monocytoid B cells. Necrotic foci are adjacent to, or encroach upon, follicle centers or are adjacent to the subcapsular sinus (Fig. 12.4). *B. henselae* is an intracellular organism with tropism for endothelial cells, and for this reason, these foci may be found in the vicinity of small blood vessels. Sinuses may contain a mixture of immunoblasts, neutrophils, and histiocytes. Over time, necrotic foci enlarge, extending deeper into the node. They contain pus, fibrin, and debris and acquire a rim of histiocytes. The foci continue to enlarge and coalesce and may become surrounded by palisading histiocytes, replacing the node with necrotizing granulomas with the classic stellate microabscesses. Nonnecrotizing granulomas and granulomas resembling those seen in fungal or mycobacterial infections may be seen. Inflammation often involves the capsule and perinodal soft tissue. The cat-scratch bacillus is a tiny, slender, pleomorphic, weakly gram-negative rod up to 3 μm long. Its very small size makes it very difficult to see, but using a silver stain, such as a Warthin-Starry stain or a Steiner stain, the bacilli are coated with the black reaction product, making them appear larger and more readily visualized (see Fig. 12.4D). Bacilli are present singly, in chains, or large clumps, along the walls of blood vessels, in macrophages in necrotic areas, or admixed with necrotic debris. Bacilli are most numerous in the early stages of inflammation within clusters of monocytoid B cells. By the time well-developed stellate abscesses have formed, the organisms can be difficult to detect (Fig. 12.5).[93,94,99,102,105] Infrequently the lymphadenitis resembles that seen in Kikuchi disease (areas of necrosis lacking neutrophils and associated with a disorganized proliferation of histiocytes) or in toxoplasma lymphadenitis, with the classic triad of follicular hyperplasia, monocytoid B-cell hyperplasia, and small clusters of epithelioid histiocytes surrounding and invading lymphoid follicles.[99]

The tiny cat-scratch bacilli may be difficult to recognize in histologic sections and are difficult to culture. For these reasons, serologic studies and molecular assays using PCR for Bartonella are often relied upon to establish a diagnosis.[86,94,99,102,106,107] Immunohistochemistry using antibodies specific for *B. henselae* are also reported to be helpful in identifying the microorganisms.[106]

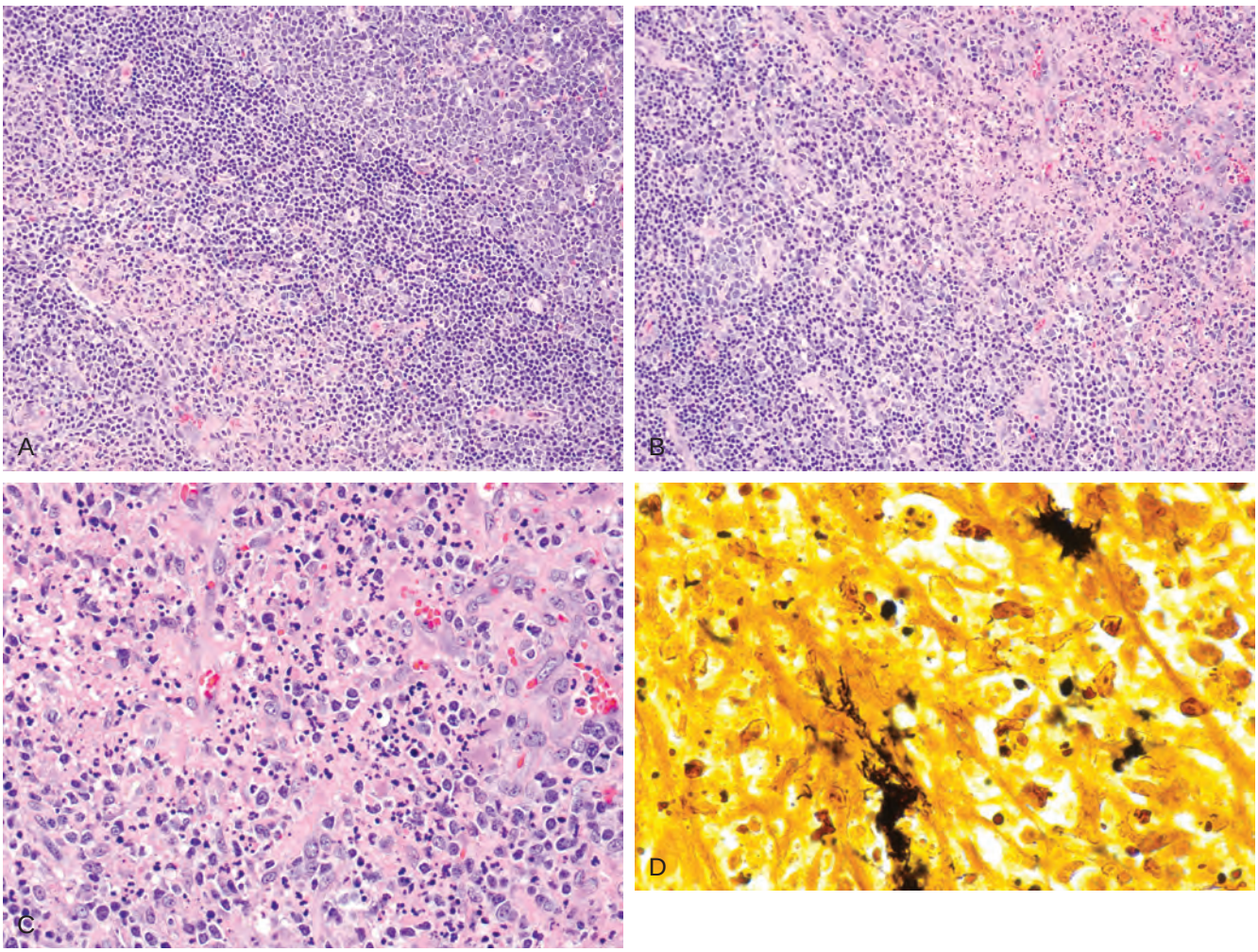
B. henselae infection in immunocompromised patients is less likely to produce a localized granulomatous lymphadenitis and is more likely to result in disseminated infection with granulomatous inflammation, bacillary angiomatosis, bacillary peliosis, or bacteremia.[94,103] Bacillary angiomatosis is the term used to describe a vasoproliferative disorder in skin, lymph nodes, and other sites, due to *Bartonella* species. When lymph nodes are involved by bacillary angiomatosis, *B. henselae* is almost always responsible, whereas *B. quintana* may cause bacillary angiomatosis in such sites as subcutaneous tissue and bone.[108] Bacillary peliosis is the term used to describe this disorder in the liver and spleen. Bacillary angiomatosis occurs mainly in HIV-positive patients. It has also been described in transplant recipients.[108] Bacillary angiomatosis in lymph nodes shows a proliferation of variably sized vascular structures lined by plump endothelial cells, sometimes without recognizable lumens. Many neutrophils, extravasated red cells, and cellular debris may be present. Microorganisms in these cases may be numerous.[103] Demonstration of microorganisms with a Warthin-Starry stain is more often successful than in immunocompetent patients. Prompt antibacterial infection therapy is required.

The florid vascular proliferation in an immunocompromised host may suggest Kaposi sarcoma, but Kaposi sarcoma typically lacks neutrophils and debris, tumor cells are HHV-8 positive, and bacteria should be absent unless there is some form of superimposed infection.

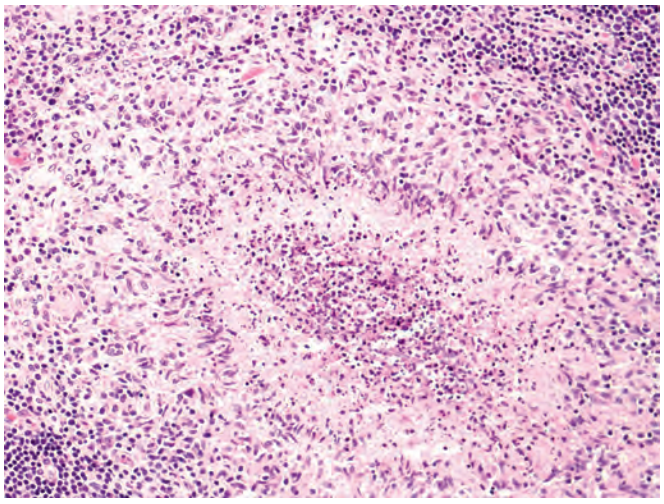
Differential Diagnosis

A variety of microorganisms cause necrotizing lymphadenitis that can mimic the lymphadenitis of cat-scratch disease. Clinical features (including age, possible exposure to various infectious agents, anatomic distribution of lymphadenopathy, and severity of symptoms), special stains on tissue sections, serologic studies, and culture are helpful in establishing a definite diagnosis. Infection by such bacteria as *Chlamydia trachomatis* (lymphogranuloma venereum), *Francisella tularensis* (tularemia),[100] *Hemophilus ducreyi* (chancroid), *Yersinia enterocolitica* (mesenteric lymphadenitis),[100] *Listeria monocytogenes* (listeriosis), *Pseudomonas mallei* (glanders), and *Pseudomonas pseudomallei* (melioidosis) can be associated with a necrotizing lymphadenitis with histologic features that may be indistinguishable from those of cat-scratch disease. These infections are all likely to be associated with significantly greater morbidity than cat-scratch disease. The florid follicular hyperplasia and monocytoid B-cell hyperplasia of early cat-scratch disease may suggest cytomegaloviral or toxoplasma lymphadenitis.

As noted previously, infection by pyogenic cocci may be associated with an acute suppurative lymphadenitis with follicular



• **Figure 12.4** Cat-Scratch disease, early stage. **A,** A hyperplastic follicle is present adjacent to a band of pale monocytoid B cells with a small amount of interstitial fibrin and scant apoptotic debris, consistent with very early involvement by cat-scratch disease. **B,** Inflammation is more advanced in this focus, which is from the same lymph node as in part A. **C,** Higher power shows an aggregate of monocytoid B cells that has been mostly replaced by neutrophils, fibrin, cellular debris, and blood vessels lined by endothelial cells with plump nuclei. **D,** A Warthin-Starry stain shows bacteria consistent with Bartonella in clumps.



• **Figure 12.5** Cat-scratch disease, late stage. This lymph node was almost entirely replaced by small and large granulomas with suppurative necrosis. Palisading histiocytes surround the central area of necrosis.

hyperplasia that can suggest cat-scratch disease except that the pyogenic cocci typically do not elicit a rim of palisading histiocytes around areas of suppurative necrosis. One exception is in cases of chronic granulomatous disease (CGD) of childhood (see later), in which palisading granulomas with suppurative necrosis may be found in association with infection due to a variety of bacteria and fungi. The appearance may resemble that of cat-scratch disease.[85,107,109]

The necrotizing granulomas of mycobacterial and fungal lymphadenitis may resemble those of cat-scratch disease. In the late stages of cat-scratch disease, the necrotic material may acquire an amorphous quality that may resemble the caseation necrosis of tuberculosis. Special stains for microorganisms, culture, and molecular genetic testing may be helpful in establishing a diagnosis.

Syphilitic Lymphadenitis

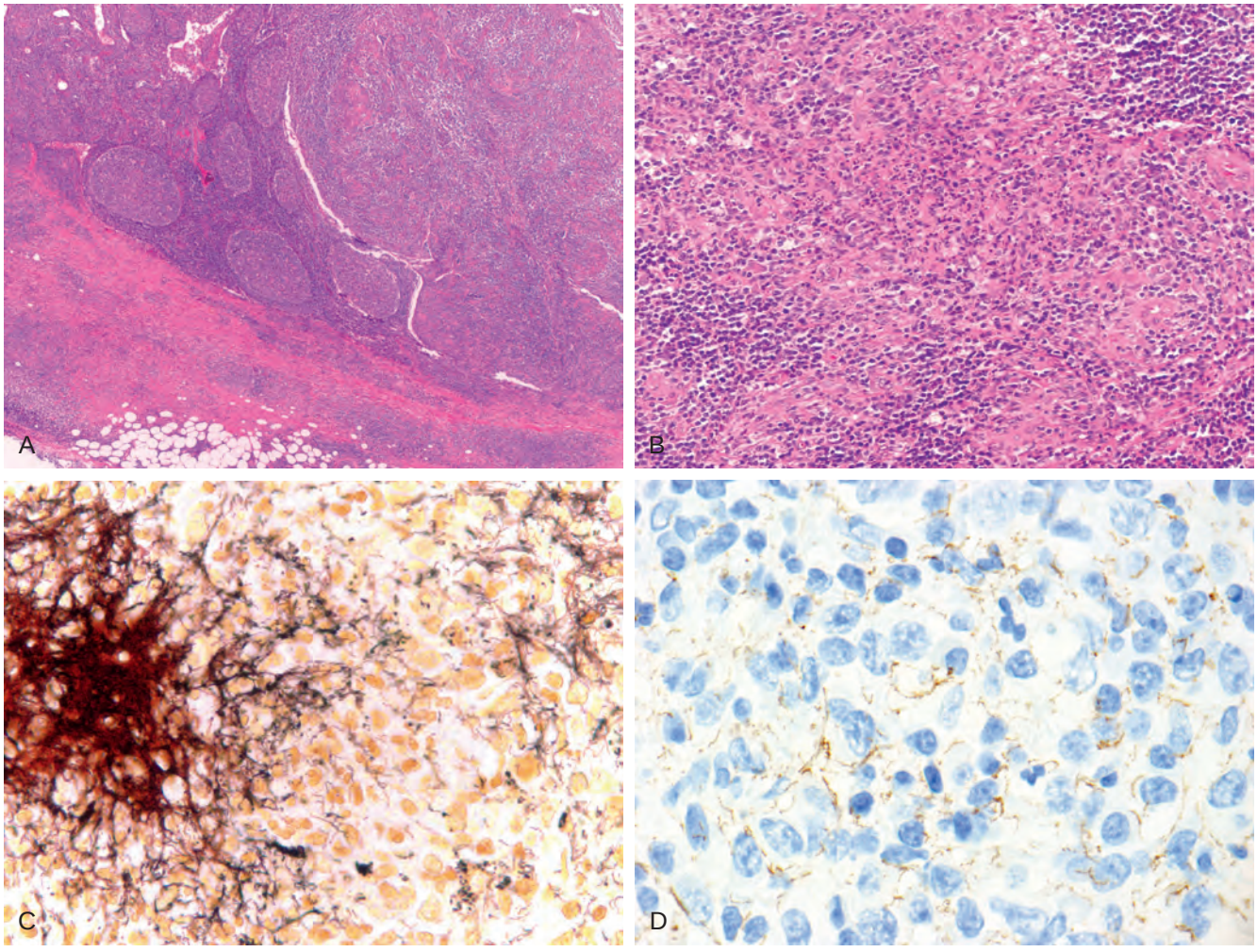
Syphilis is an infectious disease due to *Treponema pallidum*. Syphilitic lymphadenitis is uncommon but not rare. Rates of primary

and secondary syphilis have more than doubled between 2005 and 2013, most prominently among men having sex with men (MSM).[110] In primary and secondary syphilis, lymph nodes show marked follicular hyperplasia and a prominent plasmacytosis. They may also contain nonnecrotizing or suppurative granulomas and show capsular and perinodal fibrosis and endarteritis and phlebitis. Blood vessels, germinal centers, and granulomas may harbor spirochetes. The changes characteristic of syphilis are best seen in lymph nodes draining the site of the primary infection. Historically these have usually been inguinal lymph nodes (Fig. 12.6),[111-113] although cervical lymph nodes may be affected when the primary site is the oropharynx.[114,115] The tonsils may show histologic changes similar to those typically seen in the lymphadenitis; an ulcerated tonsillar chancre containing spirochetes may be present.[114] A subset of cases classified as nodal inflammatory pseudotumor in one study has been found to represent infection by *T. pallidum*. A clue to the diagnosis of syphilis in such cases is prominent follicular hyperplasia, in addition to the usual spindle cell proliferation and polymorphous inflammatory cell infiltrate of inflammatory pseudotumors.[116] Spirochetes may be detected with silver stains, such as a Steiner stain. Immunohistochemistry using antibody to *T. pallidum* is a sensitive technique for detecting microorganisms (see Fig. 12.6).[116] Although secondary syphilis may be associated with diffuse lymphadenopathy, nodes away from the inguinal region may show only nonspecific follicular hyperplasia.[111,112] Establishing a diagnosis is important to avoid spread of infection to others because infection typically is readily treated with antibiotics. In addition, among MSM, there is a high rate of coinfection with HIV and of HIV seroconversion associated with infection with syphilis.[110]

Brucellosis

Clinical Features

Brucellosis, also known as undulant fever or Malta fever, is an acute, intermittent, or chronic febrile illness due to infection by *Brucella* species. Brucella is a gram-negative facultative intracellular bacteria that infects a wide variety of domestic and wild animals.[117] Brucellosis is the most common zoonotic disease worldwide. It is endemic in certain areas, particularly in the Mediterranean region and also in the Arabian peninsula.[118] The incidence is highly variable. There are up to approximately 3.5



• **Figure 12.6** Syphilitic lymphadenitis. **A,** Low power shows a lymph node with hyperplastic follicles and fibrosis of the capsule and perinodal soft tissue. A vaguely nodular expansion of the paracortex is seen at the right of the image. **B,** Higher power of the nodular area shows increased numbers of blood vessels and focal suppurative necrosis. **C,** A Steiner stain highlights numerous spirochetes (oil immersion). **D,** Numerous microorganisms are detected with an antibody to spirochetes (oil immersion).

cases per 100,000 persons per year in some endemic areas, but brucellosis is rare in the United States. Children and adults and males and females are affected, depending on their exposure, which includes close animal contact, particularly among such individuals as shepherds or goatherds, ingestion of unpasteurized milk, or consumption of uncooked meat. *Brucella melitensis* is the most common cause of brucellosis in humans.[117-119]

The constellation of symptoms and their severity are highly variable from one case to another. Common complaints include fever, malaise, night sweats, arthralgia, myalgia, back pain, headache, abdominal pain, and gastrointestinal symptoms, such as diarrhea, nausea, and vomiting. Splenomegaly is common. Hepatomegaly and lymphadenopathy are slightly less common.[117-119] Lymphadenopathy appears to be more common in children than in adults and to be more conspicuous early in the course of the disease. Cervical lymphadenopathy is present in most children who have acquired brucellosis by oral route (consuming unpasteurized dairy products).[117] Some patients have evidence of cardiac and even central nervous system involvement. Laboratory abnormalities that may be found include anemia, leukopenia, monocytosis, and eosinophilia. The presence of splenomegaly tends to correlate with the finding of leukopenia and with fever of more than 1 week's duration.

Pathologic Features

Involved tissues show nonnecrotizing, sarcoidal granulomas; necrotizing granulomas, sometimes with suppurative necrosis; abscesses; or diffuse infiltrates of mononuclear cells. The microorganisms are gram-negative, intracellular coccobacilli.

Diagnosis can be established with blood culture, biopsy of involved lymph node (or other tissue) with microscopic examination and culture, and/or serologic studies. Brucellosis responds well to treatment with appropriate antibiotics, given for a sufficiently long course to help to prevent relapse. Proactive animal testing and treatment could decrease the incidence of disease in humans.[119-122]

Mycobacterial Lymphadenitis

Mycobacterial lymphadenitis may be found in conjunction with pulmonary tuberculosis or disseminated infection; it may also occur in isolation. The clinical picture varies widely with the type of mycobacteria and the age and immune status of the affected patients.

Tuberculous Lymphadenitis

Lymphadenitis is the most common extrapulmonary manifestation of *Mycobacterium tuberculosis*,[123,124] the most common extrathoracic site of which is the cervical area.[124-126] Tuberculous lymphadenitis is the result of lymph nodal infection by tuberculous mycobacteria, principally *M. tuberculosis* and occasionally *Mycobacterium bovis* or *Mycobacterium africanum*.[124] *M. tuberculosis* is a serious global health problem, with approximately one-third of the world's population infected by this microorganism. For example, in central Africa the most common finding among patients who undergo a superficial lymph node biopsy is tuberculous lymphadenitis; these patients were nearly all HIV positive.[127] *M. tuberculosis* was also the most common specific cause of cervical lymphadenopathy in children in a study from South Africa.[128] In a study from India, 64% of cervical lymph node biopsies showed tuberculous lymphadenitis.[124] Although tuberculosis is much less common among residents of the United States compared with those in other parts of the world, there have been increases in the number of cases in the United States due to homelessness and poor living conditions. Receiving incomplete courses of antibiotic treatment because of poor patient compliance has led to the emergence of increased numbers of drug-resistant strains.[129]

Although US patients with tuberculous lymphadenitis are typically HIV negative,[130] in Third World countries with a higher prevalence of HIV infection, patients with tuberculous lymphadenitis are commonly HIV positive,[123-125] with, in some series, the majority of patients being HIV positive.[126] Patients with lymphadenitis due to *M. tuberculosis* are usually adults who present with constitutional symptoms (fever, fatigue, and weight loss) and have associated pulmonary tuberculosis. When peripheral lymph nodes are involved, they tend to be in the supraclavicular fossa or posterior cervical triangle. Frequently, multiple lymph nodes are involved bilaterally. However, there are deviations from this classic scenario. Tuberculous lymphadenitis can also affect children[123,125] and adolescents[130] and can occur in the absence of pulmonary involvement.[123,126,130] Constitutional symptoms are not uniformly present.[126] Draining sinuses may develop. The PPD test is usually strongly positive.

Lymph nodes in immunocompetent patients show well-formed granulomas composed of epithelioid histiocytes and Langhans-type giant cells. Granulomas may be nonnecrotizing or necrotizing. Necrosis is typically caseation necrosis and only rarely suppurative necrosis.[131] Because of the risk of fistula formation with incisional biopsy, fine-needle aspiration biopsy is often used to establish a diagnosis.[124-126] A Ziehl-Neelsen stain is used to identify acid-fast bacilli; they are usually infrequent in immunocompetent patients. Among HIV-positive patients, acid-fast bacilli are more numerous, particularly in the presence of necrosis. The number of microorganisms inversely correlates with peripheral blood $CD4^+$ T-cell count.[131] PCR or culture is needed to diagnose cases in which organisms cannot be identified in tissue sections and to distinguish definitively between infections by *M. tuberculosis* and nontuberculous mycobacteria.[129]

Nontuberculous Mycobacterial Lymphadenitis

Nontuberculous mycobacterial lymphadenitis is due to infection by the "atypical" mycobacteria, most often *Mycobacterium avium-intracellulare* complex, but also *Mycobacterium scrofulaceum* or *Mycobacterium kansasii*.[124] Among immunocompetent individuals with atypical mycobacterial lymphadenitis, the majority are children who are younger than 5 years of age. Some studies report boys and girls to be affected equally; others report a higher proportion of female patients. Atypical mycobacterial infection is the most common cause of chronic lymphadenitis among previously healthy children, although it should be noted that in some developing countries, tuberculous lymphadenitis is more prevalent than atypical mycobacterial lymphadenitis. The annual incidence is estimated to be 1.2 to 2.1 cases per 100,000 persons, or slightly higher in some studies.[128,132-134]

Patients present with firm, nontender cervical lymphadenopathy that is typically unilateral. In general, constitutional symptoms are absent. Lymph nodes other than those from the cervical area are rarely affected. The mycobacteria are believed to gain access to the patient via the oropharynx, skin, or conjunctiva; involved lymph nodes correspond to those with lymphatic drainage from these sites. Over time, necrosis develops within the infected nodes, and the lymph nodes become fluctuant. Discoloration of the

overlying skin and sinus tracts may develop. The PPD test is typically negative or only weakly positive, and the chest x-ray is typically normal.

Patients have enlarged, sometimes matted lymph nodes with necrotizing and nonnecrotizing granulomas, often with Langhans-type giant cells. The necrosis may be caseous or noncaseous. Necrotic areas may contain neutrophils and nuclear debris focally or diffusely. Diagnosis can be established by complete excision of affected lymph nodes, with special stains for microorganisms and culture. Complete excision is usually also effective treatment. Biopsy or aspirate of a lymph node can be performed, but this may be complicated by draining sinuses and scarring. Ziehl-Neelsen stain typically reveals small numbers of acid-fast bacilli in some cases, particularly at the periphery of the necrotic area, but they may be very infrequent and very difficult to identify. The organisms are slow growing, such that diagnosis by culture can require a protracted period of time. In selected instances, as for infection for *M. tuberculosis*, PCR may be used to subclassify the organisms.

Antibiotics do not typically influence the course of the disease. Surgical excision of the abnormal lymph nodes is effective therapy, associated with a very low rate of recurrence. If incomplete removal of the lymph nodes is undertaken, by incision and drainage or curettage, the infection often persists. It may result in sinus tract formation and additional surgery may be required. A delay in therapy increases the chance for development of sinus tracts and scarring, leading to a permanent cosmetic defect.

Mycobacterial Lymphadenitis in Immunocompromised Patients

Disseminated nontuberculous mycobacterial infection can develop in HIV-positive patients. Lymph nodes are often involved in this setting (Table 12.1). The immune system in these patients sometimes proves unable to produce well-formed granulomas, and involved tissues may contain only loose aggregates of histiocytes containing numerous microorganisms. We have seen a remarkable case of an HIV-positive patient with splenomegaly due to concurrent infection by both atypical mycobacteria (Fig. 12.7) and pneumocystis (see later, Fig. 12.11); a case of lymphadenitis with dual infection by *M. tuberculosis* and *Pneumocystis* in an HIV-positive patient has been described.[135]

HIV-positive patients with *M. tuberculosis* or *M. avium-intracellulare* infection who initiate ART may develop "immune reconstitution inflammatory syndrome" (IRIS) in the form of an exaggerated inflammatory reaction to an infection already diagnosed and treated (paradoxical IRIS) or to an unrecognized, untreated infection (unmasking IRIS).[136] The most common manifestations are new or worsening lymphadenopathy, fever, and lung infiltrates.[136] Other types of opportunistic infections may also result in a similar phenomenon.[137]

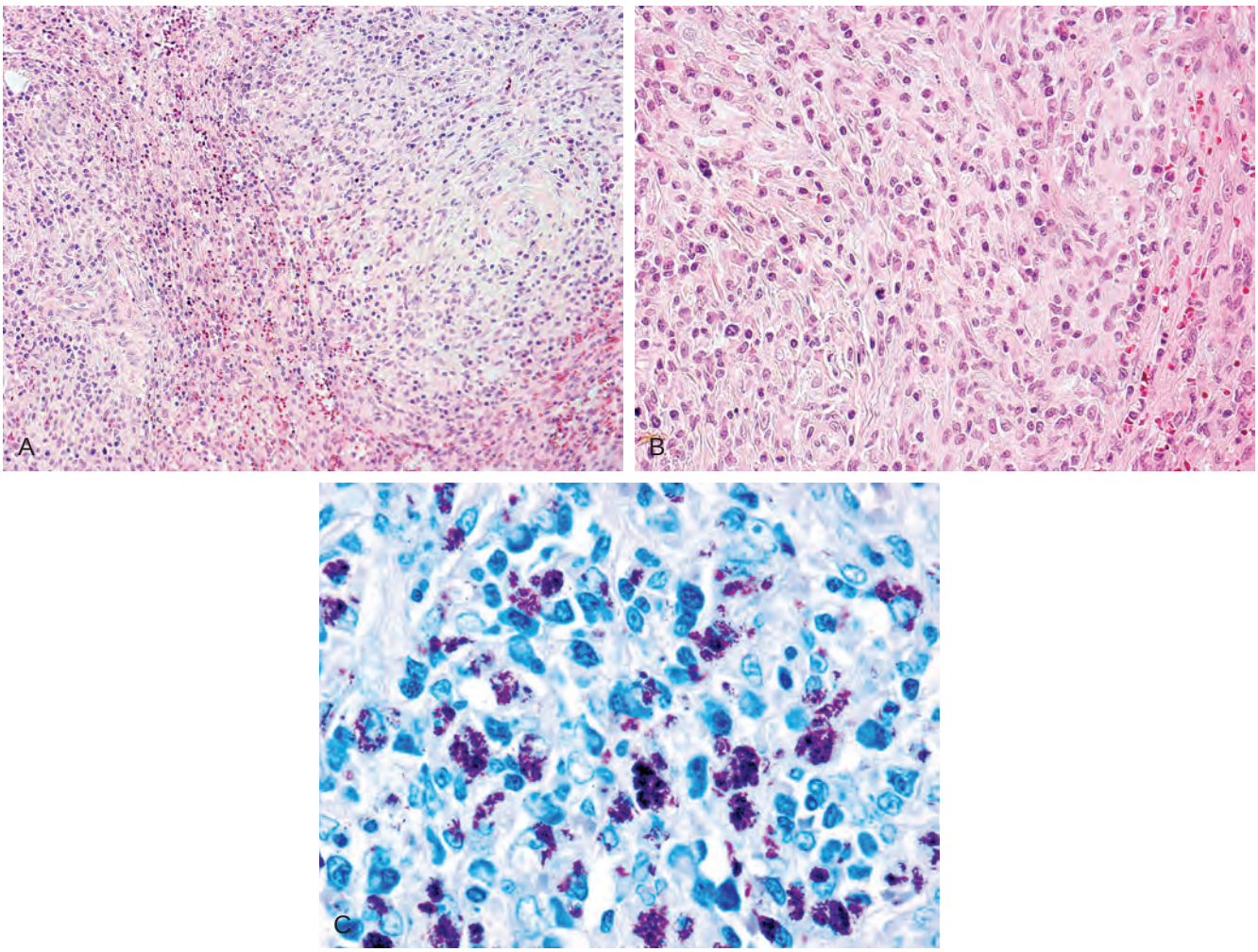
Differential Diagnosis

The differential diagnosis of mycobacterial lymphadenitis is broad; it includes a variety of infectious disorders, as well as some neoplastic processes. A wide variety of microorganisms, including fungi, *Bartonella* species, *Brucella* species, syphilis, and leishmania,[138] can be associated with granulomatous lymphadenitis. Caseation necrosis is more common in mycobacterial infections, although necrosis resembling caseation may be seen in fungal infections and in late stages of suppurative granulomatous infection, such as cat-scratch disease. In syphilitic lymphadenitis, there may be nonnecrotizing or suppurative granulomas, but they are found in the setting of marked follicular hyperplasia and plasmacytosis. Uncommon entities that may enter the differential diagnosis include CGD of childhood and leprosy, particularly the tuberculoid form.

Noninfectious granulomatous diseases may be considered in the differential diagnosis of mycobacterial lymphadenitis. Cases with little or no necrosis may mimic sarcoidosis. A Ziehl-Neelsen stain and culture are helpful in establishing a diagnosis; however, the Ziehl-Neelsen stain is often negative in mycobacterial infections lacking necrosis. Wegener granulomatosis can infrequently cause necrotizing lymphadenitis with granulomatous vasculitis and could potentially enter the differential of mycobacterial lymphadenitis.[139,140] Results of stains for microorganisms, culture, and clinical information can be helpful in such cases.

TABLE 12.1 Tuberculous Lymphadenitis Versus Nontuberculous Mycobacterial Lymphadenitis in Immunocompetent Patients

	Tuberculous Mycobacterial Lymphadenitis	Nontuberculous Mycobacterial Lymphadenitis
Definition	Lymphadenitis due to tuberculous mycobacteria (e.g., *Mycobacterium tuberculosis*, *Mycobacterium bovis*)	Lymphadenitis due to atypical mycobacteria (e.g., *Mycobacterium avium-intracellulare*)
Risk factors	Poor living conditions	None known
Age	Any age	Mostly young children
Gender	Males = females	Boys and girls affected
Constitutional symptoms	Often present	Uncommon; mild if present
Sites	Cervical nodes, mediastinal nodes, lungs	Cervical nodes, unilateral, most often submandibular, jugulodigastric or preauricular lymph nodes
Chest x-ray	Frequently abnormal	Negative, almost always
Communicable	Yes	No
PPD	Positive	Negative or weakly positive
Preferred treatment	Full course of appropriate antibiotics	Complete excision



• **Figure 12.7** *Mycobacterium avium-intracellulare* infection in the spleen of an HIV-positive patient. This patient had concurrent splenic involvement by *Pneumocystis jiroveci* (see Fig. 12.11). **A,** The markedly enlarged spleen contained multiple loose aggregates of histiocytes consistent with poorly formed granulomas. **B,** Plasma cells are scattered among the histiocytes. **C,** A stain for acid-fast bacilli shows histiocytes containing numerous microorganisms.

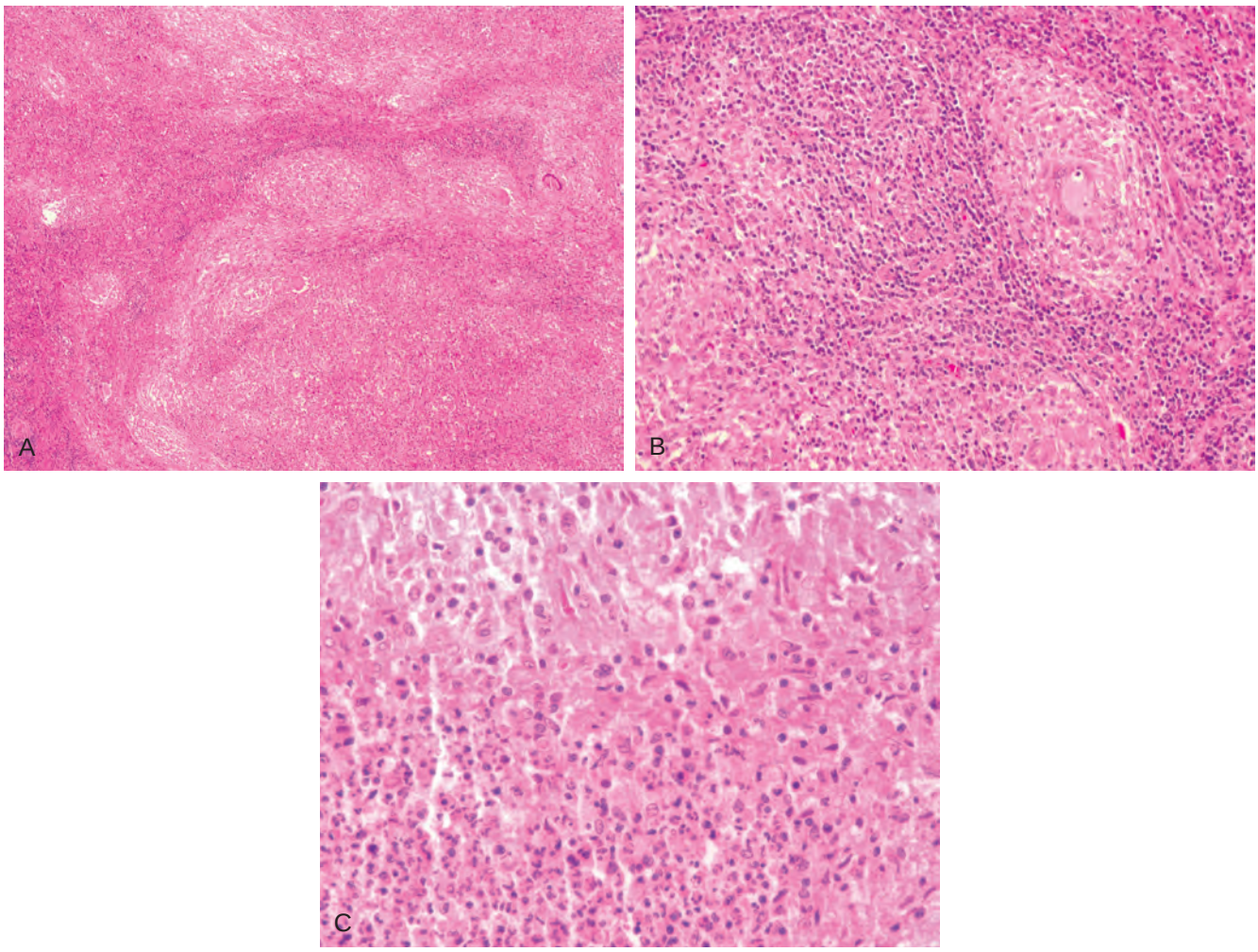
Classical Hodgkin lymphoma is often associated with necrosis and granulomatous inflammation. Identification of Reed-Sternberg cells and variants, typically found in greatest numbers at the periphery of necrotic areas, confirms the diagnosis of Hodgkin lymphoma. The presence of a polymorphous inflammatory cell infiltrate, with eosinophils and plasma cells, is more common in Hodgkin lymphoma than in mycobacterial infection. Non-Hodgkin lymphomas are also occasionally associated with granulomas, but this is less common.

Metastatic carcinoma is rarely associated with granuloma formation in lymph nodes. The granulomas may draw the pathologist's attention away from a neoplastic infiltrate if it is subtle, and the carcinoma may potentially be overlooked.

Bacille Calmette-Guérin Lymphadenitis

Clinical Features. Bacille Calmette-Guérin (BCG) vaccine is derived from a live, attenuated strain of *M. bovis*. It is administered, typically at birth, in some countries with a high prevalence of tuberculosis to decrease the risk of developing tuberculosis.[141,142] The risk of complications is related to the immune status of the patient and the BCG strain used.[141,143] BCG lymphadenitis, sometimes with abscess formation, is the most frequent complication of BCG vaccination. BCG lymphadenitis is defined as ipsilateral regional lymphadenopathy (typically axillary) occurring after BCG vaccination; it develops in approximately 0.1% to 1% of vaccinated patients.[143] Boys are affected more often than girls.[141-143] Immunocompetent individuals who develop lymphadenopathy usually do so within 6 months of vaccination. Lymphadenopathy is usually nontender. Other infrequent complications of BCG vaccination include inoculation site abscesses and inflammation involving bones, joints, and other sites. Immunodeficient individuals with such disorders as severe combined immunodeficiency, CGD, or HIV infection may develop severe BCG infection, manifest by generalized lymphadenopathy and disseminated BCG infection. BCG vaccination is thus contraindicated in immunosuppressed patients.[142,143]

Nonsuppurative lymphadenitis usually resolves spontaneously. The suppurative form of lymphadenitis is accompanied by erythema and edema of overlying skin and sometimes by ulceration and sinus tract formation. Incision and drainage of suppurative lymphadenitis can expedite healing and help to prevent development of sinus tracts. Immunodeficient individuals urgently require



• **Figure 12.8** Bacille Calmette-Guérin lymphadenitis in a 3-year-old child. **A,** Large and small granulomas replace most of the lymph node. **B,** Higher power shows well-formed granulomas with occasional Langhans-type giant cells. **C,** Some granulomas show suppurative necrosis.

prompt diagnosis and treatment. For such patients, this serious complication of vaccination may be fatal and requires antituberculous therapy, as well as treatment of the immunodeficiency.

Pathologic Features. Lymphadenitis comes in suppurative and nonsuppurative forms. Microscopic examination reveals epithelioid granulomas with or without necrosis. When necrosis is present, it is usually suppurative rather than caseous (Fig. 12.8). Acid-fast bacilli are usually present in small numbers. Among immunodeficient patients, depending on the severity of the immunodeficiency, there may be epithelioid granulomas with few acid-fast bacilli or diffuse infiltrates of histiocytes laden with numerous acid-fast bacilli.[84,144-149]

Whipple Disease

Clinical Features

Understanding Whipple disease took a major step forward when the causative infectious agent, actinobacterium *Tropheryma whippelii*, was identified.[150] In the older literature, Whipple disease was known as intestinal lipodystrophy.[151] It is a rare disorder with an estimated annual incidence of less than 1 per million persons. Whipple disease predominantly affects white men, with a male to female ratio ranging from 5:1 to 8:1. Patients can be affected from childhood to old age, but most patients are in the fourth to sixth decade of life, and the mean age at diagnosis is approximately 50 years. Transmission from one person to another has not been described.

The constellation of symptoms and signs related to Whipple disease is variable from one patient to another. The classic findings are diarrhea, weight loss, abdominal pain, and arthralgia, but anemia, cutaneous hyperpigmentation, and fever all occur in 50% or more of patients. Some patients have intermittent low-grade fever. Lymphadenopathy is found in approximately half of cases. Peripheral lymphadenopathy is common; intra-abdominal and mediastinal nodes may also be enlarged. The disease can also affect the heart and lungs, with inflammation or fibrosis of endocardium, myocardium, or pericardium, thickened, distorted cardiac valves, chronic cough, and/or pleuritic pain. Whipple disease can produce a wide variety of manifestations among patients with central nervous system involvement, including cognitive changes, motor and sensory deficits, altered consciousness, and/or hypothalamic dysfunction. Ocular involvement, almost always found in patients who also have central nervous system disease, can result in retinitis or uveitis and loss of vision. Central nervous system involvement is identified in up to approximately 50% of patients. Although symptoms related to gastrointestinal involvement are

generally the most prominent, some patients have presented with lymphadenopathy without diarrhea or malabsorption; in some of these unusual cases the diagnosis was delayed by years or was made only at autopsy.[152,153]

Whipple disease is a chronic, slowly progressive disease; untreated, it may prove fatal. It usually responds to antibiotics, but the duration must be long to avoid relapse. The antibiotic should cross the blood–brain barrier to treat any central nervous system disease and to prevent relapse in the central nervous system, which is associated with a high mortality rate.

Pathologic Features

Diagnosis is most readily established on a small intestinal biopsy, but occasionally the first tissue submitted for pathologic examination is a lymph node. In lymph nodes the distinctive finding is the presence of dilated sinuses distended by cystic-appearing, lipid-filled spaces surrounded by foreign body giant cells and lipogranulomas, with clusters of foamy and epithelioid histiocytes. The histiocytes have granular, periodic acid–Schiff (PAS)-positive, diastase-resistant cytoplasm. However, in some cases, especially when peripheral lymph nodes have been examined, nonnecrotizing granulomas, similar to those seen in sarcoidosis, are identified. In such cases the PAS stain may be negative or only focally positive, and bacilli may be rare or absent.[151,154] The high lipid content of the lymph nodes make them somewhat distinctive radiographically.[154]

Electron microscopy reveals that the PAS-positive histiocytes are filled with intact and degenerated bacilli. The Whipple bacilli are bacteria with an unusual trilaminar membrane and a cell wall similar to that of gram-positive bacteria.

PCR has been used on fluids, such as cerebrospinal fluid and blood, and on biopsy specimens to detect the presence of Whipple bacilli. However, *T. whippelii* DNA has reportedly been found by PCR in healthy individuals, so PCR should not be used in isolation to establish a diagnosis of Whipple disease. PCR is useful in confirming a diagnosis in patients with clinical and/or pathologic features suspicious for Whipple disease. PCR may also play a role in monitoring the efficacy of therapy.

Differential Diagnosis

Based on pathologic features, Whipple disease has a broad differential diagnosis. When lymph nodes have sinus histiocytosis with lipogranulomas, the appearance closely resembles the effect of a diagnostic lymphangiogram; a PAS stain can exclude this possibility. Diagnostic lymphangiograms are no longer a standard test, so the possibility of this problem arising is unlikely.

Sarcoidosis, Crohn disease, and mycobacterial infections are included in the differential when nonnecrotizing granulomas are found. Special stains for acid-fast bacilli and culture are useful in excluding a mycobacterial infection. Intestinal biopsies are helpful in distinguishing Crohn disease from Whipple disease. A PAS stain and electron microscopy may be helpful in differentiating between Whipple disease and sarcoidosis, but in some lymph node biopsies in Whipple disease, particularly peripheral lymph nodes, the appearance may be similar to that of sarcoidosis. *M. avium-intracellulare* infection in immunodeficient patients and lepromatous leprosy can both be associated with prominent infiltrates of histiocytes with finely granular or vacuolated cytoplasm containing numerous microorganisms and can enter the differential diagnosis of Whipple disease. Special stains for microorganisms and correlation with clinical features establish a diagnosis.

If clinical features suggest the possibility of Whipple disease, biopsy of the proximal small bowel should be considered to establish a diagnosis.[150,151,153,155-160]

Chronic Granulomatous Disease

Clinical Features

CGD is a genetically determined, heterogeneous disorder characterized by leukocytes with impaired intracellular killing of microorganisms after phagocytosis, leading to recurrent, severe infections by bacteria and less often by fungi. Phagocytes are able to ingest microbes normally, but microbicidal activity is diminished because of abnormal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme complex found in granulocytes, monocytes, and macrophages that normally generates the superoxide ions and hydrogen peroxide that are toxic to microbes. NADPH oxidase has membrane-bound components (gp91-phox, p22-phox, and rap1a, together composing cytochrome b_{558}) and also cytosolic components (p47-phox, p40-phox, p67-phox, Rac2). Defects in the gp91-phox of cytochrome b_{558} account for most cases; these cases have X-linked inheritance. A defect with autosomal recessive inheritance accounts for the remaining cases. Among autosomal recessive defects, an abnormal p47-phox is most common; abnormal p67-phox and abnormal p22-phox each account for a small proportion of cases. CGD is usually inherited but may occur as the result of a germ-line mutation. More than 400 mutations giving rise to CGD have been described. Approximately 95% result in complete or partial loss of protein, whereas 5% are associated with normal levels of abnormal protein.[161] The severity of the disease is dependent on the underlying genetic defect: X-linked inheritance with absence of cytochrome b_{558} expression is associated with more severe disease than other defects. CGD accounts for fewer than 10% of primary immunodeficiencies, but it is the most common inherited disorder of phagocytic dysfunction. Its prevalence is approximately 1 in 1.2 million individuals, with an incidence of approximately 1 in 250,000 live births. In those with X-linked GCD the age of onset of symptoms is often less than 1 year. Less severely affected patients may present later in childhood.[162] Boys are more often affected than girls because the most common form of CGD has X-linked transmission. There is a male to female ratio of 2:1 to 4:1 overall. Consanguinity is a risk factor for developing this inherited disease.

CGD causes recurrent suppurative infections, with lymphadenitis, pulmonary infection, upper respiratory infection, cutaneous abscesses, gastrointestinal infections, hepatic abscesses, sepsis, and/or osteomyelitis, due *S. aureus*, *Klebsiella*, *Salmonella*, *Burkholderia*, *M. tuberculosis*, *Nocardia*, *Aspergillus*, or *Candida*.

Patients also have certain noninfectious manifestations, such as poor wound healing, overly exuberant inflammatory reactions, and autoimmune-type phenomena, including inflammatory bowel disease–like symptoms and lupus-like manifestations. Prophylactic use of antibiotics can forestall a significant proportion of bacterial infections; prophylaxis for fungal infections and interferon-γ are administered to some patients. Bone marrow transplantation and gene therapy may eventually have a role in therapy. The most common cause of death is infection by aspergillus. Mortality is an estimated 2% to 5% per year.

Pathologic Features

Lymphadenopathy is common in CGD. Microscopic examination of enlarged lymph nodes reveals nonnecrotizing or

necrotizing granulomas with suppurative necrosis and occasional acute lymphadenitis.[161] Diagnosis can be established using flow cytometry, which has now largely replaced the traditional nitroblue tetrazolium reduction test. The dihydrorhodamine-1,2,3 (DHR) assay is a rapid and sensitive assay for the detection of CGD by flow cytometry; it can also detect carriers of X-linked CGD.[162] When the specific genetic defect is known, prenatal diagnosis can be performed.

Differential Diagnosis

Based on morphology in an involved lymph node, the differential diagnosis includes a variety of other types of necrotizing lymphadenitis, including Bartonella infection, atypical mycobacterial lymphadenitis, brucellosis, and others. The lack of specificity of the pathologic findings and the rarity of the disease often result in delay, sometimes for years, in establishing a diagnosis. CGD is distinctive in that there are recurrent, often severe, pyogenic infections usually beginning in the first year of life and almost always present by the age of 2 years.[161-167]

Fungal Lymphadenitis

Fungal lymphadenitis is uncommon. Patients with fungal lymphadenitis are often immunosuppressed because of HIV infection, malignancy (most often, hematolymphoid neoplasia), or iatrogenic immunosuppression. Immunologically normal individuals are occasionally affected. *Histoplasma capsulatum* and *Cryptococcus neoformans* are the most common fungi to cause lymphadenitis. Symptomatic fungal lymphadenitis is usually not an isolated finding; patients often also have pulmonary involvement or disseminated disease. Histoplasmosis involving mediastinal nodes can be associated with inflammation and severe scarring of the surrounding tissue, producing sclerosing mediastinitis. During institution of ART in HIV-positive patients harboring fungi, symptomatic fungal lymphadenitis may emerge as a patient becomes capable of mounting an immune response to the fungi (IRIS).[168,169]

C. neoformans most often gains access to the host when it is inhaled from pigeon droppings,[170] resulting in most cases in a mild or asymptomatic infection, which can become latent. In a small minority of infections, mainly in the setting of immunodeficiency, especially due to acquired immune deficiency syndrome (AIDS), cryptococcus causes a symptomatic infection involving lungs, central nervous system, skin, lymph nodes, and/or other sites. Lymph nodes in a variety of anatomic sites can be infected. In immunocompetent hosts, cryptococcus is associated with the formation of compact granulomas without suppurative or caseous necrosis. Over time the granulomas become associated with fibrosis but not with calcification. In immunodeficient and some immunocompetent patients, inflammatory response may be minimal; instead there may be clusters of encapsulated yeast in a mucoid background.[170]

H. capsulatum is endemic to the Ohio and Mississippi River valleys. It infects humans when spores from bird or bat droppings are inhaled. In immunocompetent patients, histoplasma typically causes an asymptomatic infection which may be disseminated throughout the reticuloendothelial system and become latent. Immunodeficient patients may develop a severe infection with sepsis and respiratory compromise.[171] Lymph nodes show granulomatous inflammation; in inactive disease, small numbers of yeast may be seen in a fibrotic, often calcified background (Fig. 12.9). Granulomas may be poorly formed or absent in immunosuppressed patients. The differential diagnosis is similar to that of *M. tuberculosis* and nontuberculous mycobacteria.[51,172-175]

Protozoal Lymphadenitis

Toxoplasmosis

Clinical Features

Although lymphadenitis due to other types of protozoal infection has been reported,[138] infection by *T. gondii* is the only one encountered with any frequency in the United States. Toxoplasmosis is a relatively common infectious disease due to *T. gondii*.[176] Transmission occurs via contact with cat feces containing toxoplasma oocysts or by ingestion of undercooked meat containing toxoplasma cysts.[2] Most patients are children and young adults; toxoplasma lymphadenitis is more common among females than males.

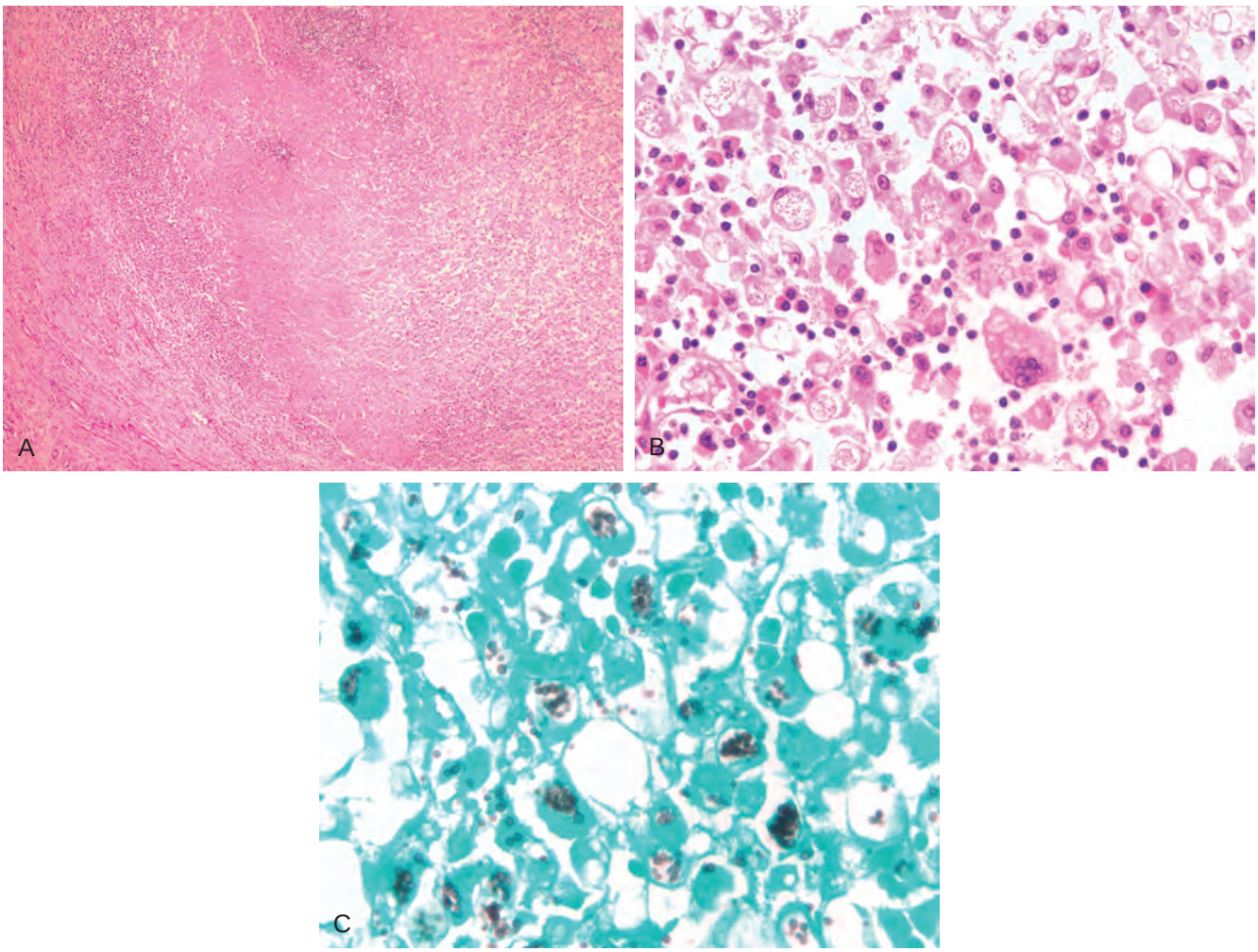
Toxoplasmosis may cause encephalitis, chorioretinitis, disseminated disease, and congenital infection, as well as lymphadenitis,[177] but lymphadenopathy is by far the most common manifestation in immunocompetent individuals.[178] Immunodeficient patients are at risk for the more severe manifestations of toxoplasmosis. Among the various clinical manifestations of toxoplasmosis, toxoplasma lymphadenitis is the most common. Patients with toxoplasma lymphadenitis typically have unilateral or bilateral cervical lymphadenopathy, especially in the posterior cervical area. They may have malaise or be asymptomatic.[179] Some patients have an atypical lymphocytosis. This type of lymphadenopathy was first described by Piringer-Kuchinka in 1952[177] and is thus sometimes called Piringer-Kuchinka lymphadenitis.

Patients with toxoplasma lymphadenitis typically have a self-limited illness and need no specific therapy. Immunodeficient patients may develop lymphadenopathy but are at high risk for severe manifestations, especially encephalitis.[178] Infection during pregnancy may be associated with fetal morbidity or mortality. Rarely, immunocompetent patients develop serious complications, such as myocarditis or encephalitis, which may be fatal. Adults with more severe manifestations may respond to drug therapy; congenital infection may cause irreversible damage.

Pathologic Features

Lymph nodes are slightly to moderately enlarged. The triad of histologic features classically associated with toxoplasma lymphadenitis include (1) florid follicular hyperplasia, (2) prominent bands of parasinusoidal and parafollicular monocytoid B cells, and (3) many small clusters of histiocytes, some of which surround and invade germinal centers (Fig. 12.10). Large granulomas and granulomas with necrosis are not features of toxoplasma lymphadenitis. The monocytoid B cells are $CD20^+$, $CD5^-$, $CD10^-$, $BCL2^-$, $BCL6^-$, $CD23^-$. Analysis of the immunoglobulin heavy chain genes of the monocytoid B cells of toxoplasma lymphadenitis has shown that they are clonally related with intraclonal variation. Preferential usage of certain V_H gene segments is also reported; it is suggested that this may be due to stimulation of the monocytoid B cells by a superantigen expressed by *T. gondii*.[180]

The microorganisms are identified in lymph nodes only rarely,[31] although finding toxoplasma DNA using the PCR has been reported.[181] The diagnosis can be confirmed using serologic studies; in 95% of cases with the triad of histologic findings described above, results of serology document infection by *T. gondii*.[176]



• **Figure 12.9** Histoplasma lymphadenitis. **A,** This lymph node shows granulomas with extensive necrosis, surrounded by fibrosis. **B,** High power shows uninucleate and multinucleated histiocytes, many containing numerous microorganisms with a dotlike appearance on this hematoxylin and eosin–stained section. **C,** The Grocott methenamine silver stain shows numerous intracellular yeast forms.

Differential Diagnosis

Other causes of reactive lymphoid hyperplasia with follicular and monocytoid B-cell hyperplasia and histiocytes form the differential diagnosis of toxoplasma lymphadenitis. Cytomegaloviral lymphadenitis, early or partial nodal involvement by cat-scratch disease, sarcoidosis or mycobacteria, or primary or secondary syphilis can result in an appearance that resembles toxoplasma lymphadenitis, except that in none of these disorders is there a tendency for epithelioid histiocytes to encroach on lymphoid follicles. Granulomas, necrosis, and necrotizing granulomas are unusual in toxoplasmosis. Leishmania can produce a lymphadenitis closely resembling that of toxoplasma; it can also be associated with a necrotizing or nonnecrotizing granulomatous lymphadenitis.[138] However, with leishmania infection, microorganisms are usually identifiable in epithelioid histiocytes. Unlike toxoplasma, leishmania have kinetoplasts[182] (see later).

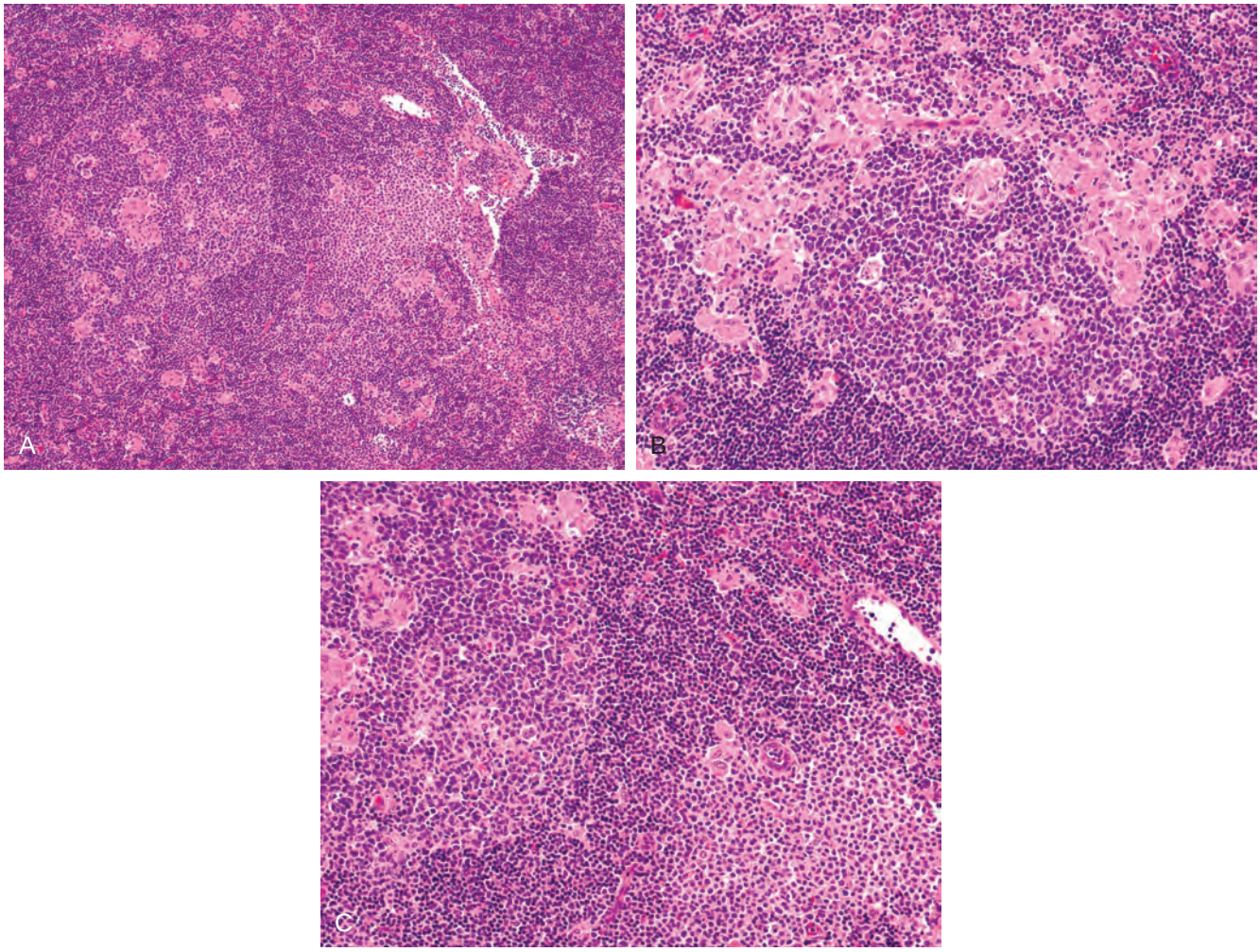
Leishmania Lymphadenitis

Clinical Features

Leishmaniasis is an infectious disease caused by *Leishmania* species and transmitted by sandflies (*Phlebotomus* species). Leishmaniasis occurs in the Middle East, parts of Africa, South America, and Asia, and in Central America; information about the patient's origin or travel history may provide a clue to the diagnosis. Leishmania can infect lymph nodes in two settings: (1) localized lymphadenopathy, often draining a focus of cutaneous infection, and (2) widespread lymphadenopathy in the setting of visceral leishmaniasis (kala-azar).

Pathologic Features

The localized lymphadenitis can show follicular and paracortical hyperplasia with necrotizing or nonnecrotizing granulomatous inflammation involving cortex and paracortex, or less often, cortex alone,[183] sometimes with numerous plasma cells. Plasma cells may contain crystalline inclusions of various shapes. The necrosis may be suppurative or caseating. The granulomas may resemble those seen in tuberculosis or can mimic the granulomas of cat-scratch disease.[138,184] Identification of Leishman-Donovan bodies establishes a diagnosis.[182] Histiocytes can occasionally be present without granuloma formation; Leishman-Donovan bodies are more numerous in such cases than in those with granulomas. In some cases, diagnosis can be established by cytologic



• **Figure 12.10** Toxoplasma lymphadenitis. **A,** Low power shows a reactive follicle with small clusters of epithelioid histiocytes outside and within follicle centers. An aggregate of monocytoid B cells with pale cytoplasm is present to the right of the follicle. **B,** A reactive follicle center is surrounded by clusters of epithelioid histiocytes; histiocytes also infiltrate the follicle center. **C,** A follicle containing clusters of epithelioid histiocytes *(left)* is seen adjacent to an aggregate of monocytoid B cells *(lower right)*.

examination of a fine-needle aspiration specimen.[182,185] Localized leishmaniasis is a relatively indolent disorder.

In visceral leishmaniasis, organisms reside in mononuclear phagocytes, but follicular hyperplasia and paracortical hyperplasia may not be prominent, and discrete granulomas are typically absent.[184,186,187] The organisms can be seen on routinely stained sections, on Giemsa stains, and on reticulum stains, although they are sometimes present in small numbers.[186-188] PCR can be useful in confirming the presence of leishmania and identifying the species.[183,185] Patients with visceral leishmaniasis are usually febrile and often have splenomegaly, elevated C-reactive protein, and elevated erythrocyte sedimentation rate, in addition to lymphadenopathy. A subset of patients with visceral leishmaniasis is immunocompromised.[185,188] Visceral leishmaniasis is a serious, potentially fatal disorder; however, it typically responds to appropriate therapy.[185,188]

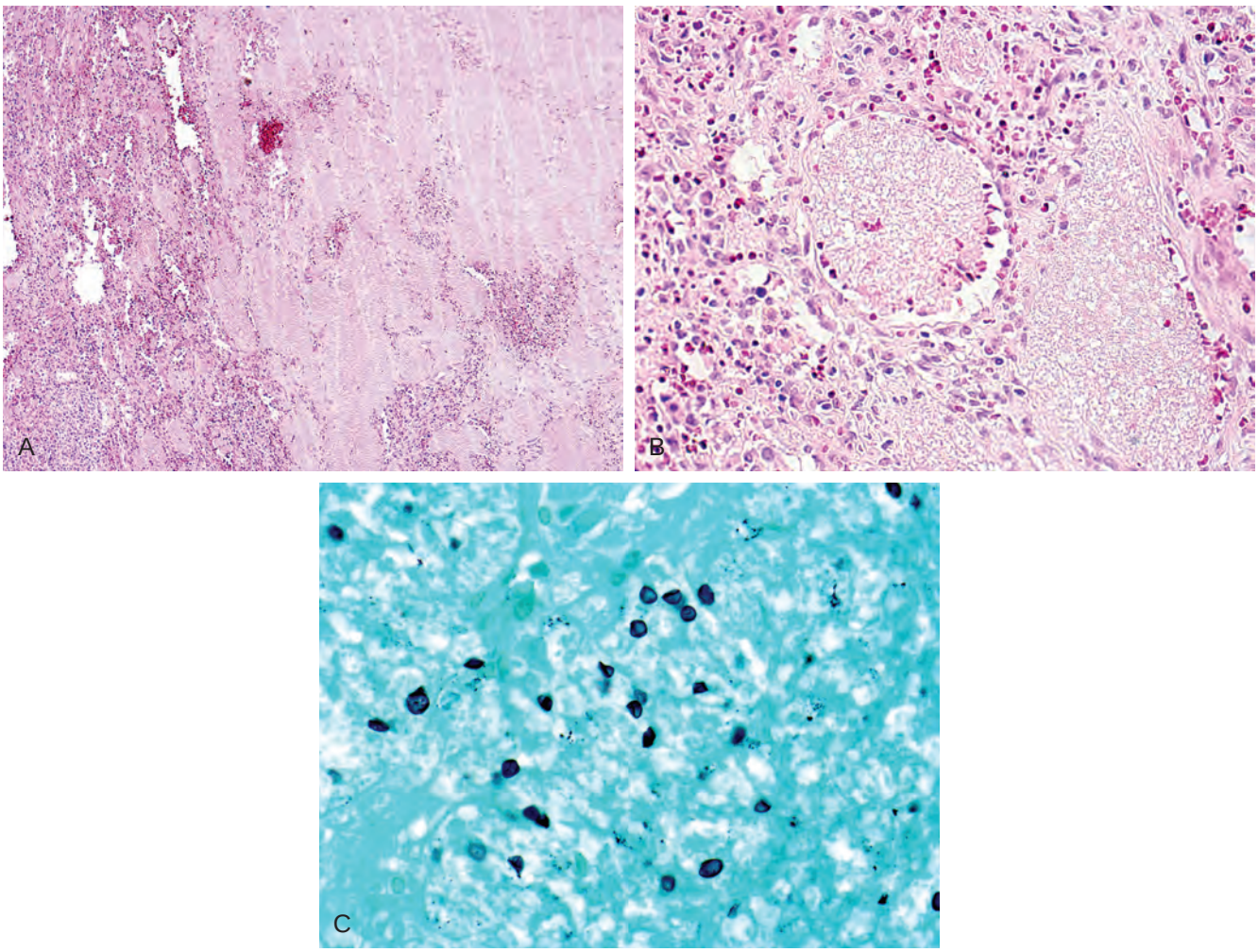
Pneumocystis jiroveci Lymphadenitis

Clinical Features

P. jiroveci (formerly *Pneumocystis carinii*) lymphadenitis is a very rare disorder. Fewer than 1% of all cases of pneumocystosis affect extrapulmonary sites. However, among extrapulmonary sites, lymph nodes are the site most commonly affected,[189,190] followed by liver and spleen (Fig. 12.11).[190] Patients are HIV-positive individuals, children with congenital immunodeficiency, organ transplant recipients, and patients with malignancies. The HIV-positive patients typically have an advanced stage of immunodeficiency and already carry a diagnosis of AIDS at the time of development of *P. jiroveci* lymphadenitis. Treatment with aerosolized pentamidine, which prevents *P. jiroveci* pneumonia, but not extrapulmonary infections, may lead to increased risk of developing *P. jiroveci* infection in extrapulmonary sites. Patients are typically febrile and quite ill. *P. jiroveci* lymphadenitis usually occurs in association with, or follows *P. jiroveci* pneumonia, or occurs in the setting of widespread extrapulmonary infection. Infection may involve multiple lymph nodes. Specific symptoms vary depending on the sites of disease. Treatment consists of prompt administration of the appropriate antibiotics. Without therapy, mortality is high.[135,189]

Pathologic Features

Diagnosis can be established with microscopic examination of a biopsy of affected tissue, although in many cases, diagnosis is only



• **Figure 12.11** *Pneumocystis jiroveci* infection of the spleen in an HIV-positive patient (same case as illustrated in Fig. 12.7). **A,** Low power shows pale pink material replacing large zones of splenic parenchyma. **B,** High power shows frothy and granular pink material. **C,** Grocott methenamine silver stain shows occasional *Pneumocystis* organisms.

made at autopsy. Involved tissues show frothy material with scattered helmet-shaped organisms that can be detected with a Grocott methenamine silver (GMS) stain.[189] Lymphadenitis with concurrent infection by *P. jiroveci* and *M. tuberculosis* has been described.[135]

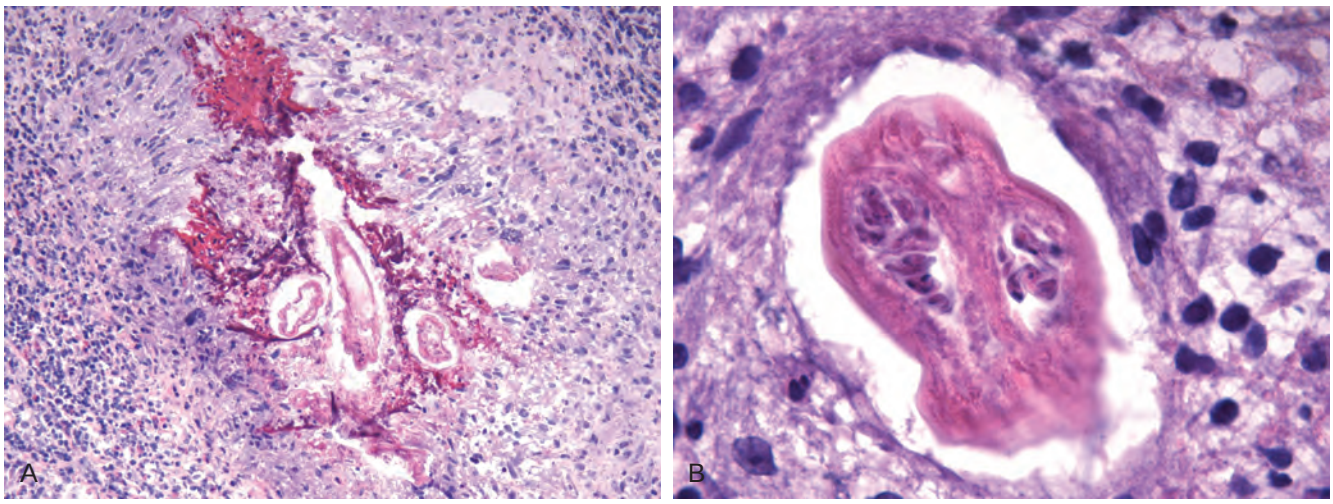
Filarial Infections

Clinical Features

Among helminthic infections, only filariasis is likely to be encountered in lymph nodes. Lymphatic filariasis affects an estimated 120 million persons worldwide. Among some, the disease is mild or asymptomatic, whereas others have a severe, disabling illness related to lymphatic obstruction. Manifestations include lymphedema, lymphadenopathy, elephantiasis of the extremities, and hydrocele.[191] Most cases are due to infection by *Wuchereria bancrofti*, which affects patients in equatorial Africa, India, the Mediterranean coast, the Caribbean, the eastern and northern coastal areas of South America, and some parts of Central America.[192,193] *Brugia malayi*, another type of lymphatic filaria, affects patients in coastal areas of Southeast Asia and India.[192,193] Another source of infection, particularly for individuals living in the United States who have not traveled to an endemic area, is one of the various zoonotic *Brugia* species. Typically, in zoonotic infections, only a single adult worm is present, and excision of the involved lymph node is curative.[192] Patients with *W. bancrofti* and *B. malayi* infection require additional antihelminthic therapy.

Pathologic Features

Histologic examination reveals adult worms in lymphatics and in lymph nodes. Within lymph nodes, the worms may be found within sinuses. Lymph nodes typically show prominent hyperplastic changes, which may include follicular hyperplasia, monocytoid B-cell hyperplasia, and a mixed infiltrate of lymphocytes, plasma cells, eosinophils, and neutrophils. When the worms present are degenerated or dead, there may be a particularly striking infiltrate of eosinophils, sometimes with formation of eosinophilic microabscesses, as well as granuloma formation. Dead worms may undergo dystrophic calcification.[192] Capsular and intranodal fibrosis may be prominent. *Brugia* species have smaller maximum midbody diameters (approximately 160 µm for females and 90 µm for males) than *Wuchereria* species (females: 250 µm; males: 150 µm).[192,193] Serial sections may be required to identify the worm[192] (Fig. 12.12).



• **Figure 12.12** Filarial lymphadenitis. **A,** Low power shows an adult worm surrounded by palisading granulomatous inflammation. **B,** High power shows a cross-section of the worm's distinct cuticle and oviducts.

Castleman Disease

The term Castleman disease encompasses a heterogeneous group of disorders with some overlapping histologic features but with different etiologies and widely differing clinical features. Castleman disease occurs in two clinical forms: localized and multicentric. Nearly all HIV-positive patients and approximately half of HIV-negative patients with MCD have evidence of HHV-8 (also known as KSHV) infection; HHV-8 is believed to underlie the development of Castleman disease in these cases.[194,195] The discussion below will focus on MCD related to HHV-8 infection, an uncommon cause of lymphadenopathy that straddles the border between an infectious disease and a lymphoproliferative disorder.

Multicentric Castleman Disease

MCD is a systemic disease historically associated with a poor prognosis, although with improvements in therapy the outlook is much more favorable.[196,197] Most patients are middle-aged to older adults; HIV-positive patients are overall younger than HIV-negative patients and show a striking male preponderance.[198-200] Patients present with multifocal peripheral and/or internal lymphadenopathy, often accompanied by hepatomegaly, splenomegaly, effusions in body cavities, and peripheral edema. Patients almost always have some systemic symptoms, including fever, fatigue, weight loss, and/or night sweats. Common laboratory abnormalities include anemia, polyclonal hypergammaglobulinemia, elevated C-reactive protein, and elevated erythrocyte sedimentation rate.[196,197,199-202] A few patients have a serum M component.[199] Infrequently, HHV-8–positive MCD unassociated with HIV infection is associated with POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes).[195] A majority of HIV-positive patients and a minority of HIV-negative patients also have cutaneous Kaposi sarcoma.[195,199,201]

The etiology of MCD appears to be related to elevated levels of human and/or viral interleukin-6 (IL-6), likely produced in the affected tissues.[203] IL-6 is believed to be responsible for the plasmacytosis seen in MCD.[201] Serum levels of IL-6 and also of IL-10 appear to correlate with disease activity.[196,199,203,204] Many MCD patients have an excellent response to rituximab monotherapy.[196,199] Single-agent chemotherapy and steroids may be of assistance in some patients, but responses are often not sustained.[196] Antiviral therapies directed against HHV-8, including zidovudine and valganciclovir, have proven effective in a subset of HIV-positive patients.[196] Newer therapies using the anti-IL-6 antibody siltuximab or the anti-IL-6 receptor antibody tocilizumab appear promising but require additional investigation.[196] It is suggested that radiographic studies including positron emission tomography scan and determining plasma HHV-8 levels can be helpful in monitoring disease activity and response to treatment.[199,200]

MCD patients are at increased risk for severe infections and also have an increased incidence of lymphoma, in particular HHV-8–positive lymphomas (e.g., primary effusion lymphoma and large B-cell lymphoma arising in HHV-8–associated MCD).[194,205,206] The prognosis of MCD patients has traditionally been considered poor, but some of the morbidity and mortality associated with this disorder, particularly among HIV-positive patients, stems from the development of lymphoma and opportunistic infections and the development or progression of Kaposi sarcoma. Immune reconstitution with ART in HIV-positive patients, in concert with more effective therapy for the MCD, has improved the outlook for MCD patients.[196,197,201,207]

HHV-8 also causes KSHV-associated inflammatory cytokine syndrome (KICS).[196,197] Patients with KICS have the inflammatory manifestations, elevated cytokine levels, and high HHV-8 viral load, as observed in HHV-8–associated MCD, but without the pathologic changes of MCD.

Cases of MCD may have the histologic features of plasma cell Castleman disease, mixed type Castleman disease, or, rarely, hyaline-vascular Castleman disease.[195] Some cases, including almost all MCD in HIV-positive patients, have pathologic features of plasmablastic Castleman disease (see later).

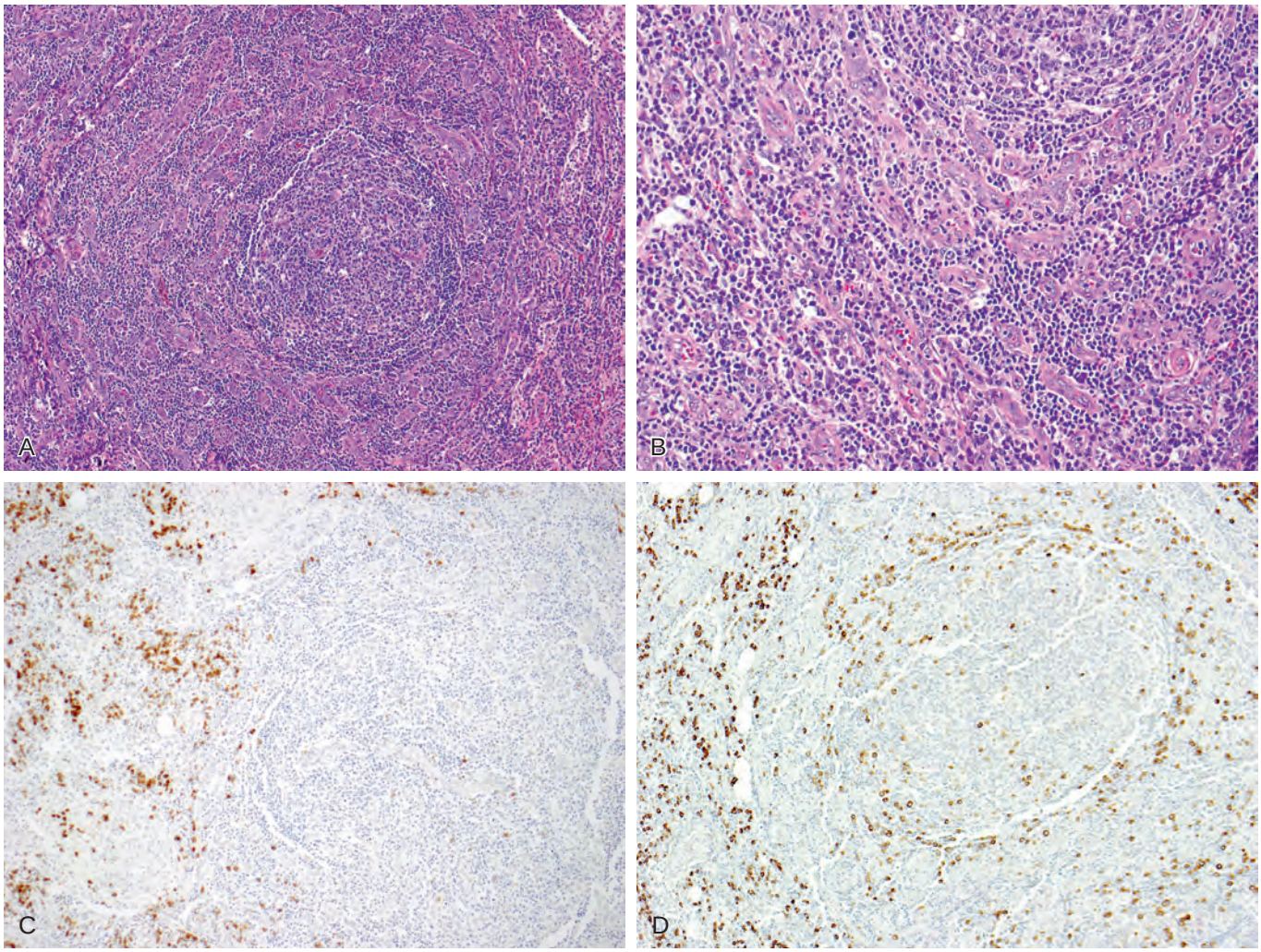
The familiar hyaline-vascular variant of Castleman disease is characterized by lymphoid follicles with small, hyalinized germinal centers and broad mantle zones. Each follicle may contain one or more than one germinal center; some follicles contain no recognizable germinal center. The germinal centers contain an increased proportion of FDCs and endothelial cells. Mantle zone lymphocytes are arranged in concentric rings ("onion skin" pattern)

around the germinal center. Follicles are often radially penetrated by a blood vessel ("lollipop follicle"). The interfollicular region shows increased numbers of high endothelial venules and of small vessels with flat endothelium and hyalinized walls. Lymphocytes are fewer in number than in a normal lymph node, and there may be an admixture of scattered plasma cells, few immunoblasts, few eosinophils, and clusters of plasmacytoid dendritic cells. Sheets of plasma cells are absent. Epithelioid histiocytes and granulomas are inconspicuous or absent. In some cases, patent sinuses may be found in residual normal lymphoid tissue at the periphery of the lesion, but they are generally absent within the lesion. Fibrosis is common peripherally and also in bands running through the lesion. There may be prominent perivascular fibrosis.[203,208-210]

The plasma cell variant of Castleman disease is characterized by enlarged lymph nodes with sheets of mature plasma cells in the interfollicular area, sometimes with Russell bodies; occasional immunoblasts may be seen. The follicles may be hyperplastic follicles of the usual type or hyaline-vascular follicles, or a mixture of the two types of follicles. Blood vessels are less conspicuous than in hyaline-vascular Castleman disease. Sinuses are usually obliterated but may be patent focally. The mixed type, or transitional type, of Castleman disease has features intermediate between hyaline-vascular Castleman disease and plasma cell variant of Castleman disease.

Plasmablastic Castleman Disease (Human Herpesvirus 8–Positive Multicentric Castleman Disease)

Subsequent to the finding of HHV-8 viral DNA in many cases of MCD,[194,195] cases of MCD in HIV-positive patients were described in which immunoblasts or plasmablasts in follicle mantles were HHV-8 positive and expressed monotypic IgMλ. The interfollicular compartment contained polytypic plasma cells and increased numbers of blood vessels (Fig. 12.13). This entity received the name of "plasmablastic Castleman disease."[196,211]



• **Figure 12.13** Lymph node with multicentric Castleman disease, HHV8+. The patient also had cutaneous involvement and involvement of the lymph node capsule by Kaposi sarcoma. **A,** Small lymphoid follicle is surrounded by a hypervascular interfollicular region. **B,** Higher power shows a portion of a follicle *(right)* with plasmablasts with vesicular chromatin and prominent central nucleoli scattered near the follicle's periphery. The interfollicular area contains numerous plasma cells *(left)*. **C** and **D,** Polytypic plasma cells (κ+, **C**; λ+, **D**) are present in the interfollicular area, while plasmablasts around the periphery of the follicle express monotypic λ light chain.

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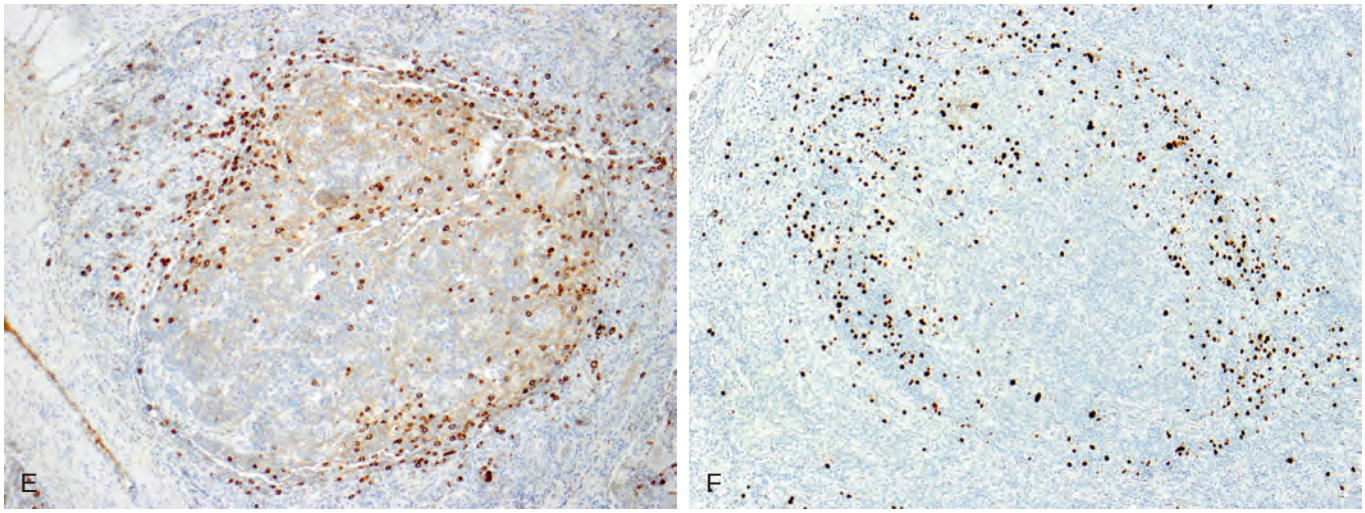

• **Figure 12.13, cont'd** **E,** Plasmablasts express IgM (monotypic IgM λ). **F,** The monotypic IgM λ express HHV8. (**C–F,** Immunohistochemistry on paraffin sections.)

Additional studies showed that the plasmablasts could also cluster and replace part of the follicle (microlymphoma) or proliferate to form sheets of plasmablasts (frank lymphoma), referred to as HHV8+ diffuse large B-cell lymphoma, NOS 2017 update to the World Health Organization (WHO) classification.[206] Surprisingly, the monotypic plasmablasts in plasmablastic Castleman disease and in microlymphomas were usually polyclonal by molecular techniques, although frank lymphomas were usually clonal.[211,212] This raises the question of whether HHV-8 preferentially infects λ-positive lymphoid cells or whether there is preferential proliferation or survival of HHV-8–infected λ-positive lymphoid cells. The plasmablasts are characteristically negative for EBV, although we have seen a case of MCD in an HIV-positive man complicated by the development of EBV-positive plasmablastic microlymphoma and then frank lymphoma.[198] In our case a hemophagocytic syndrome developed.[198] In another case report an HIV-negative male developed an HHV-8–positive, EBV-negative plasmablastic microlymphoma associated with a hemophagocytic syndrome, with a rapidly fatal disease course.[213] Hemophagocytic syndromes are typically precipitated by infections, often by EBV, and may be seen in association with EBV-positive malignancies of natural killer (NK) cell lineage, including extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia,[198,214,215] and rarely in association with HHV-8 infection.[216]

Careful examination of lymph nodes with MCD occasionally reveals concurrent involvement by Kaposi sarcoma; foci of Kaposi sarcoma are often small and tend to involve nodal capsule, trabeculae, or hilum.[217]

Differential Diagnosis

In cases of HHV-8–positive MCD in HIV-positive patients, the histologic features may not be as distinctive as in classic hyaline-vascular Castleman disease and plasma cell variant of Castleman disease in the immunocompetent host. The lymph nodes may have a depleted appearance, and in some cases only careful examination reveals a few follicles with features suggesting Castleman disease. Performing a stain for HHV-8 on lymph nodes with changes suggestive of Castleman disease will help to avoid overlooking the diagnosis.

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13

Infectious Diseases of the Bone Marrow and Spleen

ROBERT P. HASSERJIAN, LAWRENCE ZUKERBERG

Patterns of Bone Marrow Response to Infections
- Hyperplasia
- Hypoplasia
- Serous Atrophy (Gelatinous Transformation)
- Morphologic Dysplasia
- Lymphoid Aggregates
- Granulomas
- Plasmacytosis
- Hemophagocytosis

Bone Marrow Features of Specific Infections
- Parvovirus B19 Infection
- Human Immunodeficiency Virus Infection
- Epstein-Barr Virus Infection
- Cytomegalovirus Infection
- Brucellosis
- Rickettsial Infections
- Leishmaniasis
- Pneumocystis
- Fungal Infections

Infections Involving the Spleen
- Diffuse Red Pulp Reactions to Infection
- White Pulp Reactions to Infection
- Mass-Forming and Cystic Splenic Infections

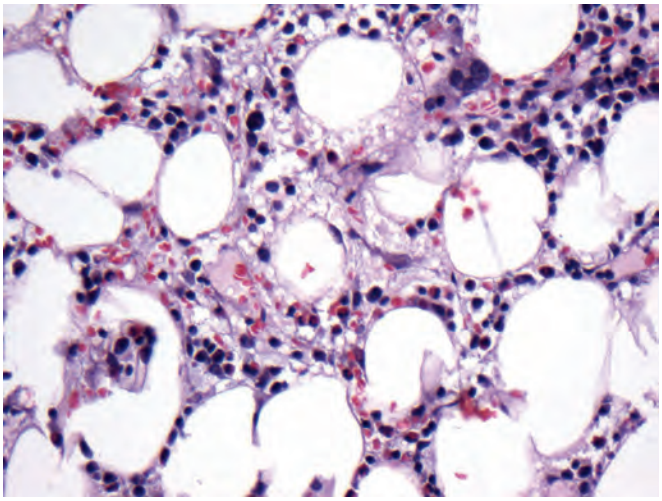
The bone marrow and spleen may be affected by a wide variety of infectious agents. Pathologists must be aware of the clinical concern for infection to initiate appropriate studies to detect microorganisms and be prepared to recognize features of infection even when it is not clinically suspected. Bone marrow sampling may be performed to evaluate fever of unknown origin (most commonly in immunosuppressed patients but sometimes in immunocompetent patients) or to evaluate for systemic infection in a patient known or suspected to harbor a particular infectious agent. Anemia and other peripheral cytopenias are the most frequent indications for bone marrow sampling and may be the presenting signs of a clinically unsuspected infection identifiable within the bone marrow.

Bone marrow morphologic changes related to infections are most often nonspecific, and confirmation of a specific infectious agent may require special stains, immunohistochemistry, microbiologic cultures, serologic studies, and molecular studies. However, it is important to understand the spectrum of morphologic changes elicited by specific infectious agents and to recognize how comparable changes can at times be caused by noninfective or neoplastic processes. The first part of this chapter discusses the various patterns of bone marrow changes that can be associated with infections; although most are nonspecific, in some instances they suggest a particular causative agent and can elicit appropriate confirmatory ancillary studies. In the second part of the chapter, the pathologies of specific infectious agents involving the bone marrow are described. Infections involving the spleen are discussed at the end of the chapter.

Patterns of Bone Marrow Response to Infections

Hyperplasia

Bone marrow cellularity (i.e., the percentage of cellular marrow elements occupying the hematopoietic marrow space of the bone marrow biopsy specimen) is frequently increased in infection. This may reflect either an appropriate reaction against the offending agent or ineffective hematopoiesis due to the effects of the infectious agent on the maturing hematopoietic cells and marrow microenvironment. In the former situation, the peripheral smear shows leukocytosis, often with increased band neutrophils, circulating immature myeloid elements, neutrophil toxic granulation, or some combination of these characteristics. If ineffective hematopoiesis is present, leukopenia or pancytopenia may be present despite the marrow hypercellularity. The marrow may show hyperplasia of all lineages, or it may show relative myeloid, erythroid, or megakaryocytic hyperplasia. Although the type of hyperplasia is not specific for any particular infectious agent, marked myeloid hyperplasia is often seen in sepsis, and the bone marrow in a region affected by osteomyelitis may show a marked predominance of neutrophils.[1,2] Megakaryocytic hyperplasia is frequently observed in human immunodeficiency virus (HIV) infection (see later discussion).



• **Figure 13.1** Acute marrow injury due to sepsis. The marrow is hypocellular, with interstitial eosinophilic fibrillar material representing products of intramedullary cell death. The marrow in sepsis may be either hypercellular or hypocellular (hematoxylin and eosin stain).

Hypoplasia

Global marrow hypocellularity may occur in severe, overwhelming infections, such as sepsis; in such cases of acute marrow injury, a necrotic background of eosinophilic granular material, reflecting products of massive cell death, may be observed (Fig. 13.1).[1,2] Marked erythroid hypoplasia or pure red cell aplasia (defined as <5% red cell precursors in the bone marrow) can be produced by a variety of infectious agents but is commonly associated with parvovirus B19 infection. Dengue hemorrhagic fever, caused by a single-stranded RNA flavivirus that can infect hematopoietic cells, is associated with cytopenias and marked bone marrow hypocellularity, often with a maturation arrest in erythroid and myeloid lineages.[3,4] Aplastic anemia may occur after acute hepatitis (including hepatitis A, B, and C viruses); the mechanism of marrow failure and definite implication of a viral etiology are uncertain in these cases.[5]

Serous Atrophy (Gelatinous Transformation)

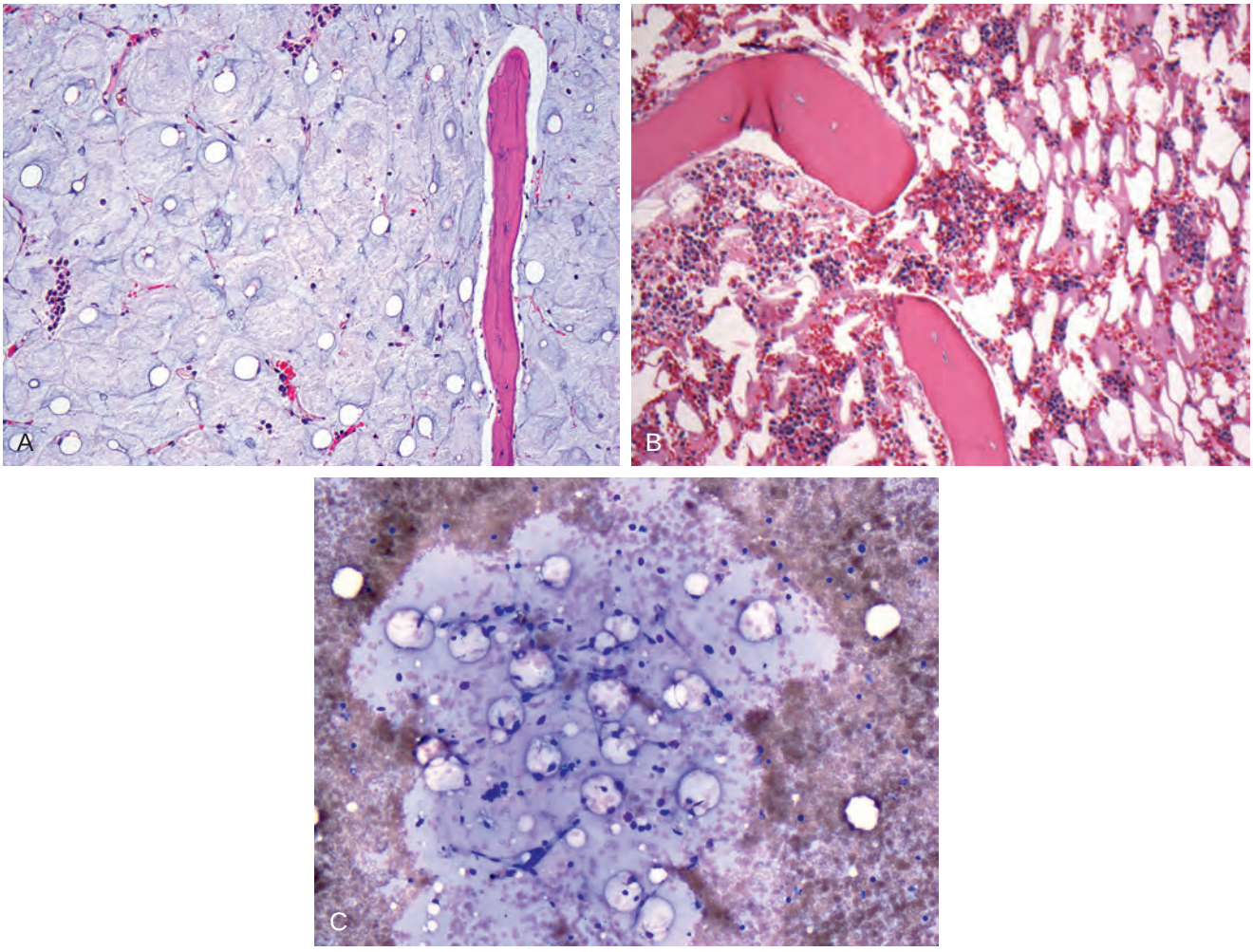
Serous atrophy of the bone marrow represents an alteration of the bone marrow stroma whereby the adipocytes and hematopoietic cells are replaced by pale pink, amorphous, extracellular ground substance. The adipocytes are often smaller in size than normal marrow adipocytes.[6] Serous atrophy can be observed in late stages of HIV infection; in that context, it is always associated with anemia (usually severe, with a median hemoglobin concentration of 7.9 g/dL) or, less often, with other cytopenias.[7] The bone marrow aspirates are typically difficult to obtain and may result in a dry tap. If an aspirate is obtained, gelatinous pink material may be present in the background of the Wright-Giemsa–stained smears.[7] In the trephine biopsy on hematoxylin and eosin (H&E) stain, the material appears amphophilic or eosinophilic and homogeneous, although a fibrillar or granular appearance may be observed on high power (Fig. 13.2). This contrasts to the eosinophilic background material of acute marrow injury (such as that observed after cytotoxic chemotherapy), which is granular at low power and appears to be more eosinophilic than serous atrophy material. The material in serous atrophy stains with periodic acid–Schiff (PAS) stain and is also positive for alcian blue at acidic pH (2.5 and 1.0). Because predigestion with hyaluronidase abrogates the alcian blue staining, serous atrophy material appears to comprise the extracellular ground substances hyaluronic acid and sulfated glycosaminoglycans.[8,9] Disruption of the normal extracellular matrix in the marrow microenvironment likely explains the ineffective erythropoiesis and marked anemia typically associated with serous atrophy. The marrow is hypocellular in approximately half of the cases and may occasionally be hypercellular. Serous atrophy may be either focal or diffuse (see Fig. 13.2B). When associated with HIV infection, megakaryocytes are usually increased and show the typical abnormal features observed in HIV-infected patients (see later discussion).

Serous atrophy may also occur in HIV-negative patients. Indeed, some reports have suggested that, since the advent of highly active antiretroviral therapy (HAART), HIV infection has become a relatively uncommon cause of serous atrophy[10]; cachexia due to tumors or malnutrition was a more common cause in one series. Other infections associated with serous atrophy include bacterial endocarditis, pneumonia, and tuberculosis.[10] In such cases the histologic features are similar to those of HIV-associated serous atrophy, but serous atrophy in HIV-negative patients is associated with less marked anemia.[7]

Morphologic Dysplasia

Myelodysplastic syndromes (MDS) characteristically exhibit morphologic abnormalities of the erythroid, myeloid, and megakaryocytic lineages, reflecting abnormal maturation of the clonal, defective hematopoiesis. These changes include nuclear irregularities, nuclear fragmentation, binucleation, megaloblastoid change, and cytoplasmic vacuolation of erythroid elements; hypogranularity, vacuolization, and abnormal nuclear lobation of myeloid cells; and small size and nuclear hypolobulation of megakaryocytes. Reactive conditions that disrupt the marrow microenvironment, including many inflammatory and infectious processes, may produce morphologic changes resembling those of MDS. In particular, the erythroid cells of HIV-positive patients often exhibit nuclear irregularities, megaloblastic change, vacuolation, and, occasionally, ring sideroblasts on iron stain (Fig. 13.3A). The megakaryocytes in HIV-positive patients are frequently small and increased in number (see Fig. 13.3B). The megakaryocytes have very sparse cytoplasm (so-called naked or bare megakaryocyte nuclei) but do not typically show the round nuclei of MDS.[11] In contrast to many cases of MDS, the myeloid lineage is usually spared. Hematopoietic elements also show a lower rate of proliferation and apoptosis in HIV infection than in MDS.[12] These morphologic changes are reversible after treatment of HIV infection, because they reflect a disrupted bone marrow microenvironment rather than an intrinsic, clonal hematopoietic stem cell abnormality as seen in MDS. Nevertheless, HIV infection should be considered in the differential diagnosis of bone marrow samples showing dysplastic changes, particularly if the dysplasia is most prominent in the erythroid lineage, if it is accompanied by numerous bare megakaryocyte nuclei and increased plasma cells, and if it occurs in young patients, in whom MDS is uncommon.

Similar to HIV infection, a reactive morphologic dysplasia of erythroid elements may be observed in other infections involving the bone marrow, such as leishmania.[13] The morphologic dysplasia in HIV and other infections is most likely due to effects of the infection on histiocytes, macrophages, and marrow stromal cells; these cells provide important microenvironmental structural and



• **Figure 13.2** Serous atrophy (gelatinous transformation). **A,** The marrow biopsy is usually hypocellular, with background amorphous pale eosinophilic material; hematopoiesis is confined to islands within the background material (hematoxylin and eosin [H&E] stain). **B,** Serous atrophy may be focal, and in this photomicrograph from the bone marrow biopsy of a patient with widespread carcinoma, it is present on the right-hand side. The fat cells within the area of serous atrophy are smaller than normal and have clearly demarcated cell borders (H&E stain). **C,** Serous atrophy in the aspirate smear appears as pale, basophilic, sparsely cellular material admixed with fat cells (Wright-Giemsa stain).

cytokine factors required for normal maturation of hematopoietic elements.[14]

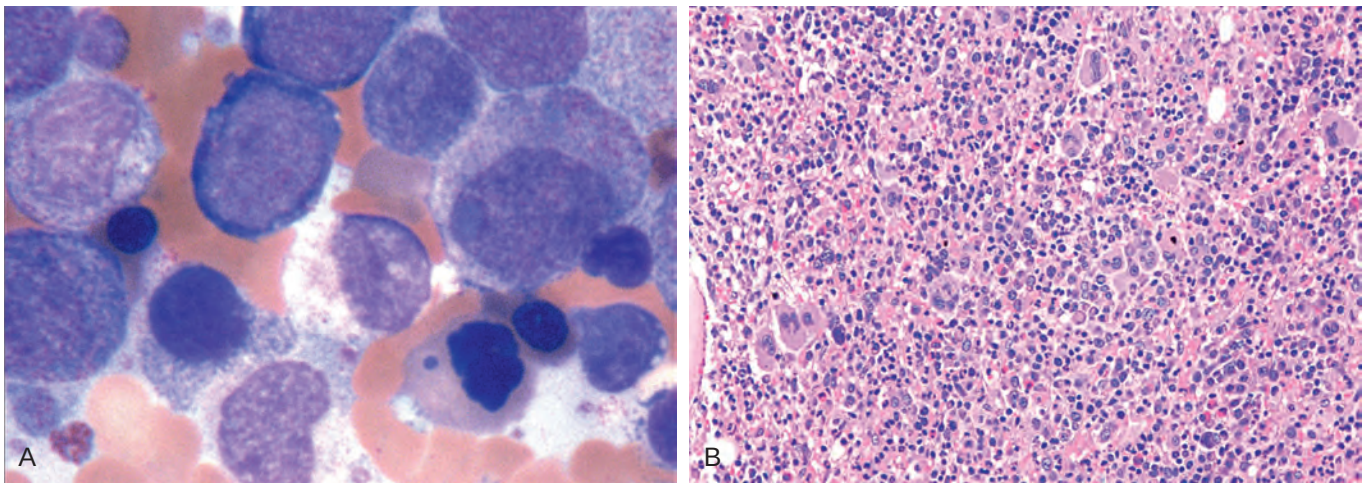
Lymphoid Aggregates

Aggregates of small, mature lymphocytes (that may or may not contain germinal centers) are nonspecific and can occur in normal individuals, as well as in a variety of neoplastic and nonneoplastic conditions. Lymphoid aggregates in the setting of HIV infection are often large, are admixed with histiocytes and plasma cells, and have ill-defined borders; they may occasionally be paratrabecular, mimicking lymphomatous aggregates.[15,16] Flow cytometry and/or immunohistochemistry on bone marrow can be helpful in such cases to exclude the possibility of lymphomatous involvement of the bone marrow.

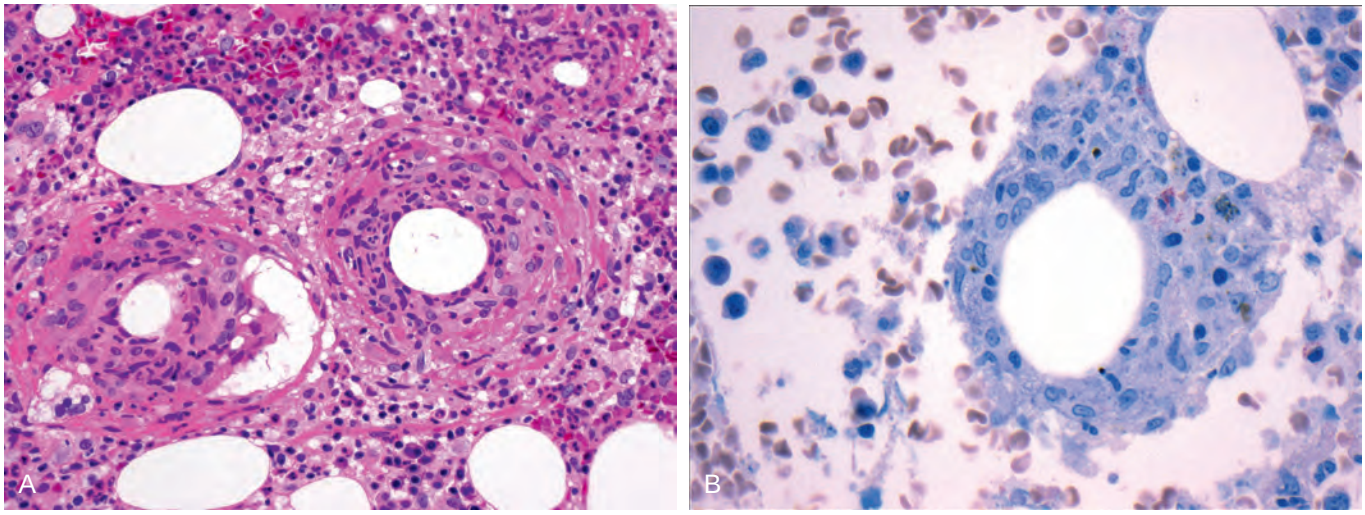
Granulomas

Granulomas are identified in approximately 1% of bone marrow samples and in approximately 20% of samples taken to evaluate a fever of unknown origin.[17] Granulomas are readily identified in trephine biopsy sections but are only infrequently identifiable in the aspirate smears.[18] Granulomas are usually not well represented in the aspirate smear due to associated reticulin fibrosis that limits aspiration of the telltale histiocytic and giant cell aggregates.[19] An associated infection is diagnosed in only approximately 30% of bone marrow granuloma cases. In many cases, bone marrow granulomas are the result of noninfectious processes, including sarcoidosis, drug reactions, and paraneoplastic phenomena caused by hematologic or nonhematologic malignancies. In up to 5% of cases, bone marrow granulomas are idiopathic with no known cause identified.[17]

The pattern of granulomas, including the ratio of histiocytes to lymphocytes and the presence or absence of multinucleated giant cells, eosinophils, and plasmacytosis, does not appear to be predictive of an infectious versus a noninfectious etiology.[17] However, granuloma localization within the marrow can be helpful because the granulomas in *Mycobacterium tuberculosis* infections are often paratrabecular.[2] *M. tuberculosis* infection in the marrow may also demonstrate bone marrow necrosis.[20]



• **Figure 13.3** Findings in bone marrow from human immunodeficiency virus–infected patients. **A,** Erythroid elements in the aspirate smear often show morphologic dysplasia, such as cytoplasmic vacuolization and nuclear irregularities, features that may lead to an erroneous diagnosis of a myelodysplastic syndrome (Wright-Giemsa stain). **B,** The bone marrow biopsy is usually hypercellular, often showing erythroid predominance with increased megakaryocytes, including small forms and bare megakaryocyte nuclei (hematoxylin and eosin stain).



• **Figure 13.4** Fibrin ring granulomas in a bone marrow biopsy, characterized by a central empty space surrounded by histiocytes and neutrophils that may or may not be encircled by a concentric ring of fibrin (**A,** hematoxylin and eosin stain; **B,** Giemsa stain).

Granulomas are most commonly associated with mycobacterial and fungal infections but can also occur with other infections, including Epstein-Barr virus (EBV),[21] leishmaniasis, toxoplasmosis,[22] brucellosis,[23] and Q fever.

Diffuse histiocytosis in the absence of granulomas may occur in immunosuppressed patients with mycobacterial infections. Acid-fast bacilli (AFB) stain should be performed on samples from any immunosuppressed patient with increased bone marrow histiocytes or unexplained lymphohistiocytic aggregates. Whipple disease can rarely involve the bone marrow as histiocytosis containing PAS-positive inclusions and occasionally PAS-positive *Tropheryma whipplei* organisms.[24] Whipple disease can also present with noncaseating granulomas in the bone marrow[25] Although the presence of PAS-positive inclusions in bone marrow macrophages is nonspecific, it may raise the differential diagnosis of Whipple disease, which can be confirmed by polymerase chain reaction (PCR) or additional clinical investigations.[24]

Bone marrow fibrin ring granulomas (also called doughnut granulomas) represent a distinctive type of granuloma associated with cytomegalovirus (CMV), EBV, and Q fever (caused by the rickettsial organism, *Coxiella burnetii*), and *Rickettsia typhi.*[26-28] Fibrin ring granulomas represent annular granulomas composed of concentric rings of neutrophils and epithelioid histiocytes surrounding a central empty space (Fig. 13.4). The granulomas are often encircled by a lamellated ring of fibrin (highlighted by Masson trichrome or fibrin stains).[29] The nature of the central clear space is uncertain; in the bone marrow and liver, the fibrin ring granulomas are typically located adjacent to blood vessels or sinusoids and have been termed a perivasculitis. However, the

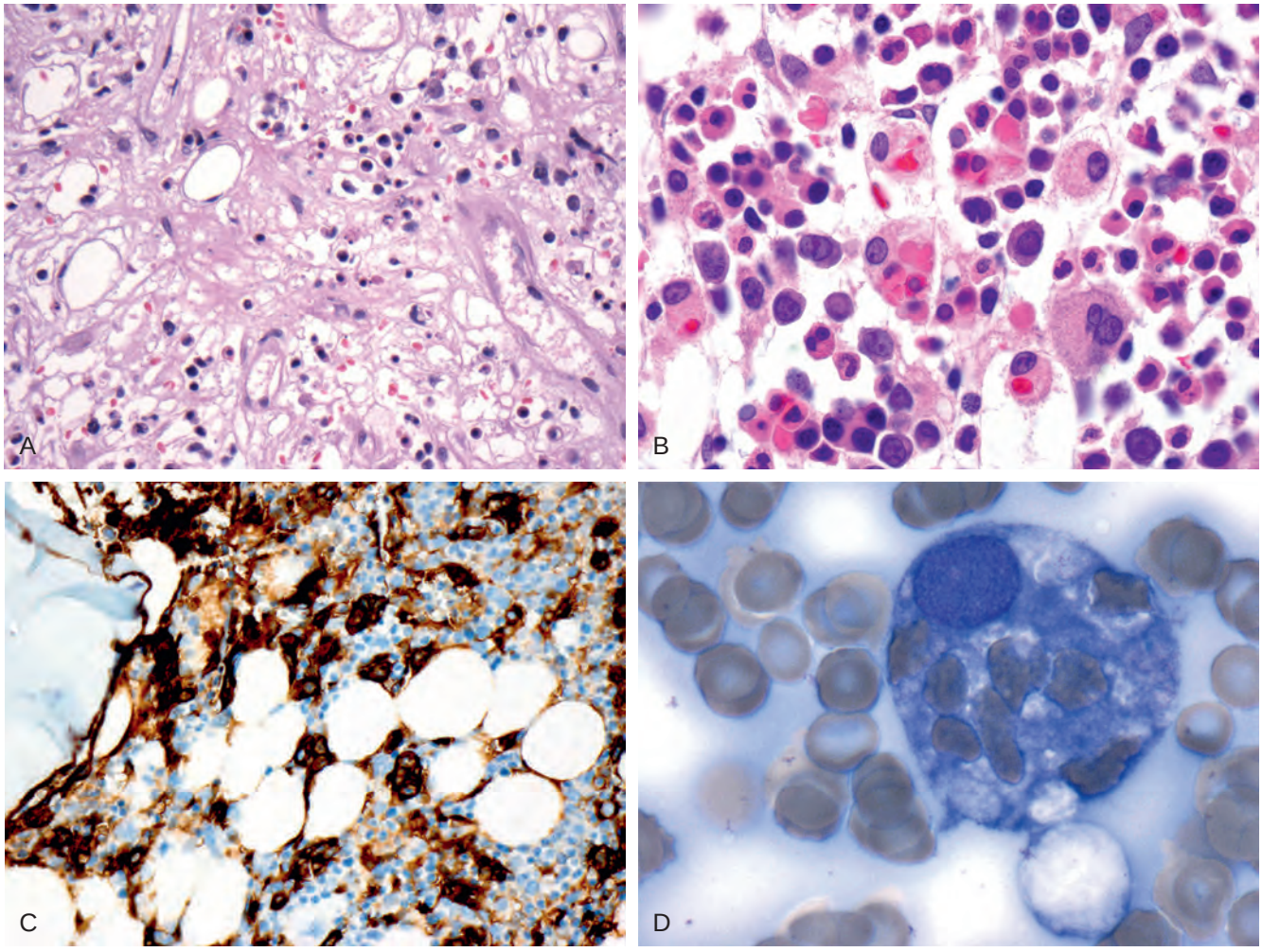
central material appears to be lipid rather than a vascular lumen because vascular components have not been identified within ring granulomas.[30] Of note, fibrin ring granulomas are not specific for an infectious process because they also may occur as a paraneoplastic phenomenon in lymphomas.[26,31] Hodgkin lymphoma must always be considered when granulomas are present in the marrow or spleen.

Plasmacytosis

Bone marrow plasma cells (normally comprising <2% of all marrow cells) may increase, often to prominent levels, in a wide variety of infections and inflammatory conditions. In such cases the plasma cells are usually perivascular and can be demonstrated to be polyclonal by immunoglobulin light chain immunohistochemistry or in-situ hybridization; this is particularly important to avoid a misdiagnosis of plasma cell myeloma in HIV-positive patients, who may have serum paraproteins. Sheets of mature, polyclonal plasma cells replacing the marrow space may be seen in chronic osteomyelitis.

Hemophagocytosis

Hemophagocytosis represents the presence of intact hematopoietic cells (erythrocytes, leukocytes, or both) within the cytoplasm of phagocytic histiocytes in any tissue; hemophagocytic histiocytes can be readily identified in both bone marrow biopsy sections and aspirate smears (Fig. 13.5). Note that phagocytic histiocytes that contain pyknotic cellular debris, and are common in germinal centers and other tissues with high cell turnover, are not equivalent to hemophagocytic histiocytes. Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is an inherited or acquired histiocyte activation syndrome that is potentially fatal. The identification of hemophagocytic histiocytes in bone marrow (or, less commonly, in spleen or lymph node tissue) represents a key diagnostic feature of HLH. However, hemophagocytosis is nonspecific and in isolation cannot confirm a diagnosis of HLH; conversely, a diagnosis of HLH can be made even if hemophagocytosis is not present in biopsied tissues.[32] The diagnostic criteria of HLH were revised in 2004 and are shown in Box 13.1.



• **Figure 13.5** Bone marrow findings in hemophagocytic lymphohistiocytosis. **A,** The bone marrow biopsy specimen may show hypercellularity or, as in this case, marked hypocellularity (hematoxylin and eosin [H&E] stain). **B,** Histiocytes with engulfed red cells and nucleated hematopoietic elements may be identified in the biopsy (H&E stain). **C,** Immunostain for CD163 can help to identify the histiocytes containing cells within the cytoplasm (CD163 immunohistochemistry). **D,** Phagocytic histiocytes contain intact red blood cells (or other hematopoietic elements) within the cytoplasm (bone marrow aspirate; Wright-Giemsa stain).

• BOX 13.1 Diagnostic Criteria for Hemophagocytic Lymphohistiocytosis

The diagnosis of HLH can be established if either criterion A or B is fulfilled.
Criterion A: A molecular diagnosis consistent with HLH (primary HLH)
Criterion B: At least five of the following eight diagnostic criteria for HLH fulfilled

1. Fever
2. Splenomegaly
3. Cytopenias (affecting at least two of three lineages in the peripheral blood)
   a. Hemoglobin <90 g/L (in infants <4 weeks of age, hemoglobin <100 g/L)
   b. Platelets $<100 \times 10^9$/L
   c. Neutrophils $<1.0 \times 10^9$/L
4. Hypertriglyceridemia and/or hypofibrogenemia
   a. Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dL)
   b. Fibrinogen ≤1.5 g/L
5. Hemophagocytosis in bone marrow, spleen, or lymph nodes
6. Low or absent NK-cell activity (according to local laboratory reference)
7. Serum ferritin ≥500 μg/L
8. Soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/mL

HLH, *Hemophagocytic lymphohistiocytosis;* IL-2, *interleukin-2;* NK, *natural killer.*
Modified from Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. *2007;48:124-131.*

• BOX 13.2 Secondary Causes of Hemophagocytic Lymphohistiocytosis

I. Infections
   A. Viruses
      1. Herpesviruses (HSV, VZV, CMV, EBV, HHV-6, HHV-8)
      2. HIV
      3. Other viruses: adenovirus, hepatitis viruses, parvovirus, influenza
   B. Other infections
      1. Bacteria, including mycobacteria and spirochetes
      2. Parasites, particularly *Leishmania* and *Plasmodium vivax*, as well as *Toxoplasma gondii* in patients post stem-cell transplant
      3. Fungi
II. Malignancy (most commonly lymphomas)
III. Macrophage activation syndrome (associated with autoimmune disease)

CMV, *Cytomegalovirus;* EBV, *Epstein-Barr virus;* HHV, *human herpesvirus;* HIV, *human immunodeficiency virus;* HSV, *herpes simplex virus;* VZV, *varicella zoster virus.*
Modified from Rouphael NG, Talati NJ, Vaughan C, et al. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. *2007;7:814-822, 898; Verbsky JW, Grossman WJ. Hemophagocytic lymphohistiocytosis: Diagnosis, pathophysiology, treatment, and future perspectives.* Ann Med. *2006;38:20-31; and George MR. Hemophagocytic lymphohistiocytosis: review of etiologies and management.* J Blood Med. *2014;5:69-86.*

Bone marrow sampling is often performed to evaluate for clinically suspected HLH. Patients typically present with fever, splenomegaly, and severe cytopenias. These cases require careful review because hemophagocytic cells may be rare: the median number in confirmed cases of HLH is only 1 in 500 cells in bone marrow aspirates. As mentioned earlier, the lack of hemophagocytosis does not exclude a diagnosis of HLH, and this diagnosis can be made based on clinical and molecular features in such cases. Although the sensitivity of the first bone marrow sample to document HLH is only approximately 60%, serial bone marrow samples in suspected disease yield a higher sensitivity.[32] Thus repeat bone marrow sampling is indicated if HLH is suspected but cannot be diagnosed on the initial sample nor confirmed on clinical and molecular criteria alone. Immunostaining for CD163 may help to facilitate the identification of the phagocytic histiocytes in the bone marrow biopsy specimen.[33] Of note, in one study, the amount of hemophagocytosis identified in the bone marrow did not appear to correlate with disease probability and lacked specificity for HLH, whereas highly elevated ferritin, fever, and cytopenias showed better correlation with a diagnosis of HLH.[34] Aside from the presence of hemophagocytic histiocytes, the bone marrow appearance of HLH is variable. The marrow can range from hypocellular to hypercellular and the myeloid to erythroid ratio is variable. Because HLH commonly occurs secondary to infection or a neoplastic process, the bone marrow may be concurrently involved by the inciting process, such as a lymphoma (Box 13.2). Other abnormal clinical and laboratory findings that can be clues to the diagnosis of HLH but do not represent diagnostic criteria are cerebromeningeal symptoms, lymphadenopathy, jaundice, edema, skin rash, hepatic enzyme abnormalities, hypoproteinemia, and hyponatremia.[32]

Primary (familial) HLH is a rare, autosomal recessive disease that usually manifests in infancy or early childhood.[35] The main gene defect in familial HLH (25% to 50% of cases) is a mutation in the perforin gene *(PRF1)*.[36] Defects in other genes, including *UNC13D*, *STXBP2,* and *STX11*,[37] have been shown to cause HLH in smaller subsets of patients. These mutations result in impaired cytotoxic T-cell and NK-cell function that leads to inappropriate activation of macrophages. Uncontrolled macrophage phagocytosis of hematopoietic elements, erythrocytes, and platelets ensues, with resulting severe and often life-threatening pancytopenia. Primary HLH may also occur during the course of inherited immunodeficiency syndromes, such as Chédiak-Higashi and X-linked lymphoproliferative syndrome.[38]

Secondary HLH is an acquired disorder characterized by a similar histiocyte activation induced by an infection, neoplasm, or chronic inflammatory condition; overall, infections are the underlying cause of approximately half of secondary HLH cases.[39] Causes of secondary HLH are shown in Box 13.2.[40] It is important to note that infections, especially EBV, can precipitate the first episode of hemophagocytosis in patients with familial HLH.[41] Therefore the presence of an infection concurrent with HLH does not necessarily exclude a genetic or familial etiology because these may not manifest until adolescence or even adulthood.[42] The identification of unexplained hemophagocytosis in a bone marrow sample in which some clinical features of HLH are present should precipitate a search for possible infectious, neoplastic, or inflammatory causes, as well as consideration that this may be a manifestation of familial HLH. Infection-associated HLH can occur in either immunocompetent or immunodeficient patients. However, in cases associated with EBV infection, viral titers are usually very high compared with uncomplicated infectious mononucleosis and even fulminant infectious mononucleosis, suggesting an underlying immune abnormality even in patients lacking a known immunodeficiency syndrome.[43,44]

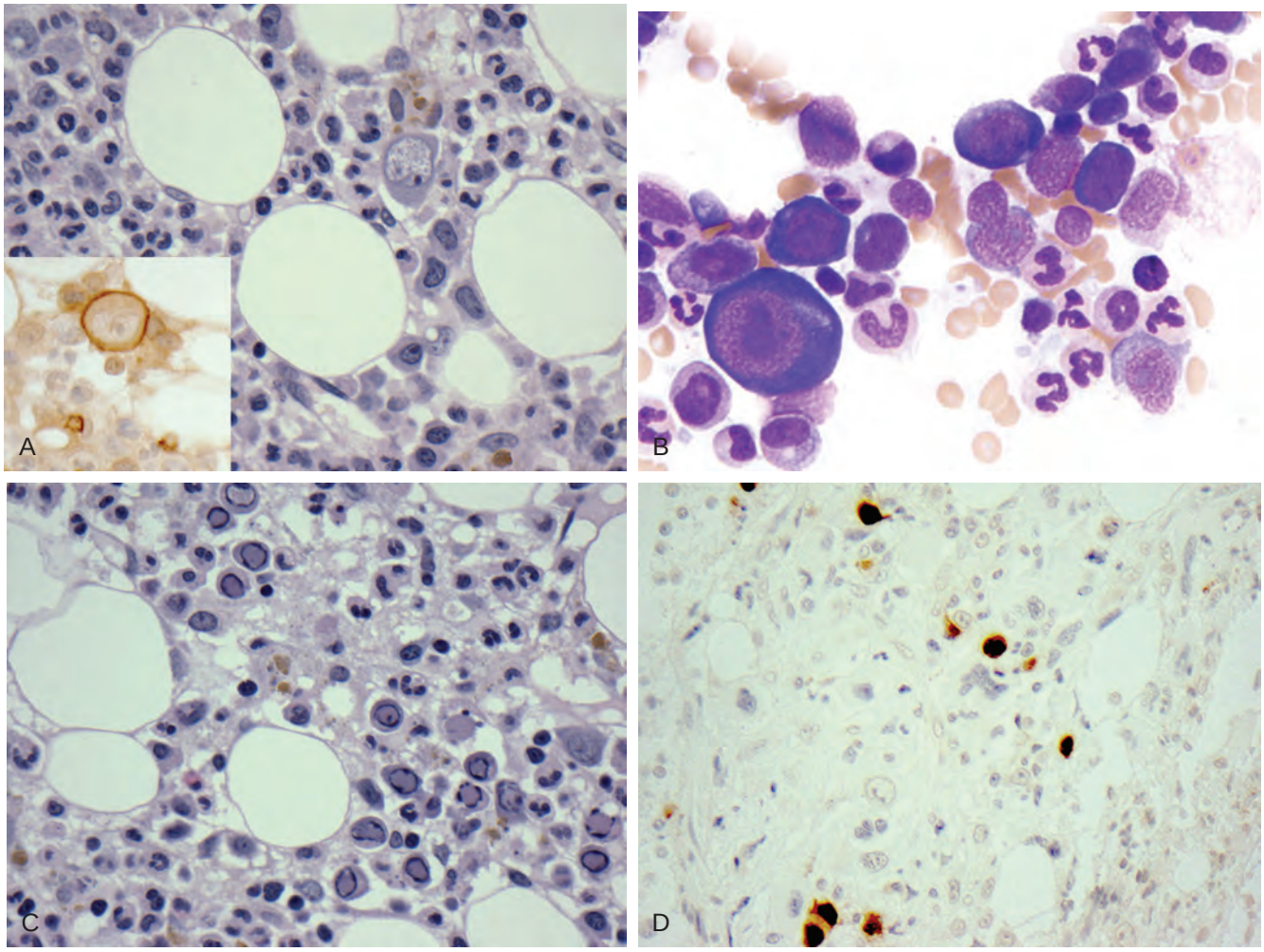
Hemophagocytosis is not specific for HLH and can be identified in up to 50% of patients who undergo bone marrow examination as part of a workup for fever and also in bone marrow after blood transfusions.[45,46] Hemophagocytic histiocytes are less frequent in these nonspecific settings than in most cases fulfilling diagnostic criteria for HLH.

Bone Marrow Features of Specific Infections

Parvovirus B19 Infection

Parvovirus B19 is a small, unenveloped DNA virus that causes erythema infectiosum, a childhood infection characterized by rash and sometimes arthralgias, hydrops fetalis and intrauterine fetal death in pregnancy, and anemia. The virus is highly transmissible through respiratory secretions. Parvovirus B19 infects erythroid precursors and arrests red blood cell production.[47] In immunocompetent patients, this arrest is usually asymptomatic except in patients with markedly decreased red blood cell survival, in which case severe anemia (so-called transient aplastic crisis) may result. Transient aplastic crisis is most commonly associated with sickle cell disease but has also been reported with hereditary spherocytosis, thalassemia, pyruvate kinase deficiency, and autoimmune hemolytic anemia.[48,49] Unlike the severe but short-term anemia of infected immunocompetent individuals with hemolytic anemia, parvovirus-infected immunodeficient patients may develop chronic anemia in spite of normal red cell survival due to a prolonged, chronic infection. These patients may remain transfusion-dependent for up to 12 months.[50,51] Parvovirus-related anemia has been described in patients with acquired immunodeficiency syndrome (AIDS), congenital immunodeficiencies, or chronic lymphocytic leukemia and in patients undergoing chemotherapy; in such immunocompromised patients, the myeloid to erythroid ratio may be preserved or even increased.[47,50]

The bone marrow in transient aplastic crisis typically shows marked erythroid hypoplasia or aplasia with preserved megakaryopoiesis and granulopoiesis. The few erythroid forms that are present are large, early precursors termed giant pronormoblasts; they may be identified in the bone marrow aspirate or biopsy specimen (Fig. 13.6). Viral inclusions usually are not identifiable by routine microscopy in the bone marrow of immunocompetent



• **Figure 13.6** Bone marrow findings in parvovirus infection. **A,** The bone marrow biopsy specimen shows a marked paucity of maturing erythroids; the rare erythroid forms present are giant pronormoblasts (hematoxylin and eosin [H&E] stain), which are positive for the erythroid marker glycophorin by immunohistochemistry *(inset)*. **B,** Giant pronormoblasts can also be identified in the bone marrow aspirate and exhibit prominent nucleoli (Wright-Giemsa stain). **C,** In immunosuppressed patients, eosinophilic intranuclear inclusions may be seen on routine histology within early erythroid forms (H&E stain). **D,** An immunostain for parvovirus highlights the inclusions and aids in confirming the diagnosis. (**B,** Courtesy Dr. Tracy George, University of New Mexico.)

individuals with transient aplastic crisis.[52] Transient aplastic crisis typically lasts only 7 to 10 days, but the anemia may be severe enough to require transfusions and can be fatal if not treated.[53] Parvovirus infection in patients with known underlying hemolytic anemia is in the differential diagnosis of acute-onset severe anemia in such patients and can be confirmed by PCR identification of virus in peripheral blood or serologic tests. In such a setting, bone marrow sampling is now rarely performed. The pathologist is more likely to encounter a bone marrow sample from an immunosuppressed patient with chronic anemia, in whom parvovirus infection may not be suspected.

In immunocompromised patients the bone marrow in parvovirus infection is usually hypercellular and commonly shows preserved erythropoiesis; bicytopenia and pancytopenia are more commonly observed in such patients than in immunocompetent patients.[54] Although giant pronormoblasts are infrequent, maturing erythroid forms contain prominent intranuclear eosinophilic inclusions with peripheral condensation of the nuclear chromatin.[50] These inclusions are better demonstrated in the bone marrow biopsy sample than in the aspirate. Fixation artifact may sometimes produce nuclear changes resembling parvovirus inclusions; however, in such cases the nuclear abnormalities are present in all hematopoietic lineages rather than being limited to the erythroid series.[50] Parvovirus infection can be confirmed by demonstration of viral protein on immunohistochemistry (see Fig. 13.6D) or of viral RNA sequences by in situ hybridization.[55] These confirmatory studies may be necessary because serologic studies (antiparvoviral immunoglobulin [Ig]M and IgG) may be negative due to immunosuppression or early-stage infection.[50] Moreover, only approximately half of immunocompetent infected patients have viral DNA detectable by PCR in blood.[54] Transmission electron microscopy also demonstrates electron-dense amorphous intranuclear material representing viral particles, but this technique is rarely required for diagnosis.[50]

Human Immunodeficiency Virus Infection

Bone marrow biopsies are performed in HIV-positive patients to evaluate cytopenias that may occur during the course of disease, to stage lymphomas and other neoplasms that develop, or to assess for the presence of suspected systemic infections (often evidenced by unexplained fevers). HIV infection causes changes in the bone marrow that appear to be independent of concurrent infections or the effects of antiretroviral or other drugs.[56] These changes most likely relate to both disruption of the bone marrow microenvironment and HIV infection of hematopoietic elements. Normal maturation of hematopoietic elements depends on cytokines secreted by local T cells and macrophages, both of which are affected by HIV.[57] HIV also has been shown to infect hematopoietic precursors, megakaryocytes, and macrophage-myeloid progenitors, potentially with direct effects on their maturation.[58,59] The retention of iron within histiocytes and the paucity of sideroblastic iron (so-called reticuloendothelial iron blockade) resembles that of anemia of chronic disease and may represent another mechanism of HIV-associated anemia. There is also increased collagen deposition in the bone marrow.[60] The changes observed in the bone marrow of HIV-positive patients are summarized in Box 13.3, and examples are shown in Fig. 13.7.

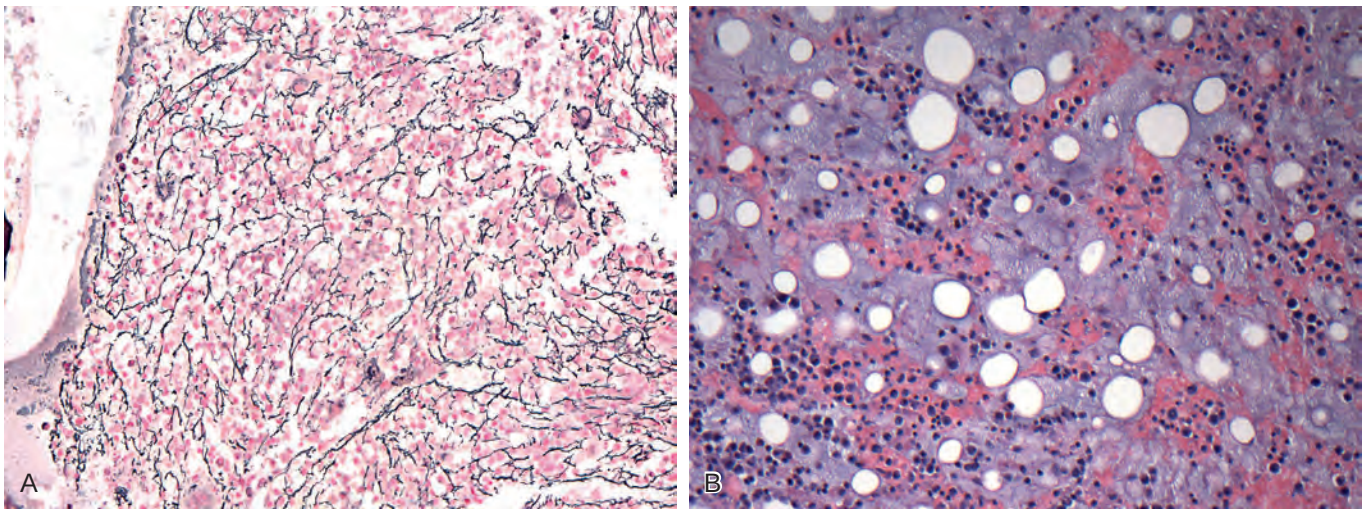
The morphologic changes in the maturing hematopoietic cells were termed "dysplastic" in older literature. Although these morphologic abnormalities overlap with some findings in MDS, they are reactive in nature and, unlike MDS, are not associated with increased risk of developing acute myeloid leukemia. In particular, there is no increase in myeloblasts, and dysplasia of the myeloid lineage (hypogranulation and pseudo–Pelger-Huët neutrophils) is uncommon in HIV infection.[11] Moreover, the megakaryocytic abnormalities differ from those of MDS: the megakaryocytes in HIV infection typically have hyperchromatic nuclei lacking discernable cytoplasm (bare or naked megakaryocyte nuclei) or with relatively scant cytoplasm, as opposed to the small megakaryocytes with round nuclei or with separated nuclear lobes that typify MDS (see Fig. 13.3B).[12,15,61] The morphologic "dysplasia" observed in HIV infection is associated with ineffective hematopoiesis and, analogous to the ineffective hematopoiesis of MDS, leads to cytopenias. Anemia is the most common manifestation; unlike most MDS cases which are macrocytic, HIV-associated anemias are usually normocytic.

Although HIV patients often receive drugs that can affect the bone marrow and cause cytopenias, the characteristic changes described here are observed independent of drug therapy, concurrent infection, or concurrent neoplasm and therefore are attributable to infection by HIV itself.[11,62] Moreover, abnormal maturation of erythroid and granulocytic elements is associated with anemia and leukopenia, respectively, as well as with more advanced AIDS. In contrast, megakaryocyte abnormalities are not associated with the occurrence of thrombocytopenia in HIV-positive patients.[11,62] Peripheral platelet destruction mediated by antiplatelet antibodies (idiopathic thrombocytopenic purpura) represents a common mechanism of thrombocytopenia in HIV-positive patients,[63] and antibodies to granulocytes also occur.[64] In the thrombocytopenic HIV-positive patient, the main role of bone marrow biopsy is to exclude an infectious or infiltrative process as a cause for the thrombocytopenia. When confronted with a bone marrow specimen from a cytopenic HIV-positive patient, provided that infections or neoplasms are excluded, the presence of the morphologic abnormalities described here implicates HIV infection (with or without the effects of any concomitant drugs) as the cause for the cytopenias.

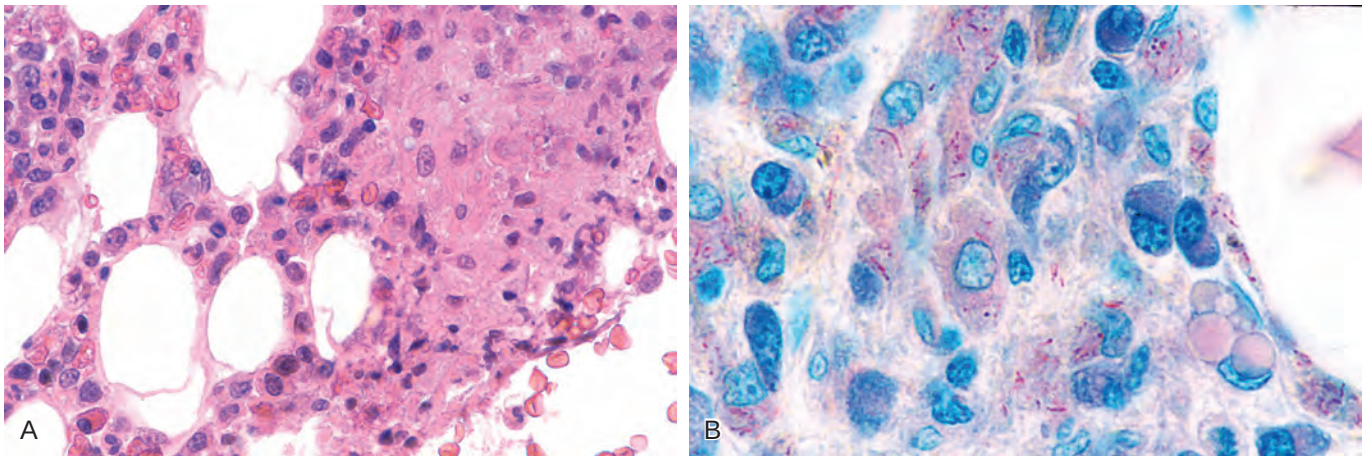
• BOX 13.3 Changes Commonly Described in the Bone Marrow of HIV-Infected Patients Directly Attributed to the Viral Infection[7,51,52]

1. Hypercellularity (majority of cases)
2. Hypocellularity (5%-15% of cases)
3. Serous atrophy* (10%-30% of cases)
4. Megakaryocyte abnormalities (clustering, bare nuclei, small size)
5. Erythroid lineage abnormalities* (left shift, megaloblastic changes, morphologic dysplasia); the erythroid lineage is the lineage that most often shows dysplastic changes
6. Myeloid lineage abnormalities* (left shift, nuclear hypolobation or hyperlobation, giant metamyelocytes)
7. Increased plasma cells
8. Lymphoid aggregates (often with admixed plasma cells and histiocytes)
9. Increased reticulin fibrosis*
10. Increased iron stores within bone marrow histiocytes*
11. Ring sideroblasts (rarely)
12. Granulomas (10%-15% of cases, not necessarily associated with identifiable opportunistic infections)

**Features that are associated with advanced acquired immunodeficiency syndrome (Centers for Disease Control and Prevention class IV-C).*



• **Figure 13.7** Examples of histologic changes in the bone marrow of human immunodeficiency virus–infected patients (in addition to morphologic dysplasia and abnormal megakaryocytes, shown in Fig. 13.3). **A,** Increased reticulin fibrosis (reticulin silver stain). **B,** Serous atrophy, usually seen in advanced infection (hematoxylin and eosin stain).



• **Figure 13.8** *Mycobacterium avium-intracellulare* infection involving the bone marrow of a human immunodeficiency virus–positive patient. **A,** Discrete granulomas often are not present, but histiocytes are increased and form some vague clusters (hematoxylin and eosin stain). **B,** Acid-fast bacilli (AFB) are identified in the cytoplasm of histiocytes (AFB stain).

Unexplained fever is a common clinical scenario in which a bone marrow biopsy is performed in HIV-positive patients; in such cases a combination of bone marrow biopsy and aspirate morphology with bone marrow culture yields a specific infectious agent in 30% to 40% of cases.[65,66] The use of acid-fast and fungal stains is important in this scenario because it often reveals an infectious agent more rapidly than cultures and has a similar diagnostic yield.[67] The most common organisms identified in the bone marrow of HIV-positive patients with unexplained fever in one study were *Mycobacterium avium-intracellulare*, *M. tuberculosis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*.[67] Lymphoid aggregates and increased interstitial T cells represent a nonspecific finding in HIV-positive patients and do not correlate with the stage of disease or the presence of opportunistic infections involving the marrow. The lymphocytes are predominantly T cells with varied CD4 to CD8 ratios. In contrast, granulomas in the bone marrow usually correlate with opportunistic infections; even in the setting of low $CD4^+$ counts, infectious agents can incite granuloma formation in the bone marrow of HIV-positive patients. However, in some cases, granuloma formation may be impaired. In these cases, organisms may be present in large numbers within the marrow, often within the cytoplasm of histiocytes. In particular, abundant intrahistiocytic mycobacteria can give the impression of pseudo-Gaucher cells due to the cytoplasmic striations (Fig. 13.8). The specific organisms commonly identified in infection of the bone marrow of HIV-positive patients and the associated morphologic patterns of marrow reaction are shown in Table 13.1.[70]

Mycobacterial cultures of blood and bone marrow are more sensitive at detecting systemic mycobacterial infections in HIV-positive patients than AFB stain of bone marrow, and AFB stain is positive in only approximately half of culture-positive cases.[71,72] For these reasons, some investigators have questioned the utility of special stains or even histologic examination of the

TABLE 13.1 Pathologic Patterns of Bone Marrow Opportunistic Infections in HIV-Positive Patients[57,60,68,69]

Infectious Agent	Pattern	Special Stain
Mycobacteria (*Mycobacterium tuberculosis* or *Mycobacterium avium-intracellulare*)	Granulomas Diffuse histiocytic proliferation with intracellular organisms	AFB and FITE
Histoplasma capsulatum	Diffuse histiocytic proliferation with intracellular organisms Occasionally granulomas	GMS or PAS
Cryptococcus neoformans	Granulomas	GMS or PAS
Leishmania	Diffuse histiocytic proliferation with intracellular organisms	Giemsa Direct examination of Wright-Giemsa–stained bone marrow aspirate
Pneumocystis jiroveci	Diffuse reticulin fibrosis Granulomas	GMS Immunohistochemistry
Cytomegalovirus	Viral inclusions	Immunohistochemistry

AFB, Acid-fast bacilli; *GMS*, Grocott methenamine silver; *PAS*, periodic acid–Schiff.

bone marrow in the workup of AIDS patients with suspected infections.[73] However, AFB staining (as well as PAS or Grocott methenamine silver [GMS] staining for suspected fungi) of a bone marrow biopsy sample allows a more rapid diagnosis of infection and prompt institution of therapy.[71,74,75] Acid-fast organisms may be detected in rare cases in which granulomas are lacking, so the absence of granuloma formation does not exclude mycobacterial infection in HIV-positive patients.[74] Conversely, granulomas or lymphohistiocytic aggregates may be present in the absence of any demonstrable infection or neoplasm.[72] Both fungal cultures and PAS or GMS stains are generally effective at detecting fungal infections, although fungal cultures are more sensitive.[71,72]

Aside from HIV-related bone marrow abnormalities and opportunistic infections, HIV patients undergoing bone marrow biopsy for fever or other nonspecific systemic symptoms may have a neoplasm. The most common neoplasms involving the bone marrow in HIV-positive patients are Burkitt lymphoma, diffuse large B-cell lymphoma, classical Hodgkin lymphoma, and Kaposi sarcoma. Lymphomas in HIV-positive patients are more likely to involve the marrow than lymphomas in immunocompetent patients, and they more commonly manifest with cytopenias and bone marrow infiltration lacking identifiable lymphadenopathy. For example, the bone marrow may be the initial site of presentation of HIV-positive patients with Hodgkin lymphoma.[76] The neoplastic cells in HIV-associated bone marrow lymphomas usually exhibit infection of the neoplastic cells by EBV (but not HIV).

Epstein-Barr Virus Infection

Bone marrow sampling is only rarely performed in infectious mononucleosis, particularly in the pediatric and young adult age group most commonly infected. However, given the rarity of infectious mononucleosis in adults (<3% of patients are >40 years old),[77] bone marrow sampling may be performed in older individuals with fever, constitutional symptoms, or atypical lymphocytosis, for which infectious mononucleosis is not seriously considered. Patients with infectious mononucleosis may also develop hemolytic anemia, neutropenia, or pancytopenia, prompting a bone marrow biopsy.[47] The bone marrow biopsy in infectious mononucleosis is typically normocellular or mildly hypocellular and frequently contains small lymphoid aggregates and small, nonnecrotizing granulomas.[78] It is unknown whether in situ hybridization for EBV-encoded RNAs (EBER) is positive in the bone marrow of infectious mononucleosis affecting immunocompetent patients.

Hemophagocytic syndrome (HLH) is a potentially fatal complication of EBV infection and is present in most cases of fatal infectious mononucleosis.[79] Fatal cases usually occur in patients with inherited or iatrogenic immunodeficiency but can occasionally occur in immunocompetent individuals. Patients usually develop pancytopenia, and the bone marrow often exhibits massive marrow necrosis and diffuse marrow histiocytic infiltrates in addition to hemophagocytosis.[68,79]

Cytomegalovirus Infection

CMV may produce an infectious mononucleosis–like picture similar to that of EBV infection in immunocompetent individuals, and in such cases the bone marrow findings are similar to those of infectious mononucleosis (discussed earlier); the CMV viral inclusions are often not detected. In immunosuppressed patients, the viral inclusions may be readily detected in the bone marrow endothelial cells and may precede positive antibody titers.[68] Viral inclusions can also be identified within the cytoplasm of circulating endothelial cells in the blood of infected immunosuppressed patients.[80] Small, nonnecrotizing granulomas and fibrin ring granulomas in the bone marrow have been associated with CMV infection in immunosuppressed patients.[27]

Brucellosis

Adults and children with brucellosis may present with pancytopenia eliciting bone marrow examination; fever and hepatosplenomegaly are commonly present concurrently.[81] The bone marrow

in patients with brucellosis is usually hypercellular (but can rarely be hypocellular) and shows nonnecrotizing granulomas or aggregates of histiocytes lacking giant cells; hemophagocytosis may be present.[82,83]

Rickettsial Infections

In Q fever the bone marrow characteristically shows nodular areas of fibrinoid necrosis, small epithelioid granulomas, and characteristic fibrin ring granulomas (see earlier discussion and Fig. 13.4). The organism typically cannot be demonstrated on microbacterial stains, and the diagnosis requires confirmation by serologic studies.[29,30]

Leishmaniasis

Visceral leishmaniasis is a parasitic disease endemic to the Mediterranean basin, but since the advent of AIDS, it has been seen more frequently in other geographic regions. *Leishmania* usually affects HIV-positive patients with advanced disease and is most common in AIDS patients in southern Europe, where it affects approximately 1% of AIDS patients over the course of their disease.[84] Visceral leishmaniasis also can affect immunocompetent infants and children.[84] Visceral leishmaniasis affects the liver, spleen, and bone marrow and is most commonly diagnosed by splenic aspiration and bone marrow sampling.[69]

In biopsy sections, the *Leishmania* amastigotes are present as extracellular organisms and also within histiocytes, occasionally associated with granulomas.[85] Direct examination of the bone marrow aspirate and Giemsa-stained bone marrow biopsy specimens for *Leishmania* amastigotes is an effective method of diagnosing visceral leishmaniasis, with a sensitivity of 90% (Fig. 13.9).[86] Bone marrow culture and serologic studies are somewhat less sensitive but do improve the diagnostic yield of morphologic examination.[84] PCR of peripheral blood or bone marrow has been shown to rapidly detect species-specific *Leishmania* in both HIV-positive and immunocompetent patients, with excellent specificity and sensitivity superior to bone marrow morphologic examination.[86]

Pneumocystis

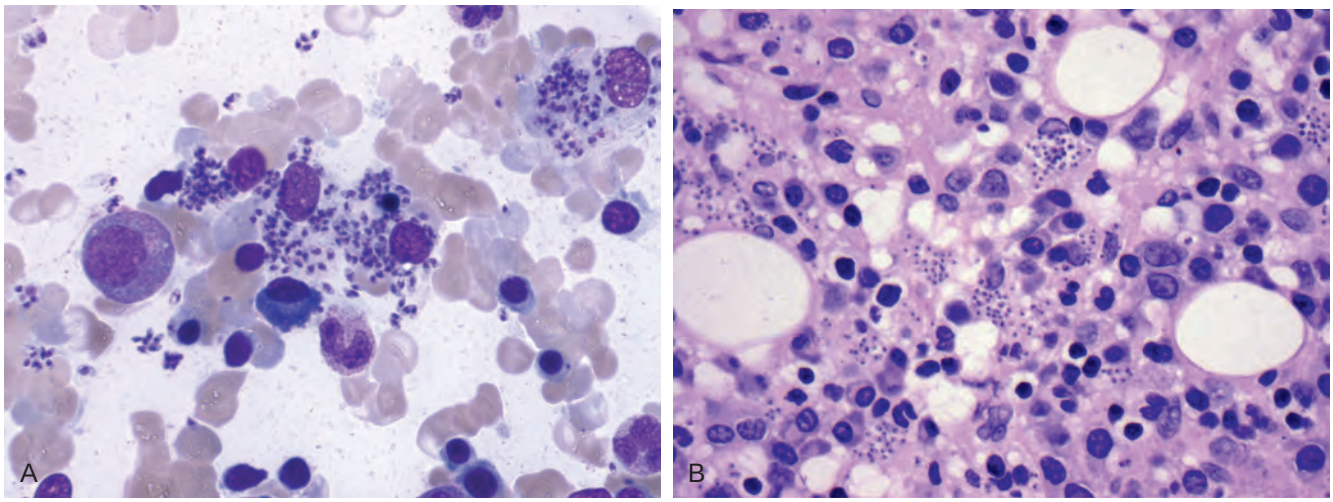
Pneumocystis jiroveci (formerly *Pneumocystis carinii*) only rarely involves the bone marrow, but marrow involvement has been reported in AIDS patients and in patients with immunosuppression due to chemotherapy.[87,88] The bone marrow may appear fibrotic or hypocellular, and interstitial amorphous, frothy eosinophilic material similar to that seen in the lung may be present.[87] The organism can be visualized by GMS stain of the bone marrow specimen, which reveals the characteristic cyst wall. Immunohistochemistry using a monoclonal antibody to *Pneumocystis* appears to be more sensitive than GMS stain in detecting extrapulmonary disease, and it stains the *Pneumocystis* trophozoites and cyst walls.[89,90]

Fungal Infections

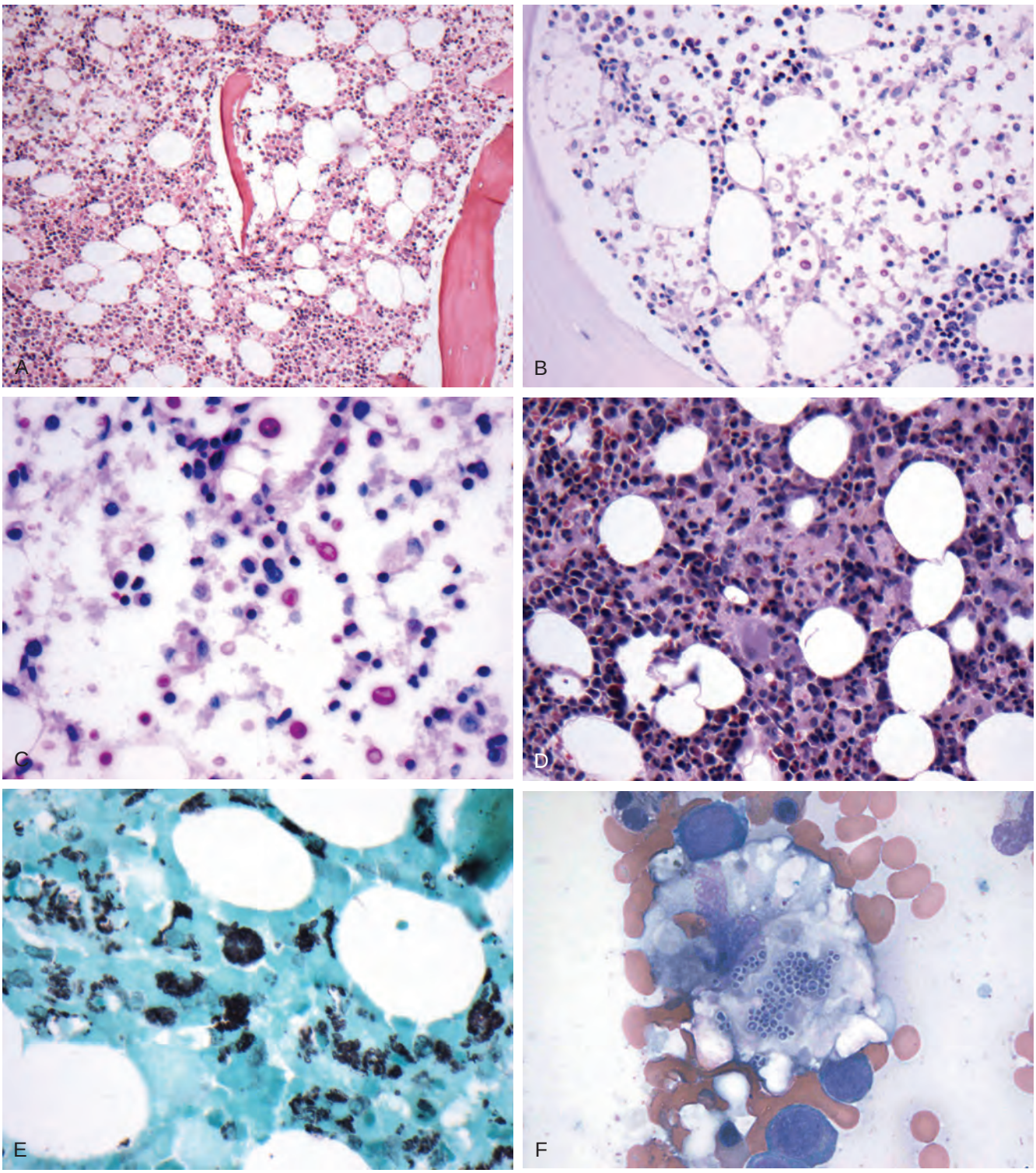
C. neoformans involves the bone marrow mainly in the setting of HIV infection[91] and may be the presenting manifestation of AIDS.[92] Patients usually present with concurrent cryptococcal meningitis and cytopenias.[93] The bone marrow almost always contains granulomas, but these may be poorly formed, and the bone marrow may also exhibit loosely cohesive histiocytic aggregates or gelatinous change (Fig. 13.10).[93] The cryptococcal organisms can be seen in biopsy sections on routine H&E staining; the capsule can be visualized by mucicarmine or PAS staining. Intrahistiocytic organisms can also be identified on Wright-Giemsa–stained aspirate smears. *H. capsulatum* involvement of the marrow is usually limited to immunosuppressed patients, but it may rarely be present in the marrow of immunocompetent individuals.[94] In rare cases, fungal forms of *Cryptococcus* or *Histoplasma* may be identified within megakaryocytes.[95]

Infections Involving the Spleen

The spleen has two major anatomic compartments, the red pulp and the white pulp. The red pulp comprises a network of vascular sinuses, blood vessels, and associated accessory cells, such as histiocytes, and serves largely as a filter and reservoir of blood



• **Figure 13.9** Bone marrow leishmaniasis. **A,** The *Leishmania* amastigotes in this aspirate smear are both extracellular and within histiocytes (Wright-Giemsa stain). **B,** Intrahistiocytic amastigotes can also be visualized on a thin trephine biopsy section (hematoxylin and eosin stain). (**A,** Courtesy Dr. Laurence de Leval, Lausanne, Switzerland.)



• **Figure 13.10** Fungal infections involving the bone marrow. **A,** In cryptococcal infection, well-formed granulomas may not be evident, but there is often a diffuse increase in histiocytes (hematoxylin and eosin [H&E] stain). **B,** On special staining of the bone marrow biopsy sample, the yeast forms are visualized (periodic acid–Schiff [PAS] stain). **C,** The yeast forms show characteristic narrow-based budding (PAS stain). **D,** In disseminated histoplasmosis involving the bone marrow, there is usually a diffuse proliferation of histiocytes (H&E stain). **E,** Special stains reveal the numerous intracellular and extracellular organisms; unlike *Leishmania*, the *Histoplasma* yeast forms are Giemsa negative (Grocott methenamine silver stain). **F,** Intracellular *Histoplasma* organisms may also be seen in the bone marrow aspirate smear (Wright-Giemsa Stain) (Courtesy Dr. Aashiyana Koreishi, Seattle).

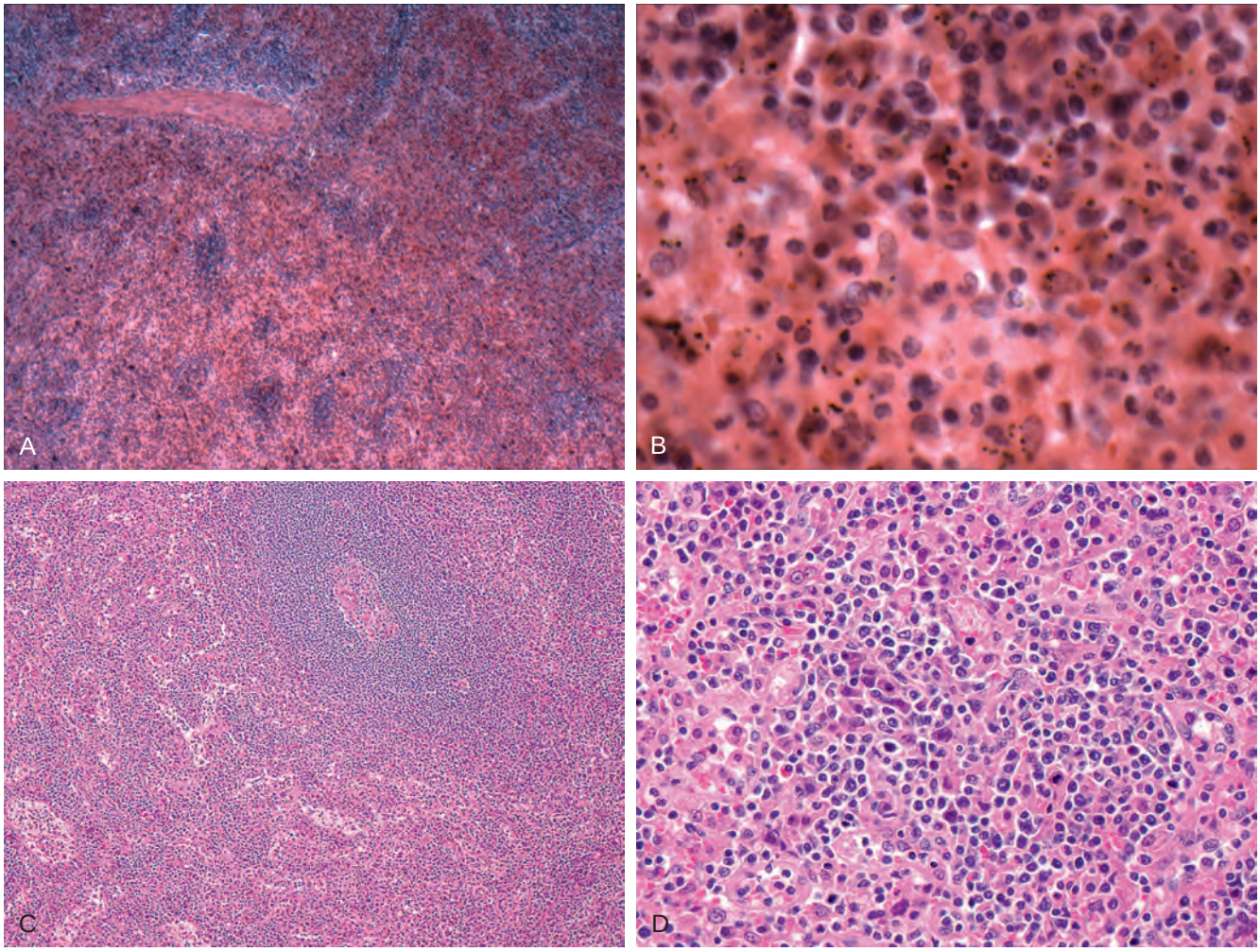
cells. The white pulp consists of specialized lymphoid tissue (B cells and T cells) organized around splenic arterioles. The splenic red pulp often manifests infections that involve the blood and bone marrow, whereas the white pulp typically reflects changes of infections similar to those observed in lymph nodes. Rare infections involving the spleen may form masses detected on radiologic studies.

Diffuse Red Pulp Reactions to Infection

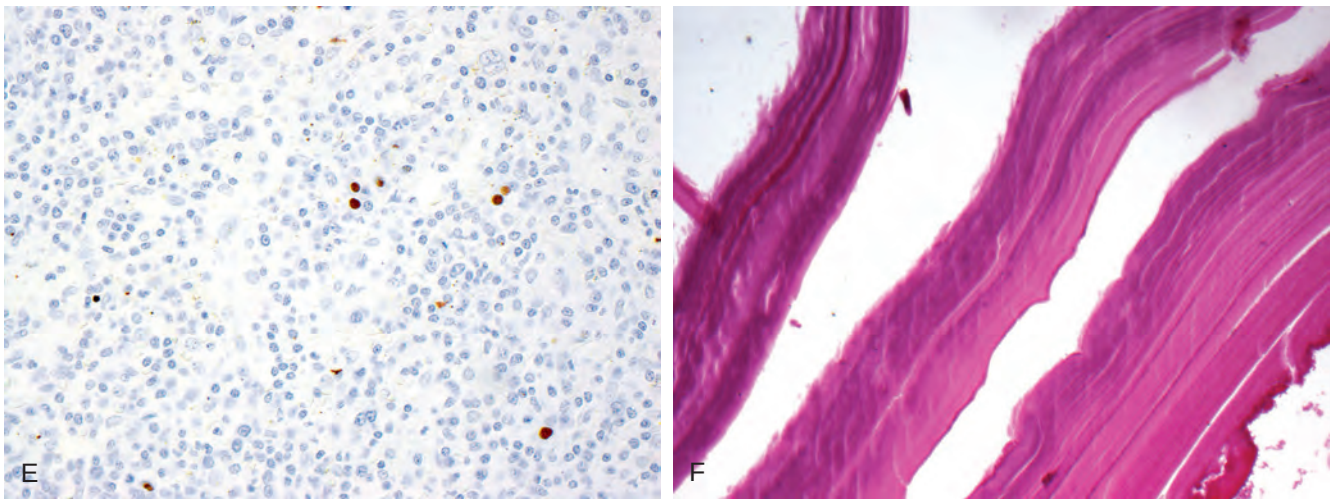
The term *splenitis* refers to massive infiltration of the red pulp sinusoids with neutrophils and is usually associated with typhoid fever; it is only rarely seen in typical bacterial infections occurring in the Western world. The spleen in sepsis is often enlarged, with red pulp hyperplasia caused by increased activated macrophages. Postmortem autolysis of the macrophages can lead to massive lysis of the red pulp, as observed at autopsy in patients dying of sepsis. However, massive red pulp autolysis at autopsy is not specific to sepsis and can result from splenomegaly of any cause.[96]

Splenic red pulp macrophages filter red blood cell debris and remove *Plasmodium* parasites from red cells passing through the sinusoids in malaria patients. The spleen is almost always enlarged in such cases; in a series of patients dying of malaria in Vietnam, the median spleen weight was 300 g.[97,98] Malaria is a common cause of splenic rupture.[99] Spleens affected by malaria show expansion of both red and white pulp, with red pulp congestion and disrupted B- and T-cell architecture in the white pulp (Fig. 13.11).[98] Chronic malaria infection may produce massive splenomegaly, known as the tropical splenomegaly syndrome or hyperreactive malarial splenomegaly. This is an important differential diagnosis of massive splenomegaly in areas endemic for malaria.[100] The median spleen weight in hyperreactive malarial splenomegaly is greater than 3 kg, and patients are cytopenic due to hypersplenism. On histology, the affected spleen shows marked sinusoidal dilatation with foci of extramedullary hematopoiesis.[101]

The spleen in hemophagocytic syndromes is enlarged, with prominent erythrophagocytosis observed in the red pulp; the red



• **Figure 13.11** Infections involving the spleen. **A,** The spleen in malaria shows disorganization of the white pulp and red pulp congestion (hematoxylin and eosin [H&E] stain). **B,** High-power view of a spleen from a patient with malaria. Malarial pigment (hemozoin) is visible within histiocytes in the red pulp cords (H&E stain). **C,** Splenectomy due to spontaneous splenic rupture in a patient with infectious mononucleosis; there is increased cellularity of the red pulp (H&E stain). **D,** High-power view of the red pulp shows a polymorphous admixture of small lymphocytes, immunoblasts, and plasma cells (H&E stain).



• **Figure 13.11, cont'd** **E,** In situ hybridization for EBV-encoded RNAs (EBER) is positive in scattered cells within the red pulp. **F,** The wall of an echinococcal (hydatid) cyst is acellular and eosinophilic with a characteristic lamellated appearance (H&E stain).

pulp histiocytes also contain intracellular neutrophils and lymphocytes. If the hemophagocytic syndrome is secondary to lymphoma, the spleen may be concomitantly involved by the lymphoma, particularly peripheral T-cell lymphomas.[102]

The spleen is often enlarged in infectious mononucleosis and may rupture spontaneously or with minimal abdominal trauma, representing a rare but potentially fatal complication of the disease.[103] Although infectious mononucleosis is associated with lymphadenopathy and usually does result in splenic white pulp hyperplasia (see later discussion), the splenic red pulp shows more prominent expansion. The red pulp cords and sinuses contain numerous immunoblasts with prominent nucleoli (see Fig. 13.11C-E).[104] Some cells may resemble Reed-Sternberg cells, mimicking Hodgkin lymphoma, and frequent large nucleolated immunoblasts may also mimic a non-Hodgkin lymphoma. EBER staining and a clinical history of infectious mononucleosis are helpful in avoiding misdiagnosis as lymphoma. EBV infection may also be associated with hemophagocytic syndrome in the spleen.

White Pulp Reactions to Infection

Follicular hyperplasia is the most common white pulp reaction that can occur as an incidental finding or as a nonspecific finding secondary to a variety of inflammatory or infectious conditions. Hyperplasia of the white pulp without prominent germinal centers can also occur in infectious mononucleosis and other viral infections, such as herpes simplex and HIV infection.[102,105]

Mass-Forming and Cystic Splenic Infections

Mycobacterial infections in HIV-positive patients may produce solid spindle cell nodules within the spleen. Special stains to demonstrate the mycobacteria within the spindle-shaped histiocytes are helpful in avoiding misdiagnosis as a splenic inflammatory pseudotumor or sarcoma.[106] Infections involving the spleen that manifest as a splenic cyst are uncommon. Splenic abscesses are rare in developed countries and usually occur in the setting of bacterial endocarditis, immunosuppression, intravenous drug use, or splenic trauma.[107,108] Bacterial agents, such as *Staphylococcus* or *Streptococcus* species, are the most common pathogens.[109] The diagnosis may be made with splenic aspiration or splenectomy and microbiologic cultures, but splenectomy is recommended for successful therapy.[107]

Hydatid cysts due to *Echinococcus granulosus* infection can rarely occur in the spleen. They manifested as a splenic cyst in only 3% of cases in one large series (see Fig. 13.11F).[110]

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14

Infection of Bone

ANDREW E. ROSENBERG, SUSAN V. KATTAPURAM, G. PETUR NIELSEN

Pathophysiology

Bacterial Osteomyelitis
- Bacterial Osteomyelitis From Hematogenous Spread
- Bacterial Osteomyelitis From Contiguous Spread
- Bacterial Osteomyelitis From Direct Inoculation

Mycobacterial Osteomyelitis
- Tuberculous Osteomyelitis
- Nontuberculous Mycobacterial Osteomyelitis
- Lepromatous Osteomyelitis

Treponemal Osteomyelitis
- Skeletal Syphilis
- Yaws Bone Infection

Fungal Osteomyelitis
- Candida Osteomyelitis
- Aspergillus Osteomyelitis
- Cryptococcal Osteomyelitis
- Sporotrichosis Osteomyelitis
- Coccidiomycosis Osteomyelitis
- Paracoccidioidomycosis Osteomyelitis
- Histoplasmosis Osteomyelitis
- Blastomycosis Osteomyelitis

Mycetoma

Helminth Osteomyelitis
- Echinococcal Osteomyelitis

Viral Osteomyelitis
- Variola Osteomyelitis
- Rubella Osteomyelitis
- Other Types of Viral Bone Infection

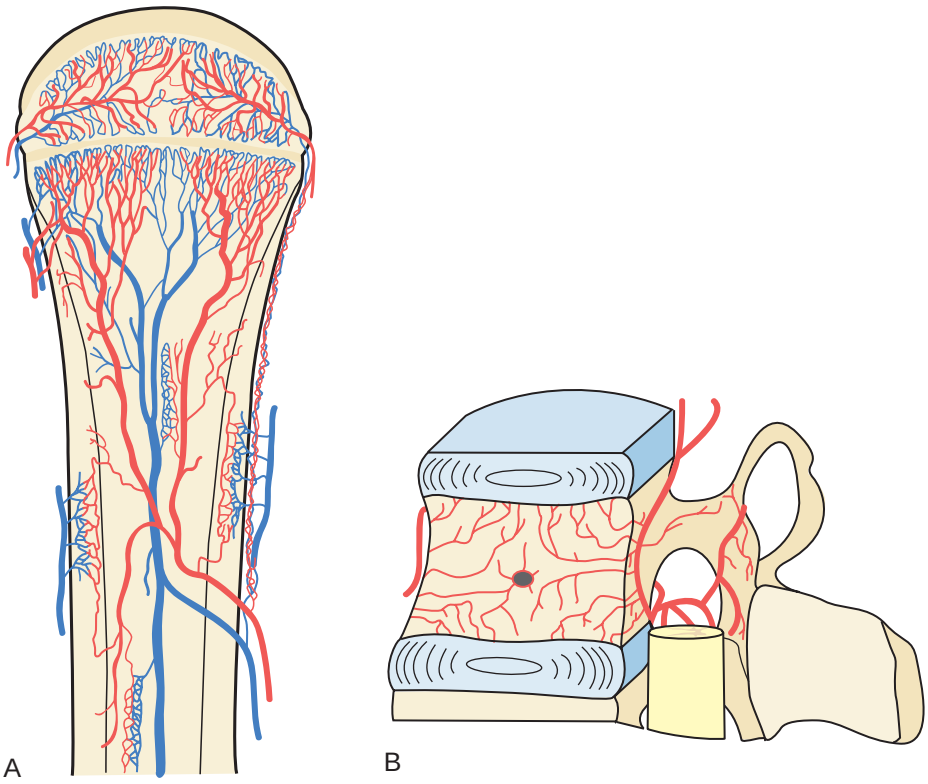
Differential Diagnosis of Osteomyelitis

Osteomyelitis is defined literally as inflammation of bone and marrow, albeit, the common use of the term virtually always implies infection. Infection of the skeleton, the largest organ system of the body, is important because it is a major site of microbial infestation that is often morbid and sometimes fatal. All types of organisms, including bacteria, mycobacteria, fungi, viruses, and parasites, can cause osteomyelitis, but infections associated with certain pyogenic bacteria and mycobacteria are the most common. The microbes and their interplay with the host produce a wide spectrum of clinical manifestations that are further influenced by the causative event, bone involved, specific pathogen and its virulence, medical condition of the host, type of therapy, and clinical course of the disease.[1] Many of the molecular mechanisms underlying these biologic interactions have been elucidated, and this knowledge has facilitated advances in the stalwarts of therapy, namely, surgery and antibiotics. Regardless, the complexities and problems in diagnosing and treating osteomyelitis have become ever more challenging because of the increase in the numbers of older adults and joint replacements, widespread prevalence of diabetes, high frequency of antibiotic-resistant pathogens, and expanding population of immunocompromised patients and their exotic opportunistic infections.

Pathophysiology

The fundamental biologic processes active in bone infection are independent of pathogen type. Entry of the organism into bone is the first step in osteomyelitis and occurs by three main mechanisms: (1) hematogenous seeding, (2) extension from a contiguous site, and (3) direct implantation (Box 14.1).

Hematogenous osteomyelitis is usually caused by bacteria and most commonly affects rapidly growing long bones. In children, it likely results from occult bacteremia associated with seeding of a site susceptible to bacterial invasion.[2] It frequently presents as a primary solitary focus of disease but, importantly, may be a complication of any localized or systemic infection. Within a long bone, the anatomic site infected depends on whether the patient is an infant, child, or adult, and the relationship between age and localization is based on the vascular anatomy of the bone. Briefly, the nutrient artery supplies the majority of blood flow to bone, and during growth and development after the first year of life, its terminal branches end as capillaries at the base of the growth plate (Fig. 14.1A and B). As the capillaries approach the epiphyseal plate, they reverse direction by forming a loop and then merge into a network of sinusoidal venous blood lakes, which in turn empty into the venous drainage system of the medullary cavity. The blood flow in the capillary-sinusoid loop region is sluggish and turbulent, and phagocyte function is suboptimal; together, these features facilitate deposition of the organism into the metaphyseal tissue.[3,4] In infants younger than 1 year, some of the terminal branches of the nutrient artery penetrate the growth plate, and as they end near the base of the articular cartilage their diameter expands, forming large venous lakes resembling metaphyseal sinusoids.[3,4] Thus in neonates, microbes may settle in the epiphysis or quickly spread into this region from a metaphyseal focus of disease.[5] In neonates and children, transphyseal blood vessels facilitate direct extension into the adjacent joint because



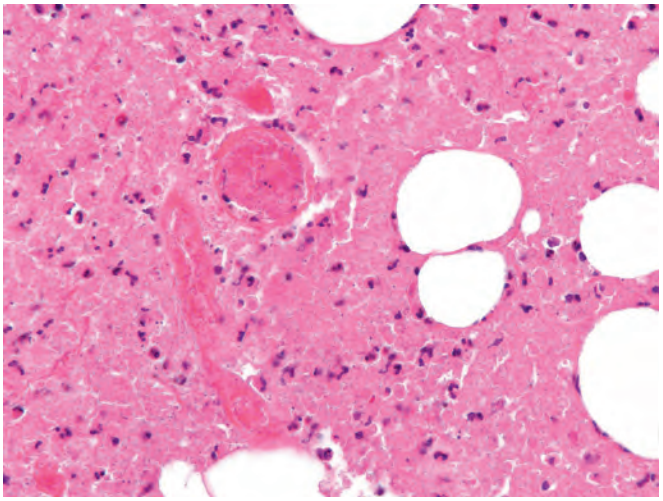
• **Figure 14.1** **A,** Vascular supply to a long bone in a child. The nutrient artery arborizes to small capillary loops at the base of the growth plate. **B,** Vascular supply to vertebral body. The subchondral region has the richest capillary network.

• BOX 14.1 Routes of Entry of Infection Into Bone

Hematogenous spread
Contiguous extension
Direct implantation

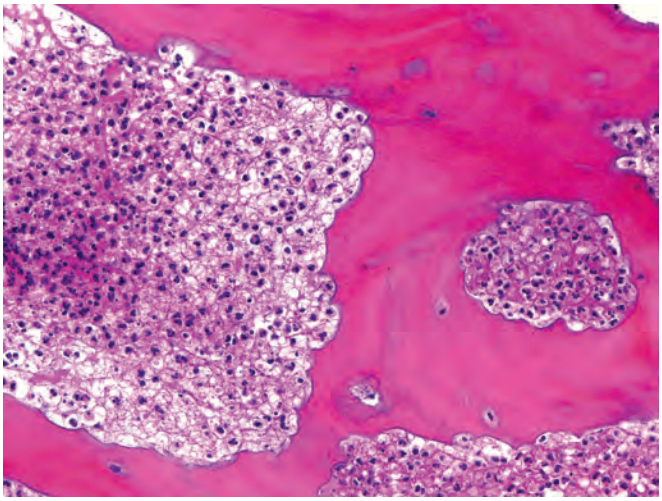
the metaphysis is intra-articular in this age group.[2] In adults, after closure of the growth plate, the metaphyseal and epiphyseal vessels establish reconnections so bacteria entering the nutrient artery are directed to the vascular loops beneath the articular cartilage. Accordingly, acute hematogenous osteomyelitis in infants and adults often affects the epiphysis, whereas in children the growth plate acts as an effective barrier and the infection is usually limited to the metaphysis.

After microbes seed the tissue, they adhere to the organic matrix or invade into cells, where they reproduce, forming growing colonies. The enlarging morass of organisms can obstruct blood flow, causing thrombosis and infarction, which creates an environment conducive to further propagation. Structural elements of the microbes or their secreted by-products that are detected by the host trigger the innate and adaptive immune responses, which may vary according to the type of microbe. After being initiated, the immunologic reaction causes the contaminated area to become inundated with fluid, fibrin, degranulated platelets, extravasated red blood cells, and inflammatory cells (Fig. 14.2).[6] This phlegmon and developing abscess increases intraosseous pressure, which forces the inflammatory reaction through the medullary cavity and into haversian systems and Volkmann canals, causing necrosis



• **Figure 14.2** Early stage of acute osteomyelitis with thrombosis, neutrophils, fibrin, platelets, hemorrhage, and necrosis.

of sometimes a sizable segment of bone—the devitalized bone is known as a sequestrum (Fig. 14.3). In infants and young children, the periosteum is not tightly bound to the cortex, so it may be mechanically lifted off the bone surface over large distances, disrupting periosteal feeding vessels and significantly enlarging the region of bone necrosis. The pus also may perforate the periosteum and extend into neighboring soft tissues, forming an abscess that can burrow to the skin surface, creating one or more sinus tracts that drain discolored exudates and necrotic tissue. Lastly, the pus



• **Figure 14.3** Sheets of neutrophils filling haversian systems. The bone is viable but shows scalloping of the surfaces indicative of previous osteoclastic activity.

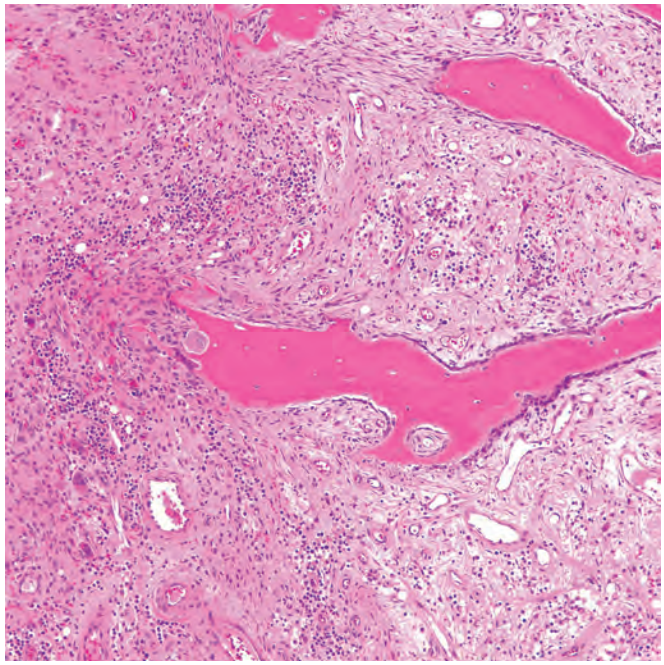
• BOX 14.2 Cytokines and Molecules Implicated in Stimulating Bone Cells in the Immune Response to Infection

RANKL	Interleukin-8
RANK	Interleukin-10
OPG	Interleukin-12
Macrophage colony-stimulating factor	Interleukin-15
Tumor necrosis factor	Interleukin-17
Interleukin-1	Interleukin-18
Interleukin-6	Interleukin-23
Interleukin-7	Interferon

OPG, *Osteoprotegerin;* RANK, *receptor activator of nuclear factor-κB;* RANKL, *receptor activator of nuclear factor-κB ligand.*

may extend into the epiphysis, where it can penetrate the articular surface or spread along capsular and tendoligamentous insertions into the joint. Consequently, septic or suppurative arthritis develops that can be associated with severe destruction of the articular cartilage and eventual permanent disability. This complication most frequently affects articulations whose joint capsules insert into cortices beyond the confines of the epiphysis.

As the acute phase intensifies over a period of several days to weeks, the inflammatory cells and their released enzymes and cytokines (Box 14.2) form a biologic broth that has powerful effects on the resident cells and biomaterials that compose the bone tissue. Accordingly, osteoclasts are generated, and in this setting their numbers and activity are more extreme than in any other disease process. The osteoclasts target and resorb necrotic bone, thereby creating space for the expanding inflamed granulation tissue (Fig. 14.4). The evolving response leads to the arrival of lymphocytes, plasma cells, macrophages, collagen-producing fibroblasts, and newly formed blood vessels, which herald the so-called chronic phase of osteomyelitis. Mesenchymal stem cells located in the medullary cavity, cortex, and periosteum are stimulated simultaneously to produce osteoblasts that manufacture and secrete newly formed viable bone, usually woven in architecture. The living bone forms a subperiosteal shell, known as the involucrum that envelops the necrotic cortical sequestrum, as well as a

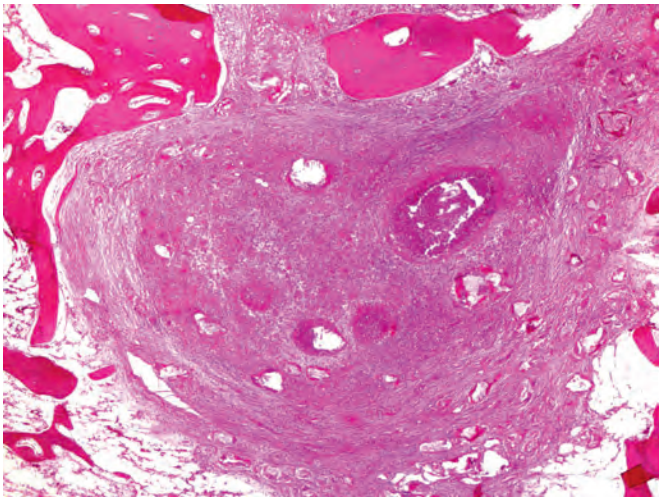


• **Figure 14.4** Acute and chronic osteomyelitis with inflammation filling the medullary cavity. Osteoclasts are resorbing the preexisting trabecular bone.

• **Figure 14.5** Medullary cavity filled with inflammation extending through the necrotic cortex (sequestrum) and associated with abundant viable subperiosteal reactive bone (involucrum).

zone of sclerosis around the intramedullary extent of infection (Fig. 14.5). Over time, bone remodeling continues and the nidus of infection may become walled off by the host reaction, forming an intraosseous abscess, also known as a Brodie abscess (Fig. 14.6).[7] Some infections, especially those in the jaw bones, are associated with extensive new bone formation that obscures much of the underlying osseous structure, and this form of disease is known as sclerosing osteomyelitis of Garré.

Hematogenous osteomyelitis also affects other portions of the skeleton. More common locations include metaphyseal equivalent sites in long bones (adjacent to the femoral trochanters and tibial tuberosity) and flat and irregular bones (ilium adjacent to the triradiate cartilage, ilium adjacent to the acetabular roof, ilium



• **Figure 14.6** Brodie abscess composed of an intraosseous abscess walled off by reactive bone.

adjacent to the anterior inferior spine, ilium adjacent to the sacroiliac joint, and ilium adjacent to the crest, etc.).[8] The organisms preferentially seed these regions leading to an inflammatory reaction and bone destruction.

In the spine, blood-borne pathogens usually localize to the subchondral regions of the vertebral body. These areas have the richest vascular supply and are fed by branches of the spinal artery and drained by a venous plexus.[9] The proliferating organisms and inflammatory reaction digest and transgress the hyaline cartilage end plate and extend into and destroy the intervertebral disk. Spread to adjacent vertebral bodies is common. If the inflammatory process violates the periosteum, inflammation can accumulate in the surrounding soft tissue, such as the epidural space or along or in the iliopsoas and paravertebral muscles.

Osteomyelitis resulting from contiguous spread occurs after organisms from a neighboring anatomic site gain direct access to the bone. This may affect any bone adjacent to a soft tissue abscess and is more commonly seen in the setting of periodontal and sinus disease, cellulitis of diabetic feet, epidural abscess, decubitus ulcer, or septic arthritis. In these situations the inflammatory process must first destroy the periosteum or articular surface before it accesses the bone. Unlike hematogenous osteomyelitis, the cortex instead of the medullary cavity is initially infected, and the site at which this occurs influences the rapidity of spread and extent of disease.

Direct implantation of pathogens into bone takes place in a variety of circumstances. Common scenarios are contamination of an open fracture, penetration of fomites, such as shrapnel or orthopedic hardware into bone, or bone that is exposed during surgery. In these situations the causative event and the extent of associated hemorrhage, tissue fragmentation, and necrosis influence important aspects of the infection.[10]

The balance between the biologic characteristics of the pathogen and host determines the extent of the osseous manifestations of the infection and the ability of the host to confine and eradicate the organism. All too frequently, the pathogens remain viable and continue to proliferate in an environment of necrotic tissue inaccessible to the immune response and therapeutic antibiotics. In this fashion a smoldering chronic infection is generated, which maintains the potential to cause future havoc when conditions are permitting.

Bacterial Osteomyelitis

Bacterial infection of the skeleton accounts for the vast majority of microbial diseases of bone. It causes a variety of different clinicopathologic conditions that are related to the mode of infection, anatomic site, type of organism, and condition of the host. Approximately 20% of cases of bacterial osteomyelitis are hematogenous in origin, with the remainder secondary to contiguous spread and direct inoculation.

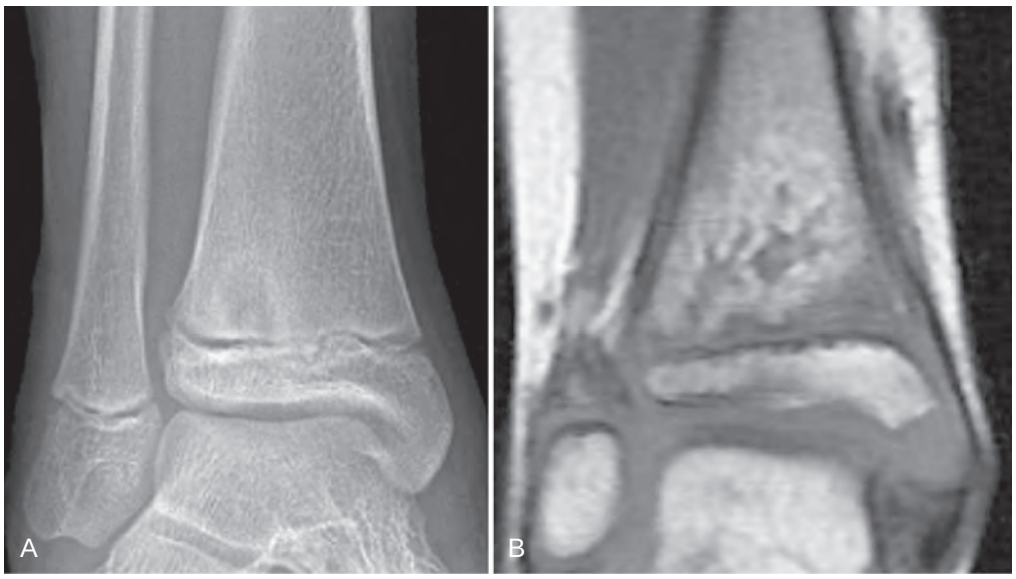
Bacterial Osteomyelitis From Hematogenous Spread

Bacterial hematogenous osteomyelitis is typically acute and most often develops in children. Indeed, in 50% of cases the patients are younger than 5 years, and in more than 85% of cases the patients are 16 years or younger. The annual incidence has remained relatively constant over the past several decades and is estimated to affect 1 in 5000 to 10,000 children annually.[2,11] Males are affected almost twice as frequently as females. Predisposing factors include indwelling catheters; infections of the skin, throat, ears, lung, heart, and genitourinary tract; nonpenetrating trauma; and such underlying diseases or conditions as diabetes, renal failure, hemoglobinopathies, and immunosuppression. More often than not the host is healthy and the source of the bacteria is obscure with the initiating bacteremia possibly complicating trivial occurrences, such as occult injury to the intestinal mucosa during defecation, vigorous chewing of hard foods, or minor infections of the skin.

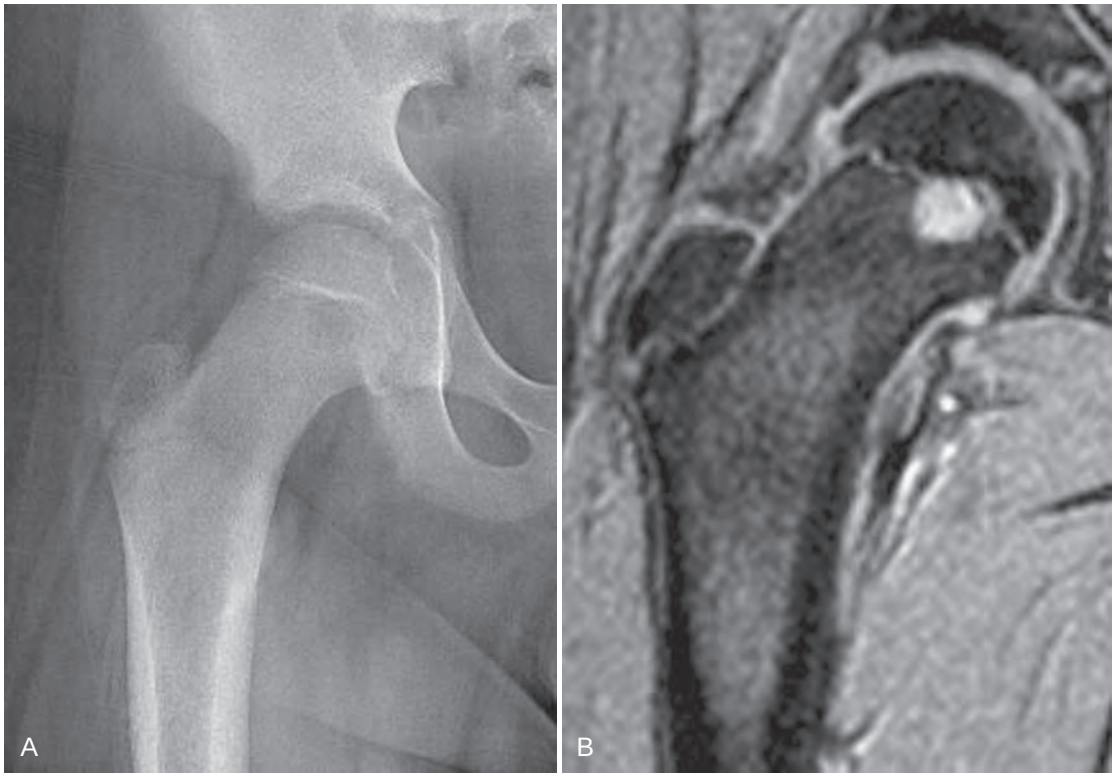
The bones most commonly affected in children are the long tubular bones of the extremities, especially the femur (36%), tibia (33%), and humerus (10%).[11] In most instances the infection involves a single osseous site, but in 5% to 20% of cases it is multifocal in distribution.[12] In adults the spine is most commonly involved, accounting for 2% to 4% of all bone infections. Most cases originate in the lumbar vertebrae (58%), followed by the thoracic (30%) and cervical vertebrae (11%).[13,14] The spine is also the most frequent site of infection in intravenous drug abusers. In patients with sickle cell disease, the osteomyelitis usually occurs in the long bones, followed by the vertebrae.[15,16]

Clinically, hematogenous osteomyelitis may manifest as an acute systemic illness with malaise, fever, chills, leukocytosis, elevated sedimentation rate and C-reactive protein level, and edema, swelling, warmth and marked-to-intense throbbing pain over the affected region. The presentation may be more subtle with only unexplained fever, particularly in infants, or only localized pain in the absence of fever in the adult.

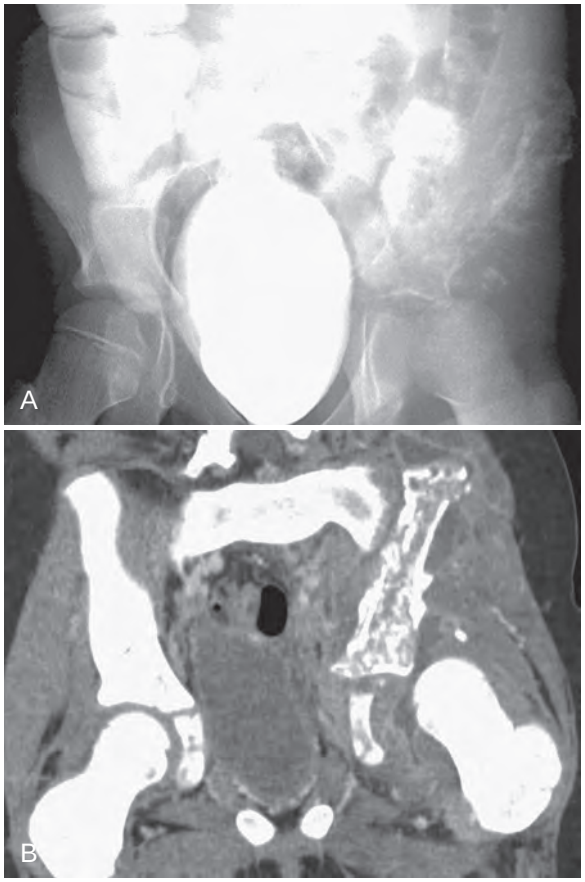
Imaging studies reveal changes that vary with the stage of infection. Technetium bone scan becomes positive within 24 to 48 hours after the development of symptoms. X-rays may reveal deep soft tissue swelling after several days; however, it takes 10 to 21 days for changes to manifest in bone because 30% to 50% of the osseous tissue must be resorbed before the infection becomes apparent on plain films.[17] The focus of infection first presents as a poorly defined area of radiolucency in the metaphysis that increases in breadth over time and in children may extend to the growth plate by an irregular channel (Figs. 14.7A, 14.8A, and 14.9A). Subsequently, reactive new bone is deposited both beneath the raised periosteum and within the medullary canal at the boundary of disease. Extraosseous spread may be associated with a cortical cloaca and a contiguous abscess (Fig. 14.10). Computed



• **Figure 14.7** Acute osteomyelitis of distal tibia. **A,** Anteroposterior radiograph shows poorly defined area of osteolysis with minimal surrounding sclerosis in the distal tibial metaphysis. **B,** Coronal T1 magnetic resonance imaging demonstrates irregular dark area composed of inflammatory tissue surrounded by a dark zone of edema adjacent to the plate medially.



• **Figure 14.8** Acute osteomyelitis of proximal femur. **A,** Anteroposterior x-ray showing central area of bone lucency adjacent to the growth plate. **B,** The area is very bright on the coronal T2-weighted magnetic resonance imaging.



• **Figure 14.9** Acute and chronic osteomyelitis of pelvis. **A,** Anteroposterior radiograph of left pelvis in a child showing marked destruction of bone with irregular periosteal reactive bone. **B,** Coronal computed tomography illustrating extensive bone lysis and new bone formation and multiple low-density areas (abscesses) in the gluteal and iliopsoas muscles. There is a joint effusion.

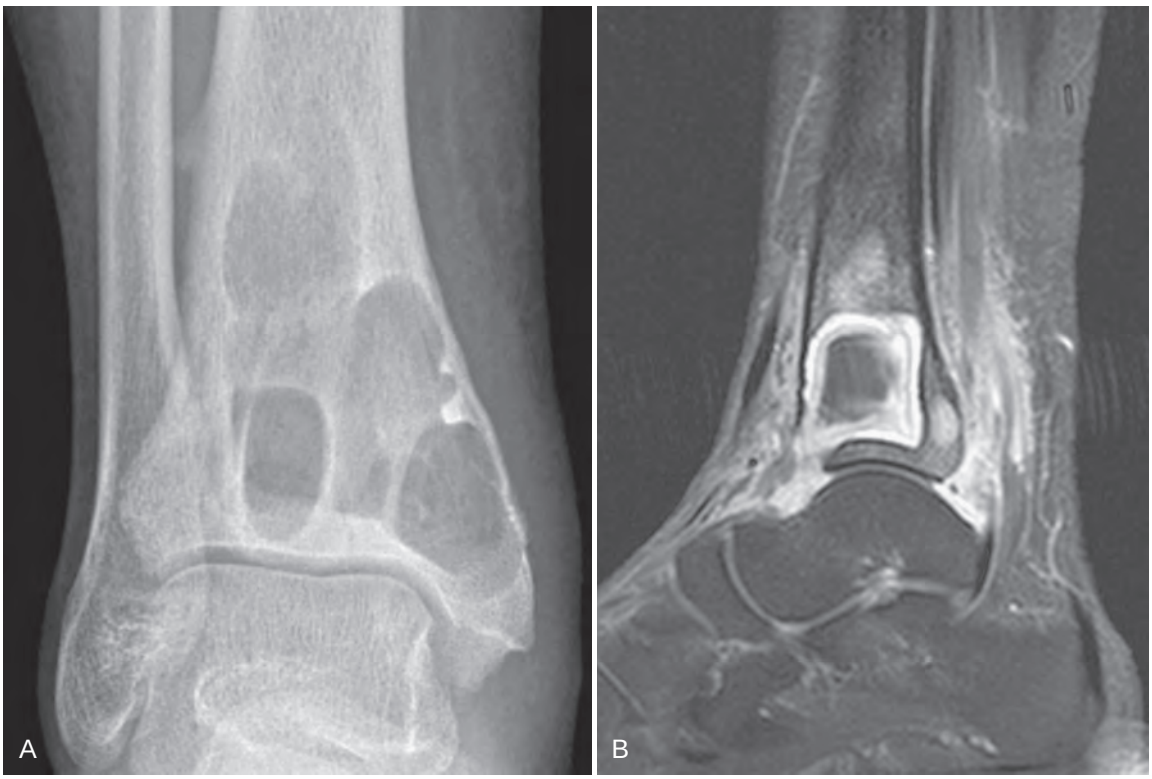
• **Figure 14.10** Acute and chronic osteomyelitis with soft tissue abscess and sinus tract. **A,** Lateral radiograph of the distal femur shows large area of lysis and sclerosis. Irregular subperiosteal new bone deposition is present. **B,** Sagittal reformatted image from computed tomography scan demonstrates marked bone destruction and sclerosis. Cortical cloaca is present which is adjacent to the soft tissue abscess sinus tract. **C,** Axial T2 magnetic resonance imaging illustrates the intraosseous inflammation, which extends into the soft tissues through the sinus tract and exits through the skin.

tomography (CT) scans more precisely delineate the presence of gas, extent of bone destruction, the formation of sequestra and involucra, and soft tissue involvement (see Figs. 14.9B and 14.10B).[17] Magnetic resonance imaging (MRI) detects edema and the inflammatory changes 3 to 5 days after the beginning of infection. It does not demonstrate the bony changes as well as CT but is better in revealing the extent of disease, the presence of intraosseous and soft tissue abscesses, zone of surrounding edema, and joint effusions and joint involvement (see Figs. 14.7B, 14.8B, and 14.10C).[17] If confined over time an intraosseous abscess (Brodie abscess) may develop and can mimic a neoplasm (Fig. 14.11).

Vertebral infection usually localizes to the anterior subchondral portion of the vertebral body in close proximity to the intervertebral disc. Within several weeks the disc becomes involved and is associated with loss of height and blurring of the normal contour of the subchondral bone plate. Progressive destruction of the bone and disk ensues, and the neighboring vertebrae and soft tissue may become involved (Figs. 14.12 to 14.14).

Pathologically, bacterial infection usually elicits a suppurative inflammatory response with edema, fibrin, hemorrhage, and necrosis. Necrosis of bone manifests as osseous tissue with empty lacunae, and in the setting of infection many of its surfaces are either covered by osteoclasts or are pockmarked by Howship lacunae (Fig. 14.15). Colonies of bacteria may be found in the inflammatory infiltrate and necrotic tissue (Fig. 14.16). After several weeks the marrow becomes replaced by reactive fibrovascular tissue and the inflammatory infiltrate becomes enriched with lymphocytes, plasma cells, and macrophages (Fig. 14.17). In some cases the plasma cells are so numerous (plasma cell osteomyelitis) they can cause confusion with plasma cell neoplasms (Fig. 14.18).[18] Intraosseous abscesses consist of granulation tissue admixed with inflammatory cells. The reactive bone that forms is frequently woven and is deposited beneath the periosteum and within the medullary cavity de novo or on the surfaces of preexisting living or necrotic trabecular bone (see Fig. 14.5).

Importantly, some bacteria are associated with granulomatous inflammation, including multinucleated Langhans-type giant



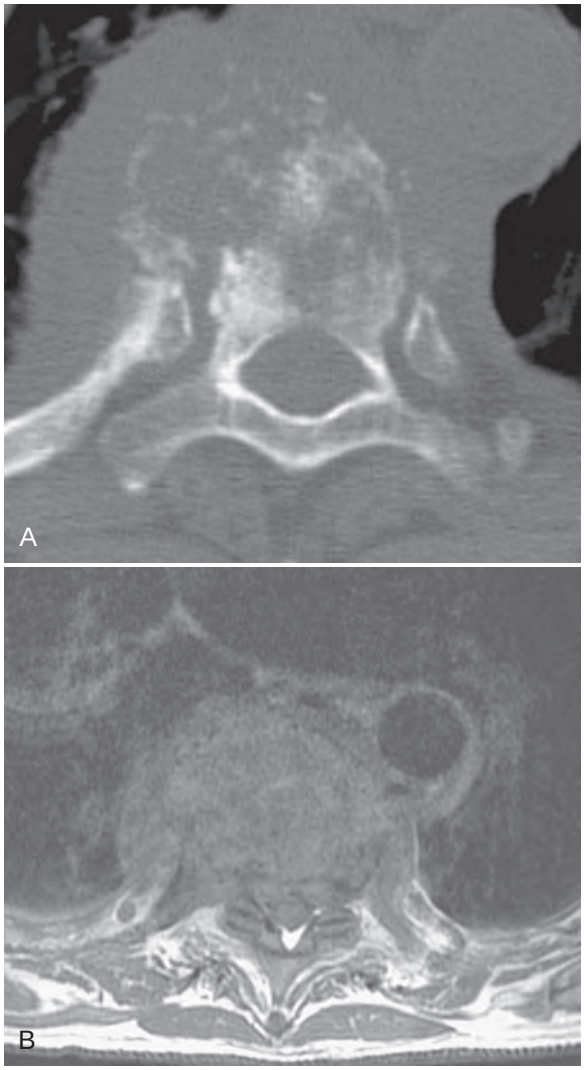
• **Figure 14.11** Brodie abscess. **A,** Anteroposterior view of the ankle shows a multiloculated lucent lesion in the distal tibia. The margins are fairly well defined. No internal matrix is visible. **B,** Sagittal, contrast enhanced T1-weighted sequence shows a characteristic appearance with a nonenhancing low signal intensity central area (abscess) surrounding hyperintense granulation tissue. There is also enhancement of the surrounding marrow and the synovium.

• **Figure 14.12** Osteomyelitis of spine. Lateral x-ray showing loss of subchondral bone plate and adjacent bone lysis.

cells. This type of inflammatory response can be associated with infections by *Salmonella*, *Brucella*, *Bartonella*, *Coxiella burnetii* (Q fever), and *Burkholderia pseudomallei* (melioidosis).[19-24]

Most specimens from bacterial hematogenous osteomyelitis are derived from curettage specimens, so the gross findings may be difficult to appreciate but may contain green-tan discolored pus that can be putrid. Resection specimens are typically derived from patients with longstanding chronic disease. In these instances the necrotic tissue is discolored green-tan-brown, and the fibrotic marrow is white gray. The cancellous bone in the center of the infection is largely destroyed by the fibrous tissue, and the surrounding bone is sclerotic, having been rebuttressed. The cortex is frequently thickened in an irregular fashion by subperiosteal reactive bone, and a cloaca appears as a round or oval hole in the cortex that is in continuity with soft tissue involvement (Fig. 14.19).

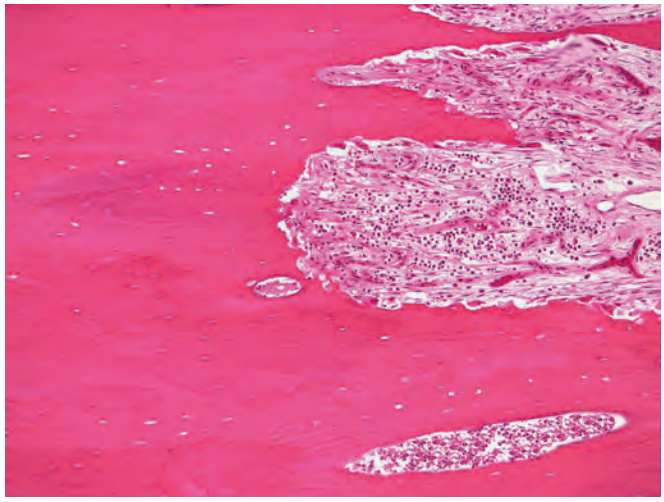
Staphylococcus aureus is responsible for 80% to 90% of the cases of bacterial hematogenous osteomyelitis in which an organism is recovered.[25] Aspects of the infection may be related to virulence factors elaborated by different clonal types.[26] Group A and B streptococci and gram-negative organisms are important etiologic agents in neonates. *Haemophilus influenzae* has become less important following the introduction of the vaccine to this organism. *Salmonella* complicates sickle cell disease, and *Escherichia coli*, *Pseudomonas*, and *Klebsiella* are more frequently isolated from patients with genitourinary tract infections or who are intravenous drug abusers.



• **Figure 14.13** Osteomyelitis of spine. **A,** Axial computed tomography demonstrating irregular destruction of bone with extension into the anterior soft tissues. **B,** T1 magnetic resonance imaging shows extensive marrow involvement and extensions into the soft tissues.

The diagnosis can be strongly suggested by the characteristic clinical and imaging studies. However, in some cases, especially in children, the destructive process may mimic a primary malignancy, such as Ewing sarcoma or osteosarcoma. In many untreated cases, blood cultures are positive, but biopsy and bone cultures are required to identify the pathogen in most instances. The combination of antibiotics and surgical drainage is usually curative; however, in 5% to 25% of cases, acute osteomyelitis fails to resolve and persists as chronic infection. Chronicity may develop when there is delay in diagnosis, extensive bone necrosis, abbreviated antibiotic therapy, inadequate surgical débridement, and weakened host defenses. Acute flare-ups may mark the clinical course of chronic infection and are usually spontaneous, have no obvious cause, and may occur after years of dormancy.[27,28] Other complications of chronic osteomyelitis include pathologic fracture, secondary amyloidosis, endocarditis, sepsis, development of squamous cell carcinoma in the sinus tract, and rarely sarcoma in the infected bone.

• **Figure 14.14** Osteomyelitis of vertebral body with epidural abscess. Sagittal T2 magnetic resonance imaging showing involvement of several cervical vertebral bodies with an epidural abscess and involvement of anterior soft tissues.

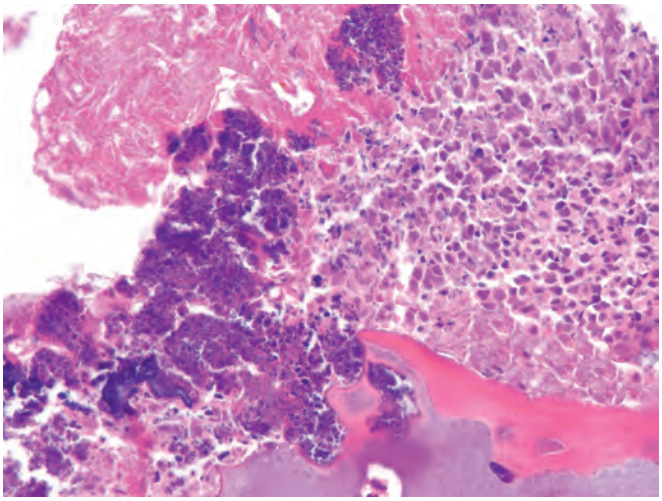


• **Figure 14.15** Necrotic cortical bone being resorbed by osteoclasts in expanded haversian canal.

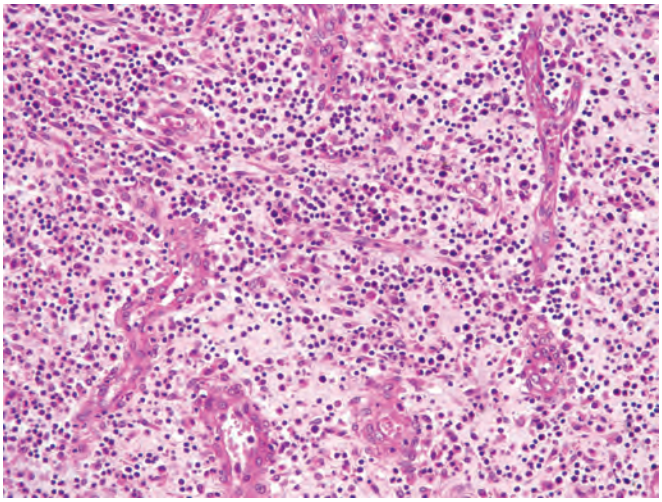
Bacterial Osteomyelitis From Contiguous Spread

Bacterial infection can spread from a poorly controlled neighboring juxtaosseous infection to involve bone. In this situation the proliferating bacteria devitalize the periosteum or digest the articular cartilage so that they gain entry to the cortex or medullary cavity. Patients with common conditions (such as periodontal disease, caries, sinusitis, cellulitis of the feet and toes), patients with severe peripheral vascular disease (especially diabetics), and individuals who are immobile and suffer from decubitus ulcers are prone to this type of infection.

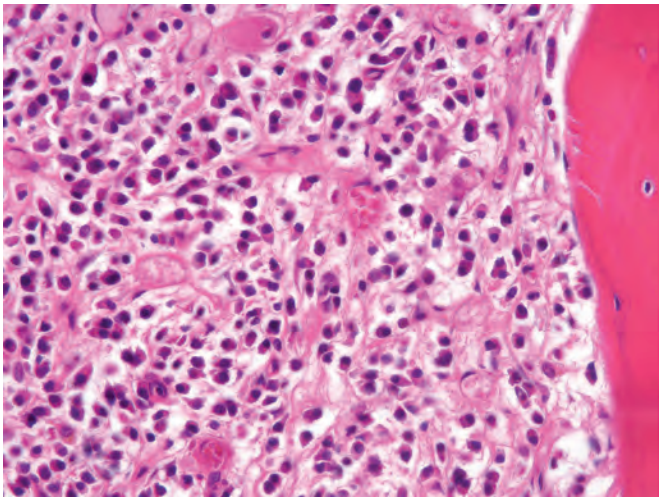
Osteomyelitis of the jaws frequently follows periodontal disease and caries (Fig. 14.20). Many patients also have a history of



• **Figure 14.16** Acute osteomyelitis involving primary spongiosa adjacent to the growth plate. Dark purple aggregates of bacteria are adjacent to dying inflammatory cells.



• **Figure 14.17** Acute and chronic osteomyelitis with neutrophils, lymphocytes, plasma cells, macrophages, and capillaries.



• **Figure 14.18** Chronic osteomyelitis with numerous plasma cells. Sometimes this can be confused with myeloma.

previous radiation, which compromises the gingiva and induces necrosis of the underlying bone. The exposed bone acts as a good medium for bacterial pathogens and frequently becomes infected by multiple organisms, including *S. aureus*, *Streptococcus* spp., and *Actinomyces*.[29]

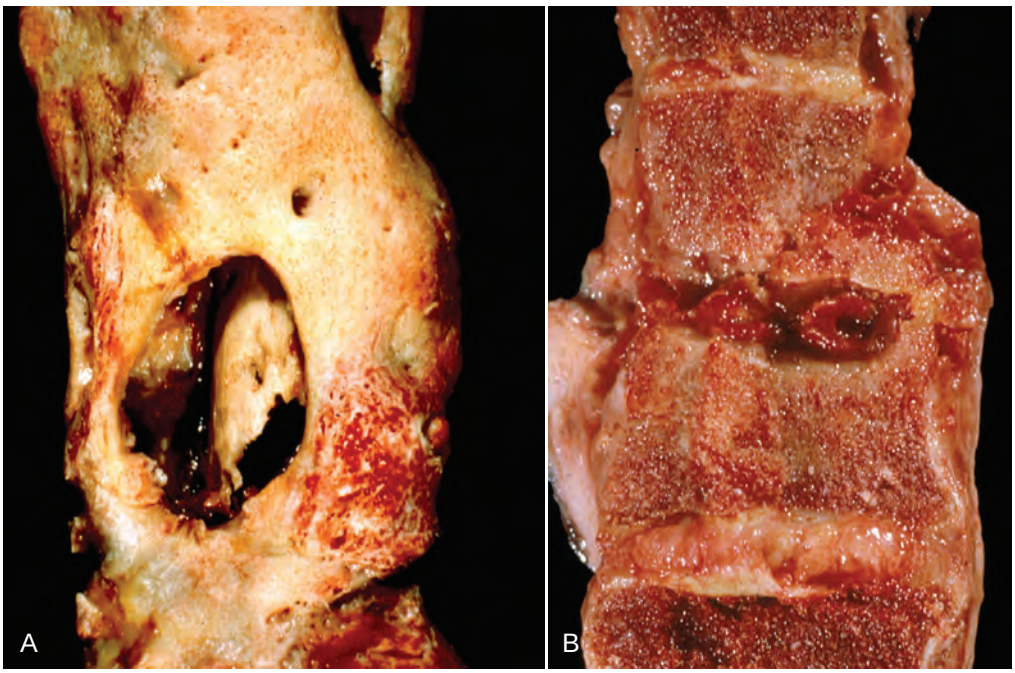
Actinomycosis most commonly occurs in the jaws and is often associated with chronic infection, including draining sinuses.[30] It is often seen in the setting of osteoradionecrosis and in patients who have been treated with bisphosphonates.[31]

Bisphosphonate-induced osteonecrosis has recently been the focus of attention because of the widespread use of bisphosphonates and the controversy regarding the pathophysiology of its genesis.[32,33] The first large series of bisphosphonate-induced osteonecrosis was described in 2003 and occurred in patients who had a malignancy, frequently myeloma, and who were receiving intravenous bisphosphonates.[34] Patients typically have a history of a recent dental procedure and present with exposed bone that is necrotic but also infected with actinomyces (Fig. 14.21). It is unclear whether the infection is the primary causative event or is secondary and whether it begins in the gingiva or in the bone. Pathologic examination of surgical specimens shows gingival inflammation and ulceration and necrotic bone infiltrated by actinomyces and neutrophils (Fig. 14.22). The organisms are pleomorphic, branching, filamentous gram-positive bacteria that frequently aggregate into sulfur granules and exhibit the Splendore-Hoeppli phenomenon. The disease can be chronic and recurrent, and treatment commonly includes surgical débridement and antibiotics.[31,34,35]

The development of osteomyelitis as a complication of bacterial sinusitis is uncommon. Patients range from adolescents to older adults and present with headache, swelling, fever, and discharge. The most common site of involvement is the frontal sinus.[36] In this region, osteomyelitis may produce a subperiosteal abscess that may present as a soft tissue mass of the forehead known as Pott puffy tumor.[37,38] The more common pathogens include *S. aureus*, nonenterococcal streptococcus, and oral anaerobes.[36]

Osteomyelitis of the feet in patients with vascular insufficiency, particularly diabetics, is a serious problem. The ulcers develop because of the combination of neuropathic, ischemic, and local biomechanical changes that subject the skin and soft tissues to excessive pressure, friction, breakdown, and necrosis while eliciting sometimes only minimal local symptoms (Fig. 14.23). The development of osteomyelitis in these patients is usually a complication of a nonhealing ulcer that gradually involves the deeper soft tissues until the periosteum is breached. After the bone is exposed, the cortex may undergo necrosis with the infection spreading along its length and into the medullary cavity via the haversian systems. Joint involvement is relatively common in severe disease (Fig. 14.24). As the bone is resorbed, it may undergo secondary fracture with callus formation. Bone cultures frequently identify *S. aureus*, which may be methicillin resistant, followed by *Staphylococcus epidermidis. E. coli*, *Klebsiella pneumoniae*, and *Proteus* spp. are followed by *Pseudomonas aeruginosa* as the most common pathogens among *Enterobacteriaceae*.[39,40]

Infection of bone beneath a decubitus ulcer typically develops when a pressure sore fails to resolve and the bacteria and inflammation extend into the underlying osseous tissue. This complication develops in 17% to 32% of infected decubiti.[41] The patients usually lack sensation in the affected region and are immobile and poorly nourished. Common sites for this problem are the sacrum, posterior calcaneous, ischial tuberosities, greater trochanters, elbows, scapulae, and lateral malleoli.[41] The infection



• **Figure 14.19** Gross specimens of osteomyelitis. **A,** Chronic osteomyelitis of long bone with cortical sequestrum and its cloaca, and well-formed involucrum, which also has a cloaca. **B,** Vertebral bodies removed at autopsy. Disk space is replaced by hemorrhagic inflammatory tissue and the adjacent vertebral bodies show replacement of the marrow by tan tissue with focal disruption of the bone.

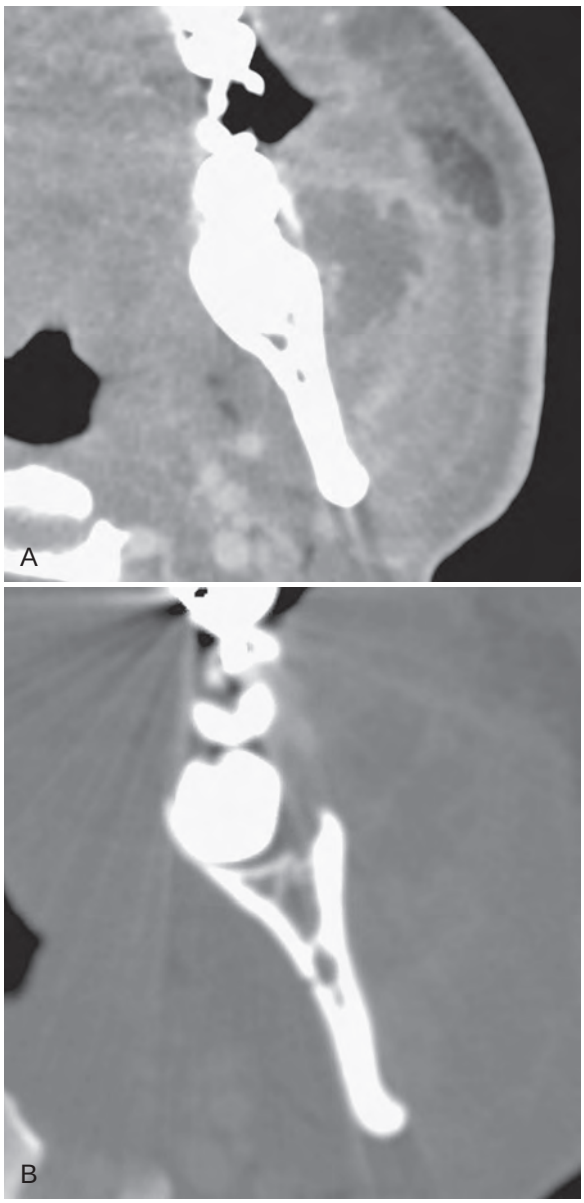
is polymicrobial and includes aerobes (*Proteus mirabilis*, *E. coli*, *Enterococci*, staphylococci, and *Pseudomonas* spp.) and anaerobes (*Peptostreptococcus* spp., *Bacteroides fragilis*, and *Clostridium perfringens*).[42,43] The infection may involve only the superficial portion of the bone or be more extensive.[44] The histologic findings frequently show the features of chronic osteomyelitis.[44]

Bacterial Osteomyelitis From Direct Inoculation

Bone infection caused by direct implantation of bacteria into bone has diverse etiologies. The common thread to all is that the bone is either exposed or violated, giving bacteria direct access to osseous tissue.[45] Degloving injuries or open fractures in which the fractured ends of the bone pierce the skin and become contaminated are serious injuries. The extent of the trauma, amount of tissue necrosis, degree of contamination, amount of available soft tissue coverage, rapidity and skill of surgical intervention, and state of the host influence wound healing.[46] Unfortunately, 3% to 50% of open fractures eventually become infected, with bacteria residing in pieces of necrotic bone.[47] Many patients are young and the long bones of the lower extremities, especially the tibia and fibula, are frequently affected. A host of different bacteria may behave as pathogens, including those that normally colonize the skin, the contaminating soil, or nosocomial organisms acquired during hospitalization and restorative surgical procedures. The most frequent offenders are staphylococci and aerobic gram-negative bacilli; however, enterococci are also infrequently implicated. The osteomyelitis often presents as a nonunion with local and systemic symptoms, such as swelling, fever, and pain. Crucial to preventing this complication are successful débridement and washout of the wound, fixation of the bone, appropriate use of antibiotic beads, and closure of the surrounding soft tissue envelope. Ideally this should be accomplished within 6 hours after the injury.[48-50]

Orthopedic implants, including prosthetic joints and internal fixation devices, are being used at a steadily increasing rate. More than 1 million reconstructive prosthetic joint arthroplasties and significantly more trauma-related implant surgeries are performed annually in the United States. The impetus for this rise in orthopedic implant surgery is the longer lifespan of the population, which is associated with a higher prevalence of significant osteoarthritis (joint replacement) and osteoporosis (pathologic fracture), active life styles, improved prosthetic materials and hardware, and more refined and safer surgical techniques, especially for older adults. Orthopedic implants have great success rates; however, they may fail because of metal fatigue with fracture, corrosion and generation of wear debris, and infection. Although there have been great strides in preventing and treating implant-associated infections, the total number is increasing because the devices have a lifelong risk of becoming seeded by bacteria. The risk of developing infection within 2 years after primary joint replacement is less than 1% for hip and shoulder prostheses, less than 2% for knee prostheses, and less than 9% for artificial elbows.[51-55] The rate increases to 5% to 40% with revision joint arthroplasties.[52,55] Approximately 5% of internal fixation devices become infected, with the infection rate after closed fractures being 0.5% to 2% and rising to 30% after reconstruction of open fractures.[52]

Orthopedic implant-associated infections can be classified into those that are perioperative or early (developing less than 3 months after surgery), delayed (3 to 24 months after implantation), and late (greater than 24 months after surgery).[54] The early and delayed infections are believed to result from contamination occurring at the time of implantation surgery, whereas the late infections are hematogenous in origin.[54]



• **Figure 14.20** Peridontal abscess of mandible. **A,** Axial computed tomography of mandible with soft tissue windows showing low-density abscess in the soft tissues. **B,** Bone window shows a small focus of cortical disruption with a lytic focus within the bone.

Infections developing soon after implantation are usually acute in onset and present with warmth, swelling, and pain at the operative site with the systemic findings of fever, leukocytosis, and elevated sedimentation rate and C-reactive protein. The organisms usually responsible are virulent and include *S. aureus* and gram-negative bacilli. Delayed infections are more subtle clinically, and the patients may present with loosening of the implant and pain (Fig. 14.25). The presentation may be very similar to loosening caused by prosthetic particulate wear debris. The cause of the loosening is stimulation of osteoclastic resorption of the bone adjacent to the implant by cytokines released by the inflammatory reaction to the pathogen. The normally thin layer of reactive fibrous tissue between the host and implant or its anchoring cement, which is frequently designated the "membrane" by orthopedic surgeons, becomes thickened by

• **Figure 14.21** Patient treated with intravenous bisphosphonate who after an extraction developed exposed bone infected with actinomycosis, which is also necrotic.

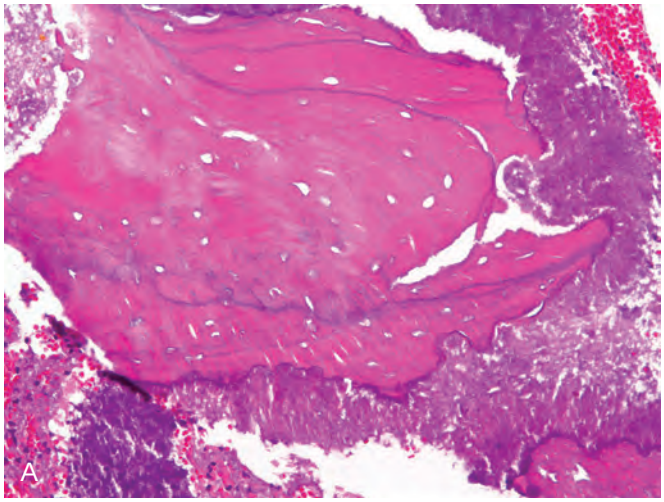
• BOX 14.3 Histologic Criteria for Diagnosis of Osteomyelitis Complicating Prosthesis

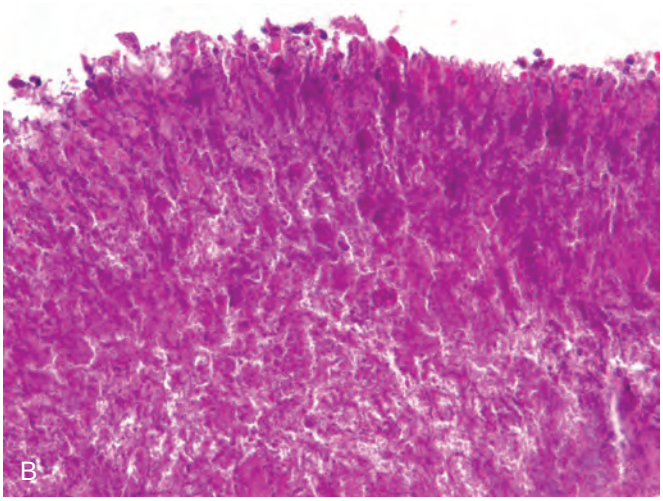
Placement
Five neutrophils in five consecutive high-power fields
Neutrophils enmeshed in fibrin
Exclude surface exudates

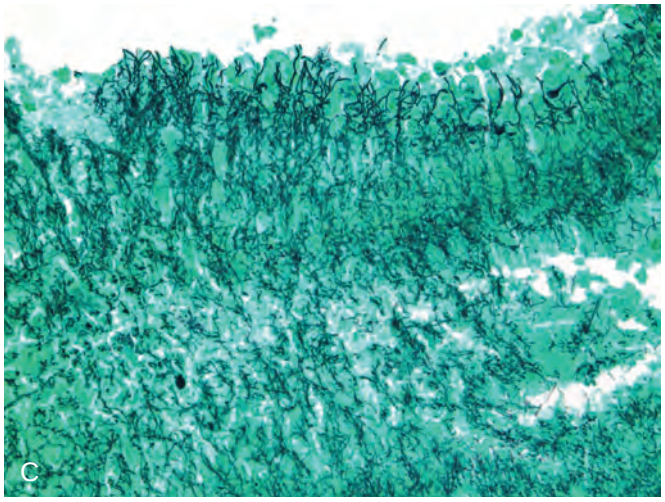
inflammatory cells, collagen, and blood vessels. In severe cases, pus develops. The organisms are usually not very virulent and include coagulase-negative *Staphylococcus* and *Propionibacterium acnes*. Although these pathogens are acquired at the time of implantation, they attach to the devices by proteinaceous cell wall and capsular polysaccharide-associated adhesins and remain protected from antibiotics and the host immune response by secreted biofilms.[45,56]

The distinction between delayed infection and failure related to prosthetic wear debris can be very difficult to detect. In many instances it requires correlation of the clinical findings, imaging studies, and laboratory tests, such as the sedimentation rate and the level of C-reactive protein. All too frequently these data are nonconclusive, and the next step is frozen section histologic examination of the tissue adjacent to the implant at the time of joint replacement.[57,58] Studies have shown that the identification of five neutrophils in five consecutive separate high-power fields excluding surface exudates and neutrophils enmeshed within fibrin aggregates correlates with culture-proven infection (Fig. 14.26, Box 14.3). This technique has relatively low sensitivity (29%) but high specificity (95%).[57] Practical suggestions for performing this technique include the following: (1) prepare a minimum of three cassettes per specimen and (2) identify the surface of the tissue that abutted the prosthesis (is shiny and frequently smooth) and section it in a perpendicular plane—if neutrophils are present they are most numerous in the superficial portion of the tissue.

The treatment of an infected prosthesis frequently involves surgical and medical therapy. The surgical procedures range from reimplantation either at the time of resection (one-stage replacement) or in a second surgery (two-stage replacement), excision of diseased tissue with preservation of the prosthesis, complete resection with or without arthrodesis, to amputation. The type of



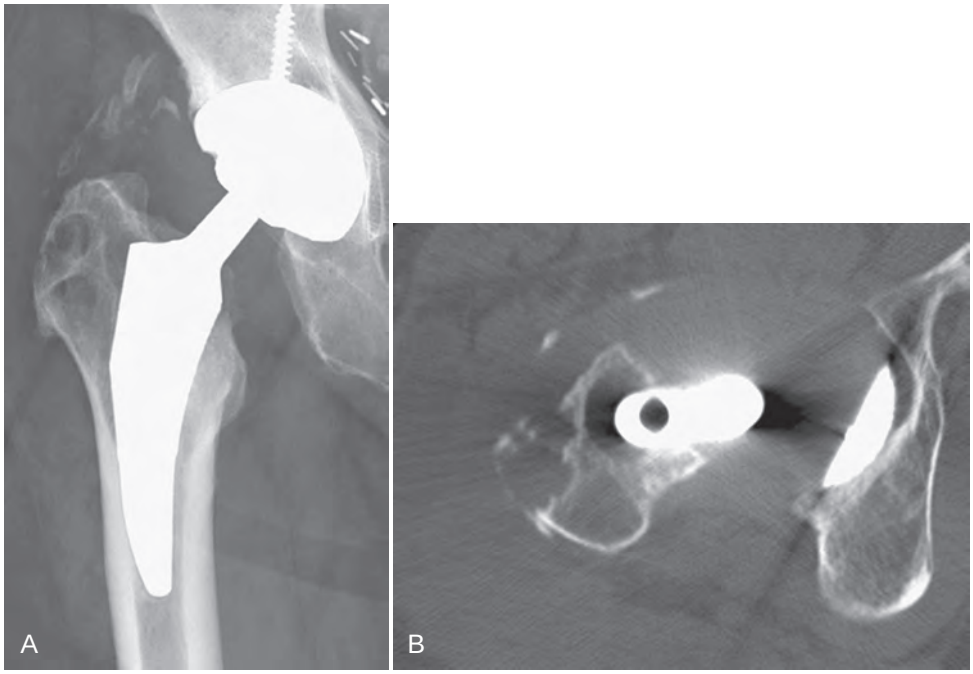




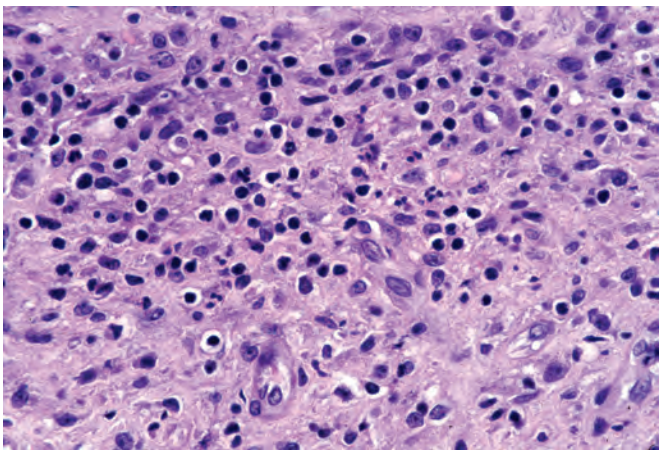
• **Figure 14.22** Actinomycosis of mandible. **A,** Necrotic bone infiltrated by colonies of actinomyces. **B,** High power of actinomyces in tissue exhibits the Splendore-Hoeppli phenomenon. **C,** Actinomyces stained with Grocott methenamine silver stain highlighting the filamentous nature of the bacteria.

• **Figure 14.23** Great toe of elderly diabetic patient. The toe is swollen and erythematous, with several ischemic ulcers.

• **Figure 14.24** Lateral radiograph of the first metatarsophalangeal joint shows osteopenia, loss of joint space, and destruction of the adjacent bones, especially the metatarsal head.



• **Figure 14.25** Hip prosthesis with heterotopic bone in soft tissues and infection of the femoral component. **A,** Plain radiograph shows large radiolucencies in the greater trochanter. **B,** Axial computed tomography shows destruction of bone around the implant and involving the greater trochanter.



• **Figure 14.26** Removed synovial-like tissue from the region around the femoral component. The neutrophils are indicative of infection.

pathogen, amount and condition of the residual bone and soft tissues, antimicrobial susceptibility of the infecting microorganism, and the patient's medical condition are factors that influence the surgical treatment strategy. The two-stage reconstruction is optimal, and after removal of the implant the structural integrity of the site can be temporarily maintained by an antibiotic-impregnated spacer or cement.[59] The patient is also treated with parenteral antibiotics and after 4 to 6 weeks after the site is sterilized a new prosthesis is implanted. The cost for the treatment of this complication is more than $50,000.

Mycobacterial Osteomyelitis

Mycobacteria are aerobic and usually nonmotile bacteria that are acid-alcohol fast and have thick cell walls that are hydrophobic and rich in mycolic acids. Within the genus *Mycobacterium*, greater than 120 different species are currently recognized.[60] The majority are not pathogenic and are ubiquitous in the environment, with their natural reservoirs being water, soil, aerosols, dust, animals, and foods. The major human pathogenic mycobacterium is *Mycobacterium tuberculosis*; however, *Mycobacterium leprae* and *Mycobacterium ulcerans* are also responsible for significant human disease. The major differences between *M. tuberculosis* and the nontuberculous mycobacteria are the virulence of the organisms and the potential for human to human transmission. Nontuberculous mycobacteria are generally of low virulence and are not associated with human to human spread. The successful pathogens have the capability to reside and proliferate within host macrophages despite the bactericidal properties of these cells. The macrophages are a component of the complex immune response involving both innate and adaptive systems that sequesters the microorganisms within granulomas. Overall, mycobacterial infection of the skeletal system is much less frequent than bacterial osteomyelitis.

Tuberculous Osteomyelitis

Tuberculosis is defined as an infectious disease caused by *M. tuberculosis*. It affects 1.8 billion people annually and is the leading cause of death associated with an infectious disease and is responsible for the death of approximately 3 million people annually.

Tuberculous osteomyelitis has plagued mankind for millennia. Evidence of bone disease has been found in Egyptian mummies and the skeletal remains dating from the Iron Age. A resurgence of tuberculous osteomyelitis has occurred in industrialized nations that have been attributed to the influx of immigrants from Third World countries and greater numbers of immunosuppressed people. In developing countries the affected individuals are usually adolescents or young adults, whereas in the indigenous population

of the United States, the victims tend to be older, except for those who are immunosuppressed. In the United States the incidence of tuberculosis is currently on the decline but remains an important health problem. Approximately 1% to 3% of patients with pulmonary tuberculosis also have osseous disease, and 10% of all cases of extrapulmonary tuberculosis are caused by bone infection.

Mycobacteremia is the mechanism that underlies most cases of bone disease, with the organisms originating from foci of active visceral disease that are in the initial stages of primary infection. Direct extension (e.g., from a pulmonary focus into a rib or from tracheobronchial nodes into adjacent vertebrae) or spread via draining lymphatics or Batson venous plexus from the lung to the spine may also occur. The bone infection is usually solitary and in some cases may be the only clinical manifestation of the disease and may fester for years before being recognized. Patients with acquired immune deficiency syndrome (AIDS) frequently have multifocal bone involvement, which results from widespread hematogenous dissemination.

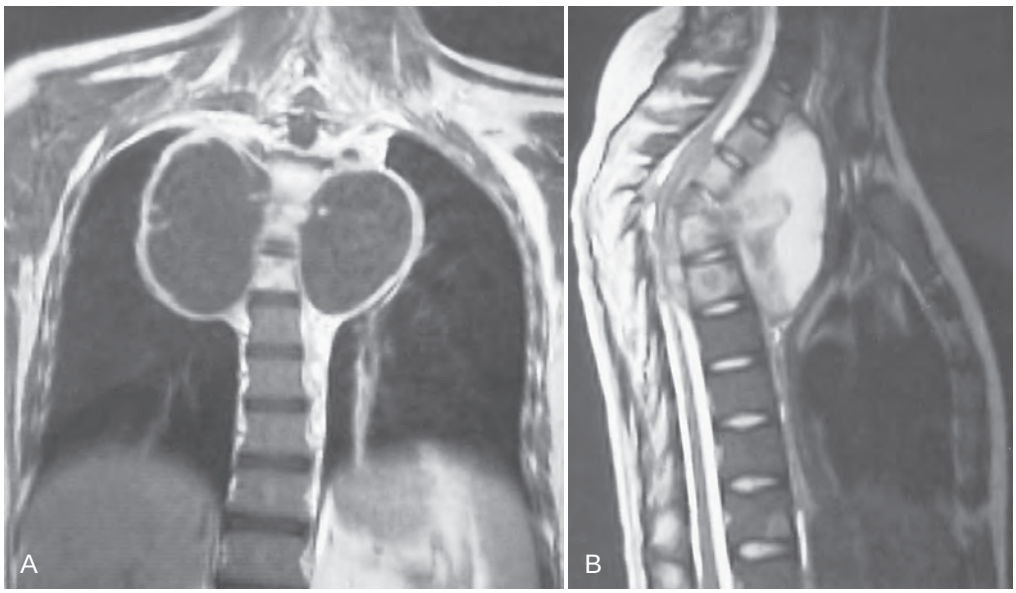
The spine is affected in 40% to 50% of cases of tuberculous osteomyelitis and is followed in frequency by the hips and knees in 25% and 20% of cases, respectively. Spinal infection occurs 2 to 10 times more frequently in HIV-infected patients. In the spine, 98% of infections are centered in the anterior column, and the involved level is usually located in the region of T6 through L3.[61-63] Thoracic disease is more frequent in children, whereas in adults the lumbar area is more often involved.

Patients present with insidious symptoms that develop over a period of months to a year or longer. Common manifestations are low-grade fevers, weight loss, pain that is regional and ranges from mild to severe, swelling, local warmth without erythema, muscle spasms and atrophy, limitation of range of motion, limping, gibbus formation, and soft tissue masses. The latter is typically a manifestation of a cold abscess.

Radiographically, nuclear magnetic resonance is the modality of choice for assessing spinal tuberculosis because plain films and CT are not very sensitive and do not adequately demonstrate marrow involvement. The disease first begins in the anterior subchondral region of the vertebral body (Fig. 14.27). It may initially appear as a localized area of osteoporosis with loss of the subchondral bone plate. On MRI the inflammatory bone marrow changes manifest as patchy high signal intensity on T2-weighted images and low signal intensity of T1-weighted images.[64] Continued expansion of the infection produces disruption of the cortex and spread into the neighboring disc. In comparison with purulent bacterial discitis, the disc space in tuberculosis may show only limited narrowing on imaging studies despite the presence of extensive bone destruction—this is an important diagnostic feature of tuberculous spondylitis. Progressive disease causes erosion of the anterior and lateral cortices, leading to angular collapse with posterior projection and deformity (Pott disease). The angle of the projection is most severe when only one or two vertebral bodies have collapsed (Fig. 14.28). If the bony collapse involves one side of the vertebral body greater than the other, then lateral angulation may also occur. Within the bone, bright signal intensity on T2-weighted images surrounding a central region of low signal intensity on T1-weighted images reflects the presence of an intraosseous abscess. The rim of the abscess shows intense enhancement after the administration of contrast agents, such as gadolinium. Craniocaudal spread beneath the anterior longitudinal ligament produces involvement of multiple vertebral bodies, and sometimes this is associated with the sparing of one or

• **Figure 14.27** Tuberculosis of thoracic spine demonstrating destruction of adjacent vertebral bodies and the intervening disc. The anterior portions of the bodies are most severely affected.

• **Figure 14.28** Severe kyphosis caused by collapsed vertebral body as a result of tuberculosis. (Courtesy Dr. W. Putschar.)



• **Figure 14.29** Tuberculosis spine. T-2 weighted MRI of tuberculosis of vertebrae with abscess extending bilaterally. **A,** Coronal T1-weighted image shows a large hypointense mass on both sides of the upper thoracic spine. **B,** Sagittal T2-weighted sequence without fat saturation shows significant anterior wedging of T6 vertebral body, leading to the gibbus deformity. Also note the large anterior hyperintense collection and the epidural mass causing pressure effect on the spinal cord. The hypointense foci within the anterior mass represent debris.

intervening vertebrae—skip lesions.[64] Extensive disease may be associated with involvement of the posterior elements and epidural space and the development of large paravertebral abscesses (Fig. 14.29A and B). In exceptional cases the abscesses may dissect along tissue planes and produce large distant masses that can compress organs, erode bones, and present as soft tissue masses. Those growing along the psoas fascia can extend beneath the inguinal ligament and present as a thigh mass (Fig. 14.30). The wall of abscess may calcify in longstanding disease and the abscess can continue to burrow and form fistulas and draining sinuses.

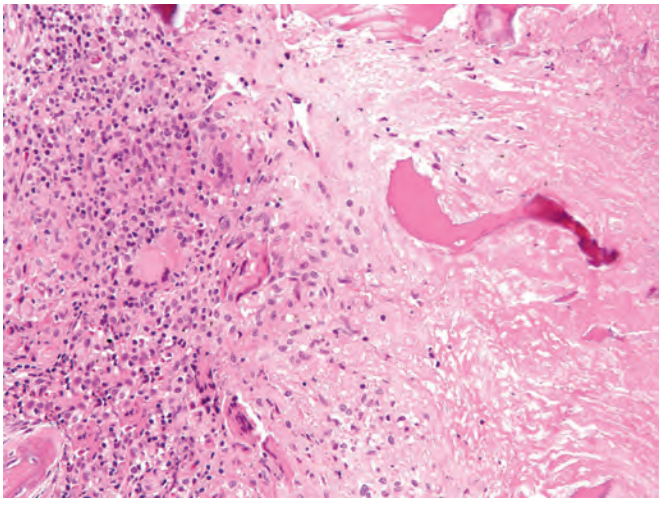
Tuberculous osteomyelitis of the appendicular skeleton usually begins in the metaphysis and extends into the epiphysis, with joint involvement being commonplace. As a result, subchondral bone destruction and periarticular erosions occur on both sides of the joint and are characteristic of the disease (Fig. 14.31). Large regions of bone may be destroyed with poorly defined margins, sequestrate, and periosteal reaction.[65] Accompanying spread into the soft tissue and abscess formation may also develop.

Grossly, infected bone is discolored pale tan—yellow-white-gray, and the involved area is poorly defined and may extend through the cortex into the soft tissues. In miliary disease the individual granulomas appear as pinpoint, round, white-gray nodules. In regions of bone destruction, firm fibroinflammatory tissue replaces the bone except in areas of abscess formation. Abscess cavities are filled with liquid of various viscosity and range in color from light green-yellow to brown and sometimes contain pieces of bone that may be finely granular ("bone sand").

Histologically the sine qua non of tuberculous osteomyelitis is necrotizing granulomatous inflammation (Fig. 14.32). The granulomas or tubercles are round to oval and composed of aggregates of polyhedral macrophages that have oval or kidney bean–shaped vesicular nuclei and abundant eosinophilic cytoplasm. Admixed are multinucleated giant cells, including Langhans-type giant

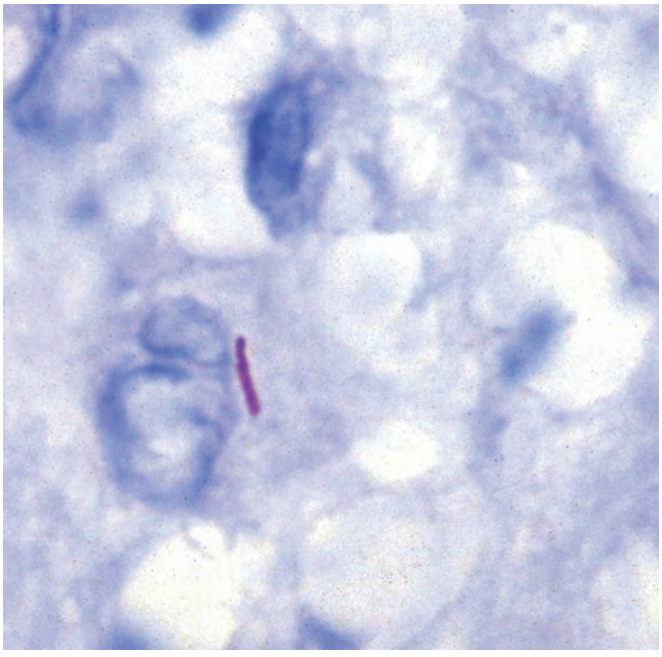
• **Figure 14.30** Tuberculosis of spine with formation of large abscess that dissected along the psoas. The wall of the abscess is calcified. (Courtesy Dr. W. Putschar.)

• **Figure 14.31** Tuberculosis shoulder. Axillary view of the shoulder shows subchondral and marginal erosions of the glenoid and the adjacent humeral head. Note the osteopenia.



• **Figure 14.32** Tuberculosis osteomyelitis. A large necrotizing granuloma involves the medullary cavity. The dead trabeculum is surrounded by caseous debris. Scattered Langhans-type giant cells, lymphocytes, and plasma cells are present in the viable portion of the granuloma.

cells. Scattered around the granulomas are small mature-appearing lymphocytes, all of which are enmeshed in fibrovascular connective tissue. Areas of necrosis vary in size from microscopic, occurring in the centers of the granulomas, to large grossly visible regions. The type of necrosis is caseous in nature being an admixture of coagulative and liquefactive devitalization. Histologically the necrotic tissue is amorphous and eosinophilic and contains scattered neutrophils, nuclear debris, and coagulated fibrin and is



• **Figure 14.33** Acid-fast stain showing beaded rod shape of *Mycobacterium tuberculosis* in cytoplasm of histiocyte.

bordered by histiocytes. The surrounding tissue consists of granulation tissue with variable numbers of granulomas. The involved bone is necrotic and shows evidence of previous osteoclastic resorption. The neighboring uninvolved bone is sclerotic with reactive new bone formation, and loose granulation tissue fills the intertrabecular spaces.

The microorganisms are usually most numerous in the areas of necrosis; however, they can also be found in the cytoplasm of macrophages. Tuberculosis are gram positive, and using acid-fast histochemical stains, such as the Ziehl-Neelsen stain, they appear as red rod-shaped structures that are 2 to 4 μm in length and 0.2 to 0.5 μm in diameter (Fig. 14.33).

Spinal tuberculosis causes significant bone destruction, which can result in permanent severe scoliotic or kyphotic deformities with subluxations, contractures, and neurologic deficits, including paralysis secondary to spinal cord and nerve compression. Tuberculous osteomyelitis tends to be more destructive and resistant to control than pyogenic osteomyelitis. Treatment is usually a combination multidrug therapy and surgery and is not always successful.

Nontuberculous Mycobacterial Osteomyelitis

Nontuberculous mycobacteria are relatively nonvirulent and are present throughout our environment. Nontuberculous mycobacterial infection in humans has been well established since the 1930s; however, the introduction of immunosuppressive therapy and the development of HIV infection have created the conditions for a significant increase in the frequency of this type of disease. There are more than 150 species, approximately 60 have been documented to cause human illness, and new species are being reported on a continual basis.[66-68] Despite this, relatively few species are responsible for the majority of infections, with *Mycobacterium avium-intracellulare* causing 61% of cases, followed by

Mycobacterium fortuitum (19%) and *Mycobacterium kansasii* (10%).[66-68] Currently, nontuberculous mycobacterial infection accounts for 0.5% to 30% of all mycobacterial diseases, and this percentage is on the rise.[68] A strong relationship exists between the type of infecting mycobacteria species and the geographic area of the population. The gamut of clinical infections caused by these organisms is broad, and they can be classified into chronic pulmonary infection, lymphadenitis, soft tissue and osteoarticular infection, disseminated disease, and iatrogenic illnesses.[65] Abnormalities with interferon-γ and the interleukin-12 pathway are hypothesized in predisposing individuals to infection.[67]

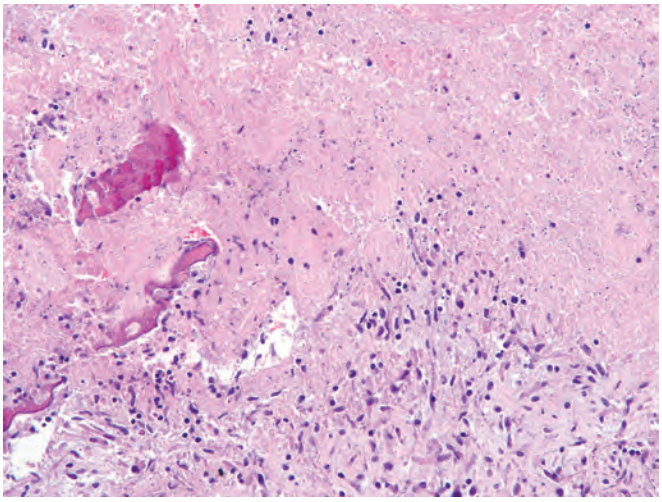
Approximately 5% to 10% of patients with nontuberculous mycobacterial disease have involvement of the musculoskeletal system.[69] The predisposing factors of bone infection are trauma, surgery, and immunocompromise. The patients tend to be young and are infected by inoculation of the organism into the soft tissues, with secondary spread into the adjacent bone or directly into osseous structures as a result of penetrating trauma, contamination of open wounds, or introduction into the body via medical devices, such as catheters, shunts, injections, and surgical procedures. The organisms responsible for this type of infection are usually *Mycobacterium abscessus*, *M. fortuitum*, *Mycobacterium chelonae*, *Mycobacterium haemophilum*, *Mycobacterium marinum*, *M. avium-intracellulare*, *M. kansasii*, and *Mycobacterium terrae*. Patients who are immunosuppressed often have disseminated disease, and the mycobacteria spreads to the skeleton hematogenously, causing multiple lesions that tend to affect the metaphyseal and diaphyseal regions of the long bones. Patients with osteomyelitis caused by *M. chelonae* and *M. haemophilum* are usually immunosuppressed.

Several clinical associations are well known. (1) *M. marinum* produces a condition known as swimming pool granuloma or fish tank granuloma in people exposed to forms of a marine environment. Local trauma, especially in the extremities, provides a pathway of access for the organism into the tissues. (2) *M. ulcerans* produces chronic necrotizing skin lesions of the distal extremities known as Buruli ulcer.[70] It is associated with secondary bone involvement in approximately 15% of cases and is most prevalent in West Africa and Australia.

Patients with nontuberculous mycobacterial osteomyelitis frequently present with localized pain, swelling, erythema, and induration of the involved site. They may also exhibit low-grade fever, sweats, chills, anorexia, malaise, and weight loss. In Buruli ulcer the lesion is nonpainful and associated with deep ulcers, which undermine the skin edges.

The radiographic findings of nontuberculous mycobacteria are similar to those caused by acute pyogenic bacterial osteomyelitis, but the rate of progression is slower; in the spine the changes resemble tuberculous spondylitis.[69] The infection results in bone resorption, sequestrum formation, deposition of reactive new bone (involucrum), and the development of sinus tracts. In some cases, the plain films may show a honeycomb pattern of bone dissolution or sometimes lytic cystic-appearing areas of bone destruction with limited surrounding sclerosis. In immunosuppressed patients the lesions are usually multifocal and lytic.

The histologic reaction to the mycobacteria is variable and depends to some degree on the immune status of the host. The infection may elicit caseating and nonnecrotizing granulomas, nonspecific chronic inflammation with lymphocytes, plasma cells and macrophages, and areas of tissue necrosis with little inflammation (Fig. 14.34). Immunosuppressed patients frequently have no granulomatous inflammation, and innumerable organisms may be present in histiocytes. The mycobacteria stain with Ziehl-Neelsen and Fite special stains.



• **Figure 14.34** Necrotizing granulomas with pieces of dead bone in *Mycobacterium avium-intracellulare* osteomyelitis of tibia.

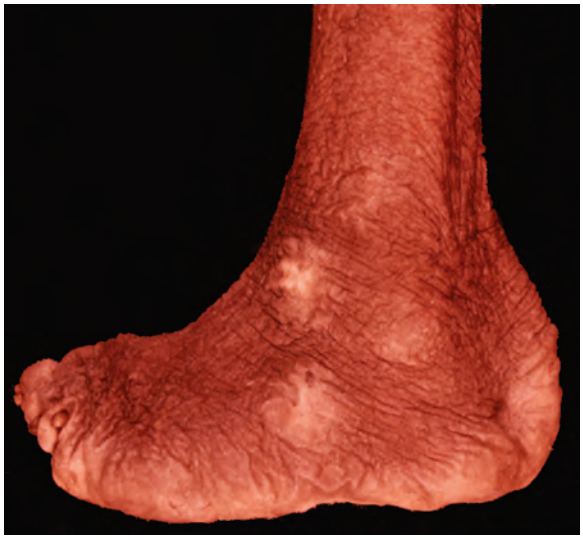
The treatment of nontuberculous mycobacterial osteomyelitis is usually a combination of surgery and multidrug antimycobacterial chemotherapy. Excision of infected should be performed if feasible, and contaminated prostheses or hardware should be removed.

Lepromatous Osteomyelitis

The bone changes associated with leprosy may result from direct infection of the organism or the complications of the disease, including deep ulceration and superimposed infection and neuropathic changes. It is estimated that 3% to 5% of hospitalized leprosy patients have bone involvement.[54] The bones most commonly affected are the small bones of the face, especially the nose, hands, and feet. The slowly progressive and relentless infection spreads from the skin, mucosa, and soft tissues into the underlying bone, producing lytic lesions and gradual resorption of the involved bone. Those bones that tend to show the greatest destruction (Figs. 14.35 to 14.38).[71] The pathology reveals bone necrosis and resorption, marrow fibrosis, and histiocytes, sometimes forming granulomas and occasional Langhans giant cells (Fig. 14.39). Bone involvement is usually indicative of chronic disease and is treated with antibiotics and surgery.

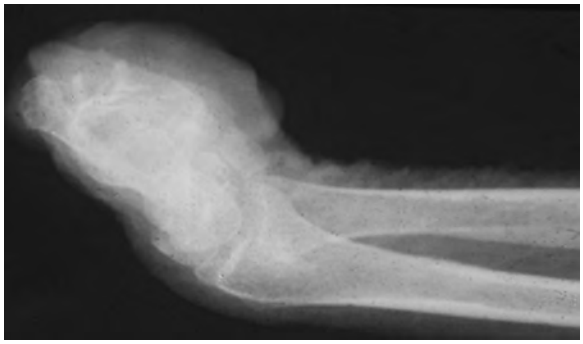
Treponemal Osteomyelitis

Treponema are anaerobic host-associated spirochetes that represent one of six genera of the phylum *Spirochetes*. Spirochetes are gram-negative bacteria that are helical or spiral-shaped and are long and thin. The organism can be visualized in tissue with silver stains, such as the Warthin-Starry stain, immunofluorescence, immunohistochemistry, and DNA analysis. The treponemes produce venereal and endemic (nonvenereal) disease, and those that most commonly cause bone infection are venereal syphilis (*Treponema pallidum* subspecies *pallidum*) and yaws (*T. pallidum* subspecies *pertenue*).

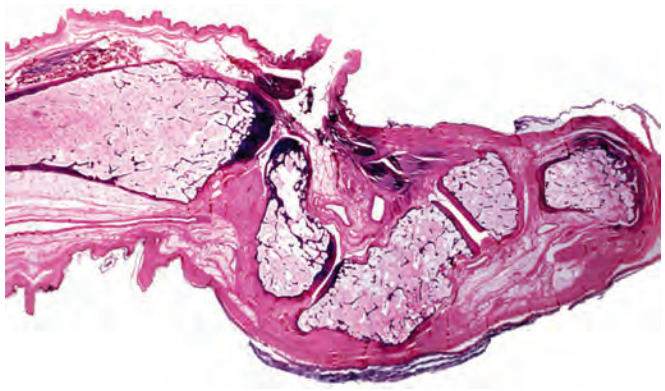


• **Figure 14.35** Leprosy of foot. The toes have been largely resorbed. The skin shows areas of depigmentation. (Courtesy Dr. W. Putschar.)

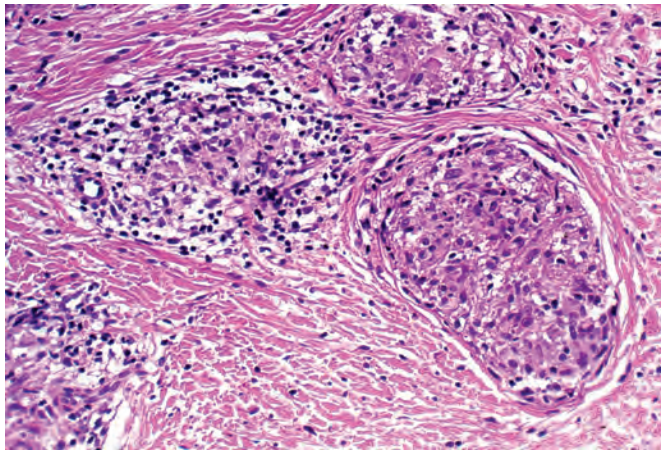
• **Figure 14.36** Leprosy. Lateral radiograph of the foot shows resorption (mutilans) of the phalanges and the distal metatarsals and the soft tissues. There is atrophy of the remaining soft tissues. (Courtesy Dr. W. Putschar.)



• **Figure 14.37** Oblique lateral radiograph of the wrist and hand shows resorption of the phalanges and the distal metacarpals and the soft tissues. There is atrophy of the remaining soft tissues. No significant demineralization is evident. (Courtesy Dr. W. Putschar.)



• **Figure 14.38** Whole mount section of hand in severe leprosy. The bones of the fingers have been resorbed with only carpal bones remaining. (Courtesy Dr. W. Putschar.)



• **Figure 14.39** Tuberculoid leprosy with well-formed granulomas.

Skeletal Syphilis

The incidence of syphilis has waxed and waned over the past several decades and this fluctuation has correlated with changes in sexual behavior associated with drug abuse and homosexuality, and the reaction of the medical community to these behaviors. Currently, syphilis is experiencing resurgence as a result of homosexual physical contact.[72] Despite this, bone involvement remains infrequent because the disease is readily diagnosed and treated before this complication develops.

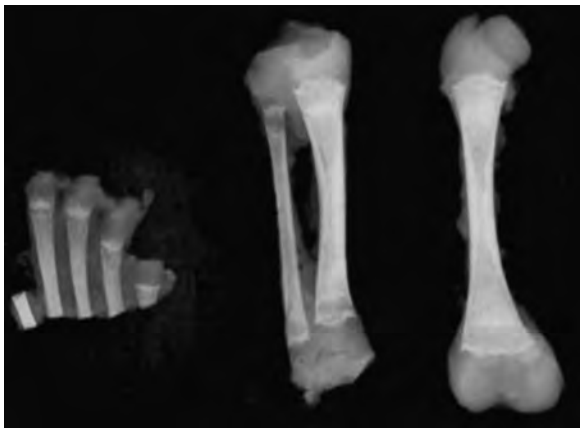
Syphilis can be acquired by physical contact, transmission through the placenta, blood transfusions, and accidental inoculation. The vast majority of cases result from direct contact with active lesions during sexual intimacy. Hematogenous dissemination leads to skeletal involvement, and the manifestations of bone disease vary according to whether the infection is congenital or acquired.

Congenital syphilis or infection of the fetus in utero may complicate the pregnancy of any untreated or inadequately treated woman and usually occurs during the early stages of the disease. The osseous lesions characteristic of congenital syphilis are osteochondritis, diaphyseal osteomyelitis, and periostitis. The lesions do not appear before the fifth month of gestation and are often fully developed at birth. They tend to be distributed in a bilateral and symmetrical fashion but with variable severity from one bone to another. The spirochetes localize to areas of greatest growth,

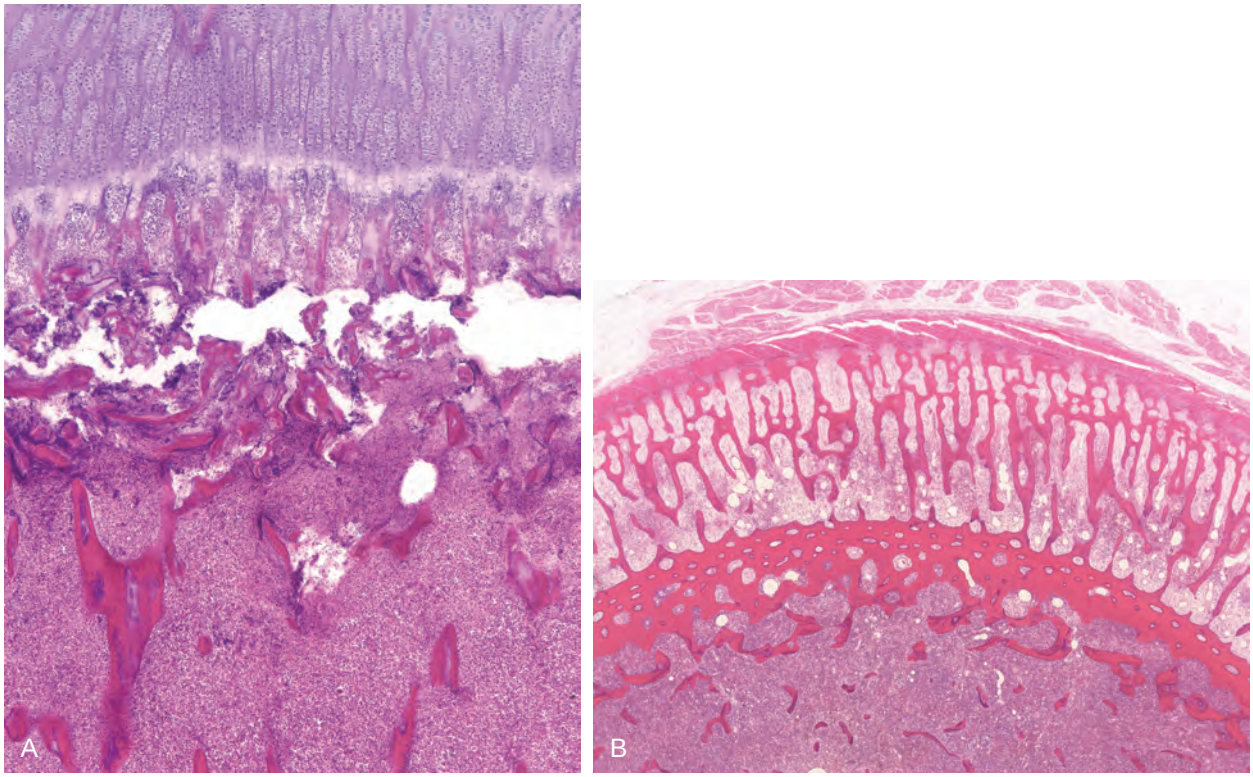
namely, regions of active enchondral ossification (osteochondritis) and in the cellular layer of the periosteum (periostitis). The sites commonly involved are the metaphyseal regions of long tubular bones and costochondral junctions. In syphilitic osteochondritis the zone of mineralization and the region of primary spongiosa are first affected. The organisms infect osteoblasts which cause a significant decrease in their number and the amount of new bone formed. As a consequence, the struts and latticework of partially resorbed mineralized cartilage elongate and occupy a greater amount of the metaphysis. Grossly, this appears as an irregular jagged white line at the junction of the metaphysis and base of the growth plate cartilage and in severe cases is discolored yellow, is 1 to 2 mm thick, and becomes visible on radiographs (Fig. 14.40). Filling the spaces between the bars of cartilage and bony trabeculae is granulation tissue with perivascular lymphocytes, plasma cells, macrophages, and neutrophils. The granulation tissue progressively occupies a greater amount of the metaphysis with small groups of preserved chondrocytes remaining as isolated islands in the juxtaepiphyseal portion of the metaphysis. With more severe involvement, much of the metaphysis is replaced by the inflamed granulation tissue. This zone of connective tissue produces the lytic regions on x-rays at the ends of the long bones and can cause separation of the epiphysis and pathologic fracture with displacement and impaction of the terminal fragment and attached epiphysis into the shaft (Fig. 14.41A). All of these changes are a manifestation of chronic infection and are part of the generalized proliferation of connective tissue that is responsible for the characteristic lesions of syphilis wherever they occur in the body; they are not pathognomonic and may occur in the setting of other chronic infections. Following treatment, the lesions may heal completely.

During the first months of infancy, distinctive foci of osteomyelitis may involve the diaphysis. The granulation tissue and inflammation may originate from the metaphysis or develop de novo through seeding of the organism. Pathologically, there is necrosis, granulation tissue, and acute and chronic inflammatory cells with osteoclastic resorption of the adjacent bone.

Periostitis also develops in infants surviving intrauterine infection. In this situation the infected periosteum deposits layers of reactive new bone that encircle the shafts of the long bones either completely or partially (see Fig. 14.41B). This produces the so-called saber shin of the tibia (Fig. 14.42). The flat bones, such



• **Figure 14.40** Congenital syphilis radiographs of costochondral region, femur, tibia, and fibula. The metaphyseal-epiphyseal junctions are mildly widened, serrated, and radiopaque.



• **Figure 14.41** Congenital syphilis. **A,** Osteochondritis showing metaphysis with fracture and separation of the epiphysis. **B,** Marked periostitis with parallel struts of subperiosteal new bone deposited on the surface of the cortex.

• **Figure 14.42** Radiograph of tibia in 8-year-old child showing "saber shin" deformity with abundant periosteal bone deposited along the anterior surface of the tibia. (Courtesy Dr. W. Putschar.)

• **Figure 14.43** Syphilis of foot. Anteroposterior view of the foot shows destruction of the adjacent bones along the naviculocuneiform and second and third tarsometatarsal joints. Small erosions are noted along the medial cuneiform and the adjacent base of the first metatarsal. A large lytic focus is seen along the base of the third metatarsal.

as the ilium and scapula, may also be affected. The maximum thickness of the reactive bone is in the midportion of the bone and gradually tapers down toward each end. The reactive bone is initially woven and then becomes remodeled into lamellar bone and disappears soon after effective treatment is initiated. The underlying cortex may remain intact or become osteoporotic with dilated haversian systems. During active disease, the organism can be found in the cambium layer of the periosteum, as well as within the bone.

In acquired disease, bone involvement may begin early in the tertiary stage, which is usually 2 to 5 years after the initial infection, and less frequently in the secondary stage. The bones most frequently involved are those that are superficial, such as the nose (saddle deformity), palate, skull, clavicle, and the long bones of the extremities, especially the tibia (Fig. 14.43). Multiple lesions are often present.[73] The lesions may vary drastically in size and are associated with a prominent inflammatory periostitis, which causes the saber shin of adults (Fig. 14.44A and B). Involvement of the underlying medullary cavity often occurs and manifests as lytic destructive areas surrounded by regions of sclerosis. The lesion causing the destruction is known as the gumma. Grossly the gumma, named after its resemblance to Arabic gum, is yellow and has a dry crumbly consistency. Histologically it consists of eosinophilic necrotic inflammatory cells and connective tissue surrounded by granulation tissue containing lymphocytes, numerous plasma cells, macrophages (which may be epithelioid), and occasional Langhans giant cells. The gumma forms as the organism proliferates in the walls of small vessels and cause an obliterative endotheliitis and thrombosis. Consequently, the tissue undergoes necrosis and, in the early stages, contains many neutrophils surrounded by numerous lymphocytes, plasma cells, and epithelioid histiocytes and can resemble the granulomas seen in tuberculosis. As the lesion enlarges, the neutrophils undergo apoptosis and viable mononuclear cells surround the periphery of the large areas of necrosis (Fig. 14.45A and B). Organisms may be difficult to identify in this stage of infection.

The treatment of syphilitic bone disease is antibiotics, which are usually effective in curing congenital disease. The large destructive lesions of tertiary disease can be irreversible and surgical intervention may be required.

Yaws Bone Infection

Yaws is an endemic form of treponemal infection caused by *T. pallidum* subspecies *pertenue*. The name of the disease may come from the Carib word "yaya" which means sore. Yaws is a contagious chronic relapsing disease that is found in the remote humid tropical rural settings of South America, Asia, Africa, and Oceania. Most patients are young and range in age from 2 to 15 years old. The disease is contracted by direct, nonsexual, physical skin contact with infected material. In most instances a child with a scratch or cut comes in contact with the exudate or serum of an infected ulcerated lesion from another.[74] After gaining entry into tissue the organism disseminates hematogenously throughout the body.

Bone infection as a component of yaws is seen in untreated patients in the secondary and uncommon tertiary stages of the disease. It usually manifests as prominent periostitis and osteomyelitis that often affect the long bones of the extremities, including

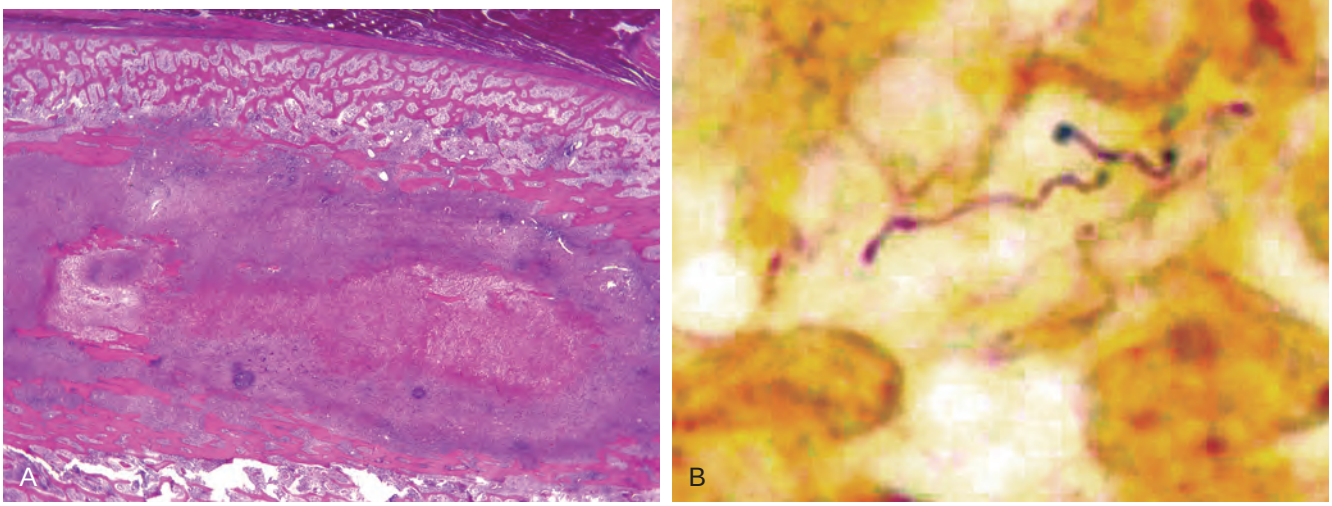
• **Figure 14.44** Macerated femur from migrant 55-year-old man with tertiary syphilis. **A,** The entire shaft of the bone is distorted by a prominent periostitis. **B,** The reactive new bone produces an irregular surface. (Courtesy Dr. W. Putschar.)

those of the hands and feet, forearm, leg, pelvis, skull, and face, and is the cause of the tibial saber shin (Fig. 14.46).[75] The bone disease may be associated with gummas that can be large and destructive, arthritis mutilans with marked resorption of bones similar to what may occur in leprosy, and severe contractures with pathologic fractures (Fig. 14.47). Histologically the inflammatory reaction is similar to that of venereal syphilis. Microscopically, the organism is identical in appearance to *T. pallidum* subspecies *pallidum*.

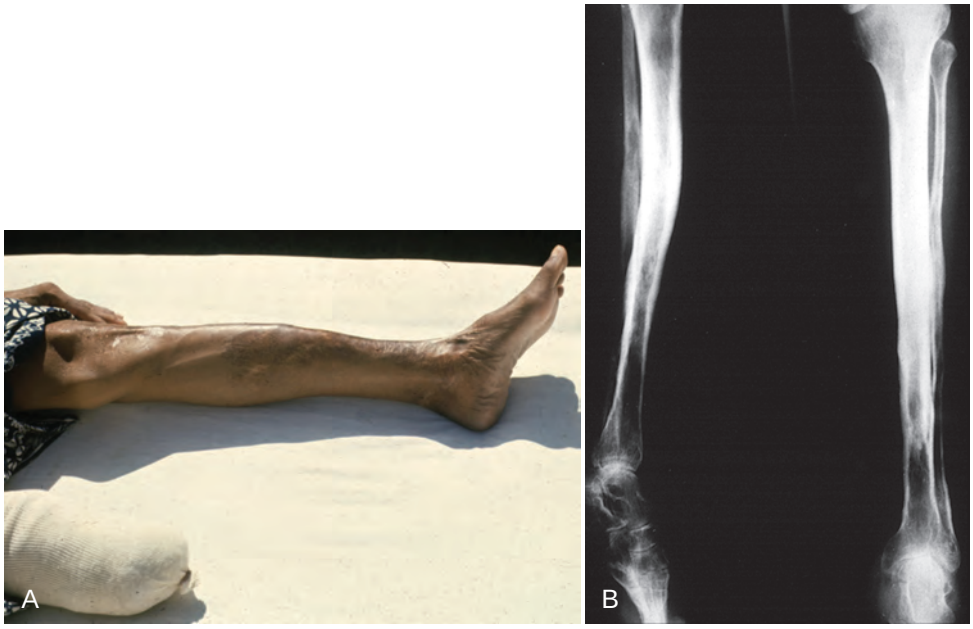
Fungal Osteomyelitis

Fungi occur worldwide and reside within the soil and dead tissue, and have a symbiotic relationship with plants, animals, and other fungi. Certain species are restricted to specific geographic conditions and locations, which has implications for human disease.

Fungi cause a variety of different human afflictions which range from insignificant to life-threatening infection. Fungal osteomyelitis is a serious illness and is most virulent in individuals with depressed immune systems. Fungus causes bone infection by hematogenous dissemination, direct inoculation, and spread from a neighboring site of disease.[76] Fungal osteomyelitis may occur as a component of a multisystem infection or as an isolated condition.[77] *Candida* spp., *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans* commonly spread to bone via the vascular system as a complication of pulmonary or other type of deep internal infection. *Histoplasma*, *Blastomyces*, and *Coccidioides* bone infections are usually endemic and are secondary to hematogenous dissemination in immunocompetent people. Opportunistic mycotic osteomyelitis is usually caused by *Candida*, *Aspergillus*, and cryptococcal organisms. *Aspergillus* spp., *Sporothrix schenckii*, and the fungi that cause mycetomas usually access bone by first forming a soft tissue infection and then spread via direct extension into the adjacent bone.



• **Figure 14.45** Gumma of tertiary syphilis. **A,** The inflammatory focus has destroyed the preexisting trabecular bone and is surrounded by reactive new bone. **B,** Steiner stain showing coiled *Treponema palladium*.



• **Figure 14.46** Tertiary yaws in adult. **A,** Clinical photograph showing tibial "saber shin." **B,** Anteroposterior and lateral radiographs of the lower leg demonstrate anterior bowing, marked periostitis, medullary sclerosis, and cortical thickening of the tibia. Similar changes are also noted in the fibula. (Courtesy Dr. W. Putschar.)

• **Figure 14.47** Radiograph of tertiary yaws with lytic destruction and pathologic fracture through diaphyses of ulna and radius. (Courtesy Dr. W. Putschar.)

The diagnosis of fungal osteomyelitis can be difficult as the disease may evolve slowly and masquerade as other conditions, such as tuberculosis, myeloma, and metastatic disease. The clinical presentation and outcome varies according to the specific pathogen, location of the infection, and host factors. Invariably the infection causes pain, localized swelling, bone destruction with reactive changes, and a prominent inflammatory reaction. Definitive diagnosis usually requires identification of the organism in tissue specimens or cultures. Treatment consists of a combination of antibiotics and surgical débridement.

Candida Osteomyelitis

The genus *Candida* includes almost 200 species, of which approximately 20 cause disease in humans and animals. In humans, *Candida albicans* is the major pathogenic species, although over the past decade there has been an increase in nonalbicans species, including *Candida glabrata*, *Candida guilliermondii*, *Candida lambica*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida stellatoidea*, and *Candida zeylanoides*. Traditionally considered to be commensal organisms and part of the normal flora of humans, *Candida* spp. can cause significant disease in the circumstance of impaired host immunity or repeated intravascular cannulation. *Candida* is the fourth leading organism causing bloodstream infections in the United States and accounts for 5% to 10% of such infections.[78] In 1% to 2% of these cases, osteomyelitis becomes a clinical complication. Accordingly, candidal osteomyelitis is often seen in the background of invasive candidiasis, and the patients are frequently immunosuppressed, debilitated, or critically ill; other risk factors include exposure to broad-spectrum antibiotics, indwelling catheters, diabetes, malnutrition, cirrhosis, and parental drug abuse. The skeletal disease may not manifest until several months or years after the candidemia has been treated. Occasionally, candida osteomyelitis results from

contamination during a surgical procedure, such as coronary bypass or spine surgery.[79] If it occurs in an individual who has no known predisposing factors, then conditions associated with polymorphonuclear leukocyte dysfunction should be suspected.

Most patients are adults and range in age from 40 to 80 years; younger patients often have a history of intravenous drug abuse. Pain localized to the site of disease is a common complaint, but constitutional findings may be minimal or absent because less than half of affected patients have fever or elevated sedimentation rates.[77] Disease progression is usually insidious and slow. Common sites of involvement include the spine, especially the lumbar vertebrae (two adjacent vertebral bodies), a long bone such as the femur, and the sternum. Most cases are solitary, but, in approximately 10% of cases, two or more sites may be involved.[80] Drug addicts have an increased incidence of costochondral involvement.

Radiographic studies show destruction of bone with surrounding sclerosis. Vertebral infection is frequently accompanied by discitis and at other sites such as the sternum, sinus tracts may form. Histologically the oval budding yeast, which are 5 to 12 µm in diameter, and hyphal forms elicit a dense acute and chronic inflammatory infiltrate and abundant granulation tissue. Treatment is antibiotics, and surgical débridement is frequently required. The prognosis is good with the infection usually being curable, but some patients who are severely ill die from the fungemia.

Aspergillus Osteomyelitis

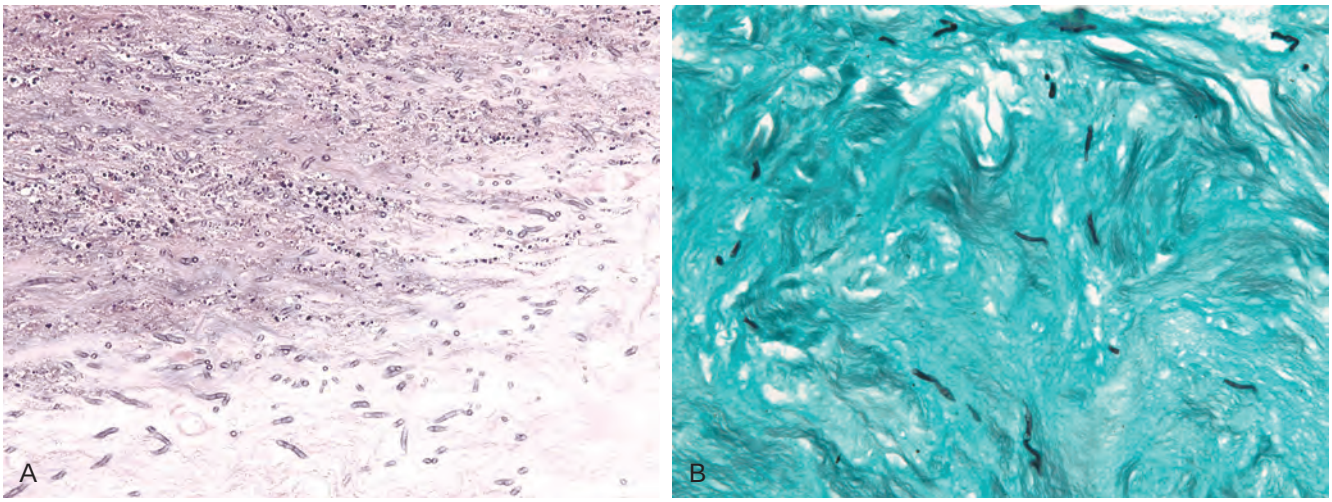
Aspergillus spp. normally reside in soil and are ubiquitous in the environment. There are more than 250 different species but only a few act as pathogens in humans, and they include *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, *and Aspergillus terreus*. Aspergillosis is the most common invasive fungal infection; however, bone involvement is very uncommon and accounts for 1.8% to 5.6% of bone infections.[81] The most important route of infection in aspergillosis is inhalation of the spores, which eventually settle in the pulmonary alveoli and on the surfaces of the sinus mucosae.[81] Additional factors leading to infection are trauma, severe burns, indwelling catheters, and intravenous drug use. Many patients have predisposing conditions associated with immune system dysfunction, including chronic granulomatous disease in children and immunosuppressive drug therapy in adults.

Affected individuals are usually adults who present with pain and symptoms related to the site of involvement. Imaging studies show that the infection destroys bone and induces surrounding reactive changes. Sometimes an associated soft tissue mass is present. In children the thoracic vertebrae are the most common locus of disease as the infection spreads from a contiguous focus in the lung. The vertebral column, especially the lumbar vertebrae, is the most frequent site of osteomyelitis in adults, and most cases are hematogenous in origin or result from direct inoculation during previous spine surgery (Fig. 14.48A and B).[82,83] Spine infection is often associated with diskitis and involvement of both neighboring vertebral bodies. Other bones reported to be described include the skull, sternum, ribs, tibia, and ilium.[77] The tissue response to the growing dimorphic septate organism is usually a purulent reaction with many neutrophils, plasma cells, lymphocytes, and macrophages. Granulomatous inflammation is uncommon but may be seen in patients with chronic granulomatous disease. The treatment of aspergillus osteomyelitis is antibiotics and surgical débridement.

Cryptococcal Osteomyelitis

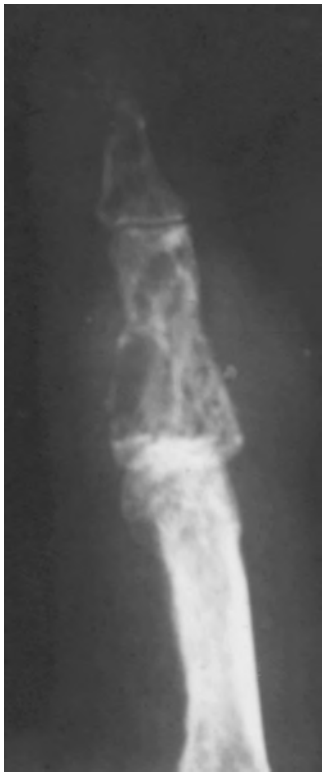
Cryptococcus is a genus of fungus that includes almost 20 different species. The encapsulated organism is found worldwide in the soil, trees, and pigeon dung. The most common species that is pathogenic to humans is *C. neoformans*. Although most patients infected with *Cryptococcus* are adults and immunosuppressed, approximately 20% to 34% are otherwise normal hosts.[83,84] Common underlying conditions include AIDS, treatment with steroids, organ transplantation, cancer, diabetes, and sarcoidosis.

The organism usually gains access to the body via the respiratory system, with direct inoculation occurring in a small number of cases. Cryptococcal osteomyelitis is frequently hematogenous in origin and develops in approximately 10% of patients with disseminated disease.[58] Patients commonly present with localized pain and swelling, and the bones involved include the vertebrae,

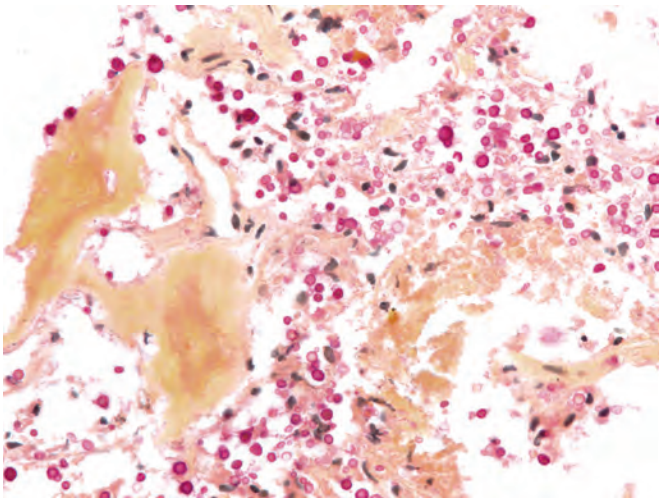


• **Figure 14.48** Aspergillus discitis. **A,** Necrotic annulus fibrosus of intervertebral disc with neutrophils, inflammatory debris, and infiltrating organisms. **B,** Grocott methenamine silver stain demonstrating septated hyphae growing between collagen fibers of the annulus fibrosus.

femur, tibia, rib, humerus, clavicle, and scapula.[83,84] In approximately 75% of cases, only one bone is involved, and, in 25%, multiple sites are involved. Radiographically the infection presents with bone destruction and sometimes an accompanying soft tissue abscess (Fig. 14.49). The histologic findings show round, yeastlike cells that are 3 to 6 μm in diameter, and the tissue reaction varies with the immune status of the host and ranges from a very limited inflammatory response to numerous granulomas with caseous necrosis (Fig. 14.50). Treatment requires antibiotics and surgical débridement.



• **Figure 14.49** Cryptococcosis involving finger. Anteroposterior view demonstrates lytic lesions involving the middle phalanx. Note the osteopenia in the distal phalanx. (Courtesy Dr. W. Putschar.)



• **Figure 14.50** Mucicarmine stain showing cryptococcal budding yeast forms surrounded by inflammatory cells and trabeculae of woven bone.

Sporotrichosis Osteomyelitis

S. schenckii, the pathogen causing sporotrichosis, is the only active member of the genus *Sporothrix*. The thermally dimorphic fungus is found worldwide in tropical zones and inhabits soil and plant material. *S. schenckii* infection varies in frequency with regard to geographic location and tends to be related to occupation because it most often develops in farmers, gardeners, fruit and vegetable pickers and packers, and other related industries.[85] Classically the infection is acquired by minor penetrating trauma, such as by a rose thorn, and manifests as skin and subcutaneous disease with spread in regional lymphatics. Osteoarticular infection is the most common form of extracutaneous disease and may result from direct spread from an abscess in the soft tissue, septic arthritis, or via the vascular system. Uncommonly, the infection can originate in the lungs or gastrointestinal tract and from there disseminate throughout the body, causing severe life-threatening disease, especially in persons who are immunosuppressed.

S. schenckii osteomyelitis may take several forms. It can manifest as eccentric erosions from a subcutaneous or synovial focus of disease or appear as solitary or multiple lytic lesions secondary to hematogenous spread. The bones frequently affected include the tibia, fibula, femur, humerus, and short tubular bones of the hands and feet.[85] The patients are adults and present with pain and swelling. Radiographically the lesions are destructive and lytic with periarticular erosions and osteoporosis. Histologically the branching hyphae induce a granulomatous reaction with necrosis and multinucleated giant cells. The treatment is antibiotics and surgical débridement.

Coccidiomycosis Osteomyelitis

Coccidioides is a genus of fungi that is endemic to the arid southwest United States and portions of Mexico and South America that have high summer temperatures, low rainfall, and low altitude.[86] The only species, *C. immitis*, lives in alkaline soil and accesses the human body via inhalation of arthroconidia. Most afflicted individuals are immunocompetent and are employed in jobs that involve working in or with the soil. Immunosuppressed individuals who develop infection are at risk for severe disseminated disease.

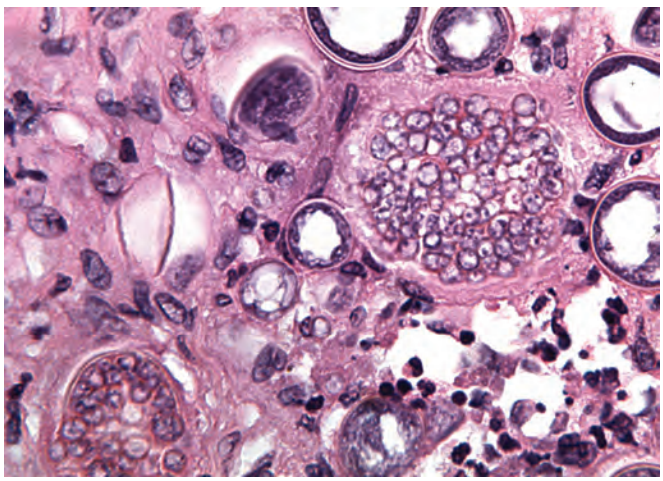
The vast majority of *C. immitis* infections are pulmonary in nature. Less than 5% of infections are extrapulmonary, and many of these occur in immunocompromised patients. Of these, 20% to 50% involve the skeletal system.[87] It is believed that most cases of coccidiomycosis of the skeleton follow hematogenous dissemination from a primary focus of disease in the lung even if it has completely resolved by the time the bone infection is diagnosed.

The patient presents with pain, swelling, limitation of function, and sometimes sinus tracts. The bone involvement is solitary in approximately 60% of cases and multifocal in the remainder. Any bone can be infected, but most cases involve the spine, skull, sternum, ribs, and pelvis. The metaphyses of long bones and carpal and tarsal bones are affected less commonly. In the spine the disease is frequently multifocal with extension into the soft tissues and development of a paraspinal abscess and relative sparing of intervening disks. In the ribs the lesions are often marginal in location and are associated with subpleural abscesses. Infection of the tubular bones frequently involves bony prominences where the disease often extends into neighboring joints. On x-ray the infected site ranges from well-demarcated to poorly defined lytic foci with limited surrounding reactive bone

formation (Fig. 14.51).[87] Histologically the thick-walled spherules of organisms elicit a granulomatous reaction that may be associated with neutrophils, and eosinophils are limited in number or absent.[88] Grocott methenamine silver (GMS) and periodic acid–Schiff (PAS) stains highlight the fungus (Fig. 14.52).

The infection is chronic, progressive, and destructive. Treatment consists of antibiotics and surgery and has a high rate of disease control and cure, except for immunocompromised patients.

• **Figure 14.51** Radiograph of proximal tibia in a patient with coccidiomycosis. In the proximal tibia there is a permeative lytic lesion with surrounding sclerosis. (Courtesy Dr. W. Putschar.)



• **Figure 14.52** Coccidioides with numerous spherules containing endospores. Hematoxylin and eosin–stained section.

Paracoccidioidomycosis Osteomyelitis

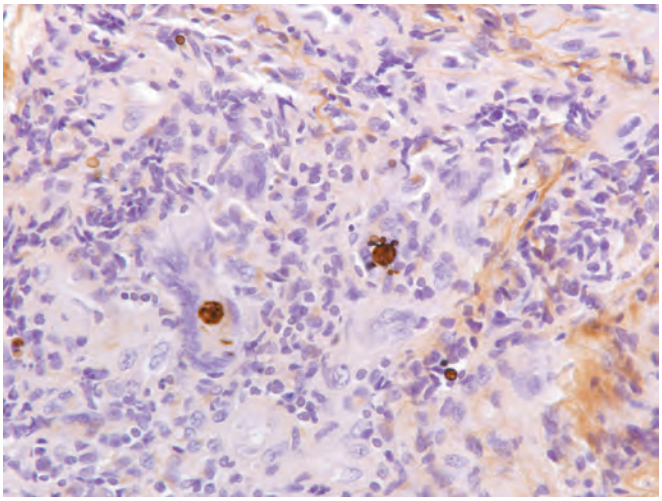
Paracoccidioidomycosis is a systemic fungal disease caused by *Paracoccidioides brasiliensis*. *P. brasiliensis* is the only member of the genus *Paracoccidioides* and is endemic to Central and South America. *P. brasiliensis* is a saprophyte and a natural inhabitant of the protein-rich soil native to this hot and humid geographic region. In this environment the fungus grows in the mycelial phase producing airborne conidia, which, if inhaled, results in inoculation. Consequently, pulmonary disease is the most common manifestation of paracoccidioidomycosis and is most often observed in male agricultural workers in rural communities.[89] The disease has its highest incidence in Brazil.

Paracoccidioidomycosis produces a variety of clinical manifestations, which can be been classified into acute and chronic forms. Chronic paracoccidioidomycosis is the most common (>90% of cases) and is usually diagnosed in adults. The disease typically begins in the pulmonary parenchyma where the fungus propagates and enters draining lymphatics, resulting in regional lymphadenitis. Depending on the host immune response, the infection may then be cleared, remain dormant, or become active. In chronic cases the disease progresses slowly, and in many instances the burden of infection is confined to the lungs, where it may produce varying degrees of parenchymal damage or become quiescent. However, in some individuals, the organism spreads (chronic multifocal form) through bronchogenic, lymphatic, or hematogenous routes, producing symptomatic involvement of one or more organs. The pathophysiology underlying most cases of bone paracoccidioidomycosis is believed to be hematogenous spread of the organism from a primary site of infection in the lung.[89] Less frequently, but more commonly in the jaw area, the bone becomes involved by contiguous spread from a neighboring site of infection, such as the gingiva. Most cases of joint involvement are believed to represent direct extension from a focus of adjacent osteomyelitis.

Bone and joint involvement in paracoccidioidomycosis is relatively uncommon and, based on radiographic studies, ranges from 5.9% to 27%. The vast majority of patients are adult males who have the chronic form of the disease. In children, the acute type of disease is more frequent and skeletal involvement has a higher incidence. The bones commonly affected are the scapula (including the acromion), clavicle, rib, humerus, radius, and phalanges. Infection centered in the long bones is usually located in the diaphyseal or metaphyseal-epiphyseal regions. Radiographically, paracoccidioides of bone produces lytic destruction that may have well-circumscribed or permeative margins with usually limited surrounding sclerosis and periostitis (Fig. 14.53). On CT scan the lesions are lytic and well demarcated, and on MRI they have the signal intensity greater than skeletal muscle on T1-weighted images and show surrounding edema and heterogeneous enhancement following administration of gadolinium.[90,91] Joint involvement should be suspected when periarticular erosions are present.

Histologically the fungus elicits an inflammatory response containing loose and compact granulomas with scattered multinucleated giant cells, acute and chronic inflammatory cells, and foci of caseous-type necrosis. The organisms are round, are 5 to 30 μm in greatest dimension, have refractile double walls, and exhibit multiple budding daughter cells that protrude from and surround the mother cell forming a "ship's wheel" configuration. They are most numerous in the loosely formed granulomas and are easily identified on PAS and GMS histochemical stains (Fig. 14.54).

• **Figure 14.53** Anteroposterior view of the knee in paracoccidiomycosis shows a poorly defined area of lysis in the femoral condyles. An erosion is also seen in the medial proximal tibia.



• **Figure 14.54** Paracoccidioides with budding yeast producing "ship's wheel" configuration seen on silver stain.

The treatment of paracoccidioidomycosis bone disease includes antibiotics and surgery when necessary. The outcome is generally good, but relapses occur and can be problematic.[92]

Histoplasmosis Osteomyelitis

The genus *Histoplasma* contains one species, *H. capsulatum*, which is composed of two varieties: *H. capsulatum var. capsulatum* and *H. capsulatum var. duboisii.* The organisms live in soil with high nitrogen content contaminated by bird droppings or bat guano. Histoplasma is endemic to midwestern and south central United States and portions of western, central, and southern Africa.[93] Histoplasmosis is usually initiated by inhalation of conidia from contaminated soil which is then followed by pulmonary infection. Hematogenous dissemination results in bone infection in a very small percentage of cases and is most common in the duboisii variant. Adult males are most frequently affected. Bone involvement may be multifocal often affects the pelvis, skull, ribs, and the short tubular bones (Fig. 14.55).[94] Histologically the infection induces a granulomatous reaction with budding yeast surrounded by the inflammatory infiltrate.

• **Figure 14.55** *Histoplasmosis duboisii* involving the skull in an African male. Lateral view of the skull shows multiple lytic foci of varying size. (Courtesy Dr. W. Putschar.)

Blastomycosis Osteomyelitis

B. dermatitidis is the only species included in the genus *Blastomyces*. *B. dermatitidis* includes two distinct serotypes, which are endemic to the Mississippi, Ohio, and St. Lawrence River valleys. Cases have been reported around the world, including Africa, South America, and Asia. Blastomycosis is a chronic infection with protean clinical manifestations initiated by inhalation of air-borne conidia and the subsequent development of pulmonary disease.[95] The fungus then disseminates via the vascular system and frequently involves the cutaneous and skeletal systems. In 10% of patients the primary presenting symptoms are related to the bone manifestations.

Approximately one-third of patients with disseminated disease develop bone lesions. Affected patients are usually middle age males. The anatomic regions most commonly affected include the axial skeleton and lower extremities; however, any bone may be targeted, and the most frequent are the vertebrae, ribs, facial bones, skull, metaphyses of long bones, small bones, pelvis, and scapula.[95] In most cases, only one bone is involved; however, almost 20% of patients have disease affecting two or more osseous sites. Patients with multifocal disease frequently have accompanying septic arthritis.[96] The osteomyelitis causes pain, swelling, and in many cases adjacent soft tissue abscesses and draining sinuses. Radiographically the lesions produce either well-circumscribed areas of bone destruction with sclerotic margins or a more diffuse pattern of bone resorption with moth-eaten margins that can mimic a primary bone neoplasm.[95] In the spine the changes may

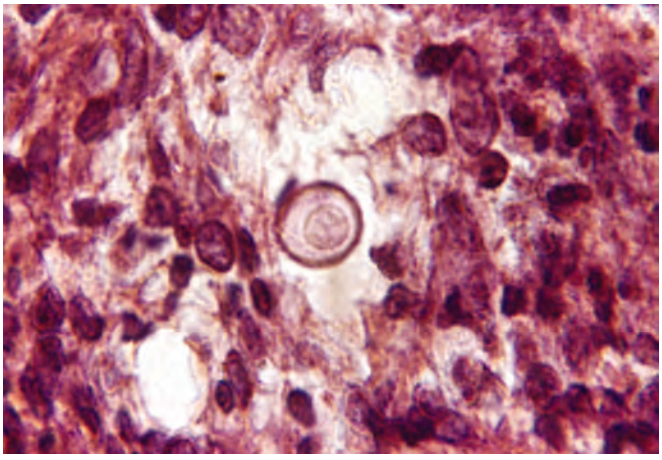
mimic tuberculosis with extensive involvement of the vertebral body, loss of disk space, and paraspinal soft tissue abscess formation. Histologically the fungus is frequently associated with neutrophilic microabscesses and granulomatous inflammation with numerous lymphocytes and plasma cells.[97] The organism is optimally visualized with PAS and GMS stains and appears as multinucleated yeast 8 to 15 μm in diameter and has a thick refractile wall (Fig. 14.56).[98] Budding occurs singly, and the bud is attached by a broad base.

The treatment of skeletal blastomycosis is usually antibiotics and surgical débridement. Outcome is generally good.

Mycetoma

Mycetoma is a chronic, frequently subcutaneous, granulomatous infection that commonly affects the feet, where it is also known as Madura foot.[99] The infecting organism is either actinomycetes bacteria (*Nocardia brasiliensis*, *Nocardia asteroids*, *Streptomyces somaliensis*, *Actinomadura madurae*, *Actinomadura pelletieri*, and *Actinomyces*) or eumycetes fungi (more than 20 different fungi, including *Madurella mycetomatis*, *Madurella grisea*, *Fusarium* spp., and *Acremonium* spp.).[99] The disease usually occurs in tropical and subtropical areas and is endemic to Latin America, India, and Africa.[100] It develops when organisms that are usually saprophytes in the soil are traumatically inoculated into tissue, such as occurs on the plantar aspect of the feet of persons who are frequently barefoot or the extremities or backs of workers carrying contaminated vegetation or other objects. Patients are typically poverty stricken, young or middle-aged adult males who present with tumefactive indurated areas of infection of the subcutis with draining sinuses.[100] Exiting from the sinuses are pus-containing grains (color depends on infectious agent), which are composed of aggregates of organisms. Long-term infection is associated with spread to the neighboring bones, with resultant osteomyelitis. The osteomyelitis often manifests as multifocal lytic bone destruction with minimal reactive new bone formation (Fig. 14.57).[101] Histologically the organisms elicit a purulent and granulomatous reaction with fibrosis and granulation tissue (Fig. 14.58A and B). The disease can be progressive and very destructive and spread via draining lymphatics.

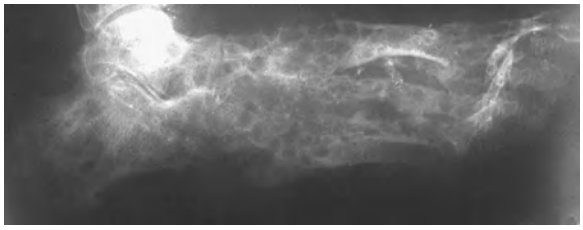
Treatment consists of identifying the pathogen and then administrating the appropriate antibiotics and surgical débridement as required. In some cases, amputation is necessary. Superinfection with other types of bacteria can result in sepsis and death.



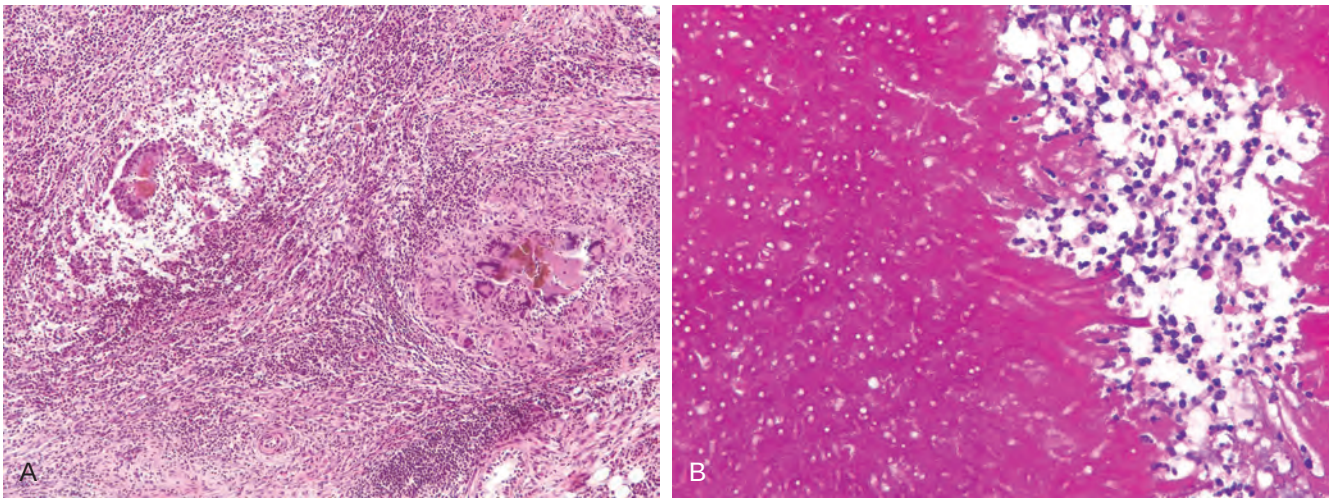
• **Figure 14.56** Thick refractile wall of blastomyces seen in periodic acid–Schiff stain.

Helminth Osteomyelitis

Helminthic infection of humans is worldwide and causes significant disease-related morbidity. The organisms can infect many



• **Figure 14.57** Madura foot. Lateral radiograph of the foot shows osteopenia with lytic foci within the tarsals, metatarsals, and phalanges. There is atrophy of the soft tissues. (Courtesy Dr. W. Putschar.)



• **Figure 14.58** Madura foot. **A,** Numerous granulomas with giant cells surrounding organisms pigmented fungi. **B,** Periodic acid–Schiff stain demonstrating branching septated fungi.

different organ systems; however, involvement of the skeleton is extremely uncommon. The worm that has the greatest propensity to infect bone is echinococcus.

Echinococcal Osteomyelitis

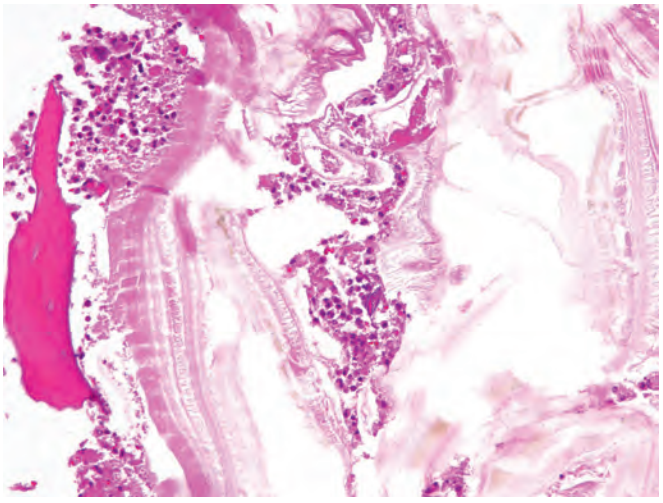
Echinococcus, a parasitic tapeworm, includes 12 different species; however, *Echinococcus granulosus* is the most important human pathogen and the only one that causes significant bone disease.[102] Human echinococcal infection occurs across the globe and results from the ingestion of contaminated water and food or handling live animals that act as definitive hosts, such as dogs, or working with their pelts. The swallowed eggs hatch in the small intestine and release minute hooked embryos that burrow through the bowel wall and are then transported by the bloodstream to various sites, such as the lung, liver, brain, skeletal muscle, and eyes. Involvement of the skeletal system is rare and occurs in 0.2% to 4% of cases. After organisms reach their final destination, they produce hydatid larval cysts. These unilocular cysts are filled with fluid and lined by an inner germinal membrane that produces brood capsules. The inner wall of the brood capsules facilitates an asexual budding process that generates thousands of new larval tapeworms (protoscolices) in daughter cysts. The hydatid cysts can achieve large size (2 to 30 cm), have a thick wall, and contain clear or pale yellow fluid that has a neutral pH and contains sodium chloride, proteins, glucose, ions, lipids, and polysaccharides. The fluid is antigenic.

The sites of bone disease commonly include the spine, pelvis, and long bones. After being lodged within bone, the hydatid cyst fills the spaces between the bony trabeculae and eventually results in their resorption and that of the neighboring cortex, such that it extends into the adjacent soft tissues. These changes cause the patient to complain of pain, swelling, and sometimes pathologic fracture, with other symptoms specific to the anatomic site of involvement.[103] Radiographically the cyst appears as an expanded portion of bone with irregular areas of bone destruction that may have sclerotic well-defined or moth-eaten margins (Fig. 14.59). On MRI the mass is bright on T2-weighted sequences because of the prominent water content of the cyst.

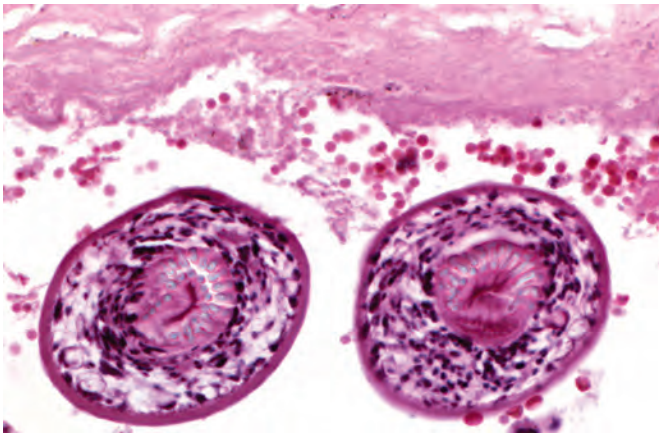
Grossly, echinococcus of bone is centered in the medullary cavity and frequently distorts the bone by expansion and destruction. On cross-section the bone contains many small thin-walled cysts that range in size from a millimeter to more than several centimeters in greatest dimension—a mother cyst is not present. The cysts percolate between the trabeculae, eventually resulting in their resorption by osteoclasts and the production of reactive fibrous tissue infiltrated by a dense population of lymphocytes, eosinophils, plasma cells, and scattered granulomas with multinucleated histiocytes, all of which surround the cysts (Fig. 14.60).[103] The nucleated germinal cyst wall is thin and eosinophilic, and rarely are protoscolices attached to its inner surface (Fig. 14.61).

The treatment of hydatid disease of bone is antihelminthic chemotherapy and surgery, which is either in the form of curettage and packing with cement or en bloc excision.[104] Because the disease evolves slowly, many patients have extensive osseous changes by the time they are diagnosed. This complicates adequate therapy and results in a high rate of local recurrence.[102]

• **Figure 14.59** Ecchinococcosis involving the pelvis and femur. Multiple lytic lesions are seen within the proximal femur and acetabulum. There is protrusio acetabuli with a pathologic fracture through the iliopectineal line.



• **Figure 14.60** Echinococcal cyst walls infiltrating medullary cavity and surrounded by inflammatory cells and necrotic bone.



• **Figure 14.61** Echinococcal cyst wall with protoscolicies.

Viral Osteomyelitis

Viral osteomyelitis is an uncommon clinical problem, with arthritis being a more common complication. The viruses that most commonly cause bone changes include smallpox, vaccinia, and intrauterine rubella and cytomegalic inclusion disease.

Variola Osteomyelitis

The variola virus, one of the most virulent pathogens of humans, is the cause of smallpox, a disease that is now considered extinct. Smallpox osteomyelitis is a well-recognized phenomenon and complicates 2% to 5% of children with infection and has been termed osteomyelitis variolosa. There is no relationship between the severity of initial smallpox infection and either the occurrence or degree of severity of bone involvement.[105] The patients usually complain of pain and swelling one to several weeks after the smallpox becomes clinically evident. Musculoskeletal involvement can affect both the joints and bones, frequently simultaneously, and in a symmetrical fashion. Tubular bones, especially those about the elbow, and those of the hands and feet are commonly affected.[106] The spine and pelvis may also be targeted. The radiographic findings include juxtametaphyseal lysis and destruction with extension into the epiphysis, which sometimes separates from the bone, and prominent periosteal reactive new bone formation (Fig. 14.62).[107] Histologically the bone undergoes necrosis with medullary fibrosis and osteoclastic activity. In severe cases, draining sinuses, pathologic fractures, contractures, growth deformities, and secondary bacterial infection may develop. Treatment is supportive, unless there is secondary infection, and uncomplicated cases usually heal and resolve over time.

• **Figure 14.62** Three-year-old child with small pox. Radiograph of the legs shows metaphyseal lysis and sclerosis of proximal and distal tibiae and prominent periostitis of fibula. (Courtesy Dr. W. Putschar.)

Rubella Osteomyelitis

Rubella (German measles) infrequently involves the skeleton, and when it does it is in the setting of intrauterine infection. Radiographically the findings are most notable along the distal femoral and proximal tibial metaphyses, where radiolucent horizontal bands may be present. In addition, linear "celery stalk–" type radiolucencies, which begin adjacent to an irregular zone of calcification of the growth plate and extend into the diaphysis, and are delineated by a thin zone of radiodensity and oriented parallel to the long axis of the bone, are sometimes evident.[107] Periostitis is absent. Histologically there is reduced bone formation with marrow fibrosis and increased numbers of plasma cells in the marrow and periosteum.[108] The changes usually resolve spontaneously but infrequently persist or progress.

Other Types of Viral Bone Infection

Infrequently, other types of viral infection can involve the skeleton. Intrauterine cytomegalic inclusion disease can produce changes similar to rubella, and rarely vaccinia and varicella can induce alterations in bone.

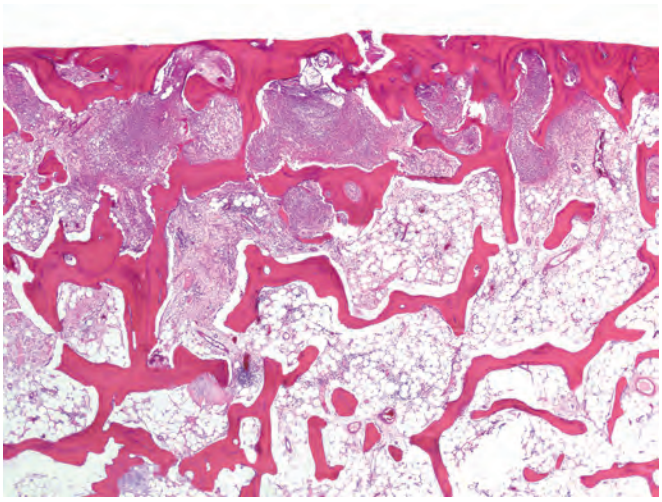
Differential Diagnosis of Osteomyelitis

The differential diagnosis of osteomyelitis includes other conditions in which an inflammatory infiltrate is present in bone. Based on histologic findings alone it can be difficult, if not impossible, to determine whether inflammation is a manifestation of infection versus some other etiology. Features that support the possibility of infection include the density of inflammation and its distribution. Situations prone to potential misinterpretation include subchondral acute inflammation in severe arthritis, acute inflammation associated with acute fracture, autoimmune-related inflammatory diseases (chronic recurrent multifocal osteomyelitis [CRMO]), sarcoidosis, hematopoietic malignancies, and histiocytic proliferations (Box 14.4).

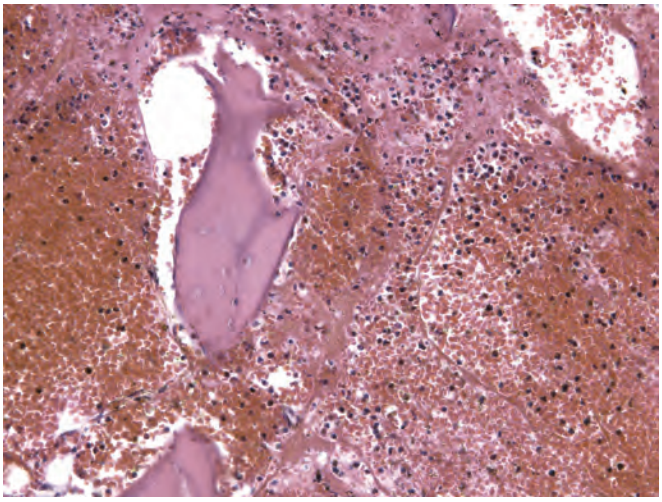
Subchondral acute inflammation in severe arthritis may be secondary to disruption of the articular surface, giving access to synovial fluid with an acute inflammatory exudate to the subchondral tissues (Fig. 14.63).[109] This process is usually identified as an incidental finding in large joint arthroplasty specimens. Patients frequently have a history of rheumatoid arthritis, osteonecrosis, or osteoarthritis. Two distinct patterns of subchondral inflammation may be present. In specimens demonstrating osteonecrosis, the suppurative inflammation diffusely fills the marrow space between necrotic cancellous bone. Because the trabecular bone is necrotic, the histologic resemblance to acute bacterial osteomyelitis can be striking. The inflammation does not extend beyond the confines of the necrotic tissue and is sharply demarcated from the

• BOX 14.4 Radiographic and Histologic Mimics of Osteomyelitis

Subchondral acute inflammation
Acute fracture
Autoinflammatory diseases (chronic recurrent multifocal osteomyelitis), sarcoidosis
Hematopoietic malignancies
Histiocytic proliferations



• **Figure 14.63** Femoral head with severe osteoarthritis. Neutrophils fill subchondral cysts. The adjacent marrow is relatively unremarkable.



• **Figure 14.64** Recent fracture with abundant hemorrhage containing many neutrophils and pieces of focally necrotic bone.

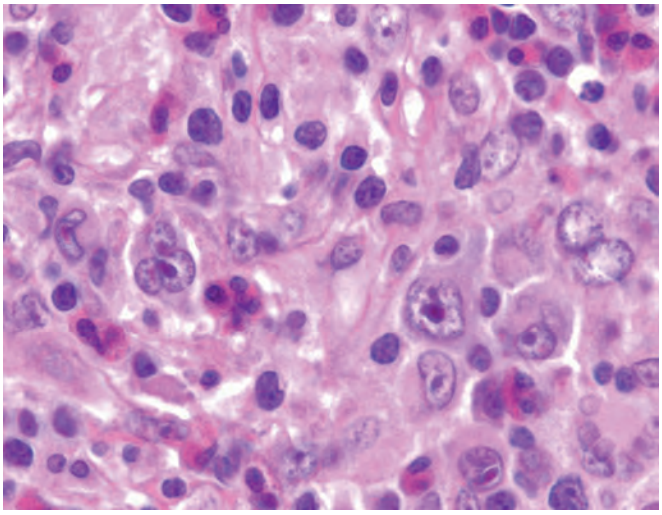
surrounding viable bone. The second and more frequent type of inflammation is centered on subchondral cysts (geodes). In these examples, the center of the cyst is distended by sheets of neutrophils associated with a fibrinous exudate. The suppurative exudate is admixed with granular eosinophilic to basophilic debris resembling bone dust. The fibrous lining of the "cyst" is edematous, contains a mild chronic inflammatory infiltrate, scattered foamy or vacuolated histiocytes, and occasional multinucleated giant cells within its walls. The nodular nature of the acute inflammatory process, in addition to the frequent presence of histiocytes within the wall, can create a distinctly granulomatous appearance. However, the adjacent sclerotic subchondral bone is viable, and the surrounding marrow spaces may show mild fibrosis that extends for only a short distance from the edge of the inflammatory infiltrate. In contrast, untreated osteomyelitis is frequently associated with a dense inflammatory infiltrate that involves a large area of bone and has a poorly defined margin with abundant reactive fibrosis at the periphery.

The acute stages of fracture can contain abundant neutrophils at the traumatized site, and the involved bone may be necrotic if enough time has passed to permit breakdown of osteoblast and osteocyte nuclei (Fig. 14.64). This combination of features can resemble osteomyelitis. However, an important distinguishing feature is extensive hemorrhage, which is present in fracture and generally not nearly as prominent in infection.

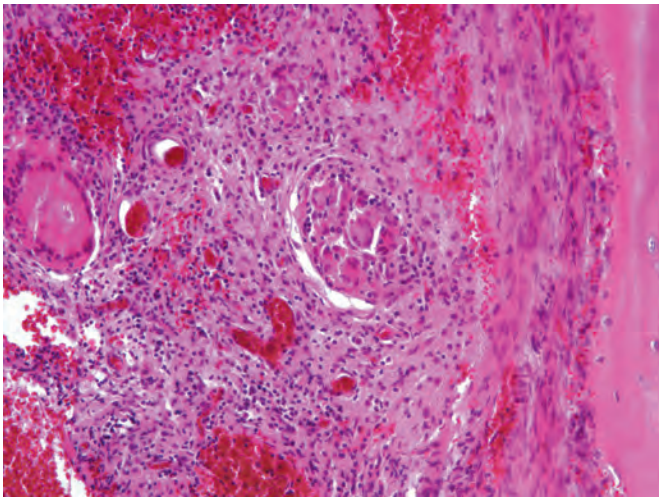
Autoinflammatory conditions of bone, especially CRMO, can be confused with infectious bone disease on clinical, radiographic, and histologic features.[110] Diagnostic criteria for CRMO include (1) multiple bone lesions, (2) extended clinical course which spans years with remissions and exacerbations, (3) no response to antibiotics, and (4) manifests on x-rays as foci of lysis surrounded by sclerosis.[110] The etiology is unknown but is unlikely to be infectious in nature. Studies suggest that genetic abnormalities may be important in its genesis. CRMO usually presents in children (median age 10 years) who complain of pain and tenderness; fever and swelling, although common, are sometimes absent. Some patients have been reported to have other conditions, such as palmar-plantar pustulosis, psoriasis vulgaris, generalized pustulosis, severe, acne, Sweet syndrome, and inflammatory bowel disease. It is also a component of SAPHO syndrome (*s*ynovitis, *a*cne, *p*ustulosis, *h*yperostosis, and *o*steitis). Frequent laboratory abnormalities include an elevated erythrocyte sedimentation rate, increased concentration of tumor necrosis factor-α, and sometimes a high white blood cell count. Patients have one or more sites of active bone disease, which is usually localized to the metaphyseal region of long bones, followed by the clavicle, but may involve essentially any bone, including the pelvis, spine, and craniofacial skeleton.[111] The lesions are avid on bone scan, and on x-ray are irregular, radiolucent, and surrounded by a zone of sclerosis. Tissue from biopsy specimens resemble those from bacterial infection with regard to the inflammatory infiltrate and bone changes. In the early stage of the disease, neutrophils predominate and over time are replaced lymphocytes, plasma cells, and scattered macrophages. The bone shows areas of necrosis, osteoclastic activity, and reactive bone formation. Cultures of the tissue and blood are negative. The clinical course is characterized by cycles of waxing and waning with new lesions developing. Treatment usually consists of nonsteroidal antiinflammatory drugs because antibiotics are ineffective.

Hematopoietic malignancies containing large numbers of mature-appearing inflammatory cells can cause confusion with osteomyelitis. This is especially relevant when the population of inflammatory cells is small lymphocytes, plasma cells, or the mixed inflammatory infiltrate that characterizes some forms of Hodgkin disease. The monomorphous nature of the cells in the setting of lymphoma or plasma cell malignancies is a key finding that helps to separate them from infection. When doubt persists, immunohistochemistry may be helpful in demonstrating clonality of the infiltrating cells. Regarding Hodgkin disease, the identification of Reed-Sternberg cells and their variants is crucial for accurate diagnosis. Occasionally, some cases of Hodgkin disease contain so many neutrophils and eosinophils that the distinction from infection is challenging and requires immunohistochemistry (Fig. 14.65).

Sarcoidosis is a granulomatous systemic disease of uncertain etiology. It involves most frequently the pulmonary parenchyma and mediastinal lymph nodes, but any organ system can be affected. Skeletal disease is present in 1% to 13% of cases and is frequently accompanied by pulmonary and skin lesions. Bone involvement is often bilateral, and bones commonly affected include the middle and distal phalanges (producing "sausage finger"), wrist, skull vertebral column, and long bones (Fig. 14.66). Radiographically



• **Figure 14.65** Hodgkin disease with Reed-Sternberg cells and scattered inflammatory cells.



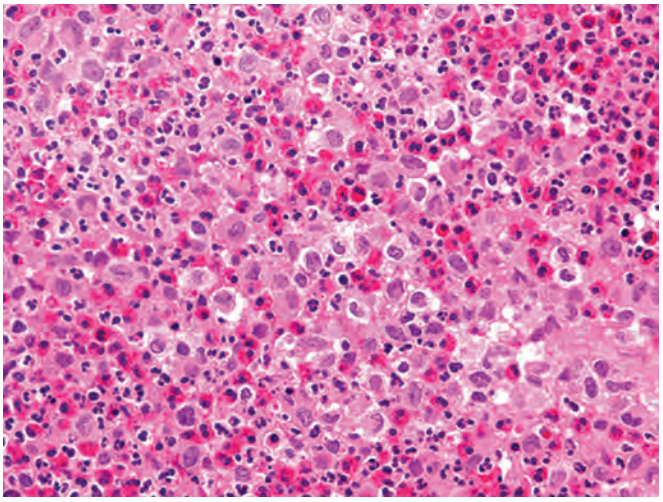
• **Figure 14.67** Sarcoidosis of bones. Well-formed granuloma with giant cells enmeshed in reactive fibrous tissue with scattered lymphocytes.

• **Figure 14.66** Sarcoidosis of spine. Sagittal T1-weighted post-gadolinium image with fat saturation shows multiple enhancing lesions in several vertebral bodies.

the lesions are lytic or "lacelike," frequently involve the cortex, and may have moth-eaten margins. Sarcoidosis is characterized histologically by compact, round or oval granulomas composed of epithelioid histiocytes that have oval or kidney bean–shaped pale nuclei (Fig. 14.67). Langerhans and conventional multinucleated giant cells may be present. The granulomas may contain limited amounts of central fibrinoid necrosis, but caseation is absent. Lymphocytes are frequently distributed around the periphery of the histiocytes, and the inflammatory infiltrate is enmeshed in reactive fibrous tissue. Special stains for microorganisms are negative. The differential diagnosis includes granulomatous osteomyelitis caused by mycobacteria, fungi, and bacteria. Helpful distinguishing features are the clinical findings and histologic features, such as the cohesive aggregation of the epithelioid histiocytes and the absence of caseous necrosis and demonstrable microorganisms.

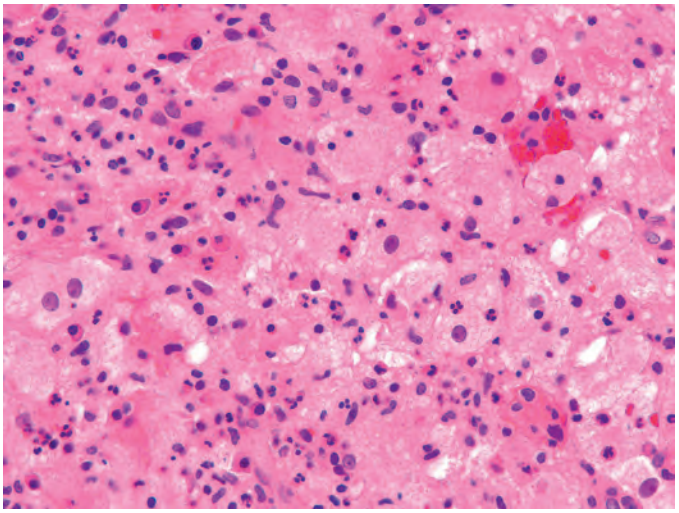
Langerhans cell histiocytosis of bone, previously known as histiocytosis X, is defined as an intraosseous mass of proliferating Langerhans cells. Langerhans cell histiocytosis is associated with a variety of clinical syndromes that vary according to the number, site, and size of the tumors. Single or multiple lesions restricted to the skeleton have traditionally been termed "eosinophilic granuloma." Langerhans cell histiocytosis is usually diagnosed during the first three decades of life, and males are affected approximately twice as frequently as females. The disease usually manifests in the skeleton, and solitary bone lesions are encountered twice as often as multiple bone lesions. The tumors can develop in any bone, but most commonly originate in the skull and jaw, followed by vertebral bodies, ribs, pelvis, and long bones. The lesions are well defined and lytic on radiographs; however, in a minority of cases, they have ill-defined and permeative margins (Fig. 14.68). Cortical involvement may elicit a periosteal reaction. Histologically the proliferating Langerhans cells are ovoid or round, histiocyte like, 10.0 to 15.0 μm in diameter, and arranged in aggregates, sheets, or individually, within a loose fibrous stroma. The cells have eosinophilic cytoplasm and contain central ovoid "coffee bean–" shaped nuclei that have pale chromatin and inconspicuous nucleoli. The coffee bean appearance is produced by deep indentations, clefts, and folds of the nuclear membranes, which form linear grooves that traverse the length of the nuclei (Fig. 14.69).[112] One of the morphologic hallmarks of Langerhans cell histiocytosis is an accompanying infiltrate of eosinophils that may be so dense that the underlying Langerhans cells are obscured. The eosinophils are distributed evenly or arranged in clusters, producing eosinophilic abscesses. Other types of inflammatory cells, including lymphocytes, plasma cells, macrophages, neutrophils, and osteoclast-type giant cells, are also frequently present. The diagnosis of Langerhans cell histiocytosis is principally based upon the light microscopic appearance of the Langerhans cells and

• **Figure 14.68** langerhan cell histiocytosis. Radiograph of the distal femur shows an eccentric destructive lesion in the lateral metaphysis with significant reactive sclerosis in the adjacent bone and overlying periosteal new bone formation.

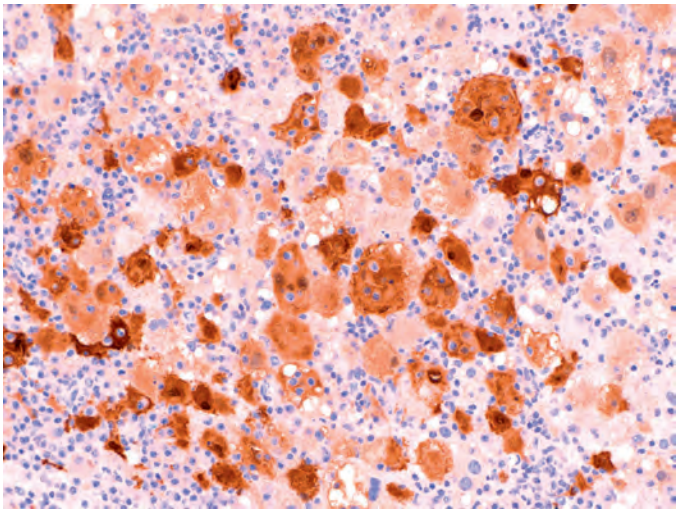


• **Figure 14.69** Langerhan cell histiocytes associated with many eosinophils.

the appropriate mixed cellular background; however, adjuvant diagnostic techniques, including electron microscopy and immunohistochemistry, help to confirm their presence. Langerhans cells strongly express S-100 protein, CD1a, and Langerin. In many cases the lesions also have a BRAF V600E mutation. Langerhans cell histiocytosis can be distinguished from osteomyelitis because the Langerhans cells grow in clusters and are not randomly distributed as single cells in the setting of infection. Other helpful features are



• **Figure 14.70** Rosai Dorfman histiocytes with abundant histiocytes demonstrating emperipolesis of acute and chronic inflammatory cells.



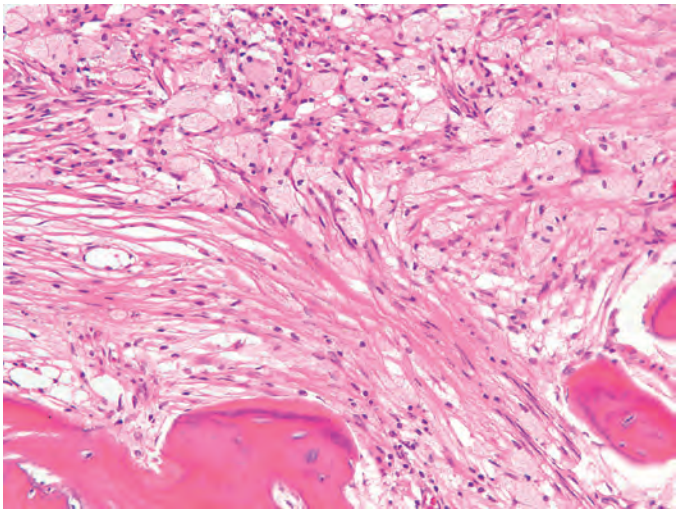
• **Figure 14.71** Rosai Dorfman histiocytes staining strongly for S100 protein.

the predominance of neutrophils in acute osteomyelitis and the relative paucity of eosinophils in most types of bacterial infection.

Rosai Dorfman disease (RDD), also known as sinus histiocytosis with massive lymphadenopathy, is a type of histiocytosis of unknown etiology. Extranodal disease can involve a variety of organ systems, including the skeleton. RDD can affect any bone, where it presents as a lytic lesion.[113] It is composed histologically of large histiocytes that have oval nuclei with fine chromatin and abundant eosinophilic cytoplasm. These large cells often exhibit emperipolesis, which is phagocytosis of inflammatory cells (Fig. 14.70) and, unlike most other types of histiocytes, are strongly positive for S100 (Fig. 14.71). The stroma is fibrotic and often contains a mixed inflammatory cell infiltrate that is rich in lymphocytes, plasma cells, conventional macrophages, and neutrophils. The inflammatory infiltrate can be interpreted as osteomyelitis, and the distinction rests on identifying the large histiocytes with emperipolesis.

Erdheim-Chester disease (ECD) is a variant of histiocytosis that is associated with a BRAF V600E mutation, which can be

• **Figure 14.72** Erdheim-Chester disease manifesting as sclerosis of the femur and tibia.



• **Figure 14.73** Sclerosis of the trabecular bone, marrow fibrosis, and numerous foamy macrophages admixed with lymphocytes and plasma cells are characteristic of Erdheim Chester disease.

detected in the lesion or the patient's blood.[114] The disease can affect multiple organ systems and often presents with bone involvement. Typically the lesions are bilateral and symmetrical and often affect the diaphysis of long bones, where they are sclerotic on imaging studies (Fig. 14.72). Histologically the lesions are composed of foamy macrophages associated with a mature lymphoplasmacytic infiltrate and fibrosis. The bone is sclerotic, caused by newly deposited woven bone (Fig. 14.73). The distinction from chronic osteomyelitis is based on the clinical findings and the prominence of foamy macrophages, which are not common in osteomyelitis.

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15

Infections of Joints, Synovium-Lined Structures, and Soft Tissue

ELIZABETH G. DEMICCO, SUSAN V. KATTAPURAM, RICHARD L. KRADIN, ANDREW E. ROSENBERG

Bacterial Arthritis
- Gonococcal Arthritis
- Meningococcal Arthritis
- Lyme Arthritis (Borreliosis)
- Tuberculous Mycobacterial Arthritis
- Nontuberculous Mycobacterial Arthritis

Fungal Arthritis
- Candidiasis
- Cryptococcosis
- Coccidioidomycosis
- Blastomycosis
- Histoplasmosis
- Sporotrichosis
- Aspergillosis
- Mycetoma

Viral Arthritis
- Parvovirus B19
- Rubella
- Hepatitis C
- Hepatitis B
- Human Immunodeficiency Virus
- Alphavirus

Reactive Arthritis
- Acute Rheumatic Fever
- Poststreptococcal Reactive Arthritis

Soft Tissue Infections
- Necrotizing Soft Tissue Infections
- Gas Gangrene
- Nonnecrotizing Cellulitis

Myositis
- Pyomyositis
- Psoas Abscess
- Bacterial Myositis
- Fungal Myositis
- Parasitic Soft Tissue Infection
- Viral Myositis

Other Unusual Soft Tissue Infections
- Mycetoma
- Chromoblastomycosis and Phaeohyphomycosis
- Protothecosis
- Emerging Nosocomial Fungal Infections
- Surgical Site Infections
- Soft Tissue Infection After Military Trauma

Pathogenic involvement of joints and other synovium-lined structures, including tendon sheaths and bursae, is a relatively uncommon but important form of infection. Its significance is related to the severe destruction of musculoskeletal structures it can cause, which often results in permanent disability and, in some instances, even death.

Synovial tissue is vital to the normal functioning of the musculoskeletal system. It helps maintain joints, tendons, bursae, and ligaments; is the source of nourishment for articular cartilage; and participates in the immune surveillance and activity of these tissues.[1] The synoviocytes secrete the fluid that provides lubrication, protection, and nourishment to the adjacent structures. Articular cartilage, which lacks its own vascular supply, is wholly dependent on synovial fluid for the provision of oxygen and other essential metabolites. The fluid is also rich in glycoproteins, hyaluronic acid, and lysosomal enzymes that are important in the homeostasis of chondrocytes, fibroblasts, and the structural matrix. Histologically, synoviocytes are cuboidal to elongate and are arranged in a layer, two to four cells thick, which is supported by loose fibrofatty connective tissue containing abundant blood vessels, lymphatics, and nerves. The synovial lining lacks a protective basement membrane, and this, in conjunction with its rich vascular network, makes it a prime target for invasion by a wide variety of microorganisms (Table 15.1).[1]

Mechanistically the initial entry of an organism into a joint or synovial structure occurs by the same pathways involved in osteomyelitis; namely, hematogenous seeding, extension from a contiguous site, and direct implantation. In many situations, hematogenous seeding is the most common etiology, followed in frequency by spread from a neighboring infection in bone or soft tissue. Trauma with direct implantation is a common cause of tenosynovitis and bursitis, which often affects the digits

TABLE 15.1 Organisms That Cause Septic Arthritis

Gram-Positive Bacteria (60–70%)	
Staphylococcus aureus	44%
Streptococcus pyogenes	7%
Streptococcus pneumoniae	7%
Coagulase-negative staphylococci	4%
Streptococcus agalactiae	3%
Other β-hemolytic streptococci	4%
Gram-Negative Bacteria (20–30%)	
Mycobacterium tuberculosis	4%
Escherichia coli	4%
Haemophilus influenzae	4%
Neisseria gonorrhoeae	3%
Pseudomonas aeruginosa	2%
Neisseria meningitidis	1%
Salmonella spp.	1%
Other gram-negatives	5%
Polymicrobial/Anaerobic Bacteria (2–5%)	
Bacteroides	
Fusobacterium	
Peptostreptococcus	
Clostridium	
Fungal (4%)	
Candida spp.	
Sporothrix schenckii	
Coccidioides immitis	
Blastomyces dermatitidis	
Cryptococcus neoformans	
Histoplasma capsulatum	
Miscellaneous (1%)	

Data from Ross JJ. Septic arthritis. *Infect Dis Clin North Am.* 2005;19:799-817.

of the hands and feet and may less frequently involve larger joints. Because tendon sheaths may communicate with bursae, infection of these structures can produce complicated patterns of extension.

After an organism infects the synovium, there is usually an acute inflammatory response with vascular dilatation and increased permeability, resulting in tissue edema and hyperemia. The inflamed synovium undergoes architectural changes to accommodate its increased girth by forming papillary structures lined by hypertrophied and hyperplasic synoviocytes. The synoviocytes increase their secretion of fluid, and this, in conjunction with serum leakage from reactive vessels and fibrin production, results in effusion of the affected joint, bursa, or tendon sheath. Both pathogenic infiltration and the reactive host inflammatory reaction can trigger necrosis of synoviocytes, chondrocytes, and tendinocytes and digestion of crucial structural components, with subsequent destruction of the tissues. The closed physical nature of joints, tendon sheaths, and bursae limits their expansion, so, after they are inundated by the infectious process, secondary ischemia and its complications (e.g., compartment syndromes) may occur. Over time, chronic inflammatory cells appear and are accompanied by reactive fibrosis and scarring. If located near bone, the infection can initiate osteoclastic activity and erosion of the bone; this frequently manifests as juxtaarticular erosions in joint infections. Eventually, there can be severe and permanent destruction of the joint and periarticular structures, resulting in significant dysfunction. In some instances the infectious agent, because of molecular mimicry between the host and the pathogen, initiates an autoimmune reaction that may be short lived or chronic and may cause further significant damage and disability.

Bacterial Arthritis

Bacteria are the most common cause of infection of the synovium. Large weight-bearing joints, such as the knee, are particularly prone to hematogenous seeding. Small joints of the hands and feet are more likely to develop septic arthritis secondary to penetrating trauma.[2] Iatrogenic causes of infection include arthroscopy, corticosteroid injection, surgery, and implantation of prosthetic joints.

The primary risk factor for septic arthritis is preexisting joint disease, and up to 47% of patients have prior joint problems or rheumatologic disorders, including rheumatoid arthritis, systemic lupus erythematosus (SLE), osteoarthritis, gout, pseudogout, trauma, or prior joint surgery.[2,3] Rheumatoid arthritis is most frequently associated with septic arthritis and tends to have worse outcomes. Chronic breach in skin integrity (e.g., psoriasis, chronic skin ulcers, intravenous drug use) is also a risk factor. An immunocompromised state (e.g., malignancy, corticosteroid use, chronic disease, acquired immune deficiency syndrome [AIDS]) is strongly linked to increased risk for infectious arthritis, in particular with atypical microorganisms, such as *Listeria*, *Salmonella*, and *Actinobacillus*, as well as fungal and mycobacterial arthritides.[4]

Most cases of bacterial arthritis are due to occult bacteremia with gram-positive organisms, typically *Staphylococcus aureus*. Gram-negative bacilli account for 10% of community-acquired septic arthritis and are associated with intravenous drug use.[4,5] Gram-positive bacteria are thought to more efficiently bind extracellular matrix proteins, thereby facilitating adhesion to affected tissues. A number of bacterial adhesion genes that may be involved in this process have been identified, including those encoding fibrinogen-binding proteins *(fib, cflA, fbpA)*, fibronectin-binding proteins *(fnbA, fnbB)*, an elastin-binding protein *(ebpS)*, a collagen receptor *(cna)*, and a less specific binding protein *(map)*.[5] Bacterial invasion and proliferation result in direct soft tissue and cartilage damage through the production of bacterial enzymes and toxins (e.g., staphylococcal TSST-1, enterotoxins, α-hemolysin). The host inflammatory response further damages articular cartilage through activation of matrix metalloproteinases (MMPs) and release of reactive oxygen species, lysosomal enzymes, and proinflammatory cytokines, including interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α.[5] Articular cartilage is also subjected to ischemia as accumulation of pus

within the joint space leads to increased joint pressure and tamponade of synovial blood flow.[4-6] If left untreated, infection may spread to adjacent bone and soft tissues, leading to the formation of sinus tracts, osteomyelitis, and possible destruction of tendons and ligaments.

S. aureus accounts for 40% to 50% of cases of septic arthritis, followed by *Streptococcus pyogenes* (8%), *Streptococcus pneumoniae* (7%), *Haemophilus influenzae* (4% to 7%), *Escherichia coli* (4%), coagulase-negative staphylococci (4%), *Neisseria gonorrhoeae* (3%), *Streptococcus agalactiae* (3%), *Pseudomonas aeruginosa* (2%), *Neisseria meningitidis* (1%), and *Salmonella* spp. (1%).[4] Other gram-negative rods and β-hemolytic streptococci are responsible for 5% and 4% of cases, respectively, and 1% to 2% of cases are polymicrobial. *Staphylococcus* and *Streptococcus* are the primary bacterial pathogens in pediatric septic arthritis, whereas vaccination against *H. influenzae* has significantly reduced the incidence of arthritis associated with that organism.[7]

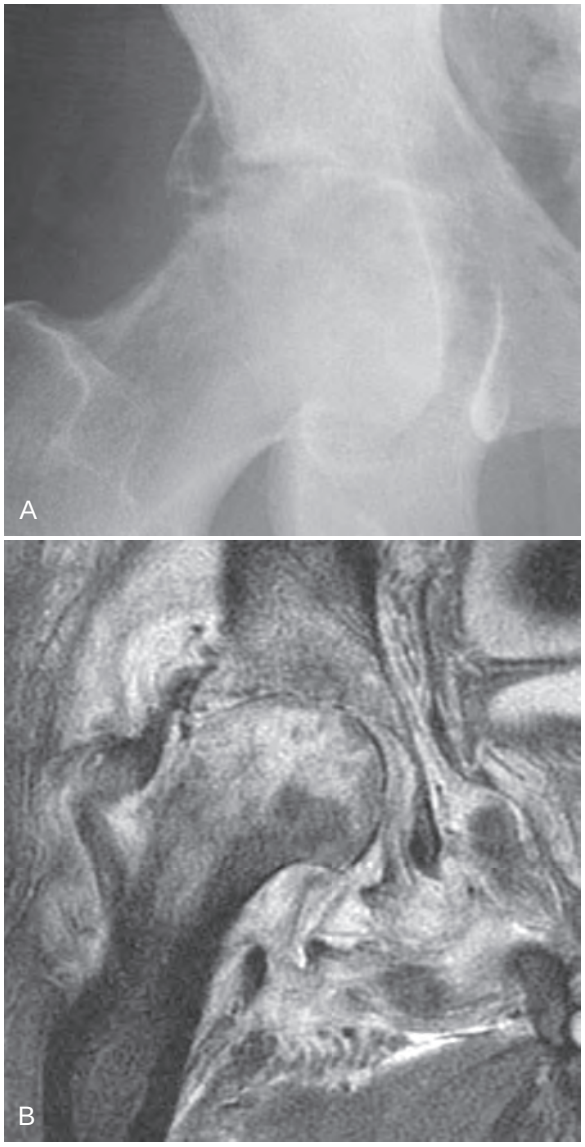
Kingella kingae, a β-hemolytic aerobic/facultative anaerobic gram-negative bacillus that forms part of the normal pharyngeal flora of infants is now recognized as an important pathogen in children younger than 2 years of age. Median age of presentation is 18.6 months, and infection is rare in patients older than 4 years. Infection typically occurs after a breach in mucosal integrity (often a result of upper respiratory illness) that leads to occult bacteremia. Clinical systemic symptoms are mild, and disease is typically monoarticular or oligoarticular, with large weight-bearing joints being the most common sites of involvement. Osteomyelitis and spinal discitis have also been reported.[8] Since the advent of polymerase chain reaction (PCR)-based diagnostic testing, *K. kingae* has been reported to account for 48% of proven bacterial joint infections in patients under 2 years, and is now thought to be the single most common bacterium responsible for septic arthritis in children younger than 3 years.[8,9]

Anaerobic pathogens are fastidious and difficult to culture and therefore are often overlooked as a cause of septic arthritis.[10] The infections are often polymicrobial, and their true incidence is uncertain. *Bacteroides*, *Peptostreptococcus*, *Fusobacterium*, *Clostridium*, and *Propionibacterium acnes* are the primary culprits, and pigmented *Prevotella*, *Porphyromonas*, and *Fusobacterium* are all associated with bite infections. *Bacteroides fragilis* has a predilection for hand and foot infections.[10] Risk factors for anaerobic infection include collagen vascular disease, bites, trauma, prosthetic joints, prior surgery, and peripheral neuropathy. Adults are more frequently affected than children.

Bacterial arthritis is usually monoarticular. The knee is the primary site for bacterial arthritis, with 30% to 50% of cases, followed by hip (15% to 22%), ankle (6% to 9%), elbow (8% to 9%), wrist (4% to 6%), and shoulder (5% to 12%).[2,3,11] In the pediatric population the most common sites of septic arthritis are the hip and knee, and 90% of cases are monoarticular.[12] Polyarticular disease occurs in approximately 15% of cases and is associated with immunocompromise.[3] Gonococcal, pneumococcal, group B streptococcal, and gram-negative infections also may result in polyarthritis.[13] Septic polyarthritis is asymmetric, and 72% of cases involve the knee.[13] The cartilaginous joints of the axial skeleton are relatively resistant to infection, although an association with intravenous drug use has been established.[14]

The classic presentation of septic arthritis is fevers and rigor accompanying a swollen, warm, erythematous, and painful joint. High-grade fever is present only in 58% of cases, whereas low-grade fever is present 90% of the time.[15] Fewer than 60% of patients present with a serum leukocytosis,[3,15] and immunocompromised patients may not report pain. Therefore a high index of suspicion should be maintained, even in the absence of classic symptoms, and arthrocentesis should be performed. Synovial fluid chemistries, including protein, glucose, and lactate dehydrogenase concentrations, as well as synovial white blood cell (WBC) counts, are relatively nonspecific for infection. Infectious etiologies should be considered in all synovial fluids with a WBC count greater than 50,000 cells/μL; however, a low WBC count does not exclude septic arthritis (Table 15.2). Gram staining and culture should be routinely performed whenever infection is suspected, as well as PCR or serologic testing where indicated.

Historically, plain films have been of use only in late-stage disease, demonstrating joint effusions, cartilage loss, or bone destruction (Fig. 15.1).[16] Magnetic resonance imaging (MRI) may



• **Figure 15.1** Septic arthritis of the hip joint. **A,** Plain radiograph of the hip shows near-complete loss of the joint space, destruction of the superomedial acetabulum and the superolateral femoral head, and acetabulum with bone fragments in the soft tissues. **B,** Coronal T2-weighted magnetic resonance image demonstrates bright signal intensity within the acetabulum and femoral head and in and around the joint, representing inflammation and edema. Note the destruction of the cortical bone along the femoral head and adjacent acetabulum.

TABLE 15.2 Synovial Fluid Analysis by Etiology*

Parameter	Normal	Noninflammatory	Inflammatory, Viral, or Reactive	Bacterial	Fungal	Crystal	Trauma
Color	Yellow	Yellow	Yellow	Gray-green	Variable	Yellow-white	Red-brown
Clarity	Clear	Slightly cloudy	Cloudy	Cloudy	Variable	Cloudy	Bloody
Viscosity	High	Reduced	Low	Low	Low	Low	Reduced
Clot	No	Rare	Rare	Frequent	—	Rare	Yes
WBCs/μL	0–200	0–2000	2000–100,000	20–200,000	10,000–100,000	500–200,000	50–10,000
% Neutrophils	<25	<30	>50	>75	>50	<90	<50
Serum glucose concentration (mg/dL)	0–10	0–10	0–40	20–100	10–20	0–80	0–20
Crystals	No	No	±	±	±	Yes	±
Culture	Negative	Negative	Negative	Most organisms	Some organisms	Negative	Negative
PCR/Serology	Negative	Negative	Positive	Some organisms	Some organisms	Negative	Negative
Stains	Negative	Negative	Negative	Gram stain, AFB, silver stain	Silver stain, PAS, Gram stain	Negative	Negative

*Synovial WBCs, glucose, protein, and lactate dehydrogenase concentrations are relatively nonspecific indicators of infection. Presence of crystals does not exclude septic arthritis. Gram stain, culture, PCR, or serologic studies remain essential for definitive diagnosis and organism identification.
AFB, Acid-fast bacteria stain; *PAS*, periodic acid–Schiff stain; *PCR*, polymerase chain reaction; *WBCs*, white blood cells.

be helpful in diagnosing early lesions, including those that involve tendon sheaths, with findings such as effusion, synovial thickening and enhancement, bone marrow edema, and perisynovial edema and enhancement (Fig. 15.2).[17] Reactive bone marrow edema in septic arthritis can be mistaken on imaging for periarticular osteomyelitis. However, in osteomyelitis, the edema demonstrates markedly low signal on T1-weighted images and is more diffuse and robust than that seen in association with septic joints, although these findings are not specific.[16] Ultrasound may also be used to detect early effusion. Echo-free effusions, resulting from clotting hemorrhagic fluid collections, are thought to be characteristic of septic arthritis.[18] Imaging studies can also be used to monitor the response to treatment or to demonstrate underlying conditions such as osteoarthritis.[5]

Histologic examination of infected synovial tissue from native joints is rarely performed, because diagnosis relies on clinical findings, arthrocentesis, and analysis and culture of synovial fluid. Animal models have shown that joint injury is rapid, with synovial hypertrophy visible within 24 hours after intravascular bacterial injection, whereas bone erosions and pannus formation may develop within 3 days. Early septic arthritis is dominated by dense neutrophilic infiltration of the synovium, which may coat articular cartilage and result in its necrosis and digestion (Fig. 15.3). Eventually, lymphocytes, plasma cells, and histiocytes appear, and an acute and chronic papillary synovitis with fibrin aggregates develops. These changes resemble those seen in noninfectious inflammatory arthritides, such as rheumatoid arthritis.[19] In long-standing disease the entire articular cartilage may be eroded, resulting in osteomyelitis (Fig. 15.4).

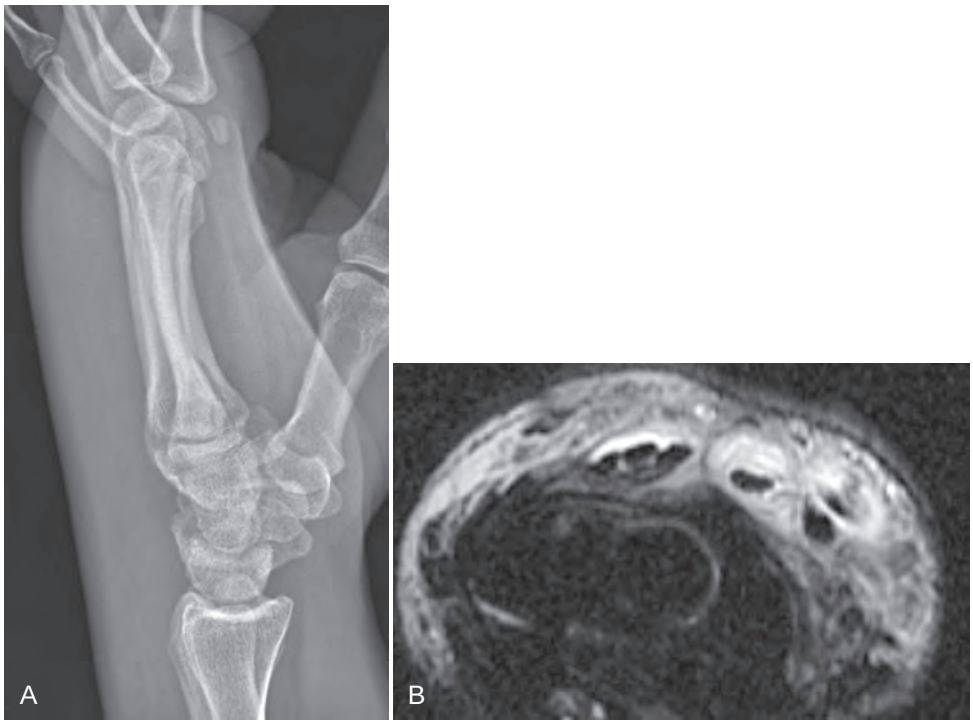
Treatment of septic arthritis should be prompt and involves appropriate antimicrobial therapy. Intraarticular antibacterial therapy is not usually indicated. Drainage should be performed by needle aspiration or open drainage and may be repeated. Débridement may be required.[5]

Outcomes in septic arthritis may be poor, especially if the infection accompanies systemic sepsis, with an overall mortality rate as high as 11%.[3,15] Morbidity is also high, with secondary osteomyelitis occurring in 8% and poor functional outcome in 24% to 40% of cases.[3,15] Poor outcome is associated with polyarticular arthritis, age greater than 65 years, delay in presentation, existence of medical comorbidities, chronic illness, and rheumatoid arthritis.[3,4]

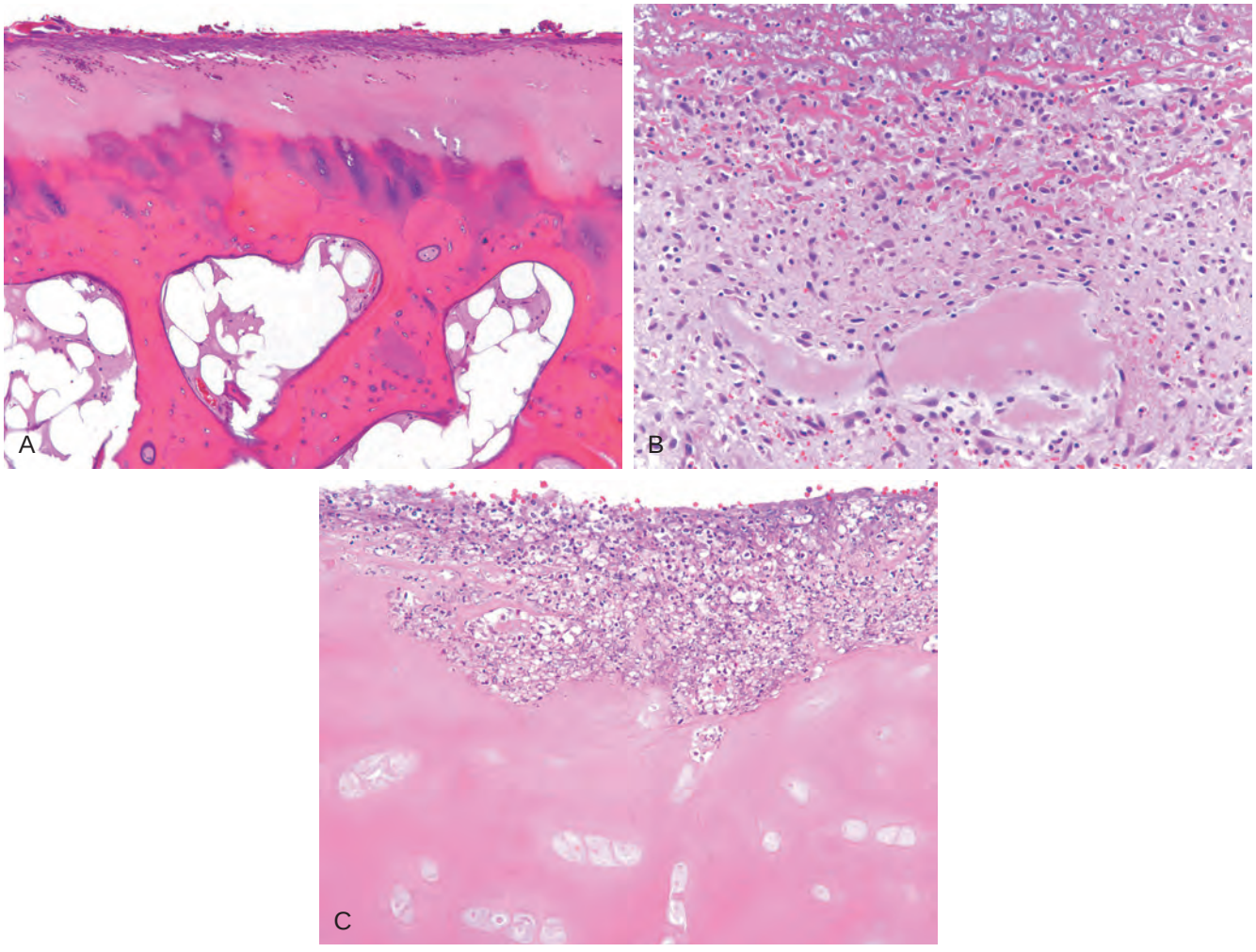
Gonococcal Arthritis

N. gonorrhoeae, a gram-negative diplococcus, is the most common sexually transmitted bacterium associated with infective arthritis. In the 1970s through the 1980s it was the most common cause of infective arthritis in the United States.[20] Safer sex practices resulted in a steep decline in disease prevalence through the 1990s. More recently, gonococcal infections have been on the rise, primarily due to high-risk sexual behavior among young men who have sex with men. Of further concern is the appearance of antibiotic-resistant strains. These two trends ensure that gonorrhea will continue to be of clinical importance for the foreseeable future.

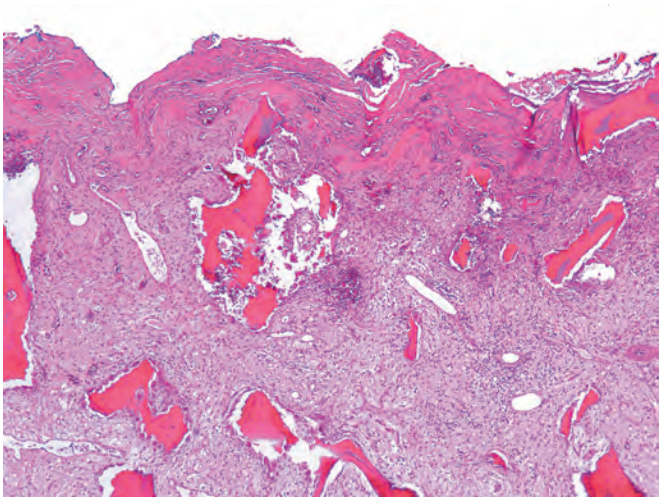
Patients with gonococcal arthritis are usually young, healthy, and sexually active. Gonococcal arthritis may take one of two forms: a classic suppurative arthritis similar to the septic arthritis seen with other bacterial infections or a syndrome of disseminated gonococcal infection (DGI) including tenosynovitis, skin lesions, and polyarthralgias without true septic arthritis.[20] Some authors have postulated an immunologic basis for DGI. However, the



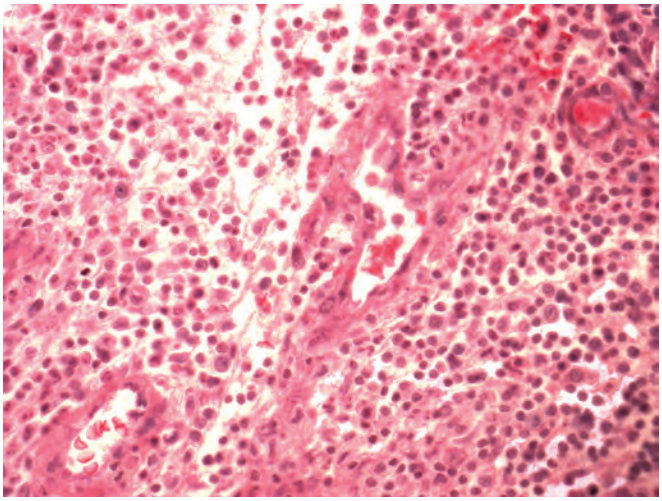
• **Figure 15.2** Septic tenosynovitis. **A,** Lateral radiograph shows edema of the dorsal soft tissues. **B,** Axial magnetic resonance image demonstrates marked T2-intense edema around the dark tendon.



• **Figure 15.3** Septic arthritis. **A,** Neutrophils coat the articular cartilage surface. **B,** Synovial surface covered by neutrophils and fibrin. Note embedded fragment of articular cartilage. **C,** Neutrophils digesting necrotic articular hyaline cartilage.



• **Figure 15.4** Articular surface with destruction of cartilage. The irregular bone surface is covered by a fibrous synovial pannus.



• **Figure 15.5** Gonococcus causes a periarticular inflammation with granulation tissue, neutrophils, and mononuclear cells.

presence of positive blood cultures in up to 50% of cases and an inability to identify circulating immune complexes argue against this theory.[20]

Gonococcal infection begins with localized mucosal disease. In fewer than 3% of cases, the organism rapidly enters the circulation and produces DGI.[21-23] Risk factors associated with DGI include delayed diagnosis, SLE, deficiency of the complement system, menstruation, and pregnancy, as well as socioeconomic factors, promiscuity, urban residence, male homosexuality, and educational status.[5] Bacterial strains also play a role, with higher risk associated with piliated *N. gonorrhoeae* strains that are capable of phase variation. Women have four times the risk of men for development of DGI,[24] most likely because of asymptomatic infection and delayed diagnosis.

Patients with DGI present with migratory arthralgias, fever, chills, dermatitis, and tenosynovitis. Most are found to have asymptomatic mucosal gonococcal infection involving the genitals, anus, or pharynx.[21] Skin lesions are classically limited to the extremities and trunk and begin as small, inconspicuous erythematous papules, which progress to vesicular or pustular lesions. The papules may overlie affected joints. Tenosynovitis causes pain, swelling, and erythema. Polyarthralgias may involve knees, elbows, and distal joints, whereas arthritis and tenosynovitis are polyarticular and preferentially involve the distal joints, including those of the hands and fingers, wrists, elbows, knees, and ankles.[5,20]

Suppurative gonococcal arthritis is monoarticular or oligoarticular and involves the knees, wrists, ankles, and elbows. Symptoms of bacteremia, skin lesions, or prior polyarthralgias are not present.

The radiographic and histopathologic findings in gonococcal arthritis are similar to those seen in other septic bacterial arthritides. However, imaging studies and synovial biopsy are rarely performed. In rare biopsied cases the periarticular soft tissues show granulation tissue with mixed polymorphonuclear and mononuclear cell infiltrates (Fig. 15.5).

Synovial fluid analysis and culture are required for diagnosis. Treatment requires appropriate antibacterial therapy in conjunction with aspiration. Surgery is rarely necessary. Patients should be tested for concomitant infection with *Chlamydia trachomatis*. Appropriate treatment is associated with a good outcome.

The differential diagnosis of DGI includes *N. meningitidis* infection, which manifests with similar skin lesions and arthralgias. Other bacterial infections also must be ruled out and can usually be distinguished by their clinical features or by culture.

Meningococcal Arthritis

Infection with *N. meningitidis* is associated with a sterile monoarticular or oligoarticular arthritis that is thought to be secondary to a hypersensitivity reaction with immune complex deposition. Recovery from this form of arthritis is usually complete. Infrequently, meningococcus may lead to a monoarticular septic arthritis.

Lyme Arthritis (Borreliosis)

Lyme borreliosis was first described in 1975 in the United States, after the perplexing outbreak of juvenile rheumatoid arthritis in the vicinity of Lyme, Connecticut.[25] The causative agent, the spirochete *Borrelia burgdorferi*, was not identified until 1982.[26,27] Lyme disease is now recognized as the most common vector-borne disease in North America and Europe, with annual incidence rates as high as 31.6 cases per 100,000 population in endemic areas.[28] In the United States it is localized to three endemic areas: the East Coast (Maine to Maryland), the Midwest (Minnesota and Wisconsin), and the Northwest (Northern California and Oregon). However, these regions continue to enlarge as the geographic range of the vector ticks infected with *B. burgdorferi* expands.

Three members of the *Borrelia* genus are known to cause Lyme disease in humans: *B. burgdorferi* (endemic to North America), *Borrelia garinii*, and *Borrelia afzelii* (endemic to Europe and Asia). More recently, *Borrelia lonestari* has been implicated in a Lyme disease–like illness in the southern United States, and *Borrelia miyamotoi* is a recently recognized borreliosis transmitted by *Ixodes persulcatus*, which presents with Lyme disease–like symptoms, including myalgia and arthralgia in 30% to 60% of patients, in the absence of a preceding rash.[29] Borreliosis is a vector-borne disease, with pathogens transmitted by the bite of infected *Ixodes ricinus* ticks. In the larval and nymph stages of the tick life cycle, ticks feed primarily on small rodents—predominantly

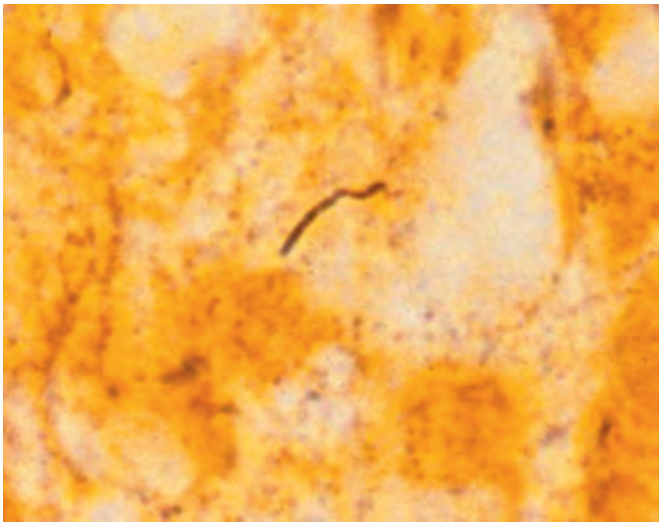
the white-footed mouse in the United States and the vole in Europe and Asia. These animals then serve as a disease reservoir for horizontal transmission between nymphs and larval ticks. In contrast, larger mammals, such as deer or humans, preferred as a food source for adult ticks, are dead-end hosts for *Borrelia*.[30,31] For *Borrelia* transmission to occur, the tick must be attached to its host for at least 24 hours[32]; this allows the bacteria to migrate from the tick midgut into the salivary gland, a process mediated by cell surface adhesion molecules, including OspA and Ospc.[33]

A number of factors have been implicated in disease transmission. Tick saliva is thought to contain vasodilators, anticoagulants, and immunomodulators, which may facilitate *Borrelia* transmission to the host.[31,34] Factors on the spirochete membrane function as adhesion molecules, such as p66, which binds to β_3-chain integrins, and BBK32, which binds to fibronectin. *B. burgdorferi* then binds to host plasmin and urokinase, resulting in plasmin-mediated MMP activation and proteolysis of extracellular matrix, which allows dissemination of bacteria throughout the host.[30,31] Additional factors assist in immune evasion, by modulating host response or downregulation of immunogenic cell surface markers.

The pathognomonic lesion of early acute Lyme disease is the erythema migrans rash, which appears at the site of tick bite within 5 to 14 days, then spontaneously resolves after several days to weeks.[35] The rash is erythematous, often with a central clearing, or bull's-eye, and is usually painless and nonpruritic. Erythema migrans has been reported to have an incidence of up to 90% in some studies of Lyme disease patients.[36,37] Within a few weeks of infection, untreated patients develop symptoms of early disseminated disease, including fever, malaise, aseptic meningitis, facial palsy, radiculoneuropathy, or carditis, as well as migratory arthralgias and myalgias. True arthritis typically develops weeks or months after the initial infection.

Acute Lyme arthritis occurs in up to 50% to 60% of untreated patients infected with *B. burgdorferi*[38]; the incidence is lower with *B. garinii* or *B. afzelii* infection.[39] Lyme arthritis is usually a monoarticular or oligoarticular arthritis that typically affects the knee or other large joints.[40] Joints of the digits are occasionally involved, and tendonitis and bursitis may also occur. In most patients, arthritis occurs in flares, which resolve over a period of years. In a minority of patients, chronic synovitis may develop despite appropriate antibiotic treatment. Chronic synovitis is accompanied by joint injury and results in permanent joint damage in 2% of patients.[40]

Chronic Lyme disease is considered to be a controversial diagnosis, and theories on its pathogenesis range from autoimmunity, to lingering *B. burgdorferi* cell bodies or bacterial DNA in tissue provoking an immune response. The crux of this controversy is the persistence of arthritis despite eradication of spirochetes in the affected joint. Evidence for persistent disease has been controversial at best, with the majority of studies finding no serologic or culture evidence of *B. burgdorferi* within synovial tissues after appropriate antibiotic therapy. The argument for an autoimmune response leading to arthritis is more convincing. Mechanistically this immune response is thought to be caused by focal homology between the *B. burgdorferi* membrane antigen OspA and hLFA-1α, an adhesion molecule expressed by most lymphoid and myeloid cells that is upregulated during an inflammatory response. An association with major histocompatibility complex (MHC) class II human leukocyte antigens HLA-DRB1*0401 and 0101 has also been identified. T cells are thought to be an important mediator of subsequent inflammatory changes, along with secreted proinflammatory cytokines.[31,41]



• **Figure 15.6** Lyme disease with Warthin-Starry stain demonstrating coiled *Borrelia burgdorferi* spirochete.

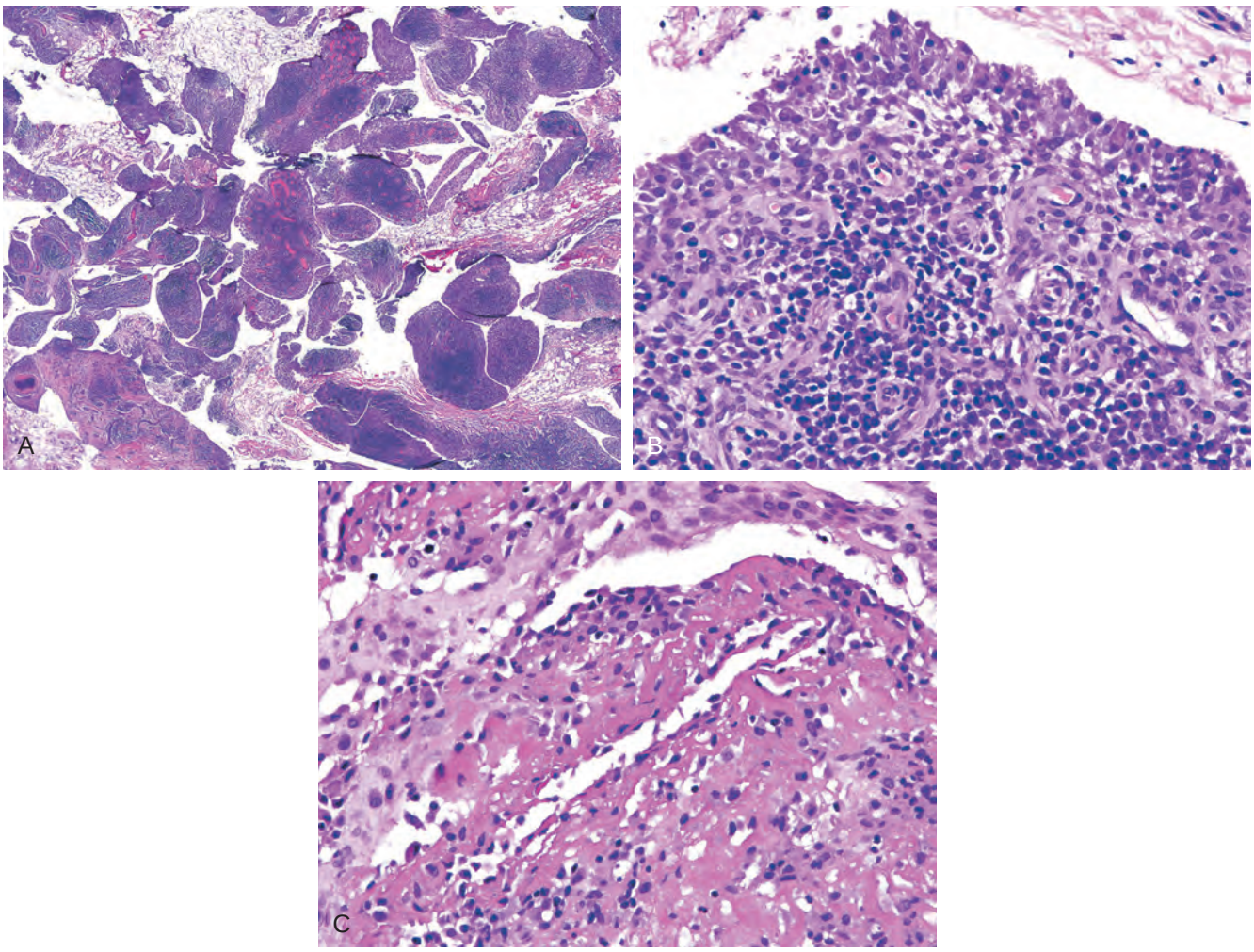
On imaging, acute Lyme arthritis shows effusion of the knee joint. Intraarticular edema can be seen, along with a spectrum of soft tissue changes involving the infrapatellar fat pad, periarticular soft tissues, and entheses. In chronic Lyme arthritis, an inflammatory arthritis is seen, with juxtaarticular osteoporosis, loss of cartilage, and bony erosions. Rarely, degenerative changes, such as subarticular sclerosis and osteophyte formation, may be seen.[42]

The histologic features of acute Lyme synovitis are similar to those observed in other septic arthritides, with edema and abundant neutrophilic infiltration of synovium and adjacent soft tissues. Organisms have been demonstrated in synovial tissues in the early stages of infection by Warthin-Starry silver stain (Fig. 15.6), and PCR has been shown to improve detection.[43] Histologic examination of synovial tissue from chronic Lyme arthritis demonstrates nonspecific features of chronic inflammatory or rheumatoid arthritis. Fibrin deposition is seen, along with synovial hypertrophy, neovascularization, and infiltration by histiocytes, plasma cells, and a subsynovial mixed lymphocyte population (Fig. 15.7).[44] In this setting the morphologic changes overlap with those of other, noninfectious inflammatory arthropathies.

Diagnosis of Lyme arthritis should be considered in patients who have recently been in areas endemic for *B. burgdorferi* and present with acute or subacute monoarthritis. A history of erythema migrans is helpful, but not required, to proceed to serologic or synovial fluid testing. Treatment of acute Lyme arthritis involves oral or intravenous antibiotic therapy. If arthritis does not resolve with two courses of an appropriate antibiotic regimen and PCR testing of synovial fluid is negative for *B. burgdorferi*, chronic Lyme arthritis should be considered, and it should be treated with immunosuppressive therapy.

Tuberculous Mycobacterial Arthritis

Joint involvement follows infection of the spine as the most common manifestation of musculoskeletal tuberculosis. Peripheral large and medium-sized joints, especially the hip and knee, are most frequently affected.[45] Risk factors for development of tuberculous arthritis include female gender and older age; children



• **Figure 15.7** Lyme disease. **A,** Low-power view of chronic papillary synovitis. **B,** Hyperplastic synoviocytes overlie a dense infiltrate composed of lymphocytes and plasma cells. **C,** Synoviocytes cover a layer of fibrin.

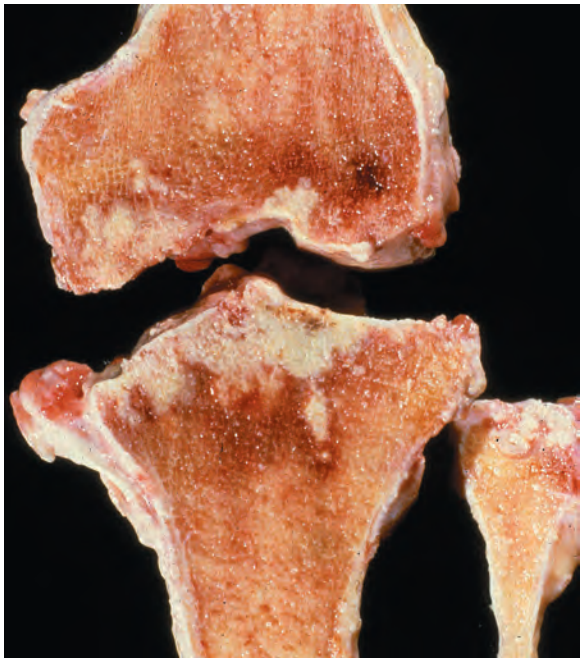
rarely develop this form of infection.[46,47] Seeding of the joint space is thought to occur as a complication of bone infection or through direct inoculation.[45] Hematogenous spread can also result in multifocal joint involvement, especially in immunocompromised patients.[48]

Pain and local swelling are common presenting complaints. Fever and weight loss are rarely present. Tuberculous arthritis has an indolent course, with the gradual development of a nonspecific synovitis and joint effusion.

Radiographically the Phemister triad is characteristic. It consists of juxtaarticular osteoporosis, osseous erosions, and slight narrowing of the joint space (Fig. 15.8).[49] In contrast, the MRI findings of synovitis with pannus formation, effusions, and peripheral and central erosions are relatively nonspecific.[50] On T2-weighted images, tuberculous synovitis demonstrates low signal in areas of granuloma formation.[51]

Grossly, the inflamed synovial tissue is tan-yellow, with destruction of the articular cartilage and possible erosion into bone (Fig. 15.9). Histologically the synovium shows acute and chronic inflammation with necrotizing granulomas. The infection frequently extends into bursae and synovial tendon sheaths.

• **Figure 15.8** Anteroposterior radiograph of the pelvis showing tuberculous arthritis of the right hip. There is mild narrowing of the joint space, and round lucencies are seen in the femoral head and acetabulum. Sclerosis surrounds the lytic foci.



• **Figure 15.9** Gross specimen of tuberculous arthritis involving the knee. The articular cartilage is eroded, and caseous necrotic tissue has infiltrated into the subchondral bone of the distal femur and the proximal tibia and fibula.

Treatment consists of local débridement with multidrug antituberculosis chemotherapy.

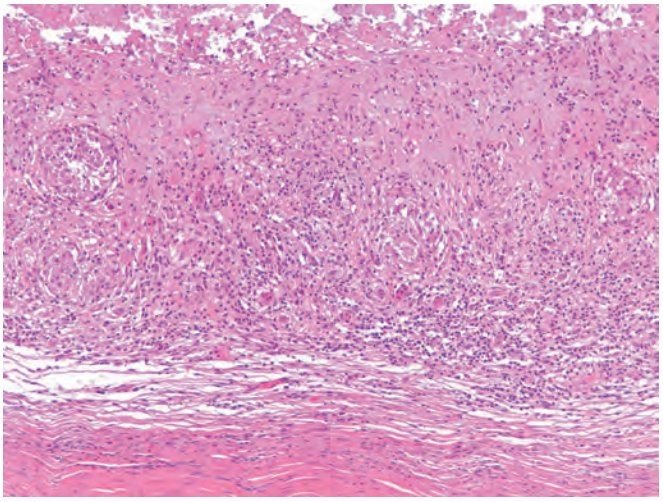
Nontuberculous Mycobacterial Arthritis

Nontuberculous mycobacterial infections rarely affect the joints and are usually associated with underlying immunocompromise or rheumatologic disorders. Direct inoculation or trauma is occasionally the route of infection[52,53] Hematogenous spread from a primary pulmonary lesion may also occur. Nontuberculous mycobacterial arthritis has also been seen in association with foreign bodies, including prosthetic joints.[45] Among the nontuberculous mycobacteria, *Mycobacterium kansasii* is the most common cause of monoarthritis, whereas *Mycobacterium marinum* and *Mycobacterium avium* complex are the most frequent causes of tenosynovitis. *Mycobacterium chelonae*, *Mycobacterium fortuitum*, *Mycobacterium abscessus*, and *Mycobacterium terrae*, among others, have also been implicated.[53,54]

Patients with nontuberculous mycobacterial arthritis typically present with an insidious onset of localized swelling and pain over the affected joint. A joint effusion may be present, and erosive changes may be seen on imaging.[52,55,56] Nontuberculous mycobacterial tenosynovitis and arthritis most frequently involve the hands, although other sites, including the feet, shoulder, and knee, have been reported.[57] Histologically the findings range from chronic synovitis with scattered macrophages to florid necrotizing granulomas (Fig. 15.10).

The differential diagnosis for nontuberculous mycobacterial arthritis includes sarcoidosis, especially if the small joints are involved; however, the histopathologic changes, special stains for microorganisms, and culture can establish the correct diagnosis.

Treatment of nontuberculous mycobacterial arthritis usually involves a prolonged course of multidrug antimycobacterial therapy with or without local débridement.



• **Figure 15.10** Atypical mycobacterial bursitis. The synovial surface is replaced by granulomas with scattered lymphocytes and fibrin deposition on the surface.

Fungal Arthritis

Fungal (mycotic) arthritis is a rare disease that arises mainly from hematogenous dissemination, although direct inoculation or extension from an adjacent region of infection may also occur.[58] As with bacterial arthritis, predisposing factors include chronic disease, immunosuppression, and prolonged use of intravenous antibiotics, as well as the presence of indwelling catheters and joint prostheses.[59,60] Since the advent of the human immunodeficiency virus (HIV) epidemic, the incidence of fungal arthritis has markedly increased. Although the true incidence is unknown, candidal infection is thought to account for most cases. However, other species, including *Sporothrix schenckii*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*, are also known to cause articular disease.[59]

Mycotic arthritis tends to manifest indolently, with an extended course between symptom onset and diagnosis. Although any joint may be affected, the knee and other large joints are the most common sites. Clinical symptoms are nonspecific, and common complaints are decreased range of motion, pain, erythema, and joint effusion.[58,60]

Radiographic findings demonstrate soft tissue swelling, periarticular bone erosions, effusions, and narrowing of the joint space.

Synovial fluid analysis may be suggestive of an inflammatory aseptic arthritis, although *Candida* and *Cryptococcus* are associated with purulent synovial fluid. Routine cultures are frequently negative. Histologically, there is an acute and chronic synovitis, and in some cases necrotizing granulomas are present. Special histochemical stains are useful in identifying the offending organism.

Treatment invariably involves intravenous antifungal therapy, and surgical drainage or débridement is often required.

Candidiasis

Nine species of *Candida* are known to be pathogenic in humans. *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, and *Candida zeylanoides* have been identified in cases of candidal arthritis.[59,60]

Joint infections involving *Candida* spp. occur in the setting of disseminated disease in most cases. Other factors predisposing to Candida infection are indwelling catheters, intravenous drug use, parenteral hyperalimentation, malignancy, multiple surgeries, use of broad-spectrum antibiotics, and prosthetic joints.[59,61] Candidal arthritis may also occur secondary to intraarticular corticosteroid injection.[59]

Candidal arthritis most frequently involves the knee (67% of cases), although hip, shoulder, ankle, and other joints have been reported to be affected.[58,59] Candidal infection from hematogenous spread usually causes a monoarticular or oligoarticular arthritis.[62,63] Patients are febrile and may have leukopenia or leukocytosis. There may be a long latency between fungemia or joint surgery and onset of arthritis, and in rare cases symptoms have been reported for up to 4 years before diagnosis.[59,60,64] Patients may present with a normal WBC count and no fever. Clinical symptoms include pain, inability to bear weight, and restricted range of motion.[65] The affected joints are swollen, painful, and warm.

Radiologic examination reveals nonspecific soft tissue swelling, joint effusion, and destructive joint changes. If a prosthesis is present, there may evidence of loosening.[59]

Candidal arthritis begins as a synovitis and spreads to adjacent bone. Grossly the synovium may be thickened and hyperemic, with scarring and erosion of articular cartilage.[66] Histologic examination of infected synovium reveals histiocytic and mononuclear cell infiltration and fibrosis. Granulomas are rarely present.[60,67] Silver stain or tissue Gram stain may demonstrate fungal organisms, but culture of synovial fluid or tissue is more sensitive. Blood cultures are often negative.

Treatment usually involves intravenous antifungal chemotherapy, with or without local débridement and synovectomy. Infected prostheses must be removed. Use of intraarticular antifungal chemotherapy is controversial.[58] The mortality rate in cases involving a systemic fungal infection may be as high as 50%.[59]

Candida is the prototypical fungal pathogen involved in pediatric arthritis. Affected patients are primarily neonates and infants younger than 6 months of age. Joint infections are typically a result of hematogenous spread in the setting of systemic invasive candidiasis.[58] Risk factors include prematurity, low birth weight, respiratory distress syndrome, aspiration pneumonia, gastrointestinal defects, surgery, malnutrition, and use of broad-spectrum antibiotics.[58] Maternal vaginal candidiasis may also be a factor. Patients present with fever and a swollen, tender joint, with abnormal positioning of the infected limb. Thirty percent of cases are polyarticular, and the knee is most frequently involved. There may be associated metaphyseal osteomyelitis, either by direct extension from synovium or by concurrent hematogenous spread. Radiographic findings include joint effusion, joint dislocation, or metaphyseal abnormalities.[63] Intravenous antifungal therapy is the treatment of choice.

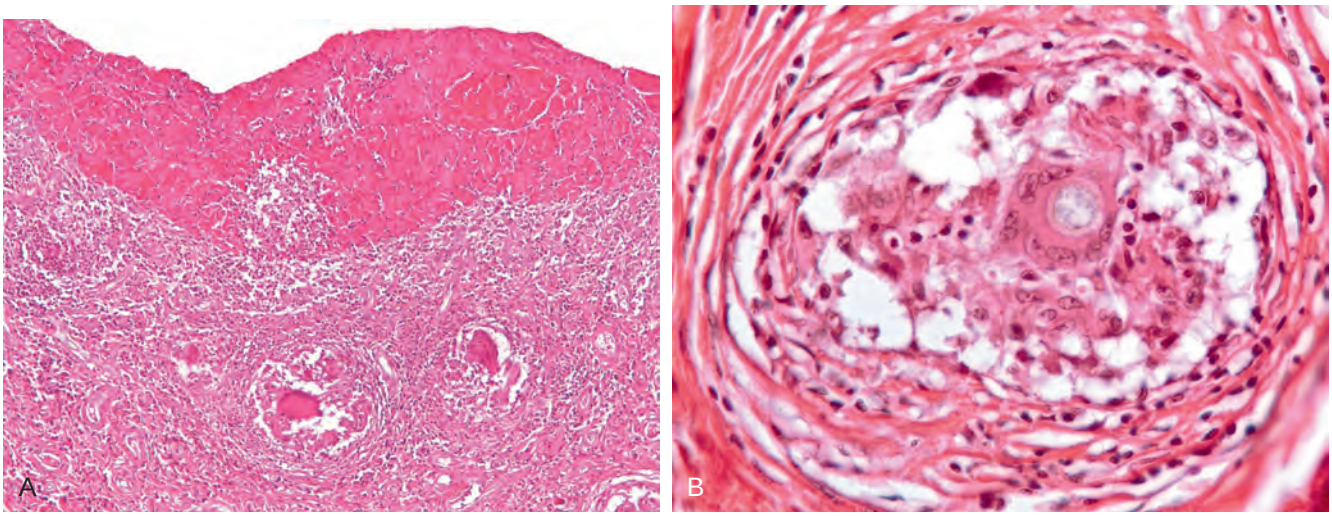
Cryptococcosis

C. neoformans infection primarily involves the lungs and central nervous system. Disseminated disease is thought to occur by hematogenous spread from infected lungs.[68] Cryptococcal joint infection is an extremely rare event that usually occurs by direct spread from adjacent infected bone.[68] Cryptococcal arthritis may affect one or several joints, and the knee is the most common site of involvement.[69] In one case, cryptococcal infection was documented in tendonitis and tenosynovitis of the forearm.[69] Patients are usually immunocompromised as a result of organ transplantation, malignancy, corticosteroid use, or AIDS.[58]

Patients present with warmth, swelling, tenderness, and limited range of motion in the affected joint. Symptoms of systemic disease may also be present. Radiographs show narrowing of the joint space, along with bony changes of osteomyelitis. MRI demonstrates synovial thickening and enhancement if tenosynovitis is present.[69]

Histologic examination reveals epithelioid granulomas with multinucleated giant cells, moderate lymphocytic infiltration, and areas of necrosis. Intracytoplasmic and extracytoplasmic spheroidal budding organisms ranging in diameter from 5 to 20 µm can be seen on silver or periodic acid–Schiff (PAS) stains (Fig. 15.11).[68] Organisms may be cultured from synovial tissue or fluid.

Treatment requires débridement and intravenous antifungal chemotherapy. Despite treatment, patients may develop postinfectious degenerative arthritis.[68]



• **Figure 15.11** Cryptococcal synovitis. **A,** Synovial tissue is covered by a thick layer of fibrin. Well-formed granulomas are surrounded by reactive fibrous tissue. **B,** Granuloma containing organism. Note refractile capsule.

Coccidioidomycosis

C. immitis infection begins with a pulmonary infection in immunocompetent individuals. In fewer than 1% of cases, infection disseminates, with involvement of meninges, skin and subcutaneous tissues, lymph nodes, and bones and joints.[60,70] Acute polyarticular arthritis with fever, rash, eosinophilia, and bilateral hilar lymphadenopathy may develop as part of a hypersensitivity syndrome in 30% of cases during primary infection.[71] The arthritis is migratory, and joints are tender and painful on motion. Effusions are generally not present. The ankle and knee are the primary joints affected, and the arthritis resolves within 1 month without permanent sequelae. In disseminated disease, arthritis arises from hematogenous seeding or direct extension from areas of osteomyelitis. Symptoms begin as intermittent, painful swelling of one joint and progress to large effusions, swelling, and development of draining sinus tracts.[60] The knee is affected in 50% to 70% of cases, but any joint may be involved. Tenosynovitis may also occur.

Imaging findings include effusion, joint space narrowing, and bone destruction. Histology demonstrates villonodular synovitis, or pannus formation with nonnecrotizing granulomas, and spherules containing coccidioidal endospores. Treatment is with antifungal chemotherapy. Drainage and débridement may be necessary.

Blastomycosis

B. dermatitidis arthritis is a monoarticular arthritis that usually affects men from the third to fifth decade of life. The knee is the main site of involvement, followed by ankle, elbow, wrist, and hand. Joint infection may occur by hematogenous seeding or by spread from a focus of osteomyelitis. If left untreated, infection may spread to additional joints. The clinical onset may be abrupt, and pain and swelling are associated with fever and weight loss. Synovial fluid can be purulent. Diagnosis requires evidence of fungus, in synovial fluid or in any other site, and can be made on wet mount microcopy. Treatment with antifungal agents results in resolution of arthritis. Drainage of infected fluid may be necessary.[58,60]

Histoplasmosis

Joint manifestations of *H. capsulatum* infection occur as part of a hypersensitivity reaction during primary infection. This self-limited arthritis is migratory and polyarticular and may coexist with other hypersensitivity reactions, such as erythema nodosum, pleuritis, or pericarditis.[72] Disseminated disease rarely results in more serious articular involvement, which is associated with immunocompromise. *Histoplasma* arthritis manifests as a monoarthritis, typically of the knee. Tenosynovitis involving the flexor tendon sheaths of the hand has been reported. Radiologically, destructive joint lesions, erosions, sclerosis, and joint space narrowing may be present. Diagnosis depends on detection of fungal organisms or on serology. Treatment is with antifungals, with or without débridement.[60]

Sporotrichosis

Infection with *S. schenckii* occurs after cutaneous inoculation or, less commonly, after inhalation of spores. Dissemination develops infrequently but may involve any organ system. The arthritis is chronic and can be monoarticular or polyarticular; it primarily involves the knee or, less often, the wrist or joints of the hands and feet.[60] Symptoms are chronic, with an insidious onset. The diagnosis is often delayed, sometimes up to several years from symptom onset, and requires culture of synovial fluid or tissue. Radiographic findings include joint space narrowing, effusions, periarticular erosions, soft tissue swelling, and periarticular osteopenia.[58] Treatment is with intravenous or intraarticular antifungal agents. Synovectomy may also be effective.

Aspergillosis

Aspergillus fumigatus and *Aspergillus flavus* are the most frequently identified *Aspergillus* spp. in human infection. *Aspergillus* infection in an immunosuppressed host very rarely causes arthritis and is usually associated with adjacent osteomyelitis. *Aspergillus* arthritis manifests with fever, chills, or malaise, as well as pain, swelling, and tenderness of the affected joint. A synovial effusion is present, and there is a restricted range of motion. On imaging, soft tissue swelling and osteomyelitis may be present. Diagnosis requires isolation of fungus from tissue or synovial fluid. Treatment requires both débridement and intravenous antifungal agents.[60]

Mycetoma

Pseudallescheria boydii is a saprophyte found in soil, polluted water, and sewage. Although infection may result in a wide variety of clinical manifestations, mycetoma is by far the most common. The infection typically is seen in men living in rural areas who have a prior history of local trauma. *Ascheria boydii* infection manifests with a monoarticular arthritis, usually involving the knee, with joint pain, swelling, and restricted range of motion. Arthritis develops within days to 1 year after trauma. Imaging findings consist of osteopenia, soft tissue swelling, and lytic lesions of the articular surface. Treatment involves surgery and intraarticular antifungal agents.[60]

Viral Arthritis

A wide range of viral infections can lead to reactive arthritis and subsequent rheumatic syndromes. Acute or chronic persistent viral infection can lead to direct tissue damage as a result of inflammation or the immune response. The immune response to viral infection is complicated by homology between viral and host antigen epitopes, which leads to induction of autoantibodies,[73] many of which have been implicated in rheumatologic disorders. Viral infection may result in the formation of antinuclear antibodies (ANAs), rheumatoid factor, antibodies to cardiolipin, double-stranded DNA, SS-A (Ro), SS-B (La), antineutrophil cytoplasmic antibody (ANCA), and cryoglobulin, among others.[74] Viruses may also directly infect synovium and can lead to persistent or recurrent infection.

The radiographic findings of chronic viral arthritis typically resemble those seen in rheumatoid arthritis, with bony erosions and cartilage destruction. Synovitis may be seen on MRI.

Histologically, viral infection causes a nonspecific synovitis or tenosynovitis. Biopsy is rarely performed, because the diagnosis usually can be made on the basis of clinical presentation and serology.

Treatment is usually symptomatic.

Parvovirus B19

Parvovirus B19 is a member of the nonenveloped, single-stranded Parvoviridae family of DNA viruses, which was first identified in 1975 as the cause of erythema infectiosum, or fifth disease, in children. It may also lead to nonspecific fever or, more seriously, arthritis, vasculitis, Still disease, SLE-like disorders, or aplastic crisis. Articular symptoms, usually arthralgias or joint swelling, affect 8% of infected children.[74,75] In adults, rash is rare, and B19 infection manifests with acute onset of symmetric polyarticular arthralgia or arthritis.[75] Joint involvement can mimic rheumatoid arthritis, and patients may suffer from morning stiffness. However, to date, studies have been contradictory as to the role of B19 in the etiology of rheumatoid arthritis.[74] Joints are tender and swollen and may be erythematous.[76] In immunocompetent patients, arthritis resolves with no permanent swelling or functional limitation. Immunocompromised patients may fail to clear an acute infection, and persistent infection may develop. B19 may be associated with presence of rheumatoid factor, ANA, anti-DNA antibodies, antilymphocyte antibodies, and antiphospholipid antibodies.[76] Diagnosis is by serology.

B19 may persist in synovium, as well as in other tissues, but persistent B19 infection alone is insufficient to cause arthritis, and the pathophysiology of chronic B19 arthritis is not well understood.

Treatment is usually symptomatic, although intravenous immunoglobulin therapy may be necessary to clear the virus in immunosuppressed patients. Nonsteroidal antiinflammatory drugs (NSAIDs) and supportive care with appropriate pain control are sufficient for management of chronic B19 arthritis.

Rubella

Rubella virus is a member of the Togaviridae family of enveloped, positive-sense, single-stranded RNA viruses.[77] Rubella typically infects young adults, who present with suboccipital lymphadenopathy and a maculopapular rash that begins on the face and spreads to the torso and extremities. Constitutional symptoms may be present.[75] Rubella may lead to arthritis or arthralgia in 30% of infected adults and is more common in women. Of note, vaccination against rubella has demonstrated an association with acute arthropathy and arthritis similar to that seen in rubella virus infection.[74] Rubella arthritis is symmetric and occurs in a rheumatoid arthritis–like pattern, although it may be migratory. Metacarpophalangeal and proximal interphalangeal joints are frequently affected, as are knees, wrists, ankles, and elbows. Periarthritis, tenosynovitis, and carpal tunnel syndrome may occur.[78] The arthritis usually resolves within 2 weeks, but in some cases it persists for years.[75]

Diagnosis relies on serology for antirubella seroconversion. Treatment is supportive, with NSAIDs. Steroids may also be used for symptom control.

Hepatitis C

Hepatitis C virus (HCV) is an enveloped, single-stranded RNA flavivirus that infects approximately 3% of the world's population.[79] HCV is a leading cause of chronic liver disease and is associated with numerous extrahepatic disorders, including cryoglobulinemia, as well as arthritis in a small percentage of patients. The exact pathophysiology of HCV arthritis is unclear, but several theories have been proposed, including the hypotheses that HCV initiates or exacerbates an inflammatory arthritis and that HCV directly causes a distinct arthritis syndrome of its own.[75] HCV-associated joint disease manifests as a monoarticular or oligoarticular arthritis and tenosynovitis. Radiographically, erosions are absent and the arthritis is nonprogressive.[80] Aggressive antiviral therapy may be attempted, but underlying rheumatologic disorders should also be ruled out or treated appropriately.

Hepatitis B

Hepatitis B virus (HBV), a member of the Hepadnaviridae family, is an enveloped, partially double-stranded DNA virus that affects approximately 30% of the world's population.[81] Infection is usually self-limited but may become chronic in 5% to 10% of patients. HBV is not associated with chronic arthritis, but acute HBV infection can cause rapid onset of a polyarticular arthritis mimicking rheumatoid arthritis.[75] The arthritis resolves with the onset of jaundice. Only supportive care is indicated.

Human Immunodeficiency Virus

HIV is now a worldwide pandemic, with more than 30 million infected individuals.[82] HIV is a risk factor for the development of septic arthritis and has been associated with articular symptoms, including arthralgia, spondyloarthropathy, reactive arthritis, and avascular necrosis. Arthritis may occur at any stage in HIV infection but is more prevalent later in the course of disease. The pathogenesis of HIV-associated arthritis is not well understood, but direct invasion of synovium by the virus, the host immune response, and genetic factors are all thought to play a role. Patients with HIV-associated arthritis are usually adult, although children may also be affected. There is a male predominance.[83,84]

HIV-associated arthritis in children is frequently polyarticular (40%), although spondyloarthropathy-like symptoms and oligoarthritis have been documented in 34% and 26% of cases, respectively. Systemic symptoms including fever, lymphadenopathy, hepatomegaly, and rash may be present.[84] In adults, HIV can cause nonspecific intermittent arthralgias or arthritis,[85] which are likely to be oligoarticular (47%) or monoarticular (38%).[86] Reactive arthritis may also occur, typically accompanied by extraarticular features, such as urethritis or skin lesions.[85] The knee is most commonly involved, followed by ankles, wrists, and small joints of the hands and feet.[87] Enthesial involvement may occur, usually in the Achilles tendon, plantar fascia, tibial tendons, or extensor tendons.[87] Erosive changes are not seen.

Histologically, there may be nonspecific chronic inflammation of synovium.[84] HIV may be isolated from synovial fluid,[88] and HIV p24 antigen may be detected within synovial cells and in synovial histiocytes.[89] HIV-associated arthritis usually resolves within 1 year. In a minority of cases, it may persist or recur.

Approximately 1% of HIV-infected patients develop septic arthritis, with *S. aureus* as the number one pathogen. Atypical mycobacterial or fungal infections may also occur.

Alphavirus

Alphaviruses belong to the Togaviridae family and include a number of mosquito-borne entities, including chikungunya virus, o'nyong-nyong virus, Sindbis virus, Ross River virus, and Mayaro virus, among others, which have been linked to the development of postviral arthritis in outbreaks across the world.[90]

Clinical symptoms at presentation of *Alphavirus* infection are nonspecific and similar to those of other mosquito-transmitted infections, including acute fever, rash, malaise, fatigue, myalgia, and arthralgia.

Chikungunya virus, transmitted by widespread *Aedes albopictus* and *Aedes aegypti* mosquitoes, has been gaining notoriety over the past six decades since its initial isolation in 1952. Outbreaks have occurred worldwide, the most severe epidemic to date occurring between 2004 and 2011.[90] Since 2010 chikungunya has become widespread in South America and the Caribbean. Acute infection with chikungunya often results in symmetrical polyarthritis of the large joints of the extremities, as well as myalgia involving the arms, thigh, and calves.[90,91] Children may also experience pain along ligament insertions and swelling of the joints of the hands.[91] Diagnosis typically relies on serology and/or genetic analysis (PCR, next-generation sequencing).

Chikungunya has been has been linked to a broad spectrum of chronic disorders of joints, tendons, and bone, including nonspecific postviral arthritis, rheumatoid arthritis, seronegative spondylitis, and persistent arthralgia which may persist for months to years after the acute phase, and is a major cause of morbidity for this viral infection. Symptoms usually affect the same joint(s) involved in the acute phase of infection, and follow a persistent or relapsing course.[91] An association with rheumatoid arthritis–associated alleles HLA-DRB1*01 and HLA-DRB1*04 has been reported.[92] In a systemic literature review, the pooled prevalence of post-chikungunya chronic inflammatory rheumatism (defined as patients with noncrystalline polyarthritis meeting diagnostic criteria for rheumatoid arthritis, spondyloarthritis, or undifferentiated polyarthritis) was 40%, with 14% prevalence of chronic arthritis. The most conservative estimates put a quarter of all chikungunya cases at risk for chronic inflammatory rheumatism and 14% risk for chronic arthritis.[93]

Chikungunya virus enters the human host following a mosquito bite. The virus initially proliferates in skin fibroblasts before spreading systemically. In nonhuman models the virus has been found to replicate in joints, myotendinous insertions, and skeletal muscle satellite cells, where it is associated with a marked inflammatory reaction, including mononuclear cells, which are thought to play a role in viral dissemination and pathogenesis of post infectious chronic inflammatory rheumatism. Inflammatory cytokines, such as IL-17, IL-6, and granulocyte-colony stimulating factor (G-CSF), have been detected in serum of patients with chronic disease up to 2 to 3 months after disease onset.[91] In humans, muscle biopsies in acute phase infection show myositis accompanied by an infiltrate rich in monocytes/macrophages and T cells.[91]

Mouse models of infection have shown acute chikungunya infection to be associated with histologic evidence of arthritis, synovitis, tendonitis, myositis, and myocyte necrosis, with infiltrating macrophage- and neutrophil-predominate inflammatory populations with scattered lymphocytes.[94] In the chronic phase of arthritis, the inflammatory population was found to be predominately composed of histiocytes and lymphocytes.[94]

O'nyong-nyong is a disease that originated in Uganda and is characterized by fever, rash, and arthritis. Ross River virus is a source of epidemic polyarthritis in Australia and the South Pacific. The virus attacks the knees and the small joints of the hands and feet. Systemic symptoms including fever and rash may be present. Barmah Forest virus is found only in Australia and may cause a polyarthritis similar to that of Ross River virus. Pogosta disease occurs in epidemics every 7 years and causes joint symptoms in 93% of patients, with polyarthritis in 40%. Kokobera, Alfuy, Edge Hill, Kunjin, Stratford, dengue, and Gan Gan viruses may also be associated with arthritis.[74,75] Alphaviral arthritis usually resolves within weeks, but severe cases may be symptomatic for month to years. Treatment is supportive with NSAIDs, although aspirin is contraindicated.

Emerging arthritis-inducing pathogens, such as the alphaviruses, are an evolving problem worldwide. It is thought that increased prevalence of these diseases is due to a combination of factors, including climate changes, increased international travel, urbanization, changes in land use, as well as changes in vector biology, and adaptation of the viruses themselves.[90] Given the problems inherent in addressing any of these factors, and lack of preventative vaccines, it is certain that outbreaks will continue to occur for the foreseeable future. Thus it is imperative to understand and address the risk of long term consequence of infection, including postviral arthritis.

Reactive Arthritis

In addition to septic arthritis, bacterial infection may result in a postinfectious reactive arthritis. The prototype for this manifestation of disease is poststreptococcal reactive arthritis, which is part of a spectrum of reactive syndromes that includes acute rheumatic fever. Reactive arthritis occurs as a result of the formation of antibodies against bacterial or viral antigens, which then cross-react with synovial antigens, leading to the deposition of immune complex in the synovium. Immune complex deposition stimulates a local inflammatory reaction that may damage tissue through release of inflammatory cytokines, cytolytic enzymes, and reactive oxygen species. Host genetic factors are important, and reactive arthritis has been linked to MHC class II HLA-DRβ1*16 and β1*01. Bacteria known to be associated with reactive arthritis include *N. meningitidis*, *Salmonella*, *Shigella*, *Yersinia enterocolitica*, and *Campylobacter jejuni*.[95] Rare parasitic infections, such as with the helminth *Strongyloides stercoralis*, may also result in a reactive arthritis syndrome.[96]

Acute Rheumatic Fever

Formerly one of the most common vascular diseases of children, acute rheumatic fever has waned in importance in developed countries since the early 20th century. The disease is exemplified by arthritis, carditis, Sydenham chorea, and skin manifestations such as erythema marginatum and subcutaneous nodules. Acute rheumatic fever arises after an untreated infection with group A streptococci in genetically susceptible individuals; it has been linked to the HLA-DRβ1*16 allele.[97] In this syndrome, arthritis develops approximately 3 weeks after streptococcal infection. Immune response to bacterial antigens is thought to result in formation of cross-reactive antibodies or cell-mediated processes that recognize human antigens within affected tissue, leading to tissue damage. Arthritis may, on occasion, be the single major manifestation of acute rheumatic fever. Large joints are involved by an asymmetric migrating polyarthritis; the axial skeleton is rarely affected. Pain in a joint may resolve within 24 hours, only to be followed by arthritis in a different joint.[95] Some patients experience only mild arthralgias. Articular symptoms typically resolve within a few weeks, without permanent sequelae. Treatment with aspirin leads to complete resolution of joint pain.

Poststreptococcal Reactive Arthritis

The symptoms of poststreptococcal reactive arthritis occur acutely, within a few days to 2 weeks after streptococcal infection. Fever and rash may be present during the initial infection, but they disappear with the onset of arthritis. In contrast to acute rheumatic fever, poststreptococcal arthritis is nonmigratory, is associated with morning stiffness, and may affect both large and small joints. Knees, ankles, wrists, and interphalangeal joints are frequent sites of involvement. The axial skeleton is affected in up to 20% of cases. The clinical course is chronic, and symptoms may persist for up to 8 months.[97] Poststreptococcal arthritis responds poorly to treatment with NSAIDs.

Soft Tissue Infections

Soft tissue infections are a common problem after local trauma or systemic infection. Superficial infections of the skin, such as impetigo or folliculitis, may develop into cellulitis with local spread or may hematogenously seed distant tissue. More severe infections, including necrotizing fasciitis, gas gangrene, or myositis, may occur in the setting of severe trauma, immunocompromise, vascular insufficiency, or prior infection (Table 15.3). In many parts of the world, parasitic soft tissue infections continue to pose a significant health problem, resulting in myositis, lymphadenitis, and lymphedema, among other conditions. Less commonly, fungi may also contribute to soft tissue infections, especially in the setting of immunocompromise (e.g., HIV infection).

Necrotizing Soft Tissue Infections

Necrotizing soft tissue infection (fasciitis) is a serious but uncommon, rapidly progressive bacterial infection involving dermis, subcutaneous tissue, fascia, or muscle. Historically, necrotizing lesions of the soft tissue have been subclassified into such entities as streptococcal gangrene, Fournier gangrene, Meleney bacterial synergistic gangrene, necrotizing fasciitis, and clostridial cellulitis. However, the initial diagnosis and management of each entity is identical, and subclassification is best left until after identification of the causative organism or organisms. Characteristic of this disease is its rapid spread along fascial planes, frequently with only minimal involvement of adjacent tissues, such as muscle or subcutaneous fat. Accordingly, necrotizing soft tissue infections cause significant morbidity and are associated with a relatively high mortality rate, ranging between 20% and 28%.[98-100] Speedy diagnosis and treatment are essential to avoid serious complications.

Infection commonly develops from a cutaneous lesion and usually originates in the extremities or perineum (Fournier gangrene), although any site may be affected. The primary risk factors are immunocompromise, smoking, diabetes mellitus, intravenous drug use, and vascular insufficiency, as well as older age and chronic renal insufficiency.[98,101] Trauma, burns, dermal abscess, or recent surgery may be antecedent events.[101,102] Risk factors for death include age older than 60 years, female gender, delay in diagnosis or débridement, extent of tissue involvement, degree of organ system dysfunction, and other medical comorbidities. In addition, infections in the perineum and those associated with burn wounds or perforated bowel are all linked to higher mortality. Myonecrosis is not linked to increased mortality.[98]

Most cases of necrotizing infection are polymicrobial, with as many as six pathogens being present simultaneously. Only 30% are monomicrobial, with group A β-hemolytic streptococci the most common culprit.[101] Most polymicrobial necrotizing infections involve a mixed population of aerobes and anaerobes. Other common organisms involved in monomicrobial and polymicrobial infections are *Bacteroides* spp., staphylococci, enterococci, *E. coli*, *Proteus* spp., *Peptostreptococcus*, *Clostridium perfringens*, *Fusobacterium*, and *Prevotella* spp., as well as fungi and other aerobic and anaerobic organisms (Table 15.4).[98,103] Pure clostridial myonecrosis (gas gangrene) is rare and highly lethal.

Clinically, necrotizing fasciitis is divided into three subtypes based on the microorganisms involved. Type 1 is a polymicrobial infection that usually affects diabetic patients or those with peripheral vascular disease. It occurs after operations for abdominal or perineal infectious disease and is caused by non–group A streptococci in combination with anaerobes or facultative anaerobes. Enterobacteriaceae may also be involved. Type 2 infection usually arises in the extremities and involves group A β-hemolytic streptococci, such as *S. pyogenes*, with or without staphylococcal involvement.[104] Infection with methicillin-resistant *S. aureus* in type 2 necrotizing fasciitis is becoming an increasingly serious problem. Type 3 necrotizing fasciitis is caused by marine *Vibrio* spp., such as *Vibrio vulnificus* or *Vibrio parahaemolyticus*, and often is the result of a puncture injury.[105]

The pathogenesis of necrotizing soft tissue infection is hypothesized to be related to the relative paucity of blood supply to the fascia. Local toxin-induced thrombosis is believed to induce fascial

TABLE 15.3 Bacterial Soft Tissue Infections

Infection	Site	Type	Organism	Treatment
Cellulitis	Extremities	Monomicrobial	*Staphylococcus aureus*	Antibiotics
Necrotizing fasciitis	Extremities, abdomen	Polymicrobial	*Streptococcus*, *Bacteroides*, enteric species	Débridement, antibiotics
Gas gangrene	Extremities	Monomicrobial or polymicrobial	*Clostridium*, anaerobes	Débridement, antibiotics, hyperbaric oxygen
Pyomyositis	Lower extremities	Monomicrobial	*S. aureus*	Drainage, antibiotics
Psoas abscess	Psoas muscle	Monomicrobial or polymicrobial	*S. aureus*, *Mycobacterium tuberculosus*, enteric species	Drainage, antibiotics
Necrotizing myositis	Extremities	Monomicrobial or polymicrobial	*Streptococcus*, *Peptostreptococcus*	Débridement, antibiotics

TABLE 15.4 Causative Organisms in Necrotizing Soft Tissue Infections

Pathogen Group	% of Cases
Bacteroides	40–50
Streptococcus	40–50
Enterococcus	30–40
Peptostreptococcus	30–35
Staphylococcus	30–40
Escherichia coli	30–35
Proteus	20–25
Gram-negative rods	40–45
Clostridium	20–25
Other anaerobes	20
Fungal	5

Data from Edlich RF, Cross CL, Dahlstrom JJ, Long WB 3rd. Modern concepts of the diagnosis and treatment of necrotizing fasciitis. *J Emerg Med*. 2010;39(2):261-265.

• **Figure 15.12** Necrotizing fasciitis. Coronal T2-weighted magnetic resonance image of thigh shows diffuse edema within all compartments of the right upper thigh, hyperintense deep fascia, and focal stranding of the subcutaneous fat.

ischemia,[101] whereas other bacterial toxins, such as hyaluronidase, contribute to tissue breakdown.[106] The host inflammatory response causes extravasation of fluid that becomes entrapped between the layers of infected fascia and adjacent tissues. This undermines the effectiveness of the immune response by impeding phagocytosis. Together, inadequate perfusion and ineffective phagocytic clearance of damaged tissue and microorganisms produce an ideal milieu for pathogen proliferation. The polymicrobial nature of the necrotizing infection is also thought to play an important role because there is a synergistic interaction among certain bacterial species, such as β-hemolytic streptococci and *S. aureus*.[101]

The clinical manifestations vary with the stage of disease. Initially, patients typically present with high fever, tachycardia, erythema and swelling of involved skin, and pain out of proportion to physical findings. As the infection progresses, skin lesions evolve into a reticulated pattern of purpura, with anesthesia over the affected area. Blistering, cutaneous hemorrhage with purpuric bullae, or gross necrosis may eventually develop.[102] Crepitus may be apparent on palpation. As the region of local spread expands, bacteria invade the local vasculature, leading to bacteremia. As a result of hematologic spread of infection, there is an accompanying increase in the severity of systemic symptoms, with a markedly elevated WBC count, signs of sepsis, and, eventually, septic shock leading to multiorgan failure.[101] Disease progression can be extremely rapid, with death ensuing mere days after presentation.

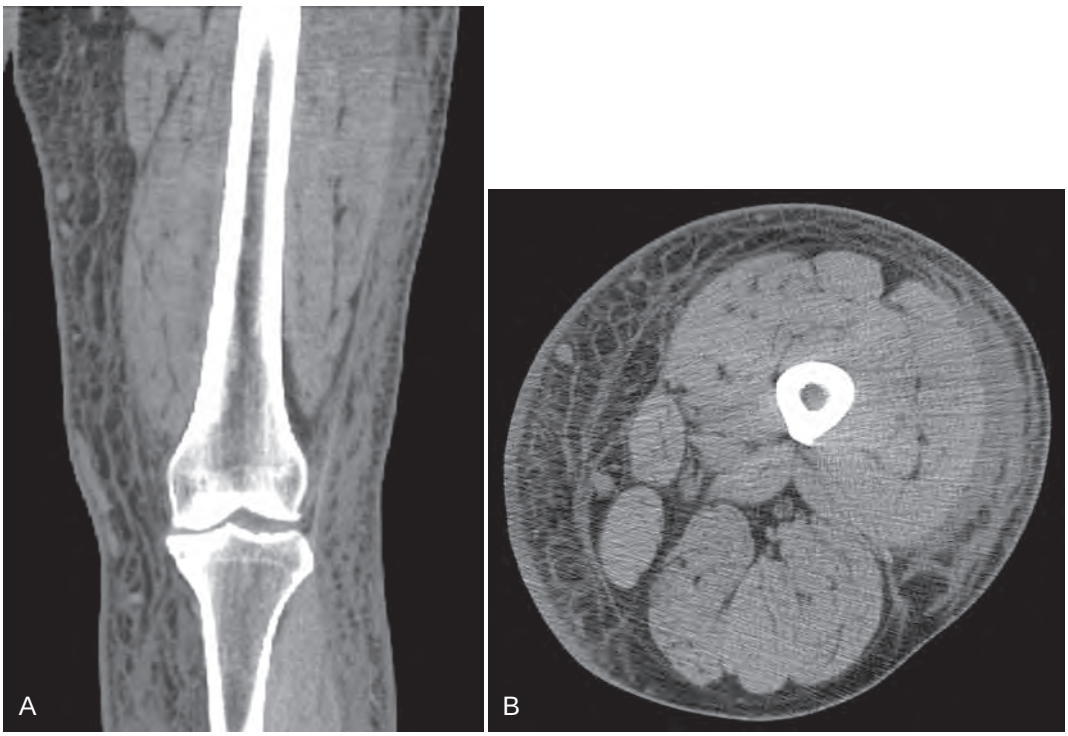
MRI findings include irregular, hyperintense fascial signals on T2-weighted scans of affected areas, although there is some debate as to the specificity of this finding (Fig. 15.12).[106] Computed tomography (CT), MRI, and ultrasonography are helpful in detecting fluid accumulations along fascial planes and tissue edema in soft tissue around the fascia (Fig. 15.13). However, it is important to note that the presence of edema and fluid are not specific for necrotizing infections.[100,106]

Grossly, incision of the infected area in necrotizing fasciitis yields a murky, "dishwater" exudative fluid. Diseased tissue is discolored gray-white to yellow. Fascia may be easily separated from subcutaneous tissues using only a finger, whereas in healthy tissue, fascia tightly adheres to adjacent tissues.[101,107,108]

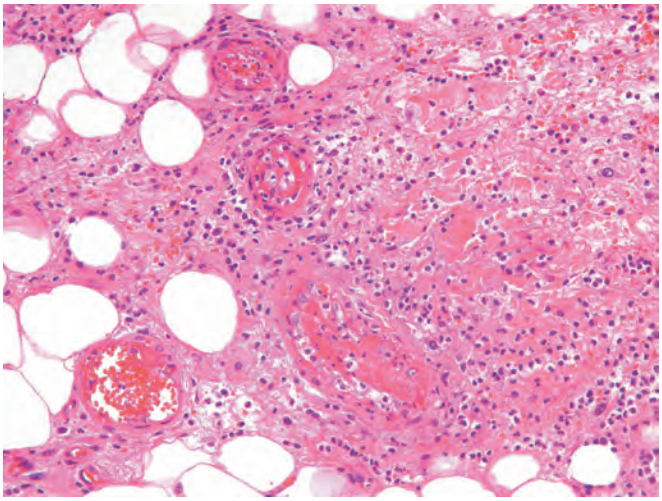
In the early stages of necrotizing fasciitis, histopathologic examination of infected tissue demonstrates edema of the subcutaneous soft tissue and superficial fascia, with obliterative vasculitis and thrombosis of the dermal vasculature with scattered neutrophils (Fig. 15.14). The overlying skin may appear uninvolved, although it is invariably affected by progressive necrosis. As the lesions progress, liquefactive necrosis of surrounding fascia and subcutaneous tissue develops, and acute neutrophilic inflammation and hemorrhage extends into the dermis (Fig. 15.15). Accompanying the neutrophils are T lymphocytes, macrophages, and monocytes.[109] Full-thickness necrosis may extend from the skin surface to the superficial fascia. Infection may then rapidly spread along the horizontal plane of the superficial fascia, undermining normal-appearing skin.[106] The thick fascia of the underlying muscle may be spared until late in the course of infection, when a vertical infectious phase may develop (Fig. 15.16).[101]

Necrotizing fasciitis may be diagnosed on frozen section using criteria developed by Stamenkovic and Lew (Box 15.1).[110] According to this diagnostic scheme, infected tissue is characterized by a marked neutrophilic infiltrate of the fascia, with accompanying liquefactive necrosis, and fat necrosis. Thrombosis and obliterative vasculitis with fibrinous necrosis of vessel walls is present. Abundant bacterial forms are seen within the necrotic tissue.[110]

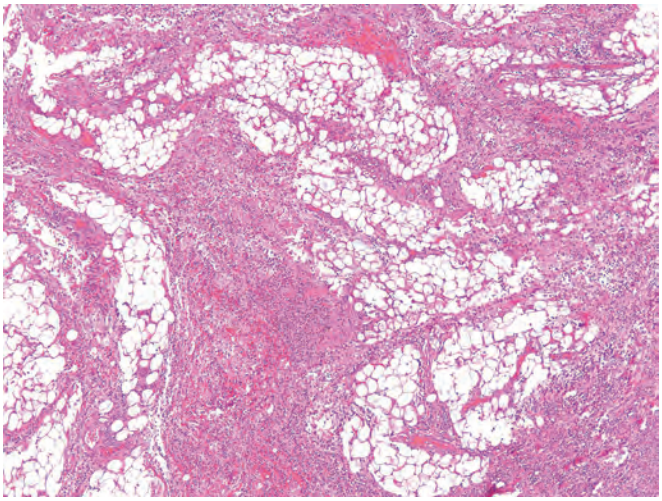
Treatment of all necrotizing soft tissue infections requires early diagnosis, aggressive surgical débridement, and wide-spectrum antibiotic therapy. Hyperbaric oxygen therapy has also been shown to be beneficial in accelerating wound closure and reducing mortality in some studies,[98] although its use remains controversial in nonclostridial infections.[111] Repeated débridement or amputation is often necessary to halt disease progression. Patients are frequently in critically unstable condition due to sepsis and must



• **Figure 15.13** Necrotizing fasciitis. **A,** Coronal computed tomogram demonstrates extensive stranding of the subcutaneous tissues of much of the leg. **B,** Axial CT scan shows that the stranding is almost circumferential around the thigh.



• **Figure 15.14** Necrotizing fasciitis with obliterative thrombosis of several small vessels. Focal fibrinoid necrosis is present with surrounding neutrophilic infiltrate.



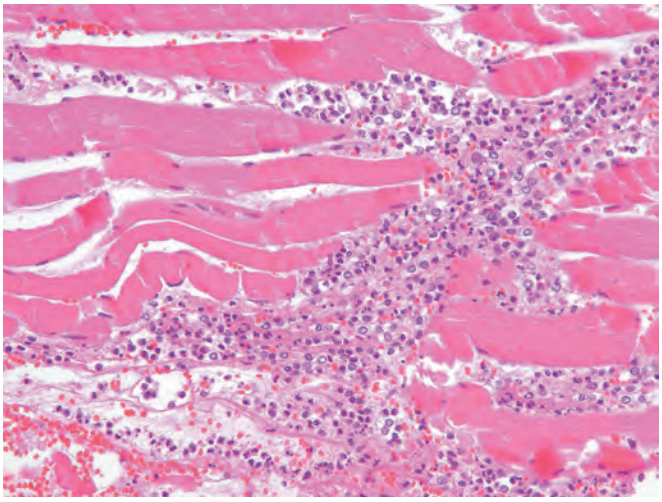
• **Figure 15.15** Necrotizing fasciitis with extensive necrosis of the subcutaneous adipose tissues, which contain a dense neutrophilic infiltrate dissecting between the septa.

be managed in the intensive care unit with hemodynamic, respiratory, and nutritional support as required. Delay in diagnosis or treatment may result in serious morbidity or death.

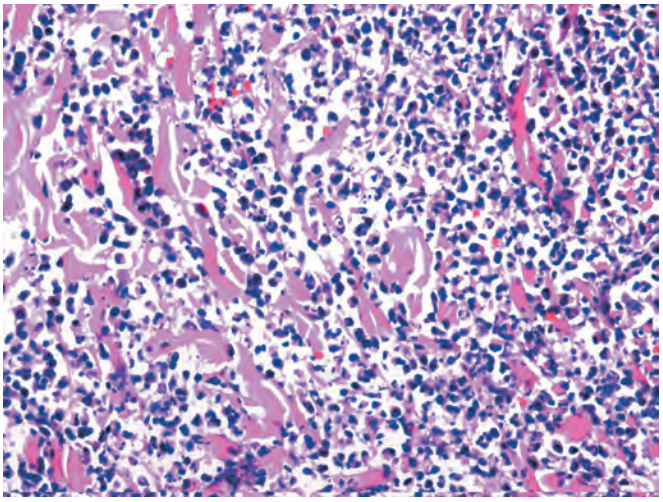
Care must be taken to exclude mimickers of necrotizing fasciitis, such as acute necrotizing neutrophilic dermatosis (ANND), a recently described variant of Sweet syndrome arising in conjunction with hematologic malignancy or granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy (Fig. 15.17). This entity should be suspected, in the appropriate clinical setting, if necrotic lesions fail to respond to débridement and antibiotic therapy and no organisms can be isolated from the suspected site of infection. Unlike necrotizing fasciitis, ANND rapidly resolves with corticosteroid therapy, and débridement is not necessary.[112]

Gas Gangrene

Clostridial myonecrosis (gas gangrene) is a type of rapidly progressive necrotizing soft tissue infection caused by *Clostridium* spp.; *C. perfringens*, *Clostridium septicum*, *Clostridium histolyticum*, and *Clostridium novyi* have all been identified as causative agents. The primary risk factor is severe penetrating trauma or crush injury with disruption of blood flow, because low oxygen concentration



• **Figure 15.16** Necrotizing fasciitis extending into edematous and viable skeletal muscle. Numerous neutrophils and macrophages infiltrate between individual myofibers.



• **Figure 15.17** Sweet syndrome in a patient receiving granulocyte-macrophage colony-stimulating factor therapy. The microscopic appearance suggests an acute pyogenic infection with large numbers of infiltrating neutrophils, but no organists were isolated, and findings resolved with corticosteroids.

• BOX 15.1 Histopathologic Findings in Necrotizing Fasciitis

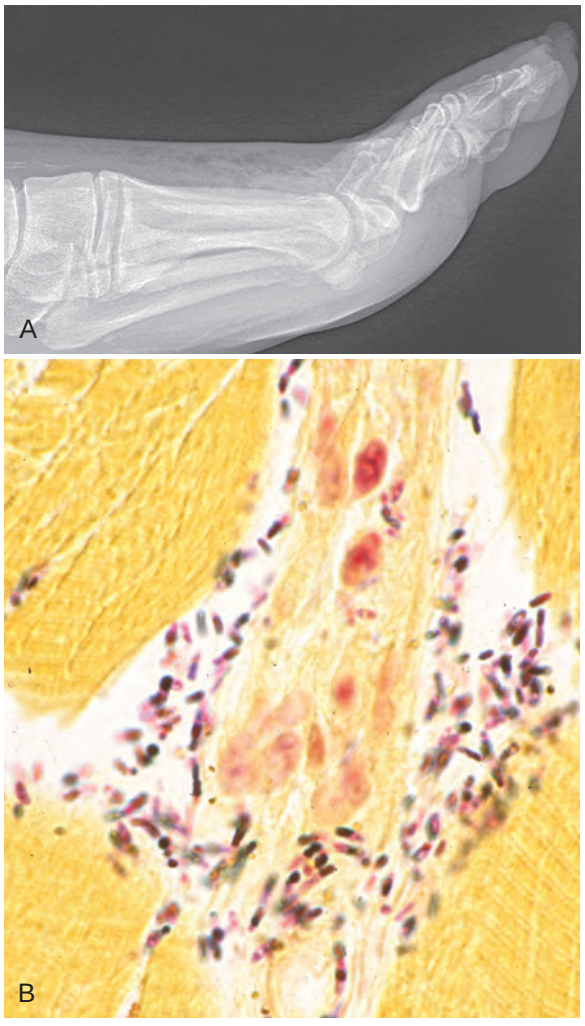
Variegated appearance at low power
Dermal edema and necrosis
Fascial necrosis and interstitial fibrin deposition
Lobular panniculitis
Sparing of deep striated muscle
Neutrophils
Macrophages and lymphocytes
Vascular thrombi

is required for clostridial infection to produce the toxins that lead to tissue damage and systemic symptoms.[102] *C. perfringens* is the most frequent pathogen in this setting. Infections have also been reported at sites of injection in intravenous drug users and secondary to malignancy or colonic perforation.[102] Presence of a foreign body has also been identified as a risk factor.[113]

Gas gangrene not related to trauma is most commonly caused by *C. septicum* and is associated with neutropenia and gastrointestinal malignancy.[102] Infection is usually a result of hematogenous spread, and the lesion may rapidly progress.

Cutaneous findings are the same as in other necrotizing soft tissue infections, with purpuric bullae, skin sloughing, edema, and systemic symptoms of fever, chills, tachycardia, or hypotension. Necrosis of skin, subcutaneous tissue, and muscle is often present. The palpation of gas in the infected tissues is a classic finding on physical examination and imaging studies; however, the diagnosis may be made even in the absence of this finding.[113] Radiographically, gas is seen in the soft tissue, together with edema and fluid collection along fascial planes. On histologic examination, large numbers of bacilli may be seen percolating through the interstices of the skeletal muscle, evoking a pauci-inflammatory response (Fig. 15.18).

Early recognition and extensive débridement and fasciotomy with appropriate wide-spectrum antibiotic therapy are critical. Hyperbaric oxygen therapy may be used as adjunctive treatment. Prognosis is poor, with a mortality rate approaching 25%[114]



• **Figure 15.18** **A,** Subcutaneous gas is present in the dorsal soft tissue on lateral radiograph of the foot in a patient with diabetes. **B,** Tissue Gram stain shows numerous gram-positive bacilli infiltrating muscle, with pauci-inflammatory response. Cultures were positive for *Clostridium* spp.

and significant morbidity in survivors due to extensive tissue destruction.

Nonnecrotizing Cellulitis

The term *nonnecrotizing cellulitis* refers to diffuse, spreading skin infections that are not associated with underlying abscess or necrotizing processes such as osteomyelitis or septic arthritis.[102] Classically, nonnecrotizing cellulitis has been divided into two overlapping entities: erysipelas and cellulitis. Erysipelas is characterized by lesions that are elevated above the surrounding normal skin and show a clear line of demarcation between involved and uninvolved tissue.[100] In contrast, cellulitis is a deeper infection involving the dermis and subcutaneous tissue. However, in practice, it may be difficult to distinguish between the two, and some clinicians use the term *erysipelas* to refer to both lesions.[102]

Nonnecrotizing cellulitis without an associated portal skin lesion is usually caused by group A β-hemolytic streptococci or other streptococcal species,[102] although other causative species, such as *H. influenzae*, pneumococcus, and *S. aureus*, have also been reported. Risk factors are associated with breaches in skin integrity and include ulcers, fungal infections, and inflammatory dermatoses, as well as alterations in vascularity or lymphatic drainage (e.g., prior surgery, lymph node dissection, venous insufficiency). Immunocompromise is another significant risk factor.[100] Erysipelas is more commonly seen in infants, young children, or older adults.[102]

Both erysipelas and cellulitis manifest with rapidly spreading areas of edema, erythema, and heat. Lymphadenitis may be present,[100] and regional lymph nodes may be enlarged. Superficial skin edema may result in a peau d'orange appearance. Vesicles, bullae, and petechiae or ecchymoses may eventually form.[102] Systemic manifestations include fever, tachycardia, hypotension, and leukocytosis. Signs of severe toxicity with widespread cutaneous lesions should raise suspicion for a more severe underlying process, such as necrotizing fasciitis.

Histopathologic changes in cellulitis are notable for edema of subcutaneous fat and a mixed inflammatory infiltrate, including a preponderance of neutrophils. The vasculature within adipose tissue may be dilated and may contain focal thrombi.[108]

Diagnosis is usually based on clinical symptoms; blood cultures are rarely positive. Care must be taken to exclude a necrotizing process or more serious condition. The differential diagnosis includes acute dermatitis, gout, herpes zoster, and acute lipodermatosclerosis.[102] Treatment is antibiotic therapy based on empirical diagnosis. Any underlying disorder or chronic skin condition that may have contributed to infection should also be treated.

Myositis

Bacterial infections of the muscle are uncommon and may result from penetrating trauma, contiguous spread from an adjacent site of infection, vascular insufficiency, or hematogenous spread.[113] The causative organisms are related to the mechanism of infection. Trauma most commonly results in a polymicrobial infection, whereas myositis secondary to hematogenous infection usually is due to *S. aureus*.

Pyomyositis

Pyomyositis, also known as pyogenic myositis, suppurative myositis, tropical myositis, temperate myositis, myositis purulenta tropica, or epidemic abscess, is a rare and frequently misdiagnosed infection of skeletal muscle. It is often mistaken for a variety of other conditions, including thrombophlebitis, osteomyelitis, malignancy, hematoma, and muscle strain. Severe complications, such as compartment syndrome, osteomyelitis, sepsis, or death, may occur if diagnosis and treatment are delayed.[107] The lower limbs and pelvic girdle muscles are most frequently affected, and the quadriceps is the most commonly involved muscle, followed by the gluteal and iliopsoas muscles, although any muscle may be affected.[107] Most cases involve only one site; however, multisite infection is present in 16%.[115]

Pyomyositis most often occurs as a result of hematogenous spread.[113] Although the exact pathophysiology of disease is unknown, infection is thought to develop secondary to skin compromise and the resultant bacteremia in the setting of muscle injury. Risk factors consist of infections, chronic inflammatory skin disease, local injections, and immunocompromised condition (e.g., HIV infection, diabetes).[102,113] In tropical areas, any age group may be affected, although the disease is more prevalent in children and immunocompetent adults. In contrast, in temperate regions, pyomyositis affects younger adults and immunocompromised patients. Men are more frequently affected than women and often have a history of local trauma. *S. aureus* is identified in 90% of cases; streptococci, *H. influenzae*, *Aeromonas hydrophila*, *Fusobacterium*, *Bartonella*, *Klebsiella pneumoniae*, other gram-negative bacteria, and anaerobes, as well as fungi and mycobacterial species, are reported less frequently.[107,113]

In the initial stages, pyomyositis manifests with fever, anorexia, and nonspecific local symptoms, such as muscle cramps and swelling. There may be restricted range of motion and pain on weight bearing. In the second, suppurative phase, patients develop high fever and chills along with muscle swelling, pain, and fluctuance of the affected region due to abscess formation. Erythema may be absent. Without treatment, patients eventually become septic.[107]

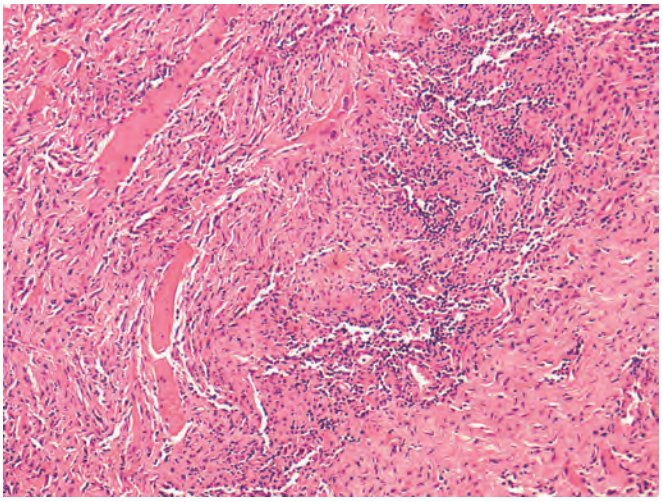
Radiographic findings in early stages of pyomyositis are nonspecific. Ultrasonography demonstrates hyperechogenicity caused by muscle swelling and edema, intermixed with areas of hypoechogenicity reflecting muscle necrosis.[116] CT imaging demonstrates muscle swelling and fluid attenuation, with ring enhancement in areas of abscess formation.[117] MRI is the most sensitive imaging modality and is useful for defining the extent of disease and tissue damage.[107]

Diagnosis requires Gram staining and culture of infected tissue or aspirated fluid. Histologically, pyomyositis is defined by the presence of pus within individual muscle groups. Examination of affected tissue reveals muscle edema with separation of myofibers, along with a lymphocytic and plasmacytic inflammatory infiltrate. Muscle necrosis with disintegration of myofibers is present.[102,118]

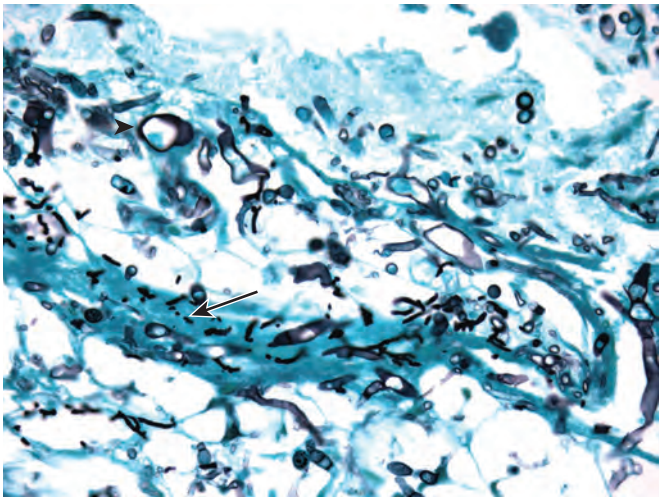
Treatment requires appropriate antibiotic coverage. If an abscess is present, drainage must be performed. The prognosis is generally good, and the mortality rate is usually less than 4%. Most patients experience a complete recovery, although recurrence may occur in immunocompromised patients.[113]

Psoas Abscess

A psoas abscess is an uncommon variant of pyomyositis in which a purulent infection affects the psoas muscle. Infection is normally unilateral and usually arises by hematogenous spread or by contiguous spread from local infection. Primary abscess arising from hematogenous spread is commonly caused by occult *S. aureus* bacteremia and usually is associated with intravenous drug use or



• **Figure 15.19** Granulomatous inflammation and degenerating skeletal muscle fibers in a patient with soft tissue swelling of the anterior chest wall. Cultures were positive for *Mycobacterium tuberculosis*. No contiguous lesion was present.



• **Figure 15.20** Soft tissue infection due to mixed infection by zygomycetes *(arrowhead)* and *Candida* spp. *(arrow)* is demonstrated in a Gomori methenamine silver–stained section.

immunocompromise. Other pathogens, including *S. pneumoniae*, *Streptococcus milleri*, group A streptococci, *E. coli*, *Pseudomonas*, *Haemophilus*, *Proteus*, and *Pasteurella* spp., have been reported. Historically, secondary psoas abscess was most often due to *Mycobacterium tuberculosis* infection occurring after Pott disease, with local spread from adjacent involved vertebral bodies, or, less frequently, after blood-borne infection (Fig. 15.19).[113] In recent decades, with the decline in tuberculosis in the developed world, there has been a shift to psoas abscess arising from genitourinary or gastrointestinal infections, especially in immunocompromised patients. Any age group may be affected, and there is no gender bias. Secondary psoas abscesses arising from intraabdominal infection are more likely to be polymicrobial and to involve enteric species such as *E. coli*, *Enterobacter*, *Salmonella*, and *Klebsiella*, as well as anaerobic species. Psoas abscess arising from adjacent osteomyelitis may involve *Salmonella*, *Brucella*, *Nocardia*, or fungi such as *B. dermatitidis* or *C. immitis*.[113]

Clinically, patients may present with fever or other systemic symptoms, such as malaise, anorexia, or weight loss. Pain and limp are only occasionally present, and the pain may involve the back or flank. A mass or fullness may be apparent in the groin. Immunocompromised patients may be afebrile with minimal symptoms.

Blood cultures may be positive, and cultures of abscess fluid should be performed. Imaging is the gold standard for detection of psoas infection. CT and MRI are the preferred modalities and clearly demonstrate the abscess. Ultrasonography is less effective. Treatment involves drainage and intravenous antibiotics. Adjacent or concomitant infection elsewhere in the body should be identified and treated appropriately with débridement, resection, or antibiotics, as indicated. The prognosis is often good, especially in primary or early disease. Secondary psoas abscess has a less favorable outcome depending on the type and extent of adjacent infections.

Bacterial Myositis

Bacterial myositis is a diffuse muscle infection without abscess formation. It occurs less frequently than pyomyositis and tends to be seen more often in adults rather than children. *Streptococcus* is the primary pathogen, although many other organisms have been reported. If *Staphylococcus* is involved, infection may rarely result in diffuse myositis with rhabdomyolysis.[113]

Group A streptococci may cause a spectrum of muscle infections, ranging from pyomyositis to necrotizing myositis, also known as spontaneous streptococcal gangrenous myositis. This disease is rare but very aggressive and often fatal. The portal of disease entry is usually unknown, with no prior trauma or illness. Clinically, streptococcal necrotizing myositis manifests with flulike symptoms, including rash and myalgia, which progress to severe muscle pain with local swelling and fevers. Overlying skin lesions such as those found in fasciitis are not typically present. Multiple sites may be involved. The course is rapidly progressive and can lead to septic shock within days. Compartment syndrome may occur as a result of rapid muscle swelling.[113]

Histologic examination demonstrates muscle necrosis with abundant streptococcal organisms. Treatment requires repeated débridement and high-dose antibiotics. Fasciotomy may be necessary to prevent compartment syndrome.

Fungal Myositis

Fungal myositis is an uncommon infection predominantly affecting immunocompromised patients. *Candida* spp. are the most common fungal pathogens reported in myositis, although *C. neoformans*, *H. capsulatum*, *Coccidioides*, *Aspergillus*, and *Pneumocystis jiroveci* have all been reported.[113] Symptoms are similar to those seen in bacterial infection, with fever and muscle tenderness the most frequent presenting symptoms.

Diagnosis is by tissue biopsy and culture. Histologic examination typically reveals diffuse microabscesses or granulomatous inflammation, with rare larger fungal abscesses. Fungal organisms may be identified within tissues (Fig. 15.20). Treatment requires débridement and antifungal therapy. Mortality rates are often high.

Parasitic Soft Tissue Infection

A variety of parasites have been identified in soft tissue infections. Most result in myositis and are only rarely found in subcutaneous

soft tissues. *Trichinella*, *Taenia solium* (cysticercosis), and *Toxoplasma gondii* are the most commonly reported causes of parasitic myositis. Many other parasites are less frequently implicated, including *Trypanosoma cruzi*, *Plasmodium falciparum*, *Echinococcus*, *Entamoeba histolytica*, *Sarcocystis*, *Microsporidia*, *Toxocara*, *Schistosoma*, and *Onchocerca volvulus*.[113] Parasitic soft tissue infections are uncommon in the United States and are usually acquired during travel abroad. Typically parasitic larvae or ova are ingested then migrate out of the gastrointestinal tract and into the bloodstream, from whence systemic dissemination occurs. Many parasites display a tropism for certain organs, such as muscle, skin, or the central nervous system. On entry into muscle or soft tissue, organisms usually encyst and remain dormant in preparation for the next stage of their life cycle. Many infections, such as cysticercosis or trichinosis, manifest with subclinical symptoms or as an incidental finding. When present, symptoms usually result from an inflammatory response to waste or shed material from parasites, or to dead and degenerating organisms, and only rarely from direct tissue damage caused by migrating parasites.[113,119]

Patients with symptomatic parasitic infection of muscle present with myalgias, muscle swelling, and weakness.[113] Some parasites, such as *Toxoplasma* or filarial worms, display a tropism for lymphatics and can cause acute or chronic lymphadenitis. Chronic lymphadenitis in filarial infection may result in lymphedema and eventual elephantiasis.

Radiographs may demonstrate calcified, encysted organisms within affected tissue. For example, cysticerci are often found incidentally as 5- to 7-μm, spindle-shaped, calcified cysts within infected tissue.[120] Other aberrantly migrating cestode larvae, including spargana and coenuri, also cause disease. The sparganum (Fig. 15.21) is distinguished by the absence of protoscolices, although so-called racemose cysticerci also lack protoscolices. Inflammatory reactions with prominent eosinophils may be present.

Viral Myositis

A number of viruses have been implicated in myalgia, myositis, or viral-associated rhabdomyolysis. In the United States, influenza A and B are most frequently reported; other responsible pathogens include enterovirus, human T-cell lymphoma virus, HBV, and HCV. However, a wide range of other viruses have also been reported as causes of myositis.[113]

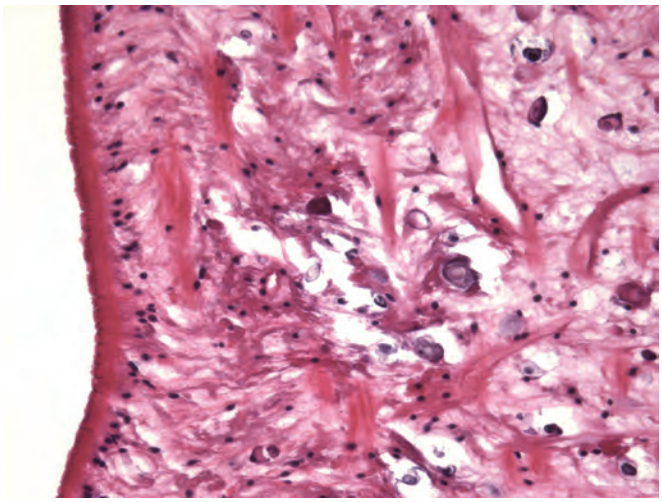
Myalgias are a common symptom of influenza infection and may be diffuse and transitory. In contrast, myositis is less common, occurs later in the course of infection, and is characterized by a more focal, sudden onset of severe pain. Myositis in influenza infection tends to affect children but may also be seen in adults. The calf is the most frequent site of involvement. Enterovirus targets costochondral muscles, resulting in severe chest pain. Rhabdomyolysis is a rare complication of infection by influenza viruses, coxsackieviruses, and enteric cytopathologic human orphan (ECHO) viruses, among others.

Muscle biopsy in viral myositis is rarely performed; however, histologic examination of affected muscle demonstrates focal muscle fiber degeneration and necrosis with a mild inflammatory infiltrate. Although viral particles have been isolated from muscle tissue, many studies postulate an immune-mediated etiology for viral myositis. Treatment is symptomatic, and myositis spontaneously resolves within 1 month.

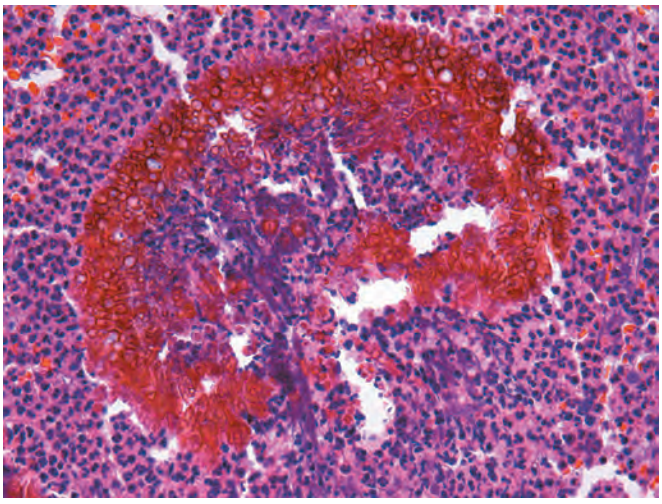
Other Unusual Soft Tissue Infections

Mycetoma

As described in Chapter 14, both bacteria and true fungi can produce localized infections of the soft tissues. The most common soft tissue mycetoma in the United States is caused by *P. boydii*, but a variety of other fungi can yield mycetomas (Fig. 15.22). Tissue Gram and silver stains, such as the Gomori methenamine silver (GMS) stain, are required to identify the cause of the granule, and culture is required for confirmation. These infections are characterized by the presence of exudate granules—proteinaceous structures that can be white, yellow, or black, depending on the nature of the infection. Organisms are present at the core of the "granule," which consists of a cortical shell of plasma proteins, the Splendore-Hoeppli phenomenon. Treatment includes surgical excision.



• **Figure 15.21** This sparganum was removed as a soft tissue tumor in the breast. It shows a tegument with loose internal structure and multiple calcareous bodies that are expressed by cestode larval forms. There is no evidence of a protoscolex.



• **Figure 15.22** Mycetoma due to *Pseudallescheria boydii* showing prominent Splendore-Hoeppli phenomenon. The diagnosis was confirmed by isolation of the fungus in culture.

Chromoblastomycosis and Phaeohyphomycosis

Soft tissue infections due to pigmented dematiaceous fungi can be caused by pigmented yeast or hyphal fungi. The subcutaneous infections form fluctuant, painless masses, often in the extremities. The generic diagnosis depends on whether yeast or hyphate fungi are detected. Infection in these cases is often the result of introduction of a foreign body (Fig. 15.23A). In chromoblastomycosis, the pigmented yeast forms show septation (sclerotic bodies or Medlar bodies) (see Fig. 15.23B). The histologic responses are often granulohistiocytic, with microabscess formation. In phaeohyphomycosis, cyst formation is common. Pigmentation of the fungi is generally apparent on hematoxylin and eosin (H&E)-stained sections (see Fig. 15.23C), but a Fontana-Masson stain can be applied if uncertainty persists. Specific identification of the causative fungus requires microbiologic culture because a large variety of fungi can produce these syndromes.

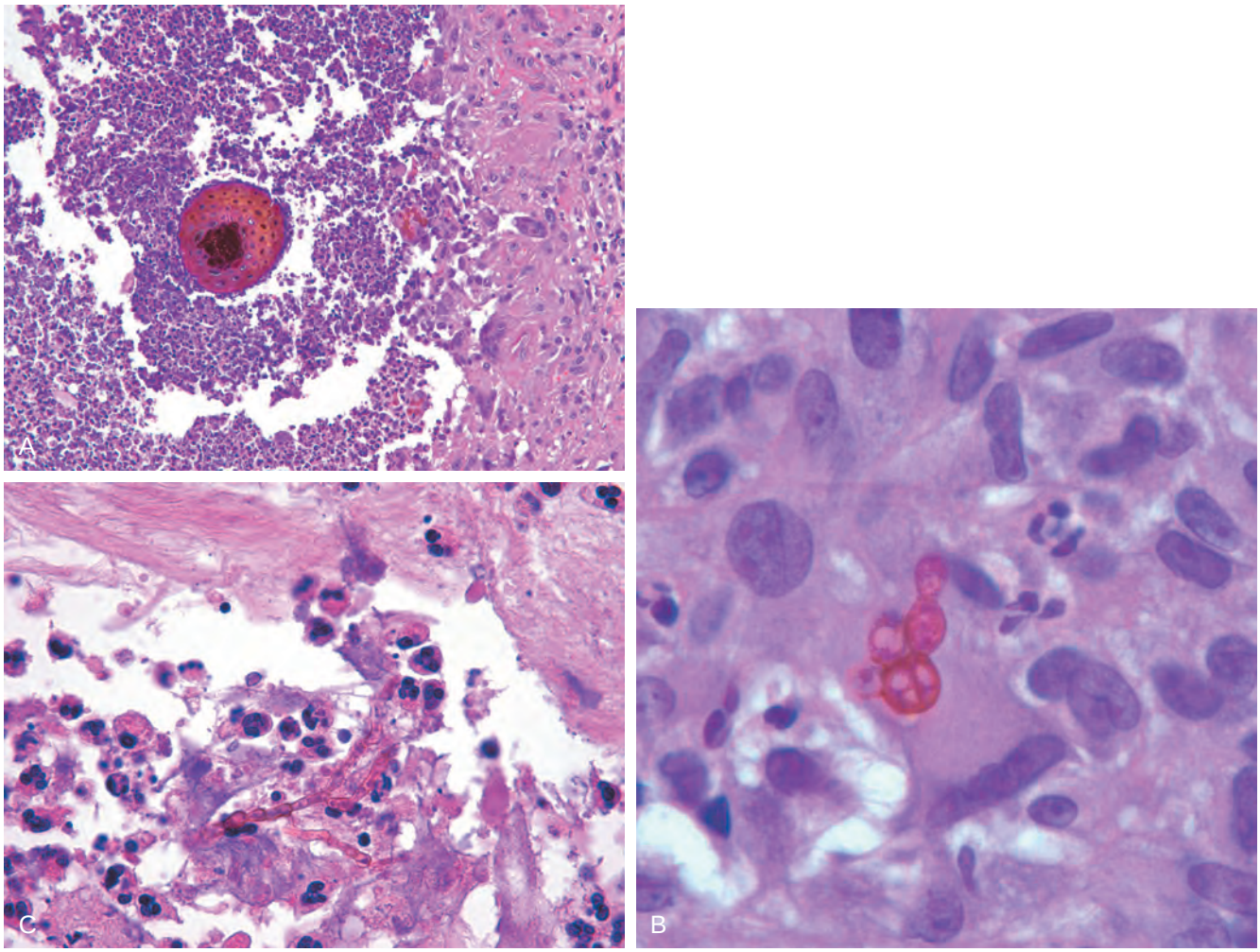
Protothecosis

This disease is caused by a nonpigmented algae of the genus *Prototheca*. Two members of the species are pathogenic in humans, *Prototheca wickerhamii* (2 to 12 μm) and *Prototheca zopfii* (10 to 25 μm). *Prototheca* organisms produce two major syndromes in humans—a cutaneous hyperkeratotic maculopapular eruption and olecranon bursitis—although tenosynovitis at other sites has been reported. The host response to infection includes histiocytic and granulomatous inflammation with fibrinous exudates (Fig. 15.24A). The organisms can be seen in H&E-stained sections and are highlighted to advantage by both PAS and GMS stains (see Fig. 15.24B). The diagnostic morphologic finding is a morula sporangium that includes a central endospore surrounded by multiple encapsulated and molded endospores (see Fig. 15.24C). The diagnostic sporulating forms can be sparse and difficult to identify.

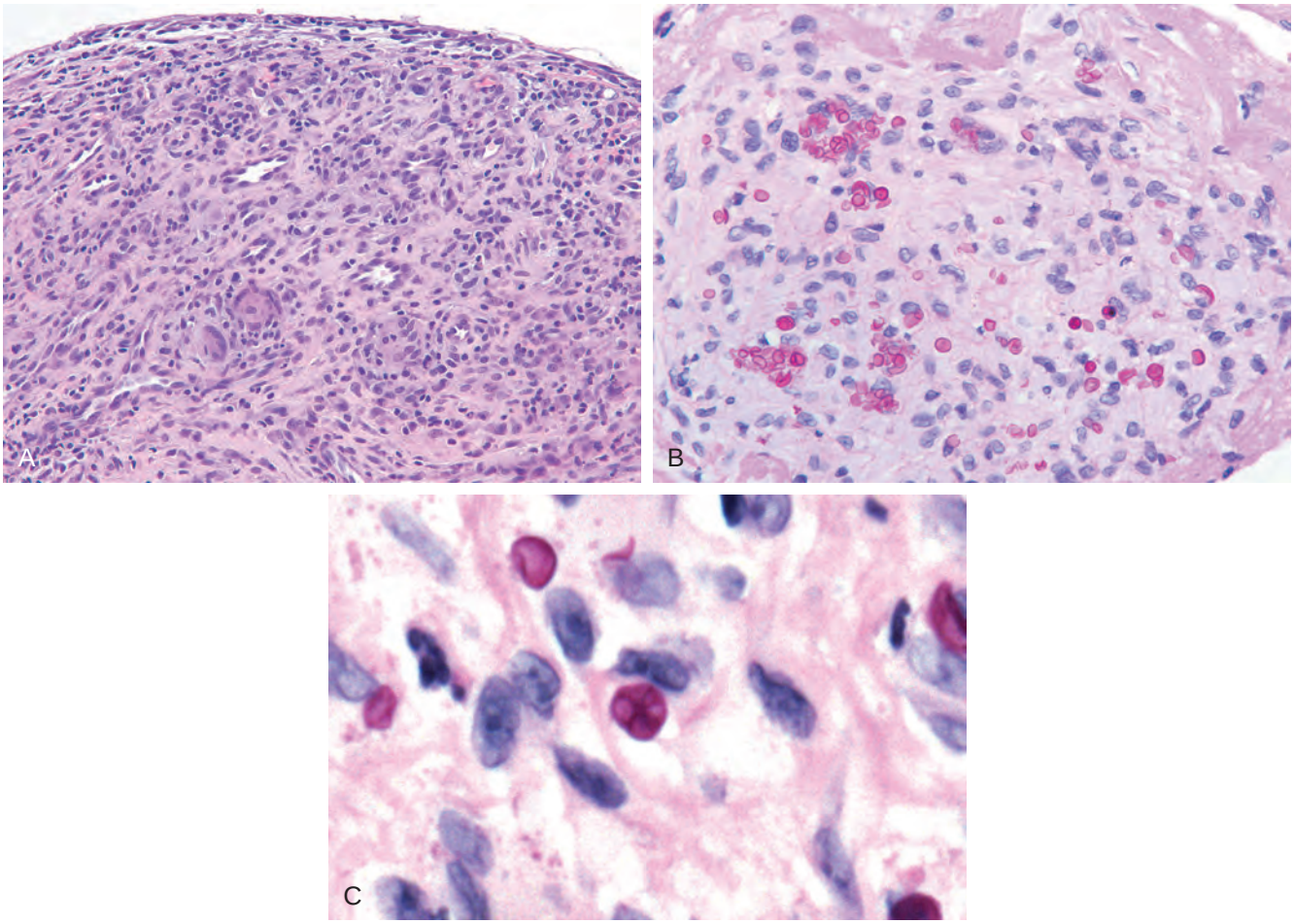
Green algae of the genus *Chlorella* rarely cause soft tissue infections. They are difficult to distinguish from *Prototheca* by light microscopy but characteristically produce green, discolored lesions; contain chloroplasts that can be seen on electron microscopy; and can be specifically identified by immunohistochemical methods.

Emerging Nosocomial Fungal Infections

Nosocomial fungal soft tissue infections have been increasing since the 1980s are associated with immunosuppression in the



• **Figure 15.23** **A,** Splinter with microabscess and adjacent pigmented yeast surrounded by granulomatous response. **B,** Chromoblastomycosis shows a septated sclerotic (Medlar) body. **C,** Phaeohyphomycosis shows pigmented hyphal fungi and microabscess formation. Definitive classification of the offending organisms in these disorders requires fungal culture and isolation.



• **Figure 15.24** **A,** *Prototheca* infection showing granulomatous synovitis. **B,** Multiple periodic acid–Schiff-positive, septated, nonsporulating forms are seen in an area of fibrin exudation. **C,** The diagnostic morula shows a central endospore surrounded by molded and septated spores.

setting of organ and stem cell transplantation, chemotherapy, debilitation, and an immunocompromised state.[121] Although *Aspergillus* spp. and *Candida* spp. remain the most common, other molds and yeastlike fungi are also reported. Among the more common of these are *Rhizopus*, *Mucor*, *Fusarium*, and *Scedosporium* spp., whereas species of *Rasamsonia*, *Verruconis*, *Tricosporon*, and *Alternaria* represent emerging pathogens.[121] Infections frequently involve skin and soft tissue but may spread to lungs or systemically in severe disease. Mortality rates of nosocomially acquired fungal infections remain high.

Surgical Site Infections

Surgical site infections (SSIs) are the most common adverse events occurring after surgical procedures; they account for 38% of nosocomial infections in surgical patients.[122] The frequency of SSI is highly dependent on the type of operation, with high-risk and contaminated surgeries carrying the highest risk. SSIs can be categorized by the depth of involvement.[122]

- Superficial SSIs extend into the subcutaneous space but do not penetrate the underlying muscular fascia. They occur within 1 month after the operation.
- Deep incisional SSIs extend into the fascia and muscle and can occur as late as 1 year after the operation. Deep SSI may be associated with prosthetic implants.
- An organ or space SSI may also occur up to 1 year after an operation and involves any organ or space that may have been exposed during surgery.

Most SSIs do not develop until at least 5 days after operation, although *S. pyogenes* or clostridial infection may occur within 2 days of surgery. The type of organism found in an incisional SSI tends to depend on the operation: abdominal procedures may lead to SSIs with polymicrobial mixed gram-positive and gram-negative species, whereas extraabdominal procedures more frequently result in *S. aureus* or streptococcal infection.[102]

Diagnosis of an incisional SSI is usually based on the appearance of the incision. Both superficial and deep SSIs are characterized by purulent incisional drainage, positive culture of wound fluid or tissue, and local pain, tenderness, swelling, and erythema.[102]

Treatment involves opening of the incision, with débridement and drainage of infected material. Antibiotic therapy has not been shown to be of use.[102]

Soft Tissue Infection After Military Trauma

Military trauma resulting from gunshot wounds, shrapnel, burns, blast injuries, or other penetrating or crushing injuries incurs a high risk of infection. Not only are wounds often deeply contaminated with soil and foreign material, but the injury may be extensive and associated with tissue devitalization. This allows

microbes to proliferate beyond the bounds of the immune response. The type of organisms responsible for posttraumatic infection largely depends on the site of and the timing of injury. Abdominal wounds are most frequently infected with enteric gram-negative species, whereas burns and extremity wounds are most likely to be initially contaminated by *S. aureus* when tested in the field.[123] Following evacuation, colonization by *Pseudomonas* or *Klebsiella* is more frequently present.[123,124] Of note, late infectious complications in such instances have been noted to be due to *S. aureus*.[123] Clostridial infections are now uncommon, primarily because of early surgical débridement and appropriate antibiotic therapy.[124] In many cases, the infections are polymicrobial,[125] and they may include unusual multidrug-resistant organisms, such as *Acinetobacter* spp..[125-127] *Acinetobacter* infection preferentially affects the extremities and manifests as a well-defined, edematous, and erythematous cellulitis arising at the edges of a contaminated wound. As the disease progresses, the skin develops a sandpaper appearance, followed by the development of vesicles that may be hemorrhagic.[127] Bacteremia may ensue, leading to life-threatening sepsis. Treatment requires débridement and intravenous antibiotics. The mortality rate approaches 12%.[127]

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16

Genitourinary Infectious Disease Pathology

ALTON B. FARRIS III, G. PETUR NIELSEN

Genitourinary Cutaneous Infections
- Bacterial Infections
- Fungal Infections
- Viral Infections
- Parasitic Infections

Kidney Infections
- Acute Interstitial Nephritis
- Acute Pyelonephritis
- Focal Bacterial Nephritis (Lobar Nephronia)
- Emphysematous Pyelonephritis
- Chronic Pyelonephritis
- Transplant-Related Renal Infections
- Other Infections

Bladder Infections
- Bacterial Cystitis
- Fungal Cystitis
- Viral Infections
- Parasitic Infections
- Special Bladder Conditions With an Infectious Association

Infections of the Urethra

Infections of the Male Genitourinary System
- Prostate
- Epididymis
- Testis
- Penis and Scrotum

Infections of the genitourinary tract can be caused by a number of viral, bacterial, fungal, and parasitic organisms (Table 16.1). Although many of these organisms are also seen in other locations, some of them have a predilection for the genitourinary area. Their clinical manifestations, in conjunction with their gross and microscopic appearances, are useful in characterizing these infections, and ancillary tests are often useful in their diagnosis.

Genitourinary Cutaneous Infections

Bacterial Infections

Erythrasma is a chronic bacterial infection of the genitourinary region caused by *Corynebacterium minutissimum*, particularly in humid cutaneous recesses, such as the vulva and inguinal folds, and especially on the inner aspect of the thigh just below the crural fold. Diabetics and institutionalized patients are predisposed. Clinically, it is characterized by a sharply demarcated, brown to red, scaly plaque that fluoresces under a Wood lamp because of the presence of bacterial porphyrins, often giving a coral-red or orange-red color. Corynebacteria are pleomorphic, small, gram-positive rods, also referred to as diphtheroid.[1,2] The diagnosis is often one of exclusion, after fungal infections are ruled out. Fungal organisms may be visualized with the use of potassium hydroxide (KOH) on various microscopic preparations or with periodic acid–Schiff (PAS) and methenamine silver stains.[2-6]

Trichomycosis pubis and axillaris is an asymptomatic colonization of hair follicles by various corynebacteria (particularly *Corynebacterium tenuis*), which can lead to a red, cream, yellow, or, less commonly, black coating around pubic or axillary hairs and produce an offensive odor. Pubic involvement is less common than axillary involvement. Microscopically, large bacterial colonies are present and may invade the superficial hair cortex. *C. tenuis* was once thought to be the primary offending organism, but now multiple species are recognized. The so-called corynebacterial triad is the combination of erythrasma, trichomycosis axillaris, and pitted keratolysis. However, pitted keratolysis usually affects the plantar surface of the feet, leading to multiple asymptomatic pits and superficial erosions; it is less common in the genitourinary region.[2,3,5-8]

Cellulitis of the scrotum and penis can result from bacteria (particularly β-hemolytic streptococci but also *Escherichia coli*, *Vibrio vulnificus*, *Vibrio cholerae*, *Pasteurella multocida*, and others) that gain an entry after a disruption of the epidermal surface, such as after circumcision. Genital skin in this condition is red, hot, and tender, and histologically there is prominent edema with dermal perivascular and interstitial acute inflammation. *P. multocida* cellulitis may occur after animal bites.[2,3,9,10]

Staphylococcus aureus produces primary lesions in the genital tract, including impetigo, bullous impetigo, furuncles, and folliculitis. Impetigo usually occurs on the face or extremities but may also spread to the genital area; although it was previously

TABLE 16.1 Summary of Some of the Important Genitourinary Infectious Disorders

Disorder	Microbe
Chancroid	*Haemophilus ducreyi*
Dermatophytosis	*Trichophyton*, *Epidermophyton*, and *Microsporum* spp.
Encrusted cystitis	*Corynebacterium urealyticum*
Granuloma inguinale	*Klebsiella granulomatis*
Lymphogranuloma venereum	*Chlamydia trachomatis*
Malakoplakia	*Escherichia coli* (frequently)
Pityriasis versicolor (tinea versicolor)	*Malassezia furfur*
Syphilis	*Treponema pallidum*

thought that group A β-hemolytic streptococci most commonly caused impetigo, *S. aureus* is currently the most common cause. Bullous impetigo consists of 0.5- to 3-cm in diameter flaccid bullae, with thin roofs and erythematous rims and erosions; the bullae may coalesce. The roof may rupture, forming a thin crust. A furuncle (boil) is an infection of the pilosebaceous unit that occurs at sites of friction, such as the inner aspects of the thighs and the buttocks. Histologically the furuncle consists of an abscess in the deep dermis centered on a hair follicle. Folliculitis, usually caused by *S. aureus*, has a number of manifestations that clinically consist of small pustules developing around follicular ostia, sometimes pierced by hair. Histologically, folliculitis consists of a subcorneal pustule with abundant neutrophils and some macrophages and lymphocytes overlying the follicular infundibulum. *S. aureus* can also cause secondary infections in atopic dermatitis, psoriatic plaques, and lichen simplex chronicus, as well as superinfections of candidiasis, genital herpes, or syphilitic chancres.[2,3]

Group A or B β-hemolytic streptococci can cause superficial erosive intertrigo.[3] In addition, perianal streptococcal dermatitis can be caused by group A β-hemolytic streptococci. Perianal streptococcal dermatitis occurs most commonly in children and is recognized by a perianal erythema with a well-demarcated border that may desquamate as a scale and subsequently heal.[2]

Hidradenitis Suppurativa

Hidradenitis suppurativa (also known as acne inversa or apocrine acne) is caused by bacterial infection of the apocrine glands, usually in the axilla, but it can also involve apocrine glands in the perineum and vulvar region.[2] Some studies have suggested that coagulase-negative staphylococci are the most common cause,[11] but others have found the microbiologic flora to be variable.[12-14]

Fournier Gangrene

Fournier gangrene is a necrotizing subcutaneous infection of the genital and anorectal regions caused by a mixture of aerobic and anaerobic enteric organisms. Risk factors include genitourinary trauma, periurethritis, diabetes, immunosuppression, poor nutritional status, septic injection into the penile vein, and, rarely, *trans*-retinoic acid treatment for hematologic malignancies. A rapidly developing cellulitis, fasciitis, myositis, and systemic toxicity may result; however, the external physical findings may not be prominent and may be out of proportion to the severity of the infection. A localized vasculitis results from a Schwartzman-like reaction. The Schwartzman reaction is the inflammatory process brought about by injection of endotoxin, which on first injection results only in mild inflammation but on second injection results in a thrombohemorrhagic lesion, similar to that seen in Fournier gangrene.[15] The mortality rate ranges from 10% to 30%. Treatment is typically with débridement and broad-spectrum antibiotics, as necrotizing fasciitis in other locations.[3,16-20]

Pseudomonal Cellulitis (Ecthyma Gangrenosum)

Pseudomonal sepsis may be complicated by a cellulitis known as ecthyma gangrenosum, which starts with an erythematous or purpuric cutaneous macule that may rapidly develop into a bulla, which sometimes ulcerates. This is caused by a bacterial vasculitis that may eventually result in cutaneous infarction. Penile ecthyma gangrenosum has been reported in patients with a history of drug abuse and neutropenia.[3,21-23]

Mycobacterial Disease

Genital tuberculosis can result from sexual contact with individuals who have active oral or genital tuberculosis. *Mycobacterium tuberculosis* is the most common organism, but infections due to *Mycobacterium celatum* have also been reported.[24] Clinically, subacute or chronic, painful ulcers with or without lymphadenopathy may be present, and the lesions may even mimic tumors grossly, notably in scrotal lesions.[25] Lupus vulgaris, which is the most frequent form of reinfection tuberculosis, can affect the penis. Confluent papules may coalesce into plaques; when viewed by diascopy, these plaques have an "apple-jelly" deposit representing cellular infiltrates. Involvement of the glans may occur. Bacillus Calmette-Guérin (BCG) balanitis after instillation for bladder carcinoma has rarely been reported.[3,25,26]

Syphilis

The spirochetal organism, *Treponema pallidum*, is the causative agent of syphilis. The anogenital area may be affected by primary, secondary, tertiary, or congenital lesions of syphilis. Three weeks after initial exposure, a primary syphilitic chancre occurs at the site of inoculation, usually the inner prepuce, coronal sulcus, penile shaft, vulvovaginal area, or anus.[3,4]

The painless, buttonlike papule ulcerates centrally, and adjacent "kissing" lesions may also be present. Histologically, there is a prominent lymphoplasmacytic infiltrate in an edematous ulcer bed with prominent capillary proliferation containing swollen endothelial cells. Spirochetes may be identified with the use of Warthin-Starry or Steiner stains or immunohistochemically.[27] Smears can be analyzed by darkfield examination or by immunofluorescence with antitreponemal antibodies.[28] Secondary syphilis occurs if the primary lesion is left untreated and the spirochete spreads systemically. It typically forms 3 to 6 weeks after the chancre but may arise up to 6 months after healing of the primary lesion. Clinically, large, red to pale, moist, flat-topped or verruciform papules may develop in the anogenital region (condyloma lata). In 80% of patients, there is a diffuse, nonpruritic, papulosquamous eruption. In dark-skinned patients, annular polycyclic lesions may occur. Microscopically, many spirochetes are present, explaining the highly contagious nature of secondary syphilis. Pseudoepitheliomatous hyperplasia may be prominent. The composition of the inflammatory cells is similar to that of the primary lesion; in addition, a neutrophilic infiltrate may be present.[3,4,29]

Tertiary syphilis targets bones, the central nervous system, the cardiovascular system, and skin with ulcerative and nodular lesions. In tertiary syphilis, the classic lesion is a gumma, a type of granuloma with a focus of necrobiosis surrounded by multinucleated giant cells, histiocytes, plasma cells, lymphocytes, and fibroblasts. Obliterative endarteritis may be present. A nodular presentation consists of tuberculoid granulomas with a mixed inflammatory cell reaction including plasma cells. Treponemes cannot usually be detected with silver stains, but polymerase chain reaction (PCR) may be useful.[3,29]

Chancroid

Haemophilus ducreyi is the gram-negative, facultative anaerobic bacterium that causes chancroid. It is uncommon in the United States but is a common cause of genital ulcers in Africa. Chancroid is transmitted by sexual contact during the active phase of lesions, which in untreated patients lasts approximately 6 weeks. A papule or pustule appears at the exposure site after approximately 1 week. This usually develops into a painful ulcer or ulcers with a gray-yellow exudate and sharp, undermined, ragged erythematous borders. Many lesions can arise in the location of the original lesion through autoinoculation, and lesions may merge with serpiginous borders. After 1 to 2 weeks, adenopathy with suppuration (buboes) occurs in approximately 25% to 50% of untreated patients, sometimes leading to inguinal abscesses and draining sinuses. Microscopically, microvascular and endothelial proliferation with overlying surface ulceration and necrotic debris is typically seen. Vessels may be thrombosed. There is often a dense lymphoplasmacytic infiltrate. Special culture medium is required for isolation. Chancroid is treated with antibiotics. Large lymph nodes may need to be drained.[3,4,30]

Lymphogranuloma Venereum

Chlamydia trachomatis, an obligate intracellular parasite, particularly serovars L1, L2, and L3, cause lymphogranuloma venereum; the organisms spread through sexual transmission, travel through lymphatic channels, and multiply within draining lymph nodes of the genital and anorectal region. Serotypes D to K are associated with sexually transmitted oculogenital disease.[3,4,29]

In the primary stage, eroded papules or groups of small herpetiform ulcers form at the inoculation site (coronal sulcus, frenulum, prepuce, shaft, glans, scrotum, or vulva). Urethritis with a mucopurulent discharge may be present. In the secondary stage, occurring several weeks later, inguinal lymphadenitis and formation of buboes (enlarged lymph nodes, from the Greek *boubôn*, "groin") occur, often with concurrent cordlike lymphangitis of the dorsal penis and suppurative inguinal bubo formation. The so-called groove sign may be present, which results from a cleft formed by the inguinal (Poupart) ligament between enlarged involved femoral and inguinal lymph nodes. Proctocolitis may occur.[3,4,29]

A third stage comprises various sequelae from inflammation and includes fistula formation, fibrosis, and scarring.[3,4,29,31] This may be complicated by sinuses and tracts between the skin and urethra and deforming scars of the penis. In the anogenital syndrome, proctocolitis and hyperplasia of the perirectal lymph nodes may occur, and fistulas and rectal strictures may form. Obstruction of chronic lymphedema may lead to elephantiasis.[3,4]

Histologically the ulcers of lymphogranuloma venereum consist of a chronic inflammatory process with scattered giant cells, plasma cells, lymphocytes, necrosis, and granulation tissue. Nonnecrotizing granulomas composed of epithelioid histiocytes may be present. Fibrosis and, eventually, prominent scarring occur as the process becomes more chronic. Pseudoepitheliomatous hyperplasia may be present. Lymph nodes show accumulation of neutrophils, often with prominent necrosis, particularly in the early stages of the infection. Plasma cell infiltration and lymphocytic hyperplasia follow. Eventually, what are referred to as stellate abscesses form; these are suppurative foci that have coalesced and are surrounded by epithelioid cells and multinucleated giant cells.[4,29]

Culture of the lesions is the best method to identify the organisms in oculogenital chlamydial infections. The obligate intracellular organisms can typically be identified in vacuoles of vacuolated macrophages.[32] The organisms stain faintly blue with hematoxylin and eosin (H&E), are gram-negative, and can be identified with Warthin-Starry stain. Electron microscopy, immunohistochemistry, and PCR for the 16S ribosomal DNA of the organisms are sometimes needed for a definitive diagnosis. Serology may be useful in confirming the diagnosis.[4,29,31]

Granuloma Inguinale (Donovanosis)

Klebsiella granulomatis (formerly called *Calymmatobacterium granulomatis*), a gram-negative bacillus, leads to donovanosis, a sexually transmitted genital ulcer disease. It is rare in the United States and occurs primarily in the southern states.[33] Clinically, well-defined, single or multiple, painless ulcers with beefy and red granulation tissue at the base and elevated, rolled, serpiginous, or hyperplastic borders form the primary lesion. The ulcer may heal with prominent fibrosis. Lymphedema with eventual elephantiasis of the penis and scrotum can result in chronic disease. Subcutaneous nodules (pseudobuboes) can simulate lymph node involvement. Lesions may be nodular, hypertrophic, cicatricial, and ulcerovegetative. Microscopically, there is prominent granulation-type tissue with an inflammatory infiltrate including plasma cells, neutrophils, and histiocytes that contain 0.6- to 2-μm Donovan bodies, which are encapsulated rods with bipolar granules (Fig. 16.1). Pseudoepitheliomatous hyperplasia may be prominent. The gram-negative coccoid to bacillary bacteria may be difficult to identify, requiring Wright/Giemsa, toluidine blue, or Warthin-Starry stains. Cytologic identification of Donovan bodies in granuloma inguinale (see Fig. 16.1) can be accomplished with Papanicolaou-stained smears. Ultrastructural studies have identified the organism in greater detail, demonstrating it in the phagosomes of macrophages.[3,4,34-38] Squamous cell carcinomas of the vulva secondary to granuloma inguinale have been reported.[4,29,39,40]

Fungal Infections

Certain superficial fungal infections, such as dermatophytosis, pityriasis versicolor, and candidiasis, occur in the genitourinary area and are discussed in this section. Deep mycotic infections, including blastomycosis, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis, also occur in this area. Diagnosis is aided by identification of hyphae in skin scrapings treated with KOH. Fungal infections are usually superficial, but deep infections may occur, and phycomycosis (an older term for zygomycosis) of the vulva has been reported in diabetic women.[3,4,41,42]

Dermatophytosis

Dermatophytosis (tinea) is a superficial fungal infection of keratinized tissues caused by species such as *Trichophyton*, *Epidermophyton*, and *Microsporum*. Tinea cruris is an infection of the

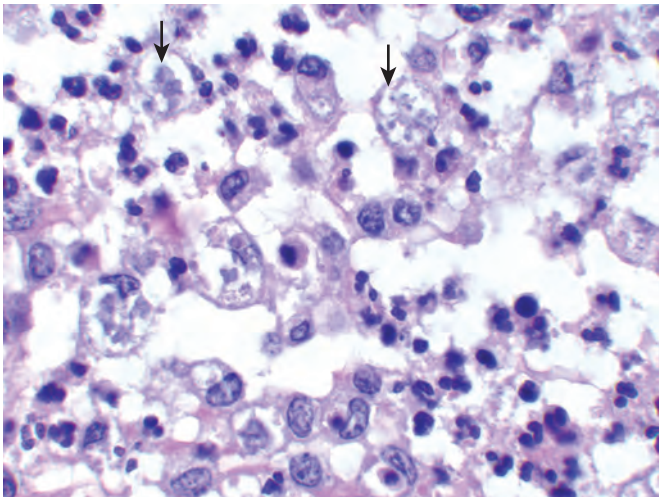
inguinal area. An infection of the genital area can result from a transfer by hand from a dermatophytosis of the feet (tinea pedis).[3,4]

Pityriasis Versicolor (Tinea Versicolor)

Although pityriasis versicolor is usually located on the upper trunk or upper arms, this superficial fungal infection, caused by the lipophilic yeast of the genus *Malassezia*, can cause hypopigmented or hyperpigmented, white to red-brown, macular eruptions in genital areas, notably on the penile shaft.[43,44] Scales show pale yellow fluorescence with a Wood lamp. Pityriasis versicolor is usually considered to be noncontagious. The organism to which this disorder is usually attributed is *Malassezia globosa*, which was formerly classified as *Malassezia furfur*.[2,3]

Candidiasis

The normal flora of the intertriginous skin, prepuce, and perineal area includes *Candida albicans*. Candidal balanoposthitis is the most common fungal infection of the penis.[45] Obesity and uncontrolled diabetes mellitus are predisposing factors, as are other immunocompromised states. The infection can be sexually transmitted. More than 75% of women experience at least one symptomatic episode of vulvovaginal candidiasis. Small pustules may be present. Microscopically, there is epidermal thickening and spongiosis with dermal chronic inflammation. Yeast and pseudohyphae are present on the surface, together with degenerated squamous cells and neutrophils.[3,4]



• **Figure 16.1** Granuloma inguinale. Granuloma inguinale, also known as donovanosis, is characterized histologically by the presence of dense acute inflammation in lesional ulcers, which are usually painless. Donovan bodies *(arrows)*, intracellular inclusions of engulfed bacteria, are present within macrophages.

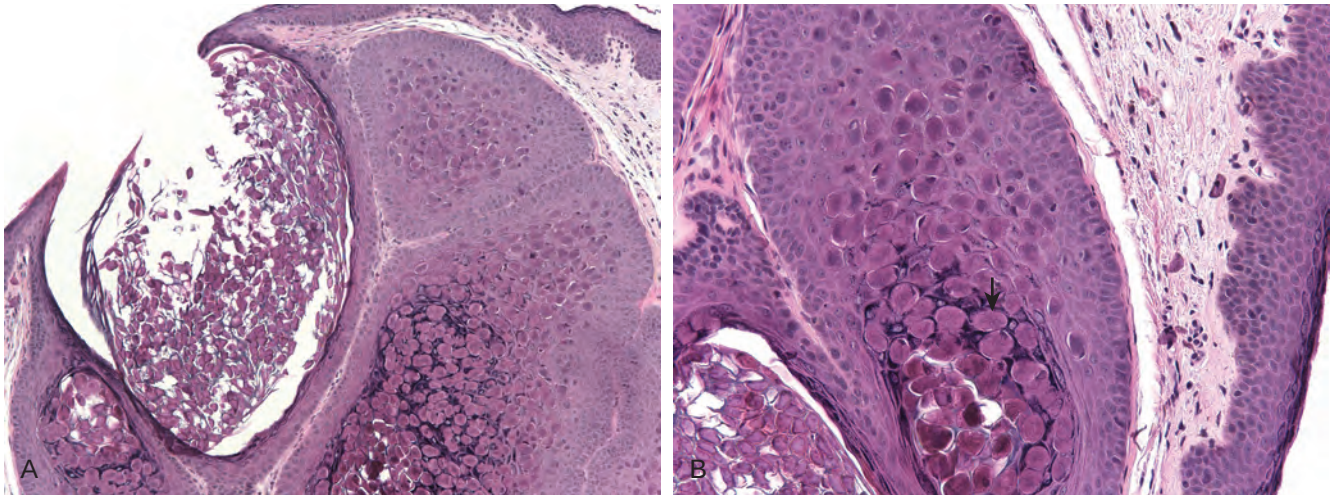
Viral Infections

Molluscum Contagiosum

Molluscum contagiosum is caused by a large poxvirus (molluscum contagiosum virus), which is a double-stranded, brick-shaped DNA virus. It manifests as solitary or multiple, 2- to 8-mm, dome-shaped, pearly, centrally umbilicated papules that may cluster, become more keratotic with trauma, and simulate condyloma acuminata. The lesion may express a white caseous material (caseum). Transmission is via fomites or sexual contact. Children often have a primary inoculation in the anogenital region, and autoinoculation may lead to additional lesions. Microscopically (Fig. 16.2), a lobular epidermal acanthosis is present and causes an inverted pattern of epidermal hyperplasia. Intracytoplasmic eosinophilic inclusions, referred to as Henderson-Paterson bodies, can be identified in the stratum spinosum and granulosum. The lesions usually regress spontaneously within 1 year, although scarring may occur. Approximately 60% of individuals with skin lesions have antibodies to the virus, but this fraction is less among patients with acquired immunodeficiency syndrome (AIDS), who, along with other immunocompromised patients, may have numerous lesions, some of which may manifest as tumor-like lesions. Ultrastructurally, large, brick-shaped viral particles can be seen.[2,3,29,46-51]

Herpetic Infections

Herpes simplex virus type 2 (HSV-2), a double-stranded linear DNA virus, is the most common organism that leads to sexually transmitted genital herpes; however, HSV-1 has been shown to cause almost half of genital herpes cases in developing countries.[52]



• **Figure 16.2** Molluscum contagiosum. **A,** Medium-power view shows a crateriform lesion with an inverted pattern of epidermal hyperplasia and epidermal acanthosis. **B,** High-power view shows characteristic viral inclusions, the intracytoplasmic eosinophilic inclusions of which are known as Henderson-Paterson bodies.

The incubation period is approximately 5 days, and the virus is harbored for life in sensory ganglia. Vesicles consisting of clear lesions with an erythematous base form the primary lesions, which may rupture and leave behind erosions. The primary lesion may be accompanied by lymphadenopathy, but the lymphadenopathy usually subsides and does not recur. Histologically the lesions start with epidermal cell peripheral nuclear chromatin clumping, ballooning degeneration, and homogeneous ground-glass changes with eosinophilic nuclear inclusions. Cytoplasmic vacuolization may also be present. Eventually, affected cells swell, losing their attachment to and separating from adjacent cells (a process referred to as secondary acantholysis). Cellular cytoplasm becomes homogeneous and intensely eosinophilic, and multinucleated cells (Tzanck cells) may be present. The basal layer of the epidermis may be destroyed, leading to the formation of a subepidermal vesicle. Reticular degeneration, a process whereby the epidermal cells undergo progressive hydropic swelling and cytoplasmic clearing with peripheral cytoplasmic strands remaining, can occur. Eventually, these break, and further vesicle formation occurs. Vesicles may contain neutrophils, and accompanying vasculitis may be present. Late lesions are often ulcerated, with ghosts of acantholytic, multinucleate epithelial cells. Primary infection and viral excretion last approximately 3 weeks. Extragenital lesions occur in approximately 20% of cases, and aseptic meningitis in approximately 10%.[2,3,51]

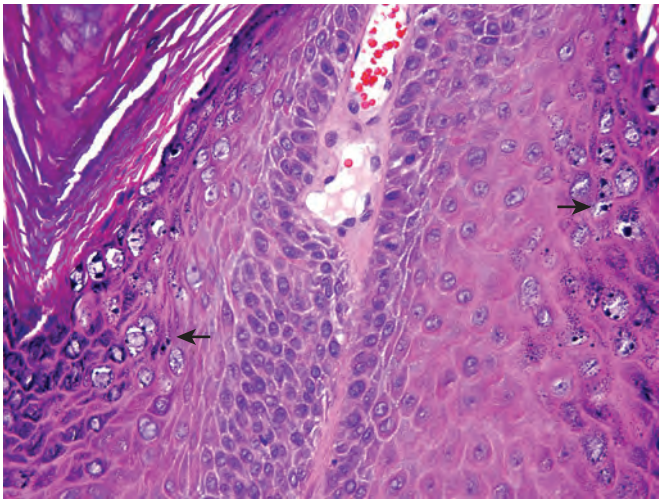
Recurrences are less severe. Burning and tingling may manifest 1 to 2 days before a recurrence. Vesicles are limited in number and heal in approximately 10 days, and virus is shed for only a few days. The glans, prepuce, and shaft are the primary sites of involvement; however, the scrotum may be involved. Perianal lesions may also be present, particularly in men who have sex with men.[3] Chronic HSV disease manifests as painful ulcers with rolled borders. Chronic persistent herpes lesions may suggest and help define the presence of AIDS if they last for longer than 1 month, and they may also occur in other immunosuppressed states (e.g., history of transplantation, chemotherapy, hematologic disorders).[3] The herpes varicella-zoster virus (VZV) may infect the anogenital area in a dermatomal distribution through involvement of the third and fourth sacral sensory nerves.[3,51]

Immunohistochemical stains may be useful in distinguishing HSV from VZV in biopsy specimens. Monoclonal antibodies to the VZV envelope protein gp1 are sensitive and specific in distinguishing between HSV and VZV.[53-56] Cultures and molecular probes for viral antigens can also be useful. Viral antigen can be detected in inflamed nerve twigs. Electron microscopy may reveal the 90- to 130-nm viral particles.[2,3]

Human Papillomavirus

Human papillomavirus (HPV) is a member of the Papovaviridae family, which consists of circular, double-stranded DNA viruses. HPV is involved in a number of pathologic lesions, from the benign end of the spectrum, the common wart (verruca vulgaris) (Fig. 16.3), to frankly malignant squamous cell carcinoma. More than 100 genotypes are known.[2,3,51,57] Relationships between different HPV types and particular lesions have been recognized (Table 16.2).

HPV was first recognized to have oncogenic potential in patients with epidermodysplasia verruciformis. This is an autosomal recessive condition, associated with a gene locus on chromosome 17, that leads to decreased defenses against several HPV subtypes (HPV-2, 3, 5, 10, and 8), resulting in exophytic lesions on the trunk and upper extremities which appear in the first decade of life. These lesions may undergo malignant transformation into squamous cell carcinoma during young adulthood. Unlike common warts caused by HPV, epidermodysplasia verruciformis lesions are not transmissible to healthy individuals. Lesions resembling epidermodysplasia verruciformis are sometimes seen in organ transplant recipients.[57]



• **Figure 16.3** Verruca vulgaris. High-power image shows characteristic keratohyaline granules in a lesion associated with human papillomavirus *(arrows)*.

Condyloma acuminatum is one of the most common sexually transmitted diseases. In males, the frenulum, corona, glans, shaft, and scrotum are affected. Multiple lesions may become confluent, forming a cobblestone appearance. Microscopically, condyloma acuminatum typically has some degree of epidermal hyperplasia or hyperkeratosis, acanthosis, parakeratosis, and numerous koilocytes which cytologically are characterized by perinuclear clearing and wrinkled or crenated ("raisinoid") nuclei[3,51] (Fig. 16.4). Condyloma acuminatum is usually caused by HPV types 6 and 11, and there is little risk of squamous cell carcinoma from infection with these HPV types. HPV types 16 and 18 are the high-risk types, leading to anogenital squamous intraepithelial lesions and squamous cell carcinomas (e.g., penile squamous cell carcinoma, cervical carcinoma; Fig. 16.5). HPV 16 and 18 account for more than 70% of all malignancies in the anogenital region and are present in more than 98% of cases of high-grade dysplasia and squamous cell carcinoma of the cervix. After treatment, latent HPV in perilesional skin may lead to recurrent lesions.[3,57]

Generic intraepithelial neoplasia terminology has been defined by consensus recommendations in the Lower Anogenital Squamous Terminology (LAST) Project, which was cosponsored by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. In this terminology, "-IN" can be used to refer to intraepithelial neoplasia of particular sites. Thus for particular sites the following designations are used: anus = AIN; perianus = PAIN; penis = PeIN; cervix = CIN; and vagina = VaIN; and, for example, the corresponding designation of -IN3 of each of these sites would be AIN 3, PAIN 3, PeIN 3, CIN 3, and VaIN 3, respectively. In this system, low-grade dysplasia is referred to as grade 1, which is also termed low-grade squamous intraepithelial lesion (LSIL); and grade 2 or 3 is considered to precancerous or high-grade dysplasia, which is also

TABLE 16.2 Human Papillomavirus Types and Associated Lesions

Human Papillomavirus Type	Lesion
1	Plantar, common (verruca vulgaris) warts (see Fig. 16.3) and anogenital warts
2	Common warts; also plantar, oral, and anogenital lesions
3	Plane warts (verruca planae) and epidermodysplasia verruciformis
4	Plantar and common warts
5	Epidermodysplasia with subsequent development of carcinoma Psoriatic skin lesions (detected in research setting)
6	Anogenital warts and epidermodysplasia Condyloma acuminatum (other types detected include HPV-2, 6, 11, 16, 18, 31, 33, 35, 39, 41-45, 51, 56, 59, and others) (see Fig. 16.4) Oral and laryngeal squamous papilloma Verrucous carcinoma
7	Warts in meat and fish handlers
8, 9, 10, 12, 14, 17, 19, 22, 24, and others	Epidermodysplasia
10	Plane warts
11	Anogenital lesions (e.g., condyloma acuminatum) Oral and laryngeal squamous papilloma Verrucous carcinoma
16 and 18	Bowenoid papulosis (which sometimes progresses to invasive carcinoma) Anogenital warts Cervical squamous dysplasia and invasive squamous cell carcinoma (types 16, 18, 31, 33, and 35) Cervical AIS and invasive cervical adenocarcinoma (type 18 > type 16) Squamous cell carcinoma in other nongenital sites Verrucous carcinoma
13 and 32	(Oral) Focal epithelial hyperplasia (Heck disease)
57	Plantar epidermoid cysts and nail dystrophy
60	Plantar warts and epidermal cysts
63, 65, 66	Plantar warts
75, 76, 77	Common warts in immunosuppressed patients

AIS, Adenocarcinoma in situ; *HPV*, human papillomavirus.

Data from Weedon D, Strutton G. *Skin Pathology*. 2nd ed. Edinburgh: Churchill Livingstone; 2002; and Mais DD. *Quick Compendium of Clinical Pathology*. 2nd ed. Chicago: American Society of Clinical Pathology Press; 2008.

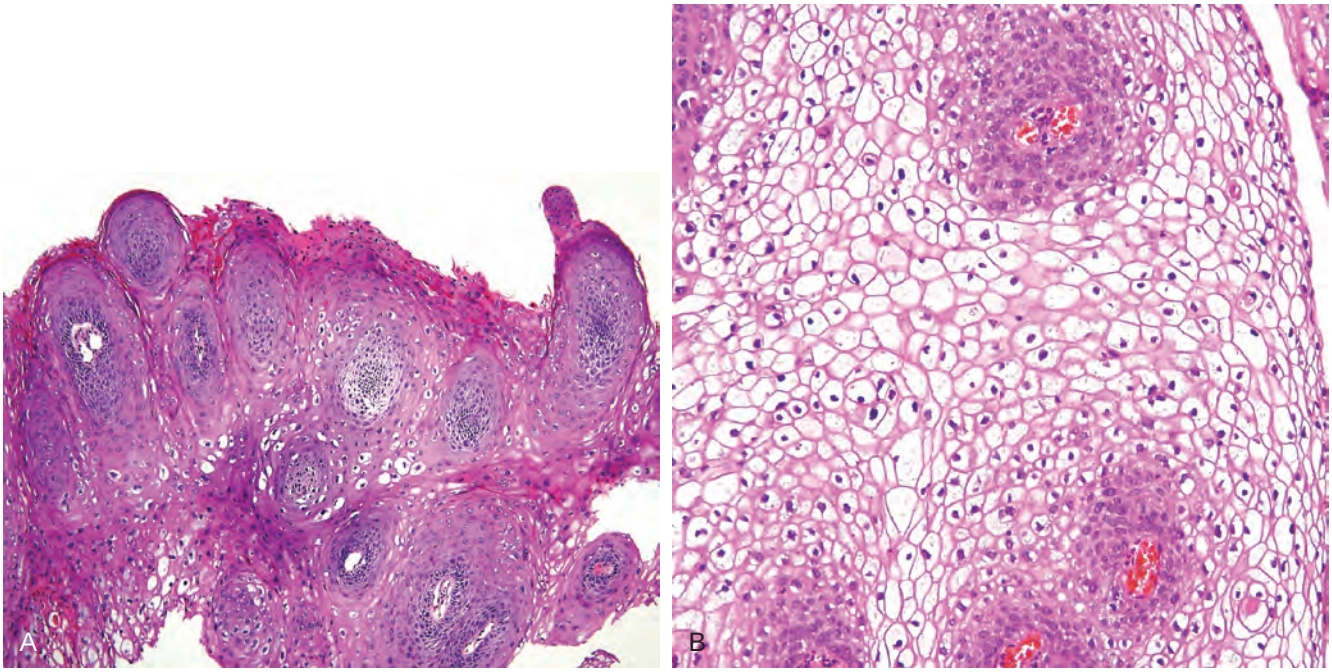
termed a high-grade squamous intraepithelial lesion (HSIL). Thus a three-tiered system formerly used ("-IN1" through "-IN3") was converted to a two-tiered system (LSIL and HSIL) by the LAST Project.[58,59]

Immunohistochemistry for p16 can be used as a surrogate marker of HPV infection and can be used in the assessment of dysplasia. So-called "block" p16 positivity, consisting of strong diffuse p16 staining, is considered indicative of an HSIL. If the H&E morphologic differential diagnosis is between negative for dysplasia and low-grade dysplasia (an intraepithelial lesion grade 1 [-IN1]), then p16 should not be used because -IN1 lesions can be p16-negative. Although p16 can be an aid for the diagnosis of HSIL, p16 positivity in and of itself does not define -IN.[58,59] Ki67 staining has been used in conjunction with p16 staining, and some studies have shown an increased specificity and sensitivity for dysplasia when these stains are used together. Studies have indicated that Ki67 positivity increases with increasing dysplasia.[60,61]

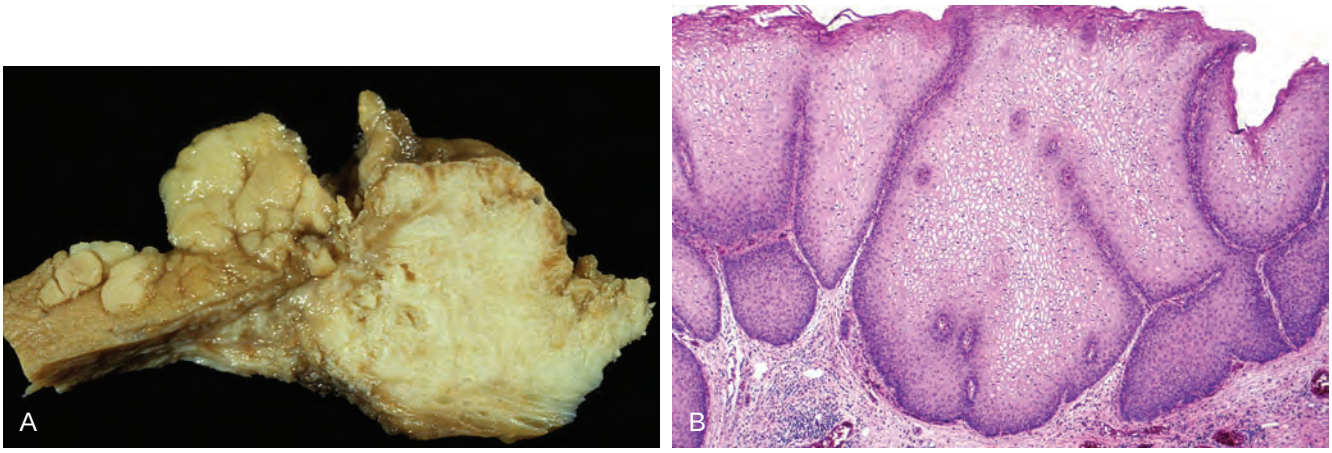
Bowenoid papulosis due to HPV (mostly type 16) manifests on the anogenital skin as multiple small (2 to 3 mm), pearly papules. Histologically, it resembles squamous cell carcinoma in situ (also known as Bowen disease). Bowenoid papulosis rarely results in invasive carcinoma and often spontaneously regresses. It can usually be distinguished clinically from Bowen disease (also known as erythroplasia of Queyrat).[57]

Verrucous carcinoma (giant condyloma of Buschke and Lowenstein) is sometimes associated with HPV. It is a slow-growing, well-differentiated variant of squamous cell carcinoma with an exophytic papillary appearance, typically 1 to 3 cm in diameter, that arises in the skin (usually the genital skin), oral cavity, larynx, and esophagus. It is defined by a broad base between the tumor and the underlying stroma with minimal atypia and mitoses at the base, although focal stromal invasion may be observed; dense chronic inflammation may also be present at this interface. Microscopically, verrucous carcinoma is hyperkeratotic and acanthotic, with orthokeratosis (the presence of keratohyaline granules), parakeratosis, and papillomatosis. Papillae may have fibrovascular cores, and papillae may also have a central keratin plug with peripherally located tumor cells. Occasional vacuolated (nonkoilocytotic) clear cells may be present. Verrucous carcinoma has been associated with HPV-6, 11, 16, and 18, although koilocytosis is not present. Verrucous carcinoma is sometimes distinguished from giant condyloma by the absence of koilocytic change,[29,57] and it has been suggested that the term "warty (condylomatous) squamous cell carcinoma" should be used for exophytic warty tumors of the penis and similar lesions that occur on the vulva and are related to HPV-16. These warty carcinomas are described as having more prominent, long, condylomatous papillae and fibrovascular cores with rounded, irregular, jagged bases, and more obvious koilocytotic atypia. Penile warty carcinoma appears to have a better prognosis than typical squamous cell carcinoma.[2,62-64]

Both in situ hybridization and immunohistochemistry are available for the detection of HPV in formalin-fixed tissue. In situ hybridization, which is typically considered to be more sensitive, has largely replaced immunohistochemistry for this purpose (except for specific diagnostic purposes in the cervix, discussed later). Immunohistochemistry detects only productive and not latent infections, and it cannot be used to determine the type of virus present, whereas other molecular techniques are useful for some of these purposes.[56,65-68]



• **Figure 16.4** Condyloma. **A,** Low-power view shows hyperkeratosis, parakeratosis, acanthosis, and koilocytes with cytoplasmic clearing. **B,** High-power view shows cytoplasmic clearing, primarily in a perinuclear location, which is characteristic of the koilocytes that make up a large proportion of cells in condylomas.



• **Figure 16.5** Bladder condyloma. **A,** Bladder condyloma with urothelial hyperplasia and koilocytic change. **B,** Squamous cell carcinoma showing an exophytic growth pattern, arising in bladder condyloma. (Courtesy Dr. Robert H. Young.)

Parasitic Infections

The perineal skin can be affected by *Entamoeba histolytica* when infection extends from the anus in a patient with *E. histolytica* colitis. This can result from spread by anal intercourse and may also involve the penis. Clinically, ulcerated, painful nodules with serpiginous borders result. An amebic ulcer may present as balanoposthitis resistant to antibiotics.[3,69]

Wuchereria bancrofti, *Brugia malayi*, and *Brugia timori* are filarial worms that can involve lymphatic vessels, resulting in obstruction, chronic lymphedema leading to elephantiasis, and chronic inflammation. Up to 25% of the male population are affected in tropical areas. The scrotum is often swollen, erythematous, and tender. Histologically, there may be thickening of the scrotal skin and verruciform epidermal hyperplasia. Vulvar elephantiasis may also occur from lymphatic obstruction by filariform worms.[3,4,70]

Schistosomiasis

Schistosoma mansoni is a fluke that can colonize pelvic veins. Adult organisms within abdominopelvic veins can involve surrounding visceral or cutaneous tissue with a granulomatous reaction. Eggs are typically conducted to the bladder lumen and are excreted during micturition. Sinuses, fistulas, and masses may

be present. Ulcers and papillary nodules may simulate condyloma acuminata.[3,4,71]

Scabies and Lice

Scabies (caused by the mite *Sarcoptes scabiei*) can form tunnels under the stratum corneum of the penis, scrotum, buttock, and waist. Immunocompromised patients may have crusted lesions (known as Norwegian scabies). Lice may also infect the genital region.[3,4,29]

Kidney Infections

Acute Interstitial Nephritis

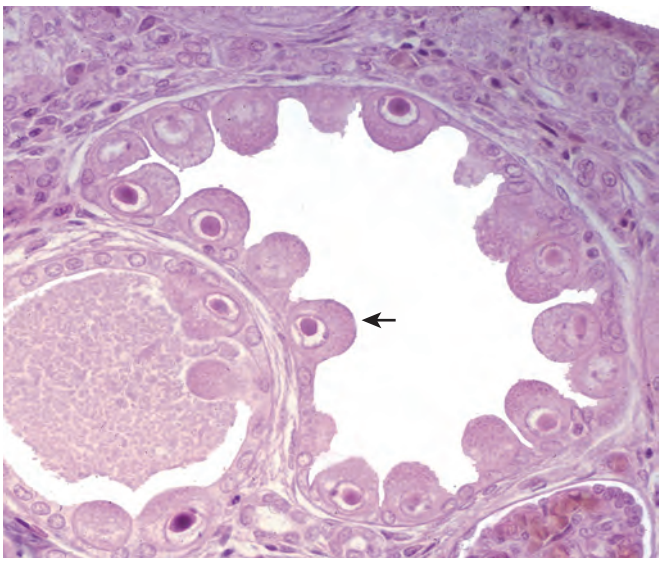
Acute interstitial nephritis was first thoroughly defined by Councilman in 1898.[72] Before the advent of widespread antibiotic use, acute interstitial nephritis was most commonly caused by bacterial infections, such as scarlet fever (scarletina, caused by *Streptococcus* spp.) or diphtheria. Currently, acute interstitial nephritis is most commonly observed in the setting of an allergic reaction (such as to a drug[73]) or acute transplant rejection; however, bacteria and viruses are still important causes of acute interstitial nephritis.[74-78] Councilman was astute in observing that many cases of acute interstitial nephritis do not arise as a direct infection by organisms filling the kidney but rather from an indirect influence in which inflammatory cells are attracted to the kidney by, as he surmised, "soluble substances that may exert a positive chemotaxis for them."[72,78]

Grossly the kidneys are enlarged, soft, and pale due to edema. Microscopically, edema expands the interstitium, and there is usually a mixed inflammatory cell infiltrate composed primarily of lymphocytes and monocytes with a smaller number of plasma cells and eosinophils. Granulomas may be present in rejection, allergic reactions, or sarcoidosis, in which they are usually noncaseating.[79] Tubulitis, a lymphocytic infiltration of tubular epithelial cells, and vasculitis may be present.[74,77,78]

Bacterial organisms, particularly *Streptococcus*, were formerly the common causes of acute interstitial nephritis. Glomerulonephritis was formerly rare compared with interstitial nephritis in streptococcal infections. However, interstitial nephritis due to *Streptococcus* declined around the beginning of the 20th century. Specific bacteria involving the kidney are discussed in the following paragraphs. In general, acute interstitial nephritis due to bacterial organisms contains a mixed infiltrate of neutrophils, plasma cells, and eosinophils amid interstitial edema and tubular injury.[74-78]

Cytomegalovirus (CMV), adenovirus, and polyomavirus produce characteristic intranuclear viral inclusions in tubular epithelial cells and occasionally in the endothelium and glomerular epithelium. These infections are particularly important offenders in transplant patients.[74,77,78,80] Adenovirus may produce smudgy intranuclear inclusions that may have a glassy appearance. Intranuclear inclusions may have a distinct halo surrounded by a ring of marginated chromatin (Cowdry A inclusion). Necrotizing tubulointerstitial nephritis due to adenovirus has been reported, notably in patients who are immunosuppressed. Mass-forming lesions and granulomatous inflammation have also been reported. Immunofluorescence or immunohistochemistry for viral antigens may aid the diagnosis.[74,77,81-85]

CMV produces large eosinophilic intranuclear inclusions with halos, most often in proximal tubules but also in the loops of Henle and collecting ducts. Microscopically, interstitial inflammation is typically observed to be composed of lymphocytes, plasma cells, and macrophages. CMV-infected renal tubular cells or stromal cells may show enlargement and a single eosinophilic nuclear inclusion surrounded by a clear halo (Fig. 16.6). CMV acute interstitial nephritis can occur as a congenital infection during the neonatal period. Another setting for CMV acute interstitial nephritis is in renal allografts. CMV may result in impairment of renal graft function and glomerular and interstitial injury. Immunohistochemistry for viral antigens may help to solidify the diagnosis.[77,78,86-88]



• **Figure 16.6** Cytomegalovirus acute interstitial nephritis. Characteristic eosinophilic intranuclear inclusions surrounded by a clear halo can be seen in enlarged renal tubular epithelial cells *(arrow)*.

Epstein-Barr virus (EBV), the cause of infectious mononucleosis, may also produce an acute interstitial nephritis. In EBV acute interstitial nephritis, there is usually a prominent lymphocytic infiltrate with plasma cells and macrophages producing tubular injury and necrosis. Lymphocytes may be atypical and are usually $CD8^+$. Granulomas may be present. It has been argued that EBV acute interstitial nephritis is simply an immunologic phenomenon. In situ hybridization for EBV RNA and immunohistochemistry findings may be negative; however, EBV DNA has been identified on PCR studies in biopsy material involved by EBV-associated acute interstitial nephritis.[78,89]

Other infectious agents that may cause acute interstitial nephritis include viruses, such as HSV, rubeola virus (measles), and human immunodeficiency virus (HIV); bacteria, including *Brucella* spp., *Legionella*, *Mycoplasma*, and *Yersinia*; and parasites, including *Toxoplasma gondii* and *Leishmania* (acute visceral leishmaniasis or kala-azar). Only rare cases have been reported of HSV and measles leading to acute interstitial nephritis. *Brucella* may produce a caseating granulomatous infiltrate with neutrophils, lymphocytes, and interstitial edema. *Legionella* often has an infiltrate rich in lymphocytes, plasma cells, macrophages, and sometimes even neutrophils. *T. gondii* can lead to an acute interstitial nephritis rich in lymphocytes and neutrophils. Acute visceral leishmaniasis results in an inflammatory infiltrate of plasma cells and lymphocytes without eosinophils. The renal parenchyma is spared. Patients with HIV infection (AIDS) can have a severe interstitial nephritis composed of an infiltrate rich in lymphocytes

and plasma cells. AIDS also predisposes individuals to infections with a number of organisms such as *Pneumocystis*.[78]

Certain disorders are in the differential diagnosis of granulomatous interstitial nephritis and other granulomatous diseases of the kidney; they include xanthogranulomatous pyelonephritis, malakoplakia, brucellosis, mycobacterial infection, fungal infection, parasitic infection, urate nephropathy, oxalosis, sarcoidosis, vasculitis, drug hypersensitivity, tubulointerstitial nephritis with uveitis syndrome (TINU), granulomatous vasculitis (i.e., Wegener granulomatosis, or the recently preferred term *granulomatosis with polyangiitis* [GPA]), and cholesterol granulomas. Many infectious agents that may produce a granulomatous interstitial nephritis are listed in Table 16.3. Ultimately, many cases are idiopathic.[90,91]

Immune complex deposits may be seen on immunofluorescence in tubular basement membranes or peritubular capillaries in cases of interstitial nephritis, but this is a nonspecific finding that merely indicates that an immunologic reaction has taken place. Immunoperoxidase stains may be useful to confirm the presence of adenovirus, CMV, or BK polyomavirus. However, in many cases of acute interstitial nephritis, a specific etiology cannot be determined.[74,77,78]

Acute Pyelonephritis

Acute pyelonephritis occurs as a result of infection of the kidney, usually from bacteria.[74,77] Most pediatric cases occur in boys, and most adult cases occur in women. Infections can be ascending, from lower urinary tract infection, or hematogenous, from sepsis or endocarditis.[74,77,92,93]

Lower urinary tract infections that can involve the kidney include *E. coli*, followed by *Proteus*, *Klebsiella*, and *Enterobacter*. Vesicoureteral reflux in cases of bacterial cystitis can lead to pyelonephritis. Congenital predisposition toward vesicoureteral reflux leads to more frequent cases of pyelonephritis.[74] Incompetence of the vesicoureteric junction causes congenital (primary) reflux.[77] Some cases of congenital obstruction are familial. Reflux may also result from neurogenic bladder, cystitis, or other causes of distal obstruction. Intrarenal reflux leads to infection of the kidney.[74,77,92,93]

Hematogenous infections involve organisms such as *S. aureus* and are usually composed of numerous small abscesses scattered throughout the parenchyma, particularly the cortex; this is sometimes termed multiple cortical abscesses, diffuse suppurative nephritis, or diffuse bacterial nephritis. This process is sometimes considered distinct from acute pyelonephritis.[77] Prolonged infectious endocarditis or sepsis is a common cause of hematogenous pyelonephritis, and gram-positive bacteria and fungi are the most common culprits in these instances. *Pyonephrosis* is the term used for near-total destruction of an obstructed kidney by acute pyelonephritis. *Pyonephrosis* or renal abscesses may lead to perinephric abscesses.[74,77,92,93]

Clinically the patients are often febrile with flank pain and have leukocytosis. Urinalysis usually shows pyuria and white blood cell casts. Treatment is usually medical with antibiotic therapy, although surgical intervention with either débridement or nephrectomy may be required in severe cases.[74,77,92,93]

Grossly the kidneys are often yellow-white with suppurative foci and sometimes frank abscesses, particularly in ascending acute pyelonephritis. The abscesses may be smaller and scattered, with a concentration in the cortex, in the hematogenous form.[74,77] Microscopically, there are numerous neutrophils in the tubules and interstitium. Neutrophils and bacteria can be seen in the tubules and collecting ducts of the cortex and medulla (Fig. 16.7). Glomeruli are affected only in severe cases in which there is extensive parenchymal destruction with abscess formation. Vascular thromboses, ischemic necrosis, and cyst formation may be seen.[74,77,92,93]

TABLE 16.3 Differential Diagnosis and Pharmacotherapy of Interstitial Nephritis*

Causes	Selected Drugs
Viral infection	Antibiotics
CMV	β-Lactams
EBV	Amoxicillin
Polyomavirus (e.g., BK virus)	Ampicillin
HIV	Nafcillin
Hantavirus	Oxacillin
Bacterial infection	Penicillin G
Scarlet fever *(Streptococcus)*	Cephalexin
Diphtheria	Cefotaxime
Typhoid fever	Others
Brucellosis	Chloramphenicol
Leptospira	Ethambutol
Rickettsia	Gentamicin
Fungal infection	Rifampin
Histoplasma	Sulfonamides
Parasitic infection	Tetracyclines
Leishmania	Diuretics
Toxoplasma gondii	NSAIDs
Ascaris	Allopurinol
Systemic disorders	Rifampicin
Connective tissue disorders	Cimetidine
Systemic lupus erythematosus	Sulfa drugs
Sjögren syndrome	Phenytoin
Sarcoidosis	
Transplant rejection	
Irradiation	
Ischemia	
Protein accumulation (e.g., amyloid, light chain, myeloma)	
Hereditary (e.g., Alport syndrome, medullary cystic disease, nephrophthisis, familial interstitial nephritis)	
Metals (e.g., cadmium, lithium, gold)	
Metabolic disorders (e.g., oxalosis, hypercalcemia, gout, cystinosis)	
Toxins (e.g., snake bite, CCl_4, mushroom)	
Anti-tubular basement membrane disease	
Tubulointerstitial nephritis-uveitis syndrome (Dobrin syndrome)	
Idiopathic	

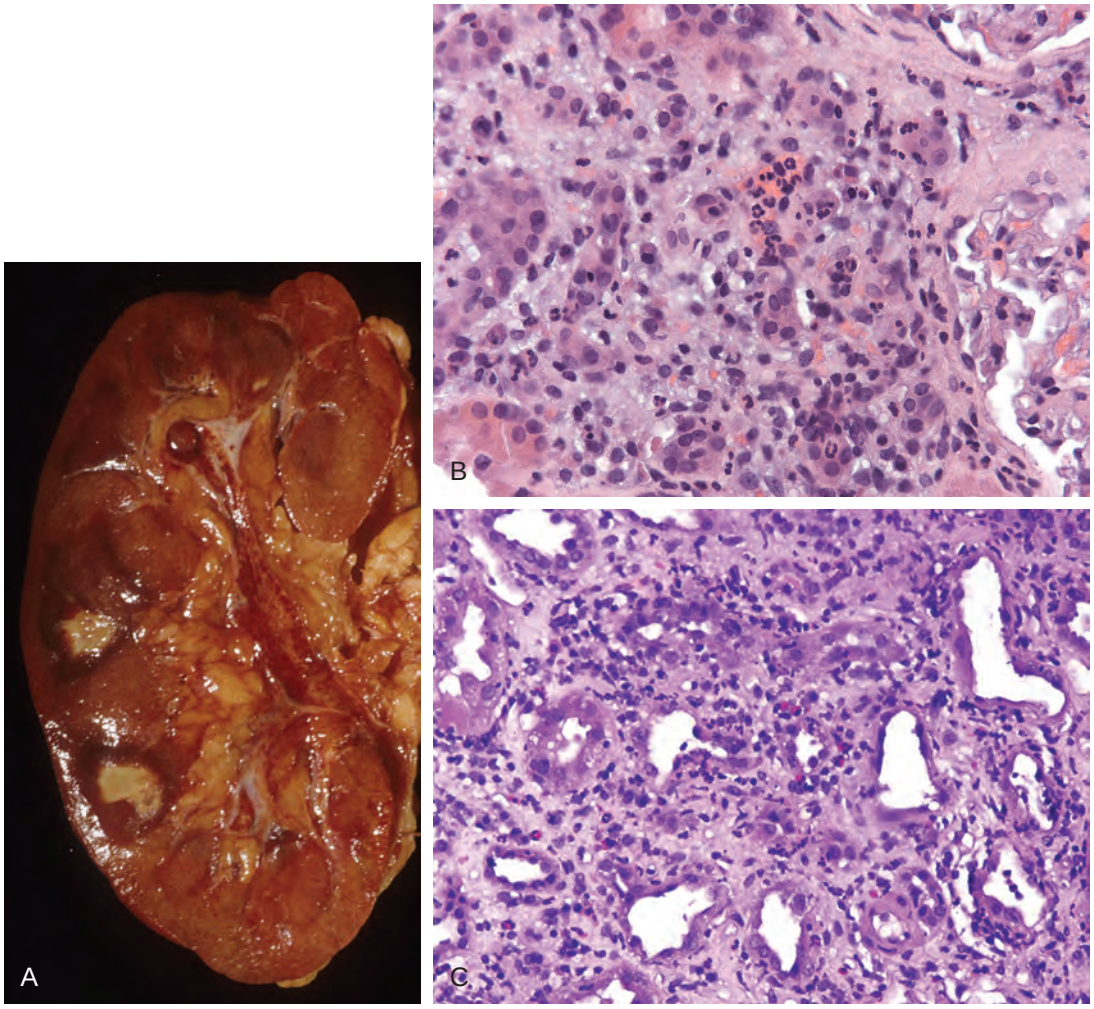
*Organisms, agents, and disorders listed include entities that affect the kidney directly (e.g., through direct infection) or indirectly (e.g., through an immunologic reaction or other reactive state).

CMV, Cytomegalovirus; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus; *NSAIDs,* nonsteroidal antiinflammatory drugs.

Data from Colvin RB, Fang LST. Interstitial nephritis. In: Tisher CC, Brenner BM, eds. *Renal Pathology with Clincial and Functional Correlations.* Vol 2. Philadelphia: J.B. Lippincott Company; 1994; and Bonsib SM. Non-neoplastic disease of the kidney. In: Bostwick DG, Eble JN, eds. *Urologic Surgical Pathology.* St. Louis: Mosby; 1997.

Focal Bacterial Nephritis (Lobar Nephronia)

Acute focal bacterial nephritis (lobar nephronia) is a rare form of renal infection. It typically involves the renal cortex and may



• **Figure 16.7** Acute pyelonephritis. **A,** Nephrectomy specimen grossly shows acute pyelonephritis with abscess formation. **B,** Acute pyelonephritis with abundant neutrophils invading tubules and filling the interstitium adjacent to a relatively normal-appearing glomerulus. **C,** Acute pyelonephritis with a mixed inflammatory infiltrate in the interstitium composed of lymphocytes, neutrophils, and abundant eosinophils. Tubulitis is multifocal.

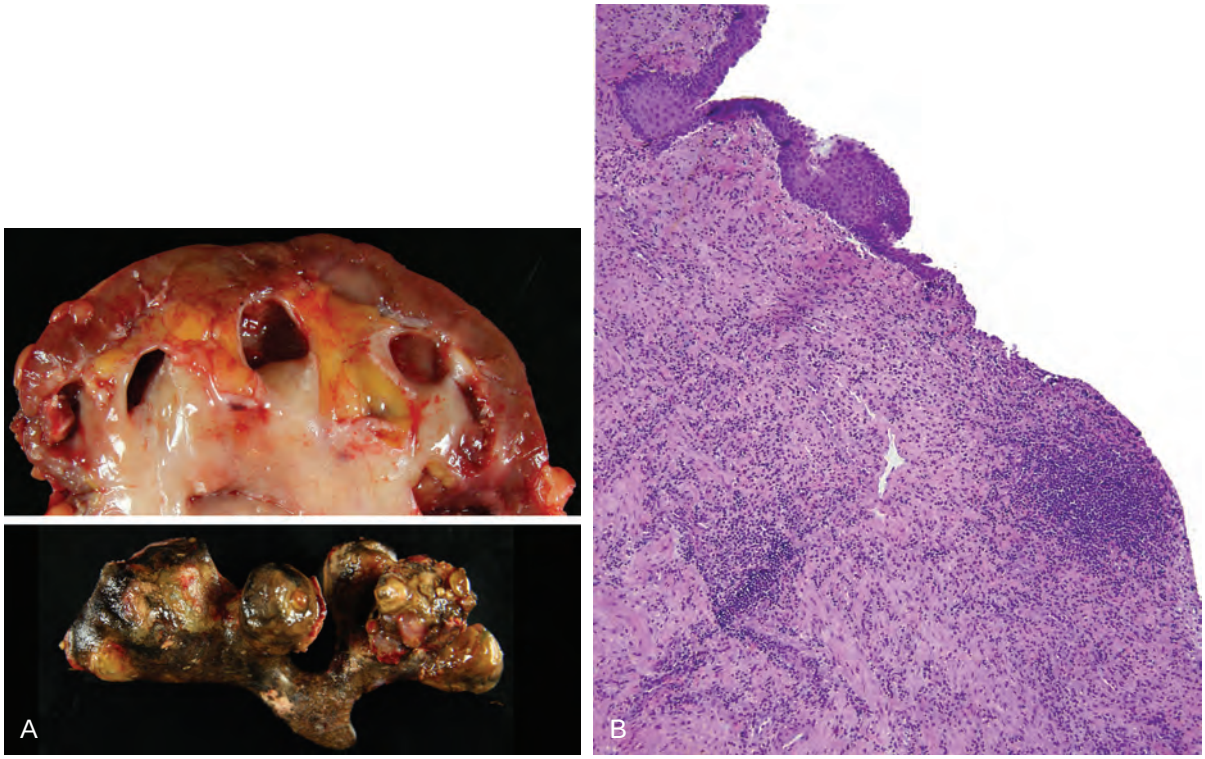
manifest as a renal mass and be mistaken for a neoplasm, even after cytologic examination. It is thought to be the early stage of an intrarenal abscess. Clinically, flank pain, fever, leukocytosis, pyuria, and bacteriuria may be present; all of these features may also be present in renal abscesses or acute pyelonephritis. Radiologic studies may detect lobar nephronia as focal imaging abnormalities. Microscopically, microabscesses with foci of hemorrhage and necrosis can be appreciated. *E. coli* is thought to be the most common causative organism, but other organisms have also been implicated, including *S. aureus* and *Klebsiella pneumoniae*. Lobar nephronia has been reported in renal allografts.[94-98]

Emphysematous Pyelonephritis

Emphysematous pyelonephritis is an uncommon, life-threatening condition in which bubble-like collections of gas develop in the renal parenchyma, particularly in areas of coagulative necrosis, and extend into the perinephric space or retroperitoneum or both. The emphysematous foci may be cystic and multiple. Approximately 90% of the patients have diabetes mellitus, and signs of diabetic glomerulosclerosis may be present. Urinary tract obstruction is present in approximately 40% of patients.[74,77,99,100] Cortical and papillary necrosis and vascular thromboses may result.[77] *E. coli* is the most common organism (approximately 68% of the cases), and *Klebsiella* is present in approximately 9% of patients. Mixed infections are detected in 19% of patients.[74] The infectious route is usually thought to be hematogenous, and gas-forming bacteria, high tissue glucose levels, decreased tissue perfusion, and an inadequate immune response are the four factors that are considered important in the development of emphysematous pyelonephritis.[77,101] Emphysematous pyelonephritis has also been described in the parenchyma of tumors.[102] Surgical intervention is ultimately required because antibiotics are usually not effective.[74,77]

Chronic Pyelonephritis

Chronic pyelonephritis is a chronic tubulointerstitial disease caused by bacterial infection, vesicoureteral reflux (also termed chronic nonobstructive pyelonephritis), or urinary tract obstruction (also termed obstructive pyelonephritis). It is responsible for 5% to 15% of cases of end-stage renal disease. Other causes of chronic interstitial nephritis include exogenous agents, such



• **Figure 16.8** Chronic pyelonephritis. **A,** Gross specimen that microscopically exhibited chronic pyelonephritis. The chronic pyelonephritis in this case was caused, at least in part, by a large staghorn calculus, which caused the dilated renal pelvis and calyces. **B,** Chronic pyelonephritis characterized microscopically by numerous mononuclear lymphocytes singly and in aggregates in the interstitium and underneath the urothelium.

as drugs, including analgesics (nonsteroidal antiinflammatory drugs), chemotherapeutic agents (cisplatinum, methyl CCNU), cyclosporine, and lithium, and heavy metals, such as lead and cadmium.[74,77,90,93]

Patients with reflux nephropathy often present with flank pain but may be asymptomatic. Uremia, polyuria, and polydipsia may result.[74,77,93]

Grossly, diffuse scarring may be present in reflux nephropathy. Microscopically, there may be tubular atrophy, interstitial fibrosis, chronic inflammation (Fig. 16.8), and glomerulosclerosis that may manifest in a focal segmental glomerulosclerosis (FSGS) pattern.[74,77,93]

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is a chronic inflammatory condition that is caused by pyogenic infection of the kidney, usually secondary to staghorn calculi (70% of cases), most commonly by *Proteus* or *E. coli*.[74] The process usually begins with suppurative inflammation that may extend into the pelvic mucosa and sinus fat with edema and fat necrosis. Eventually, the cortex, retroperitoneal tissue, and perinephric fat may be involved.[74,77,93]

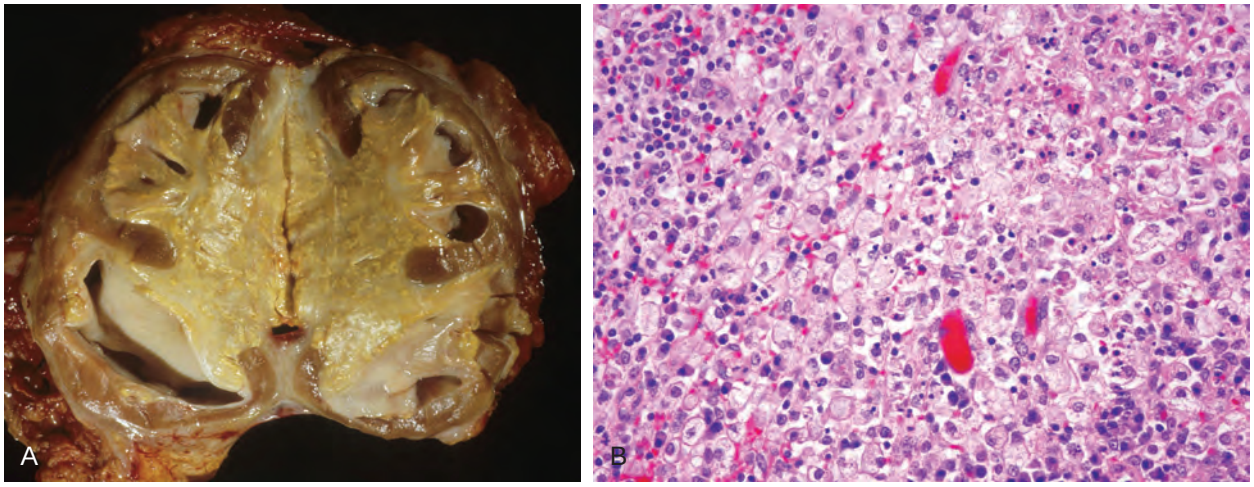
Approximately 70% of the patients are women who present clinically with fever, flank pain, and pyuria. Imaging studies demonstrate a lipid-rich mass, and calculi may be seen. The affected kidney may be completely destroyed; eventually, a nephrectomy may be required.[74,77,93]

Grossly the renal pelvis is dilated, with thickened walls containing a staghorn calculus, and there may be a yellow, tumor-like nodular mass replacing the renal pyramids. A diffuse form with widespread involvement of the renal parenchyma is most common; however, a segmental form, often involving a pole of the kidney, may be present, particularly in children. A focal form, also referred to as the tumefactive form, lacks communication with the pelvis and is not associated with pyelitis, calculi, or urinary tract obstruction. Nephrectomy is often required.[74,77,93]

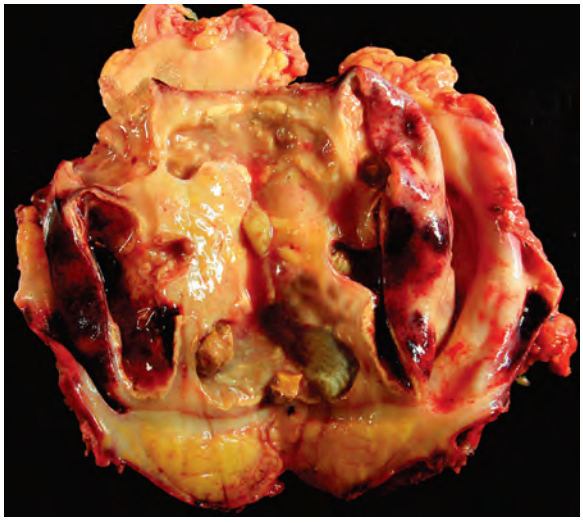
Microscopically, there is a central nidus of necrotic debris and neutrophils surrounded by a zone of foamy macrophages (Fig. 16.9). Peripherally there is a fibroblastic proliferation. Immunohistochemical stains for CD68 may be used to demonstrate the prominent population of macrophages. The reactive fibrous tissue is vimentin-positive and keratin-negative. This is helpful in ruling out carcinoma, which can mimic xanthogranulomatous pyelonephritis histologically, because the foamy macrophages can look like clear cell renal cell carcinoma. The presence of mitoses and atypia favors a diagnosis of carcinoma. The spindled areas often raise the possibility of a spindle-cell neoplasm. Xanthogranulomatous pyelonephritis also needs to be distinguished from inflammatory malignant tumors such as "malignant fibrous histiocytoma," which may contain numerous acute inflammatory cells in addition to scattered atypical neoplastic cells.[74,77,93]

Malakoplakia

Malakoplakia is an inflammatory disorder that occurs in the urinary tract[103-106] and in a number of other organs, including the prostate,[107] testis,[108] vagina, gastrointestinal tract (primarily the large intestine),[109] lung, bone, brain, and skin.[74,77] Early descriptions were by von Hansemann[110] and by Michaelis and Gutmann.[111] It is rather uncommon, and the bladder is involved



• **Figure 16.9** Xanthogranulomatous pyelonephritis. **A,** Gross specimen of a kidney with xanthogranulomatous pyelonephritis showing yellow areas in the renal parenchyma. **B,** Microscopic appearance showing abundant foamy macrophages in a case of xanthogranulomatous pyelonephritis.



• **Figure 16.10** Renal malakoplakia with yellow areas appreciated grossly that histologically were malakoplakia.

4 to 10 times more commonly than the upper urinary tract; renal involvement is relatively rare. *E. coli* is the most common agent. It is thought that megalocytic interstitial nephritis (MIN) is a precursor lesion (see later discussion).[74,77,112]

Clinically, patients have fever, flank pain, and renal dysfunction, eventually leading to renal failure in most cases of renal involvement.[74,77,113] Females are affected more commonly than males (4:1 for malakoplakia in general,[114] 3:1 for the renal form[115]). Patients from childhood to older adulthood are affected,[115,116] and involvement is bilateral in up to 50% of patients.[74,77,117]

Macroscopically the kidneys show dilated pelves and calyces due to the obstructive process. The mucosa of the bladder and, less commonly, the ureter and renal pelvis shows yellow-brown, soft *(malakos)* plaques *(plakos)*, often with a central umbilication (Fig. 16.10). Similar nodules may be present in the parenchyma. Microscopically, there are large masses of eosinophilic histiocytes in the interstitium (von Hansemann histiocytes), many of which contain granular eosinophilic cytoplasm and basophilic inclusions (Michaelis-Gutmann bodies), which can be highlighted with PAS, iron, or calcium stains. The histologic appearance is similar in the testis (Fig. 16.11). Renal tubules may be destroyed.[74,77]

Megalocytic Interstitial Nephritis

MIN is similar to malakoplakia characterized by a prominent histiocytic infiltrate, often leading to tumor formation. Unlike malakoplakia, MIN does not contain Michaelis-Gutmann bodies (Fig. 16.12).[74,77]

Grossly, MIN may be solitary, sometimes forming a cortical nodule, and tumorlike. Alternatively, MIN may consist of multiple foci, comprising numerous gray nodules. Microscopically, there are large numbers of polygonal cells with coarse granular eosinophilic cytoplasm containing PAS-positive material. Some contend that MIN is simply malakoplakia that cannot be strictly diagnosed as such, possibly because it is an early stage of malakoplakia.[74,77,118]

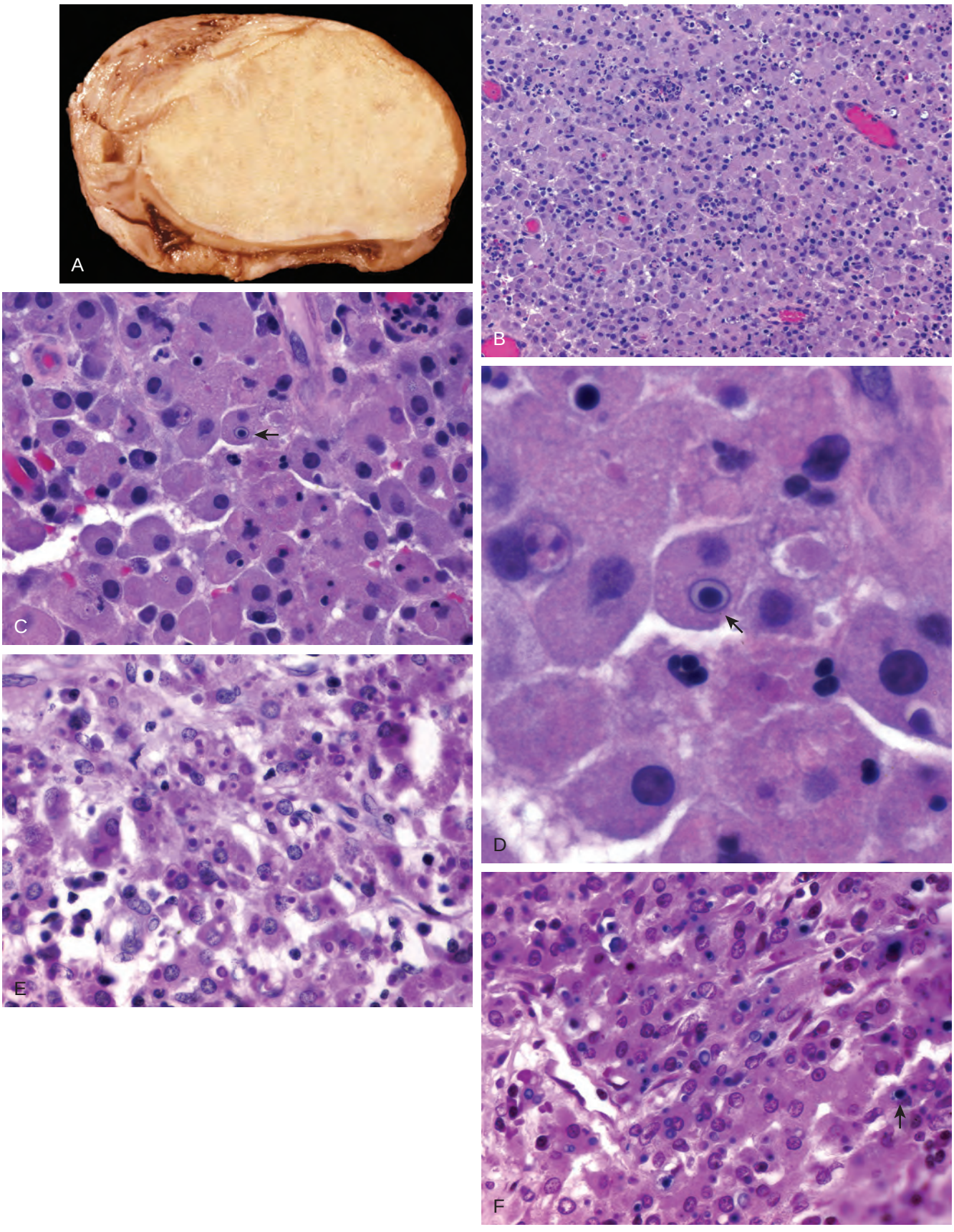
Transplant-Related Renal Infections

A number of infections occur in the posttransplantation setting. These include a variety of bacterial, viral, and fungal infections that are typically seen in any postsurgical patient or in immunocompromised patients. The most characteristic infections are BK virus nephropathy and posttransplantation lymphoproliferative disorder (PTLD) (Table 16.4).[119] As noted previously, CMV and adenovirus are infectious agents that can be seen in renal transplants.

BK Nephropathy

BK polyomavirus, a member of the Papovaviridae family composed of double-stranded circular DNA viruses, causes nucleomegaly, pale intranuclear inclusions, and karyolysis. It was first observed in 1971 in a Sudanese renal transplant recipient who had ureteral obstruction secondary to a virally induced distal ureteral mass; the name *BK* came from the initials of that first patient.[51,77,120-131]

The virus is small and nonenveloped, with structural similarities to simian virus 40 (SV40). BK virus is a normal inhabitant



• **Figure 16.11** Malakoplakia of the testis. **A,** Gross appearance showing diffuse involvement of the testis and replacement of the parenchyma by yellow, relatively homogeneous, soft tissue. **B,** Low-power view shows numerous histiocytes. **C,** At medium power, Michaelis-Gutmann bodies can be appreciated *(arrow)*. **D,** High-power view shows a Michaelis-Gutmann body with concentric lamellations *(arrow)*. **E,** Macrophages stain periodic acid–Schiff positive, at least in part because of abundant intracellular glycogen. **F,** Iron stain highlights the Michaelis-Gutmann bodies *(arrow)*.

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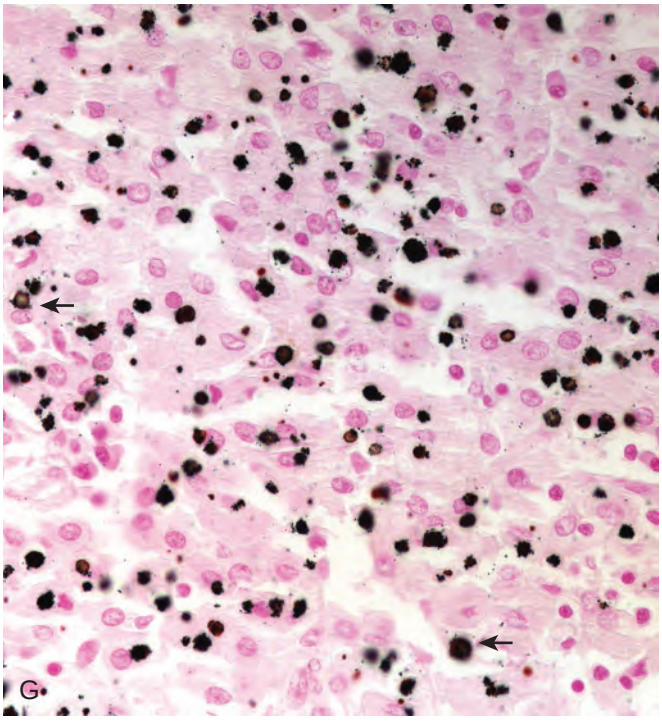
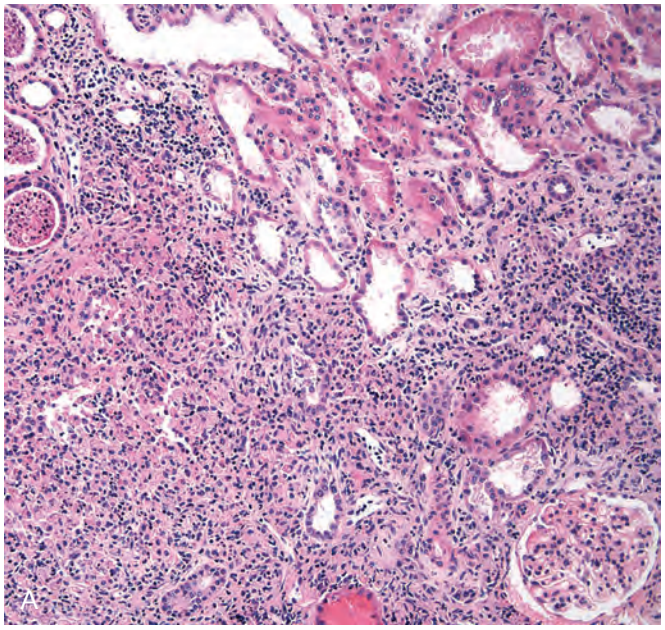


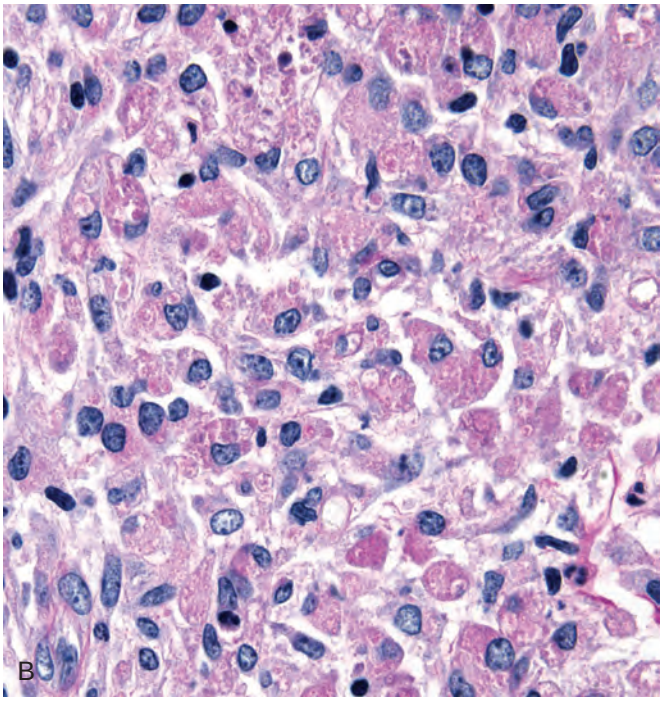
• **Figure 16.11, cont'd** **G,** von Kossa stain, which is a stain for phosphate salts (including notably calcium salts), also stains the Michaelis-Gutmann bodies *(arrows)*. (Courtesy Dr. Robert H. Young.)

TABLE 16.4 Typical Time Course of Selected Common Infections After Transplantation

Interval (Months)	Infection
<1	Bacteria (MRSA, VRE, *Clostridium difficile*, *Pseudomonas*), fungi (*Candida*, *Aspergillus*) Wound infections and other common infections Donor-derived infections: HSV, LCMV, rhabdovirus (rabies), WNV, HIV, *Trypanosoma cruzi*
1–6	BK nephropathy, *C. difficile*, HCV, adenovirus, influenza, *Cryptococcus*, *Mycobacterium tuberculosis*
>6	Community-acquired pneumonia and other bacterial and viral infections, fungi (e.g., *Aspergillus*), CMV, HBV, HCV, BK nephropathy, PTLD

CMV, Cytomegalovirus; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HSV,* herpes simplex virus; *LCMV,* lymphocytic choriomeningitis virus; *MRSA,* methicillin-resistant *Staphylococcus aureus*; *PTLD,* posttransplantation lymphoproliferative disorder; *VRE,* vancomycin-resistant enterococci; *WNV,* West Nile virus.
Data from Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med.* 2007;357:2601-2614.





• **Figure 16.12** Megalocytic interstitial nephritis. **A,** Low-power view of renal parenchyma shows a dense inflammatory infiltrate in the tubulointerstitium composed of mononuclear cells including lymphocytes and macrophages and abundant eosinophilic cytoplasm with tubular atrophy, necroinflammatory tubular debris, and a relatively preserved glomerulus (hematoxylin and eosin stain). **B,** High-power view of a periodic acid–Schiff (PAS)-stained specimen shows abundant polygonal macrophages with PAS-positive cytoplasmic material; some have convoluted nuclei with prominent nucleoli and a few admixed neutrophils. Some consider this entity to be in the spectrum of malakoplakia. In contrast to malakoplakia, megalocytic interstitial nephritis does not produce Michaelis-Gutmann bodies.

of urothelium but rarely causes morbidity in immunocompetent individuals. Polyomavirus typically has striking intranuclear viral inclusions in a background of interstitial nephritis and tubular injury (Fig. 16.13). The intranuclear viral inclusions have been classified into four types: type 1 has classic basophilic inclusions; type 2 is eosinophilic and granular with an incomplete halo; type 3 is finely granular without a halo; and type 4 has markedly enlarged nuclei with clumped irregular chromatin and prominent nucleoli.[51,77,120-132]

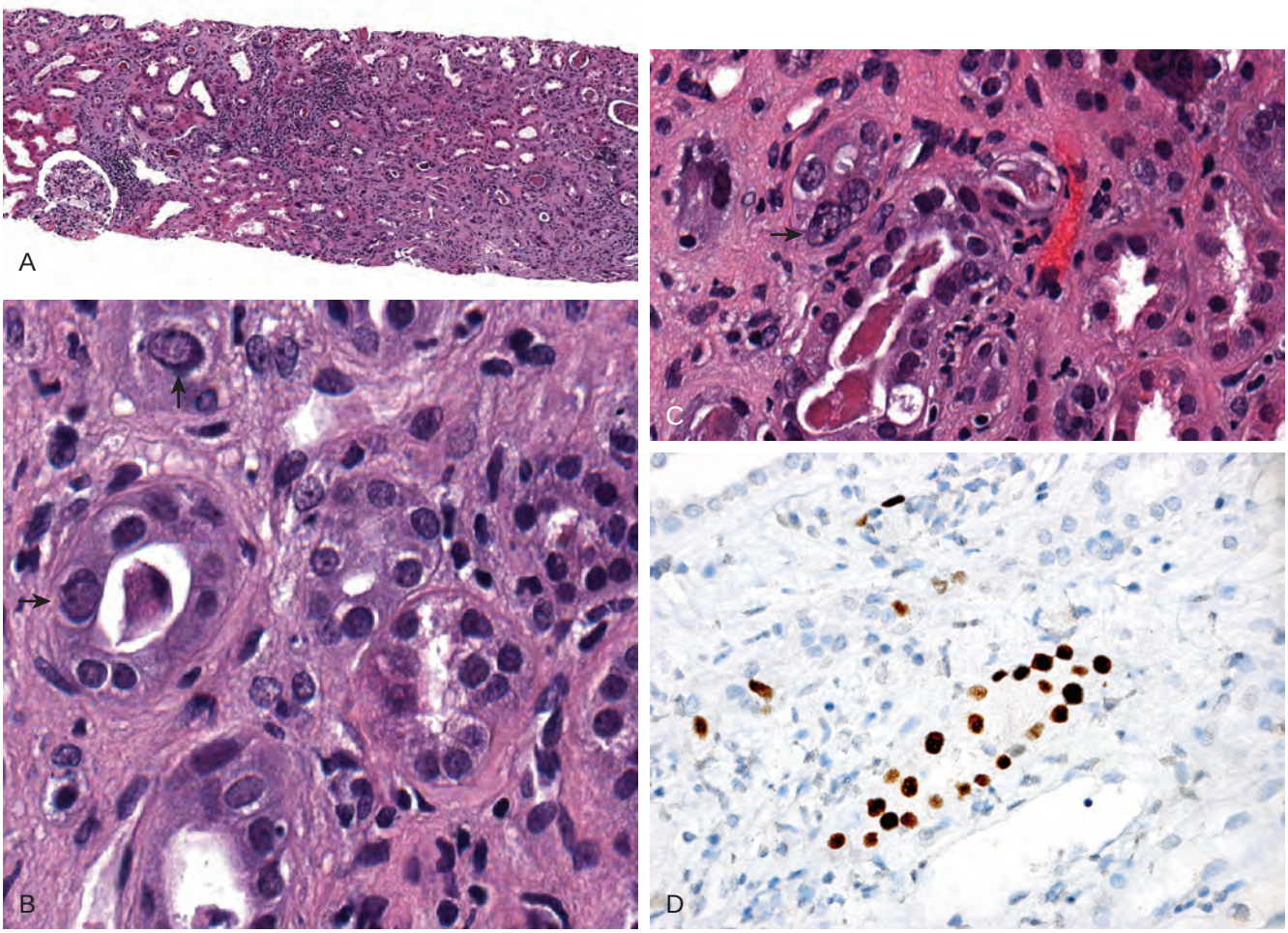
In addition to the viral cytopathic effect, which is most notably observed in renal tubular epithelial cells, there is usually prominent tubulointerstitial nephritis, composed primarily of lymphocytes. Active polyomavirus can be detected in approximately 65% of renal transplant recipients through careful microscopic examination or urinary cytology (which shows the characteristic "decoy cells" that display inclusions and mimic urothelial carcinoma in situ); however, only approximately 26% of patients show functional renal impairment.[132,133]

Immunohistochemistry, which typically uses antibodies to the large T antigen of SV40, has proved to be useful in demonstrating virally infected cells, although the SV40 antibody can cross-react with the large T antigen of BK and JC viruses. The virus can also be seen ultrastructurally. Plasma viral loads may be useful in the diagnosis of BK nephropathy.[56,77,119,132,134-138]

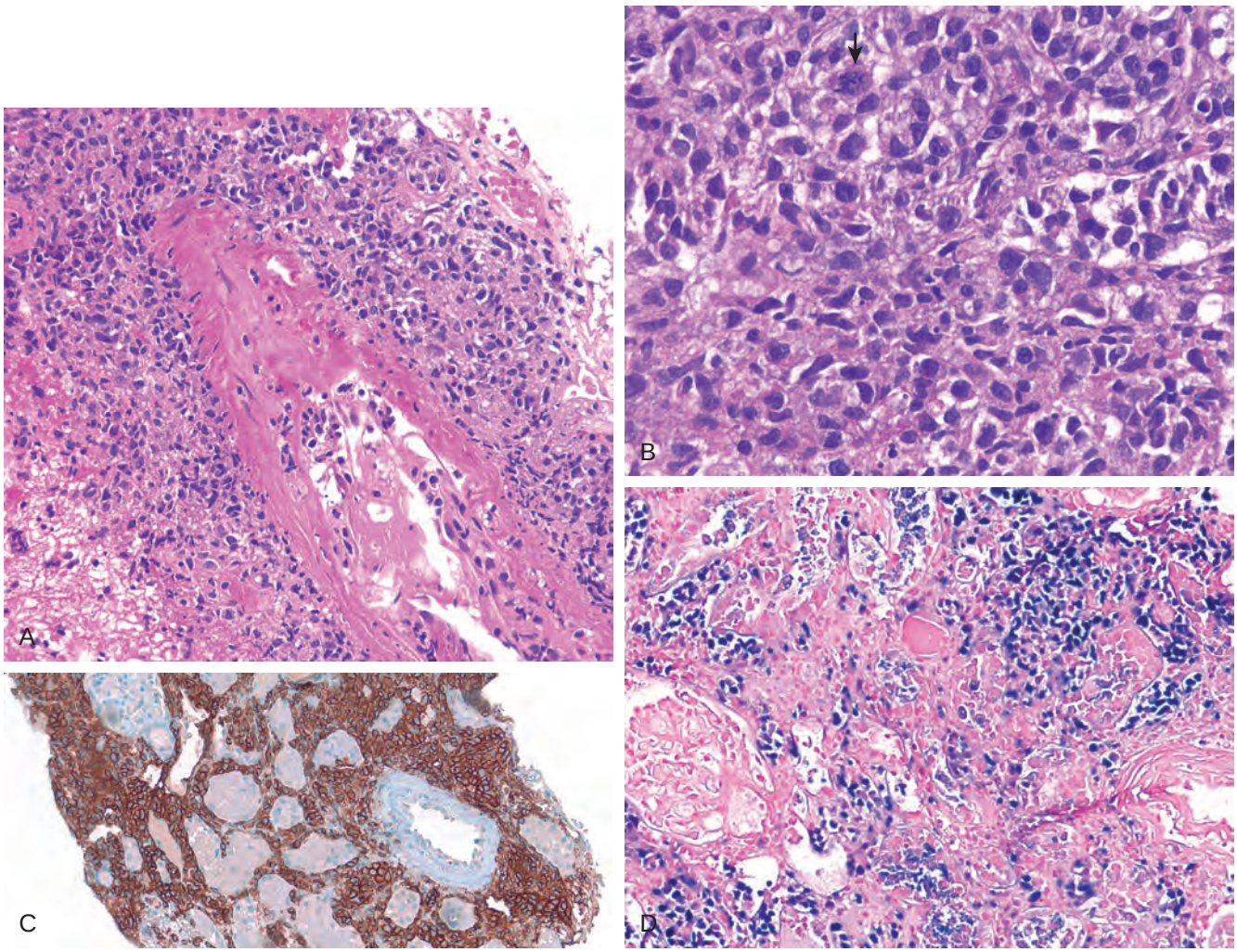
Posttransplantation Lymphoproliferative Disorder

PTLD is a lymphoproliferative disorder that in many cases manifests as a frank lymphoma; it arises in immunocompromised hosts, such as recipients of solid organ or bone marrow allografts. Many cases of PTLD are driven by EBV, and most are of the B-cell type. Some are of the T-cell type. PTLD is estimated to occur in 1% of renal allograft recipients. Among renal allograft patients with PTLD, the allograft kidney is affected in more than 30% of the cases. PTLD restricted to the kidney transplant (approximately 12% of cases) occurs early (approximately 5 months) after surgery. PTLD is most often of donor origin, and the prognosis can be favorable with treatment, which may include reduction in immunosuppression, irradiation, antiviral drugs (acyclovir, ganciclovir, interferon-α), or chemotherapy.[139,140] Chemotherapy regimens using anti-CD20 (rituximab) have been useful.[119,139,141]

Pathologic features of PTLD involving the kidney include swollen kidneys with blurring of the corticomedullary junction and diffuse petechiae. Involvement is usually vaguely nodular and composed of mononuclear cells with enlarged nuclei, prominent nucleoli, and mitoses; the cells of PTLD may be monomorphic or polymorphic (Fig. 16.14). Necrosis, often described as presenting a serpiginous pattern, may be present. Cells may have blast-like



• **Figure 16.13** BK nephropathy. **A,** Low-power view shows prominent interstitial nephritis. **B** and **C,** High-power views show nuclear viral cytopathic effect. **D,** Immunohistochemistry for the simian virus 40 antigen is used to detect the BK virus in BK nephropathy.



• **Figure 16.14** Posttransplantation lymphoproliferative disorder involving the kidney. **A,** Prominent lymphoid infiltrate is appreciable at low power. **B,** Prominent lymphoid infiltrate with mitosis. **C,** CD20 immunohistochemical stain is positive, indicating the majority of cells are B cells. **D,** In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA is positive in numerous cells, indicating that the posttransplantation lymphoproliferative disorder is EBV-driven.

features. PTLD may be confused with allograft rejection, because features mimicking rejection, such as tubulitis and endarteritis, may be present. In PTLD, there may be admixed plasma cells, but granulocytes and macrophages are not seen as commonly as they are in rejection.[119,139]

Most PTLD cases (85% to 90%) express CD20, and in situ hybridization for EBV-encoded RNA (EBER) usually shows prominent staining in the atypical lymphoid cells. By contrast, rejection usually shows a predominance of $CD3^+$ and $CD68^+$ cells.[119,139]

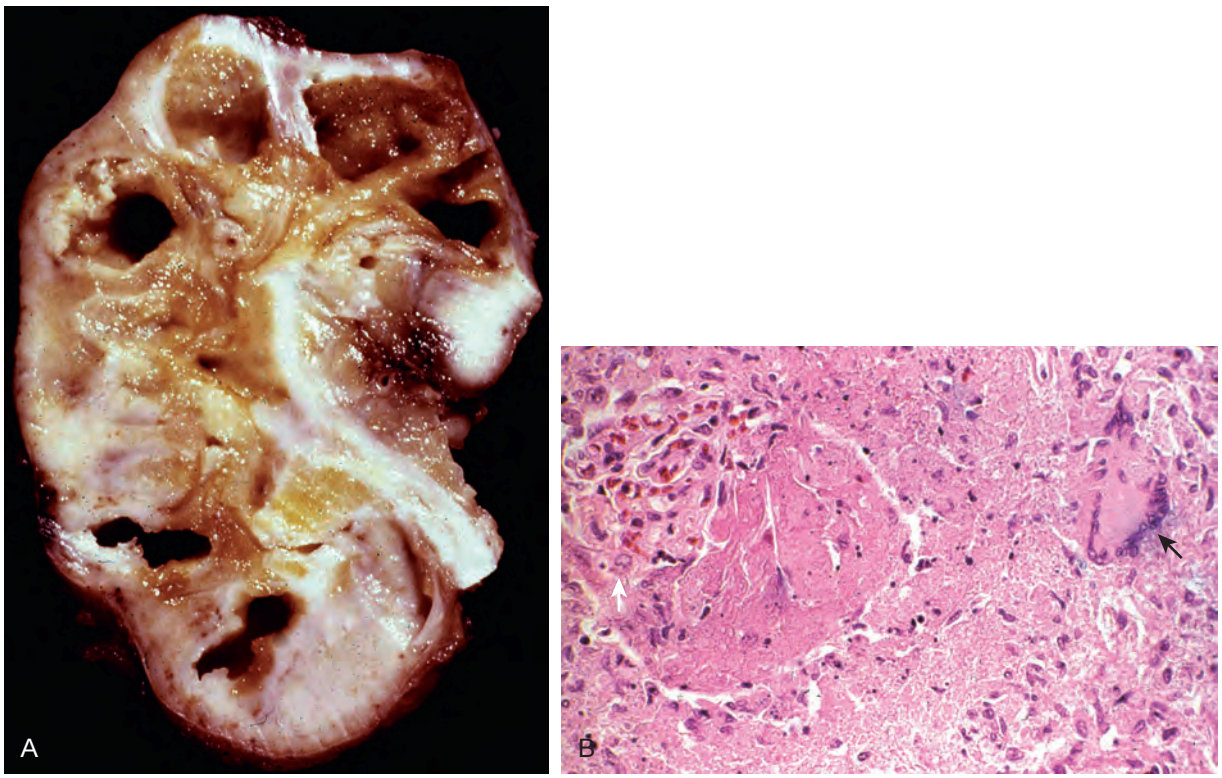
Other Infections

Direct infection of the kidney is an important cause of illness, particularly in immunocompromised patients and transplant recipients; however, various renal diseases are observed in different geographic areas around the world as disorders normally considered endemic to those regions.[77]

Mycobacterial Infections

According to studies in the United States and other Western countries, approximately 5% of patients with tuberculosis have renal involvement. However, individuals in areas of the world other than the United States appear to have a higher incidence of renal involvement, and it is possible that renal disease is underestimated as an extrapulmonary manifestation of tuberculosis infection.[77,142] In addition, mycobacterial organisms other than *M. tuberculosis* also cause renal disease, including *Mycobacterium avium-intracellulare*, *Mycobacterium bovis*, *Mycobacterium kansasii*, and *Mycobacterium leprae*. Risk factors for renal mycobacterial infection include transplantation, HIV, and other causes of immunosuppression. Renal tuberculosis may be cavitary or miliary.[77,142]

Cavitary tuberculosis of the kidney often occurs through an ascending infection from a lower urinary tract infection. The renal medulla is severely affected, with epithelioid caseating granulomas



• **Figure 16.15** Renal tuberculosis. **A,** Gross appearance of renal tuberculosis with extensive parenchymal destruction. **B,** Microscopic appearance of renal tuberculosis showing extensive caseating cortical necrosis (with the amorphous background) and a Langhans giant cell *(black arrow)* embedded in the necrosis amidst only a small amount of residual preserved renal cortical parenchyma *(white arrow)*.

that become confluent and eventually form cavities, often with associated papillary necrosis. Renal involvement is frequently unilateral, and renal function may be largely preserved. Renal failure occurs in approximately 12% of patients.[77,142-145]

Grossly, in cavitary tuberculosis, the kidney may be smaller or larger than normal, and there may be irregular surface scarring. The pelves and calyces may be dilated, constricted, or deformed. The parenchyma may show atrophy, calcification, and foci of caseating necrosis (sometimes termed tuberculous pyonephrosis or a "chalk," "cement," or "putty" kidney) (Fig. 16.15). The appearance may even mimic a neoplasm (i.e., renal cell carcinoma). Microscopically, caseating necrosis with surrounding granulomatous inflammation with plasma cells and calcification (i.e., tubercles) are observed. Langhans-type giant cells may be seen as a component of the granulomatous inflammation. Stains to detect acid-fast bacilli are often useful.[77,142,144]

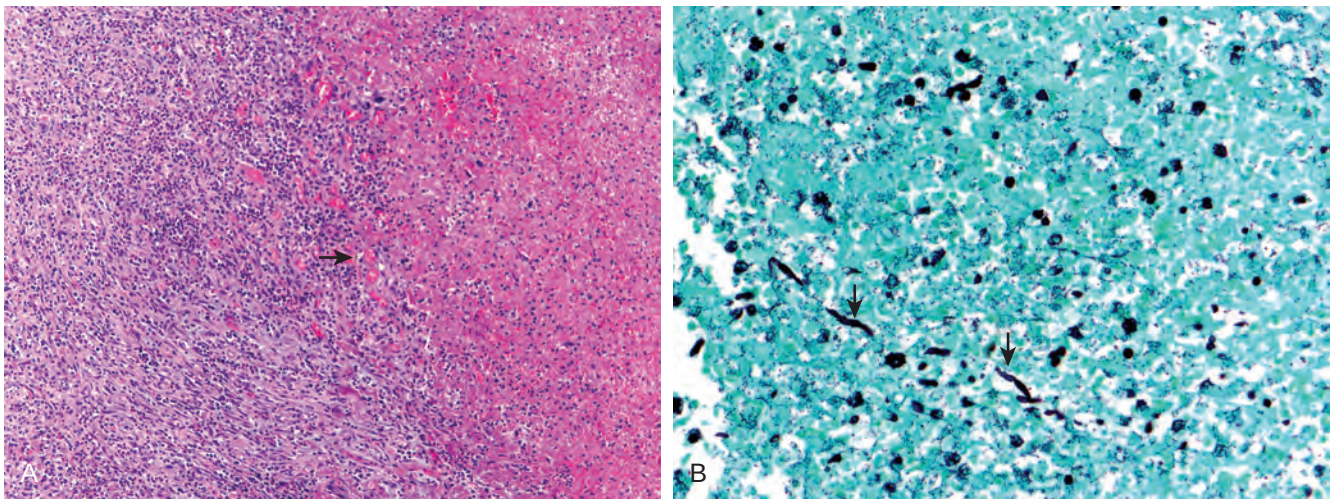
Miliary tuberculosis of the kidney occurs as a consequence of disseminated infection. Grossly, there are white nodules (tubercles) approximately 1 mm in diameter occurring at a higher density in the cortex than in the medulla. Microscopically, there is granulomatous inflammation with caseating granulomas containing epithelioid histiocytes and a mixed inflammatory infiltrate of neutrophils, lymphocytes, monocytes, and plasma cells.[77,142,144]

Tuberculoid forms of leprosy *(M. leprae)* may involve the kidney, affecting approximately 70% of patients based on autopsy studies. Microscopically, there is diffuse infiltration of macrophages and cells filled with bacilli. Chronic tubulointerstitial nephritis composed of a mononuclear infiltrate with interstitial fibrosis and tubular atrophy may be present. Coexisting glomerulonephritis and amyloidosis may be present.[77,142,146]

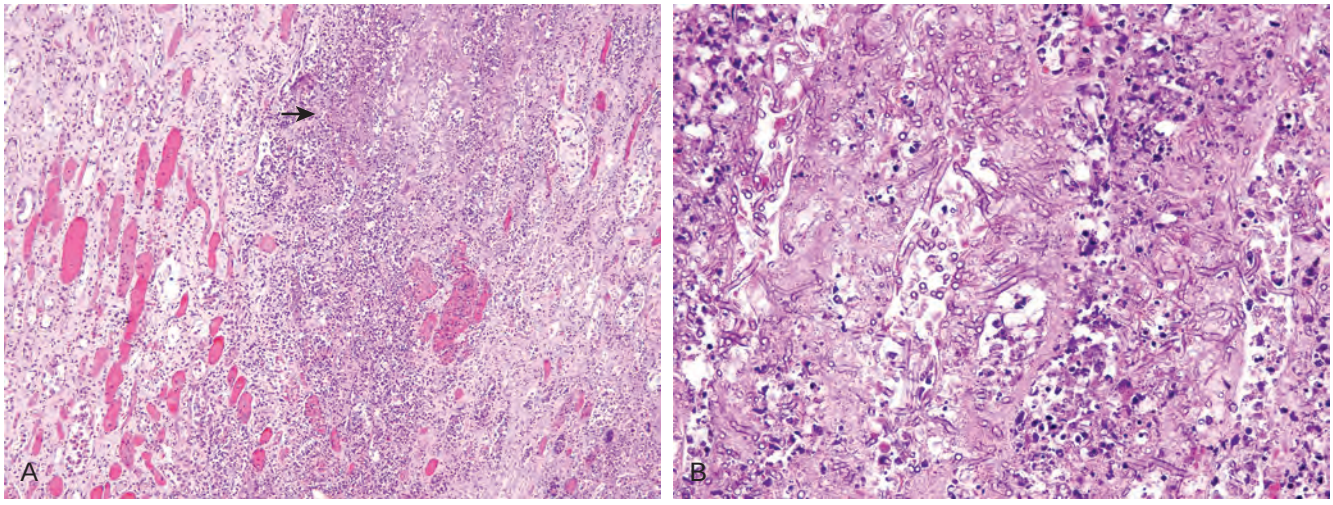
Fungal Infections

The most common fungal organisms affecting the kidney are of the genus *Candida*, including *C. albicans*, *Candida* (formerly *Torulopsis*) *glabrata*, and other *Candida* spp.. Other organisms implicated include *Aspergillus*, *Cryptococcus*, *Coccidioides*, *Histoplasma*, *Blastomyces*, *Paracoccidioides*, and *Mucor* spp. Infection of the kidney often results from hematogenous dissemination, particularly in cases of fungal sepsis. Histologically, fungal infections often show a prominent granulomatous inflammation. Because the PAS stain is frequently used as routine stain to evaluate basement membranes in renal biopsy specimens, it is also a good stain to evaluate for fungi.[77,147]

Candida infections, mostly *C. albicans*, spread to the kidney from other locations through hematogenous dissemination, particularly in immunosuppressed, newborn, diabetic, or granulocytopenic patients. Grossly, papillary necrosis may be present. Abscesses with a miliary pattern may be recognized. Fungus balls may be present in the pelves and calices. Microscopically the inflammatory response may be brisk or practically nonexistent. Cortical abscesses that destroy glomeruli may be seen. Pseudohyphae and budding yeast 2 to 4 μm in diameter can usually be identified and may be recognized with PAS or Grocott methenamine silver (GMS) stains (Fig. 16.16). *C. albicans* infection may also result in emphysematous pyelonephritis. *C. glabrata* produces manifestations similar to those of *C. albicans*. Immunohistochemistry



• **Figure 16.16** *Candida* pyelonephritis. **A,** *Candida* pyelonephritis forming an abscess cavity *(arrow)*. **B,** Grocott methenamine silver stain reveals several yeast cells (blastoconidia) and pseudohyphae with constrictions or septations between the cells *(arrows)*.



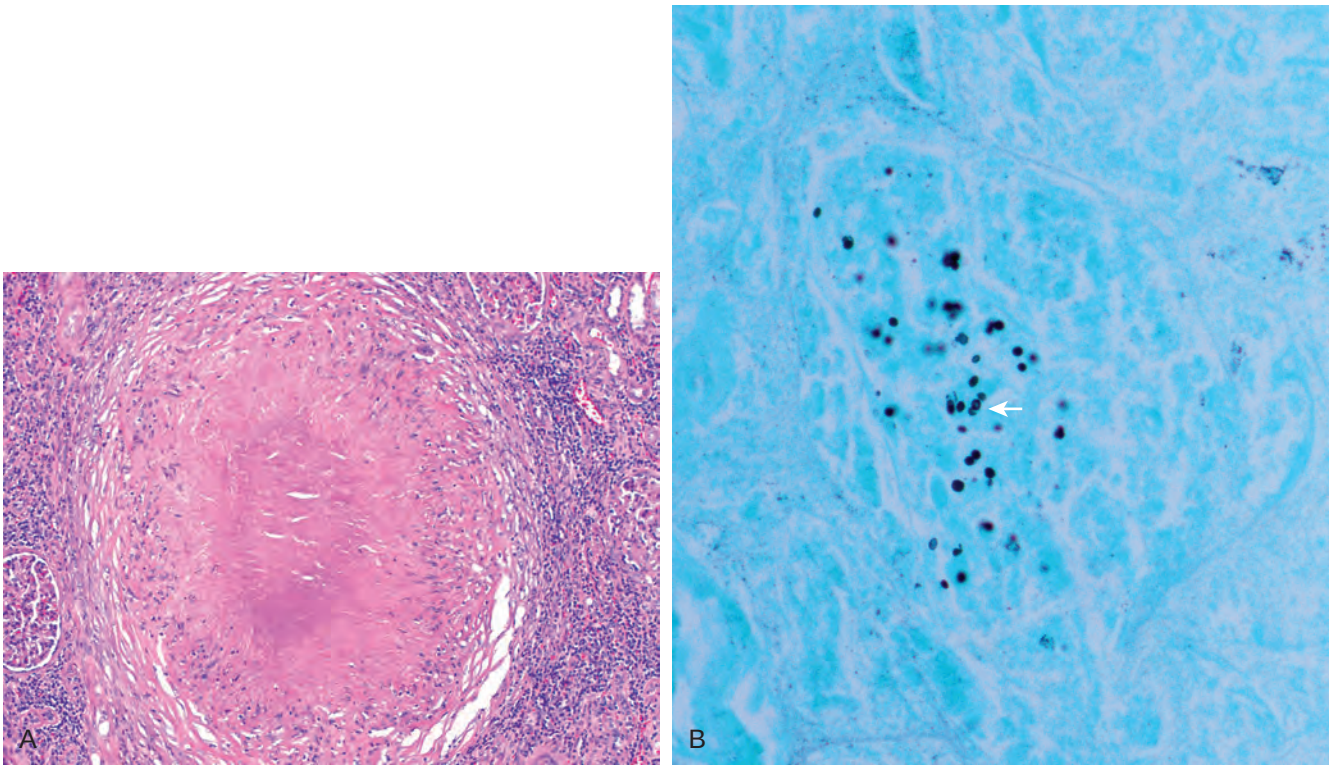
• **Figure 16.17** *Aspergillus* pyelonephritis. **A,** Low-power appearance of *Aspergillus (arrow)* causing a pyelonephritis. The infection in this case was caused by a disseminated systemic *Aspergillus* infection. **B,** High-power appearance of *Aspergillus*, showing dichotomously branching hyphae on a hematoxylin and eosin–stained section.

may be useful in the identification of *Candida* spp., which may sometimes be difficult to distinguish from *Histoplasma*, *Cryptococcus*, or *Pneumocystis*.[56,77,148-150]

Aspergillosis is caused by *Aspergillus* spp., the most common of which is *Aspergillus fumigatus*. Hematogenous dissemination is the usual route of infection. *Aspergillus* affects a similar patient population as *Candida* and in addition affects patients with invasive bronchial infections or pneumonitis and those receiving corticosteroid therapy. There is usually a mononuclear inflammation with neutrophils, abscesses, and infarcts, and branching septated hyphae 3 to 5 μm in size can be identified with PAS or GMS stains (Fig. 16.17). Immunohistochemistry may be useful in confirming the identity of the organisms, although attention should be paid to potential cross-reactivity that may be seen between various organisms.[56,77,148-153]

Cryptococcus neoformans infection usually results from hematogenous dissemination, often from the respiratory tract and classically from avian (e.g., pigeon) exposure. Immunosuppressed and neutropenic patients are particularly susceptible. Parenchymal abscesses, granulomas, and necrosis are identified in the cortex and medulla, sometimes with papillary necrosis, severe tubular injury, and destruction. However, there may be a paucity of inflammation. The organisms are 4 to 20 μm in diameter and are surrounded by a polysaccharide capsule, which stains with mucicarmine, alcian blue, and PAS. Immunohistochemistry is also available.[56,77,148-152]

Histoplasma capsulatum, the causative agent of histoplasmosis, is endemic in the Mississippi and Ohio River valleys and in South and Central America. It is usually contracted through inhalation of soil that is contaminated with bird or bat droppings containing the fungus. The kidneys are affected in 40% of patients with progressive disseminated histoplasmosis. Histologically, there is typically a significant degree of tubulointerstitial inflammation, which may be granulomatous and caseating. Macrophages show large numbers of nonencapsulated, intracellular, oval or round yeast forms measuring up to 5 μm in diameter, some of



• **Figure 16.18** Granulomatous pyelonephritis due to histoplasmosis. **A,** Caseating granulomas that were found throughout the kidney. **B,** Grocott methenamine silver stain reveals small, black-staining, 2- to 3-µm, budding yeast cells *(arrow)*.

which may display budding (Fig. 16.18). GMS stains, immunohistochemistry, and immunofluorescence are useful for their recognition.[56,77,151,154,155]

Coccidioides immitis, the causative agent of coccidioidomycosis, is endemic in the southwestern United States. The infective form consists of arthrospores from arthroconidia. The fungus also exists in nature in a mycelial form. The kidneys are affected in approximately one-third of patients who die of disseminated infection. Multiple abscesses and minute granulomas may be present, along with caseous or suppurative necrosis. Thick-walled spherules (Fig. 16.19), approximately 100 µm in diameter, contain endospores 5 to 30 µm in diameter. Special stains, such as GMS and PAS, may be useful in highlighting the organisms. Immunohistochemistry is available but is usually not needed for diagnosis.[77,149,150]

Blastomyces dermatitidis, the causative agent of blastomycosis, is present notably in the southeastern United States and in the Ohio and Mississippi River areas. It affects males approximately four times more frequently than females. Grossly the renal capsule may have perinephric abscesses and draining sinuses. Microscopically, microabscesses and epithelioid and giant cell granulomas, some of which are caseating, are present. Yeast cells measure 8 to 15 µm and may have a double-contoured appearance with broad-based budding of the daughter cells. GMS stains and immunohistochemistry may be useful in the diagnosis.[77,149,150]

Paracoccidioidomycosis (i.e., South American blastomycosis) is caused by *Paracoccidioides brasiliensis*, which is endemic to South America, Central America, and Mexico. Infection is by inhalation with pulmonary involvement and secondary spread to other organs, including the kidneys. Microscopically, there is often prominent granulomatous inflammation with giant cells, often with caseating necrosis. Nonbudding forms, approximately 10 µm in diameter, predominate, but multiple buds may radiate from a single cell, resembling the steering wheel of a ship (mariner's wheel). GMS stains and immunohistochemistry may be useful.[77,150]

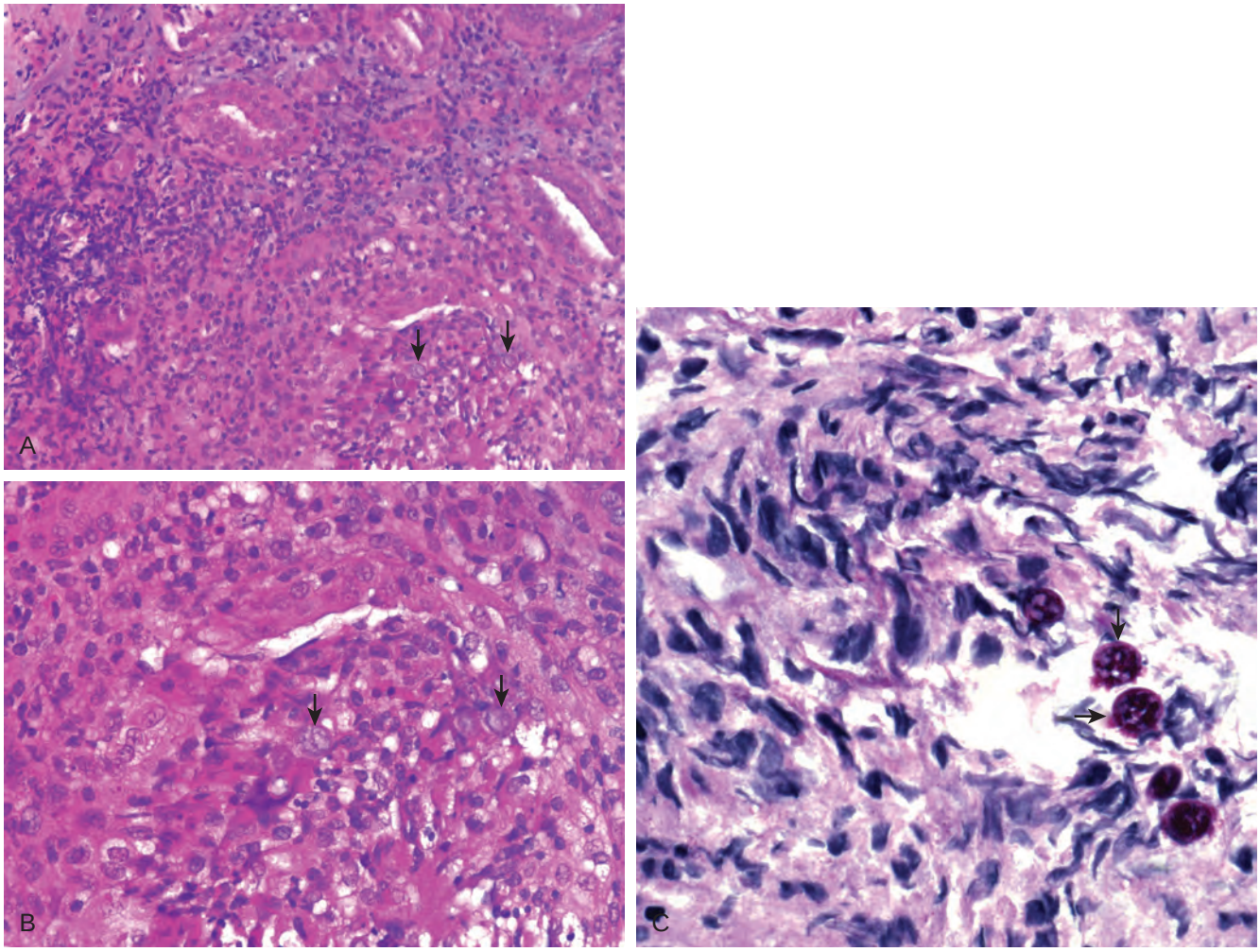
Mucormycosis infection (i.e., zygomycosis) occurs through hematogenous dissemination, usually from respiratory infections. Immunocompromised patients and diabetics are particularly susceptible to disseminated infection. On microscopic examination, there is suppurative necrotizing inflammation with interlobular and arcuate artery thrombosis. The hyphae are broad and nonseptated, with right-angle branching.[77,148,150]

Syphilis

The kidney may be affected in tertiary and congenital syphilis. Primarily glomeruli are affected, leading to membranous nephropathy with granular immune deposits in glomeruli and the tubular basement membrane and the resultant nephrotic syndrome, particularly in congenital syphilis. Microscopically, gumma and tubulointerstitial nephritis composed of mononuclear cells, plasma cells, and interstitial fibrosis may be present. Attachment of *Treponema* organisms to endothelium, as mediated by fibronectin, complement, and humoral- and cell-mediated immune responses, are thought to be important mechanisms for infection.[77,156]

Filamentous Bacteria: Actinomycosis and Nocardiosis

The filamentous bacteria from the genuses *Actinomyces (Actinomyces israelii)* and *Nocardia* (mainly *Nocardia asteroides* but also *Nocardia farcinica*) may involve the kidneys, primarily in immunocompromised individuals. Microscopically, both organisms produce suppurative infections. In *Actinomyces* infections, necrosis



• **Figure 16.19** *Coccidioides immitis* involving the kidney. **A,** Medium power shows prominent interstitial nephritis *(arrows)*. **B,** High power shows several organism spherules with vaguely defined endospores *(arrows)*. **C,** High power shows several organism spherules with endospores highlighted with a periodic acid–Schiff stain *(arrows)*.

may be present. So-called sulfur granules (bacterial macrocolonies) may be present in the urine or sinus tracts and may be appreciated on sections stained with H&E, Gram, or GMS stains or immunofluorescence. In nocardial infections, vascular thromboses may be present with solitary or multiple abscesses, diffuse pyelonephritis, or draining sinus tracts. *Nocardia* can also form granules and large colonies, which can be classified as mycetomas. *Nocardia* can be identified with Gram, PAS, or GMS stains.[77,154] Unlike *Actinomyces*, *Nocardia* also stains with acid-fast stain.[147]

Rickettsia

Rickettsial infections are arthropod-borne zoonoses that are caused by small, gram-negative, rod-shaped bacteria. These include Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*), Mediterranean spotted fever *(Rickettsia conorii)*, scrub typhus *(Rickettsia tsutsugamushi)* transmitted by mites, and epidemic typhus *(Rickettsia prowazekii)* transmitted by body lice. All cause varying degrees of tubulointerstitial nephritis and tubular injury, and the inflammation is often vasculocentric and composed of an infiltrate of lymphocytes and histiocytes. Rocky Mountain spotted fever and epidemic typhus in particular show vasculitis, sometimes with appreciable rickettsial organisms or antigens in the endothelial cells. Scrub typhus is milder, with less severe vascular changes and only rare thrombosis and necrosis. Acute renal failure may occur.[77,78]

R. rickettsii, the causative agent of Rocky Mountain spotted fever, is transmitted by the bites of ticks. Small mammals are reservoirs. In the early stage of infection, there is often fever and a rash. Histologically, in the kidney, there is often a tubulointerstitial nephritis composed of a mononuclear infiltrate with mononuclear cells, neutrophils, edema, and tubular cell necrosis. Inflammation is often vasculocentric, and endothelial cell necrosis is a characteristic feature. Death due to renal failure may occur, particularly in the elderly and in those who do not obtain rapid treatment. Immunofluorescence and immunohistochemistry can be useful in the diagnosis, because *Rickettsia* antigens may be demonstrated in the capillary endothelium; serologic methods are also helpful to detect antibodies to spotted fever group (SFG) rickettsiae.[56,77,157-160]

Leptospirosis

Leptospira spp., including *Leptospira icterohaemorrhagiae*, the causative agent of Weill disease, *Leptospira pomona*, *Leptospira bataviae*, *Leptospira canicola*, *Leptospira shermani*, and *Leptospira*

grippotyphosa, are long, thin aerobic spirochetes that can be responsible for human infection. Patients typically present with jaundice, renal dysfunction, or even acute renal failure. Leptospira are typically considered to be enzootic diseases with reservoirs in skunks, foxes, rodents, livestock, and ducks. The organism is present in the urine of these animals, and humans are infected through direct contact or via contaminated water. In Weill disease the kidneys are enlarged, edematous, and brownish yellow. Histologically, there is prominent acute tubular injury and necrosis with a mixed inflammatory infiltrate of monocytes, plasma cells, and neutrophils with petechial hemorrhages and vasculitis. A special stain that is sometimes used is the Levaditi stain, which is a special silver nitrate stain, although it requires special expertise.[77,78,161]

Parasitic Infections

Parasitic infections can cause renal dysfunction and gross and microscopic abnormalities in the kidney through direct and indirect mechanisms. Some changes result from the direct effects of parasitic infestation or deposition of parasitic eggs in the kidney, but changes may also occur due to inflammatory mediators released by or as a response to the parasites. Parasitic infections that may affect the kidney include malaria, schistosomiasis, filariasis, microsporidiosis, amebiasis, and hydatidosis. Microfilariae incite granulomatous inflammatory responses. Amebic abscesses in the kidneys (primarily caused by *E. histolytica*) may occur. Microsporidia may lead to tubulointerstitial nephritis, and spores may be deposited in the kidney. Other major parasitic infections affecting the kidney are discussed later in this chapter.[77,78]

Malaria. *Plasmodium* spp., transmitted by the bite of the *Anopheles* mosquito, lead to malaria. However, malaria may also be transmitted by blood transfusion or organ transplantation. Glomerular disease may be caused by malaria, leading to proteinuria, hematuria, and nephrotic syndrome. Glomerular disease is particularly a feature of *Plasmodium malariae*. However, *P. malariae* and *Plasmodium falciparum* may also lead to tubulointerstitial injury. Hemoglobinuric acute renal failure may result in the so-called blackwater fever, which occurs in 1% to 4% of patients in endemic areas. Histologically, acute tubular injury and necrosis are seen, along with interstitial nephritis composed primarily of mononuclear cells (lymphocytes) and edema with tubular hemoglobin casts. Parasitized red blood cells become adherent to the endothelium and platelets, leading to occlusion of the microcirculation, which explains many of the pathogenic effects of malaria.[77,162-165]

Schistosomiasis. *S. mansoni* and *Schistosoma hematobium* are the primary schistosomal organisms that affect the kidney. *S. mansoni* causes glomerulonephritis, leading to nephrotic syndrome. *S. hematobium* causes obstructive uropathy, leading to prominent interstitial inflammation with mononuclear cells and fibrosis. Schistosomal eggs (notably from *S. mansoni*, *S. hematobium*, and *Schistosoma japonicum*) may be localized to the kidney and may incite a granulomatous inflammatory reaction. The eggs of *S. mansoni* (with characteristic large lateral spines) and *S. japonicum* (with small lateral spines) are acid fast, but those of *S. hematobium* (with characteristic terminal spines) are not acid fast.[77,166,167]

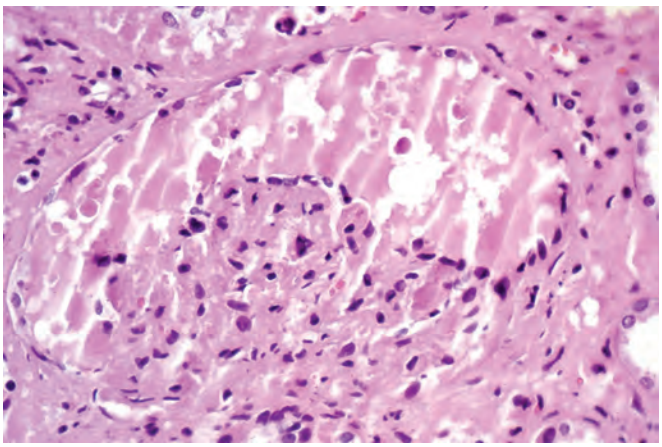
Hydatidosis. Larval forms of *Echinococcus granulosus* cause cystic hydatid disease, which is endemic in parts of Latin America, Africa, Turkey, and the Mediterranean region. Cystic hydatid disease is most common in the liver and the lungs, and renal involvement is usually accompanied by hydatid disease in other organs. Isolated disease is rare. Hydatid cysts or grapelike material may be passed in the urine, but only in a minority (5% to 25%) of cases. Renal cysts may be treated by cystectomy or marsupialization, although nephrectomy may be required.[77,168,169]

Renal Glomerular Disease Caused by Infection

A number of infections can cause renal dysfunction and various renal diseases that affect the glomeruli as a prominent pattern of injury, including bacteria (poststreptococcal and other postinfectious glomerulonephritides), viruses (particularly hepatitis), malaria, schistosomal infection, echinococcal infection (hydatid disease), and filarial infection (Table 16.5).[171,172,177,178] Clinically, these disorders often lead to nephrotic syndrome, but disease may range from mild proteinuria or hematuria to the nephrotic syndrome, the nephritic syndrome, renal insufficiency, or rapidly progressive glomerulonephritis. Organisms that produce glomerular disorders may give rise to a number of patterns of glomerular injury (Table 16.6), primarily proliferative disorders such as diffuse proliferative, membranoproliferative, or mesangioproliferative glomerulonephritis. Membranous nephropathy, FSGS (Fig. 16.20), minimal change disease, and minimal change diseaselike lesions may all be the glomerular manifestations of systemic infections.[77,179]

Postinfectious (Poststreptococcal) Glomerulonephritis. One of the classic glomerular diseases associated with infection is poststreptococcal glomerulonephritis (PSGN) and other postinfectious glomerulonephritides. Acute glomerulonephritis has been recognized as a disorder for some time, since before it was recognized histologically. Hippocrates first noticed the association between flank pain and dark urine (hematuria) which eventually leads to oliguria or anuria. The English physician Richard Bright realized the association between scarletina and swelling of the face and lower-extremity edema, and he further described the hematuria and eventual chronic renal failure in some cases of the disorder.[77,179-181]

PSGN usually occurs after exposure to group A β-streptococcus type 12, 4, or 1, such as after an upper respiratory tract infection, although other organisms may account for the glomerulonephritis.



• **Figure 16.20** Collapsing focal segmental glomerulosclerosis (FSGS) caused by acquired immunodeficiency syndrome (AIDS). The glomerular tuft is collapsed, making Bowman space appear relatively larger. This is one of many manifestations of viral and other infectious diseases that affect glomeruli. Collapsing FSGS is not observed as commonly as it once was before the advent of more effective antiviral agents for AIDS.

TABLE 16.5 Selected Infectious Diseases and Their Renal Glomerular and Other Manifestations

Organism	Pattern of Injury	Presentation and Comments
Bacteria		
Streptococcus	Diffuse proliferative GN	Nephritic syndrome
Treponema pallidum (syphilis)	Membranous GN	—
Other	Postinfectious, from organisms other than *Streptococcus* IgA nephropathy IgA-dominant postinfectious GN, often with *Staphylococcus aureus*, MRSA, and *Staphylococcus epidermidis*, particularly in diabetics[171]	IgG and C3 in a "starry sky" pattern[170] Infections of the gastrointestinal and respiratory tracts Predominance of IgA deposits in mesangium and subepithelial "humps"
Viruses		
Hepatitis B	Membranous GP[172] MPGN[173-176]	Proteinuria/nephrotic syndrome —
Hepatitis C	Membranous GP MPGN (type I) Cryoglobulinemic GP Glomerular intracapillary proliferation with or without extracapillary proliferation	Proteinuria/nephrotic syndrome Usually has the pattern of MPGN type I "Pseudolinear" deposits of IgG/IgM and C3[170] —
HIV	CGP/HIVAN	Resembles a collapsing variant of FSGS but is now thought to be a distinct entity[170]
Parvovirus B19	Non-HIV-associated CGP	—
CMV	Non-HIV-associated CGP CMV glomerulitis	Characteristic inclusions seen in tubulointerstitial disease may be present
Parasites		
Plasmodium malariae	Mesangioproliferative GN MPGN Membranous GP FSGS	Proteinuria/nephrotic syndrome Nephrotic syndrome — —
Plasmodium falciparum	Tubulointerstitial damage Mesangio-MPGN	Acute renal failure Proteinuria/nephrotic syndrome
Schistosoma hematobium	Hydronephrosis and pyelonephritis	Chronic renal failure
Schistosoma mansoni	Mesangio-MPGN FSGS Membranous GP Amyloidosis (rare) Crescentic GN	Proteinuria — Nephrotic syndrome Acute renal failure —
Leishmania donovani (kala-azar)	Mesangioproliferative GN MPGN, amyloidosis	Proteinuria Nephrotic syndrome
Trypanosoma brucei subsp. *rhodesiense* and *T. brucei* subsp. *gambiense*	Mesangioproliferative GN (rare)	Proteinuria
Toxoplasma gondii	MPGN FSGS (rare) Tubulointerstitial nephritis —	Proteinuria/nephrotic syndrome Nephrotic syndrome (especially congenital toxoplasmosis) Acute renal failure
Trichinella spiralis	Mesangio-MPGN Tubular necrosis	Proteinuria Mild renal failure
Babesia bovis, *Babesia divergens*, *Babesia microti*	Tubular necrosis	Acute renal failure

TABLE 16.5 Selected Infectious Diseases and Their Renal Glomerular and Other Manifestations—cont'd

Organism	Pattern of Injury	Presentation and Comments
Echinococcus granulosus (hydatid disease)	Cyst (usually solitary) Membranous GP (rare)	Mass effect Nephrotic syndrome
Loa loa, *Wuchereria bancrofti*, *Brugia malayi* (lymphatic filariasis), *Onchocerca volvulus* (river blindness)	Interstitial nephritis Amyloidosis (rare) Mesanigo-MPGN	Renal failure Nephrotic syndrome Proteinuria or even nephritic syndrome
Wuchereria bancrofti	Acute GN (rare)	Nephritic syndrome
Loa loa	Membranous GP FSGS (rare)	Nephrotic syndrome —

CGP, Collapsing glomerulopathy; *CMV,* cytomegalovirus; *FSGS,* focal segmental glomerulosclerosis; *GN,* glomerulonephritis; *GP,* glomerulopathy; *HIV,* human immunodeficiency virus; *HIVAN,* HIV-associated nephropathy; *Ig,* immunoglobulin; *MPGN,* membranoproliferative glomerulonephritis; *MRSA,* methicillin-resistant *Staphylococcus aureus.*

Data from Weiss M, Liapis H, Tomaszewski JE, Arend LJ. Pyelonephritis and other infections, reflux nephropathy, hydronephrosis, and nephrolithiasis. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, eds. *Heptinstall's Pathology of the Kidney.* Vol 2. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:991; van Velthuysen ML, Florquin S. Glomerulopathy associated with parasitic infections. *Clin Microbiol Rev.* 2000;13:55-66; and Barisoni L, Meehan S, Arend LJ. Introduction to renal biopsy. In: Zhou M, ed. *Genitourinary Pathology.* Philadelphia: Churchill Livingstone; 2007.

TABLE 16.6 Patterns of Glomerular Injury in Selected Infectious Diseases

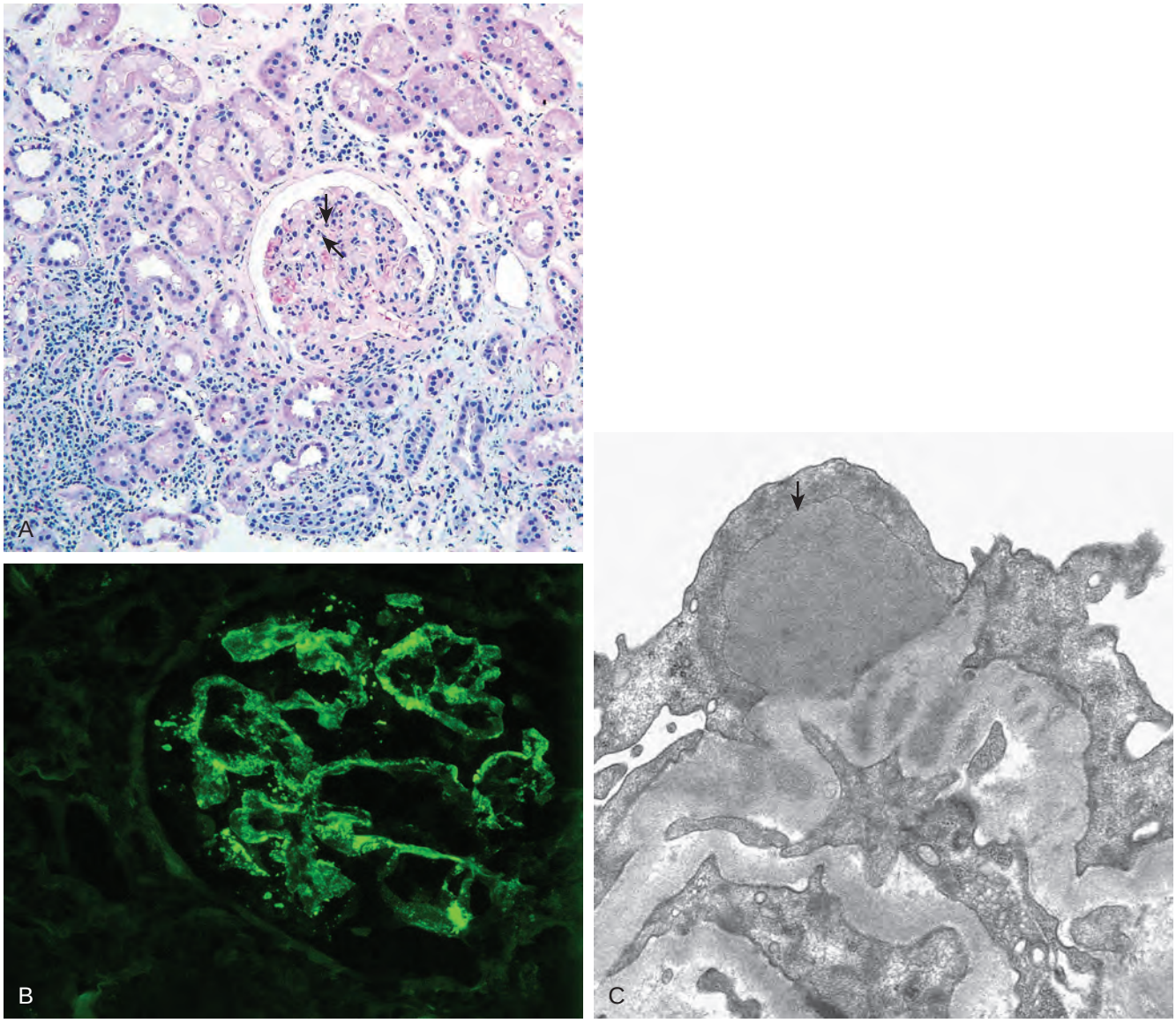
Pattern	Light Microscopy	Immunofluorescence Microscopy	Electron Microscopy
Minimal change disease	Normal; lipid in tubules	Negative	Loss (effacement) of foot processes; no deposits
Focal segmental glomerulosclerosis	Focal and segmental sclerosis and hyalinosis	Focal; IgM and C3	Loss (effacement) of foot processes; epithelial denudation
Membranoproliferative GN	Mesangial proliferation; BM thickening; splitting ("tram tracks")	IgG + C3; C1 + C4	Subendothelial deposits
Mesangioproliferative GN	Expanded mesangial matrix and proliferation of mesangial cells	Immunoglobulins in mesangium	Deposits in mesangium
Diffuse proliferative GN	Diffuse proliferation; leukocytic infiltration	Granular IgG and C3 in glomerular BM and mesangium	Subepithelial humps
Membranous GP	Diffuse capillary wall thickening with "spikes"	Granular IgG and C3; diffuse	Subepithelial deposits
IgA nephropathy	Focal proliferative GN; mesangial expansion/widening	IgA + IgG, IgM, and C3 in mesangium	Mesangial and paramesangial dense deposits, often closely apposed to mesangial cells

BM, Basement membrane; *GN,* glomerulonephritis; *GP,* glomerulopathy; *Ig*G, immunoglobulin.

Data from Haas M. IgA nephropathy and Henoch Schönlein purpura. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, eds. *Heptinstall's Pathology of the Kidney.* Vol 1. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:424; van Velthuysen ML, Florquin S. Glomerulopathy associated with parasitic infections. *Clin Microbiol Rev.* 2000;13:55-66; Nadasdy T, Sedmak D. Acute and chronic tubulointerstitial nephritis. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, eds. *Heptinstall's Pathology of the Kidney.* Vol 2. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:322; and Barisoni L, Meehan S, Arend LJ. Introduction to renal biopsy. In: Zhou M, ed. *Genitourinary Pathology.* Philadelphia: Churchill Livingstone; 2007.

Clinically, in addition to hematuria and the nephritic syndrome, patients with PSGN have elevated anti-streptolysin O (ASO) titers and low levels of serum complement. The pediatric population is the most commonly affected age group. Approximately 90% of cases resolve within a few weeks, but adults may have a less favorable prognosis. Histologically, there is typically a diffuse proliferative glomerulonephritis, often with prominent intracapillary neutrophils (Fig. 16.21). One of the most useful diagnostic features is the presence of amorphous subepithelial, electron-dense deposits on electron microscopy. The deposits are also known as subepithelial humps. In addition, immunofluorescence shows the presence of immunoglobulin (Ig)G and C3 in a granular, "starry sky" pattern in the mesangium and capillary walls.[77,179-181]

It has been increasingly recognized that a substantial proportion of infection-related glomerulonephritis is due to causes other than the classic postinfectious GN due to PSGN. For example, more cases of poststaphylococcal GN are being recognized. Notably, many of these poststaphylococcal GN have been seen in diabetic patients. Many of these cases of poststaphylococcal GN have an important difference from classic PSGN in that they have a predominance of glomerular IgA staining as opposed



• **Figure 16.21** Acute poststreptococcal glomerulonephritis. **A,** Light microscopic appearance showing increased glomerular cellularity with numerous intracapillary neutrophils *(arrows)*. **B,** Immunofluorescence microscopy showing granular positivity for immunoglobulin G, primarily along the periphery of capillary loops. **C,** Electron microscopy showing subepithelial amorphous, electron-dense deposits *(arrow)*, also known as subepithelial humps.

to the IgG seen in PSGN; many of these cases may mimic IgA nephropathy.[177,182,183]

The recognition of these poststaphylococcal GN cases, as well as other infection-related GNs, has prompted a shift in the thinking on infection-related GN. Importantly, it has been noted that many GNs caused or at least associated with infection are not all *post*infectious but are rather due to ongoing/concurrent infections; therefore, many have argued that it is more accurate to use to the term *infection-related* GN.[177,184] Furthermore, it has been recognized that individuals may have inherent defects or deficiencies that predispose them toward infection-related/postinfectious GN; specifically, these include abnormalities in the complement pathway.[185]

Membranoproliferative Glomerulonephritis. Membranoproliferative glomerulonephritis (MPGN) is a pathologic pattern of injury that may appear in association with a number of infectious disorders. One of the most important infectious associations with a MPGN-type pattern occurs in hepatitis virus infections. Hepatitis viruses can produce a number of patterns of injury, but MPGN and cryoglobulinemic glomerulonephritis are some of the most noteworthy. MPGN is characterized by *tram-tracking* or *double contours* of the glomerular basement membrane (GBM). Immune deposits (C3 in all cases and also usually IgG and IgM) are recognized by immunofluorescence, and frequently by IgG and IgM. Ultrastructurally, it can be appreciated that the double contours of the GBM are caused by mesangial matrix and cellular

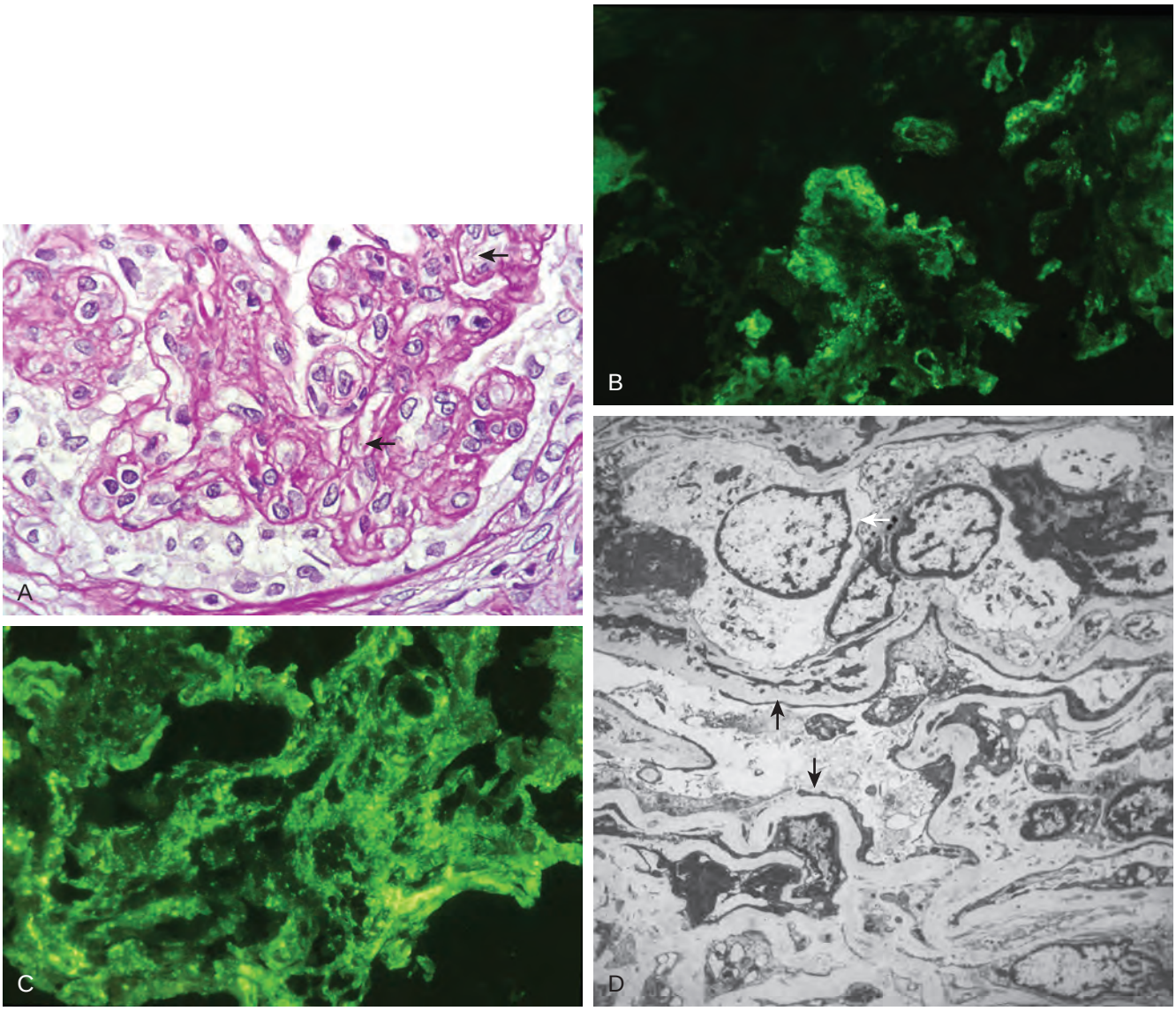
interposition and basement membrane duplication. Subendothelial, electron-dense deposits are appreciated on electron microscopy, and deposits may also sometimes be subepithelial.[77,179,170,173-176,186]

At times, an MPGN-like pattern can be associated with cryoglobulins, particularly in cases of hepatitis C; and in these special cases the glomerulonephritis is referred to as a cryoglobulinemic glomerulonephritis. In cryoglobulinemic glomerulonephritis, "pseudothrombi" composed of the cryoglobulin can be recognized in glomerular capillary lumens, particularly with PAS stains. Glomeruli also have intraluminal hypercellularity, and many of these cells can be identified as monocyte-macrophages with a CD68 immunohistochemical stain. Deposits with a somewhat granular, "wormy" substructure can be seen on electron microscopy (Fig. 16.22).[77,179,170,173-176,186]

Bladder Infections

Bacterial Cystitis

The most common population affected by bacterial cystitis includes women of reproductive age (particularly sexually active women 20 to 40 years old). These infections result most



• **Figure 16.22** Membranoproliferative glomerulonephritis associated with hepatitis C. **A,** Light microscopic appearance of membranoproliferative glomerulonephritis (type I). There is a lobulated, hypercellular glomerulus with basement membrane duplication *(arrows)*, best appreciated on periodic acid–Schiff stains (as shown here) or on silver stains such as the Jones stain. **B,** Immunofluorescence microscopy showing positivity for immunoglobulin (Ig)M in a granular pattern, primarily along the periphery of capillary loops but also in the mesangium. Immunofluorescence is typically positive for IgG and C3, but IgM may also be present. **C,** Immunofluorescence microscopy showing positivity for C3 in a granular pattern, primarily in the mesangium. **D,** Electron microscopy shows intracapillary hypercellularity *(white arrow)* and areas of basement membrane duplication with mesangial matrix and cellular interposition *(black arrows)*, corresponding to the areas of duplication seen on the PAS stain.

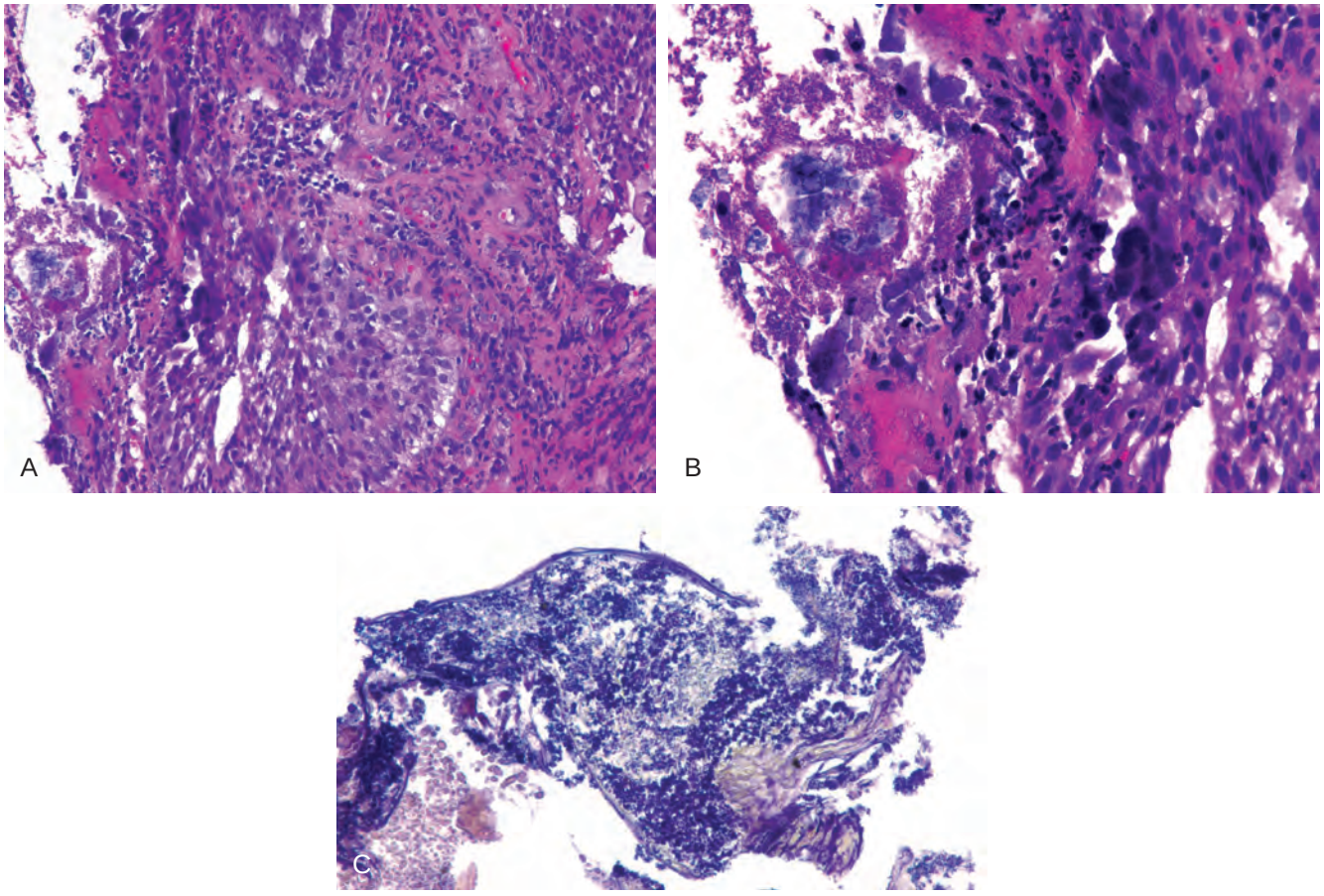
commonly from ascending bacterial infections of the distal urethra and introitus. Recurrent cystitis may occur in patients with structural (e.g., bladder exstrophy) or physiologic abnormalities or systemic disease (e.g., diabetes, immunosuppression). Symptoms include frequency, urgency, dysuria, and abdominal and suprapubic pain. Common organisms include gram-negative enteric bacteria: *E. coli*, followed by *Proteus* (especially *Proteus mirabilis*), *Enterobacter*, and *Klebsiella* and then by various organisms such as *Staphylococcus saprophyticus* and enterococci. Rare cases are caused by mycobacteria and *Actinomyces*. Typically, there is a chronic inflammatory background, although acute inflammation may predominate. Many special histologic patterns, some of which are discussed later in this chapter, may be present. Of note is granulomatous cystitis, which can occur in a number of situations, notably after BCG treatment of transitional cell (i.e., urothelial) carcinoma of the bladder, although concurrent infections with other mycobacteria cannot be excluded (Figs. 16.23 and 16.24).[3,74,187]

Fungal Cystitis

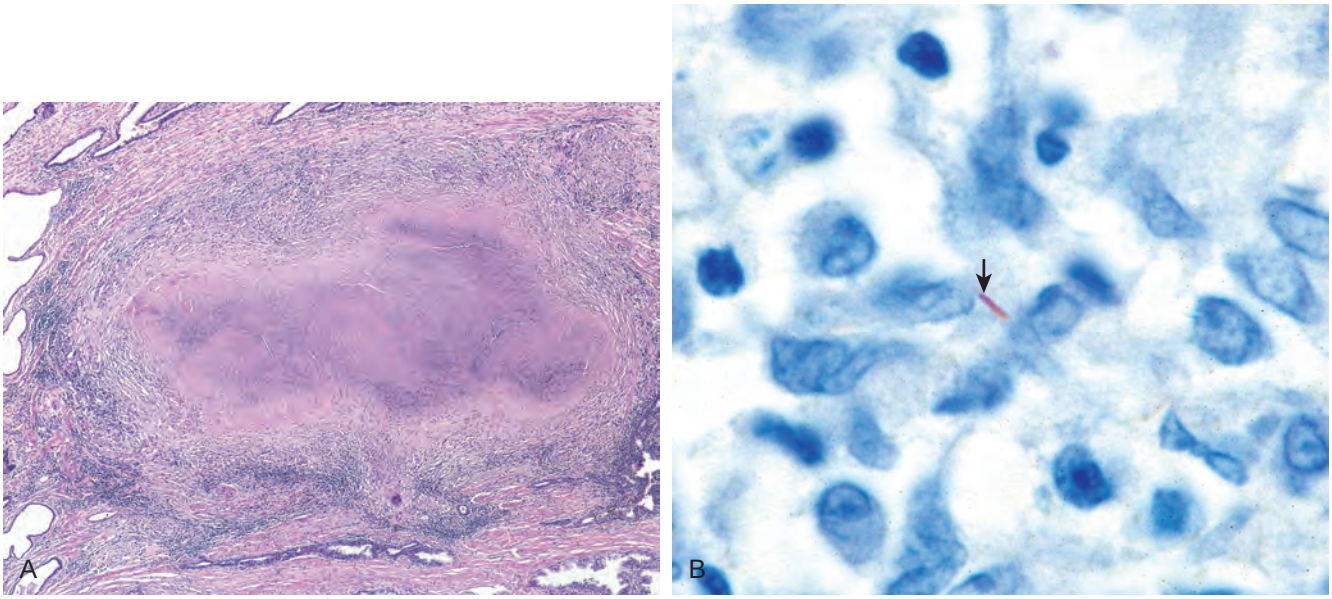
Fungal cystitis occurs in patients with immunosuppression (including those on steroid therapy), indwelling catheters, diabetes, or bladder outlet obstruction. Urinalysis may show pyuria and microhematuria. *C. albicans* is the most common cause. Fungus balls from *Candida* causing obstructive uropathy have been reported. Candiduria may result from noninvasive colonization, low-grade infection, or extensive urinary tract infection. Fungal cystitis occurs less commonly from *Aspergillus*, *Mucor*, *Blastomyces*, *Histoplasma*, *Cryptococcus*, and *Coccidioides*. Microscopically, there is inflammation in the lamina propria, and there may even be ulceration with identifiable fungal forms.[3,74] Special stains, such as silver and PAS stains, may be helpful in identifying fungal pseudohyphae and yeast forms. *Cryptococcus* may have large, clear capsules surrounding yeast forms, which can be seen most readily with India ink preparations or mucicarmine stains.[3,74]

Viral Infections

Cystitis caused by viral pathogens is rare. HSV (notably type 2), CMV, and polyomavirus are DNA viruses that affect the urinary tract most commonly. Adenovirus (specifically types 11 and 21) may lead to a hemorrhagic cystitis, particularly in patients receiving chemotherapy or after bone marrow transplantation.[3,57,74] Hemorrhagic cystitis may also be caused by polyomavirus (BK), CMV, human herpesvirus (HHV)-6, HSV, or *Strongyloides*. Viruses that can be detected in the urine, sometimes by cytologic or molecular methods, include polyomavirus, papillomavirus, and adenovirus type 2. Viral inclusions are more frequently identifiable by cytology than on tissue sections. RNA viruses and oncogenic RNA viruses (Retroviridae) are not thought to be important pathogens.[3,74]



• **Figure 16.23** Encrusted cystitis. **A** and **B,** hematoxylin and eosin-stained sections show inflammation in the bladder lamina propria and necrotic debris covered by fibrin and calcium. **C,** Brown-Hopps tissue Gram stain highlights bacteria in the necrotic debris.



• **Figure 16.24** Granulomatous prostatitis from Bacillus Calmette-Guérin (BCG). **A,** Loose, noncaseating granulomas are seen in various sites of the genitourinary tract, including the bladder, and, as shown here, the prostate. **B,** Acid-fast bacilli *(arrow)* stain positive in a granuloma of the bladder. These would be consistent with BCG infection, with the appropriate history of BCG treatment; however, mycobacterial infection of the bladder (e.g., *M. tuberculosis*) cannot be excluded.

Herpes Simplex Virus

HSV can lead to hemorrhagic cystitis, particularly in patients with genital herpes, and there have also been reports of this condition in immunocompetent patients, although it is more common in the immunocompromised. Cell enlargement, multinucleation, nuclear molding, and ground-glass nuclei are present in a background of acute inflammation, and Cowdry A, eosinophilic, irregular nuclear inclusions may be present. Concurrent BK polyomavirus and acyclovir-resistant HSV infections leading to hemorrhagic cystitis have been reported.[3,188]

Cytomegalovirus

CMV, a member of the herpesvirus family of double-stranded linear DNA viruses, can lead to hemorrhagic cystitis in patients who are immunosuppressed, such as AIDS patients and those receiving chemotherapy for bone marrow transplantation. Urine culture is not sensitive for the diagnosis of CMV in this setting. CMV-infected cells may appear like the infected renal tubular cells shown in Fig. 16.6. Intracytoplasmic inclusions can also occasionally be found.[3,51]

BK Polyomavirus

Polyomavirus, the renal disease manifestations of which have already been discussed, is usually acquired at an early age. Clinical manifestations of polyomavirus occur only with immunosuppression, such as after bone marrow transplantation, and can include cystitis and hematuria. Urine cytology can detect polyomavirus, and the cytopathic effect may persist for several months after cessation of symptoms. Recognition of polyomavirus cytopathic effect in routine surgical pathology histologic sections is more difficult than on cytology. Polyomavirus is implicated in BK nephropathy, which typically occurs in the setting of transplantation or immunosuppression and is discussed later.[3,189-191]

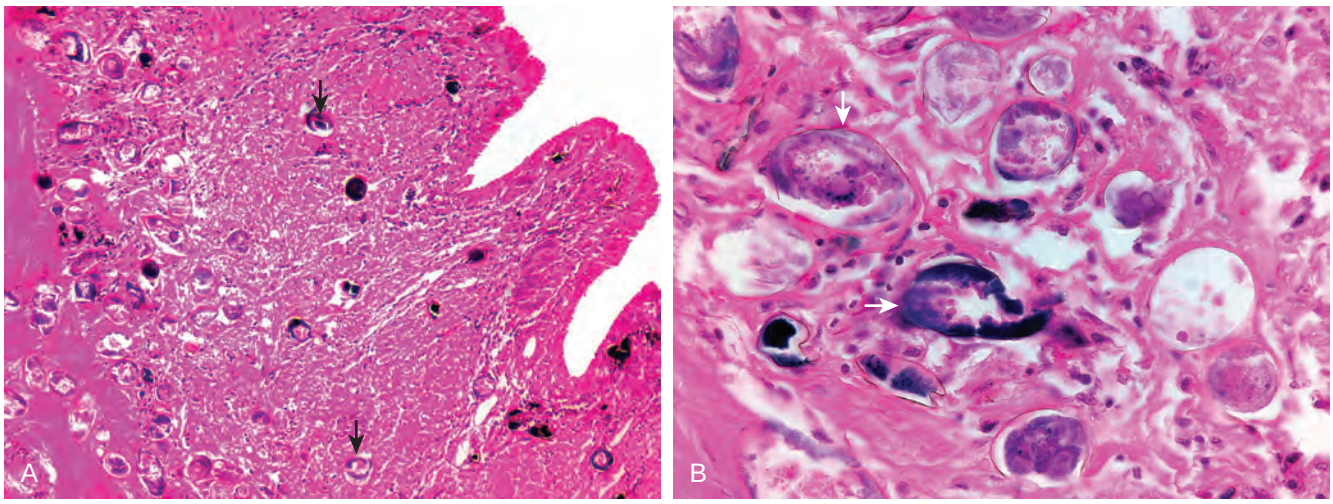
Human Papillomavirus

HPV leads to many types of lesions, both neoplastic and nonneoplastic (see Table 16.2). In men, it is the causative agent of condyloma acuminatum. Bladder condylomas may occur when anogenital condylomas extend into the urethra and bladder. Bladder condylomas are more common in women. HPV types 6 and 11 have been found in bladder condylomas. Despite the well-known role of HPV in cervical carcinogenesis, it is controversial whether HPV contributes to all urothelial carcinomas[192,193]; however, it is possible that it plays a role in urothelial carcinogenesis, particularly in the immunosuppressed. Grossly, lesions may be exophytic and may give rise to squamous cell carcinoma (see Fig. 16.5). Histologically, condyloma in the bladder consists of papillary fronds of thickened squamous epithelium that may be hyperkeratotic. Koilocytes with perinuclear halos are often present in many of the epithelial cells. Koilocytes can be detected in urine samples. Cytology of condylomas obtained through such methods as brush cytology show a higher yield than urine cytology; however, condylomas of the external genitalia and urethra should be excluded before a diagnosis of a bladder condyloma is made.[3,191]

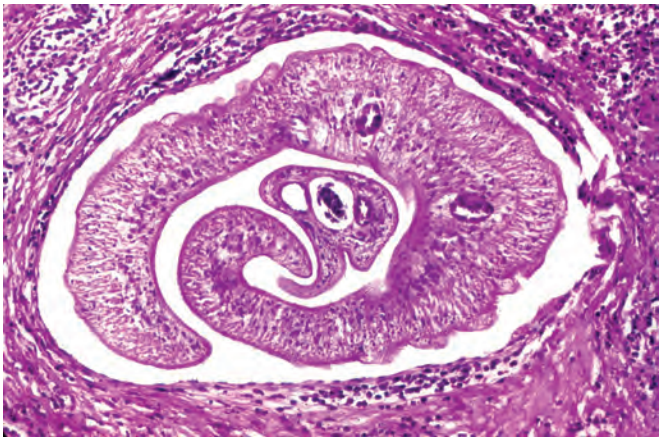
Parasitic Infections

Schistosomiasis

S. hematobium is endemic to Mediterranean Asia and Africa. It inhabits the pelvic veins and sheds its eggs into the urinary bladder, causing hematuria or chronic cystitis. Urinary egg excretion has diurnal periodicity: shedding is most prominent at midday. *S. hematobium* is recognized by its terminal spine (Fig. 16.25). Rarely, adult "worm" forms may be recognized. These forms usually are paired male and female organisms and usually reside in the venous plexus of the bladder (Fig. 16.26). Histologically, there is typically a chronic inflammatory background.



• **Figure 16.25** Schistosomiasis of the urinary bladder. **A,** Medium-power image shows schistosomal eggs *(arrows)* in the lamina propria of the bladder with a chronic inflammatory background. **B,** High-power view of schistosomal eggs *(arrows)*.



• **Figure 16.26** Schistosomiasis of the urinary bladder in worm form. The schistosomal organisms in this form usually reside in the venous plexus of the bladder and are present paired as adult male and female worms. (Courtesy Dr. Robert H. Young.)

Bladder biopsies may show urothelial squamous and intestinal metaplasia, mural granulomas, fibrosis, and calcified eggs. Chronic infection may eventually lead to fibrosis and calcification of the lower ureters, with resultant renal parenchymal damage and kidney failure. Urothelial squamous cell carcinoma may eventually develop, and this is particularly a problem in certain parts of the world where the infection is endemic, notably Egypt and other areas of Africa. Although they are typically found in the stool, *S. mansoni* eggs (identified by their characteristic lateral spines) may also sometimes be found in the urine.[3,166]

Trichomoniasis

Trichomonas can cause urethritis and cystitis, particularly if there is a coexisting genital trichomoniasis. After HPV, *Trichomonas* is probably the most common sexually transmitted infection in the United States, so recognizing this entity is important. *Trichomoniasis* is increasingly being acknowledged as a cause of nongonococcal urethritis and as a contributor to male factor infertility. Diagnosis is typically established by urine cytology.[3,194-199]

Special Bladder Conditions With an Infectious Association

Malakoplakia

Malakoplakia is a vaguely granulomatous, chronic inflammatory disease that primarily affects the urinary tract, particularly the bladder and ureters, but also other organ systems. Middle-aged women are most commonly affected. Urine culture often isolates *E. coli*. Submucosal aggregates of large histiocytes (von Hansemann cells) containing cytoplasmic Michaelis-Gutmann bodies, thought to consist of mineralized bacterial fragments, are present.[3,200]

Encrusted Cystitis

Encrusted cystitis is a condition in which inorganic salts (e.g., calcium) are deposited in the urinary bladder in areas of urothelial mucosal injury from urea-splitting bacteria, which alkalinize the urine. Necrotic debris covered with fibrin and calcium is deposited in the lamina propria (see Fig. 16.23).[74] Common causative organisms include bacteria with potent urease activity, such as *Corynebacterium* (particularly group D2, now known as *Corynebacterium urealyticum*), but many organisms have been reported. A substantial number of patients have been immunocompromised, particularly after renal transplantation. Urine may contain abundant calcified, mucopurulent debris. The main therapy is antibiotic treatment, as well as acidification of the urine. Excision of the encrusted plaques may be required. Graft nephrectomy has been required in some cases.[201,202]

Emphysematous Cystitis

Emphysematous cystitis is an inflammatory condition of the urinary bladder in which gas-filled cystic spaces (called pockets or blebs) are formed in the wall of the bladder. These can be appreciated grossly or by cystoscopy. This condition is more common in women and diabetics and is usually caused by the *E. coli. Enterobacter aerogenes* (formerly called *Aerobacter aerogenes*), *K. pneumoniae*, *Enterococcus*, *Candida*, and *Clostridium perfringens* are other offending organisms. Predisposing conditions include neurogenic bladder, chronic urinary infection, trauma, instrumentation, urinary stasis, and fistula formation. Microscopically,

there are empty cavities in the lamina propria lined by attenuated cells, including foreign body giant cells.[74,203,204] Urinary obstruction may result, requiring surgical intervention.[203,205]

Infections of the Urethra

Infections that affect the urinary bladder can affect both the male and female urethra, and vice versa. Infections often ascend the urethra to affect the bladder, particularly in women of reproductive age. In this process, bacterial cystitis usually results from bacterial infection that ascends from the introitus and distal urethra. The most common organisms are gram-negative enteric bacteria (*E. coli*, followed by *Proteus*, *Enterobacter*, and *Klebsiella*).[3]

Gonorrhea, caused by *Neisseria gonorrhoeae*, is the second most commonly reported notifiable disease that is an important cause of urethritis.[206] Nongonococcal urethritis is typically due to *Chlamydia* or *Mycoplasma* infection.[207,208] Malakoplakia can rarely involve the urethra.[209]

Urethritis is part of the classic triad—urethritis, conjunctivitis, and arthritis—that is seen in Reiter syndrome, also known as reactive arthritis, a condition of uncertain pathogenesis.[3,206-209]

Infections of the Male Genitourinary System

Prostate

Acute and chronic prostatitis are both relatively common clinical problems in routine urologic practice, but they rarely require surgery and are usually treated empirically with antibiotics.[210,211] Histologically, acute bacterial prostatitis usually shows abundant neutrophils surrounding acini, desquamated cellular debris, hyperemia, stromal edema, and sometimes microabscess formation.[210] It is impossible to distinguish chronic infectious prostatitis from nonspecific chronic inflammation seen in benign prostatic hyperplasia.[210] In the case of diffuse lymphocytic infiltrate of the prostate, the possibility of chronic lymphocytic lymphoma should be considered. Malakoplakia rarely involves the prostate; its appearance is similar to malakoplakia seen in other areas of the urinary tract.[107,210,212,213]

Mycotic prostatitis is usually caused by blastomycosis, coccidioidomycosis,[214,215] or cryptococcosis. Histoplasmosis, paracoccidioidomycosis, aspergillosis, and candidiasis have also been reported. The most common setting is the infection of an immunocompromised patient via a hematogenous route.[210,216]

Tuberculous prostatitis occurs in 3% to 12% of patients with disseminated tuberculosis. More than 90% of patients with tuberculous prostatitis have lung involvement. Surgical pathology specimens of tuberculous prostatitis are not seen as frequently as they once were, primarily because the incidence of tuberculosis has decreased. Tuberculous involvement may still be seen in the setting of BCG immunotherapy for superficial transitional cell carcinoma of the bladder. Granulomas from BCG may show positivity with acid-fast stains, as in the bladder (see Fig. 16.24); they diffusely involve the prostate, may be small and noncaseating, but can also be large and caseating.[187,210] Abscesses may occur.[217] Patients may have abnormal digital rectal examination findings or increased prostate-specific antigen levels, or both, after BCG therapy.[218] Acid-fast stains can be used to demonstrate the bacteria, but the use of stains to identify the organisms is not typically recommended.[187,210,217,219-221] Mycobacteria other than tuberculosis (MOTT) and atypical mycobacteria, such as *Mycobacterium fortuitum*, *M. kansasii*, and *M. avium* complex, may also infect the prostate. Histologically, these mycobacteria give rise to a granulomatous prostatitis similar to that seen with other mycobacteria. Acid-fast bacterial stains may be useful in establishing the diagnosis, although the concurrent presence of BCG or other mycobacterial organisms cannot always be excluded.[187,210,222-225]

Other infections that affect the prostate include brucellosis,[226] syphilis, actinomycosis,[227] *Cryptococcus*,[228] amebic prostatitis,[229] schistosomiasis (which has been associated with cases of prostatic adenocarcinoma),[230-233] echinococcosis,[234] CMV,[235] and herpes zoster.[236]

The topic of prostatitis and its relationship to neoplasia has been the subject of a great deal of research.[237-242]

Epididymis

Epididymitis is said to be the most common intrascrotal inflammatory process.[243] *C. trachomatis* and *N. gonorrhoeae* are the most frequent causes of scrotal swelling due to epididymitis in sexually active men younger than 35 years of age. Epididymitis is associated with urinary tract infections, and in that setting bacterial infections ascend via the urinary tract or lymphatics. Infections may also be spread hematogenously, leading to a number of bacterial infections of the epididymis and testis.[211,244]

Acute epididymitis consists of an enlarged epididymis that microscopically shows suppurative inflammation with fibrin covering. The epididymal duct may contain pus and may rupture, producing sperm granulomas, as classically described by Glassy and Mostofi.[245] Chronic epididymitis consists of a fibrotic process with a narrowed epididymal duct and a variable number of chronic inflammatory cells.[246]

CMV epididymitis has been reported in an immunosuppressed patient after renal transplantation and in patients with AIDS. Cytologic changes were similar to those typically seen with CMV.[247,248]

Testis

Bacterial

Bacterial infections usually spread to the testis from the epididymis, leading to epididymo-orchitis. Hematogenous and lymphatic orchitis can be caused by *E. coli*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Salmonella*,[249-251] *Pseudomonas aeruginosa*, *Brucella*, *Listeria monocytogenes*, and *Actinomyces*. Significant destruction of the testicular parenchyma may occur. Infections in neonates can occur, and mixed anaerobic and aerobic abscesses in neonates have been reported. There are a number of histologic patterns, including malakoplakia, which has virtually the same histologic appearance as in other sites (see Fig. 16.11).[244,252-255]

Mycobacterial

M. tuberculosis infection can secondarily infect the testis, leading to a granulomatous orchitis. Spread to the testis is hematogenous or via the epididymis, which can be enlarged and dilated. There is usually bilateral involvement. Scrotal fistulas may occur. Grossly (Fig. 16.27) and microscopically, caseating or noncaseating granulomas are observed, similar to those seen in other parts of the body; however, granulomatous orchitis (Fig. 16.28) has a number of causes, including autoimmune disorders and nonspecific granulomatous orchitis, a disorder of unknown but probably infectious etiology.[256-258] An infiltrate of mononuclear lymphoid cells may

be present. Acid-fast bacilli can be identified with the aid of special stains. The testis may also be infected by nontuberculous organisms, such as *M. avium-intracellulare* and *M. kansasii*.[244,258-263]

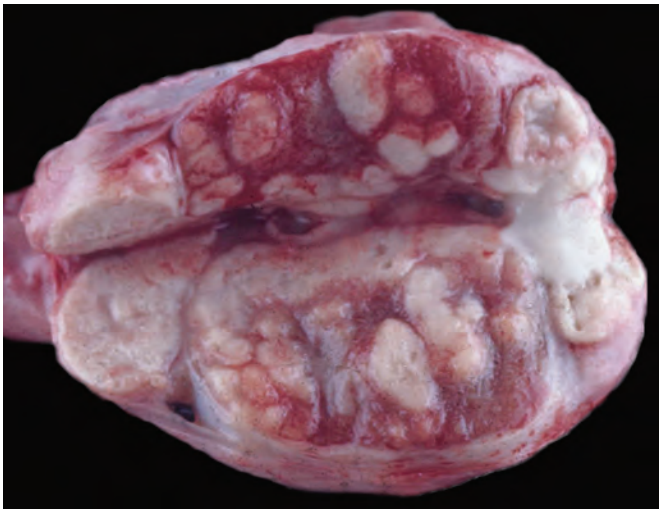
Testicular infection by *M. leprae* is more common in other parts of the world than in the United States. The infection can be divided into three phases. First, there is a vascular phase, in which cells packed with acid-fast bacilli, known as lepra cells, are observed in the interstitium and blood vessels. Second, there is an interstitial phase, with interstitial fibrosis, obliterative endarteritis, Leydig cell clusters, and variable numbers of histiocytes containing acid-fast bacilli (Fig. 16.29). Spermatogenesis may be reduced. Third, there is an obliterative phase, with complete fibrosis, severe reduction to complete loss of seminiferous tubules, diminished vascularity, and rare acid-fast bacilli. Fite stains can be useful to identify the organisms.[244,264-266] Cytology of semen or testicular aspirates may be useful in the diagnosis of testicular leprosy.[267] Involvement of the testis by leprosy can lead to reduction in testicular volume and hypogonadism, gynecomastia, oligospermia or azoospermia, and infertility.[244,264,268]

Fungal

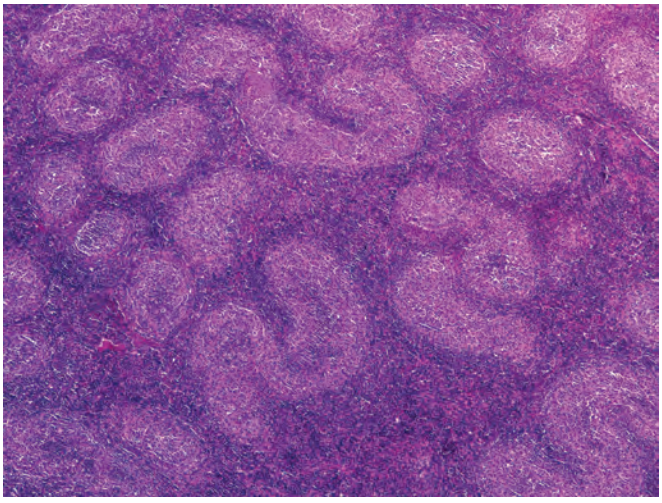
Fungal infections can result in epididymo-orchitis. Organisms such as *Candida*, *Blastomyces*, *Actinomyces*, *Aspergillus*, *H. capsulatum*, *Trichophyton mentagrophytes*, and *C. immitis* have been implicated. Notably, *Aspergillus* has been reported in a renal transplant recipient.[269] The histologic appearance can be variable and usually is that typical of the fungal infection; however, the histologic findings can be unique.[244,268-271] A case of *H. capsulatum* has been reported that gave the appearance of sperm granulomas.[271]

Viral

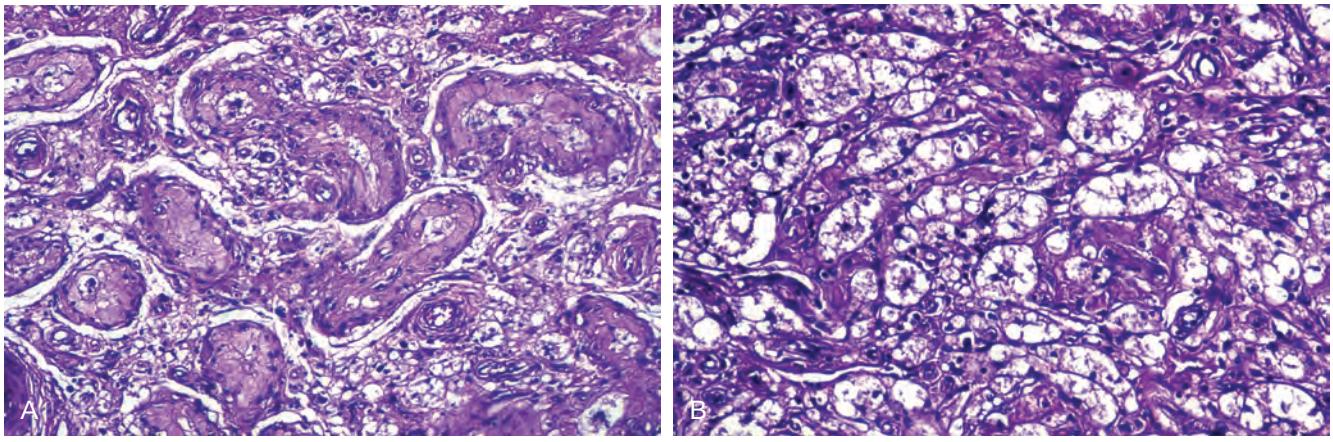
A number of viral infections can involve the testis, including mumps, variola, varicella, EBV, echovirus, lymphocytic choriomeningitis virus, group B arbovirus, influenza, West Nile virus, dengue fever, sandfly fever, and Coxsackie B virus.[244,272-274] A case of West Nile virus orchitis has been reported in a renal transplant recipient.[274]



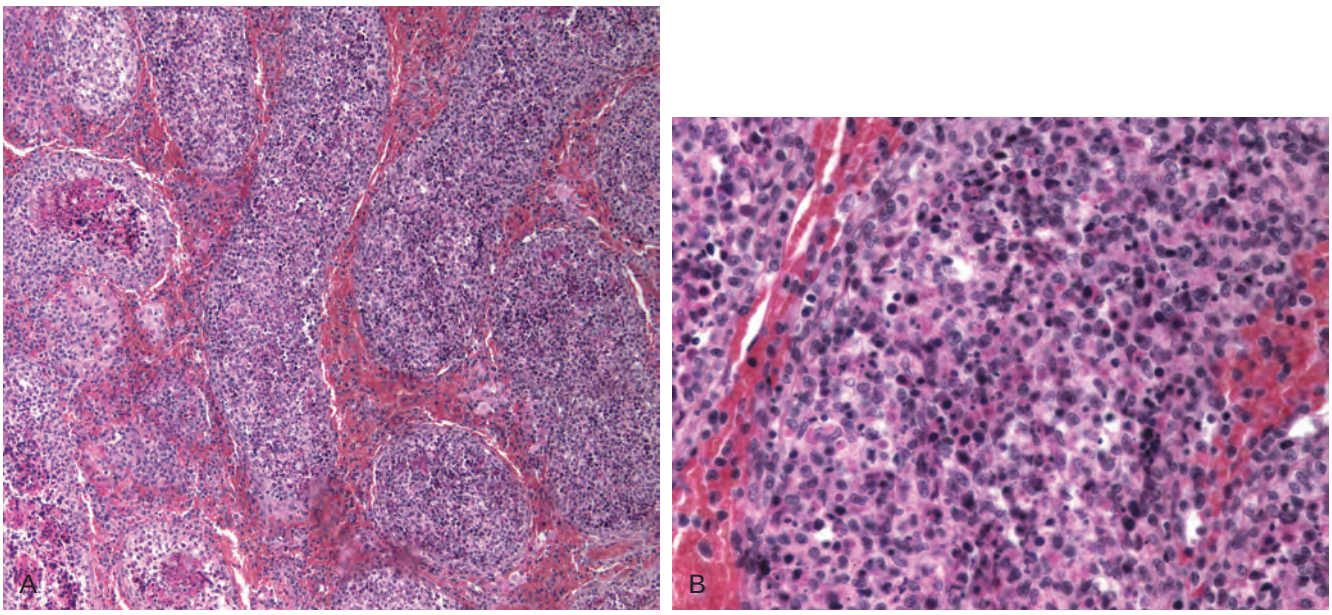
• **Figure 16.27** Tuberculous orchitis. Gross image of tuberculous orchitis heterogeneously replacing the testis parenchyma with areas of caseous necrosis. (Courtesy Dr. Robert H. Young.)



• **Figure 16.28** Granulomatous orchitis. This pattern can be seen in a number of conditions, including mycobacterial infections of the testis. Granulomas can be caseating or noncaseating.



• **Figure 16.29** Testis with leprosy. **A,** Medium power shows atrophy and obliteration of the seminiferous tubules with fibrosis of the interstitium. **B,** High power shows obliteration and loss of the seminiferous tubules and histiocytes, which may contain acid-fast bacilli. (Courtesy Dr. Robert H. Young.)



• **Figure 16.30** Mumps orchitis. **A,** Low-power view shows necroinflammatory debris in the seminiferous tubules. **B,** Medium power shows degeneration of the seminiferous tubular epithelium and vascular congestion. (Courtesy Dr. Robert H. Young.)

Mumps. Mumps is a member of the paramyxovirus group, which are spherical, single-stranded RNA viruses. It was once an important cause of orchitis, with 20% of postpubertal males infected with the virus developing orchitis, usually unilaterally. Mumps orchitis is now rare in developed countries due to immunization[51,275,276]; however, cases still persist among patients who have not been immunized, and there have been outbreaks in the United States and the United Kingdom.[277] Rare cases of orchitis have been reported with immunization.[211,243,278-280] Histologically (Fig. 16.30), edema and inflammation of the tunica albuginea, edema and inflammation with lymphoid aggregation of the interstitium, and congestion of the tunica vasculosa are observed. Inflammation is usually mixed, with lymphocytes, histiocytes, plasma cells, and neutrophils. Necrosis of spermatogenic cells occurs, leaving only Sertoli cells, which may also eventually disappear from the tubules. Infertility may result. Orchitis rarely occurs prepubertally. Atrophy develops in approximately one-third of patients but infertility in only approximately 2%.[244,281,282] Healed orchitis usually shows little or no inflammation, with foci of tubular sclerosis and decreased numbers of germ and Sertoli cells.[244]

Human Immunodeficiency Virus. The testes are noticeably affected in AIDS. Although the effects may not be appreciated clinically, autopsy studies have demonstrated a generalized atrophy with decreased spermatogenesis, tunica propria thickening, interstitial fibrosis, tubular hyalinization, and interstitial inflammation with reduced numbers of germ and Leydig cells.[283-287] It has been suggested that prolonged survival with antiviral therapy has resulted in a shift toward greater loss of germ cells. Characterization and quantitation of the testicular cells, particularly the germ cells, is thought to be important because the testis is believed to be an important location for sexual transmission of the virus.[286,287]

In addition, the testis can be involved by numerous opportunistic organisms in AIDS patients, including *M. tuberculosis*[288] and *Toxoplasma.* AIDS is also a predisposing factor for testicular neoplasia.[289-298]

Other Diseases

Orchitis has been described in patients with Q fever or typhus.[244,299] Typhus usually displays a vasculitis composed of swollen capillary endothelial cells containing large numbers of organisms and accompanying vascular thrombi.[244]

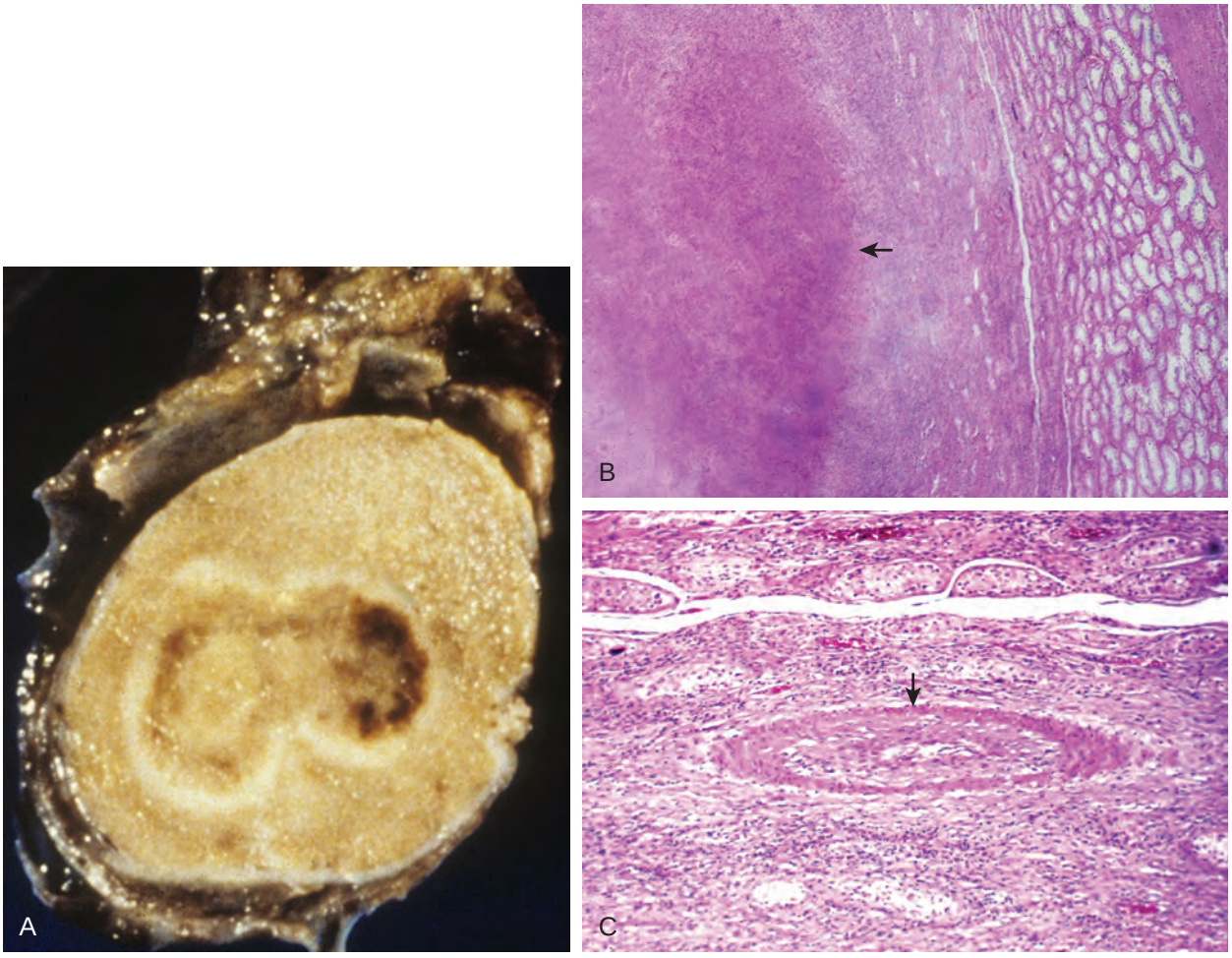
Syphilis can lead to orchitis. Fibromatous and gummatous stages have been described. An enlarged, irregular, nodular testis is usually present in the gummatous form, and discrete gummas can be seen grossly. The gummas (Fig. 16.31) contain coagulative necrosis with a peripheral zone containing a mixed inflammatory infiltrate of lymphocytes, plasma cells, and occasional histiocytes, some of which may be the giant cell form.[300] An obliterative endarteritis may be present. The fibromatous form consists of a painless, enlarged testis with peritubular inflammation and fibrosis with decreased numbers of germ cells.[244,301] Spirochetes cannot usually be identified in the fibromatous stage, but they may be present in the gummatous stage. PCR on tissue may be required to make the diagnosis.[302]

Penis and Scrotum

The penis and scrotum may be affected by genitourinary cutaneous infections, as previously discussed. In addition, there are some special infections of this region.

Bacterial Infection

The population most affected by bacterial infection of the penis and scrotum is uncircumcised male newborns, who develop inflammation of the mucosa of the glands (balanitis) and prepuce (posthitis).[3,29,303] Bacteria can more readily adhere to and colonize the mucosal surface of the prepuce, making balanoposthitis more common in uncircumcised men.[29,303] A nonspecific inflammatory infiltrate is usually found. Uncommonly, balanoposthitis may be caused by *Gardnerella vaginalis* or *Trichomonas*, and gonococcal infections have been reported to cause median raphe cysts and



• **Figure 16.31** Syphilis involving the testis. **A,** Gross image of testis involved by syphilis, showing a characteristic gumma with caseating necrosis. **B,** Medium-power image of syphilitic gumma showing caseating necrosis *(arrow)*. **C,** Medium-power image of vasculitis caused by syphilis involving the testis *(arrow)*. (Courtesy Dr. Robert H. Young.)

deeper soft tissue infections of the penis. These infections are usually transmitted sexually. Pediatric cases are caused by chronic, mixed bacterial infections, and perianal infections can occur, leading to perianal streptococcal cellulitis. Localized infections can spread and result in cellulitis, which usually is caused by streptococcal organisms. Severe cases may lead to necrotizing fascitis, which in this region is referred to as Fournier gangrene and often involves the dartos and penile fascia. Previous trauma, burns, diabetes, leukemia, and alcoholic cirrhosis may be predisposing conditions.[3,29]

The penis is often the site of primary syphilis in males, which manifests as a solitary papule that typically ulcerates and forms an indurated base, known as the chancre. The chancre is usually on the inner prepuce, coronal sulcus, penile shaft, or penile base. Additional details of primary, secondary (condyloma lata), and tertiary syphilis were described earlier in this chapter.[29]

Mycobacterial infections can affect the penis; however, this is now rare. Tuberculosis involving the penis can result from direct spread from adjacent infection or from hematogenous dissemination. Microscopically the appearance is that of mycobacterial infections in other sites.[29]

As previously discussed, granuloma inguinale *(K. granulomatis)* (see Fig. 16.1) and chancroid *(H. ducreyi)* may affect the penis in the same manner as in the genitourinary skin.[29] Granuloma inguinale is usually present on the prepuce, glans, penile shaft, or scrotum. Satellite lesions may be present and are referred to as pseudobuboes.[29]

Fungal Infection

Superficial fungal (mycotic) infections of the penis are the most common mycotic infections. Dermatophytes commonly affect the penis and often spread from other areas of the groin. Another common superficial mycotic organism that affects the penis is *C. albicans*, which is present in 15% to 20% of men. This infection is considered to be sexually transmitted and may be a source of infection and reinfection in candidal vaginosis.[29,304]

Deep penile mycotic infections are rare. An example causative organism is *C. neoformans*.[29,304]

Parasitic Infection

Lymphogranuloma venereum, caused by an obligate intracellular parasite, was discussed previously. It may also be present with

lymphadenitis with bubo formation. Scabies can also affect the penis.[29]

Viral Infection

As previously discussed in the section on infections of the genitourinary skin, the penis can be affected by herpes, molluscum contagiosum, and HPV or HPV-associated lesions (see Table 16.1).

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17

Gynecologic Infections

ROSEMARY TAMBOURET

Lower Genital Tract
Vulva and Vagina
Cervix and Vagina
Upper Genital Tract
Pelvic Inflammatory Disease
Endometrium
Fallopian Tube and Ovary

Because the female genital tract comprises both cutaneous and mucosal surfaces in contact with the external environment, exposure to a significant number of infectious agents, either commensal or pathogenic, is the norm. And because the lower genital tract is in communication with the normally sterile peritoneal cavity, ascending infections can be treacherous. In addition, close physical contact with sexual partners can lead to infectious sexually transmitted disease (STD), especially if the woman is exposed to multiple sexual partners. Since the first edition of this book, knowledge of the mirobiome of the female genital tract has greatly expanded by use of molecular techniques; the work has been supported by the National Institutes of Health Human Microbiome Project (HMP). The work has demonstrated the significant microbial diversity among women.[1] The external genitalia and the lower female genital tract are the sites of the majority of infections, which are often transitory and without serious sequelae. Most of these infections are recognized clinically without need of biopsy and are treated on the basis of the clinical presentation. However, biopsy is warranted if the prescribed treatment is not effective or if the presentation is atypical. In addition, routine cervical cytology specimens (the Pap smear), used to screen for malignant and premalignant lesions, regularly identify a limited number of potential pathogens.

The upper genital tract can be the site of silent infections with serious secondary effects, such as infertility. In the case of suspected infection, the endometrium is readily amenable to biopsy, but the fallopian tubes and ovaries are usually examined only if significant disease is present.

Immunocompromised patients often experience more severe infections or infections due to organisms not previously associated with disease. The patients most affected include transplant recipients, oncology patients, and patients with acquired immunodeficiency syndrome (AIDS).

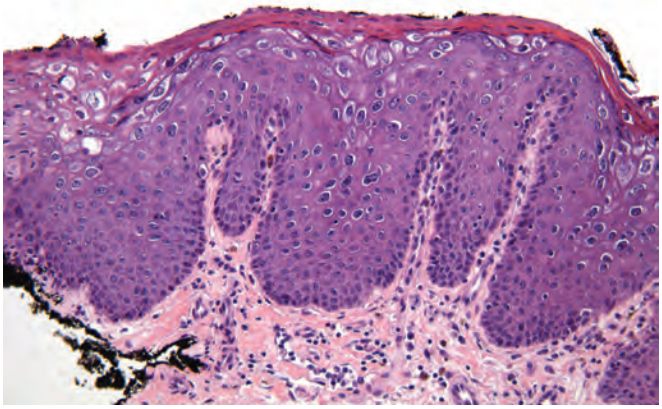
Most clinical reviews of infections of the female genital tract emphasize the anatomic site and clinical manifestations, with an added emphasis on the clinical appearance of lesions arising in the lower genital tract and the appearance on radiologic imaging for infections in the upper genital tract. For example, herpes infection of the cervix often causes small vesicles that evolve into shallow ulcers. Where appropriate, the clinical or radiologic appearance of the lesion is mentioned in the text here.

Lower Genital Tract

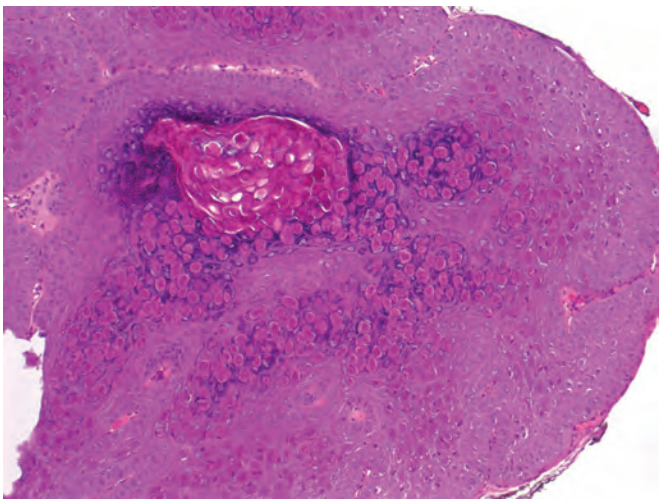
Vulva and Vagina

Viral Infections

Genital infection by human papillomavirus (HPV) is the most common sexually transmitted infection in the United States. More than 100 HPV types have been identified, at least 40 of which infect the genital tract.[2] The HPV genotypes are divided into low-risk and high-risk types depending on their oncogenic potential. All HPV types are epitheliotropic obligate intracellular viruses that promote cell proliferation in normally quiescent, more differentiated epithelial cells to produce a hyperplastic epithelial lesion. Low-risk HPV, most often types 6 and 11, produce exophytic genital warts or condyloma acuminata, usually found on the perineum and labia and, with less frequency, on the vagina, cervix, and anal region. High-risk HPV, such as HPV-16 and HPV-18, cause preneoplastic intraepithelial lesions in the lower genital tract, most often in the cervix and vagina; the oncogenic risk is highest in persistent infections. On the external genitalia, condyloma acuminata appear as exophytic, smooth to somewhat rough, flesh-colored papules, either single or multiple, that are not usually biopsied before treatment.[3] However, because cervical or vaginal lesions often accompany condyloma acuminata of the external genitalia, a careful vaginal and cervical examination should be done, usually with a speculum examination after application of acetic acid, which causes an acetowhite appearance of the HPV-induced lesions due to coagulation of cytokeratins of infected squamous cells.[4] If the condyloma acuminatum is biopsied, the histologic findings include acanthosis, parakeratosis, and hyperkeratosis, which may be associated with a branching papillomatous architecture with fibrovascular cores (Fig. 17.1),[5] but the hallmark is the HPV viral cytopathic effect, known as koilocytosis, which corresponds to a cavitation of the cytoplasm of the infected cell along with nuclear hyperchromasia and enlargement with membrane irregularity. Koilocytosis occurs as a result of the vegetative cycle of the HPV and is due to the accumulation of



• **Figure 17.1** Condylomatous lesion of the vulva with viral cytopathic effect (koilocytosis) of human papillomavirus infection (hematoxylin and eosin stain, ×20).

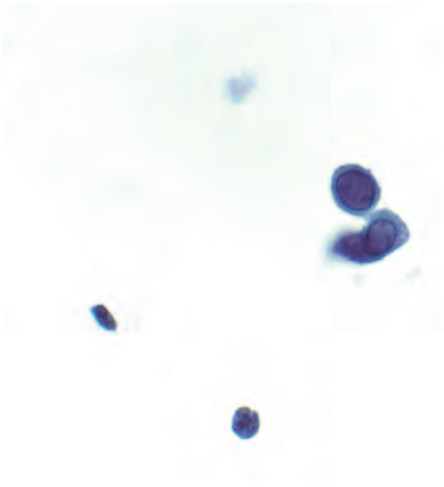


• **Figure 17.2** Molluscum contagiosum viral cytopathic effect on squamous epithelium of the vulva (hematoxylin and eosin stain, ×40).

infective virions in the nucleus with disruption of cytoplasmic keratin in infected squamous cells.[6,7] Koilocytosis is less pronounced in lesions of the perineum, vulva, and vagina than in lesions of the cervix.[8,9] Diagnosis of HPV infection can be confirmed on tissue samples by molecular testing for HPV DNA or RNA; the HPV cannot be cultured and serologic testing is not routinely performed.

Molluscum contagiosum is caused by a chronic, localized infection with poxvirus that causes flesh-colored, rounded, raised papules with an umbilicated center.[10] Diagnosis is usually based on the clinical appearance, but if a biopsy is performed to rule out other clinically similar lesions, such as those caused by cryptococcosis or histoplasmosis, the keratinocytes are found to contain large, eosinophilic cytoplasmic viral inclusions (Fig. 17.2).[11,12]

Herpes simplex virus (HSV) is common and often subclinical (manifested only by the serum antibody response), so the incidence of disease is difficult to determine.[13] Both HSV type 1 and type 2 cause genital HSV infections. HSV infections are categorized as primary if the patient has no preexisting antibodies to HSV-1 or HSV-2; as nonprimary if a new infection arises in a patient with antibody evidence of the other HSV type; or as recurrent if there is serologic evidence of the same type as found in the reactivated infection. In a survey of adults in New York City, 28% were found to be infected with HSV-2 but only 12% were aware that they were infected.[14] The most severe symptoms tend to occur during primary infection and include genital vesicles with subsequent ulcer formation, fever, and regional lymphadenopathy. Clinical diagnosis of HSV infection is confirmed by viral culture, polymerase chain reaction (PCR), direct fluorescence antibody, and type-specific serologic tests.[15] PCR and serology are more sensitive than culture.[16,17] If need be, a genital ulcer can be scraped to obtain material for cytology (Tzanck preparation) or biopsied. The Tzanck smear, reported to have a low sensitivity and specificity for HSV cytopathic effect, is prepared by scraping the base of a fresh ulcer (Fig. 17.3).[18] The slide may be air dried and stained with Wright-Giemsa stain, or the cells may be fixed with alcohol and stained with Papanicolaou stain. HSV-infected epithelial cells show either a homogenization of the chromatin (ground-glass appearance) or clumping of the chromatin on the

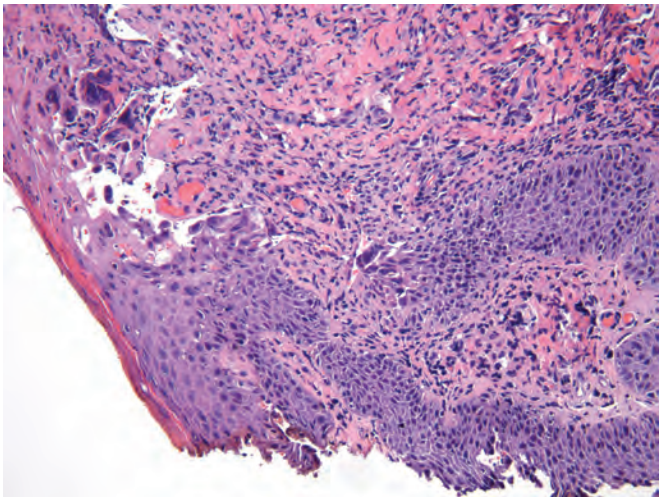


• **Figure 17.3** Tzanck-stained smear of vulvar ulcer showing ground-glass nuclear herpes simplex virus cytopathic effect in epithelial cells (Papanicolaou stain, ×100).

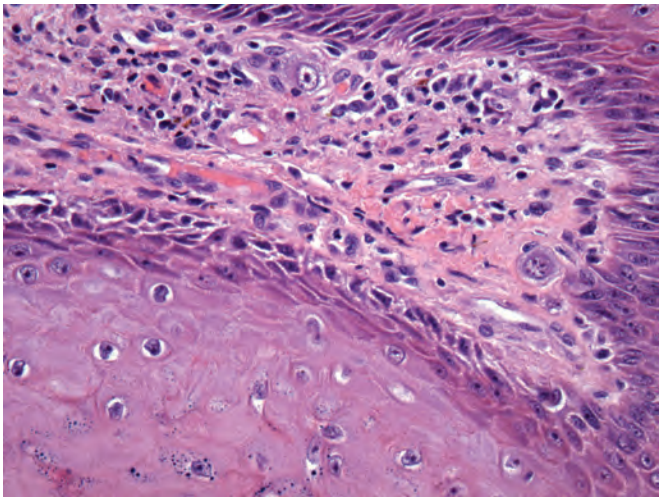
nuclear membrane and in the center of the nucleus. The infected cells are often multinucleated. Biopsy is most informative if an intact vesicle is sampled. The intraepidermal vesicle shows degenerating keratinocytes; intranuclear viral cytopathic changes are most easily found on the margin of the vesicle. With ulceration, the changes are less specific and include chronic inflammation and granulation tissue (Fig. 17.4).

HSV-2 infection in HIV-infected women may produce hypertrophic vulvar lesions that may mimic a neoplasm.[19] Biopsies to demonstrate viral cytopathic effect and culture are necessary to diagnose this uncommon condition.

Rarely, primary infection with Epstein-Barr virus (EBV) can be accompanied by genital ulcers that clinically mimic the more common ulceration caused by HSV (Table 17.1).[20] Ulceration due to EBV should be considered in young patients who have painful ulcers, lymphadenopathy in a region distant from the ulcer, and systemic symptoms. An EBV etiology should be



• **Figure 17.4** Vulvar biopsy with vesicle secondary to herpes simplex virus cytopathic effect in squamous cells (hematoxylin and eosin stain, ×20).



• **Figure 17.5** Cytomegalovirus cytopathic effect in biopsy of vulva (hematoxylin and eosin stain, ×40).

TABLE 17.1 Genital Infections Presenting as Ulcers

Organism (Disease)	Gross Characteristics
HSV	Shallow painful ulcers, initially pruritic focus evolves to blister
EBV	Ulcer similar to herpes
CMV	Ulcer similar to herpes
Treponema palladium (chancre of primary syphilis)	Painless ulcers
Chlamydia trachomatis (LVG)	Shallow, painless ulcer
Haemophilus ducreyi (chancroid)	Begins as nodule, evolves to pustule, then to painful ulcer
Klebsiella granulomatis (granuloma inguinale)	Painless ulcers evolve to hidradenitis
Escherichia coli (malakoplakia)	Papule or plaque evolving to ulcer

CMV, Cytomegalovirus; *EBV,* Epstein-Barr virus; *HSV,* herpes simplex virus; *LVG,* lymphogranuloma venereum.

considered if bacterial and viral cultures, as well as the results of syphilis serology studies, are negative.

Cytomegalovirus (CMV) infection of the vulva manifests with ulceration.[21,22] Characteristic viral intranuclear and intracytoplasmic inclusions are identified in epithelial cells and endothelial cells (Fig. 17.5).

Bacterial Infections

Erythrasma, an uncommon infection of the vulva caused by *Corynebacterium minutissimum*, is usually diagnosed by fluorescence of the bacteria under the Wood lamp.[23] Rarely, Wood lamp examination is negative, but the diagnosis can be made histologically.[24] The clinical presentation is similar to vulvar candidiasis, but, on biopsy, filamentous bacteria are present in the stratum corneum.[24]

The chancre, the primary lesion of syphilis caused by the spirochete *Treponema pallidum*, is a nontender ulcer with a clean base.[25] *T. pallidum* cannot be cultured routinely. Either serology or direct visualization of the organism in clinical specimens are the traditional testing methods. Darkfield microscopy, nucleic acid amplification (PCR), or direct fluorescent antibody is used on scrapings from the ulcer bed to diagnosis primary infection.[26] On biopsy, the epidermis is acanthotic along the rim and thinned or eroded in the center of the lesion. A dense lymphoplasmacytic infiltrate is found in the dermis surrounding blood vessels with swollen endothelial cells. Silver stains, such as Warthin-Starry stain, demonstrate many 8- to 15-μm spirochetes within the epidermis and around blood vessels.[5] Immunohistochemical staining for *T. pallidum* has been reported to be more sensitive than the silver stain.[26a]

Condyloma lata, the lesions of secondary syphilis that arise 1 to 6 months after healing of the primary chancre, appear clinically raised, somewhat flattened, and velvety. On biopsy, condyloma lata show epidermal hyperplasia and lymphoplasmacytic infiltration of dermal vessels with endothelial hyperplasia, dilatation of the vascular lumens, and thickening of their walls. Interface dermatitis and scattered epithelioid granulomas may be seen. Silver stain highlights spirochetes in a minority of cases, usually around blood vessels and within the epidermis.[5]

Bacillary angiomatosis of the vulva and cervix has been reported in a patient with AIDS; it manifested as red-purple nodules that microscopically comprised a lobular epithelioid vascular proliferation with neutrophils and aggregates of bacterial organisms (Table 17.2).[27] The responsible gram-negative bacterium, *Bartonella henselae*, can be demonstrated by blood cultures and histologically with Warthin-Starry stain.[28] The differential diagnosis in an immunocompromised patient is Kaposi sarcoma secondary to human herpesvirus 8 (HHV-8) infection.[29]

Acute inflammation or folliculitis of the hair follicles of the vulva produces an erythematous papule that evolves into a pustule, then a carbuncle through coalescence of several pustules, and even a furuncle or boil if the abscess extends into the subcutaneous tissue.[30,31] The most common causative species is *Staphylococcus aureus*, increasingly, methicillin-resistant *S. aureus* (MRSA) but other bacteria may be the cause, such as *Pseudomonas aeruginosa* secondary to hot tub or swimming pool contamination.[32,33] Less common causative agents include nontuberculous mycobacteria, *Klebsiella oxytoca*, and *Acinetobacter baumannii*.

TABLE 17.2 Genital Infections Manifesting as Raised Nodules

Organism (Disease)	Characteristics
HPV	Smooth to rough, flesh-colored papules
Molluscum contagiosum	Nodule with central crater
Cryptococcus neoformans	Nodules can evolve to painless ulcer
Histoplasma capsulatum	Nodules can evolve to ulcers
Treponema pallidum (condyloma lata of secondary syphilis)	—
Bartonella henselae (bacillary angiomatosis)	Red-purple papules
HHV-8 (Kaposi sarcoma)	Red-purple papules
Mycobacterium tuberculosis	Dermal or lamina propria nodules
Trichophyton spp. (Majocchi granuloma)	Erythematous raised plaque with prominent border
Myiasis	Dome-shaped lesion with central pore

HHV-8, Human herpesvirus 8; *HPV*, human papillomavirus.

Recurrent abscess formation leading to scarring and sinus formation is termed hidradenitis suppurativa; it is a relatively common condition, occurring in 1% to 4% of the population.[34,35] It is probably caused by obstruction of a hair follicle followed by secondary bacterial infection.[36] The condition affects more women than men and primarily the intertriginous skin, including the perianal and perineal regions. Clinically, hidradenitis suppurativa is characterized by recurrent deep, painful nodules that ultimately drain malodorous, purulent material and that often lead to scarring and sinus formation or even fistulas to contiguous organs, such as the bladder.[37,38] Central necrosis, characteristic of a furuncle, is not a feature of hidradenitis suppurativa. The lesion is excised if malignancy is suspected or to remove scarred tissue harboring sinuses.[39] The differential diagnosis includes Crohn disease of the vulva and chronic granuloma inguinale, which is distinguished by involvement of the vagina and cervix, as well as the external genitalia.

Chancroid, although rarely encountered in the United States, had been a common cause of genital ulcers in sub-Saharan Africa, Latin America, and Asia but has been reported to be in steep decline due to increased use of condoms and effective therapy.[40] The cause is *Haemophilus ducreyi*, a small, gram-negative rod that requires technically challenging culture conditions to grow.[40] Gram staining of the culture shows a parallel array of bacteria resembling a school of fish. This sexually transmitted infection produces a papule that progresses to a pustule and finally to a painful ulcer with a gray, purulent base, usually associated with painful inguinal lymph node swelling.[41] Biopsy of the lesion shows acute inflammation in the epidermis and a perivascular mononuclear infiltrate or granulomatous response within the dermis; gram-negative bacteria can sometimes be found in the tissue with special staining techniques.[5] Clinical diagnosis and Gram staining of a sample of the exudate from the bed of the ulcer have low sensitivity; culture is more sensitive, but the culture medium is not widely available. The most sensitive method of diagnosis is multiplex PCR.[42] Of note, although chancroid is on the decline, leg ulcers due to *H. ducreyi* in children and adults in Pacific and African countries are on the rise.[39]

Lymphogranuloma venereum (LVG), until recently confined mainly to the tropics, is increasingly identified in temperate climates. It is caused by the L serovars of *Chlamydia trachomatis*, which cause a painless, shallow, solitary ulcer on the vulva, vagina, or perianal region.[43] The ulcer, which spontaneously heals in 3 to 12 days, is often missed. If it is left untreated, painful regional lymphadenopathy of the secondary, or inguinal, stage ensues. If the disease continues to progress, fistulas, labial scarring, and even labial elephantiasis can develop. Diagnosis is primarily serologic, but nucleic acid detection techniques are more sensitive and specific. Biopsy findings show nonspecific, chronic inflammation or granulomatous inflammation with necrosis; Brown-Hopp and Warthin-Starry stains highlight the short rods within vacuoles of macrophages.[44]

Granuloma inguinale or donovanosis, rarely encountered in temperate climates except in travelers, is common in the tropical regions of Pacific countries, Asia, and South America. Granuloma inguinale, or donovanosis, is caused by the gram-negative rod, *Klebsiella granulomatis* (formerly called *Donovania granulomatis* or *Calymmatobacterium granulomatis* but reclassified by means of 16S ribosomal RNA analysis).[46] Initially a papule appears on the vulva, vagina, or cervix. The papule progresses to a red ulcer that bleeds easily; if left untreated, granuloma inguinale can lead to scarring similar to hidradenitis suppurativa.[47] Rarely the upper genital tract may be involved. *K. granulomatis* can be cultured only with difficulty,[48] so diagnosis relies on demonstration of the bacteria on cytology or histology by special stain. Romanowsky-stained air-dried smears from the ulcer bed demonstrate numerous intracytoplasmic, short bacilli within macrophages.[47] Rarely the organisms are identified on Pap smears.[49] Biopsy of the ulcer base, performed for smear-negative cases, should be stained with Giemsa or Warthin-Starry stain.[50] A PCR test has been developed for granuloma inguinale.[51] Ulcers caused by multiple infectious agents have been reported.[52]

Tuberculosis of the vulva is the rarest form of genital tuberculosis and is usually associated with foci of infection elsewhere in the genital tract.[53] Hypertrophic lesions with sinus formation may develop.[54,55] Surgery may be necessary in conjunction with antituberculosis drugs.[56] Necrotizing granulomas are found on biopsy. Nucleic acid techniques including real-time PCR are used to diagnose *Mycobacterium tuberculosis* in clinical samples.[57,58]

Malakoplakia, a chronic histiocytic inflammatory response characterized by calcified Michaelis-Gutmann bodies, usually occurs in the urinary tract, more often in women than in men. Malakoplakia has been reported on rare occasions in the female genital tract, including the vulva[59,60] and, more often, the vagina.[61,62] Diagnosis of vaginal malakoplakia has been made by cytology, including fine-needle aspiration cytology.[63,64]

Fungal Infections

Candida is found in the vaginal tract of up to 50% of asymptomatic women; before menopause, the majority of women will have at least one episode of *Candida* vulvovaginitis, which is not considered to be an STD.[65,66] *Candida albicans* accounts for up to 90% of infections; *Candida glabrata* and *Candida parapsilosis* are less commonly the cause.[67,68] *Candida* vulvovaginitis manifests as a pruritic, erythematous swelling of the vulva with a thick,

TABLE 17.3 Genital Infections Presenting as Flat Lesions

Organism (Disease)	Characteristics
Candida albicans	Erythematous swelling
Schistosoma haematobium	"Sandy patches" appear as tiny rice grains beneath skin; also ulcers and polyps
Sarcoptes scabiei	Pruritic, erythematous, scaly tracts; also blisters and pustules
Corynebacterium minutissimum (erythrasma)	Erythematous lesion similar to candidiasis
Malassezia furfur (pityriasis or tinea versicolor)	Oval or irregular, flat, hypopigmented or hyperpigmented, 0.5- to 2.5-cm lesions

white vaginal exudate.[69,70] Diagnosis is usually achieved in the clinic by means of microscopic demonstration of yeast forms, with pseudohyphae in the case of *C. albicans*, after application of 10% potassium hydroxide to vaginal secretions (to disaggregate squamous cells) with a normal vaginal pH of less than 4.5. Biopsy occasionally is performed on long-standing, chronically pruritic, hyperkeratotic lesions; often, only a few organisms are found in the stratum corneum. Periodic acid–Schiff (PAS) with diastase or Grocott methenamine silver (GMS) stain can be used to highlight the yeast and pseudohyphae.[71] *Candida* spp. can cause pustular lesions in immunocompromised patients (Table 17.3).[31]

With the advent of the 21st century a nationwide increase in incidence of coccidioidomycosis has been reported in the United States, most particularly in endemic regions, including Arizona and California.[72] Infection, which is four times more prevalent in men than in women, occurs after inhalation of spores. Most infections (60%) are asymptomatic, but flulike symptoms can occur; less than 1% of patients develop disseminated disease. Although vulvar lesions have not been reported, scrotal lesions characterized by necrotizing granulomatous inflammation containing Coccidioides immitis spherules have been described.[73]

Histoplasma capsulatum is a dimorphic fungus that is found worldwide but is most common in North and Central America.[74] In the United States the fungus is endemic to the Mississippi and Ohio River valleys. Soil and caves contaminated by bird or bat guano are especially rich in this fungus. Infection, usually asymptomatic, occurs by the respiratory route. Less than 1% of infected patients develop clinically recognized symptoms, mainly pulmonary. Disseminated histoplasmosis usually occurs in immunocompromised patients, such as patients with AIDS or transplant recipients. The liver, spleen, bone marrow, gastrointestinal tract, skin, and mucous membranes may be involved. Ulcers of the female genital tract due to *H. capsulatum* have only rarely been described; the organisms were recognized as small intracellular budding yeast on routinely stained smears and biopsy samples.[75,76] Disseminated histoplasmosis can also occur in nonimmunocompromised older patients, in whom the slow disease progression is usually fatal. Disease in older adults is not always accompanied by constitutional symptoms. A case of venereal spread provoking an isolated vulvar ulcer has been described.[77]

Cryptococcus neoformans, an encapsulated yeast, is found worldwide, especially in soil harboring pigeon guano, but infection usually causes symptoms only in immunocompromised patients.[78,79] With symptomatic infection, a granulomatous response develops. The fungus occurs as a yeast form in tissue and can be recognized on routine staining by its more variable size compared with other fungi (2 to 20 µm in diameter). Special stains used to highlight *Cryptococcus* include PAS and methenamine silver. The capsule can be seen in negative relief on smears with India ink and is demonstrated in tissue sections with mucicarmine stain. *Cryptococcus* contains phenol oxidase, which is involved in the metabolism of phenolic compounds to melanin. Phenol oxidase is a virulence factor, and the melanin that is produced can be detected by the Fontana-Masson stain on histologic sections.[80] Isolated lesions of the female genital tract have rarely been reported. A vaginal nodular lesion was found in a 20-year-old, nonimmunocompromised woman,[81] and a painless ulcer of the labia major occurred in a 60-year-old renal transplant recipient.[82] In both instances, biopsy was performed because the lesions were clinically suspected to be carcinoma, and in both cases, granulomatous inflammation with mucin-positive fungi was found.

Trichophyton mentagrophytes has been reported to cause a case of Majocchi granuloma of the vulva in a young woman.[83] Majocchi granuloma, or nodular granulomatous perifolliculitis, is an uncommon intradermal infection by dermatophytes that usually cause follicular lesions.

Parasitic Infections

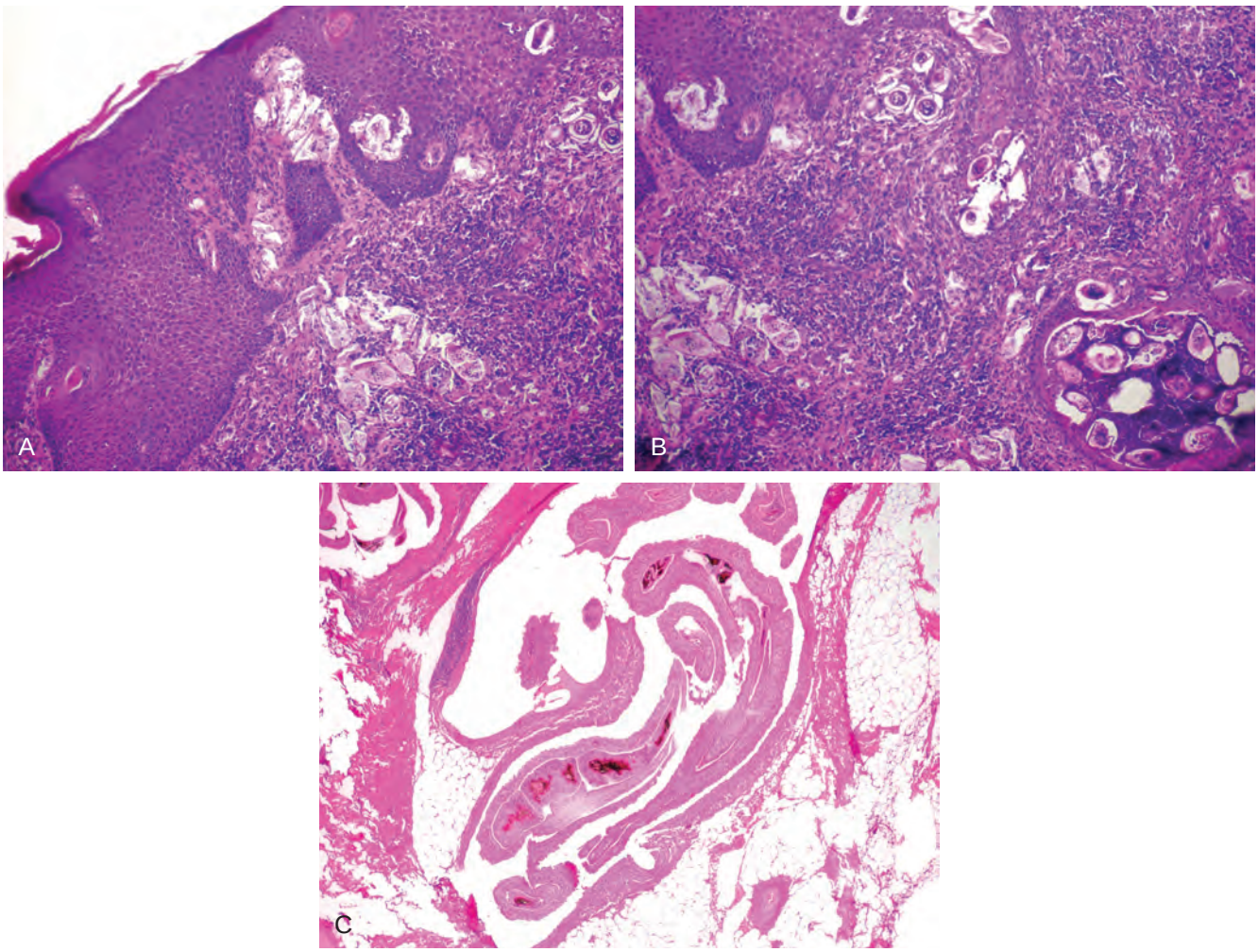
Genital schistosomiasis, usually caused by the bladder trematode, *Schistosoma haematobium*, which is endemic to Africa, has been reported to involve the vulva of women living in endemic areas, such as Malawi,[84,85] and in travelers.[86] The eggs gain access to the stromal tissue of the vulva via the venous drainage from the bladder and engender granulomatous inflammation.[87] A variety of lesions are associated with genital schistosomiasis, including polyps and ulcers, but the most common lesion is the "sandy patch," which appears as an aggregate of tiny rice grains beneath smooth mucosa.[88,89] Regional lymphadenopathy often accompanies the genital lesions.[90] The diagnosis relies on demonstration of the characteristic egg with a terminal spine in tissue biopsy.[85] It can be seen with routine hematoxylin and eosin (H&E) stain, but silver stains highlight the egg. *Schistosoma mansoni*, the cause of intestinal schistosomiasis, is found in many of the same regions of Africa as *S. haematobium*, as well as in South America and the Caribbean, and has also been associated with genital disease, although the incidence is less certain.[85,89,91] The ova of *S. mansoni* are differentiated from those of *S. haematobium* by the presence of a lateral spine; both are approximately 60 µm wide by 160 µm long (Fig. 17.6).

Vulvovaginitis secondary to *Enterobiasis vermicularis* has been reported in prepubertal girls and rarely in older women.[92]

Scabies is caused by the mite *Sarcoptes scabiei*, which is spread by person-to-person contact and rarely by fomites. The mites burrow into the epidermis to lay eggs. Clinically the burrow is linear and intensely pruritic. On biopsy, diagnostic fragments of mites and eggs are found in the epidermis, and a polymorphous inflammatory infiltrate including eosinophils is present in the epidermis and dermis.[93,94] Additional histologic findings include spongiosis and superficial fibrin thrombi.

Larval Infestations

Myiasis is the cutaneous infestation by larvae of several fly species. The larvae can infest devitalized tissue of open wounds or burrow



• **Figure 17.6** Vulvar biopsy of 7-year-old with swelling of the vulva. No purulent exudate was obtained on incision and drainage, so a biopsy was performed. It showed an intact, albeit acanthotic squamous epidermis (hematoxylin and eosin [H&E] stain) **(A)**. Within the dermis were numerous, mostly embryonated, schistosome eggs, a few of which showed the terminal spine of *Schistosoma haematobium* (H&E stain) **(B)**. Within the subcutis, a distended vein contained a pair of *Schistosoma* worms (male and female) (H&E stain) **(C)**. (Courtesy James Kitinya, MD, Tanzania.)

into healthy skin of the vulva, resulting in furuncular myiasis. The lesion is dome-shaped, with a central pore from which a variable number of larvae exit. The larvae can be plucked from the lesion for identification by a parasitologist. The infestation occurs mainly in tropical climates and is associated with poor hygiene, diabetes, and travel.[95-97]

Noninfectious Lesions in the Differential Diagnosis of Vulvar and Vaginal Infections

Fixed drug eruptions are circumscribed erythematous plaques that are usually located on the hands, feet, and external genitalia; the lesions recur in the same site with repeated exposure to the drug.[22,69] A central vesicle may develop. Biopsy of a vulvar lesion may be necessary, if the association with the offending drug is not recognized, to exclude similar-appearing infectious lesions such as HSV or secondary syphilis.[98] The histologic appearance of a fixed drug eruption consists of basal cell hydropic change, interface lymphoid infiltrate occasionally with eosinophils, and individual keratinocyte necrosis.

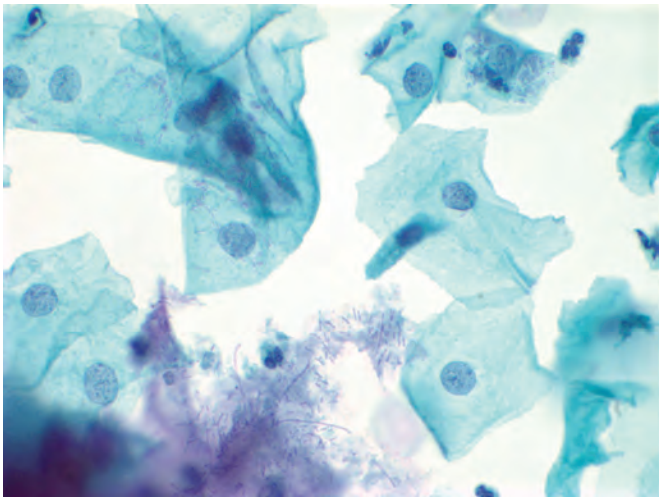
Behçet disease, a rare cause of vulvar ulcers, occurs in Japan and Eastern Mediterranean countries. The diagnosis is made in the presence of recurrent oral ulcers and at least one of the following: genital ulcers, skin pustules or nodules, and uveitis/retinitis.[99] Herpetiform or aphthous vulvar ulcers show necrotizing vasculitis of all calibers of vessels, with acute or chronic inflammation.[100] The diagnosis is one of exclusion; ulcerative lesions of the vulva may be the presenting symptom.[101]

The differential diagnosis of vulvar and perianal abscesses, sinus tracts, and fistula formation includes Crohn disease. Crohn disease can be particularly challenging to diagnose if the genital manifestations precede the gastrointestinal lesions, in which case a diagnosis of granulomatous vulvitis may be made.[102] Sarcoidosis of the vulva has been reported.[103]

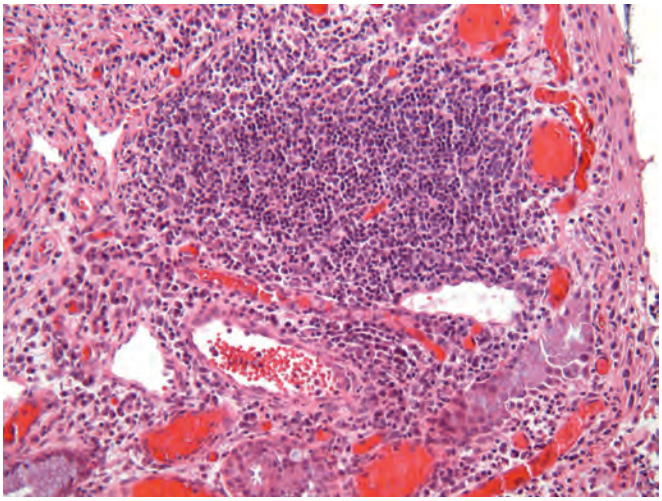
Cervix and Vagina

Normal Cervical and Vaginal Flora

The normal cervical-vaginal milieu is moist and acidic (pH 4 to 4.5), with a relatively large assortment of bacteria, the variety of



• **Figure 17.7** Lactobacilli in cervical cytology sample (Papanicolaou stain, ×100).



• **Figure 17.8** Follicular cervicitis: germinal center in the lamina propria of a cervical biopsy specimen (hematoxylin and eosin stain, ×20).

which changes with the hormonal state of the woman. The predominant bacteria under normal conditions are lactobacillus, diphtheroids, and *Staphylococcus epidermidis*.

Lactobacillus acidophilus, a facultative anaerobic, gram-positive bacterium, predominates in the normal vaginal flora and produces an acidic vaginal pH due to the production of lactic acid from intracellular glycogen. During the normal female reproductive cycle, glycogen gradually accumulates in the squamous cells of the vagina and cervix. By the end of the luteal phase, in the absence of pregnancy, abundant lactobacilli will be found on cervical cytology preparations amid marked cytolysis or cytoplasmic debris and naked nuclei resulting from the action of the bacteria on the glycogen of the squamous cells (Fig. 17.7).

Cervicitis and Vaginitis

Symptoms of vaginitis and cervicitis are very common and consist of increased and malodorous vaginal discharge, pruritus, burning, and dyspareunia. The diagnosis is usually made clinically based on the presence of an abnormal discharge and erythema of the mucosa of the vagina, the cervix, or both. In the case of vaginitis, the diagnostic workup consists of assessment of the vaginal pH in women of reproductive age. A pH greater than 4.5 suggests bacterial vaginosis or *Trichomonas* infection and tends to exclude candidiasis. In premenarchal and postmenopausal women, assessment of the pH is less helpful because it is generally higher in these age groups. A saline wet preparation of vaginal-cervical secretions may demonstrate increased polymorphonuclear leukocytes, motile trichomonads, candidal yeast or pseudohyphae or clue cells (squamous cells covered by a blanket of coccobacilli) representing bacterial vaginosis. Culture or molecular tests should be performed for bacterial vaginosis, *Candida* vaginitis, and trichomoniasis because clinical diagnosis and saline microscopy are poorly sensitive for these organisms.[70,104] Likewise, for *C. trachomatis* and *Neisseria gonorrhoeae*, molecular tests should be performed.[105]

Varying degrees of acute and chronic inflammation including a mixture of lymphocytes, neutrophils, plasma cells, eosinophils, and histiocytes are commonly found in the mucosa of the cervix as an incidental finding on cervical biopsy specimens obtained at colposcopy as part of the workup of an abnormal cytology finding. Chronic cervicitis is diagnosed if the inflammation is marked and can be caused by a variety of organisms. Well-formed germinal cells are present in follicular cervicitis (Fig. 17.8).

On clinical examination, acute cervicitis is often recognized by erythema and a mucopurulent exudate. Microorganisms, chemical injury (spermaticides), or local trauma (intrauterine device [IUD], pessary, tampons) may be the cause. Acute inflammation is exceedingly common in cervical cytology samples obtained to screen for cancer, and the underlying cause is not usually evident.

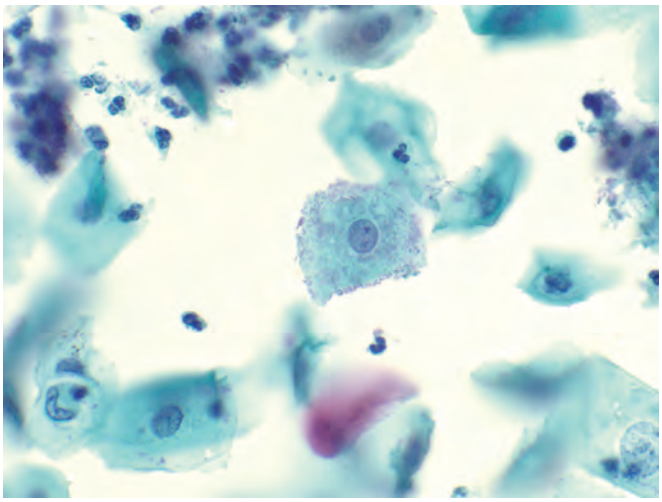
Common Causes of Symptomatic Cervicitis and Vaginitis. Tissue biopsy is rarely performed in this setting, but a cervical-vaginal cytology sample may be obtained. A number of organisms are routinely identified on cervical cytology as incidental findings. In contrast to surgical pathology, special stains are not usually used on cervical cytology samples to highlight microorganisms. However, molecular tests, either alone or in conjunction with cytologic analysis, are commonly used.

The most common causes of mucopurulent cervicitis are *C. trachomatis* and *N. gonorrhoeae. C. trachomatis* is a small, obligate intracellular, gram-negative bacterium that is transmitted by sexual contact. The life cycle of *C. trachomatis* comprises two phases. Infection begins with penetration of the small elementary body form of the bacterium into the cell, where the bacterium converts to the larger, metabolically active, reticular form, creating intracytoplasmic vacuoles. After 48 to 72 hours the cell ruptures, releasing infectious elementary bodies. The prevalence of *C. trachomatis* is 3% to 5% in asymptomatic women and as high as 20% in women attending STD clinics.[106] These figures probably reflect the successful use of screening texts, although most cases of *C. trachomatis* are not reported to the Centers for Disease Control and Prevention (CDC) from STD clinics. Reported cases are approximately three times more frequent in women than in men.[107] *Chlamydia* infection induces weak immunity and can lead to pelvic inflammatory disease (PID) in 30% of untreated patients. If untreated during pregnancy, infection can lead to early rupture of the membranes and risk of conjunctivitis and pneumonia in the newborn. Diagnosis is made primarily by nucleic acid amplification tests (NAATs) that include PCR. Samples for NAATs can be obtained from urine or

from self-collected vaginal swabs. Screening programs for sexually active women younger than 25 years of age have been instituted in various health care settings. Although the cytopathic effect of *C. trachomatis* has been described as cytoplasmic vacuoles containing small eosinophilic coccoid bodies with epithelial cells,[108-112] the morphologic recognition of *Chlamydia* inclusions within epithelial cells is unreliable on both cytology and histology.[113-116] However, immunocytochemical staining has been shown to be sensitive for chlamydial infection.[115]

A shift in vaginal flora to a predominance of mainly anaerobic coccobacilli, including *Gardnerella vaginalis* and *Mobiluncus* spp., is known as bacterial vaginosis, a condition that was formerly thought to be innocuous but in the 1980s has been found to be associated with an increased susceptibility to STDs and with preterm birth. Women with bacterial vaginosis are often asymptomatic but may present with a fishy-smelling vaginal discharge. On cervical cytology the presence of a film of small coccobacilli covering the background of the smear, as well as the entire surface of squamous cells, is suggestive of bacterial vaginosis but must be correlated with the clinical findings (Fig. 17.9). The cytologist may choose to report the shift in vaginal flora, but this will probably have little impact on the treatment of the patient because cervical cytology, by virtue of being a screening test, usually has a certain delay in being reported. The columnar epithelium of the endocervix is more susceptible to both bacterial and chlamydial infections, and this is thought to explain why young women and pregnant women with large ectropions are more susceptible to the development of acute endocervicitis.[106,117]

Trichomonas vaginalis infection is an STD that may be asymptomatic or may cause a thin, malodorous vaginal discharge accompanied by pruritus, dysuria, and other symptoms. Trichomoniasis is associated with premature rupture of membranes in the pregnant patient. On cervical cytology, the trichomonads assume a round, ovoid, or pear shape ranging from 15 to 30 µm. The cytoplasm is gray-blue, and the lancet-shaped nucleus is only slightly darker than the cytoplasm (Fig. 17.10). Occasionally, eosinophilic flagellae and cytoplasmic granules may be appreciated. The organisms occur singly or in clusters. A filamentous bacterium, *Leptothrix*, may accompany the trichomonads. The squamous cells often show small, perinuclear halos that may be the first clue to the presence of *Trichomonas* infection. Cervicovaginitis emphysematosa, a rare condition in which blue-gray subepithelial cysts form in the vagina, has been associated with trichomoniasis.[118]



• **Figure 17.9** A film of coccobacilli covers squamous cells in a cervical cytology sample. This aspect is commonly referred to as "clue cells" and is associated with bacterial vaginosis (Papanicolaou stain, ×100).

Candida spp. are identified on cervical cytology as small yeast forms; in the case of *C. albicans*, pseudohyphae are also present. The squamous cells often have inflammatory changes consisting of pseudoparakeratosis (small, superficial squamous cells with pyknotic nuclei and orangeophilic cytoplasm) and perinuclear halos. On liquid-based preparations the pseudohyphae may appear to have skewered stacks of squamous cells (Fig. 17.11).

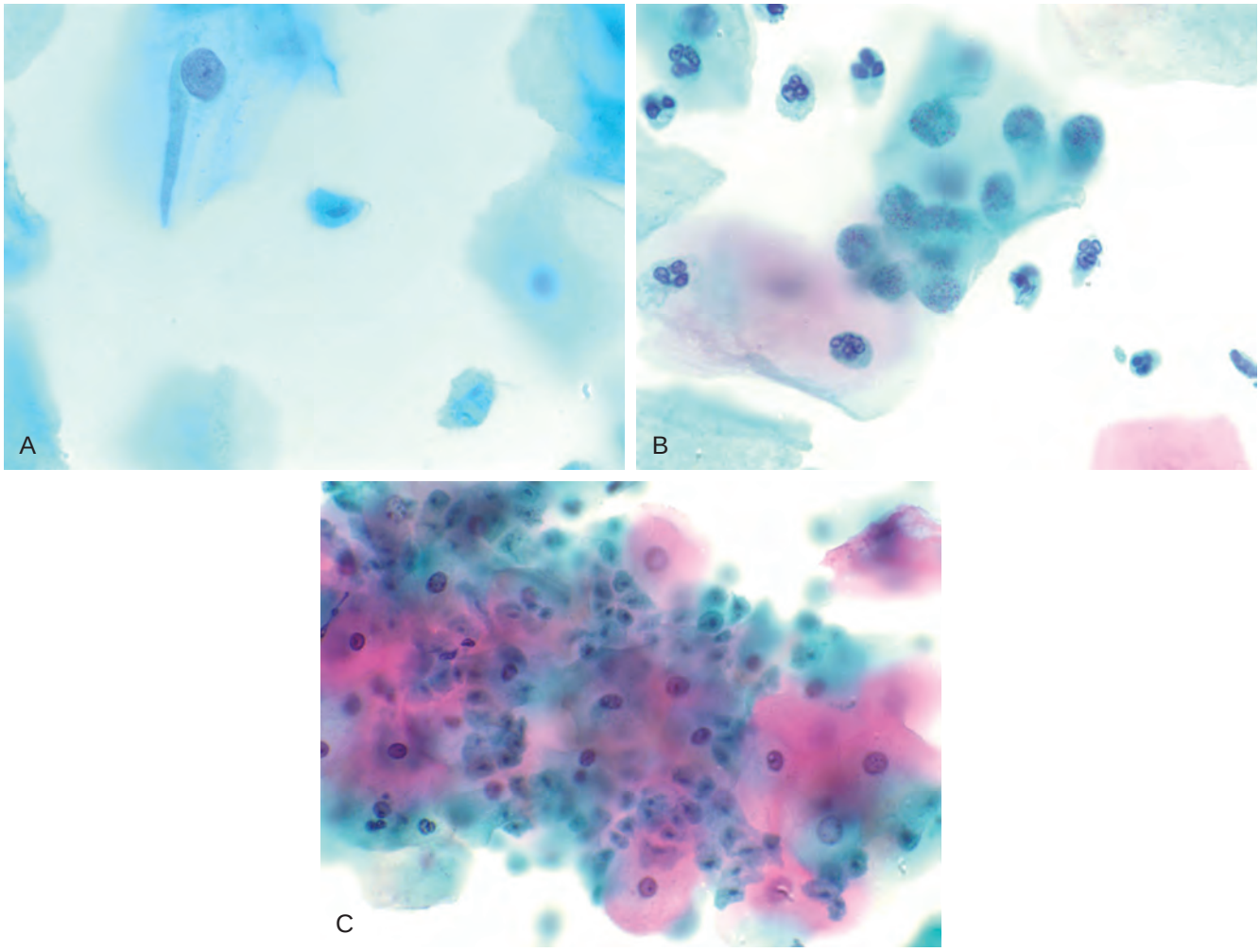
Actinomyces israelii are finely filamentous bacteria that manifest as fuzzy, densely tangled, dark blue masses easily recognized at low magnification on a cervical cytology slide (Fig. 17.12). *Actinomyces* has been determined to be commensal in the vagina.[119] The presence of aggregates of *Actinomyces* is most often associated with an IUD and has rarely led to pelvic abscess formation,[120] but usually cultures of removed IUDs do not grow *Actinomyces*.[121,122] Pseudosulfur granules have been described and are sometimes, but not always, associated with IUDs.[123-126] The pseudosulfur granules, also known as pseudoactinomycotic radiate granules (PAMRAGs), are recognized by their more crystalline appearance and failure to stain with special stains for microorganisms (Fig. 17.13).

Although in the United States serologic prevalence of HSV-1 and HSV-2 is 67% and 26%, respectively, only a fraction of seropositive patients are diagnosed with genital HSV infections.[127] Detection of the viral cytopathic effect of HSV in cervical cytology is even less common, 1% or less.[128,129] Breakdown of the primary vesicles can lead to acute, sometimes severe and necrotizing, inflammation.[130] Both squamous cells and endocervical cells show ground glass nuclei with chromatin marginated as a thin rim beneath the nuclear membrane. Clustering of multiple nuclei is common (Fig. 17.14).[131] Alternatively, a large eosinophilic inclusion appears in the nucleus surrounded by a clear halo.

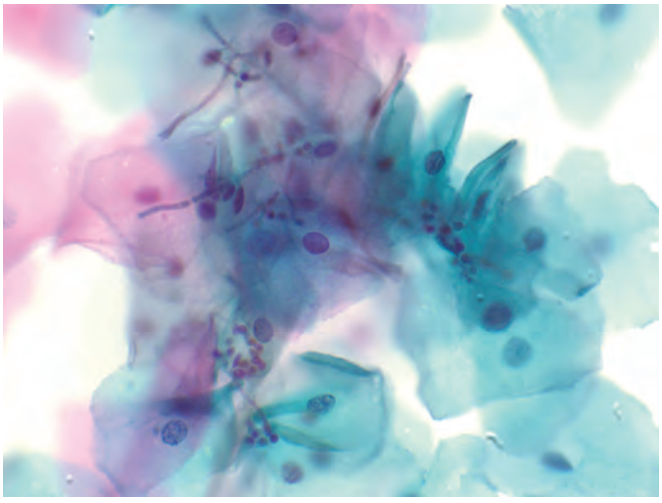
Uncommon Causes of Cervicitis and Vaginitis. CMV is less common but produces cytologic changes that are difficult to distinguish from those of HSV; it infects not only epithelial cells but also endothelial cells and stromal cells. A report of five cases of CMV cervicitis noted that all cervical biopsies were performed for atypical cells seen on cervical cytology. The biopsies showed a dense, mixed, acute and chronic inflammatory infiltrate. CMV inclusions were identified on routine stain and mainly in endocervical cells.[132] CMV infection of the cervix is more commonly determined by culture than identified by its viral cytopathic effects on cytology or histology samples.[133-136]

Similarly, certain fungi are rarely reported as true pathogens in cervical samples. Included among this group are *Aspergillus*, *Fusarium*, *Blastomyces*, *Coccidioides*, *Cryptococcus*, and *Paracoccidioides*.[137-143] In the case of *Paracoccidioides*, the organism appears in cervical cytology samples as yeast, 30 µm in diameter, with multiple budding daughter yeast forms protruding from the periphery, appearing like a "pilot's wheel." The offspring are 2 to 10 µm in diameter (Fig. 17.15). To confirm the fungal nature of the organisms, a cell block from the liquid-based cytology preparation can be obtained; special stains for fungi (PAS and GMS) are performed on the cell block to highlight the yeast.[143]

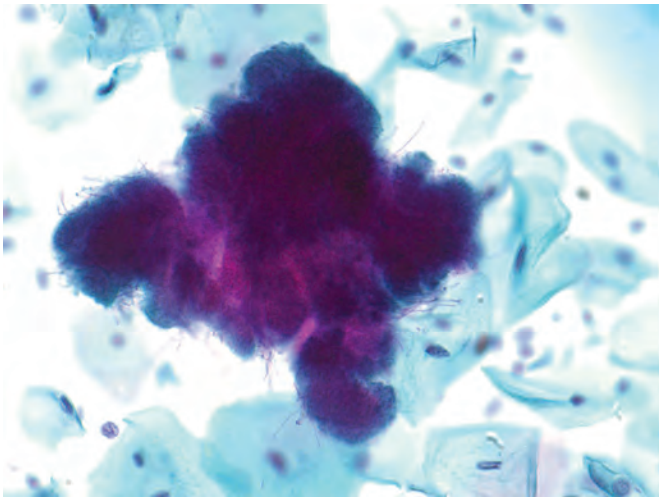
Tuberculous cervicitis is rare and usually occurs secondary to tuberculosis of the upper female genital tract. Some, but not all, patients have a history of pulmonary disease.[144] The cervix may grossly appear ulcerated or may have many small lesions



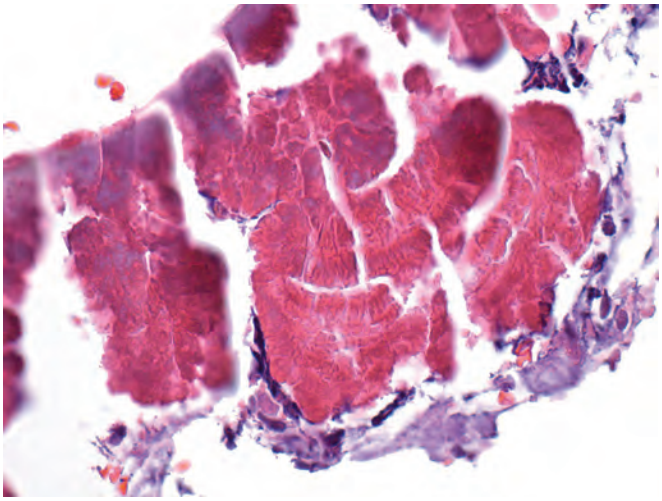
• **Figure 17.10** **A,** *Trichomonas* organisms are pear shaped and have a lancet-shaped nucleus. **B,** The organisms often demonstrate red cytoplasmic granules. **C,** The organisms may, on occasion, be so numerous as to form sheets (Papanicolaou stain, ×100).



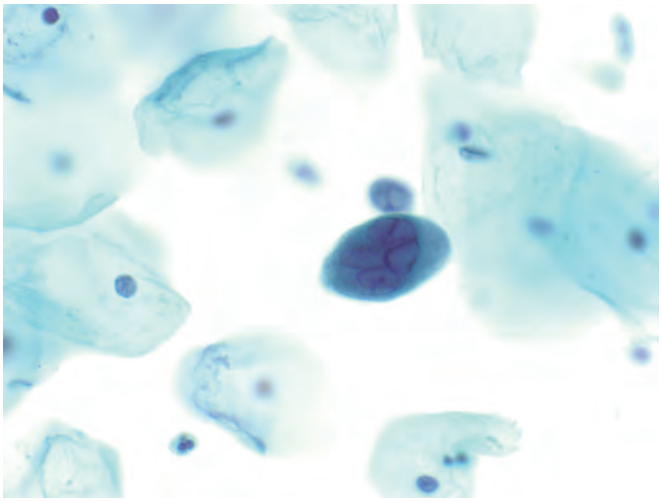
• **Figure 17.11** *Candida* spp. are present as yeast forms and pseudohyphae in association with squamous cells on cervical cytology samples (Papanicolaou stain, ×100).



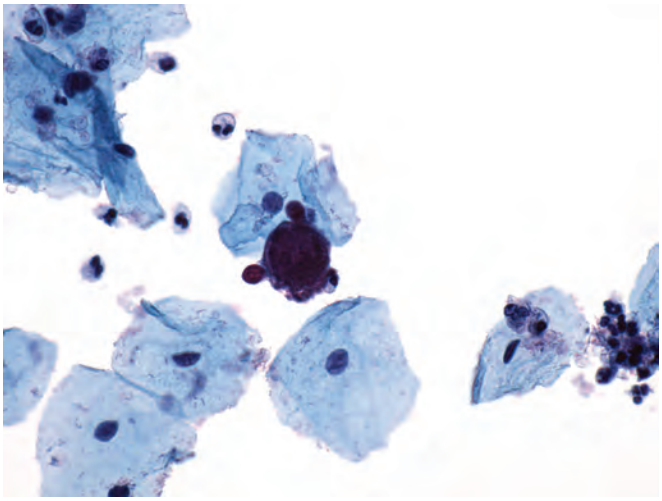
• **Figure 17.12** A mass of filamentous *Actinomyces* bacteria, known as sulfur granules, is identified in a cervical cytology sample from a woman with an intrauterine device (Papanicolaou stain, ×100).



• **Figure 17.13** Pseudoactinomycotic radiate granules are seen in an endometrial biopsy specimen (hematoxylin and eosin stain, ×60).



• **Figure 17.14** Cervical cytology sample with herpes simplex virus cytopathic effect in a multinucleated endocervical cell (Papanicolaou stain, ×100).



• **Figure 17.15** Paracoccidioidomycosis organism with a "pilot-wheel" profile, found in a cervical cytology sample (Papanicolaou stain, ×100).

or larger, tumorlike lesions.[145] Microscopically, granulomatous inflammation, often with necrosis, is present. Demonstration of the mycobacteria can be achieved by acid-fast stain or by PCR.[146] Nested PCR has been used on cells from a cervical cytology sample to confirm the diagnosis of tuberculous cervicitis.[147]

Although the external genitalia is the usual site of granuloma inguinale (donovanosis), the cervix is occasionally infected.[47] As noted in the discussion of granuloma inguinale in the vulva, multiple methods of diagnosis are possible, including special stains on smears or biopsies and PCR. Granuloma inguinale has been recognized on Papanicolaou-stained cervical cytology samples. Many short rods (Donovan bodies) are aggregated within histiocytes.[148] Granuloma inguinale of the cervix can be mistaken grossly for carcinoma.[149]

Schistosomiasis, where endemic, is a cause of cervicitis and may even be common, but, because women in countries where schistosomiasis is endemic may never get physical examinations, documentation may be poor.[89,150] In a study of women in Malawi who had ova of *S. haematobium* in their urine, more than 60% were found to have cytologic crush preparation or histologic evidence of ova also in the vagina or cervix.[88] Cervical infection may be present in the absence of urinary egg excretion.[151] Infection by *S. haematobium* causes ulcers or nodular lesions.[152] Histologically, nonnecrotizing granulomas with fibrosis are seen.[106,130] Other, rare parasitic infections of the cervix include *Echinococcus*, amebiasis, and Chagas disease.[153-156]

Cervical and Vaginal Infections Causing Scant Inflammation

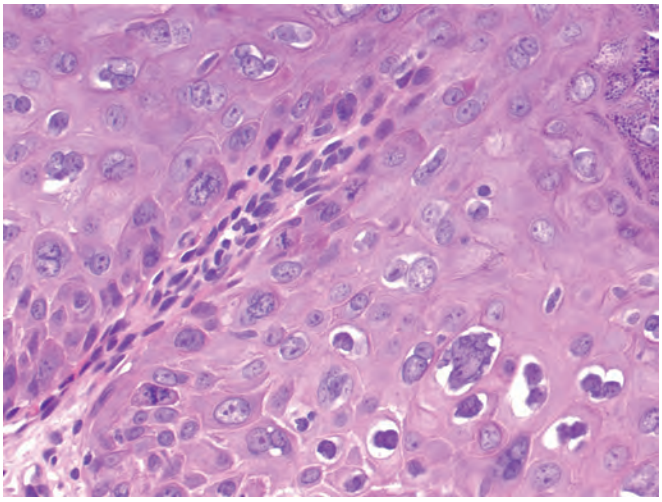
Productive infections by HPV can be recognized in cytologic preparations by the viral cytopathic effect in squamous cells. After the initial infection of the basal cell, viral replication is dependent on maturation of squamous cells. As the superficial squamous cells are sloughed, thousands of intact virions, present in the nucleus of the squamous cell, are released into the environment. The infected superficial squamous cell is characterized by the viral cytopathic effect of koilocytosis or cytoplasmic cavitation, nuclear enlargement, irregularity, hyperchromasia, and multinucleation. Although exophytic condyloma acuminatum (most often caused by HPV-6 or HPV-11) can occur on the cervix, the more usual lesion is a flat condyloma, which is caused in most cases by the high-risk types of HPV. The flat condyloma requires the magnification by the colposcope for visualization, which is enhanced by the application of acetic acid or Lugol solution. Associated inflammation is not a feature of HPV infection. Whereas many low-grade HPV lesions regress without treatment, guidelines for treatment recommend colposcopy with ablative treatment because of the risk of association with or progression to a higher-grade HPV-associated premalignant lesion (Fig. 17.16).[157]

Malakoplakia has been reported to occur in the cervix.[59,158] In certain cases the initial recognition of the disease has been on cervical cytology.[159-161]

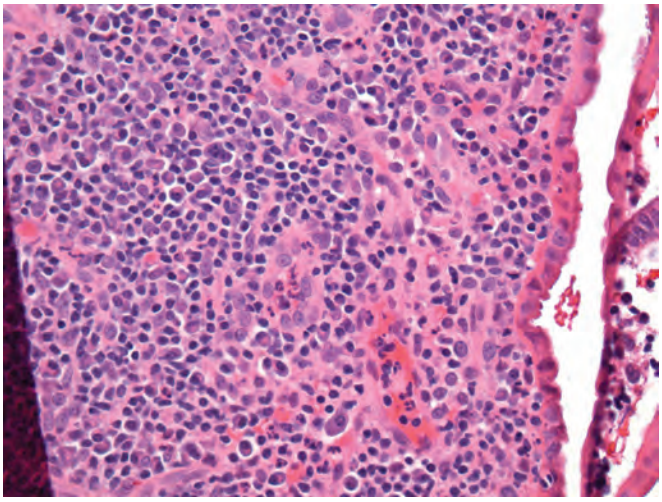
Noninfectious Lesions Mimicking Infections

Rarely, the cervix can harbor a lymphoid infiltrate so dense as to suggest the presence of lymphoma. Although lymphomas of the genital tract are uncommon, many of these cases are primary to the cervix.[162] A few cases of nonneoplastic, lymphoma-like lesions of the cervix have been described.[163]

Pemphigus vulgaris is a dermatosis that produces vesicles that mimic those produced by HSV.[164] Evidence of pemphigus can be found on cervical cytology.[165]



• **Figure 17.16** Human papillomavirus cytopathic effect (koilocytosis) in squamous dysplasia identified on a cervical biopsy specimen (hematoxylin and eosin stain, ×40).



• **Figure 17.17** Chronic endometritis is manifested by abundant plasma cells within the endometrial stroma of an endometrial biopsy (hematoxylin and eosin stain, ×20).

Upper Genital Tract

Pelvic Inflammatory Disease

PID is acute inflammation of the upper female genital tract and includes endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscess. In 2005 approximately 45,000 women were hospitalized for treatment of PID, although the number of initial visits to the physician for PID declined by more than 50% from 1997 to 2005.[107] The disease is thought to have peaked in 1982.[166] The decline is thought to be the result of increased screening and treatment of *C. trachomatis*, one of the main causative agents of PID. The normal vaginal flora is polymicrobial but is dominated by *Lactobacillus*, which, along with the barrier of the endocervical canal, protects against more potentially pathogenic bacteria. Widening of the endocervical canal with menses or immediately after IUD insertion increases the risk for PID. Another risk factor is sexual activity at a young age, with one study finding the highest rates in the 20- to 24-year-old group.[167] Additional factors include multiple sex partners, neglecting to use a barrier method of contraception, intercourse during menses, and sexually transmitted infections with *C. trachomatis* or *N. gonorrhoeae*. In more than 50% of PID cases, *C. trachomatis* or *N. gonorrhoeae*, or both, are cultured.[168] *Mycoplasma genitalium*, a common sexually transmitted pathogen, has been increasingly implicated in PID.[169,170]

Although patients are often asymptomatic, the main symptom of PID is lower abdominal pain, usually of recent onset and worsened by coitus or during menses. Vaginal discharge and fever may be present; the uterus and adnexa are tender on physical examination. PID can be associated with perihepatitis inflammation (Fitzhugh-Curtis syndrome) leading to right upper quadrant pain. There is no gold standard test to diagnose PID; cases are considered confirmed with certain positive findings, including demonstration of acute or chronic endometritis on biopsy. The CDC has issued guidelines for diagnosis of PID that include histologic evidence of endometritis, radiologic images of thickened, fluid-filled fallopian tubes, and laparoscopic abnormalities indicating PID.[171] Gram staining of vaginal discharge that shows gram-negative, intracellular diplococci suggestive of *N. gonorrhoeae* is highly suggestive of PID. In practice, a constellation of clinical findings is used to diagnose PID. Empirical antibiotic treatment is given even in the face of minimal findings because, if left untreated, PID can lead to infertility.

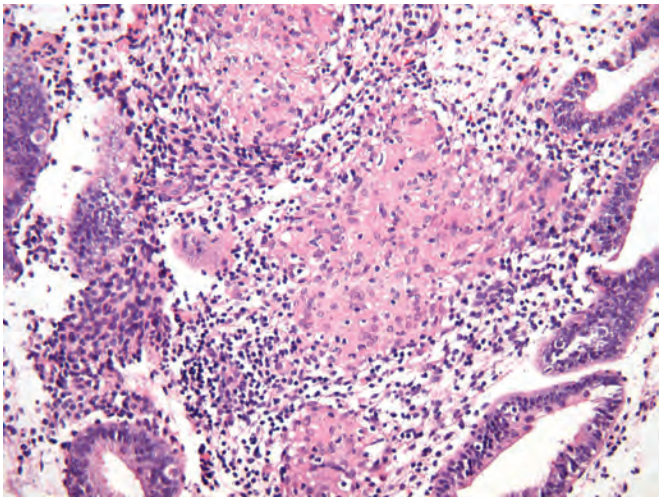
Endometrium

In women of reproductive age, certain types of inflammatory cells can be found in the endometrium, depending on the time of the woman's monthly cycle. Neutrophils are abundant in menstrual endometrium.[172] Lymphocytes, also known as large granular lymphocytes, are present, either singly or in aggregates, throughout the cycle but increase in number in the luteal phase.[173-176] Immunohistochemical studies have found that most of the lymphocytes are T cells.[177]

Endometritis

Endometritis usually results from ascending infection secondary to unobstructed passage via the normally blocked endocervical canal during menstruation, parturition, or instrumentation permitting retrograde spread of microorganisms to the endometrial cavity.

Purely acute endometritis is the presence of microabscesses involving the endometrial glands and stroma, which is a rarely reported finding outside obstetrically related endometritis. The endometritis most often associated with PID is chronic or plasma cell endometritis, although lymphocytes and neutrophils are also present.[178,179] Low-power clues to the presence of endometritis are the presence of spindled stromal cells arranged in a swirling pattern or difficulty in dating the endometrium. Histologic findings of chronic endometritis include plasma cells within the stroma near surface epithelium, glands, blood vessels, and lymphoid infiltrates (Fig. 17.17). The plasma cells may not be numerous and may be interspersed singly among lymphocytes and neutrophils. Primary or secondary lymphoid follicles may be present. Neutrophils are present in the surface and glandular epithelium and may even form microabscesses. The endometrial glands may appear inactive, and the stroma may take on a spindled appearance. Although histochemical staining with methyl green pyronin and immunohistochemical staining with syndecan-1 have been advocated for increased sensitivity in identification of



• **Figure 17.18** Granulomatous endometritis is characterized by well-formed, nonnecrotizing granulomas found within the stroma of an endometrial biopsy sample (hematoxylin and eosin stain, ×20).

plasma cells, special stains to highlight plasma cells are not recommended, because the cases so identified are not always associated with PID.[180] If the finding of a rare plasma cell is the only finding in an endometrial sample, the diagnosis of chronic endometritis may not be warranted, because a minority of control subjects in several studies of endometritis have been found to have plasma cells in their endometrial samples.[181-183]

Granulomatous Endometritis

Granulomatous endometritis is uncommon and may result from a variety of diseases, including reaction to a foreign body, or as a manifestation of sarcoidosis (Fig. 17.18). Granulomatous endometritis, either nonnecrotizing or necrotizing, resulting from systemic *M. tuberculosis* infection, has rarely been reported in the United States in recent years but is not uncommon in Asia and Africa.[184-188]

An incidental case of uterine coccidioidomycosis was diagnosed at the time of hysterectomy for squamous carcinoma of the cervix.[189] In another case an endometrial curettage performed for irregular vaginal bleeding in a 33-year-old patient contained well-formed granulomas, some with spherules, identified within the endometrium on routine stain.[190] Endometrial coccidioidomycosis was reported in a woman who had undergone chemotherapy for Hodgkin disease.[191] A rare case of chronic endometritis with giant cell formation and necrosis secondary to *C. neoformans* infection occurred in an immunocompromised patient (Fig. 17.19) (Blaise Clarke, MD, personal communication). *Blastomyces dermatitidis* has been reported to produce granulomatous endometritis as part of disseminated infection.[192] *E. vermicularis* ova were found in endometrium examined in the workup of postmenopausal bleeding.[193,194] Coccidiosis, a rare protozoan infection that usually produces disease in the gastrointestinal tract, has been reported in endometrium.[195] The 20- to 30-µm cysts of coccidiosis were identified within the endometrial glandular cells, and nonnecrotizing granulomas were present in the endometrium. Other unusual parasitic infections of the endometrium include *Echinococcus granulosus*, *Schistosoma*, and *Toxoplasma gondii*.[196-198] Malakoplakia, an uncommon reaction to bacterial infection, has been reported to occur in the endometrium (Fig. 17.20).[199]

Fallopian Tube and Ovary

Acute Salpingitis

The fallopian tube may be the site of physiologic endosalpingitis at the time of menses, presumably due to reflux of blood, but the neutrophilic response is sparse compared with an infectious process. Acute salpingitis occurs most frequently in young, sexually active women, the majority of whom are younger than 25 years of age.[200] Factors other than age that increase the risk include instrumentation and abortion.[201] As discussed in the section on PID, the bacteria most often responsible for primary acute salpingitis are *N. gonorrhoeae* and *C. trachomatis*, but cultures usually demonstrate a polymicrobial infection (facultative and anaerobic) which dictates the treatment.[202,203] In acute salpingitis, the fallopian tube is grossly edematous. Histologically the plicae initially become edematous and infiltrated by polymorphonuclear leukocytes (Fig. 17.21) and tend to adhere to one another. With more severe infection, inflammation extends through the entire wall, producing a fibrinopurulent exudate within the lumen (pyosalpinx) and beyond the tube to the ovary, resulting in tubo-ovarian adhesions and abscess. The epithelium may show reactive changes, such as loss of cilia.[200] If the infection progresses so far as to produce pyosalpinx, surgery may be necessary. Culture of surgical tissue specimens, obtained either by the operating room team or by the pathologist, is the standard. A case of *Enterobiasis* infection resulting in isolated acute salpingitis has been described.[204]

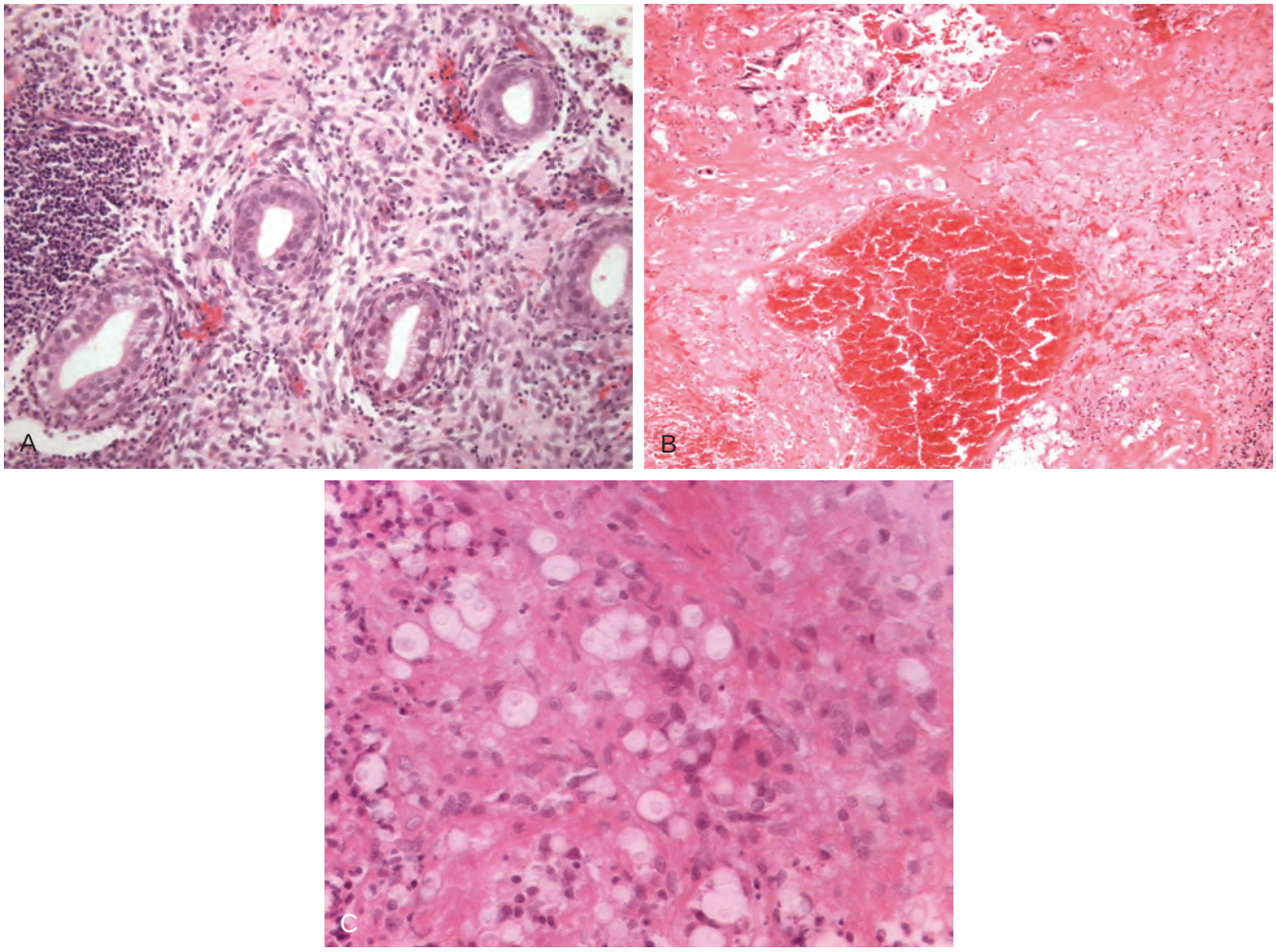
Actinomycosis may occur secondary to IUD contraception, although most PID cases secondary to IUD use are not due to actinomycosis[122] and can produce a marked acute salpingitis or tubo-ovarian abscess.[205] Rarely, sulfur granules, the grossly apparent colonies of actinomycosis, similar to those seen in tonsils, may be present within the abscess cavity. Sulfur granules identified on endometrial curettage or in cervicovaginal smears are often associated with actinomycosis of the upper genital tract. A mimic of actinomycotic granules is the noninfectious PAMRAGs, which can be distinguished by the absence of coloration with histologic stains for microorganisms (Brown and Brenn, Gomori methenamine silver, or acid-fast bacillus stain).[126]

Chronic Salpingitis

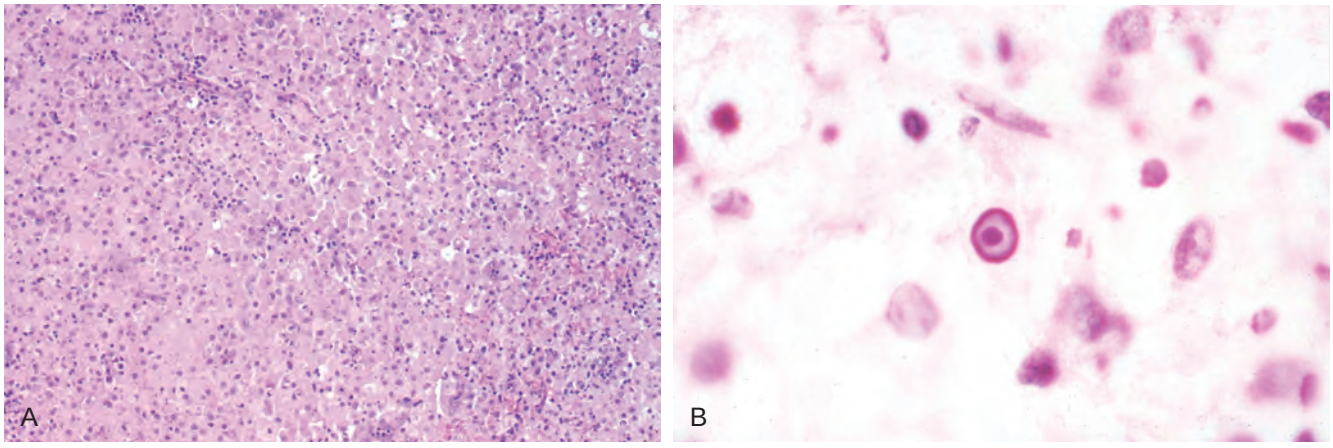
As acute salpingitis resolves, the fallopian tube may become distorted, with occlusion of the fimbriated end of the tube, dilation of the lumen, and accumulation of serous fluid creating a hydrosalpinx. Microscopically, there is, at first, chronic inflammation with shortening, blunting, fusion, and fibrosis of the plicae, which may result in pseudoglandular spaces, a condition also referred to as chronic follicular salpingitis (Fig. 17.22). Pseudocarcinomatous hyperplasia of the epithelium of the fallopian tube can occur in chronic salpingitis.[206] Persistent hydrosalpinx leads to attenuation of the fallopian tube plicae. A large hydrosalpinx may obscure the adjacent ovary and may be mistaken for an ovarian cyst. A case of *Entamoeba histolytica* associated with hydrosalpinx has been reported.[207]

Granulomatous Salpingitis and Oophoritis

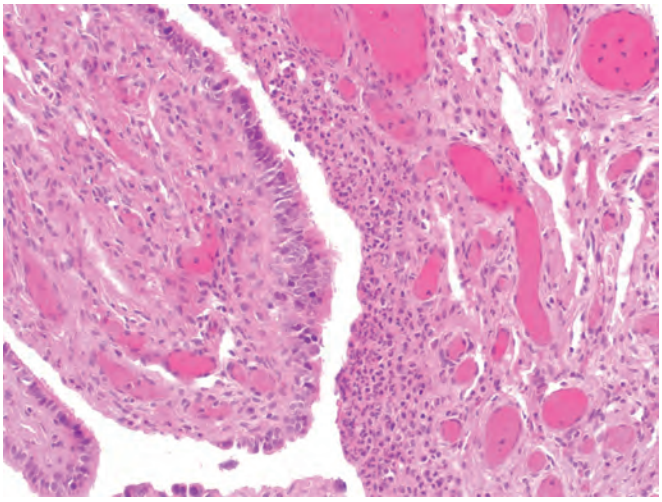
Mycobacterial disease is the most common cause of granulomatous salpingitis, which is present in 80% to 90% of women with genital tuberculosis. There is bilateral involvement in 90% of cases (Fig. 17.23). With time, the fallopian tube becomes grossly nodular, mimicking salpingitis isthmica nodosa.[205] Often, dense tubo-ovarian adhesions form.[208] However, the lumen and fimbria may remain patent, a sign considered by some to be characteristic



• **Figure 17.19** An immunocompromised woman presented with pelvic pain and menorrhagia. **A,** The endometrial curettage focally shows chronic endometritis (hematoxylin and eosin [H&E] stain, ×20). **B,** Elsewhere the tissue is necrotic with granulomatous response and giant cells at low power (H&E stain, ×10). **C,** On high power the pale, round yeast-forms of *Cryptococcus neoformans*, surrounded by a clear halo, are found embedded within the granulomatous inflammation (H&E stain, ×40). (Courtesy Blaise Clarke, MD, University of Toronto).



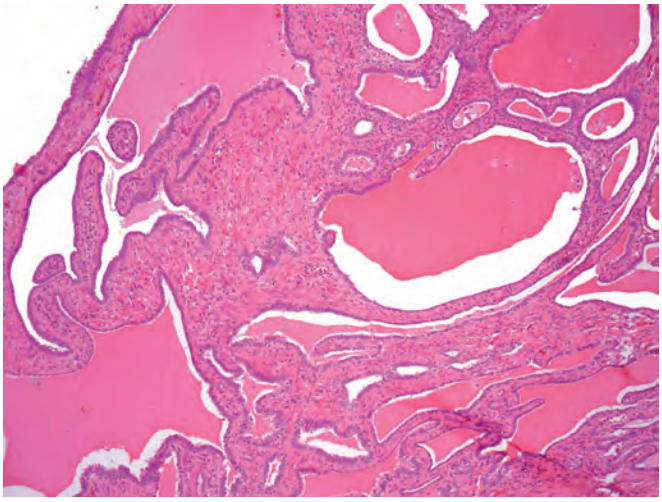
• **Figure 17.20** **A,** An endometrial biopsy sample shows marked histiocytic inflammation characteristic of malakoplakia (hematoxylin and eosin [H&E] stain, ×20). **B,** High-power view of the biopsy sample shows Michaelis-Gutmann bodies (H&E stain, ×60).



• **Figure 17.21** In acute salpingitis, neutrophils predominate. In this case, there is an impressive amount of eosinophilic infiltration of the wall of the fallopian tube (hematoxylin and eosin stain, ×20).

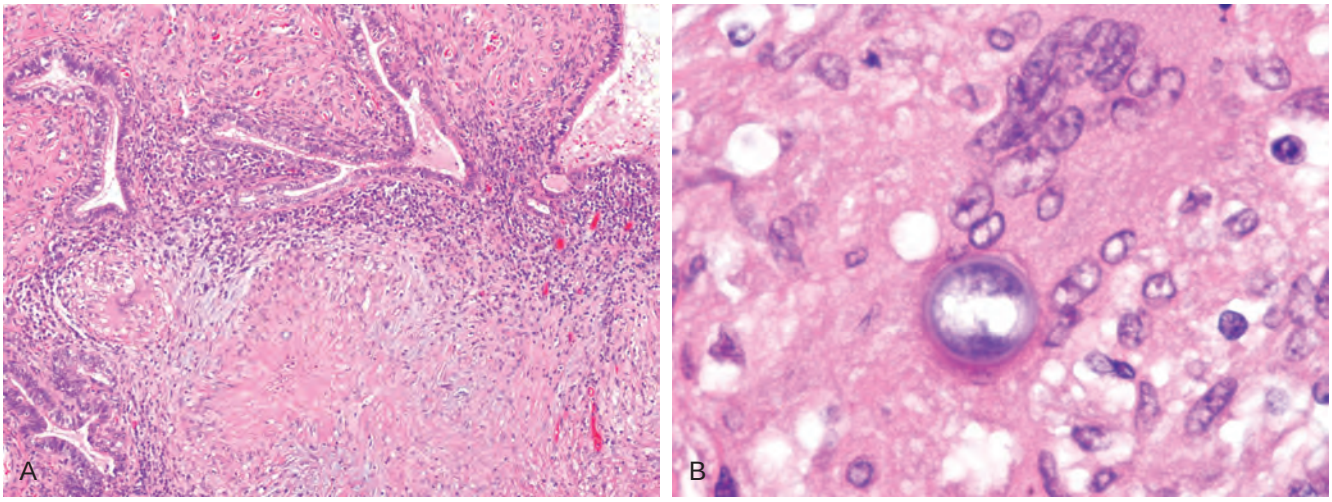
of tuberculous salpingitis.[209] Microscopically, there is prominent granulomatous inflammation, often with necrosis, which begins in the mucosa but eventually engulfs the wall and can extend into surrounding tissues. As in other forms of chronic salpingitis, fusion of plicae can produce a cribriform pattern, which should not be mistaken for adenocarcinoma.

Rarely, fungal or parasitic disease provokes granulomatous salpingitis. Coccidioidomycosis can lead to chronic granulomatous salpingitis.[140,191] Granulomatous salpingitis secondary to schistosomiasis is common in regions endemic for *S. haematobium*. Grossly, the fallopian tube may be nodular or scarred. Microscopically, there may be a granulomatous reaction to ova, which may be identified amid the inflammation.[205] Tubal schistosomiasis may lead to ectopic pregnancy.[210,211] Cysticerci associated with endometriosis have been reported to cause focal granulomatous salpingitis containing partially calcified cestode larvae.[212] *E. vermicularis* (pinworm) can cause chronic salpingitis (Fig. 17.24).

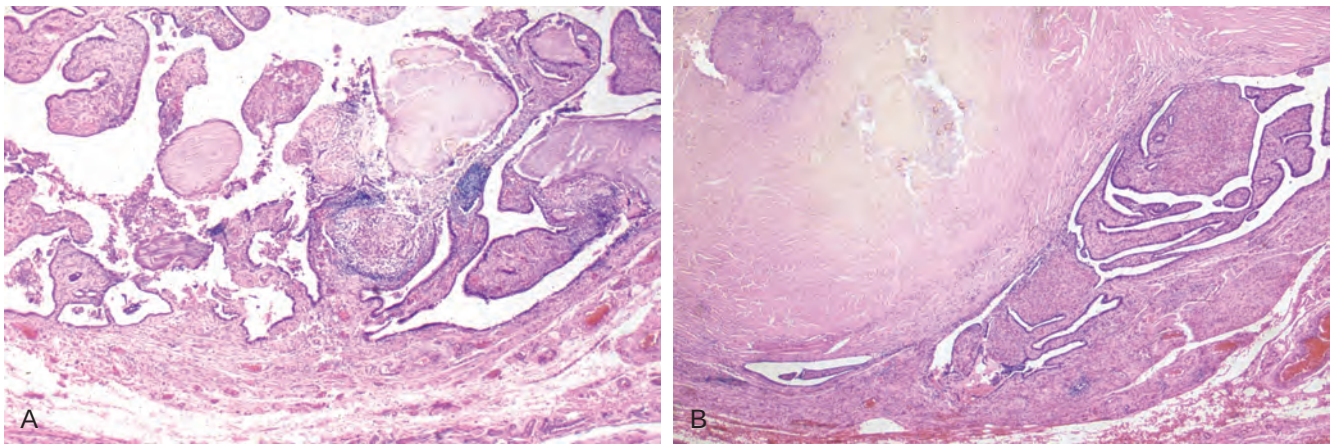


• **Figure 17.22** Chronic salpingitis with marked chronic inflammation in the distorted plical mucosa (hematoxylin and eosin stain, ×20).

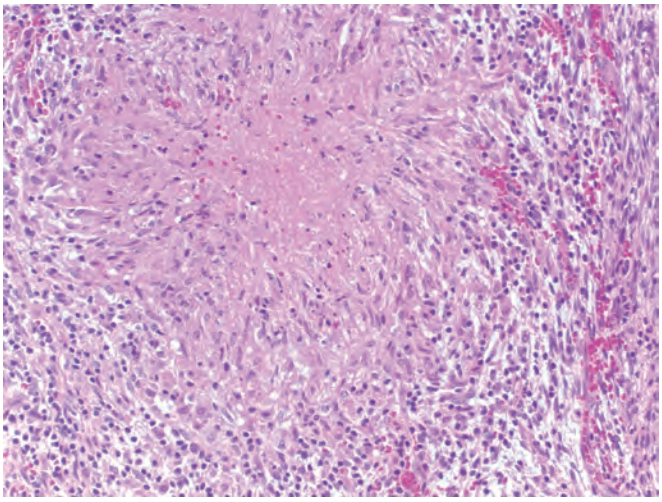
• **Figure 17.23** This massively distorted fallopian tube was found to be infected with *Mycobacterium tuberculosis.*



• **Figure 17.24** **A,** Low-power view of granulomatous salpingitis due to coccidioidomycosis (hematoxylin and eosin [H&E] stain, ×10). **B,** Higher-power view demonstrates the budding yeast forms within granulomatous inflammation (H&E stain, ×40). (Courtesy Drs. Sarah Chiang and John Carpenter, Tucson, Arizona.)



• **Figure 17.25** **A,** Granulomatous salpingitis secondary to *Enterobius vermicularis* (pinworm) is characterized by granulomas within the plicae and wall of the fallopian tube. **B,** High-power view demonstrates the organisms (hematoxylin and eosin stain, ×20).



• **Figure 17.26** Incidentally found palisading granuloma within an otherwise normal ovary. Special stains for acid-fast bacilli and fungi were negative (hematoxylin and eosin stain, ×40).

• **Figure 17.27** Gross image of an actinomycotic tubo-ovarian abscess.

Noninfectious causes of salpingitis include sarcoidosis and Crohn disease.[205,213] Pseudoxanthomatous salpingitis secondary to pelvic endometriosis shows edematous plicae with chronic inflammation and abundant foamy macrophages.[214,215]

Granulomas can be found in the ovary and often are secondary to exposure to foreign material (e.g., sutures, keratin). The granulomas may be associated with multinucleated giant cells, or they may be palisading granulomas (Fig. 17.25).

Tubo-ovarian Abscess

Bacterial infection of the ovary usually occurs as a consequence of salpingitis and PID. Ovarian involvement is typically bilateral but may be unilateral in the rare case of extension from the gastrointestinal tract (appendicitis, diverticulitis). During the acute phase, in addition to purulent exudates, tubo-ovarian adhesions are usually present (Fig. 17.26). Tubo-ovarian abscesses may heal, forming cysts, which may mimic cystic ovarian lesions.[216] Rarely, a chronic abscess develops, producing a solid, yellow, xanthogranulomatous mass.[217] A clue to the inflammatory nature of the cystic change is the presence of entrapped inflamed fallopian tubal plicae. Tubo-ovarian inflammatory disease may produce changes similar to those observed in polycystic ovarian disease.[218]

The bacteria responsible for tubo-ovarian abscesses are most commonly *C. trachomatis* and *N. gonorrhoeae*, but *M. genitalium* is increasingly recognized as a culprit.[168-170] Other, less commonly encountered bacteria include non–sexually transmitted organisms such as anaerobes (*Prevotella*, *Bacteroides*, and *Peptostreptococcus*), *Nocardia*, *Streptococcus pneumoniae*, and *S. aureus)*.

Actinomycotic tubo-ovarian abscesses are associated with IUD use (Fig. 17.27).[219] Actinomycotic granules are only rarely grossly visible. The inflammatory infiltrate may be intense, mainly neutrophils and histiocytes; a specific diagnosis is made by microscopic identification of actinomycotic granules, which comprise gram-positive, argyrophilic, filamentous bacteria radially arranged. Occasionally, a preoperative diagnosis can be achieved by recognition of actinomycotic granules on cervical cytology or endometrial curettage.[220,221]

In approximately 10% of cases of tuberculous salpingitis, the ovary is also involved, producing tubo-ovarian adhesions and granulomatous inflammation.[222] The granulomas are usually

within the cortex of the ovary.[219] *Mycobacterium leprae* has been reported to infect the ovary.[219,223] The resulting leprous oophoritis shows chronic inflammation, fibrosis, and acid-fast–positive bacilli.

As in the fallopian tube, fungal and parasitic infections can lead to tubo-ovarian abscesses. Coccidioidomycosis can cause considerable granulomatous inflammation in the female genital tract, including the ovaries, secondary to systemic disease.[140,224] A tubo-ovarian abscess due to *B. dermatitidis* manifested as an abdominal mass and was initially diagnosed on cervical cytology.[225] The first evidence of disseminated cryptococcosis was as a tubo-ovarian abscess in a 25-year-old, HIV-positive woman.[226]

Enterobiasis has rarely been implicated in tubo-ovarian abscess formation.[194,227,228] Infection by *Taenia* spp. has been reported to cause a tubo-ovarian abscess.[229] Ovarian schistosomiasis is encountered in endemic areas, along with involvement of the fallopian tube.[230]

Rarely, oophoritis results from a viral infection. CMV viral cytopathic effect may be found in stromal cells and endothelial cells of the ovary, usually as a part of systemic infection.[219,221,231,232] Mumps oophoritis occurs much less commonly than mumps orchitis.[219]

Malakoplakia has been reported to involve the ovary[62,233-235] and can manifest as a tubo-ovarian abscess.[236] The ovaries are transformed into friable, yellow masses.[219]

Noninfectious inflammatory processes in the ovaries that must be distinguished from infectious oophoritis include foreign body granulomas, necrobiotic granulomas, sarcoidosis, Crohn disease, and incidental cortical granulomas.[219]

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18

Perinatal Infections

DRUCILLA J. ROBERTS

Ascending Infections
Hematogenously Spread Infections
Specific Infectious Organisms
- Bacterial Infections
- *Mycobacterium tuberculosis*
- Fungal Infections
- Parasitic Infections
- Mycoplasmal and Ureaplasmal Infections
- Syphilis
- Viral Infections

Pregnancy-Associated Uterine Infections
Fetal and Congenital Infections

The placenta is a fetally derived organ present in the maternal uterus; it has many different functions, including serving as a barrier to infection. Maternal infectious diseases and vaginal colonization can lead to congenital infections if the placental barrier is breached. The fetus develops in a fluid-filled space surrounded by membranes composed of two epithelial-mesodermal layers, the amnion and chorion (Fig. 18.1). These membranes generally cover the endocervical os. The fetus and its fluid environment are thus isolated from the microorganisms in the cervicovaginal vault by only four layers of tissue and the cervical mucus. The human placental disk adheres to the maternal uterus via direct colonization of the uterine vascular bed beneath and is directly exposed to maternal blood. Organisms can gain entry into the placenta by direct hematogenous spread or by breaching the membranes over the endocervix, resulting in ascending infection. Fetal infection can follow, but this depends on many factors, including the type and number of microorganisms and the maturity of the fetal immunologic response.

Ascending Infections

The histologic hallmark of ascending infections is acute chorioamnionitis (ACA).[1] Organisms reach the amniotic fluid, where they multiply and stimulate an inflammatory response from the mother and fetus. Histologically, presence of inflammatory cells within the fetal membranes (Fig. 18.2) represents the maternal inflammatory response to microorganisms in the amniotic fluid. The fetal response is diagnosed by the presence of inflammatory cells derived from the fetus in the umbilical cord or chorionic plate vessels, or both (Fig. 18.3). The diagnosis of histologic ACA should be made if there is sufficient inflammation to warrant it and should be staged as to the anatomic location and source of the inflammation. Redline et al.[2] have provided a grading and staging system that is summarized in Table 18.1.

The diagnosis of ACA is established clinically when one or more of the following features are present: maternal fever, fetal tachycardia, uterine tenderness, and pus per cervical os. The most common use of the diagnosis is with maternal fever in labor. The histologic confirmation of this clinical diagnosis is poor, with less than a 25% concordance rate.

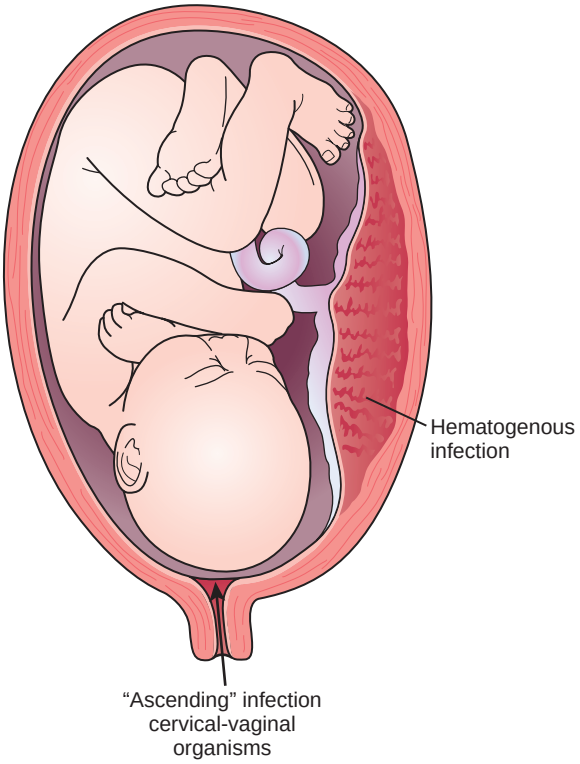
The clinical significance of ACA is, first, that it is one of the most common causes of preterm labor.[3-6] In addition, the presence of ACA stimulates the common practice of culturing a tissue sample from the neonate and treating for a presumption of infection pending results of the culture.[6,7] Newer literature suggests that the presence of a fetal response to the chorioamnionitis is associated with neurocompromise in the neonate.[8-11]

Although it is largely believed that ACA is diagnostic of amniotic fluid infection, this is still not proven. In preterm chorioamnionitis the association with infection is well substantiated.[12-16] Organisms responsible can be single species, such as a group G streptococcus,[13] but most cases of ACA are polymicrobial.[14,15,17-19] In third-trimester cases of ACA, and especially at term, the incidence of chorioamnionitis falls, as does the association with documented infection.[7,16,20,21]

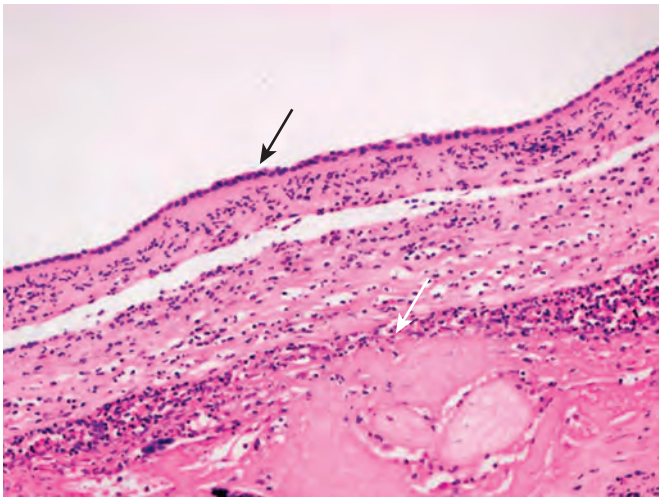
Hematogenously Spread Infections

Maternal infection can spread directly to the placenta. The hallmark of this type of placental infection is villitis, an inflammatory infiltrate of the placental villi (Fig. 18.4).[22] The dominant cell type depends on the type of organism. The most common infections that occur during pregnancy and lead to sepsis are bacterial infections of the urinary tract,[23] although these rarely cause placental infections via sepsis. The two bacterial groups that most commonly infect the placenta and result in villitis are group B streptococci (GBS)[24] and *Listeria monocytogenes*.[25-31] The villitis associated with these infections is termed *acute villitis* because the infiltrate shows a predominance of neutrophils. The classic infections that are hematogenously spread to the placenta are not bacterial but viral or parasitic and lead to *chronic villitis*, with a predominance of lymphocytes and plasma cells in the villous infiltrate.[32]

Although chronic villitis is the most common pathologic manifestation of hematogenously spread infections to the placenta, it is not specific. Chronic villitis at term is common but is thought to be very rarely caused by infection.[33,34] Most cases have

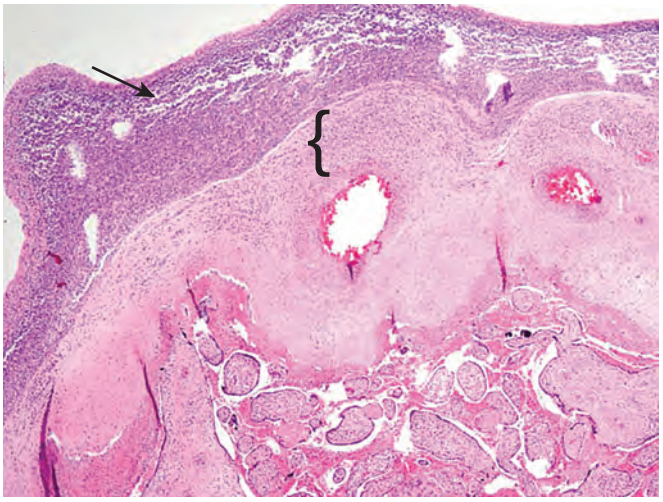


• **Figure 18.1** The routes of fetal infection. (Modified from Netter Anatomy Illustration Collection, Elsevier Inc. All Rights Reserved.)



• **Figure 18.2** Acute chorioamnionitis, in this case maternal stage 2 grade 2. *Black arrow* points to the amniotic epithelium and *white arrow* to the chorionic epithelium.

an unknown etiology and are thought to represent a manifestation of an immunologically based host-versus-graft reaction; therefore a recurrence risk is present, and the infection can potentially result in placental rejection with fetal sequelae.[35-37] In noninfectious chronic villitis, maternal lymphocytes invade the villi.[38] If infection is the cause of chronic villitis, it usually has distinctive features that help to distinguish it from the noninfectious type (Box 18.1). The source of the inflammatory cells in infectious chronic villitis has not been studied. The pathologic workup of chronic villitis should be based on the clinical history and the presence of any of the features listed in Box 18.1.

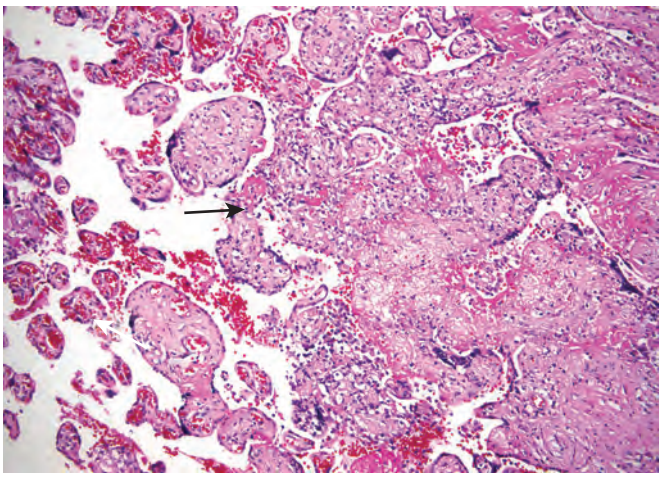


• **Figure 18.3** Acute chorioamnionitis on the chorionic plate (*arrow* points to neutrophils in chorionic plate mesoderm). This is maternal stage 3 grade 2, fetal stage 1 *(bracket)* grade 2.

TABLE 18.1 Grading and Staging of Acute Chorioamnionitis

Diagnostic Category	Definition
Maternal Inflammatory Response	
Stage (Location)	
1 (Early)	Neutrophils in subchorionic fibrin, in chorionic plate, or in chorionic epithelial layer of membranes
2 (Intermediate)	Neutrophils within chorionic or amnionic mesoderm
3 (Advanced)	Stage 2 plus necrosis of amnionic epithelium and/or neutrophils
Grade (Severity)	
1 (Mild to moderate)	Anything less than severe (grade 2)
2 (Severe)	Confluent or more than three foci ≥200 cells
Fetal Inflammatory Response	
Stage	
1 (Early)	Fetal inflammatory cells within chorionic plate vessel walls or umbilical vein vessel wall or both
2 (Intermediate)	Fetal inflammatory cells within umbilical arteries ± vein
3 (Advanced)	Necrotizing funisitis
Grade	
1 (Mild to moderate)	Not severe (grade 2)
2 (Severe)	Confluent fetal inflammatory cells with attenuation/degeneration of smooth muscle

From Redline RW, Faye-Petersen O, Heller D, et al: Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol.* 2003;6:435-448.



• **Figure 18.4** Chronic villitis. *Black arrow* points to region of clustered villi with increased intravillous and intervillous inflammatory cells and decreased to absent villous vessels.

• BOX 18.1 Features Suggesting Infectious Chronic Villitis

Clinical Features

Prematurity
Stillbirth
NICU admission
Hydrops fetalis or placentalis
Trimmed placental weight <5th percentile
Positive HIV status in mother
Suspected or confirmed maternal illness or exposure

Pathologic Features

Marked and diffuse stromal expansion of villi
Diffuse chronic villitis with any of the following:
- Plasma cells
- Giant cells
- Hemosiderin
- Fetal normoblastemia (if >20 weeks gestational age)
- Chorionic plate vascular thrombi

HIV, *Human immunodeficiency virus;* NICU, *neonatal intensive care unit.*

Specific Infectious Organisms

Bacterial Infections

Group B Streptococci/Streptococcus agalactiae

GBS infection is typically due to colonization, usually asymptomatic, of the genital and/or gastrointestinal tract. These bacteria infect the placenta either by ascending through the cervix into the amniotic cavity or via the urinary tract resulting in urinary tract infection and bacteremia.[24] GBS infection is particularly lethal to the fetus because it can cause pneumonia and sepsis. For this reason, it was the focus of a recent late pregnancy screening (35 to 37 weeks) and treatment protocol in the United States.[39-41] Infection during pregnancy can occur in the absence of ruptured membranes.[13] Classically, GBS causes a necrotizing chorioamnionitis of high maternal and fetal stage (Fig. 18.5A).[42,43] Rapid bacterial growth often allows the organisms to be readily visualized on the placental membranes (see Fig. 18.5B). In cases of retained stillbirth, especially in the second trimester, one can occasionally see organisms filling the villous vascular spaces (see Fig. 18.5C). The usual histology of ACA due to GBS amnionic fluid infection is nonspecific, but if necrotizing ACA is present, GBS should be strongly suspected, especially if there is high fetal and maternal stage. Umbilical cord involvement is usually limited to the vessels, but, in some cases, small surface abscesses may be present (see Fig. 18.5D), different from the larger abscesses seen with candidal omphalitis (discussed later).

Hematogenously spread GBS can also result in acute villitis. The histology is usually an isolated villitis without necrosis or abscess formation (see Fig. 18.5E), which helps in differentiating it from listerial placentitis (discussed later).

Fusobacterium

Fusobacterium spp. of filamentous gram-negative, anaerobic bacilli are the cause of ACA in approximately 10% of all cultured cases but in a much higher percentage of preterm ACA.[44-46] They cause necrotizing ACA (Fig. 18.6) comparable with that associated with GBS. The overgrowth of organisms makes them easy to identify on routine hematoxylin and eosin (H&E) staining (see Fig. 18.6). Silver stains or immunohistochemical studies can be used to confirm the diagnosis. The organism does not cause infection in the neonate or fetus but is associated with premature delivery due to preterm ACA.[44-46]

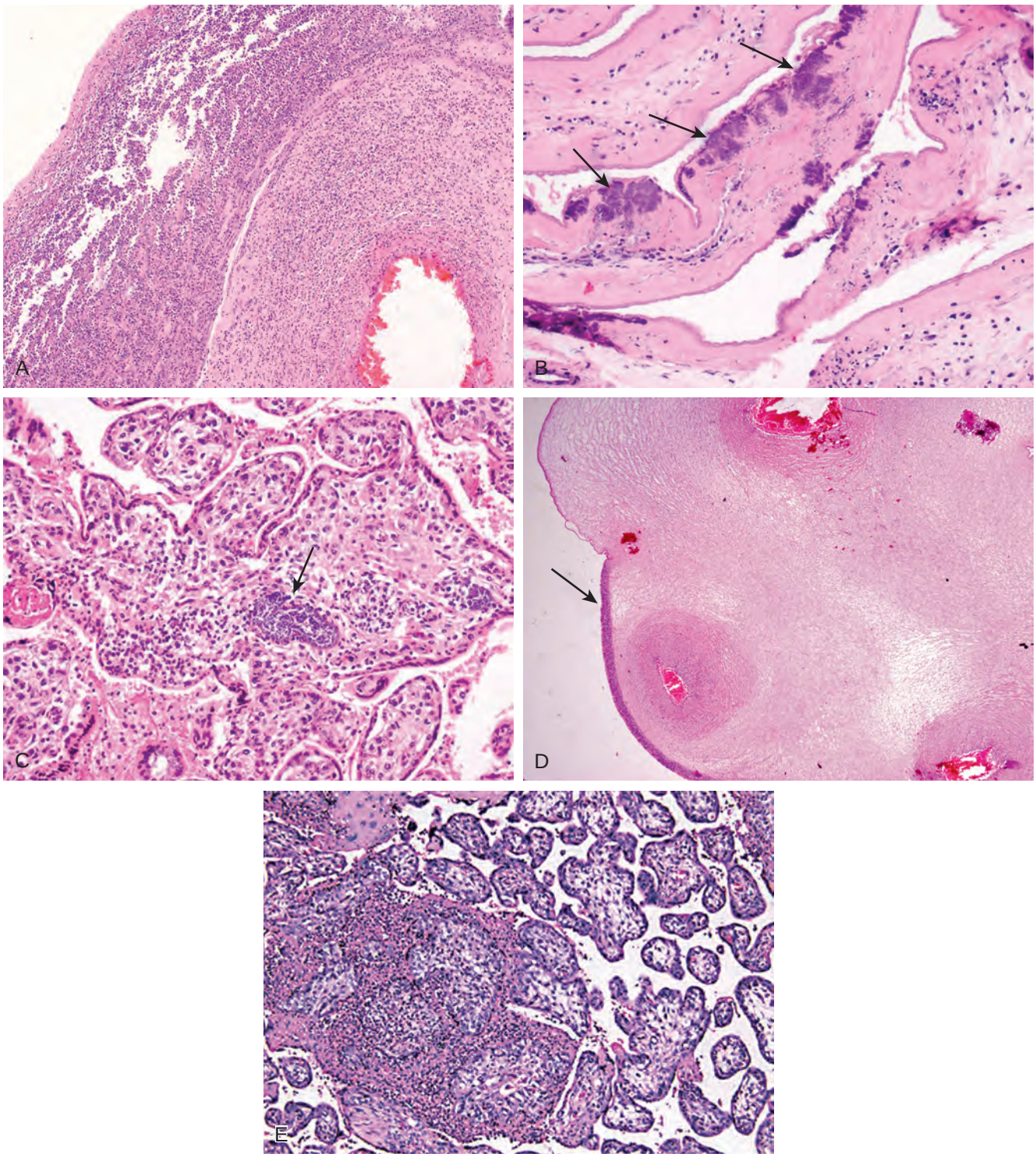
Listeria monocytogenes

L. monocytogenes, a gram-positive motile bacterium, is a well-known perinatal pathogen. It is a common cause of septic abortion in cattle and sheep, and infection occurs in a wide variety of mammals, including humans. The organism is ubiquitous and is present in soil, water, and animal feces. Human infections result from consumption of contaminated foods, most commonly processed meats.[47] Miniepidemics of infections have been reported as a result of the consumption of coleslaw and dairy products that were improperly pasteurized or unpasteurized.[48-52]

Listeria typically produces gastrointestinal illnesses or an influenza-type disease that is self-limited. In immunocompromised hosts, older adults, and pregnant women, the infection can be serious and life-threatening. In pregnancy, a mild maternal gastrointestinal syndrome or flulike symptoms may be all that is observed, yet a marked placentitis with congenital infection can occur. Congenital listeriosis is fatal in 40% to 50% of cases. Other known complications include abortion, prematurity, and stillbirth.[26-31]

The placental findings are striking and the diagnosis can be strongly suspected based on gross findings. The placenta often grossly shows infected yellow membranes (ACA) that emanate a characteristic fruity odor. Parenchymal abscesses may be large enough to be grossly diagnosed (Fig. 18.7A). The presence of placental abscesses should be considered evidence for the diagnosis of listerial placentitis until proven otherwise. The clinicians for the mother and infant should be immediately notified, and the microbiology laboratory should be alerted because specific culture methods may be needed to identify the organism, which grows best at slightly cooler temperatures.

Histologically, both microabscesses and macroabscesses are present, with acute suppurative villitis (see Fig. 18.7B) and necrotizing ACA. The organisms can be identified by silver staining or by immunohistochemistry, and they are located in the center of the abscess or in the amniotic epithelium (see Fig. 18.7C).[53]

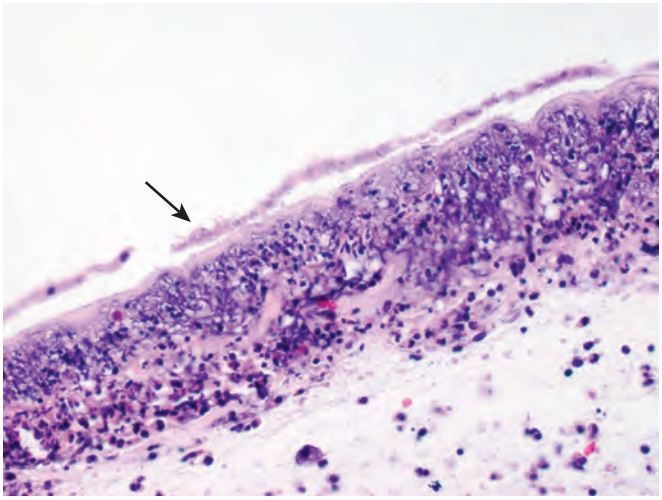


• **Figure 18.5** Group B streptococcal (GBS) placentitis. **A,** Maternal stage 3 grade 2, fetal stage 3 grade 2 acute chorioamnionitis due to GBS. **B,** Fatal case of GBS sepsis in utero with placental membranes showing overgrowth of the bacteria *(black arrows)*. **C,** Fatal case of GBS sepsis with bacteria present in chorionic villous vessels *(arrow)* and acute villitis. **D,** GBS phlebitis with abscess on the surface of the umbilical cord *(arrow)*. **E,** GBS acute villitis.

Mycobacterium tuberculosis

M. tuberculosis is currently causing a worldwide epidemic of tuberculosis, facilitated by human immunodeficiency virus (HIV) infection, resistant strains, and poverty. Women, whether pregnant or not, are at risk for common pulmonary tuberculosis but can also be infected genitally. Congenital infections result from direct extension from an infected endomyometrium[54] or from hematogenous spread that seeds the placenta. The fetus becomes infected by hematogenous infection in the fetal blood[55,56] or by rupture of a caseous lesion into the amniotic fluid and aspiration of the fluid in utero.[57]

The placental pathology in congenital tuberculosis is the same as for infection elsewhere, with caseating granulomas and miliary tubercles involving the villi, intervillous space, or basal plate (Fig. 18.8). Also reported is granulomatous deciduitis and ACA.[58]



• **Figure 18.6** Maternal stage 3 grade 2 acute chorioamnionitis with filamentous rods on amniotic surface *(arrow)*, typical for fusobacteria.

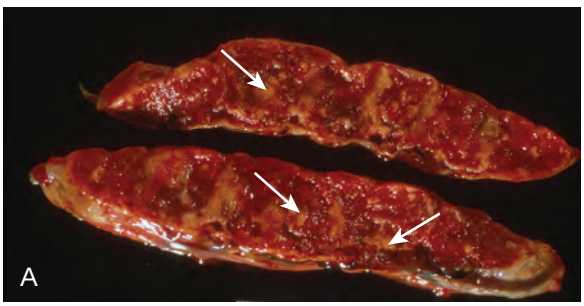
Fungal Infections

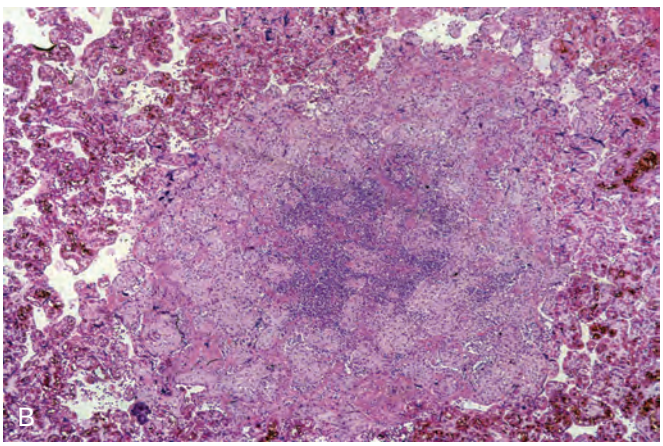
Candida

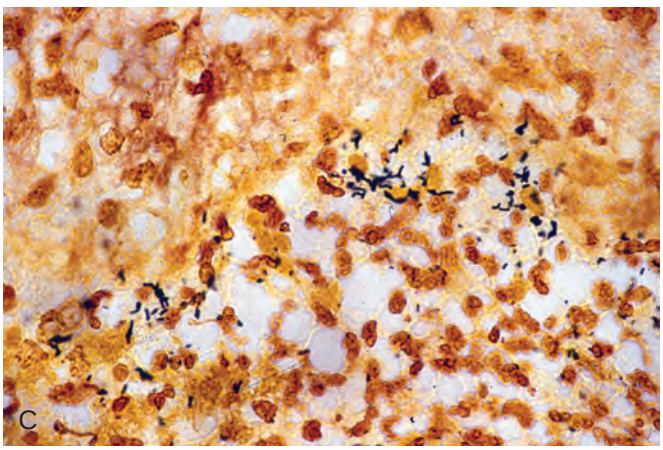
Candida spp. are the most frequent cause of fungal infections of the placenta. Although most *Candida* infections are benign and produce no clinical sequelae in mother or infant, systemic congenital candidiasis is associated with a high fetal mortality rate.[11,59-61] The risk factors for candidal placentitis include maternal glucose intolerance, maternal sickle cell disease or trait, the presence of a foreign object (e.g., intrauterine device, cerclage), and HIV infection.

The hallmark of candidal placentitis is abscesses along the surfaces of the umbilical cord that are visible grossly (Fig. 18.9A).[62-64] Microscopic examination reveals multiple abscesses on the cord, with hyphae often visible on routine H&E staining (see Fig. 18.9B). The presence of hyphae invading into the Wharton jelly is thought to increase the risk of true congenital infection,[63] although that has not been my personal experience.

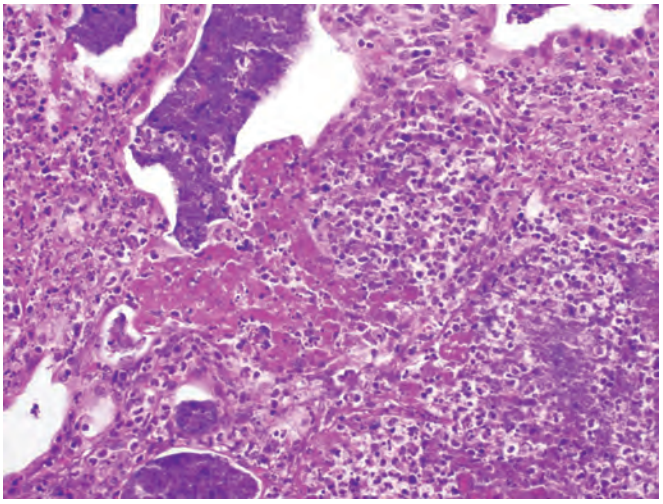
Other *Candida* spp. can infect the placenta, most commonly *Candida glabrata*.[65] The placental lesions include the presence of the organism in the membranes (see Fig. 18.9C),[66] although the placenta can be uninvolved despite congenital infection.[64] The risk







• **Figure 18.7** Listerial placentitis. **A,** Gross photograph of a term placenta with multiple yellow, soft lesions in the parenchyma *(arrows)* which are the microabscesses and macroabscesses typical of listerial placentitis. **B,** Histologic specimen of placental abscess. **C,** Steiner silver stain demonstrates the rod-shaped listerial organisms.



• **Figure 18.8** Placenta infected by *Mycobacterium tuberculosis*. A 30-week gestational age placenta with two granulomatous giant cells in a confirmed case of tuberculosis involving the placenta. (From Wong RMS, Wong KY, Lam BCC. Atypical presentation of congenital tuberculosis in a preterm infant. *Hong Kong J Paediatr*. 2007;12:133-136.)

factors for this type of infection include those for *Candida albicans* infection and immunosuppression.[65]

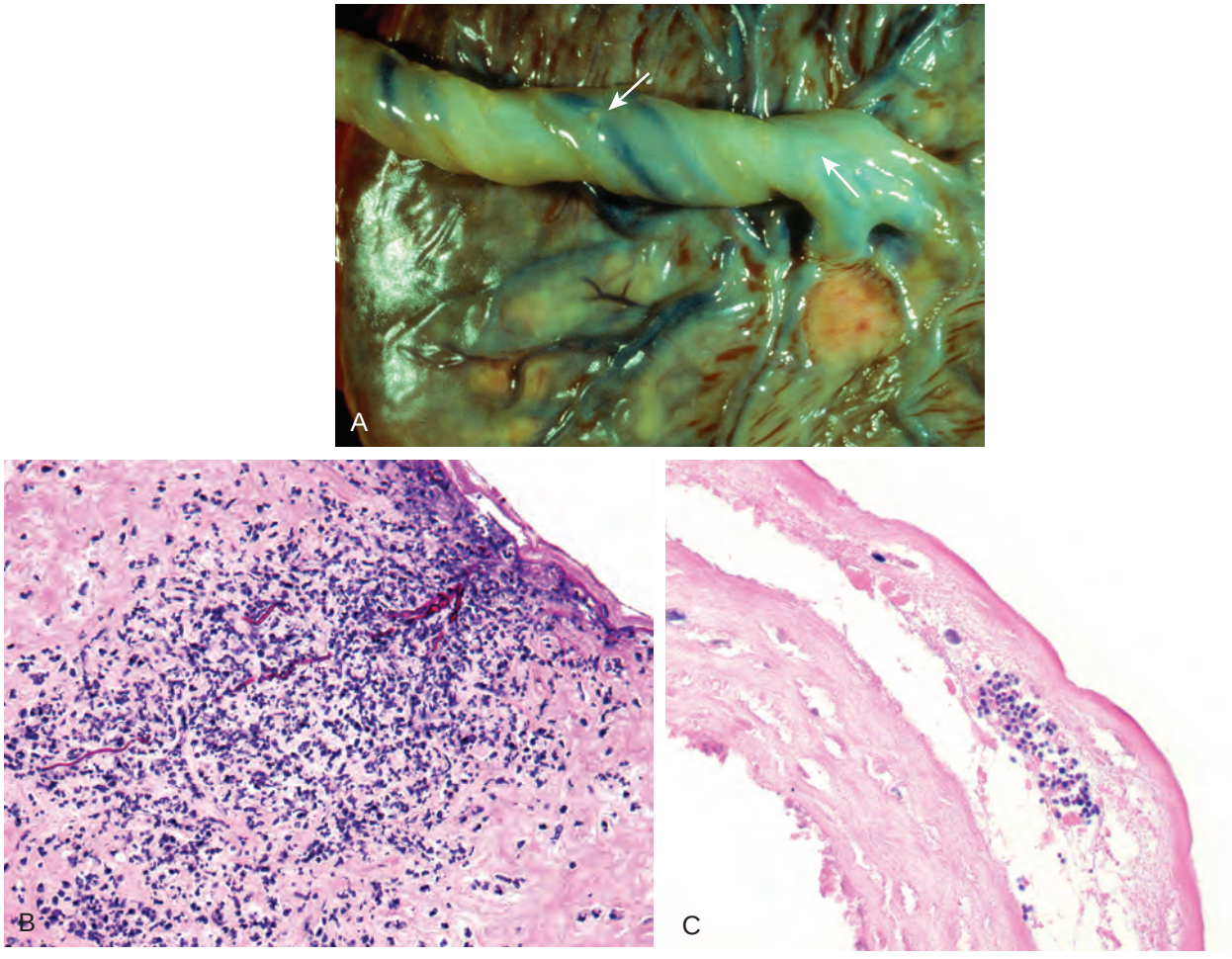
Other yeast infections have been reported in placentas, although extremely rarely and in exceptional circumstances. They manifest as yeast forms present in the intervillous space associated with maternal sepsis.[67]

Parasitic Infections

Plasmodium falciparum

Malarial infections are a devastating reality to the developing world, with a high mortality rate among children. Congenital infections are also common and lead to morbidity and mortality for both mother and infant. The fetus, in cases of maternal malarial infection, is at increased risk for prematurity, low birth weight, and death.[68,69] Maternal mortality is also increased.[69] Pregnancy increases the parasite burden in all species of malaria, irrespective of parity and maternal age.[70]

Placental pathology in malaria can be striking because the pigment-laden maternal red blood cells aggregate in the intervillous space, as do macrophages with large granules of pigment. In some cases the placenta sequesters the organisms, giving the



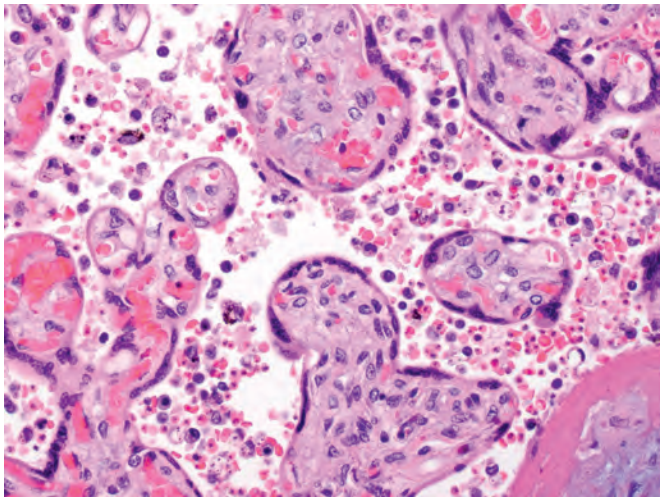
• **Figure 18.9** Candidal placentitis. **A,** Green, discolored placenta and cord with candidal abscesses *(white arrows)* on umbilical cord. **B,** Histologic examination of the abscesses from **(A)** showed deep involvement of Wharton jelly. Pseudohyphae are easily seen. **C,** *Candida glabrata* organisms within membranes with relatively little acute chorioamnionitis.

appearance of a malarial abscess (Fig. 18.10).[71,72] This infiltrate affects placental function by interfering with oxygen delivery by the infected red blood cells.[73,74] The whole effect is exacerbated by the concurrent maternal anemia caused by the infection.

Congenital malaria can be diagnosed on placental examination by the presence of the infected red blood cells or malarial pigment in hematopoietic cells in the placental circulation. Clinically the onset of disease is often delayed by several days and up to months.[75] There is a good association between placental malarial sequestration and congenital malarial infection.[76,77]

Schistosomiasis

Although infection by most *Schistosoma* spp. has been reported in animal species, only *Schistosoma mansoni* has been documented as a congenital infection in humans.[78] *S. mansoni* is prevalent in many regions of the world, yet placental involvement has only rarely been described. Reports of *S. mansoni* eggs[79] and adult worms[80,81] in the placenta in the fetal circulation have been published (Fig. 18.11). The sources include direct extension from the involved female genital tract[82] or backward migration of eggs or worms into the dilated uterine venous system.[79]



• **Figure 18.10** Malaria sequestration. Pigment and organisms are present in maternal red blood cells in the intervillous space.

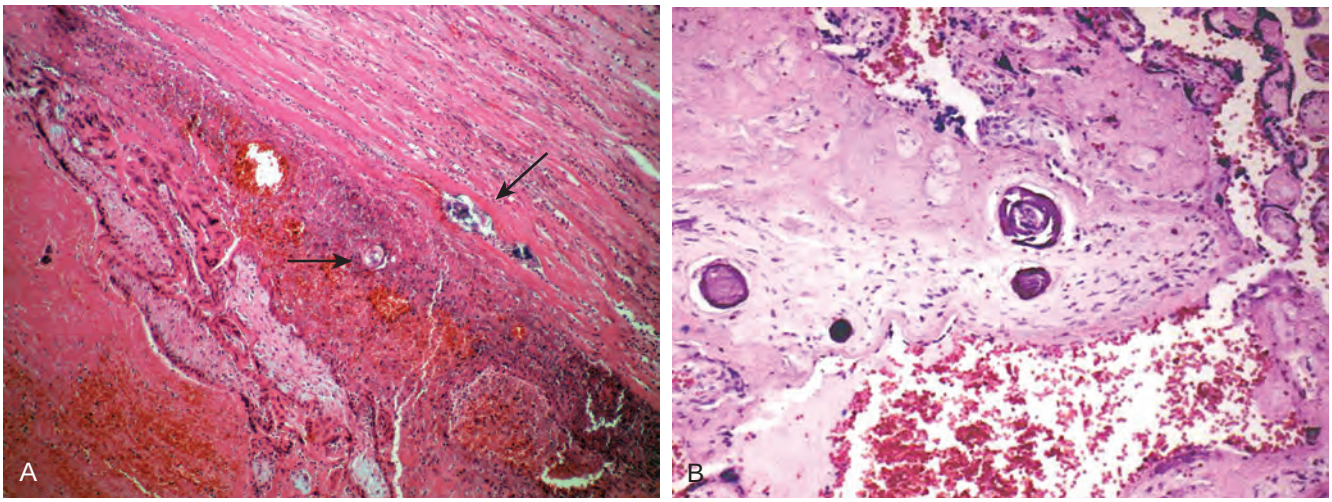
Toxoplasma gondii

The apicomplexan parasite *T. gondii* can infect any warm-blooded animal. Its preferred host is the domesticated cat. Infections in immunocompetent adults are typically silent and result in protective antibodies, although it has been estimated that only 15% of women of reproductive age in the United States have antibodies.[83] The parasite infects the placenta with a transmission rate of approximately 40% and is especially lethal to the fetus, although the lethality is lessened closer to term.[84,85] The organism adheres to and infects the villous trophoblast via migration.[86,87] *T. gondii* trophozoites and cysts can be identified in the umbilical cord in Wharton jelly (Fig. 18.12A) and within the chorionic plate amniocytes (see Fig. 18.12B), although even in documented cases of toxoplasmosis these are difficult to find. The characteristic placental finding is a granulomatous villitis (see Fig. 18.12C)[88,89] or hydrops placentalis.[90] The difficulty in finding the organism has led to molecular techniques for improved diagnosis.[83]

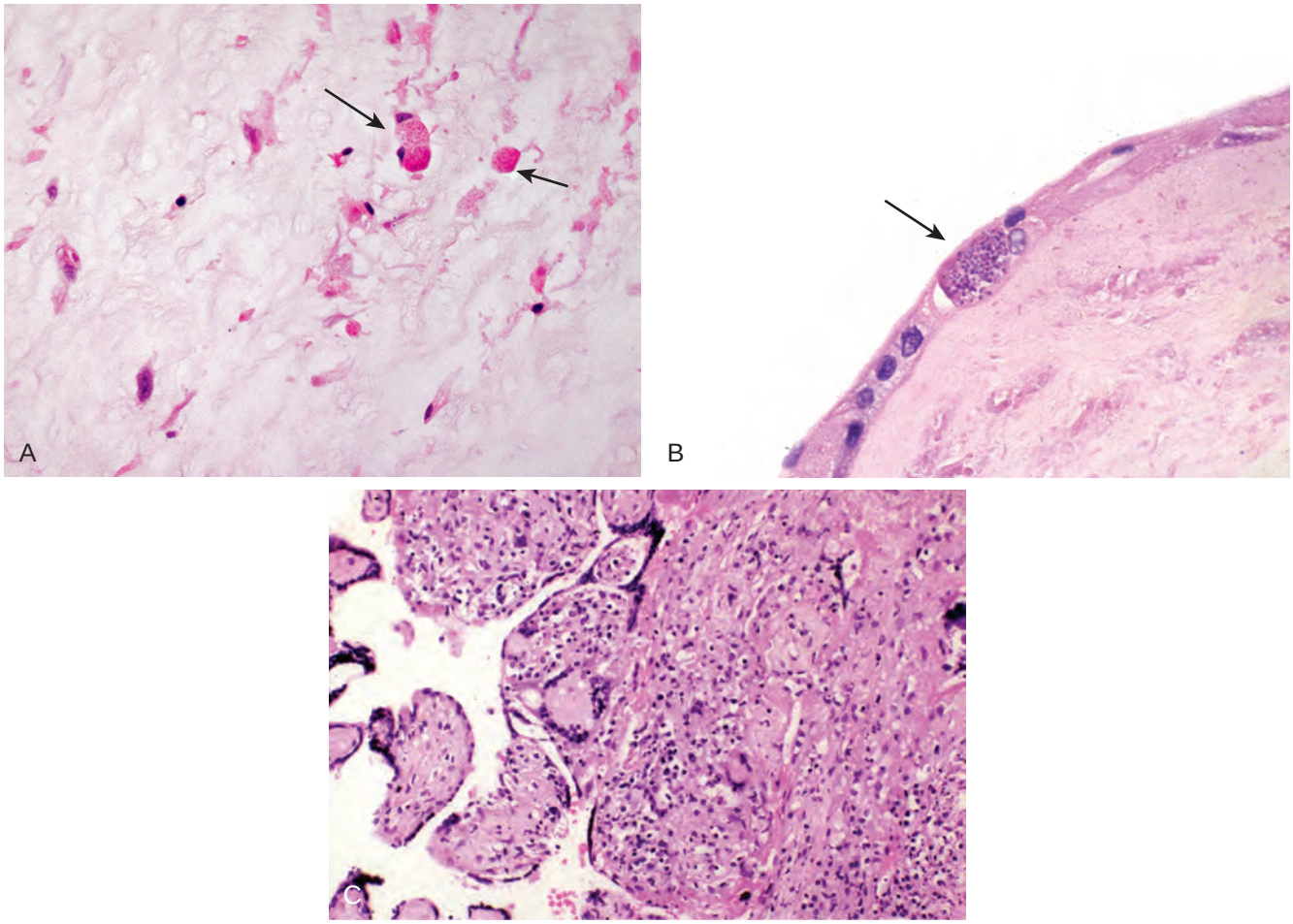
It is known that the visibility of the organism in the placenta relates to the fetal/neonatal outcome: easily identifiable cysts and pseudocysts are present in severely affected cases.[91] The severity of congenital infection and its associated morbidity and mortality are dictated by the timing of the infection in relationship to gestational age; death is most common with first-trimester infections, but infectious transmission is more common in the third trimester.[74,92] There appears to be a latency period in congenital toxoplasmosis infection, such that placental infection is present for some time before fetal infection occurs—the so-called prenatal incubation period.[93] As a result, the greatest risk to the fetus for severely damaging infection occurs during the gestational age of 10 to 24 weeks.[74]

Trypanosoma cruzi

Chagas disease, caused by infection with the protozoan *T. cruzi*, affects millions of people worldwide and has been shown to be vertically transmitted.[74] Hematogenous spread of the organism



• **Figure 18.11** Schistosomiasis in the placenta. *Schistosoma* eggs are present in the villi *(arrows)* of a preterm placenta **(A)** and in the wall and intervillous space of a tubal ectopic pregnancy **(B)**. (**A,** Courtesy Dr. A.L. Bittencourt; **B,** Courtesy Dr. J.N. Kitinya.)



• **Figure 18.12** Toxoplasmosis. Cyst forms *(arrows)* are seen in Wharton jelly **(A)** and in amniotic epithelium *(arrow)* **(B)**. **C,** A giant cell chronic villitis is present in a hydropic stillbirth at 28 weeks of gestational age.

can lead to infection of the trophoblast. Following differentiation into amastigotes, the organisms then spread to the Hofbauer cells, from which they are released into the fetal circulation. The parasites can be present in the villi or membranes (Fig. 18.13)[94]; when present, they are usually associated with intrauterine fetal demise.[95,96] Hematogenously spread placental infection occurs most often in the chronic form of the disease, and the mother is often asymptomatic.[74]

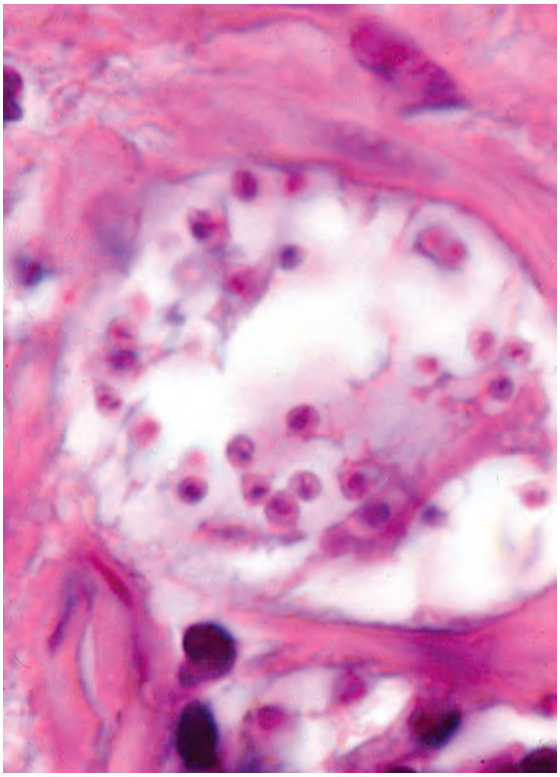
Mycoplasmal and Ureaplasmal Infections

The prokaryotes *Mycoplasma* and *Ureaplasma* are both commonly present in the flora of the lower female genital tract in sexually active women, often without causing symptoms. They have been documented in 30% to 80% of sexually active women, either singly or together.[74] Therefore these organisms and cultures of amniotic fluid or the chorionic plate are commonly associated with histologic ACA.[14,15,97-100] Although the association is much better with preterm ACA, *Mycoplasma* and *Ureaplasma* are still cultured frequently in term ACA. There are no specific histopathologic features for *Mycoplasma hominis* or *Ureaplasma urealyticum*, although they are one of the few documented infections of the amniotic cavity that can occur despite intact membranes.[99,101,102]

Syphilis

Treponema pallidum, a member of the family *Spirochaetaceae*, has a long and fascinating medical history[103] and still causes congenital infections worldwide. The morbidity of syphilis infection in pregnancy depends on the treatment the mother has received and on the stage of her disease. Untreated disease carries a high risk of fetal mortality (at any gestational age), with early syphilis being the most lethal.[104,105]

The placental findings in congenital syphilis are very subtle. There is a diffuse thickening and pallor of the disk grossly. Histologically the hallmarks include relative immaturity of the villi, very mild mixed inflammatory villitis, thickening of the villous perivascular tissues and endothelium ("onion skinning"; Fig. 18.14A), persistent normoblastemia, and a mild ACA (see Fig. 18.14B).[106,107] In the older literature, necrotizing funisitis (a distinctive pattern of umbilical cord inflammation in which the inflammatory cells cluster and break down in a mid–Wharton jelly location; see Fig. 18.14C) was considered to be specific for congenital syphilis,[108,109] but this has not proved to be true; however, the etiology has remained elusive.[110,111] Despite the details of pathologic findings associated with maternal syphilis, placental pathology in syphilis is not correlated with congenital infection.[107,112]



• **Figure 18.13** Chagas disease. The parasites are present in the chorionic villi. (Courtesy Dr. A.L. Bittencourt.)

Viral Infections

Cytomegalovirus

The most common congenital viral infection in humans is cytomegalovirus (CMV). It is associated with placental dysfunction and perinatal morbidity independent of congenital transmission.[113] As with other viral infections, placental involvement is a function of viral load and therefore occurs almost exclusively in primary infection or with immunocompromise. Reinfection or reactivation has been shown to have perinatal sequelae.[114-117] Although the CMV transmission rate is high (approximately 40%),[118] most congenital infections are asymptomatic or mild. However, CMV is one of the most common infectious causes of intrauterine growth restriction, intrauterine fetal demise, neurocompromise, blindness, and deafness.[118-121] Between 1% and 2% of all pregnancies are complicated by congenital CMV, and 10% to 20% of those will have clinical sequelae.[118-121]

CMV placentitis is typified by a thick placental disk, often visualized by antenatal ultrasound.[122] The histopathologic findings include large, "pink" villi caused by stromal expansion and a mild lymphoplasmacytic villitis (Fig. 18.15A). The villi often have focal surface necrosis with intervillositis (see Fig. 18.15B). Chorionic plate vascular thromboses are also common. In active infections the cytoplasmic and nuclear inclusions are numerous and include villous stromal and endothelial cells (see Fig. 18.15C). However, viral inclusions are rare and present only in a small minority of confirmed placental infections (approximately 10%).[123,124] Plasma cell deciduitis is common.[32]

Herpes Simplex Virus

Maternal herpes simplex virus (HSV) infection can involve the fetus or neonate transplacentally or during or after delivery.[125] The transmission rate in primary infection is high, greater than 50%. These infections characteristically occur in asymptomatic women and may occur with either viral type (HSV-1 or HSV-2). Congenital infections are frequently lethal and, if not lethal, are associated with prematurity, intrauterine growth restriction, and severe neurosequelae. Because most of these infections are not clinically apparent, the diagnosis rests on placental pathology or the obvious presence of fetal or neonatal disease (discussed later).

Placental findings are not diagnostic but include severe chronic villitis (Fig. 18.16A) with plasma cell deciduitis.[126] In one case of fetal death at term and unsuspected maternal primary HSV, the placental infection included a marked chorionic plate plasma cell infiltrate (see Fig. 18.16B).

Human Immunodeficiency Virus

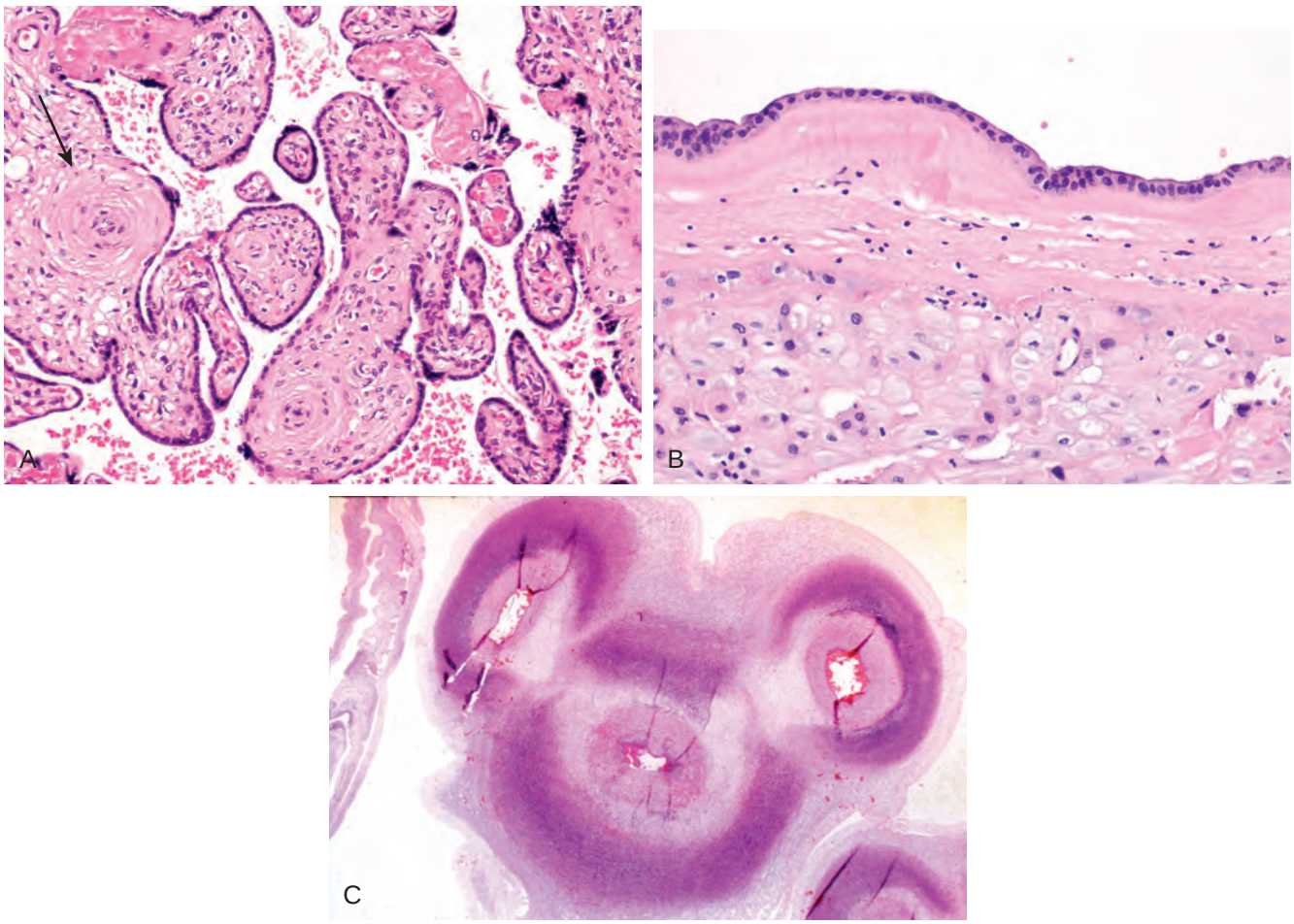
Although it is clear that the HIV virus can infect the chorionic villi (trophoblast, stroma, and Hofbauer cells all can be shown to have viral DNA),[127] there appear to be no specific histopathologic findings predictive of transmission.[128-131] There are reports that the incidence of ACA in HIV-positive mothers may be increased in some populations, but this has not been established.[132-134] What is clear is that coinfections are increased with maternal HIV infection and that there is an increased morbidity due to congenital toxoplasmosis,[135] malaria,[136,137] CMV,[117] syphilis,[138] and other infections.[139] Because of the rarity of these infections in the United States, in practice I encourage clinical consideration for HIV evaluation whenever any placental infection is demonstrated or suspected to be transplacental.

Human Papillomavirus

Although congenital infection by human papillomavirus (HPV) has been demonstrated in several studies,[140-143] placental findings or infection has only recently been described. Slatter et al.[144] describe detecting HPV in the decidua associated with ACA and in the villous trophoblast associated with chronic villitis. This is very interesting and important because the villitis they described associated with HPV has no specific features and would be diagnosed as *villitis of unknown etiology* (VUE) based on the H&E stains alone. VUE is a relatively common finding in placentas, seen in 5% to 15% of placentas.[145] HPV currently is relatively common in the reproductive ages (before the advent of the HPV vaccine), reported in 15% to 25% of pregnant women.[146-150] Perhaps many of the VUE cases are actually infectious and due to HPV. Further study is needed on this important topic.

Parvovirus

Parvovirus is a relatively common pediatric pathogen that can cause significant morbidity in the fetus if a pregnant woman becomes infected. The virus preferentially infects rapidly dividing cells, and the fetal hematopoietic system is a prime target. Mortality is high if infection occurs during the first or early second trimester, when fetal red blood cell mass is low. From mid-gestation on, the mortality rate declines, but significant fetal anemia and hydrops fetalis can still occur. The virus easily traverses through the villi into the fetal bloodstream, where it infects hematopoietic precursors. Infected cells are unable to carry oxygen well and therefore lyse easily, causing anemia and hypoxemia. The placenta does not become directly infected, and the pathology is limited to the infected fetal erythroid cells (Fig. 18.17); the hypoxic and anemic effects on the fetus include hydrops placentalis and chorangiosis.[151-153]



• **Figure 18.14** Syphilitic placentitis. Mild hypertrophy of the vessel wall **(A)** is often seen with congenital syphilis *(arrow)*. Other nonspecific findings that are common in syphilitic placentitis include a mild acute or chronic chorioamnionitis **(B)** and a necrotizing funisitis **(C)**.

Rubella

Congenital rubella is rare in developed countries but is still a risk among nonimmunized pregnant women in the developing world.[154,155] The congenital syndrome is devastating and well described and includes cardiac and ocular anomalies as well as growth restriction and neurocompromise.[156-158] Placental findings are nonspecific and depend on gestational age.[159] Chronic villitis, with or without necrosis and fibrin agglutination, has been described (Fig. 18.18). Occasionally, inclusions are present, which can assist in the differential diagnosis.[159] A publication describes the immunolocalization of the antigen in fetal and placental tissues and reports that antigen is widespread in the tissues known to be associated with malformations in congenital rubella syndrome (heart and brain) as well as in the lung and placenta.[160]

Rubeola

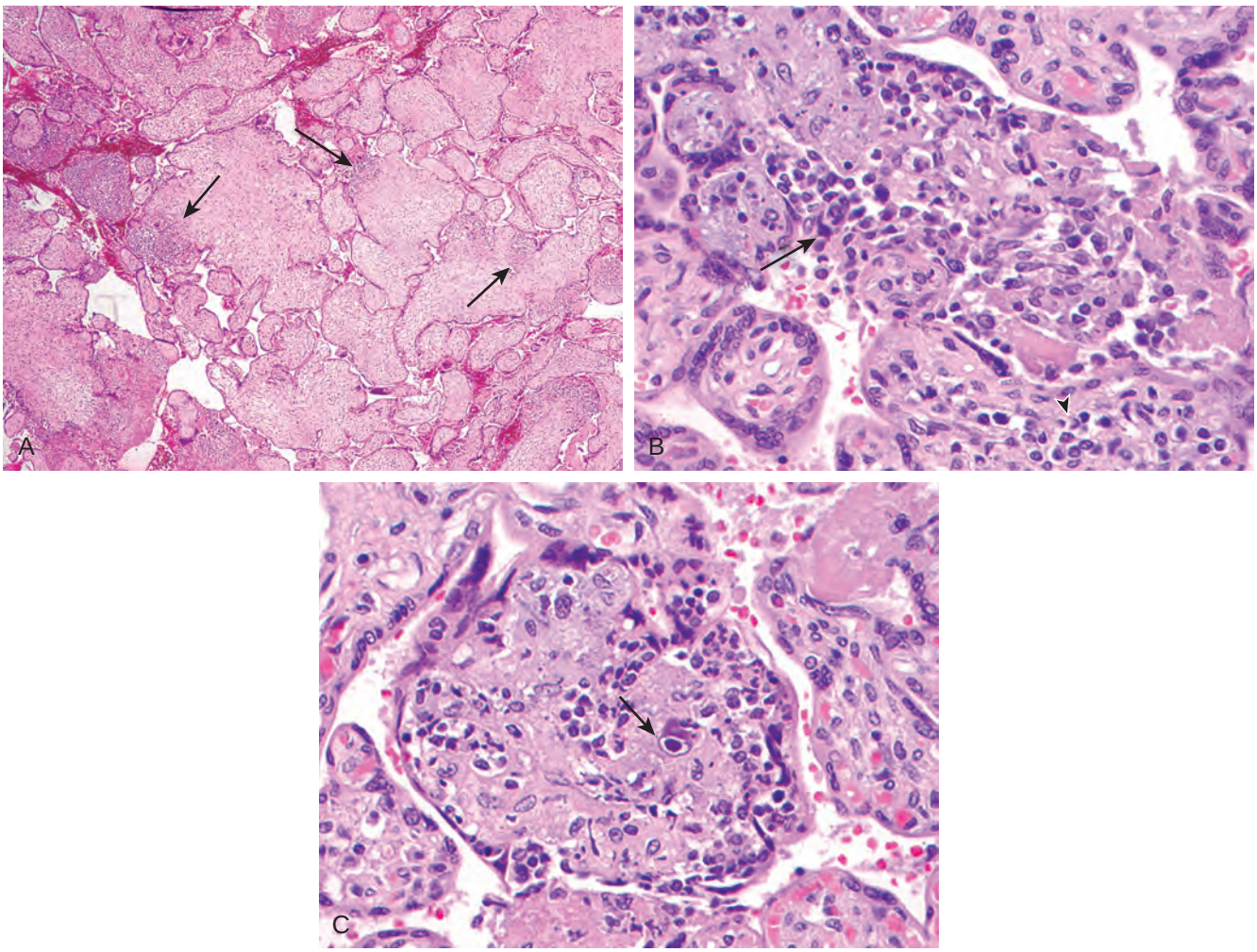
Congenital measles is extraordinarily rare. Transplacental transmission has been reported to occur, but placental findings have only rarely been described.[161,162] In one report, it appeared that the viral particles could be identified in the trophoblast cells.[162]

Varicella

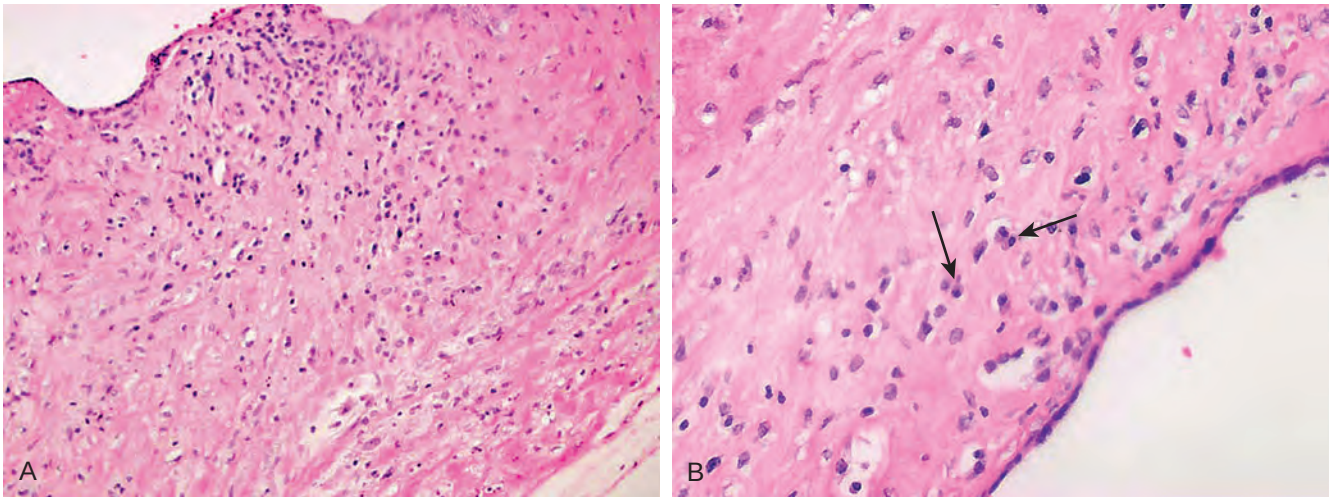
Varicella zoster infection during pregnancy often results in significant maternal morbidity.[163,164] Fetal morbidity includes congenital varicella syndrome, when maternal infection occurs during the first 20 weeks of gestation,[165] and fetal death.[166,167] Transmission is low after 20 weeks of gestation but can be associated with both fetal and neonatal morbidity.[168] Placental findings are uncommon but characteristic; they include marked granulomatous villitis with multinucleated giant cells (Fig. 18.19).[167-169]

Zika Virus

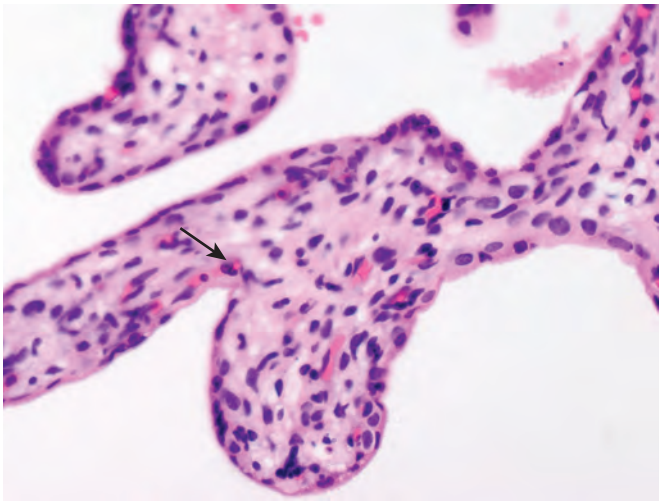
This mosquito-borne flavivirus recently associated with devastating congenital neurologic compromise often resulting in catastrophic microcephaly and perinatal mortality.[170-172] Although not definitively proven to date (March 2016 as of this writing) to be causational, most reports highly suggest the virus is the culprit. Zika is transmitted primarily by *Aedes aegypti* mosquitoes and have humans as the amplifying host. The virus is present throughout much of Africa and Asia but recent outbreaks in the Caribbean and South America are those associated with congenital findings (presumably due to immunologically naive hosts). Little has been published on the pathology of Zika virus–associated neuropathology, but the two reports describe marked necrosis of brain with characteristic perivascular calcifications.[170,171] The placental findings have been much less well described. The third trimester placentas of affected fetuses have been described as normal.[170,171] The first trimester cases resulting in spontaneous abortion are



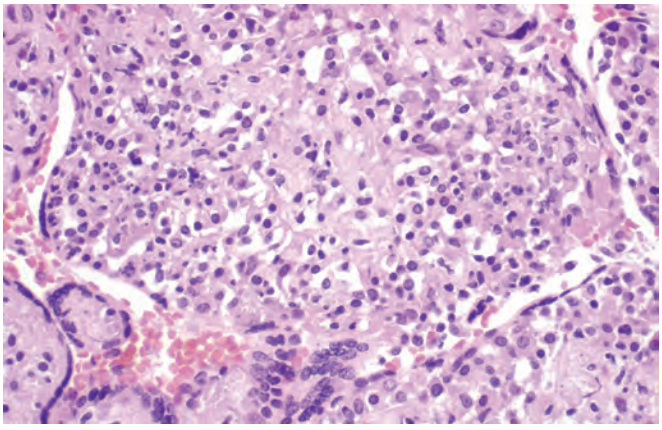
• **Figure 18.15** Cytomegalovirus (CMV) placentitis. **A,** Low-power photomicrograph of a 28-week placenta infected with CMV shows the expanded villi with necrosis of their surfaces *(arrows)*. **B,** Higher-power image shows nonspecific lymphohistiocytic intervillositis *(arrow)* and chronic villitis *(arrowhead)*. **C,** CMV virus in a villous stromal cell *(arrow)*.



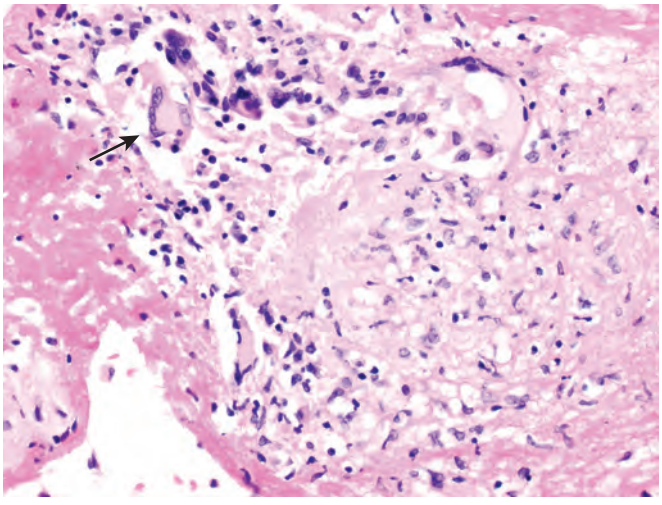
• **Figure 18.16** Herpes simplex virus placentitis. **A,** Stillborn twin gestation at term with marked fibrosis and expansion of the villi involved with a plasmacytic villitis. **B,** The chorionic plate was also involved and showed numerous plasma cells *(arrows)*.



• **Figure 18.17** Term gestation with nonfatal congenital parvovirus infection. Note the inclusion in a red blood cell circulating within the villous vessels *(arrow)*.



• **Figure 18.18** Congenital rubella. Necrotizing chronic villitis in a term placenta complicated with maternal and congenital rubella. (Courtesy H. Rosenberg.)



• **Figure 18.19** Varicella placentitis. Granulomatous villitis with giant cells *(arrow)* in a stillborn at term with maternal varicella pneumonia.

described as showing chronic intervillositis.[170] These reports have not been confirmed in larger studies. Attention to reports of these ongoing studies is necessary before any distinctive pathologic findings are confirmed.

Pregnancy-Associated Uterine Infections

Endomyometritis is a complication of pregnancy that can lead to sepsis, iatrogenic perforation of the uterus during manipulation, gravid hysterectomy, and death. The risk factors include pregnancy complicated by infection (typically bacterial), ACA, retained placenta, and uterine cavity exploration, either manually or with curettage. The pathologist may receive a curettage specimen or the gravid uterus for evaluation, and this may occur immediately or up to several days after delivery.

The histologic features of endomyometritis include inflammation with a predominance of either neutrophils and eosinophils (acute endomyometritis) or chronic inflammatory cells. Inflammation may be associated with necrosis and hemorrhage of the endometrium and myometrium. In the immediate postpartum period, these features may be confused with those resulting from normal labor and delivery. Acute endometritis with hemorrhage and necrosis is a common finding of the labored endometrium. A diagnosis of either acute or chronic endomyometritis requires the presence of myometrial inflammation.

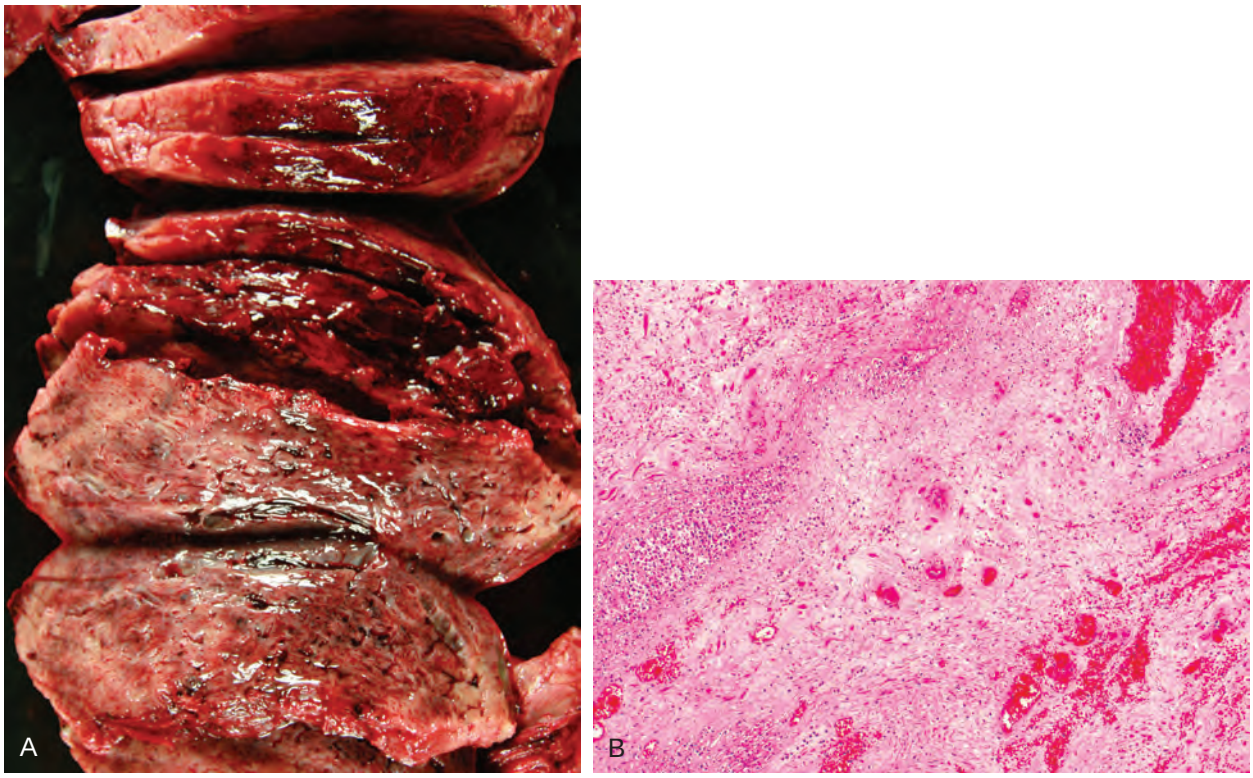
Most cases of endomyometritis are caused by bacterial infections that are common in pregnancy and have no specific histopathologic features other than those listed previously. However, group A streptococcal infection causes a specific infectious endomyometritis that is especially lethal.[173-176] The infection is often fulminant and can occur in the third trimester,[177] during labor,[178] and through the puerperium.[179] The course is rapid and follows an upper respiratory tract infection but causes relatively mild symptoms initially, other than fever.[179] However, multiorgan failure ensues, leading to both maternal and fetal or infant death. The histopathology shows necrotizing endomyometritis (Fig. 18.20).

Fetal and Congenital Infections

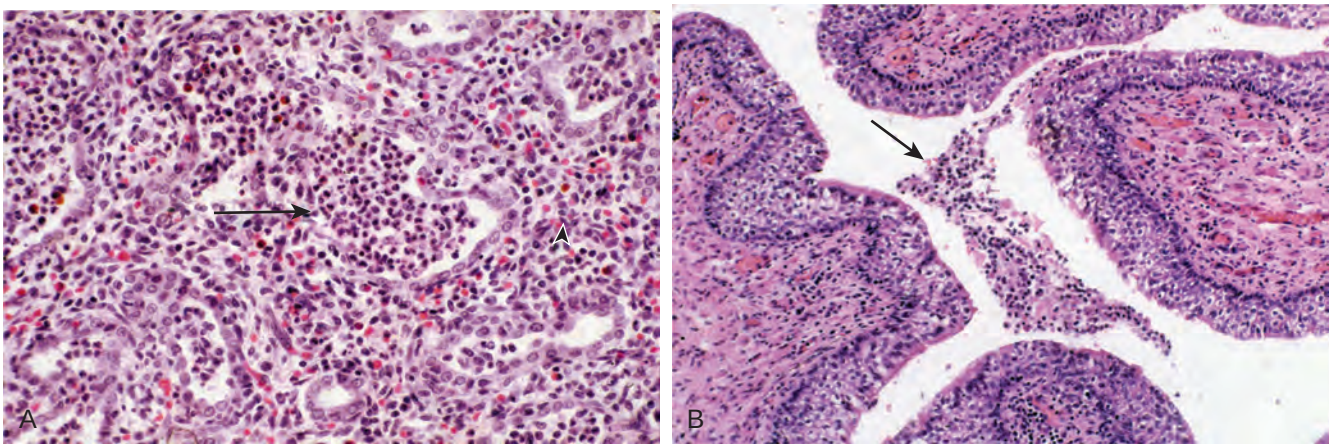
Congenital infections occur in transplacental and ascending infections and are often lethal. The histopathologic features mirror the route of infection.

Infected amniotic fluid involves the fetus by so-called aspiration, leading to aspiration pneumonia due to the presence of maternal neutrophils, with or without organisms, in the developing airways or, rarely, a true congenital pneumonia due to a response by the fetus. Both can result in sepsis (Fig. 18.21A). The fetus also may become infected by "swallowing" the contents of the amniotic fluid; this fills the lumen of the gastrointestinal tract (see Fig. 18.21B) with swallowed inflammatory cells and organisms, leading to sepsis. In addition, the fetus can become directly infected by exposure of the skin, ear canals, or eyes to the infected amniotic fluid.

The presence of ACA and intrauterine fetal demise or neonatal death (especially in prematurity) should raise the suspicion for an amniotic fluid infection–related death. Samples for microbiologic culture should be taken from the placenta (by peeling back the amnion and swabbing the exposed chorionic plate) and from the fetus at autopsy (by exposing the thoracic cavity and obtaining a small piece of lung using sterile tools). Both aerobic and anaerobic cultures should be requested. In addition, the microbiology



• **Figure 18.20** Group A streptococcal endomyometritis. **A,** Gross image of a uterus resected after a term delivery with clinical chorioamnionitis complicated by postpartum hemorrhage and septic shock. **B,** Histopathology of the uterus from the same patient as in **(A)**, showing diffuse hemorrhagic and inflammatory necrosis of the myometrium.

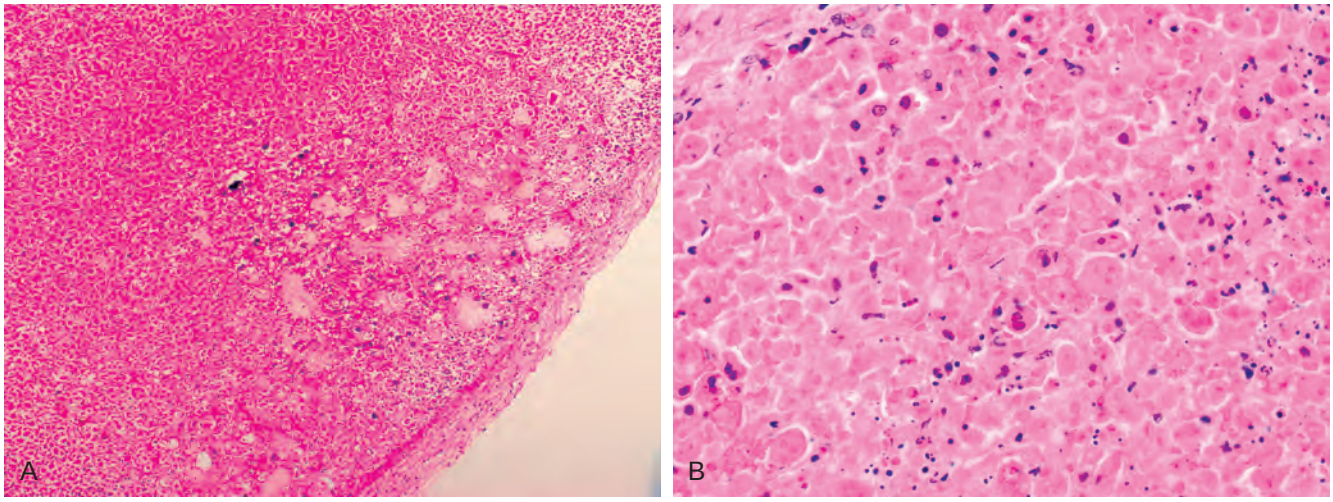


• **Figure 18.21** Congenital pneumonia. **A,** Specimen from a second-trimester fetus delivered prematurely with amniotic fluid infection (acute chorioamnionitis). The fetus died during labor. Maternal inflammatory cells were aspirated in utero and were found to be present in the developing airways *(arrow)*. The fetal response in the interstitium *(arrowhead)* documents infection before death. **B,** Mature (maternal) inflammatory cells in the lumen of the fetal esophagus *(arrow)*.

laboratory should be alerted for directed culture techniques if there is suspicion of *L. monocytogenes*, *M. hominis*, or *U. urealyticum* infection clinically or based on the gross examination of the placenta or fetus.

Hematogenous spread of maternal infection results in congenital infection through involvement of the villous stroma and endothelium, and access to the fetal blood results in fetal sepsis.

Each organism has tropism for specific organs, which it preferentially infects, although liver, lungs, and kidneys are most commonly involved. Specific patterns of infection are a function of the route of congenital infection and the tropism of the organism. For example, parvovirus preferentially infects the most rapidly dividing cells in the fetus, typically the hematopoietic elements, leading to the presence of circulating infected fetal nucleated red blood cells (see Fig. 18.17). Congenital bacterial



• **Figure 18.22** Congenital herpes simplex virus (HSV) infection. **A,** Autolyzed stillbirth at term with congenital HSV, showing classic wedge-shaped adrenal cortical infarct with calcifications. **B,** Typical HSV viral inclusions are seen in hepatic geographic necrosis.

infections from ACA typically lead to congenital pneumonia and sepsis. Most hematogenously spread congenital infections infect the fetal liver initially and then show some level of tropism to other organs. For example, HSV infects the lung and liver, yielding geographic necrosis, and can also infect the central nervous system and the cranial nerve ganglia. It produces a characteristic pattern of infection in the developing adrenal gland, targeting the subcapsular cortical regions of both the definitive and the fetal cortex, and producing a wedge-shaped infarct, often with dystrophic calcifications (Fig. 18.22A), which can be identified even in the most autolyzed cases. The inclusions are often present in the center of the necrotic area and may be recognized on routine H&E staining (see Fig. 18.22B). *T. pallidum* can be demonstrated by special stains in the liver even in very autolyzed stillbirths[180] and in my experience is easy to find in the vitreous of the eye. Parvovirus infects bone marrow and hematopoietic precursors in the liver. CMV is often disseminated diffusely but is unique in its preference for infecting the islets in the pancreas. Coxsackievirus targets myocardium.

In general, hematogenously spread infections result in nonspecific findings of intrauterine growth restriction, hydrops fetalis, normoblastemia, hepatosplenomegaly, calcifications in the central nervous system, and intrauterine fetal demise. Suspicion for such a congenital infection should be increased if two or more of these features are present, and maternal antibody status should be evaluated. Molecular or virologic studies can also be performed on placental or fetal tissue, if needed for the diagnosis, although usually the histopathology is definitive.

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19

Infections of the Nervous System

SANDRA CAMELO-PIRAGUA, E. TESSA HEDLEY-WHYTE

Introduction

Acute Inflammatory Response
- Infectious
- Noninfectious

Chronic Inflammatory Response
- Infectious
- Noninfectious

Granulomatous or Predominantly Histiocytic Inflammatory Pattern
- Infectious Etiologies
- Noninfectious Etiologies

Minimal or No Inflammatory Response
- Fungi
- Viruses
- Prions

Introduction

The central nervous system (CNS) is well protected and separated from the rest of the body by a thick physical barrier, the skull and the spine, and by a finer blood–brain barrier. Therefore infections of the CNS occur by trauma, by hematogenous spread with disruption of the blood–brain barrier, or by direct spread in more vulnerable areas such as the olfactory nerve.

If an infection of the CNS is suspected, the most common approach is to analyze the cerebrospinal fluid (CSF) for cell count and microorganisms or to examine blood cultures if a systemic disease is suspected. This will not likely be a challenge for the surgical pathologist. The challenge occurs when infection is not suspected and a brain biopsy is performed for other reasons, such as imaging abnormalities with no clear etiology. It is important to remember that, radiologically, infections can mimic demyelination, primary brain tumors, metastases, or even lymphomas.

Because infection may not be among the possibilities being considered by the clinical team, it is important for surgical pathologists to recognize or suspect an infectious etiology in certain circumstances. The objective of this chapter is to approach the various infectious entities by pattern of nervous system inflammation rather than by specific organism. The chapter also describes noninfectious entities that can produce the same type of reaction and should be considered in the differential diagnosis.

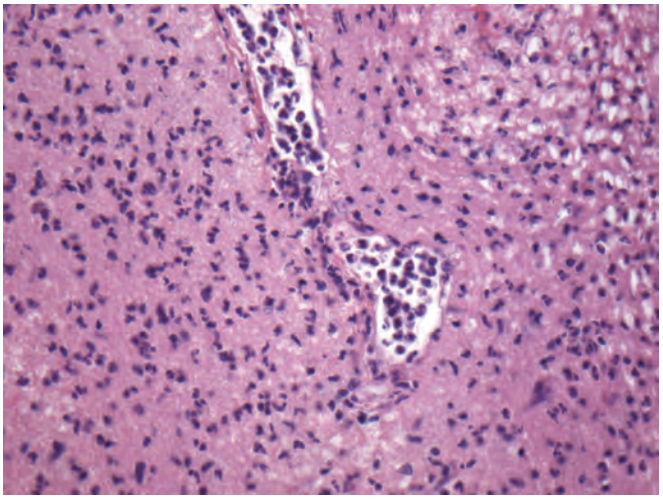
The brain has a limited number of ways in which it can respond to infections. There can be an acute inflammatory response, such as is seen in other organ systems of the body. This is usually generated by bacterial organisms but can occur in some viral infections, particularly herpes simplex. Some infectious organisms have a specific tropism in the CNS. In particular, viruses can be neurotropic (i.e., specifically targeting neurons) or pantropic (i.e., affecting the entire neuropil). Some of the neurotropic viruses, particularly rabies, may result in no or only minimal inflammatory response. Acute inflammatory reactions need to be differentiated from acute infarction, which can on occasion resemble an acute cerebritis (Fig. 19.1). Some infections generate a chronic inflammatory response including lymphocytes and plasma cells. Perivascular lymphocytic cuffing is a characteristic feature of infection of the brain (Fig. 19.2). A unique response to infection in the brain is the appearance of rod-shaped microglial cells in gray matter structures (Fig. 19.3). Specific examples are viral spirochetal infections and subacute sclerosing panencephalitis (SSPE).

Acute Inflammatory Response

Infectious

Bacterial

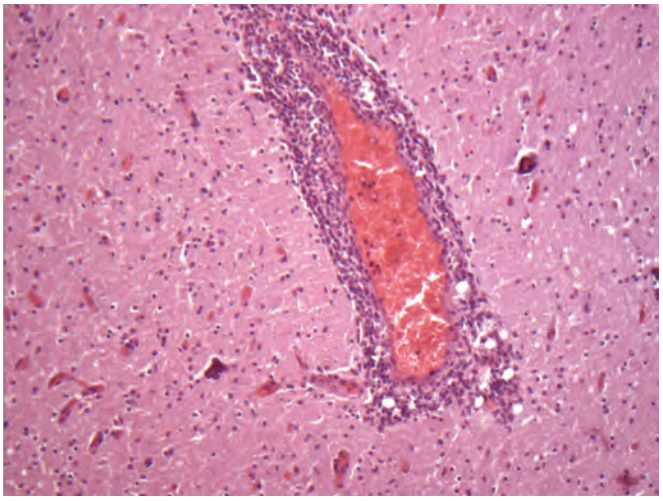
Meningitis. Bacterial meningitis is an important cause of morbidity and mortality. It is usually caused by hematogenous dissemination of bacteria from another source, although other routes of entry are also seen, such as direct extension from the skin after trauma or developmental anomalies (e.g., myelomeningoceles, congenital sinuses) that can be found in the sacral region. The most common etiologic agents of meningitis are *Haemophilus influenzae* type B, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. However, since the development of vaccination, the incidence of *Haemophilus* has decreased by more than 90% in developed countries.[1-3] These organisms tend to affect specific age groups. For instance, β-hemolytic streptococci and *Escherichia coli* are most commonly seen in the neonatal period, whereas *S. pneumoniae* is seen at the extremes of life, both infants and older adults. *N. meningitidis* is primarily a disease of children and young adults (Table 19.1). Patients present with severe headache, neck stiffness, and high fever.[4] Acute meningitis shows a massive number of neutrophils in the leptomeninges, with spread throughout the Virchow-Robin spaces. Bacteria can be found within the neutrophils or outside them in the inflammatory infiltrate (Fig. 19.4). The neutrophilic infiltrate



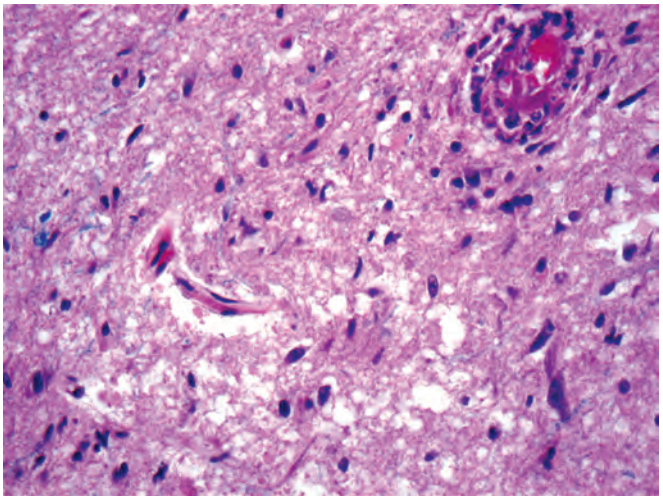
• **Figure 19.1** Cerebral infarct. Acute inflammation in the brain parenchyma of a recent infarct resembling a cerebritis. Note the neutrophils in the vessel lumen and in the brain parenchyma.

TABLE 19.1 Bacterial Meningitis Pathogens by Age Group Affected

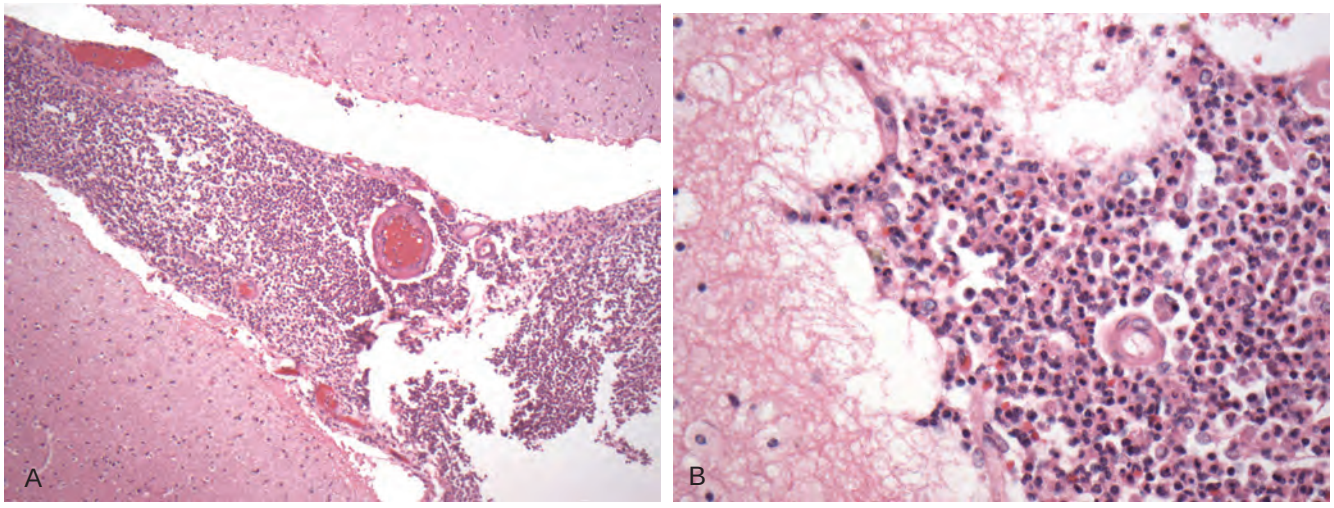
Pathogen	<1 Month	1 to 3 Months	3 Months to 50 Years	>50 Years
Group B streptococcus	X	X	—	—
Escherichia coli	X	X	—	X
Listeria monocytogenes	X	—	—	—
Haemophilus influenzae	—	X	X	—
Streptococcus pneumoniae	—	X	X	X
Neisseria meningitidis	—	X	X	X



• **Figure 19.2** Brain with perivascular lymphocytic inflammation, a common indicator of an infectious etiology.



• **Figure 19.3** Microglia distributed throughout the neuropil. Note the small, necrotic vessel with lymphocytic infiltration of its wall.

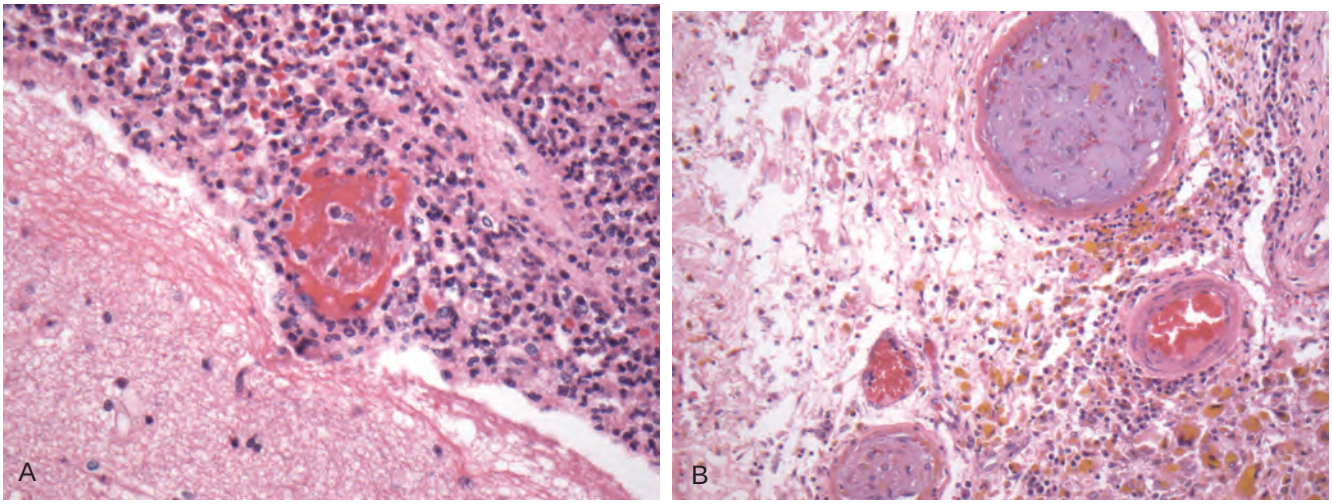


• **Figure 19.4** Bacterial meningitis. **A,** Pus is present in the subarachnoid space. Note the mild astrocytic reaction in the subpial regions. **B,** The inflammatory exudate is composed of numerous neutrophils with rare eosinophils.

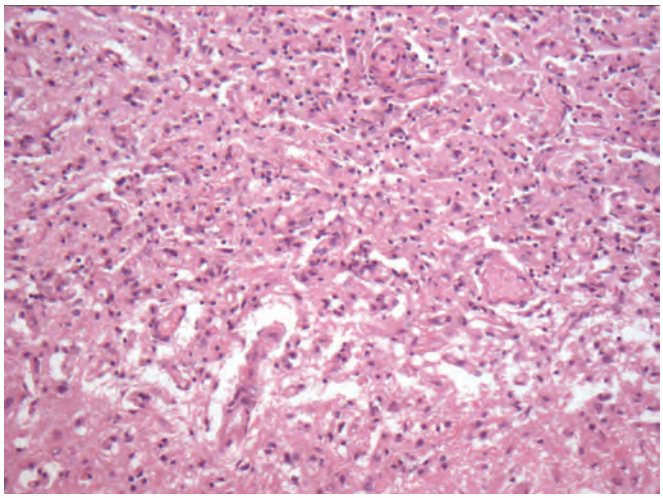
can invade leptomeningeal vessels, leading to a vasculitis with subsequent thrombosis and infarcts (Fig. 19.5). After 2 or 3 days, the inflammatory infiltrate changes to lymphocytes, plasma cells, and macrophages; eventually, it becomes an organized exudate, leading to fibrosis of the subarachnoid space that obstructs the CSF flow and causes hydrocephalus (Fig. 19.6).

Abscess. Another acute inflammatory process that is seen by the surgical pathologist is a brain abscess. Abscesses begin as a focus of acute cerebritis and progress to a sharply circumscribed, encapsulated, necrotic mass with prominent neutrophilic exudate surrounded by a fibrous capsule (Fig. 19.7). The only other cause of fibrosis within the brain is trauma, either accidental or surgical. The fibrous capsule is easily demonstrated by use of a trichrome stain (Fig. 19.8). Most brain abscesses are caused by infections with mixed flora, such as those present in the oral cavity. Patients with cyanotic congenital heart disease or a history of drug abuse are particularly susceptible to brain abscesses after dental work.[5,6] On imaging, an abscess may be hard to differentiate from a malignant tumor.[7]

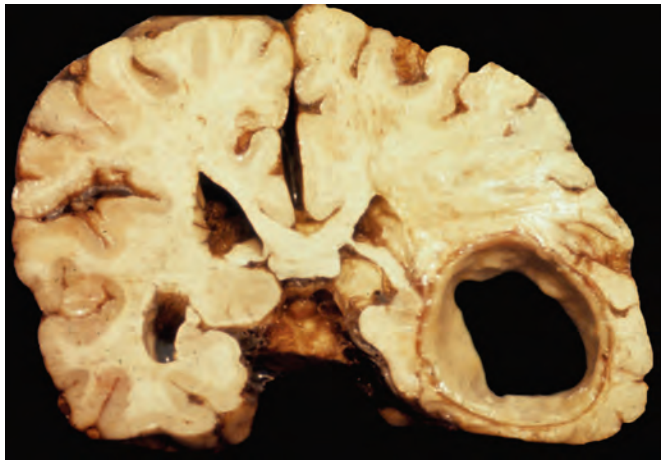
Tuberculous abscesses can be seen in immunocompromised patients. Under such circumstances, there is no granulomatous response. These lesions are characterized by a fibrous wall surrounding caseous material with neutrophils. The fibrous capsule forms 10 to 13 days after the initial abscess formation and isolates the necrotic and infected tissue from the rest of the brain parenchyma (Fig. 19.9). Dense granulation tissue with new vessel formation, lymphocytes, macrophages, and plasma cells admixed with fibroblasts start the capsule formation. A well-encapsulated abscess has a necrotic center with surrounding macrophages, vessels, and a rim of lymphocytes and plasma cells with dense fibrous tissue around it (Fig. 19.10). The brain parenchyma around the abscess is densely gliotic and may exhibit Rosenthal fibers. At this point, the abscess can be drained or excised from the brain parenchyma.



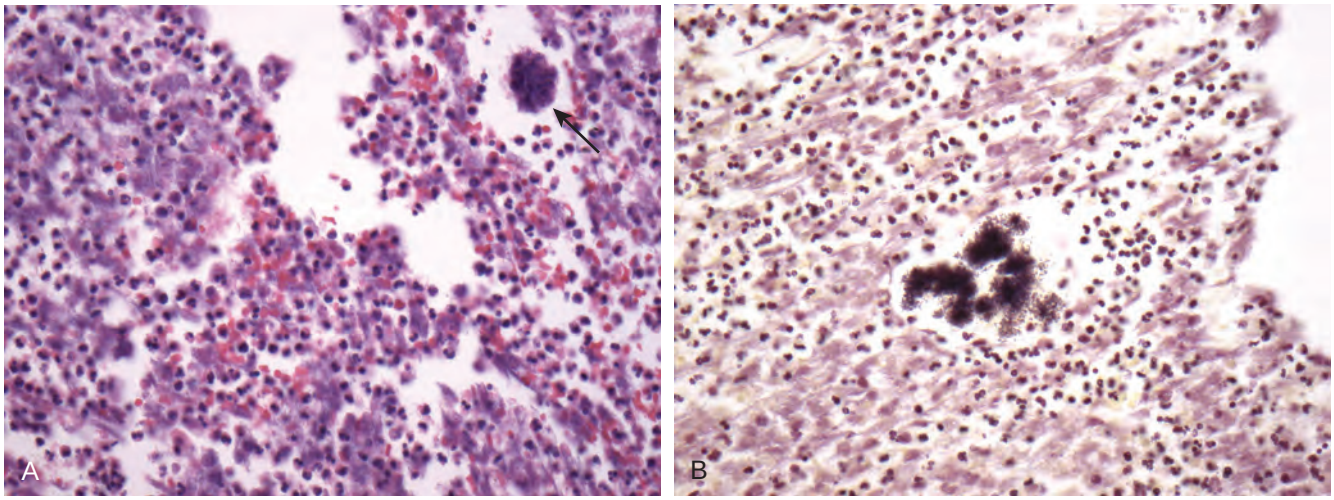
• **Figure 19.5** Bacterial meningitis. Neutrophils invading blood vessel walls can lead to vasculitis **(A)** and thrombosis **(B)**. Note the hemosiderin-laden macrophages, indicating prior hemorrhage, accompanying the inflammatory response.



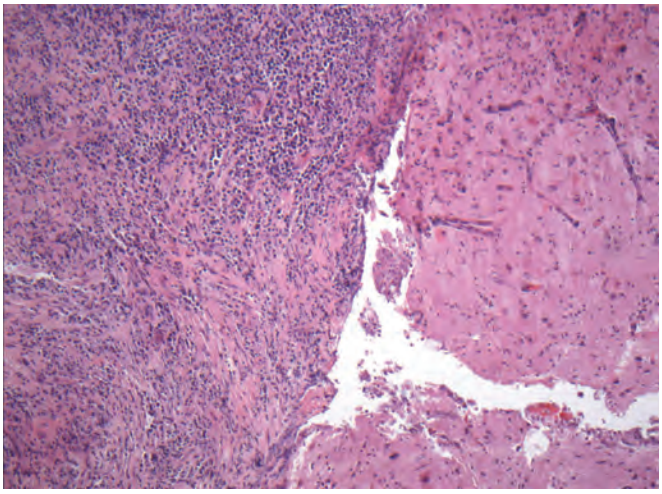
• **Figure 19.6** Bacterial meningitis, showing organizing exudate leading to fibrosis with an inflammatory infiltrate composed of lymphocytes, plasma cells, and macrophages. Note adjacent brain parenchyma with reactive astrocytes at the base of the image.



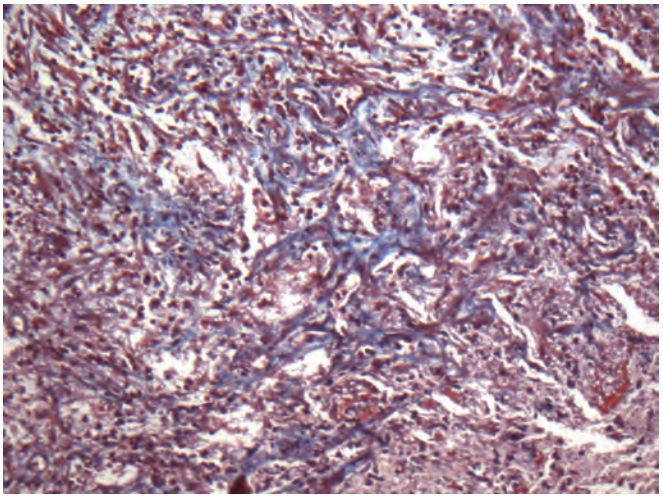
• **Figure 19.7** Abscess. Coronal section of the brain demonstrates an abscess cavity in the right temporal lobe. Note the fibrous capsule that separates the abscess from the surrounding brain parenchyma. The pus has drained out of the cavity.



• **Figure 19.8** **A,** Hematoxylin and eosin stain of a brain abscess with prominent neutrophilic exudate in its center, as well as clumps of bacteria. **B,** Gram stain highlights a colony of gram-positive cocci.



• **Figure 19.9** Abscess at 10 to 13 days. The dense fibrous capsule delineates the abscess from the inflammatory infiltrate consisting of lymphocytes and plasma cells. Note the reactive astrocytes and proliferation of capillaries in the adjacent brain.



• **Figure 19.10** Abscess. The fibrous capsule is highlighted by Masson trichrome stain.

Viral

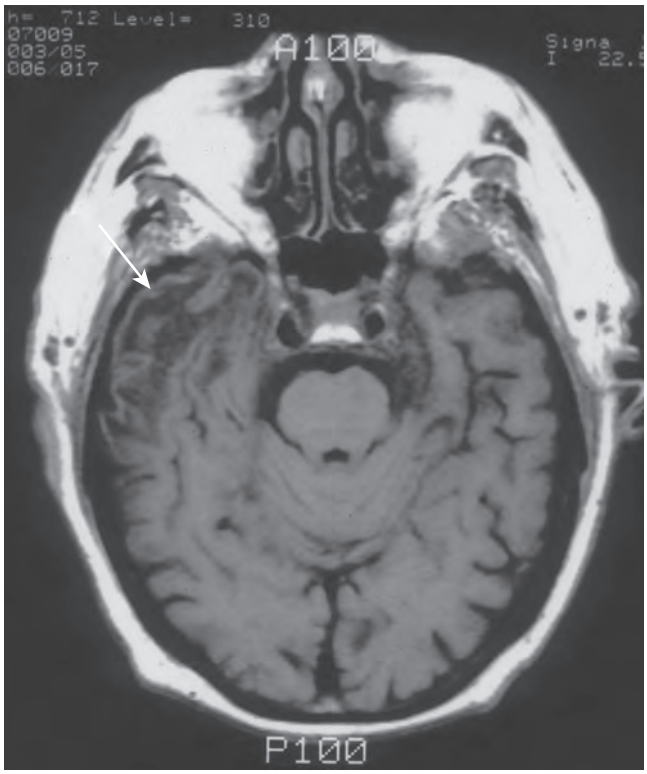
Herpes Simplex Virus. The prototypic herpes encephalitis is caused by herpes simplex virus type 1 (HSV-1). The primary infection involves the mucocutaneous and oropharyngeal mucosa. The route of entry to the CNS is controversial, but it is believed that the virus can travel by retrograde transport from the olfactory bulbs to the posterior orbital, frontal, and limbic regions or via the peripheral trigeminal sensory fibers innervating the face and dura to the trigeminal ganglion and then into the brainstem.[8,9] Up to 15% of infections occur via the olfactory nerve after a primary nasopharyngeal infection.[10]

HSV manifests as an asymmetric hemorrhagic mass of the temporal lobe or, less often, the insula, cingulate, or posterior orbital frontal cortex, accompanied by marked swelling of the temporal lobes (Figs. 19.11 and 19.12). It can be unilateral or bilateral. The earliest changes are lymphocytes and macrophages in the leptomeninges with mild parenchymal inflammation. The disease progresses from the meninges to the gray and white matter, leading to hemorrhagic necrosis of the gray and white matter with a neutrophilic reaction (Fig. 19.13). The neurons lose their nuclear outlines and their Nissl substance with dense eosinophilic cytoplasm, and there may be neuronophagia and microglial nodule formation (Fig. 19.14). Viral inclusions are best identified at the edge of the lesion and consist of eosinophilic intranuclear inclusions with marginated chromatin in neurons, ganglion cells, and glial cells (Fig. 19.15). At a later stage, the tissue collapses and the brain is left with a cystic glial scar not unlike that seen after an infarct (Fig. 19.16). Although the reaction can resemble ischemia, the differential point here is that the lesion does not fall into a vascular territory. The chronic inflammatory response can remain in the damaged tissue for many years. The virus can be demonstrated by immunohistochemistry, and viral antigens can be detected for up to 3 weeks after the onset of the encephalitis.[11] Alternative methods of detection include DNA in situ hybridization of frozen or paraffin tissue with polymerase chain reaction (PCR) amplification.[12,13]

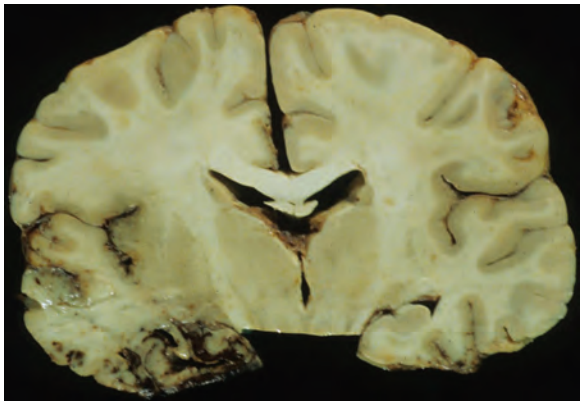
HSV encephalitis is usually fatal if not treated. Current antiviral therapy has significantly reduced morbidity and mortality. Sequelae include neurologic deficits and behavioral and memory disturbances.

Neonatal HSV encephalitis is caused predominantly by HSV-2 and is acquired during delivery when the fetus is in contact with genital lesions. The herpes encephalitis in neonates is a panencephalitis with no predilection for any particular anatomic area of the brain. It can affect cerebrum, the cerebellum, and rarely, the brainstem.[14] A similar distribution of herpes encephalitis is seen in immunocompromised people.

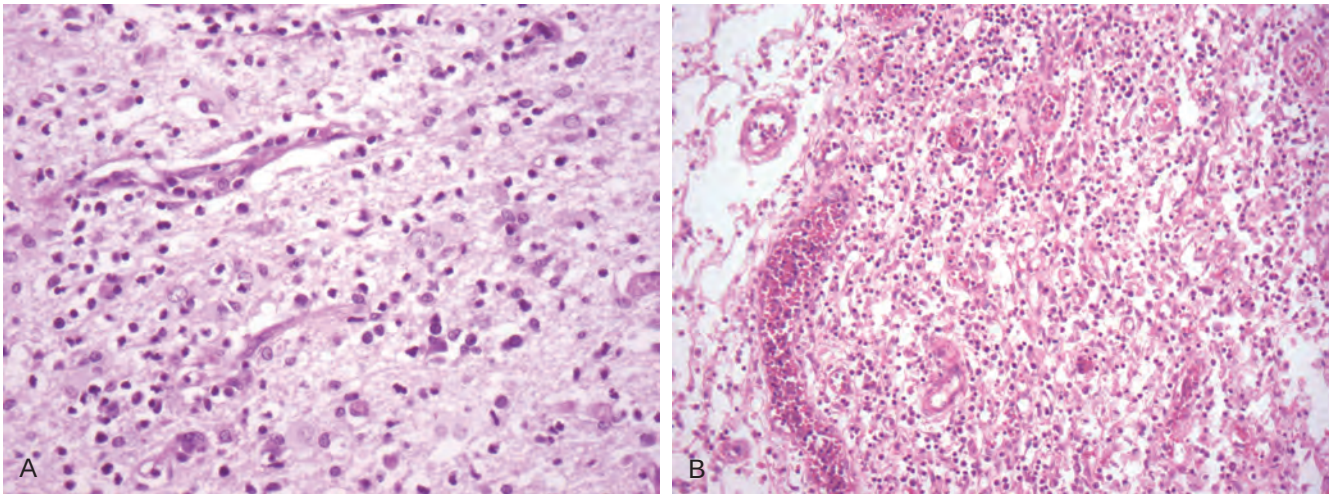
Varicella Zoster Virus. Several patterns of infection are related to varicella zoster virus, the most common being dorsal root ganglionitis leading to shingles. Other patterns include vasculitis, myeloradiculitis, brainstem encephalitis, and encephalitis involving the visual system, ventriculitis, and, in the immunosuppressed, multifocal lesions of the white matter (Figs. 19.17 and 19.18).[15,16] The white matter lesions are characterized by large areas of necrotizing inflammation, particularly of blood vessels, with perivascular and interstitial infiltration by lymphocytes and macrophages (Fig. 19.19A). Intranuclear inclusions can be detected with hematoxylin and eosin (H&E) staining and immunohistochemistry, particularly around the borders of the white matter lesions (see Fig. 19.19B). Brainstem encephalitis is characterized by prominent neuronophagia and microglial nodules. Brain biopsies are performed for the diagnosis of cerebral vasculitis. Many cases of CNS vasculitis are due to varicella zoster.[17] The vasculitis is characterized by a lymphocytic infiltrate of the arterial wall, with occasional giant cells but no fibrinoid change (Fig. 19.20). The elastic lamina is disrupted by the transgression of inflammatory



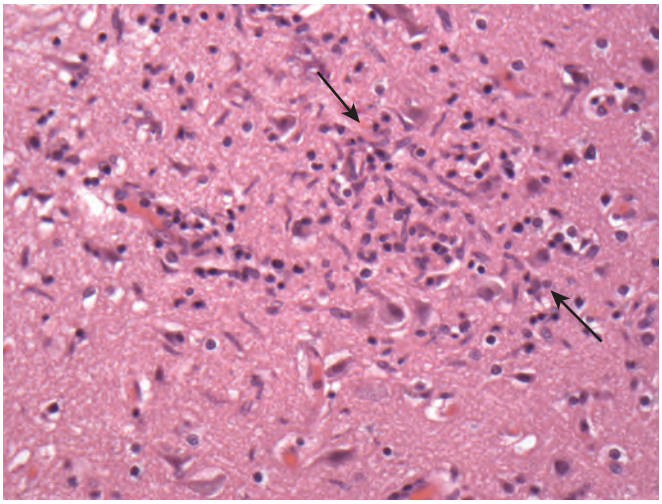
• **Figure 19.11** Herpes simplex virus infection. This radiologic image demonstrates involvement of the right temporal lobe by herpes encephalitis *(arrow)*. The disease is frequently asymmetric.



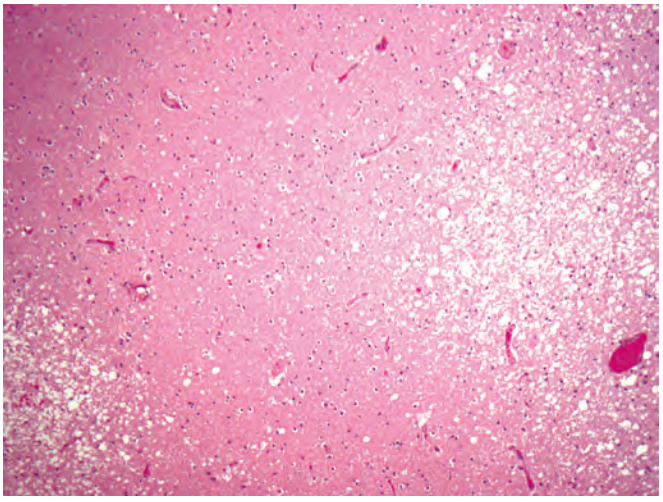
• **Figure 19.12** Herpes simplex virus encephalitis. Coronal section of the brain shows a large hemorrhagic area in the left temporal lobe, with surrounding edema, and minor hemorrhagic necroses in the right temporal lobe and left cingulate gyrus.



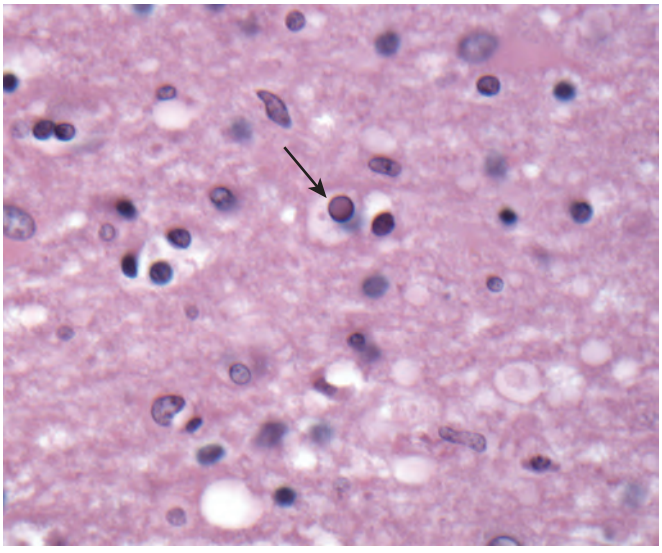
• **Figure 19.13** Herpes simplex virus encephalitis. **A,** Brain parenchyma with neutrophils, lymphocytes, capillary proliferation, and few reactive astrocytes. **B,** Note the almost total destruction of the brain tissue, with acute and chronic inflammation, rare macrophages, and vascular proliferation.



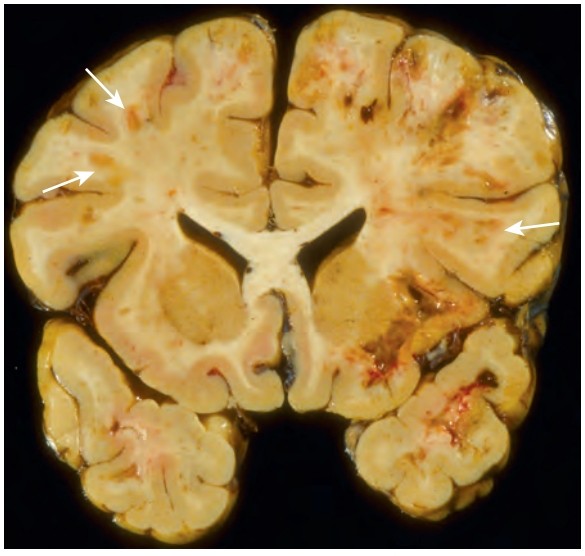
• **Figure 19.14** Herpes simplex virus encephalitis. Brain parenchyma with numerous rod-shaped microglia (between *arrows*), forming a nodule and surrounding neurons.



• **Figure 19.16** Herpes simplex virus encephalitis. Note acute infarction in adjacent brain.



• **Figure 19.15** Herpes simplex virus encephalitis. Brain parenchyma showing intranuclear viral inclusion *(arrow)* with glassy chromatin and marginated chromatin.



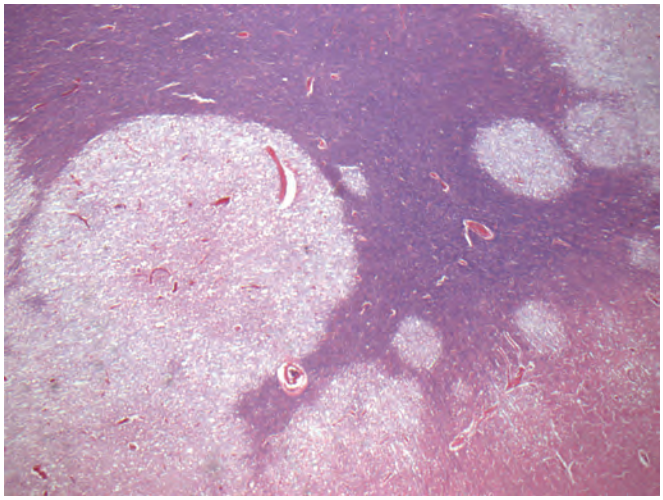
• **Figure 19.17** Herpes zoster virus encephalitis. Coronal section of the brain demonstrating multiple hemorrhagic cavities as well as sharply defined but pale round areas in the deep white matter *(arrows)*.

cells through the vessel wall (Fig. 19.21). In this situation, viral inclusions are rarely recognized, and the diagnosis is confirmed by immunohistochemistry for varicella or PCR from paraffin-embedded tissue.

Eastern Equine Encephalitis Virus. Eastern equine encephalitis (EEE) virus belongs to the arbovirus family. Encephalitis due to EEE virus has its highest incidence during the late summer. EEE is seen most commonly on the eastern and Gulf coasts of the United States, in Caribbean countries, and in South America. It affects predominantly children and older adults. It is a severe disease with a mortality rate that ranges from 50% to 80%. Patients who survive are left with significant neurologic sequelae.[18] The most severely affected parts of the CNS are the basal ganglia, thalamus, and brainstem.[19] Unlike most viral encephalitides, EEE has a prominent acute neutrophilic infiltrate (Fig. 19.22). Vasculitis with marked endothelial swelling and thrombosis is also characteristic. Necrosis is abundant and may extend to the white matter with small microabscesses (Fig. 19.23). The diagnosis is made by reverse transcriptase PCR on CSF.[20,21]

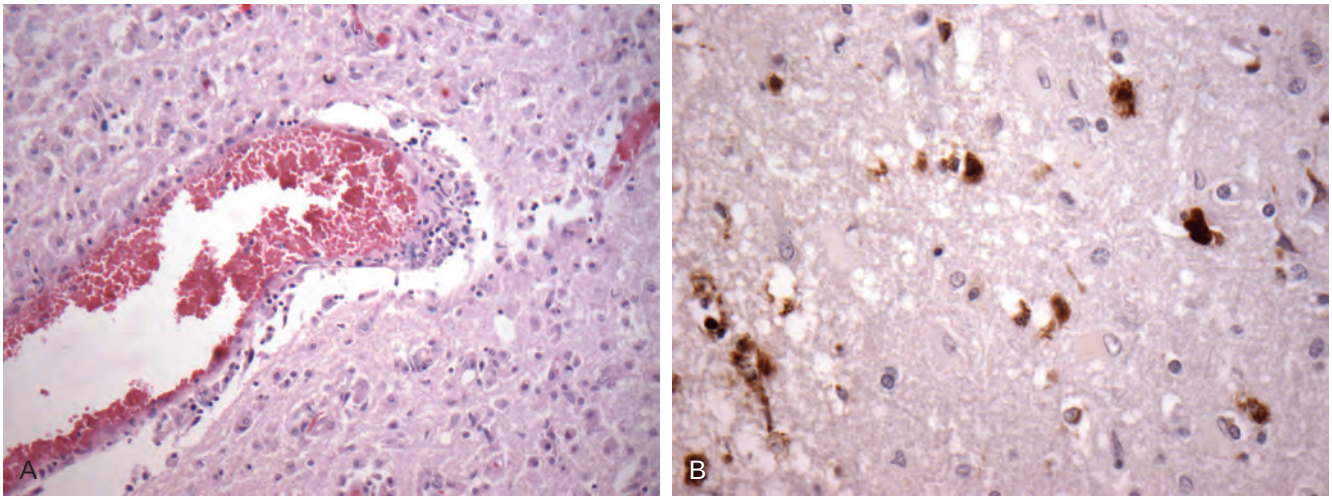
Protozoal

Toxoplasmosis. *Toxoplasma* are parasites which can infect the CNS in two different age groups. *Toxoplasma* as an in utero infection causes a necrotizing leukoencephalitis with a predilection for the periventricular white matter, whereas in adults *Toxoplasma* has a predilection for the basal ganglia, the thalamus, and the gray-white matter junction (Fig. 19.24).[22] In the adult, toxoplasmosis is most commonly thought to represent reactivation in an immunocompromised host.[23] It was a rare event until the onset of the acquired immunodeficiency syndrome (AIDS) epidemic, when it became one of the AIDS-defining diseases. Clinically, toxoplasmosis manifests with focal or nonspecific clinical signs, depending

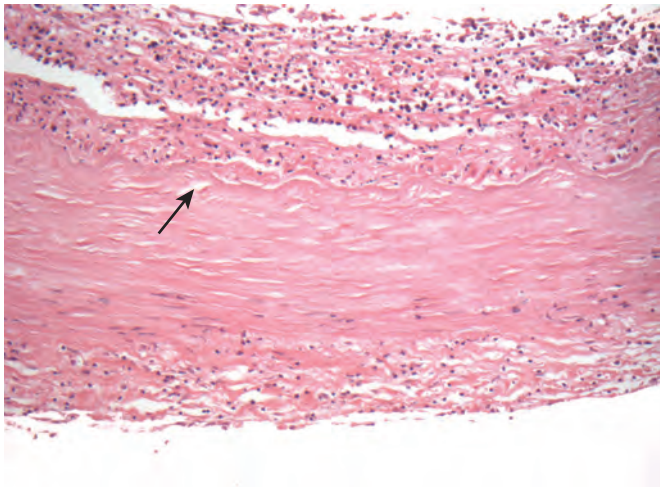


• **Figure 19.18** Herpes zoster virus. Luxol fast blue and hematoxylin stain demonstrate sharply circumscribed areas of white matter damage in a patient with acquired immunodeficiency syndrome.

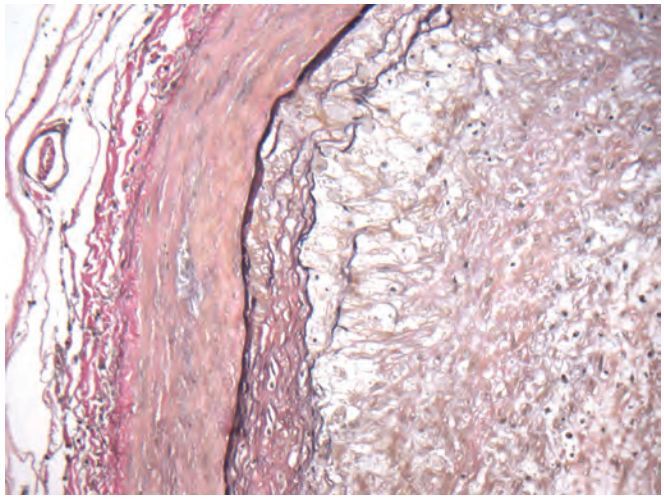
on the location of the lesions. Radiologically in the adult, it is characterized by multiple enhancing parenchymal lesions. The differential diagnosis includes CNS lymphoma or neoplasm.[24,25] The diagnosis is confirmed by biopsy or autopsy or by response to specific treatment with pyrimethamine. If the lesions disappear, the assumption is that they were due to *Toxoplasma*.[26,27] Histologically the lesions are characterized by a cerebritis with lymphocytes and plasma cells or by abscess formation with an eosinophilic acellular necrotic center surrounded by an inflammatory reaction, which can have a vaguely granulomatous appearance (Fig. 19.25). Necrotizing abscesses are poorly circumscribed, with varying degrees of associated hemorrhage. Typically, there is coagulative necrosis with abundant cellular debris. Parasites may be found in vessel walls, causing vasculitis, thrombotic occlusion, and subsequent hemorrhages. The parasites may be seen within pseudocysts (bradyzoites) or as free forms (tachyzoites 2 μm in diameter) (Fig. 19.26; see also Fig. 19.25). The latter may be difficult to recognize on H&E because the organisms resemble cellular debris, but they are easily identified with immunohistochemistry.



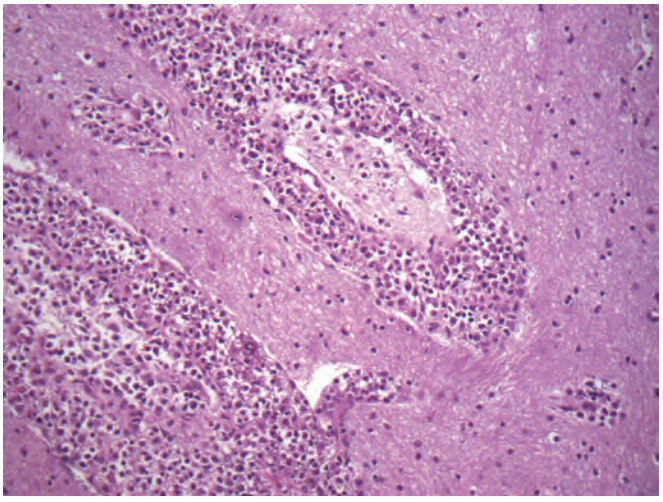
• **Figure 19.19** Herpes zoster virus. **A,** Necrotic blood vessel with a few perivascular lymphocytes and many macrophages in the white matter surrounding the necrotic areas. **B,** Immunohistochemistry using an anti–herpes zoster virus antibody highlights the viral inclusions.



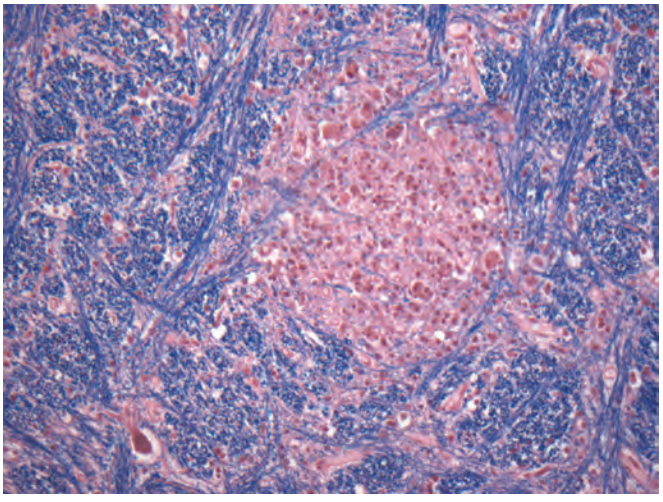
• **Figure 19.20** Herpes zoster virus. Zoster vasculitis with arterial wall invaded by inflammatory cells, predominantly lymphocytes, with no evidence of fibrinoid necrosis. Note partially destroyed elastic lamina *(arrow)*.



• **Figure 19.21** Herpes zoster virus. Elastic stain demonstrates disruption of the internal elastic lamina by the inflammatory infiltrate of the vessel wall.



• **Figure 19.22** Eastern equine encephalitis. Perivascular polymorphonuclear leukocytes and lymphocytes with migration from the vessel lumens. A few neutrophils are present also in the parenchyma.



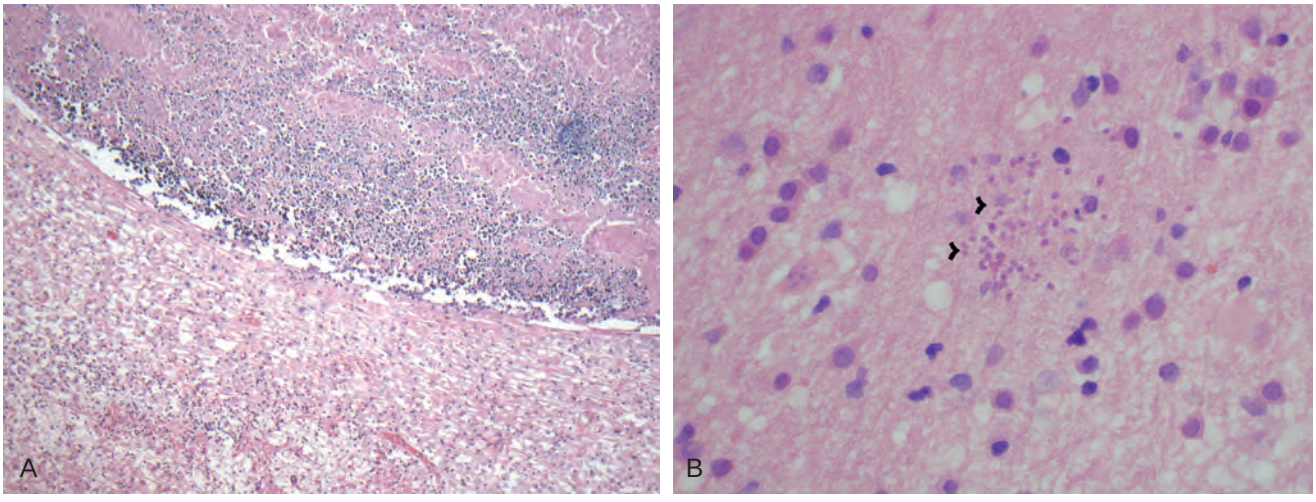
• **Figure 19.23** Eastern equine encephalitis. Luxol fast blue and hematoxylin stain. Note the microabscess in the white matter.

Fungal

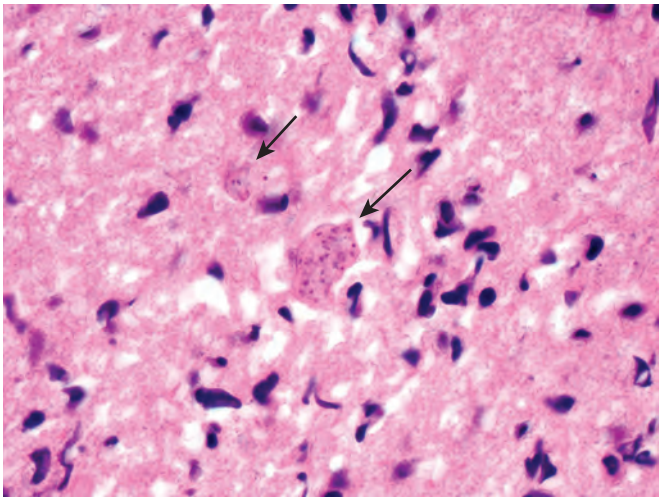
Aspergillus. Aspergillosis of the CNS is predominantly an opportunistic disease of the immunocompromised host.[28,29] *Aspergillus* usually disseminates by hematogenous spread from the lungs or gastrointestinal tract. *Aspergillus* is an angioinvasive organism that transgresses vessels, producing hemorrhages, necrosis, and dense acute inflammation (Fig. 19.27). The inflammatory response, the hemorrhage and ischemia, is so intense that there is destruction of the parenchyma. *Aspergillus* in tissue is an acute-branching, septated, hyphal organism (Fig. 19.28). The diagnosis of aspergillosis of the brain is usually made on postmortem examination. It is rare that a biopsy of the brain is done for diagnosis. The diagnosis is usually made by biopsy of another organ or by isolation of the fungus from the lung.

Mucor. Rhinocerebral mucormycosis is an entity seen primarily in diabetic patients with ketoacidosis, as a consequence of direct extension of the fungal organism from the nasal sinuses to

• **Figure 19.24** Toxoplasmosis. Healed toxoplasmic abscess *(arrow)* demonstrating predilection of *Toxoplasma* for the gray-white junction. Note the well-defined cyst with a necrotic center.



• **Figure 19.25** Toxoplasmosis. **A,** Abscess formation with an eosinophilic, acellular, necrotic center surrounded by an inflammatory reaction with lymphocytes and reactive astrocytes. **B**, Free tachyzoites. If present in necrotic tissue, the tachyzoites give the impression of "dust" or "dirty necrosis."

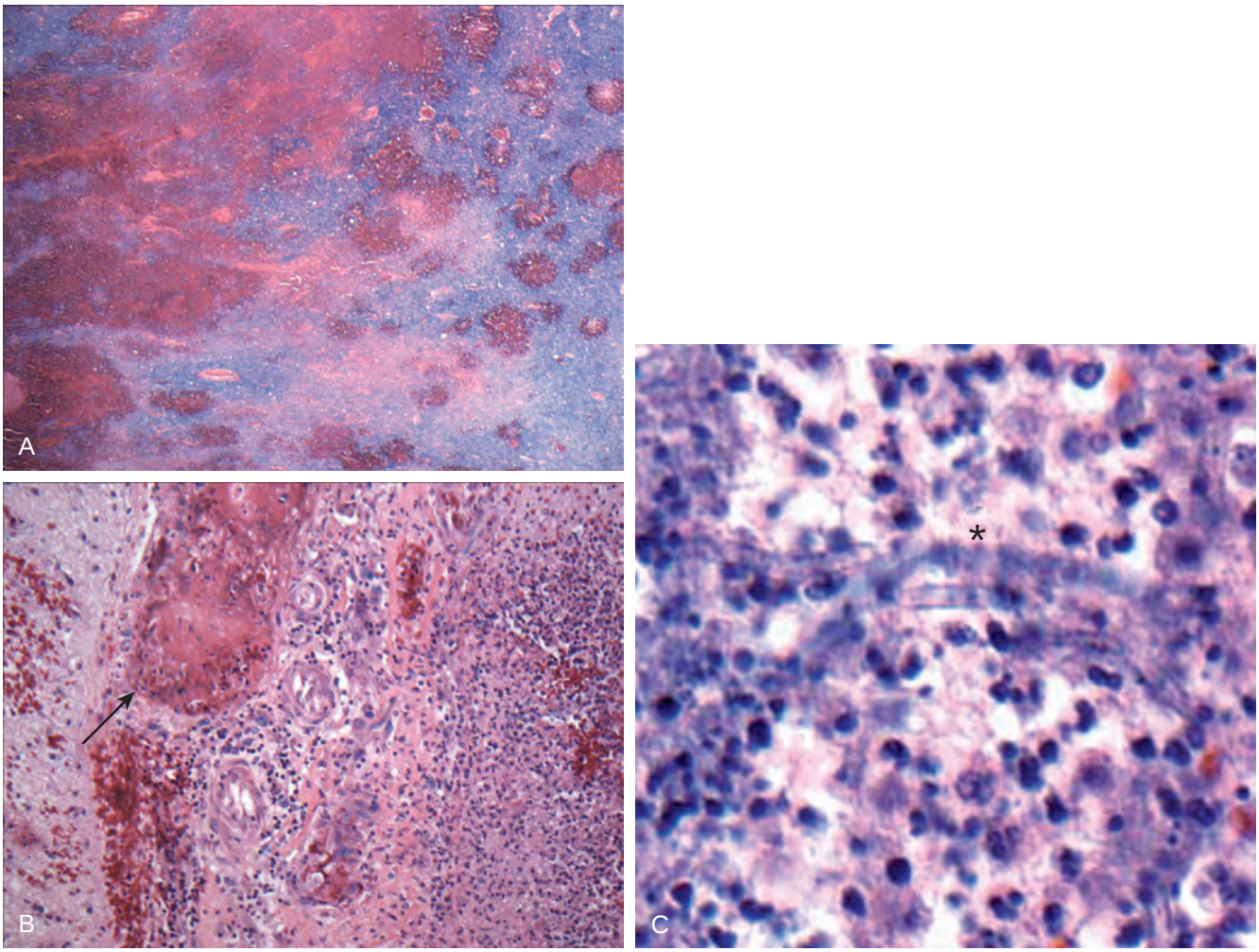


• **Figure 19.26** Toxoplasmosis. Brain parenchyma with dense reactive gliosis. Note two bradyzoites in the center *(arrows)* filled with microorganisms.

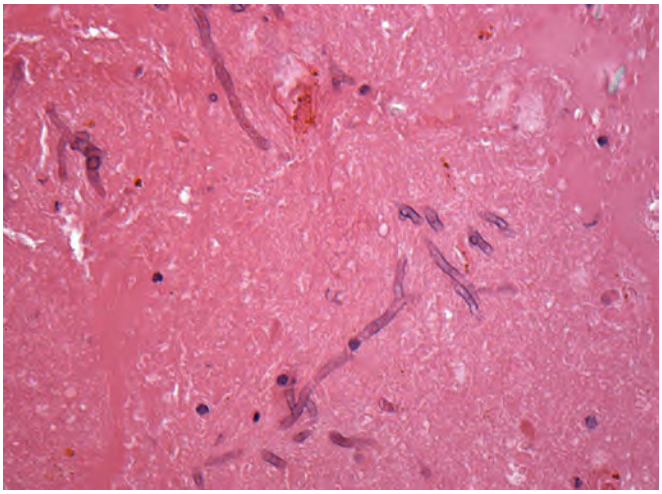
the brain, with predilection for the base of the brain and the basal ganglia. Histologically, this is a vascular destructive and invasive organism that produces poorly defined ischemic areas with necrosis and hemorrhage. Brisk neutrophilic infiltrate and areas of necrosis are common (Fig. 19.29). The course from infection to death is usually a matter of days or weeks.[30] The diagnosis can be made by biopsy of the nasal sinus. Brain biopsy is only rarely used for diagnosis. The fungus can be seen with H&E staining as a nonseptated, broad, gray-blue, ribbonlike hyphal organism, 10 µm thick, with right-angle branching (Fig. 19.30). *Mucor* can also produce an aneurysm of the circle of Willis removed from the primary site of infection in the nasal sinuses. Carotid artery involvement and cavernous sinus thrombosis are common.[31,32]

Amebic

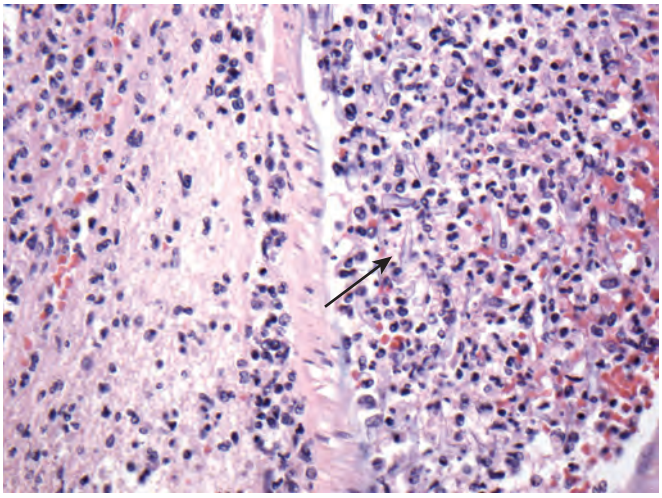
The three most common amebas involving the CNS are *Naegleria fowleri*, *Acanthamoeba*, and *Balamuthia*. *Naegleria* is typically found in warm fresh water and invades the CNS through the nasal mucosa. *Naegleria* is a free-swimming, nonencapsulated ameba. *Naegleria* produces an acute meningoencephalitis in



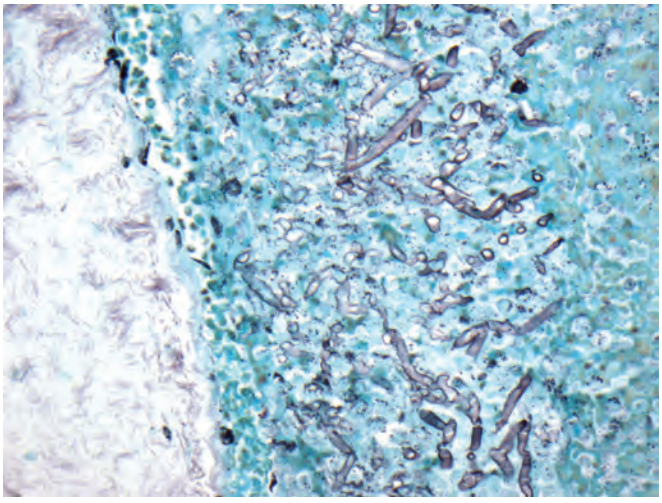
• **Figure 19.27** Aspergillosis. **A,** Note the dense hemorrhagic and necrotic areas in the white matter (Luxol fast blue, hematoxylin and eosin stain). **B,** Angioinvasion with blood vessel destruction *(arrow)*. Note the purulent exudate on the right side of the image. **C,** Note the hyphal organism intermixed with macrophages and polymorphonuclear leukocytes *(asterisk)*.



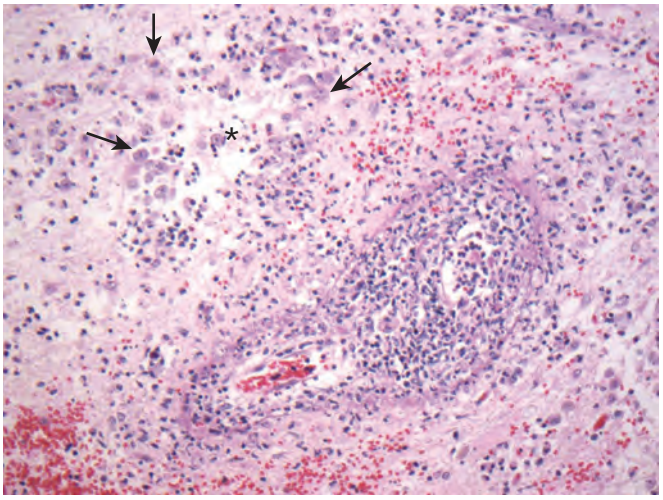
• **Figure 19.28** Aspergillosis. The organism is identified by its acute branching with septated hyphae.



• **Figure 19.29** Mucormycosis. Note the blue hyphal forms surrounded by neutrophils *(arrow)*. The adjacent brain is infiltrated by neutrophils and lymphocytes.



• **Figure 19.30** Mucormycosis. Grocott methenamine silver stain highlights the broad, nonseptated hyphae.

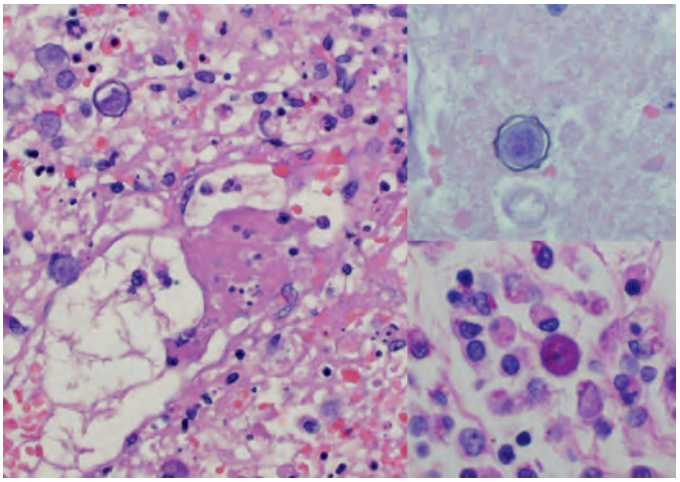


• **Figure 19.31** Amebic encephalitis. Neutrophilic infiltrate extends through the arterial wall into the Virchow-Robin spaces *(arrows)*. There is surrounded hemorrhagic necrosis and acute inflammation with multiple clusters of encapsulated amebas in the surrounding parenchyma. The amebas have a prominent nucleolus *(asterisk)*.

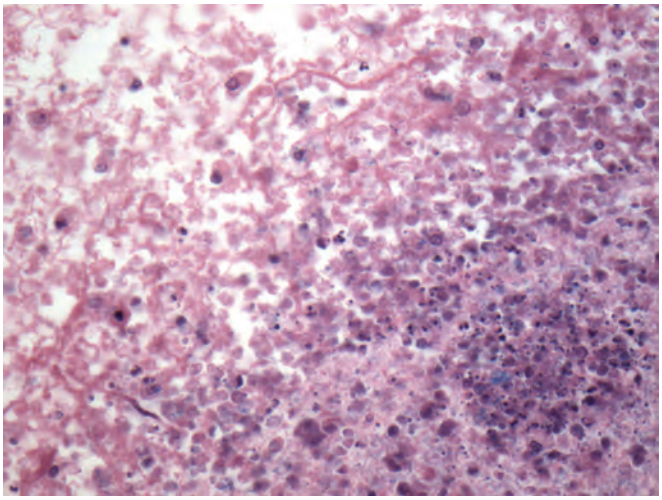
immunocompetent people. It produces hemorrhagic necrosis of the fronto-orbital cortex and the olfactory bulbs. The inflammation is composed of a dense neutrophilic infiltrate with variable degree of mononuclear cells. The inflammation tends to extend through the Virchow-Robin spaces to the gray-white matter junction and produces hemorrhagic necrosis (Fig. 19.31).[33] The diagnosis is made by finding the free-swimming ameba in the CSF. *Naegleria* is very sensitive to amphotericin B but carries a high mortality, up to 95%, because the correct diagnosis is not suspected and therefore often delayed.[34,35]

Acanthamoeba and *Balamuthia mandrillaris* are ubiquitous free-living, soil-dwelling, encapsulated amebas. *Acanthamoeba* can produce keratitis or encephalitis. It has been found in nonsterile lens solutions. Acute *Acanthamoeba* spp. meningoencephalitis occurs in immunocompromised hosts, whereas *B. mandrillaris* meningoencephalitis is seen in both immunocompetent and immunocompromised hosts. These amebas are postulated to enter the CNS through lesions in the skin or the upper respiratory tract or via inhalation of airborne cysts. Patients present with signs and symptoms of a brain mass with surrounding edema. Brain biopsy is the only way to make the diagnosis before death. Even so, the diagnosis is often missed because the organisms strongly resemble macrophages and may not be numerous in the biopsied material. The pathologic findings of *Acanthamoeba* consist of multiple areas of hemorrhagic, granulomatous inflammation that can become confluent. The initial lesion can have a necrotic center. The tissue reaction is a mixed inflammatory infiltrate with neutrophils, lymphocytes, plasma cells, and fibroblasts. The lesions are usually angiocentric. The vessels are inflamed, with areas of fibrinoid necrosis (Fig. 19.32).[36,37] Sometimes, a skin ulcer or nodule, if present, can be biopsied and the diagnosis can reflect the process in the brain.[38] The organism can be typed by PCR or immunofluorescence, although these tests may not be available in all institutions, and samples may need to be sent to the Centers for Disease Control and Prevention (CDC).

Combinations of amphotericin B with rifampin or imidazole agents may be tried. However, the mortality rate is very high, even with appropriate treatment, when the CNS is involved.[39] In rare



• **Figure 19.32** Amebic encephalitis. Fibrinoid necrosis of the vessels. Ragged cyst wall with large nuclei and prominent nucleoli (*top insert:* hematoxylin and eosin stain; *lower insert:* periodic acid–Schiff stain).



• **Figure 19.33** Infarct. Necrotic center of an infarct with scattered inflammatory cells that can be mistaken for an abscess.

TABLE 19.2 Clinical and Pathologic Features of Free-Living Amebas Involving the CNS

Feature	*Acanthamoeba* spp. and *Balamuthia mandrillaris*	*Naegleria fowleri*
Predisposition	Immunodeficient patients and those with chronic debilitating diseases	Healthy individuals; associated with exposure to contaminated water
Route of entry	Upper respiratory tract, conjunctiva, or injury to the skin; infection of CNS through the bloodstream	Nasal mucosa, traveling along olfactory nerves
Histopathologic features	Angiocentric lesions with parasites clustered around vessels, perivascular inflammation, fibrinoid necrosis, and granulomatous inflammation	Hemorrhagic necrosis of the fronto-orbital cortex and olfactory bulbs with dense neutrophilic infiltrate and variable mononuclear cells; no granulomas formed
Forms present	*Cysts*: Thick cell wall, PAS positive *Trophozoites* (14 to 40 μm): pale eosinophilic granular cytoplasm with prominent nucleoli	*Only trophozoites* (<10 μm): vacuolated cytoplasm; nuclei with prominent nucleoli
Clinical progression	Progression from infection to CNS involvement may take months; after this, there is a rapid fatal progression (approximately 1 month)	Incubation is 1 to 14 days, followed by rapidly fatal progressive course

CNS, Central nervous system; *PAS*, periodic acid–Schiff stain.

instances, patients with granulomatous amebic encephalitis have survived, usually in cases manifesting with a single lesion that could be excised surgically.[40] Distinctive features of the most common amebas involving the CNS that can be helpful in the diagnosis are highlighted in Table 19.2.[41]

Noninfectious

Infarct

Acute cerebral infarction, between 24 and 48 hours old, can have the appearance of an acute cerebritis, with numerous polymorphonuclear leukocytes permeating the tissue and margination of neutrophils around blood vessels, but special stains fail to reveal any organisms, and cultures are sterile (Fig. 19.33). The presence of intravascular thrombi can be helpful in making the diagnosis (Fig. 19.34). The surrounding brain parenchyma can show other signs of early ischemia, such as red neurons with shrunken nuclei, hyperchromatic nuclei, loss of nucleoli, eosinophilic cytoplasm with loss of the Nissl substance, and damaged glial parenchyma with a more clear eosinophilic and granular texture (Fig. 19.35).

Chronic Inflammatory Response

Infectious

Bacterial

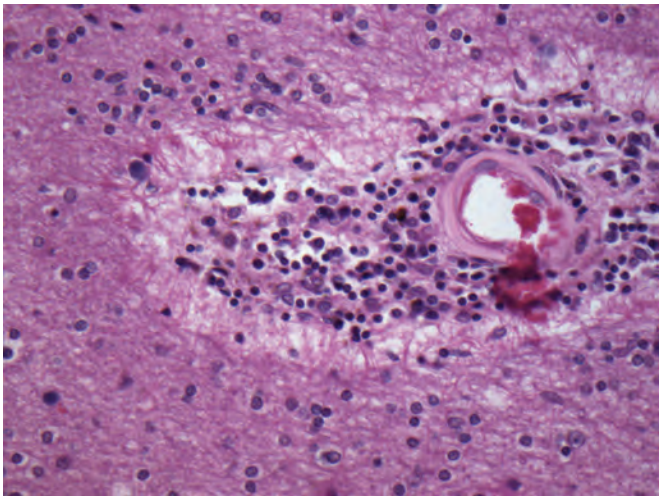
Syphilis. Symptomatic neurosyphilis can occur as the secondary (early neurosyphilis) or tertiary (late neurosyphilis) stage of the disease. The most common clinical presentation is aseptic meningitis, but syphilis can also manifest with iritis or uveitis or, less commonly, with cranial nerve paralysis and hydrocephalus. It involves meninges, blood vessels, and the CSF. Patients with vascular involvement can present with stroke. A rarer syndrome occurs if there is parenchymal involvement and can include progressive personality changes, psychiatric disorders, or dementia (also known as general paralysis of the insane).

In general, syphilis manifests as a gumma or as chronic meningitis, composed predominantly of plasma cells and lymphocytes, usually leading to fibrosis of the meninges that leads to CSF obstruction. The meningitis may be accompanied by an arteritis characterized by invasion of the arterial walls by lymphocytes and plasma cells (Heubner arteritis), which induces intimal proliferation, and thinning of the media with intact elastic lamina (Fig. 19.36). The vasa vasorum are surrounded by inflammatory cells, which may lead to ischemic lesions (Fig. 19.37).

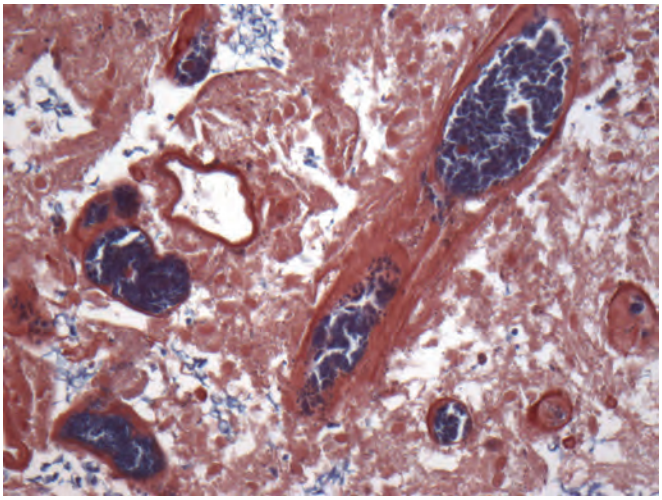
Viral

Most viral meningitides are culture-negative and are characterized by a chronic inflammatory response with a mild lymphocytic infiltrate in the subarachnoid space. The diagnosis is made by examination of the CSF and serology.

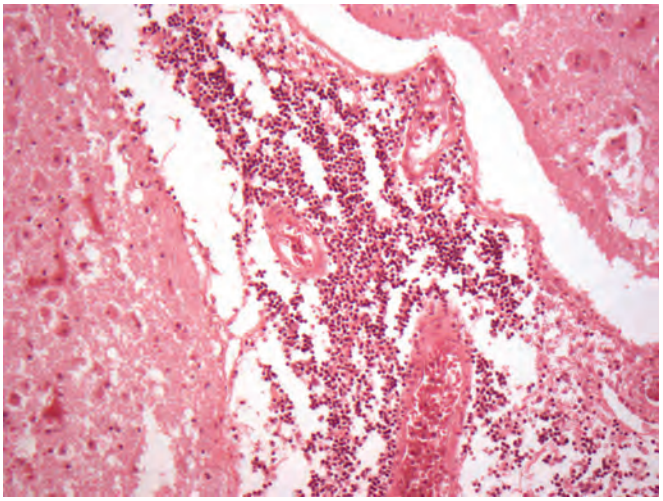
West Nile Virus. West Nile virus causes an arthropod-related encephalitis in late summer and early fall. Most of the infections occur through a mosquito bite, although the virus can also be acquired through breast milk, transplacentally, or via organ



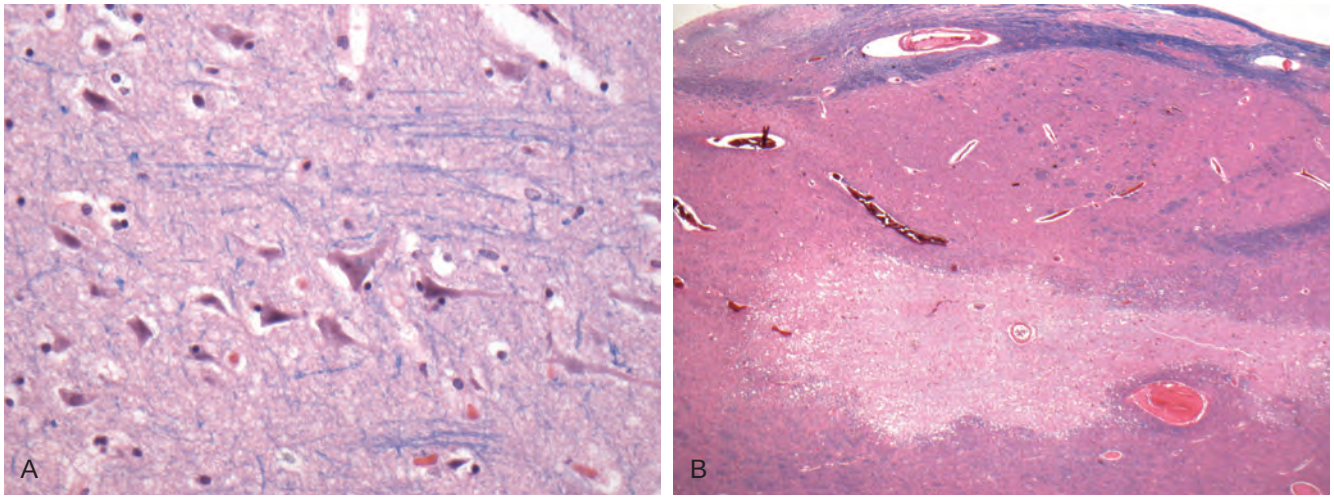
• **Figure 19.36** Syphilis. Brain with perivascular lymphoplasmacytic inflammation in a case of syphilis.



• **Figure 19.34** Edge of infarct with phosphotungstic acid hematoxylin staining highlighting the intravascular thrombi.



• **Figure 19.37** Syphilis. Dense chronic inflammation of the leptomeninges with involvement of the vessels, surrounded by infarction with macrophages and reactive astrocytes.



• **Figure 19.35** Infarct. **A,** Brain parenchyma with shrunken red neurons, a common sign of irreversible ischemia. **B,** Acute infarct. Sharply delineated area of pallor with scalloped edges and "spongy" center defines the acutely infarcted thalamus.

transplantation.[42-44] Fewer than 15% of the human infections result in aseptic meningitis or encephalitis.[45,46] Most cases of West Nile viral infection are subclinical, but in a few patients a polio-like syndrome or parkinsonism occurs.[47,48] The CDC has strong programs for reporting, control, and prevention of the disease because West Nile virus can cause many fatalities (Table 19.3). This disease is manifested histologically by the presence of non-necrotizing inflammation involving particularly motor neurons in the spinal cord, brainstem, and neurons of the substantia nigra, and Purkinje cells. There is an infiltration of $CD3^+$ lymphocytes, macrophages, and microglial nodules, associated with chromatolytic neurons and neuronophagia.[49-52] Viral inclusions are not seen by light microscopy, but the presence of the virus can be detected by immunohistochemistry in the remaining neurons involved (Fig. 19.38). The CSF has an elevated protein content and a high white blood cell count, predominantly neutrophils.[53] The diagnosis is made mainly by the detection of antibodies in CSF, although in some cases PCR of CSF is required.[54]

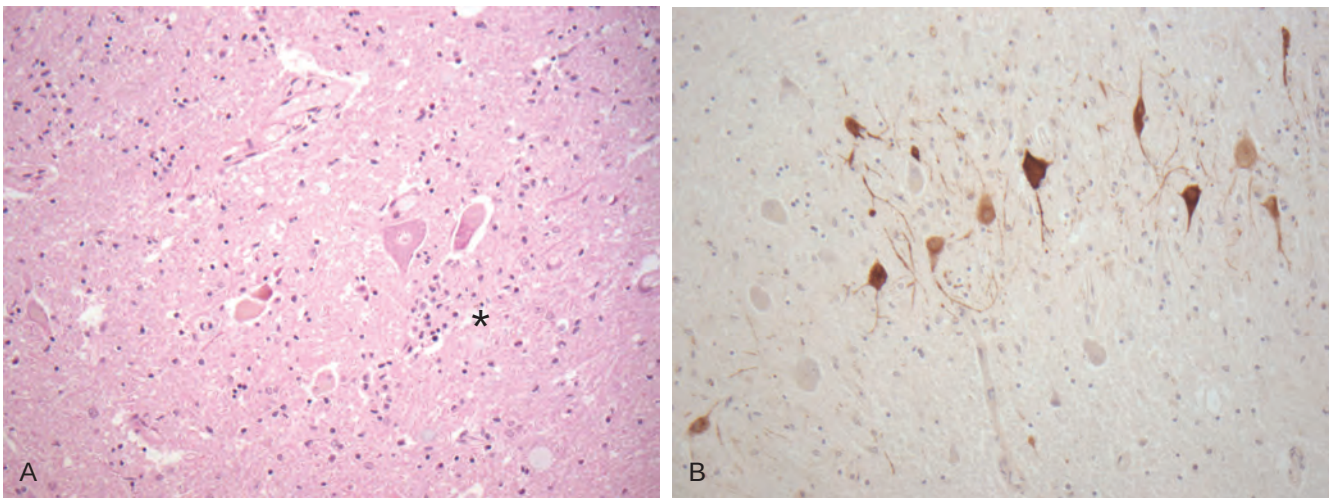
Progressive Multifocal Leukoencephalopathy. Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease of immunocompromised patients, including patients with human immunodeficiency virus (HIV) infection, leukemia, lymphoma, a transplant, or chronic immunosuppression.[55] PML is caused by the polyomavirus known as JC virus, which was first cultured from brain tissue in 1971.[56] Treatment of the underlying immunosuppression can lead to remission, but sometimes its indolent course and delay in diagnosis can prove fatal. The clinical and radiologic appearance can mimic that of tumor (Fig. 19.39). Demyelination is multifocal and often has the classic appearance of "salt on ice," with multiple small ill-defined areas of demyelination and intervening relatively uninvolved white matter (Fig. 19.40). The histologic features are characterized by areas of myelin loss with relative preservation of axons, abundant foamy macrophages, reactive astrocytes, and characteristic viral inclusions. The pathognomonic feature of PML is the presence of enlarged, glassy, dark-staining viral inclusions in oligodendrocytes, seen particularly at the edges of the demyelinating process (Fig. 19.41). Occasionally, inclusions may be seen in the granular cells of the cerebellum.[57] Reactive astrocytes are usually present and can be very large and bizarre; care should be taken not to misinterpret these bizarre large astrocytes as a high-grade astrocytoma on frozen or permanent sections, particularly of small biopsies (see Fig. 19.41).

In most cases, there is no or only mild perivascular lymphocytic infiltrate, but occasionally there is a striking inflammatory reaction with many perivascular and parenchymal lymphocytes and plasma cells. This reaction has been seen particularly in some patients with AIDS, especially after initiation of highly active

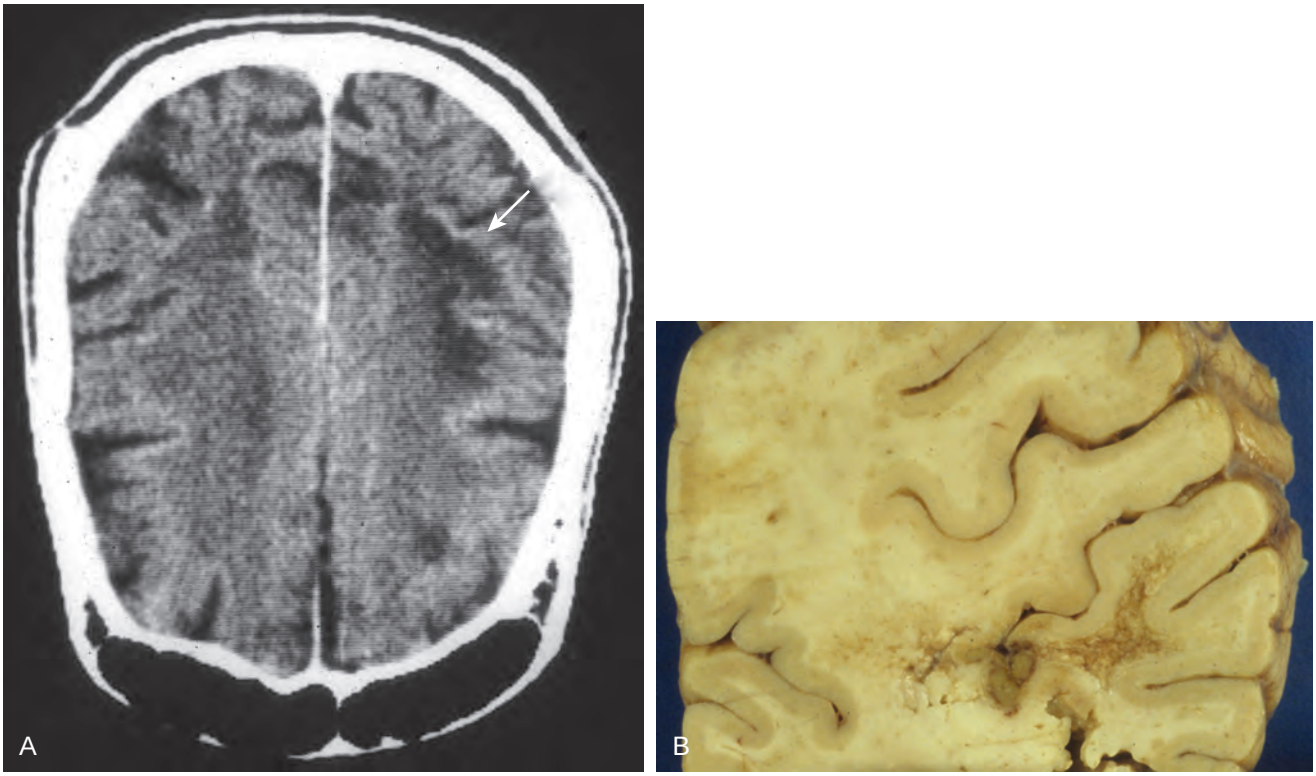
TABLE 19.3 West Nile Virus Disease Cases and Deaths Reported to the CDC From 2004 to 2015

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total of cases	3000	4269	3630	1356	720	1021	712	5674	2469	2205	2175
Total of neuroinvasive cases	1309	1495	1227	689	386	629	486	2873	1267	1347	1455
Deaths in patients with neuroinvasive disease	104	162	117	41	32	54	42	270	111	87	142
Total of deaths	119	177	124	44	32	57	43	286	119	97	146

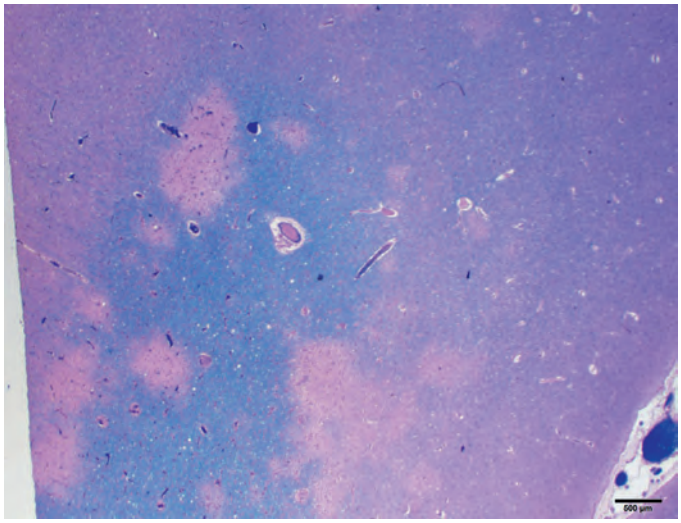
CDC, Centers for Disease Control and Prevention.
Modified from Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases: West Nile Virus: Statistics, Surveillance and Control, 2016. Available at https://www.cdc.gov/westnile/resources/pdfs/data/1-wnv-disease-cases-by-year_1999-2015_07072016.pdf.



• **Figure 19.38** West Nile virus infection. **A,** Anterior horn of the spinal cord with necrotic motor neurons. Lymphocytic infiltration, occasional macrophages, and microglial nodules *(asterisk)* are present. **B,** Immunohistochemical analysis with an antibody to West Nile virus demonstrates intense immunoreaction in the neuronal cell bodies and dendrites in the hippocampus.



• **Figure 19.39** Progressive multifocal leukoencephalopathy (PML). **A,** Radiologic appearance of PML, showing a white matter–based lesion *(arrow)*. **B,** Coronal section of brain with white matter–based lesion.



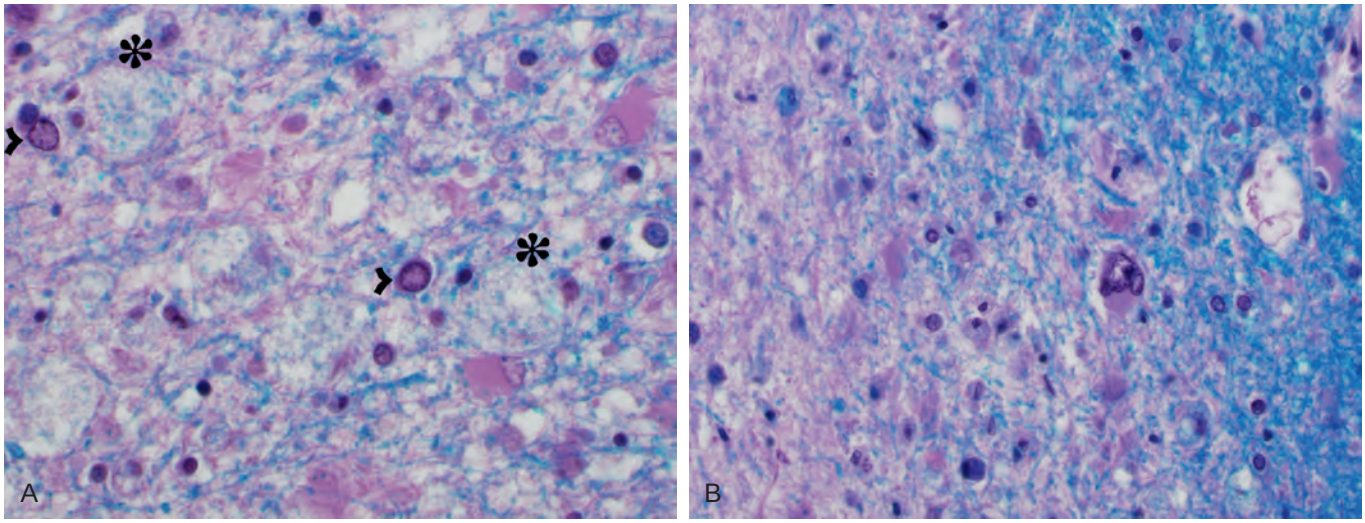
• **Figure 19.40** Progressive multifocal leukoencephalopathy. Luxol fast blue/hematoxylin and eosin staining shows irregular, multifocal, spotty demyelination "salt on ice."

antiretroviral therapy (HAART).[58,59] It has been posited that this is a sign of immune reconstitution.[60] The inflammatory response can be so intense as to raise the possibility of a second infectious agent, particularly *Toxoplasma*.[61] Therefore it is necessary to perform special stains for bacteria and fungi, as well as immunohistochemistry for *Toxoplasma*.

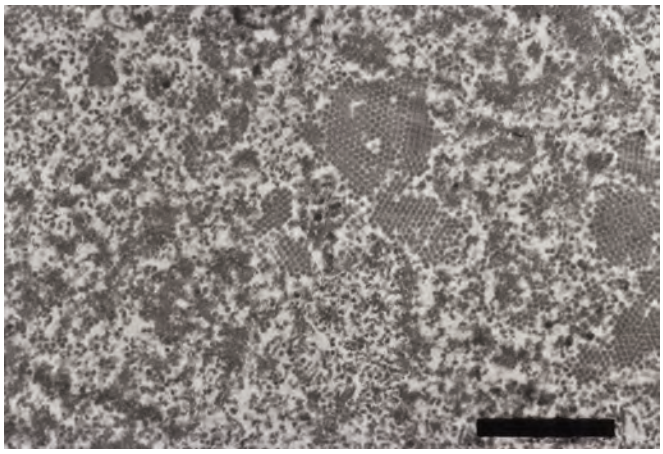
Electron microscopic examination of biopsy specimens reveals the characteristic morphologic appearance of JC virus (Fig. 19.42).[62] The diagnosis is most often confirmed with the use of immunohistochemistry against BK virus or polyomavirus (Fig. 19.43).

Human Immunodeficiency Virus. In the CNS, HIV infects microglia and T lymphocytes. The CNS involvement and pattern of inflammation vary, from a diffuse, low-grade, perivascular lymphocytic infiltrate with microglial nodules to demyelination and diffuse gliosis of the deep gray matter. Multinucleated cells surrounding capillaries with scant or sometimes abundant cytoplasm can aggregate in a granulomatous fashion and are helpful in the diagnosis (Fig. 19.44).[63,64] When faced with a brain biopsy of a patient with HIV/AIDS, it is important to remember associated infections or diseases, such as mycobacterial (especially with atypical mycobacteria), parasitic *(Toxoplasma)*, viral (cytomegalovirus [CMV], PML, herpes zoster virus), and fungal *(Cryptococcus*, *Aspergillus*, *Coccidioides*, *Histoplasma)* infections, as well as primary CNS lymphomas.[65] Biopsy of the brain may reveal only mild perivascular lymphocytic cuffing and a microglial response (Fig. 19.45).

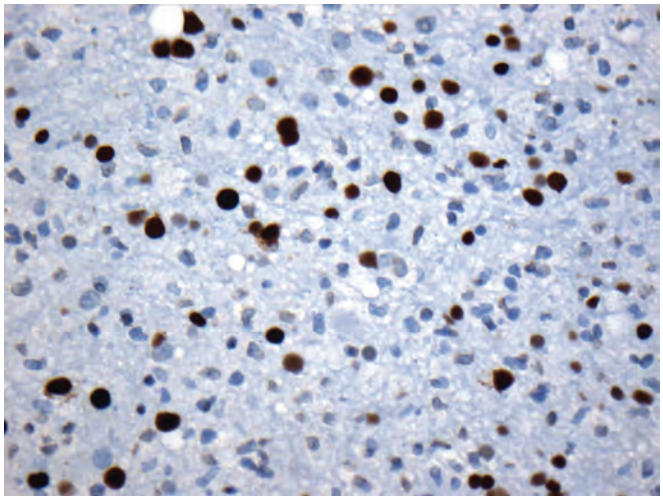
Cytomegalovirus. CMV infection of the nervous system occurs in neonates and immunocompromised patients. The virus primarily infects endothelial and ependymal cells. There are several reaction patterns, ranging from microglial nodules with cytomegalic cells and otherwise minimal inflammation to severe necrotizing encephalitis with cystic changes resembling infarcts in perinates (Fig. 19.46).[66] A common pattern of injury in immunocompromised adults is ventriculoencephalomyelitis with enlargement of the ventricles and hemorrhagic necrosis of the surrounding parenchyma.[67] CMV can also affect the peripheral nervous system with a vasculopathy and microvascular thrombi around nerve roots, producing a meningoradiculitis and myelitis.



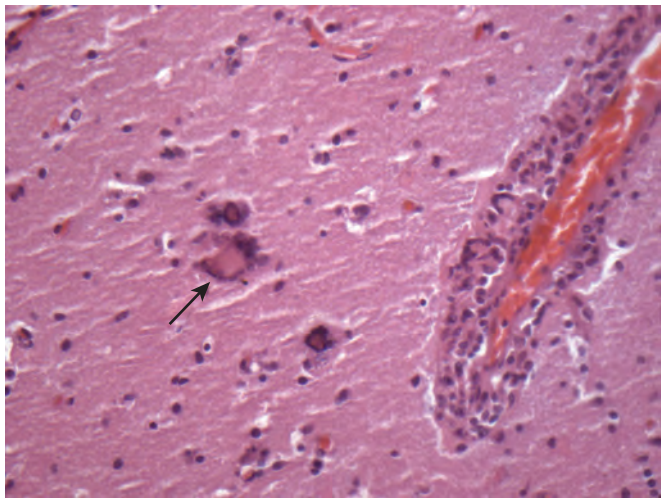
• **Figure 19.41** Progressive multifocal leukoencephalopathy. **A,** Luxol fast blue/hematoxylin and eosin staining shows dense infiltration of foamy macrophages *(asterisk)*, reactive astrocytes, and numerous oligodendroglial cells with intranuclear inclusions *(arrowheads)*. **B,** Large, bizarre astrocytes (Creutzfeldt cells) could be misinterpreted as a primary high-grade malignant neoplasm in the frozen section.



• **Figure 19.42** Progressive multifocal leukoencephalopathy. Electron microscopic appearance of the virus.



• **Figure 19.43** Progressive multifocal leukoencephalopathy. Immunohistochemistry with antibodies against simian virus 40, which cross-reacts with polyomavirus, highlights the cells infected with the virus.

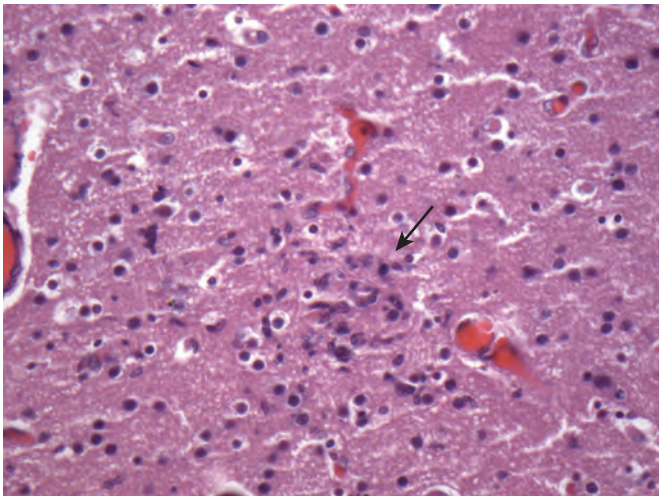


• **Figure 19.44** Human immunodeficiency virus infection. Perivascular inflammation with surrounding multinucleated giant cells *(arrow)* and focal mineral deposition in the adjacent white matter.

The diagnosis is made on tissue examination by the presence of enlarged cells with large intranuclear inclusions and less conspicuous cytoplasmic inclusions (Fig. 19.47).

Subacute Sclerosing Panencephalitis. SSPE is a chronic progressive panencephalitis that occurs several years after exposure to measles. Most patients have a history of measles in the first 2 years of life. The onset of disease usually occurs between 5 and 15 years of age, but the disease has also been reported in adults.[68-70] Clinically, patients progress from intellectual and behavioral changes to myoclonus, dystonias, spasticity, stupor, coma, dysautonomia, and, finally, death.[71] The diagnosis is usually made by the finding of high anti-measles titers in the CSF. However, in the rare instances of adult onset of disease, the diagnosis has been made by brain biopsy.

The gray matter shows perivascular inflammation with lymphocytes and macrophages, gliosis, loss of neurons with occasional neuronophagia, and eosinophilic intranuclear inclusions



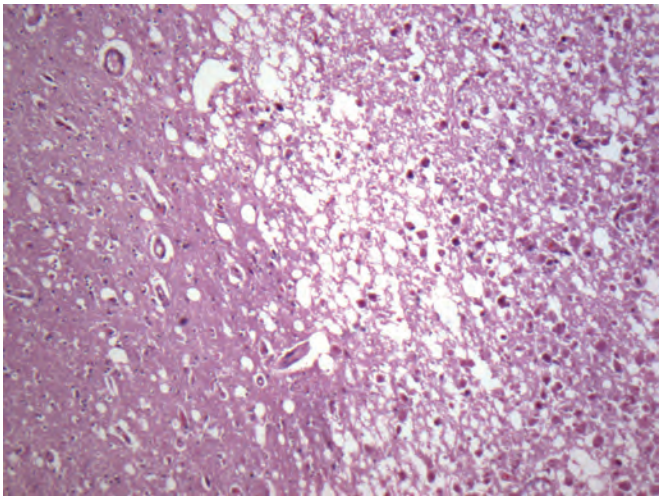
• **Figure 19.45** Human immunodeficiency virus infection. Subacute encephalitis with microglial nodules *(arrow)*.

in neurons (Fig. 19.48). The latter are very sharply defined eosinophilic inclusions with a surrounding halo. The white matter exhibits abundant gliosis, perivascular inflammation, and patchy demyelination. A striking feature in chronic cases is the presence of classic neurofibrillary tangles in the cerebral cortex, hippocampus, nucleus basalis of Meynert, hypothalamus, and raphe nuclei of the brainstem (Fig. 19.49).[72]

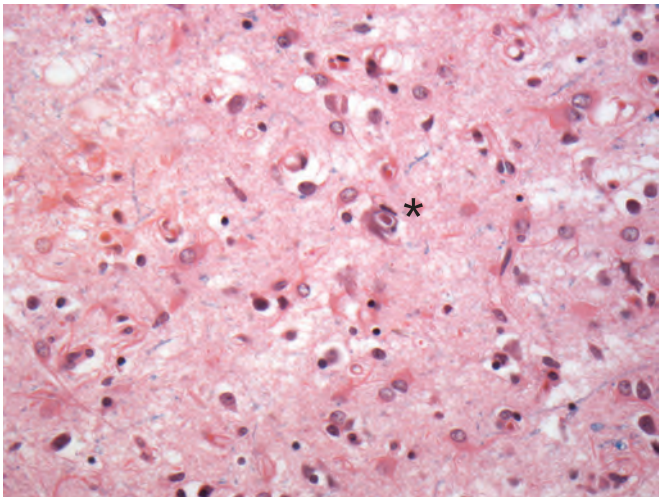
Pathologists should be aware of this chronic complication of measles, particularly because there have been several outbreaks in the United States since 2010, the largest in 2014 with 667 cases reported, mostly coming from clusters of unvaccinated communities (CDC MMWR, May 2016).

Fungal

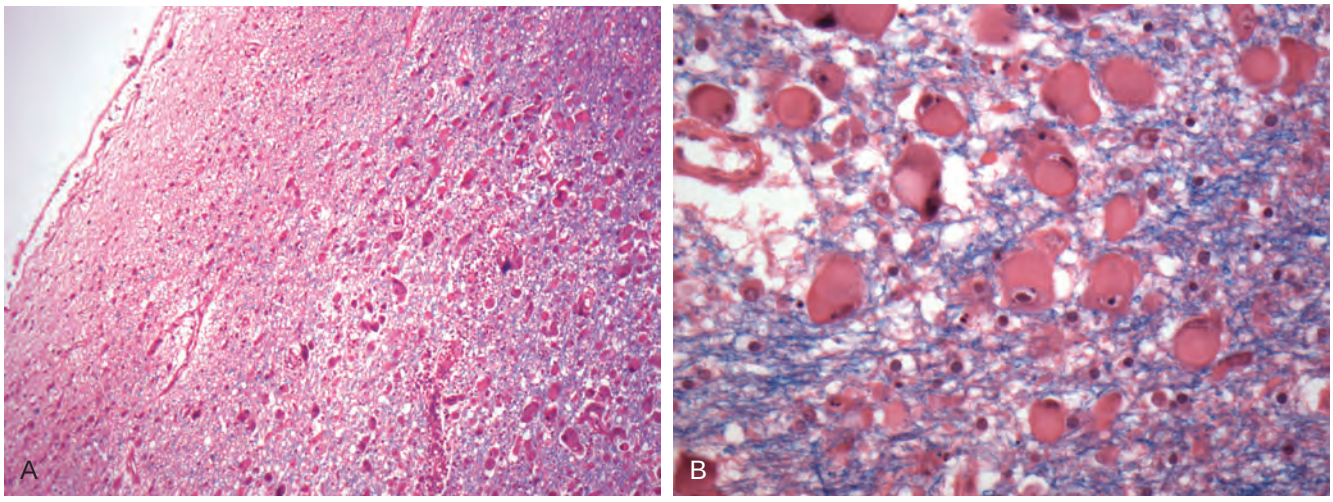
Candida. CNS involvement by *Candida* occurs mainly in patients with alteration of the immune defenses or mechanical barriers, such as patients with diabetes, lymphoma, HIV infection, intravenous drug use, ventriculoperitoneal shunt, neurosurgery, or lumbar puncture. *Candida* infections of the brain result



• **Figure 19.46** Cytomegalovirus. Spongy changes in white matter resemble an early infarct.



• **Figure 19.48** Subacute sclerosing panencephalitis. Gray matter with lymphocytes, macrophages, loss of neurons, and numerous fibrous astrocytes. Note the intranuclear viral inclusion in a neuron *(asterisk)*.

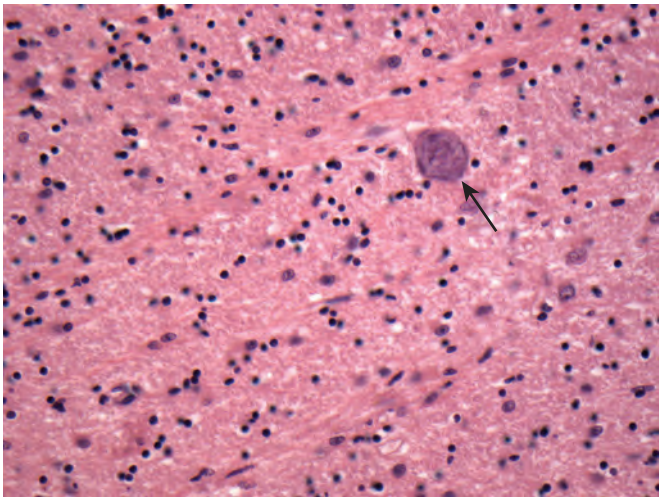


• **Figure 19.47** Cytomegalovirus ventriculitis. **A,** Cytomegalic cells with nuclear and cytoplasmic inclusions in the subependymal astrocytes. **B,** Note the large size of the inclusion-bearing cells compared with the normal oligodendrocytes and astrocytes in the background.

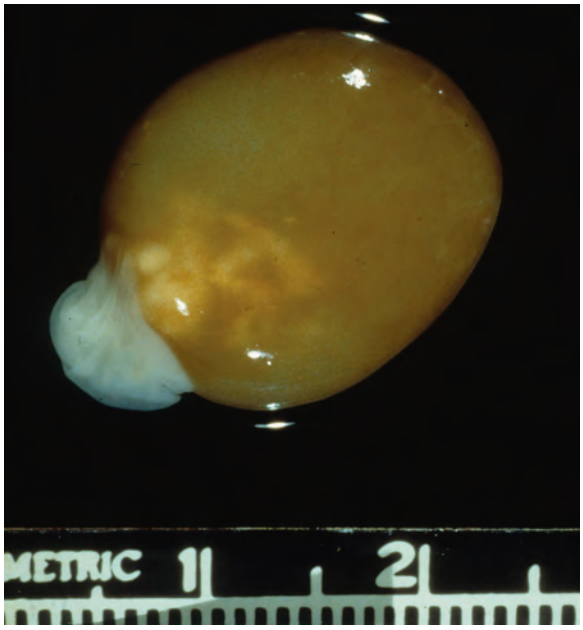
in tiny microabscesses with chronic inflammation, necrosis, and vasculitis with thrombus formation. *Candida* is identified by the presence of budding yeasts 2 µm in diameter and pseudohyphae. The organism can be seen with H&E staining and is highlighted by Grocott methenamine silver (GMS) and periodic acid–Schiff (PAS) stains (Fig. 19.50).

Helminthic

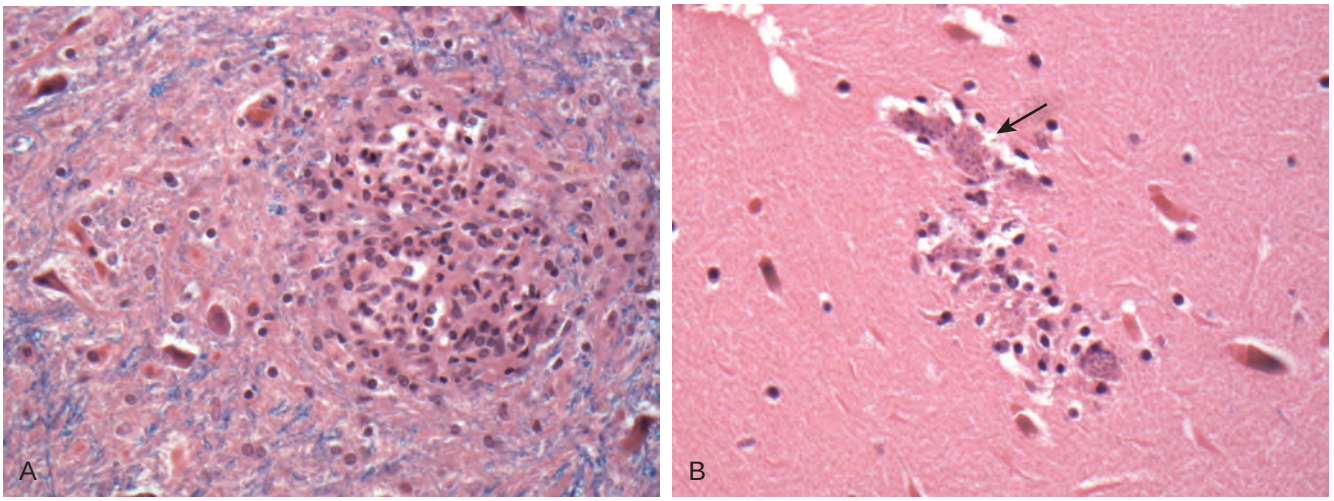
Cysticercosis. *Cysticercus* is the most common parasitic infection of the CNS. It is caused by ingestion of the egg of the pig tapeworm *(Taenia solium)* from contaminated water or undercooked meat. The egg loses its capsule when it comes into contact with the acid pH of the stomach. The larvae enter the bloodstream and spread to various organs, including the CNS, where they encyst (Fig. 19.51). In this environment, they cannot grow to adult worms, so they remain as cysts indefinitely. The cyst initially produces minimal response, and the infestation may remain silent for years. The cysts are usually found in the cerebral cortex but can occur in the meninges of the base of the brain, in the ventricles, and, rarely, in the spinal cord (Fig. 19.52).[73] Symptoms occur when the worm dies, the cyst ruptures, and the contents leak, producing a dense inflammatory response composed of lymphocytes, eosinophils, and plasma cells. With time, a fibrous capsule forms (Fig. 19.53). Patients usually present with seizures or hydrocephalus, and brain imaging reveals the calcified cyst.[74,75] The cysts may also be found incidentally when the patient has a brain computed tomography scan for a different reason. To make the definitive diagnosis histologically, one must see the parasite. The live parasite has a single scolex with four suckers and a double row of hooklets. The cyst wall has an outer cuticular layer, a middle pseudoepithelial cellular layer, and an inner reticular layer. However, in excisions or biopsies of *Cysticercus*, only the cuticular layer of the dead worm is seen with H&E stain (Fig. 19.54).



• **Figure 19.49** Subacute sclerosing panencephalitis (SSPE). Intraneuronal globose neurofibrillary tangle *(arrow)* is present in a chronic case of SSPE.

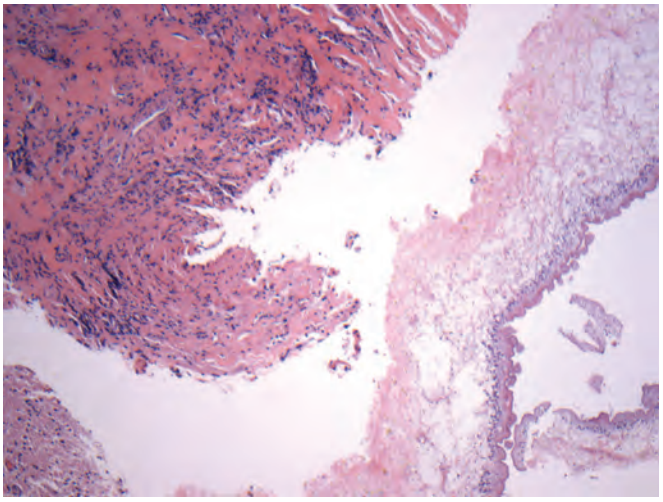


• **Figure 19.51** Cysticercosis. Intact *Cysticercus* cyst surgically removed from the fourth ventricle of the brain.



• **Figure 19.50** *Candida.* **A,** Microabscess due to *Candida* in the cerebral cortex. **B,** *Arrow* shows small yeasts in a blood vessel in the center of a microglial nodule in the molecular layer of cerebellum.

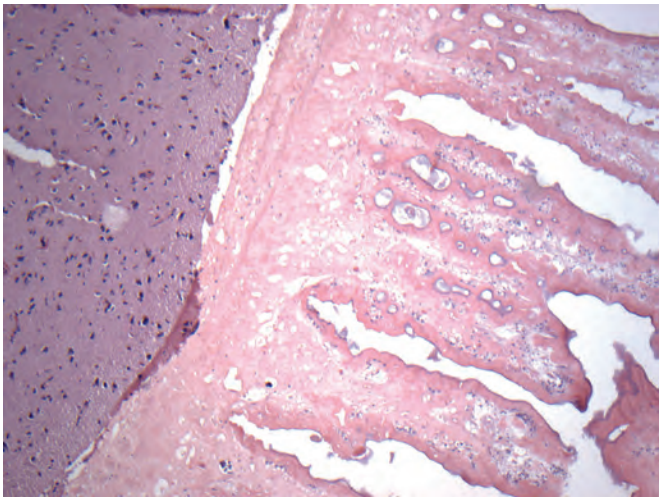
• **Figure 19.52** Cysticercosis. Radiologic image demonstrates a cyst in the fourth ventricle *(arrow)*, with obstruction of the cerebrospinal fluid flow and hydrocephalus.



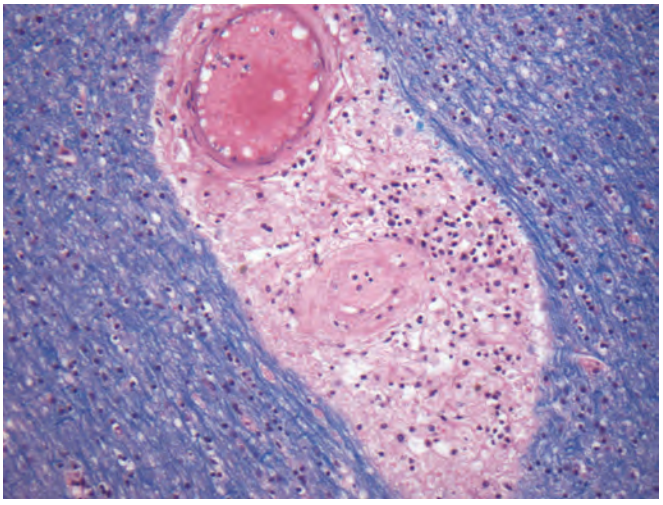
• **Figure 19.53** Cysticercosis. Wall of dead worm, with cyst wall, lymphoplasmacytic infiltrate, and dense fibrous tissue.

Rickettsial

Rickettsia are intracellular microorganisms that are transmitted by ticks or mites. After entering the bloodstream, they transgress the vessels, damaging the endothelial and smooth muscle vascular cells. In fact, vessel wall damage is one of the hallmarks of rickettsial infections. There is usually angiocentric inflammation composed of lymphocytes and macrophages, with thrombosis and microinfarcts but without fibrinoid necrosis of the vessels (Fig. 19.55).[76] The organisms can be very difficult to find but can be seen as gram-negative coccobacilli inside macrophages with the use of Brown-Hopps stain. The disease is severe and may be fatal in older adults, in patients with alcohol abuse, and in those with



• **Figure 19.54** Cysticercosis. Cuticular layer that helped to identify the dead worm in an excisional biopsy.



• **Figure 19.55** *Rickettsia.* Angiocentric inflammation composed of lymphocytes and macrophages, with thrombosis but no fibrinoid necrosis of the vessels.

glucose-6-phosphate dehydrogenase (G6PD) deficiency. The diagnosis is made by serology and PCR.[77]

Noninfectious

Vasculitis

CNS vasculitis is a rare disorder that can be primary or secondary. Most frequently, it is part of a systemic disorder, such as systemic lupus erythematosus (SLE), Sjögren syndrome, Wegener granulomatosis, or polyarteritis nodosa, but giant cell arteritis and granulomatous small vessel vasculitis limited to the brain also occur. The main histologic findings are vascular fibrinoid necrosis with inflammatory infiltration of the vessel wall and rupture of the elastic lamina.[78] The degree of inflammation varies depending on previous treatment and the chronicity of the process (Fig. 19.56).

Paraneoplastic Limbic Encephalitis

Paraneoplastic encephalomyelitis is typically encountered as a complication of small cell carcinoma of the lung, but it is also

associated with non–small cell cancer of the lung, ovarian tumors, Hodgkin lymphoma, breast carcinoma, and testicular germ cell tumors.[79] Clinically the patients present with short-term memory loss; complex, partial, or generalized seizures; confusion; and personality disturbances. Magnetic resonance imaging often shows a T2-hyperintense lesion of the medial temporal lobe, involving the amygdala, hippocampus, and entorhinal cortex.[80,81]

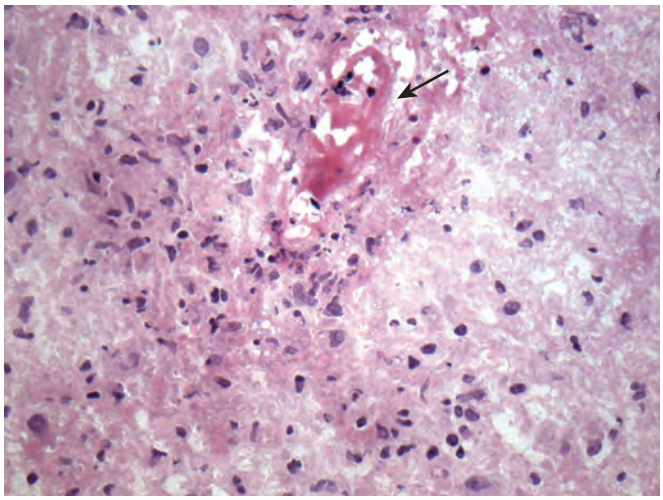
Histologic examination shows a florid reactive astrocytosis, perivascular lymphoid cuffing, and neuronophagia with microglial nodules suggestive of viral encephalitis. The detection of autoantibodies in the CSF (e.g., anti-Hu, anti-Yo, anti-Tr) is occasionally helpful in the diagnosis (Fig. 19.57).[82]

Lymphoma

Lymphoma can mimic an infectious process in the brain. The most common lymphoma involving the CNS is the diffuse large B-cell lymphoma. It diffusely involves the brain parenchyma with a perivascular pattern of growth. Histologically the cells are dyshesive with usually large, prominent nucleoli, prominent mitoses and apoptosis, and occasional macrophages. The diagnosis can be made on H&E but is confirmed with immunohistochemistry, which reveals a predominantly B cell ($CD20^+$) population with occasional T cells ($CD3^+$). In situ hybridization for κ and λ light chains can demonstrate monoclonality, although some large B-cell lymphomas have limited light-chain expression (Fig. 19.58). At the margins of the lymphoma, the diagnosis may be difficult because of the often atypical T-cell reaction to the presence of the lymphoma in the neuropil. Pretreatment with steroids can also abolish the B-cell neoplastic population, making the diagnosis difficult. In such cases, the diagnosis can be made from the immunohistochemical profile of the invading cells and the lack of infectious organisms on special stains. T-cell lymphomas are much rarer in the CNS.[83,84]

Patients with AIDS have a 50- to 60-times greater risk of developing primary CNS lymphoma than the general population. In the AIDS population the lymphoma is usually Epstein-Barr virus–driven, and this can be demonstrated by in situ hybridization (see Fig. 19.58).[85,86]

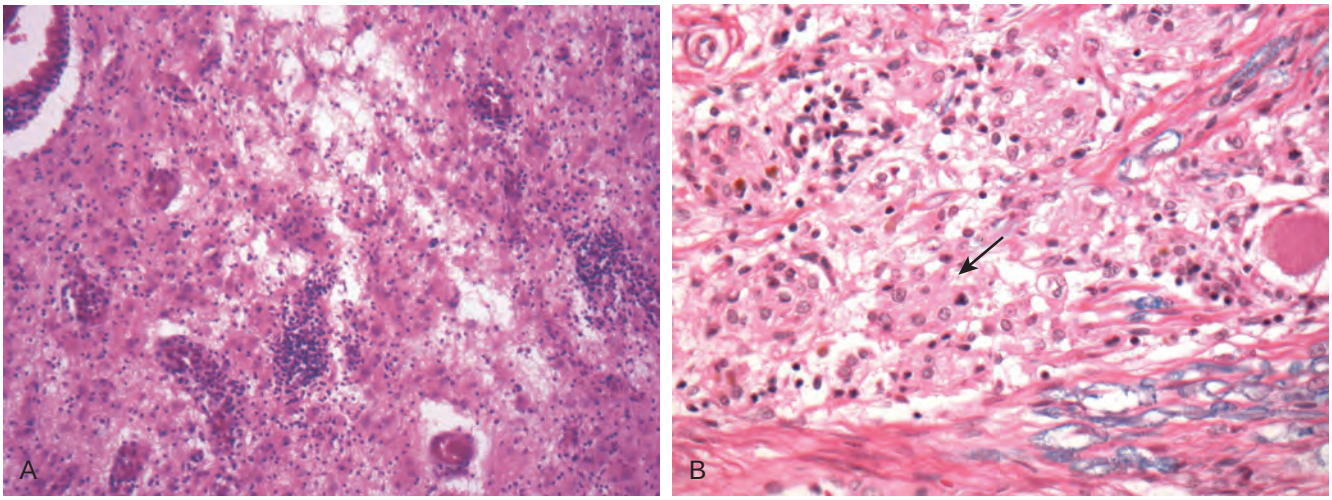
Lymphomas can also manifest as dural-based masses mimicking meningiomas. Immunohistochemical characterization may be less helpful here because the lymphomas tend to be of the more indolent type, with a mixed population of T and B cells. Therefore infectious causes have to be ruled out with stains for bacteria, spirochetes, mycobacteria, and fungi.



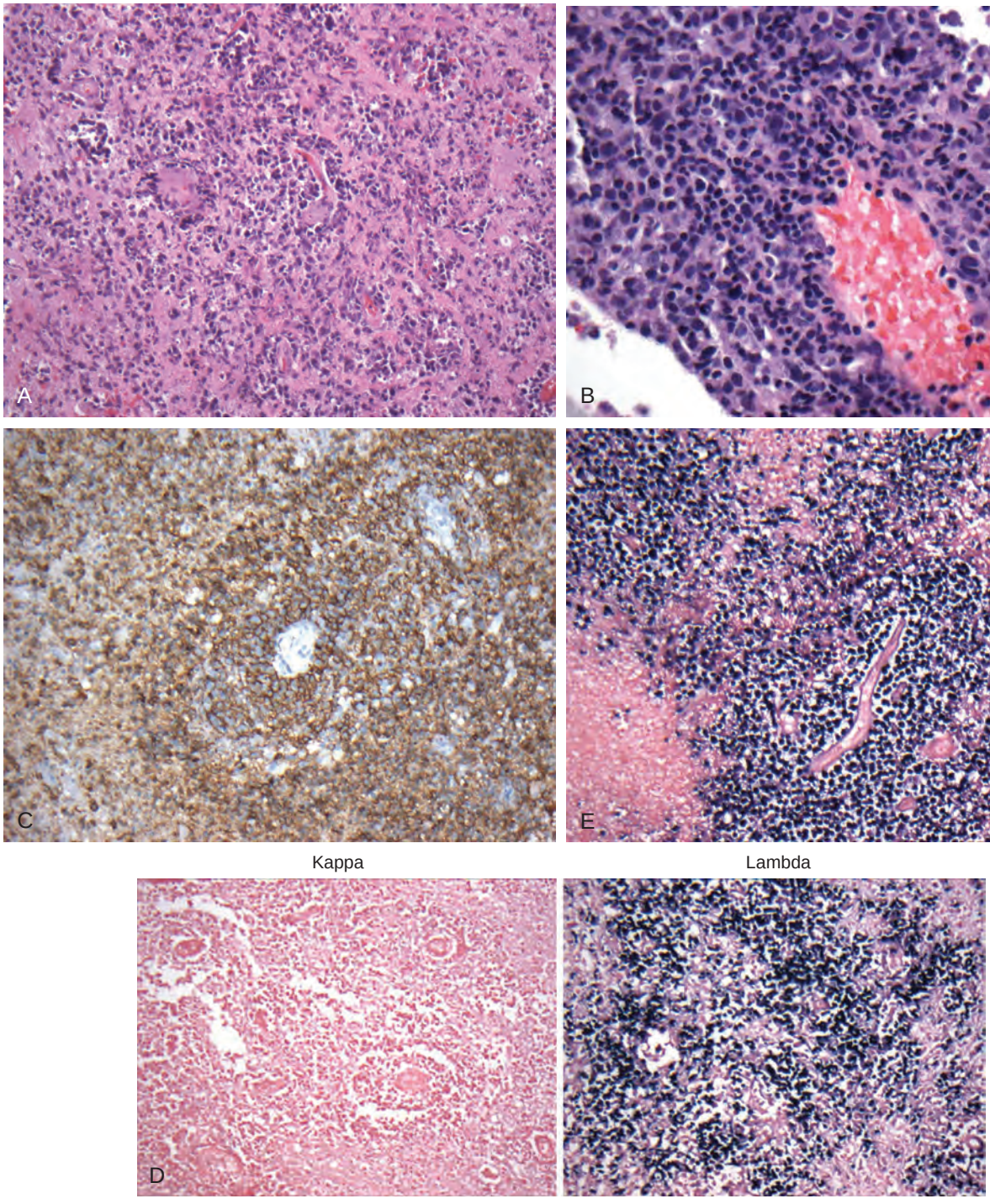
• **Figure 19.56** Vasculitis. Fibrinoid necrosis of a vessel wall *(arrow)*, showing moderate inflammatory infiltrate and reactive gliosis in adjacent tissue (frozen section, hematoxylin and eosin stain).

Extramedullary Plasmacytoma

The most common location of extramedullary plasmacytoma in the CNS is the suprasellar region, although other sites, such as the meninges, can be involved. The lesions are usually attached to the dura and radiographically resemble meningioma. Histologically, they are composed of a dense infiltrate of plasma cells. The plasma cells can have a normal mature morphology with eccentric nuclei, clock face chromatin, and a perinuclear halo. Binucleated forms, eosinophilic intranuclear inclusions (Dutcher bodies), or cytoplasmic inclusions (Russell bodies) can be seen (Fig. 19.59). Monoclonality can be proved with ancillary studies, such as immunohistochemistry or flow cytometry. Occasionally, extramedullary plasmacytoma can manifest as a pituitary adenoma, in



• **Figure 19.57** Paraneoplastic encephalomyelitis. **A,** Marked destruction of the medial temporal lobe with microglial nodules, gliosis, loss of neurons, and collections of lymphocytes. **B,** Note the loss of ganglion cells and the lymphocytic infiltrates *(arrow)*.



• **Figure 19.58** Lymphoma. **A,** Brain with dense, atypical lymphocytic infiltrate with some perivascular predilection. **B,** High power demonstrates dyshesive population of large cells with high nuclear-cytoplasmic ratio and prominent nucleoli. **C,** Immunohistochemical staining for CD20 shows a predominant neoplastic B-cell population. **D,** The κ and λ light chains are demonstrated by in situ hybridization. The monoclonality of the λ light chain supports the diagnosis of diffuse large B-cell lymphoma. **E,** Positive Epstein-Barr virus in situ hybridization in a patient with human immunodeficiency virus infection.

which case immunohistochemical studies are helpful for identifying hormonal activity or demonstrating plasma cell markers.[87,88]

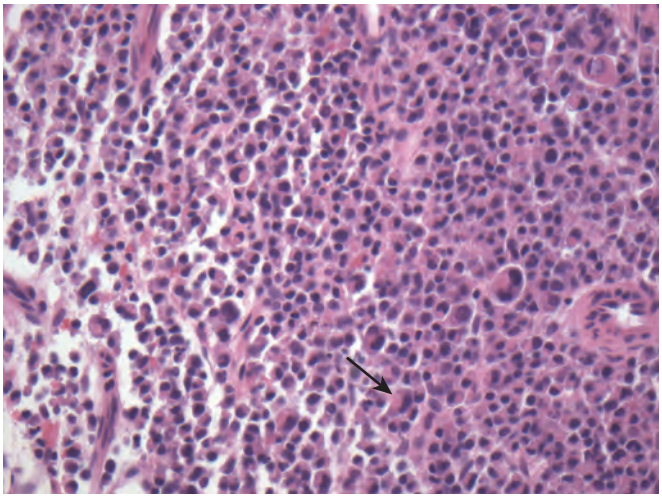
Idiopathic Hypertrophic Pachymeningitis

Idiopathic hypertrophic pachymeningitis is an inflammatory fibrosing process of the dura mater. It manifests clinically with headache, progressive cranial neuropathy, and cerebellar ataxia.[89,90] Radiologically, there is a multifocal and diffuse thickening of the dura, often involving the tentorium, the falx, peribulbar meninges, and even the spine at the exit of peripheral nerves, causing peripheral neuropathies or compressing the spinal cord itself. Biopsies reveal dense fibrosis as the hallmark, with a mixed

lymphoplasmacytic infiltrate; in some instances, necrosis and granuloma formation are present (Fig. 19.60). Note that this is a diagnosis of exclusion; other processes, such as tuberculosis, Wegener granulomatosis, sarcoidosis, rheumatoid meningitis, and other specific causes of a fibrous meningitis should be considered. Appropriate special stains, including those for infectious organisms, need to be performed.

Acute Disseminated Encephalomyelitis

Acute disseminated encephalomyelitis is an entity that usually follows an infection or recent vaccination. It may be associated with a preceding skin rash. Clinically, its manifestations can include hemiparesis, seizures, encephalopathy, visual disturbances, ataxia, cranial nerve palsies, and psychosis. The lesions are characterized by small, perivenous inflammatory infiltrates and demyelination with relative sparing of the axons. The inflammation is composed of lymphocytes, plasma cells, and some macrophages. The density of macrophages is low, and they are confined to the perivascular spaces. Frequently, perivascular hemorrhages are also present. A Luxol fast blue stain demonstrates the perivenular demyelination (Fig. 19.61). In general, these patients have a single episode with no recurrences and no or only minor neurologic sequelae.[91,92]



• **Figure 19.59** Plasmacytoma. Dense infiltrate of plasma cells, some of which have dense intracytoplasmic eosinophilic inclusions, known as Russell bodies *(arrow)*.

Germinoma

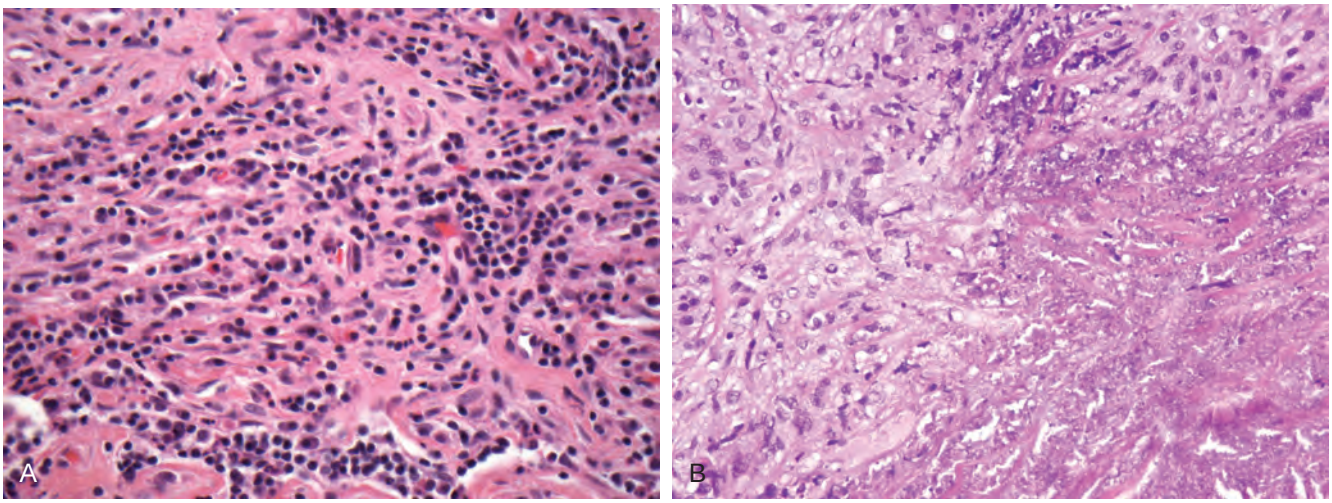
Germinomas occur in the midline and involve predominantly the pituitary and pineal regions in the CNS. The challenge of this entity is that the appearance on a biopsy specimen may resemble an infectious process because it usually consists of a dense lymphocytic infiltrate with only rare scattered large cells with clear cytoplasm and prominent nucleoli (germ cells). Smear preparations from frozen section can be helpful in identifying the neoplastic germ cell population. This is a diagnosis to keep in mind when encountering a midline inflammatory lesion, especially in adolescents and young adults because the peak incidence is between 10 and 14 years of age. Ancillary studies are helpful in arriving at the diagnosis and may include CSF germ cell markers and stains for C-kit (KIT), placental alkaline phosphatase (PLAP), and the homeobox transcription factors NANOG and Oct-4 (POU5F1) (Fig. 19.62).[93,94]

Granulomatous or Predominantly Histiocytic Inflammatory Pattern

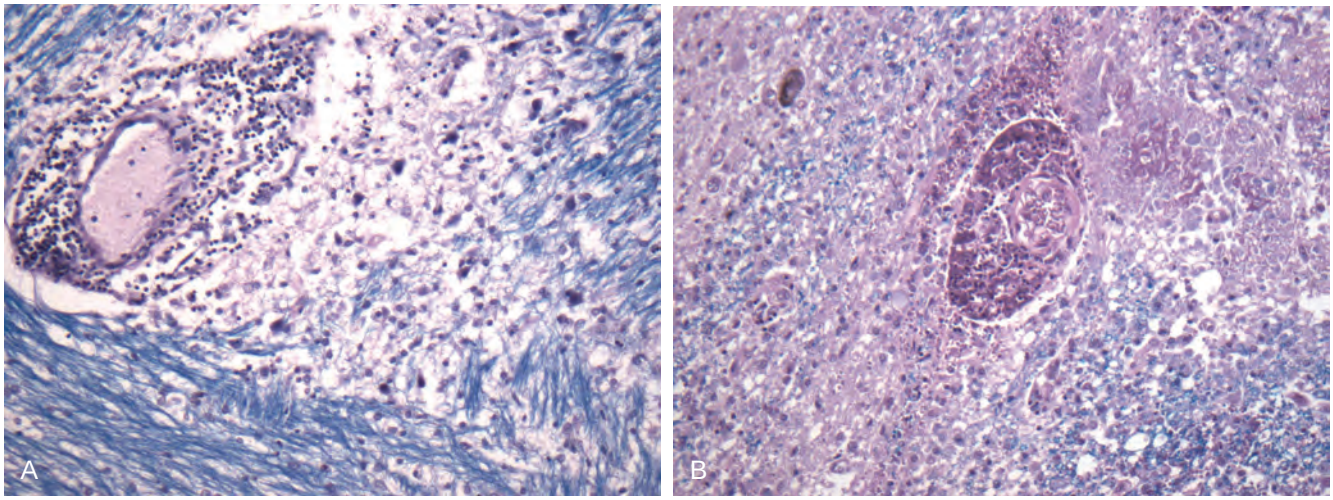
Infectious Etiologies

Bacteria

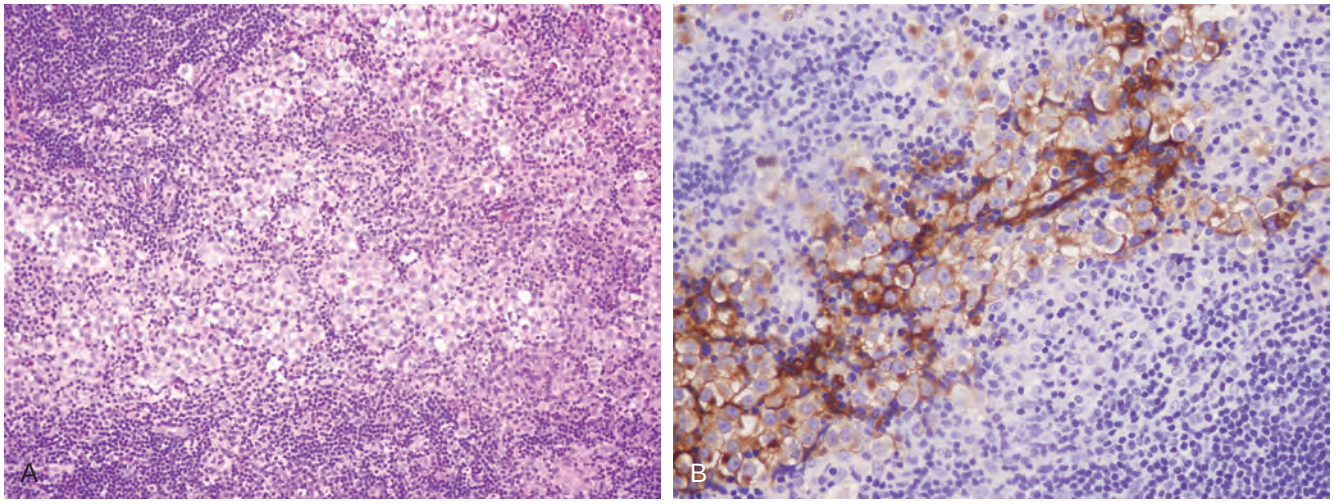
Tuberculosis. *Mycobacterium tuberculosis* causes meningitis and parenchymal caseous granulomas. Tuberculous meningitis is usually due to hematogenous dissemination of the tubercle bacillus. The meningitis predominantly involves the base of the brain but extends throughout the subarachnoid space. The inflammatory infiltrate extends into the subpial region, the ventricles, and subependymal parenchyma (Fig. 19.63). The viscous nature of the inflammatory exudate leads to the rapid development of hydrocephalus. The inflammatory infiltrate is composed of lymphocytes, mononuclear cells, epithelioid histiocytes, and occasional multinucleated giant cells (Fig. 19.64). Granulomas with epithelioid histiocytes and lymphocytes surrounding central caseous necrosis



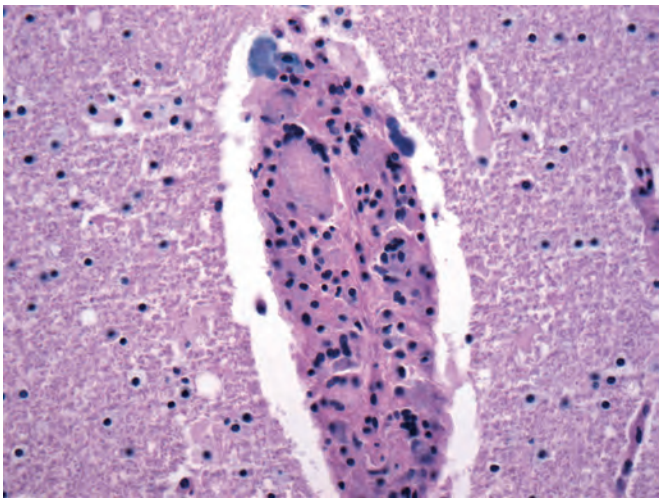
• **Figure 19.60** Pachymeningitis. **A,** Dura with dense fibrosis and lymphoplasmacytic infiltrate. **B,** Dura with chronic inflammation surrounding an area of necrosis.



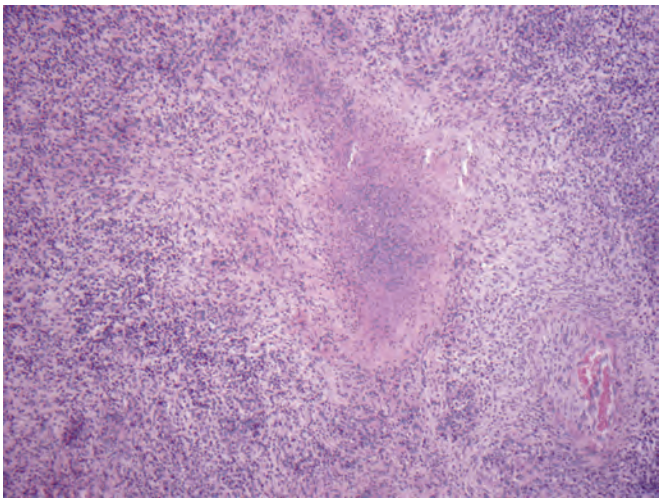
• **Figure 19.61** Acute disseminated encephalomyelitis. **A,** Luxol fast blue and hematoxylin and eosin staining of white matter emphasizes the perivenular lymphocytic infiltrate and the perivenular demyelination. **B,** Perivascular hemorrhage and demyelination.



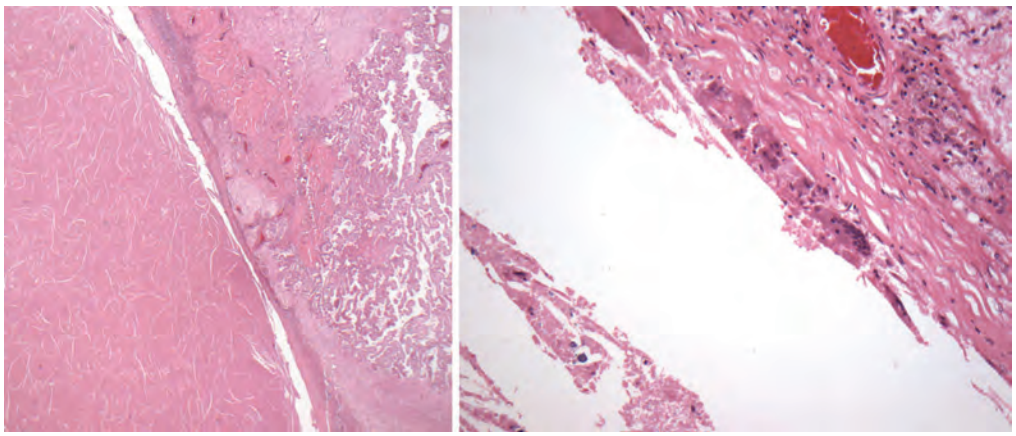
• **Figure 19.62** Germinoma. **A,** Dense lymphocytic infiltrate surrounding admixed large cells with clear cytoplasm, large nucleus, and prominent nucleoli (germ cells). **B,** Immunostaining for placental alkaline phosphatase highlights the germ cells.



• **Figure 19.63** Tuberculosis. A dense perivascular granulomatous infiltrate involves the Virchow-Robin space.



• **Figure 19.64** Tuberculosis. Tuberculous abscess with central caseous necrosis and surrounding multinucleated giant cells and lymphocytes.



• **Figure 19.65** Tuberculoma. Low- and high-power views of a tuberculoma with a fibrous capsule and a caseous center surrounded by epithelioid macrophages and lymphocytes.

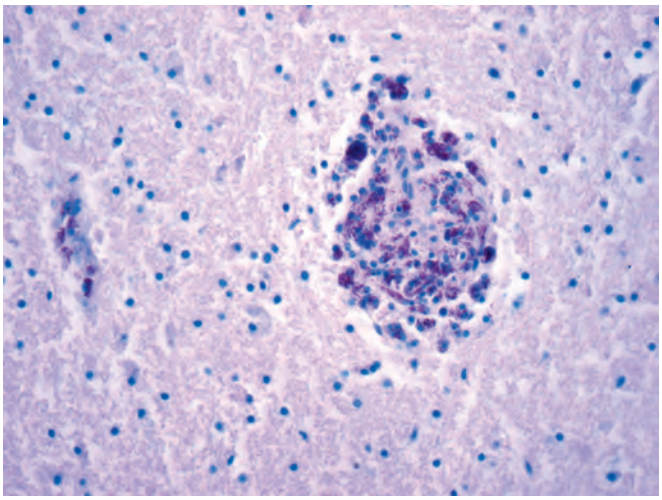
can be seen. Frequently, arterial lesions with endarteritis obliterans are present and are responsible for the development of ischemic infarcts.

Tuberculomas are the most common brain tumors in India and other areas of the world where tuberculosis is still a common infection. Tuberculomas can be multiple and can involve any lobe of the brain. Histologically, they are multiloculated, with a fibrous capsule and a caseous center surrounded by epithelioid macrophages and lymphocytes (Fig. 19.65). Old tuberculomas may become cystic or calcified with minimal inflammatory infiltrate.

If tuberculosis is suspected, an acid-fast bacterial stain should be performed and carefully examined (Fig. 19.66), keeping in mind that a negative stain does not exclude the diagnosis. If tuberculosis is clinically suspected, CSF cultures and PCR nucleic amplification should be performed.[95,96]

Whipple Disease. Whipple disease usually manifests as a systemic disease, although it may be confined to the CNS. Clinically, patients present with arthralgia, abdominal pain, malabsorption, and progressive weight loss. CNS involvement is characterized by confusion, memory loss, cranial nerve abnormalities, nystagmus, and ophthalmoplegia.[97,98] There are usually small lesions throughout the CNS, with special predilection for the subpial regions of the cortex, basal ganglia, hypothalamus, brainstem, and dentate. The response is characterized by infiltrates of foamy macrophages filled with bacilli of *Tropheryma whipplei*, which may have a gray-blue appearance on H&E staining and can be confirmed by PAS stain (Fig. 19.67). This dense macrophage infiltrate is associated with a dense reactive astrocytosis and parenchymal lymphocytes. Diagnosis of the disease in the brain in the absence of a systemic diagnosis is done by biopsy and demonstration of the bacilli on PAS staining, electron microscopic examination, or PCR analysis.[99,100]

Cerebral Syphilitic Gummas. Cerebral syphilitic gummas are seen less and less commonly in clinical practice, although one must be aware of the possibility. Syphilitic gummas can be confused clinically and radiologically with brain tumors.[101] They are firm, necrotic nodules that extend from the brain parenchyma to the leptomeninges. Microscopically, there is a central area of necrosis with ghost cells surrounded by a granulomatous reaction of epithelioid macrophages, fibroblasts, and multinucleated giant cells (Fig. 19.68). It is usually not possible to identify the spirochetes.



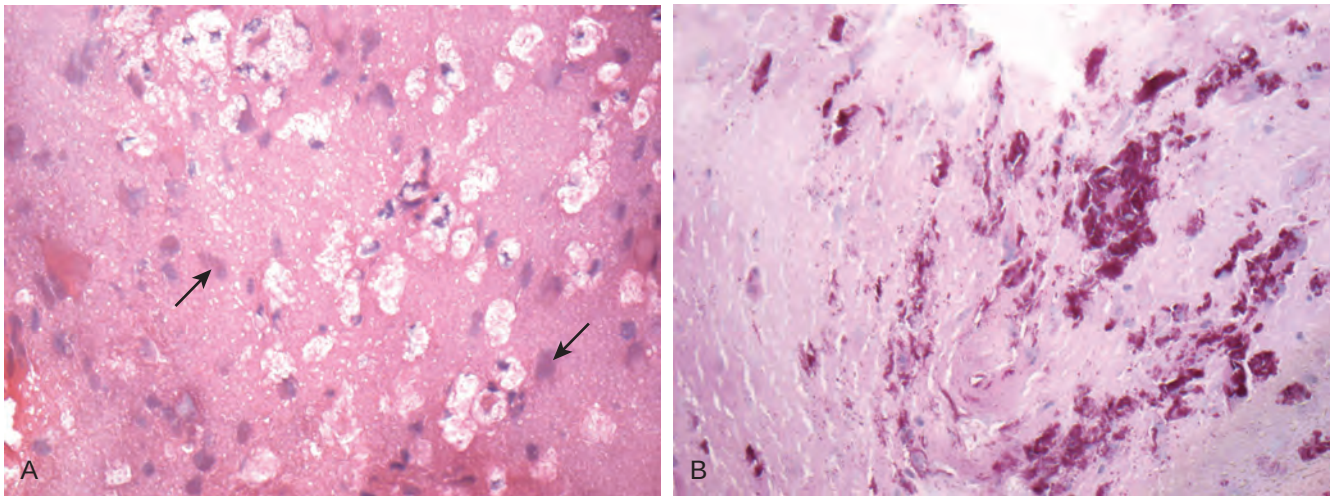
• **Figure 19.66** Atypical mycobacteria. Acid-fast stain demonstrates numerous mycobacteria. Numerous organisms are more likely to be seen in infections with atypical mycobacteria in immunosuppressed hosts.

Fungal

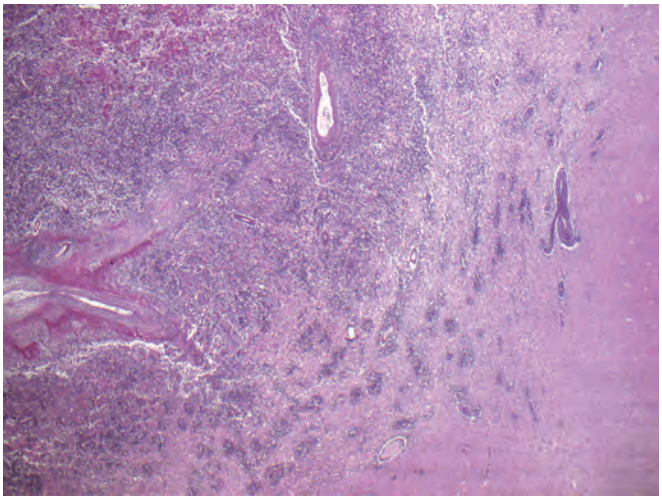
Dimorphic Fungi. The fungi that elicit granulomatous inflammation in the CNS are some of the dimorphic fungi, including *Blastomyces*, *Histoplasma*, *Coccidioides*, and *Paracoccidioides*. All of these manifest with primary lung infection and secondary dissemination to other organs, including the CNS. However, CNS involvement is rare and is more commonly seen in immunosuppressed patients.

Blastomycosis can manifest with single or multiple parenchymal abscesses or as a dural-based mass. Morphologically, *Blastomyces* is an 8- to 15-μm yeast that divides by broad-based budding (Fig. 19.69). It is usually found within macrophages, although extracellular forms are also present (Fig. 19.70).[102,103]

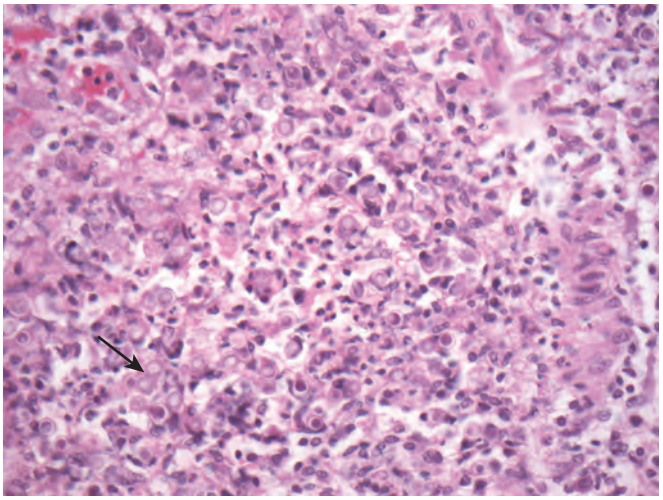
Histoplasmosis is most frequently seen as a basal meningitis with a gray-yellow exudate. Histologically, this consists of a large granulomatous lesion with caseous necrosis, giant cells, and surrounding fibrosis. As in tuberculosis, endarteritis obliterans with areas of ischemic necrosis is frequently seen. *Histoplasma* is an obligate intracellular microorganism that can be seen within macrophages. The yeasts are small, with an average diameter of 2 to 5 μm (Fig. 19.71).[104]



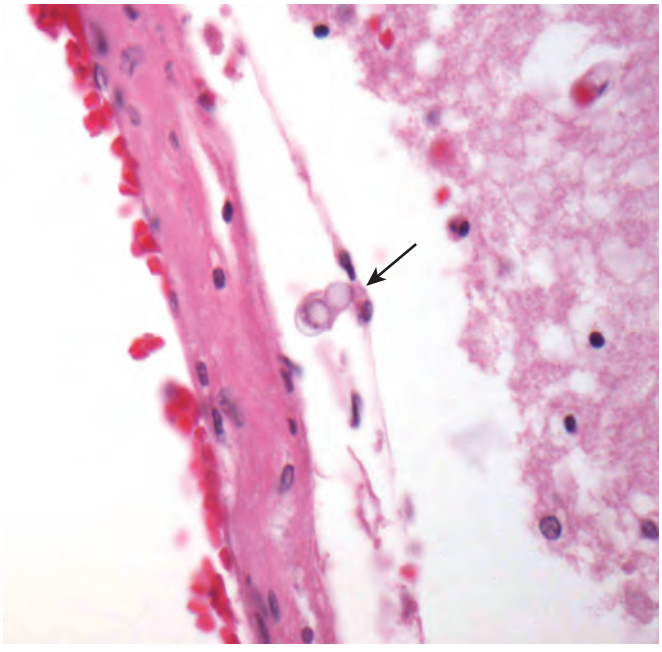
• **Figure 19.67** Whipple disease. **A,** Dense infiltrate of macrophages with foamy blue cytoplasm *(arrows)*. **B,** The microorganisms can be highlighted by periodic acid–Schiff staining.



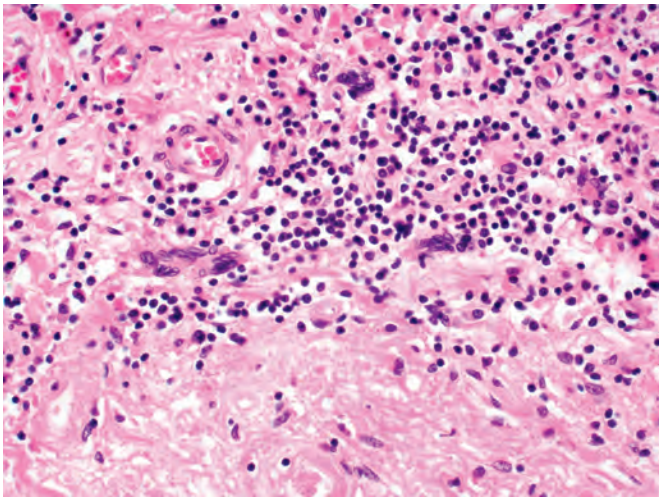
• **Figure 19.68** Syphilitic gumma. A central area of necrosis is surrounded by a granulomatous reaction of epithelioid macrophages, fibroblasts, and multinucleated giant cells.



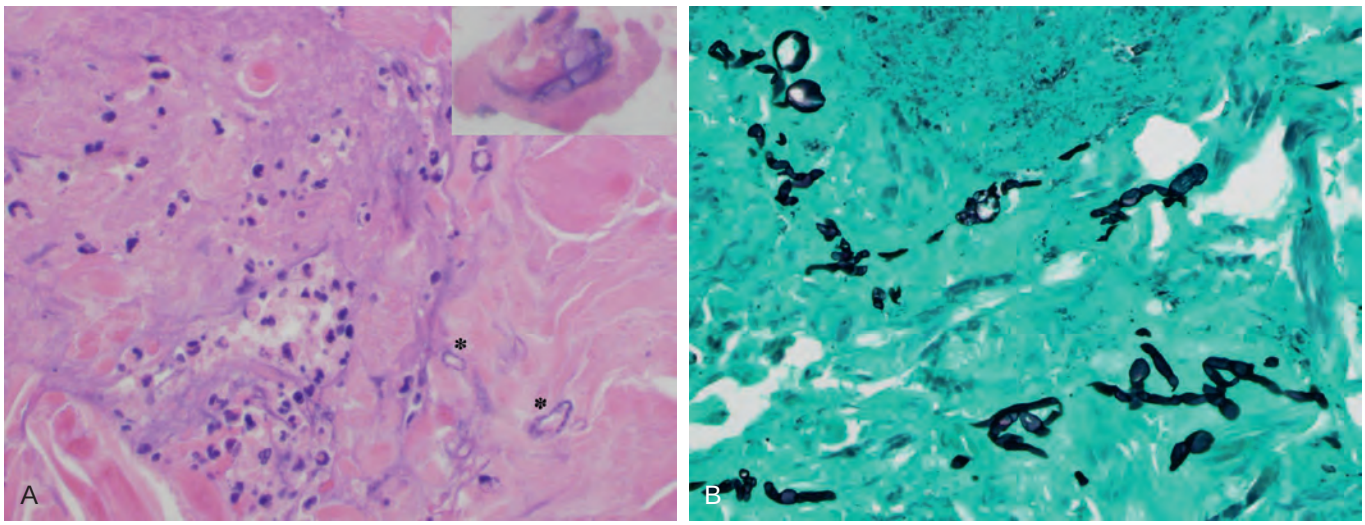
• **Figure 19.69** Blastomycosis. Brain tissue with extensive destruction and invasion by medium-sized (8 to 15 μm) yeast *(arrow)*, scattered lymphocytes, and macrophages.



• **Figure 19.70** Blastomycosis. Higher magnification demonstrates broad-based budding *(arrow)*, characteristic of this organism.



• **Figure 19.71** Histoplasmosis. Wall of caseous granuloma in the spinal cord, with multinucleated giant cells and mild chronic inflammation.



• **Figure 19.72** *Exserohilum rostratum.* **A,** Necrosuppurative inflammation with several fungal forms evident at the periphery *(asterisk)* (*inset* is from a different case). **B,** Chain of septated branching fungal hyphae, with occasional bulbous dilations (Grocott methenamine silver staining).

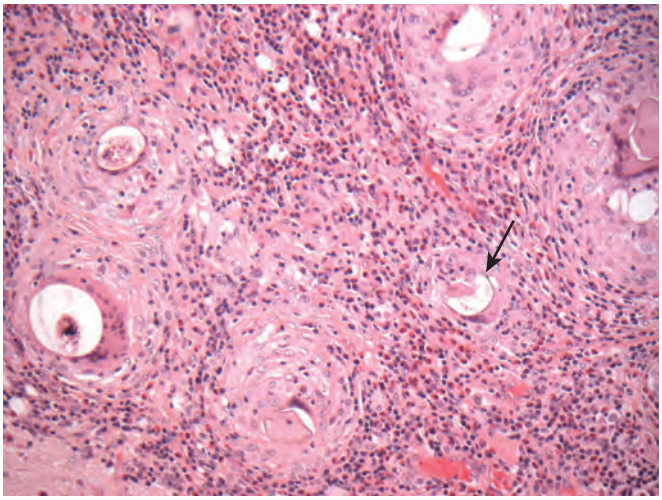
Coccidioides infection of the CNS manifests as a basal meningitis, although paravertebral abscess formation with spinal cord compression is also common. Histologically, it produces a granulomatous inflammation with mixed acute and chronic inflammation and, typically, numerous eosinophils. Chronic lesions form nodules with central caseous necrosis surrounded by granulomas and fibrosis. *Coccidioides* is a large yeast that measures up to 40 μm in diameter.[105]

Paracoccidioides infection of the CNS usually involves the cerebral hemispheres, although dural meningitis and spinal cord involvement have also been reported. The fungus elicits a granulomatous response and is identified by the presence of multiple small yeasts budding from a central medium-size yeast, the so-called steering wheel pattern.[106-108]

In September 2012 the CDC initiated an outbreak investigation of fungal infections linked to injection of contaminated methylprednisolone acetate (MPA). More than 13,000 people were exposed, and more than 750 developed fungal infections following their MPA injections. *Exserohilum rostratum* was the primary pathogen in this outbreak and was also confirmed from unopened MPA vials. *E. rostratum* is a saprobic mold found in soil and plant debris worldwide. Infections included meningitis, as well as localized epidural, paraspinal, and peripheral joint infections, complicated by arachnoiditis and abscess formation. Fatal cases had necrosuppurative granulomatous meningitis and vasculitis with secondary strokes due to fungal angioinvasion, predominately arterial circulation of the circle of Willis. The fungal organisms show chains of septated branching fungal hyphae, with occasional bulbous dilations seen on fungal stains (Fig. 19.72). Many cases were originally identified and diagnosed using broad-range PCR assay and DNA sequencing.[109-111]

Parasitic

Schistosomiasis. Schistosomiasis is uncommon in the United States but is endemic in South America, the Middle East, and Africa. *Schistosoma mansoni* and *Schistosoma hematobium* involve predominantly the spinal cord, whereas *Schistosoma japonicum* more commonly affects the brain.[112-114] Worms can migrate to unusual sites, such as the CNS vasculature. They can also get access through arteriovenous shunts in the lungs or portopulmonary connections via the azygos veins.[115] The worm elicits an initial inflammatory response, with numerous eggs surrounded by eosinophils, lymphocytes, plasma cells, and macrophages (Fig. 19.73). At a later stage, there is a granulomatous response with multinucleated giant cells. Vasculitis with rupture of the elastic lamina leading to formation of mycotic aneurysm and rupture with hemorrhage can also be seen.[116,117] The diagnosis is made by identifying the eggs in the inflammatory exudates.[118] The eggs are refractile and can be speciated by egg size and the shape and location of the spine on H&E stain. The capsule is acid-fast with Ziehl-Nielsen stain. *S. mansoni* has a prominent lateral spine.

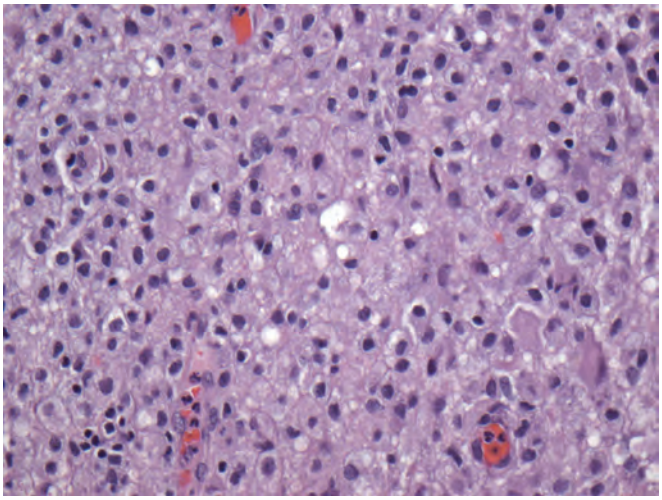


• **Figure 19.73** Schistosomiasis. Numerous eggs *(arrow)* are surrounded by eosinophils, lymphocytes, plasma cells, and macrophages.

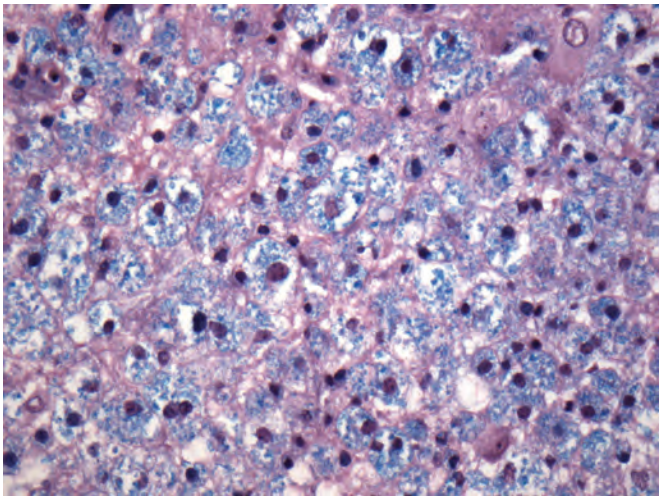
Noninfectious Etiologies

Demyelination

Demyelination is a clinical and pathologic challenge because acute inflammatory demyelination can mimic a primary CNS neoplasm



• **Figure 19.74** Acute inflammatory demyelination. Luxol fast blue stains myelin blue. In this picture, one can appreciate the edge of the demyelinating lesions where the parenchyma is devoid of myelin (Luxol fast blue and hematoxylin and eosin stain).

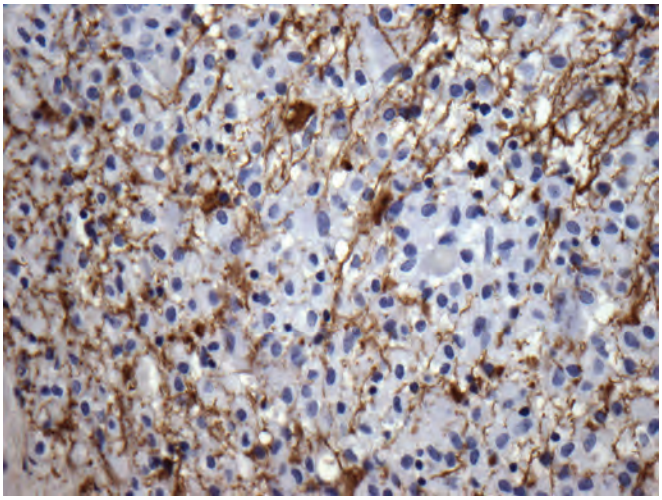


• **Figure 19.75** Demyelination. High power demonstrates that the lesion is rich in macrophages that contain myelin debris (Luxol fast blue and hematoxylin and eosin stain).

radiologically, and the dense inflammatory reaction may lead the pathologist to confuse it with an infectious disease or an infarct.[119]

By far the most common demyelinating process of the CNS is multiple sclerosis. Acute inflammatory multiple sclerosis can manifest as a mass lesion and may be biopsied with a preoperative diagnosis of neoplasm.

The active plaque of multiple sclerosis is sharply demarcated from the surrounding parenchyma; however, stereotactic biopsies may sample only the center of the demyelinating area. Affected tissue displays abundant foamy macrophages (resulting in a hypercellular appearance), mixed reactive astrocytosis, perivascular lymphocytes (predominantly T cells), and occasional plasma cells (Figs. 19.74 and 19.75). The definitive diagnosis can be made by demonstration of loss of myelin (Luxol fast blue or immunostains for myelin basic protein) with relative preservation of axons (immunostain for neurofilament or silver preparation for axons, such as Bielschowsky) (Fig. 19.76). The remnants of myelin can



• **Figure 19.76** Demyelination. Immunostaining with an antibody to neurofilament demonstrates relative preservation of the axons in a background of numerous macrophages (immunoperoxidase reaction).

be seen as a phagocytized element in the macrophages.[120-122] Caution is recommended with this entity because many mistakes can be made if the lesions are misinterpreted as neoplasms, such as oligodendrogliomas, due to the abundant clear cytoplasm of the macrophages, or astrocytomas, due to the prominent reactive astrocytes, which can be atypical and may display numerous mitotic figures. Correct diagnosis for appropriate treatment is imperative to avoid the devastating consequences of irradiating a "tumor" that is no more than a demyelinating process. Diffuse infiltration of macrophages is a rare feature of untreated gliomas and practically excludes the diagnosis of glioma.

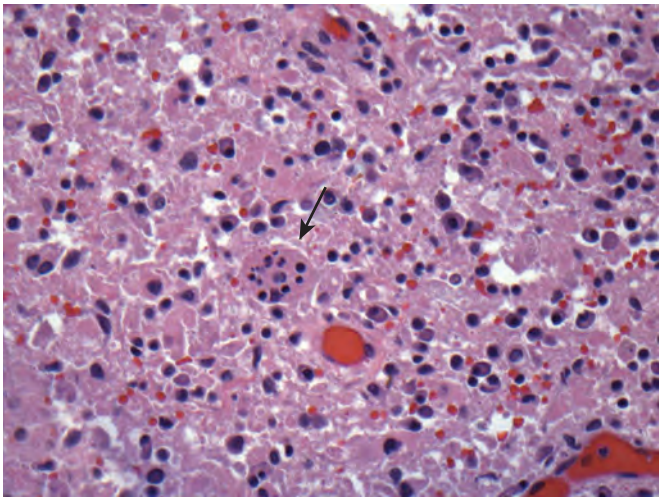
Of note, steroid-treated lymphomas also can have abundant macrophages, lymphocytes, and plasma cells without evidence of demyelination. This is an entity that must be kept in mind.[123]

Rosai-Dorfman Disease

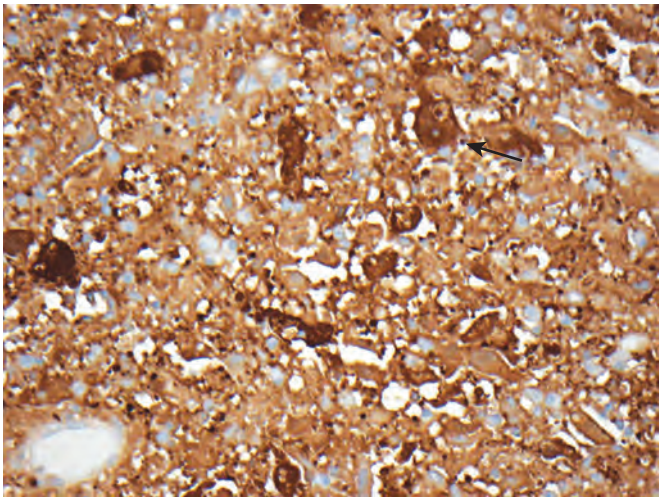
Also known as sinus histiocytosis with massive lymphadenopathy, Rosai-Dorfman disease (RDD) is an idiopathic histiocytic proliferation, usually affecting lymph nodes, that can be misinterpreted as an infection. The most common clinical presentation is painless cervical lymphadenopathy. The disease occurs most often in the third and fourth decades and may be accompanied by leukocytosis, elevated erythrocyte sedimentation rate (ESR), weight loss, and possibly hypergammaglobulinemia. Although lymph node involvement often dominates the clinical presentation, other anatomic sites are involved in 30% to 40% of cases, most commonly skin, upper respiratory tract, orbit, or testes.

RDD is uncommon in the CNS (<5% of cases) but can masquerade as a meningioma, manifesting as a dural-based lesion with contrast enhancement and with vasogenic edema in the underlying cerebral cortex and white matter, leading to a biopsy.[124,125] The dural involvement may be the only manifestation, or it may be part of multifocal disease. Approximately 75% of the CNS RDD cases are intracranial, whereas only 20% involve the spine.[126]

Histologically the lesions consist of a mixed lymphoplasmacytic inflammatory infiltrate with occasional neutrophils and eosinophils and variable numbers of pale-staining histiocytes. The histiocytes display the characteristic emperipolesis (Fig. 19.77).



• **Figure 19.77** Rosai-Dorfman disease. Dura with mixed lymphoplasmacytic inflammatory infiltrate and variable numbers of pale-staining histiocytes. The histiocytes display the characteristic emperipolesis *(arrow)*.



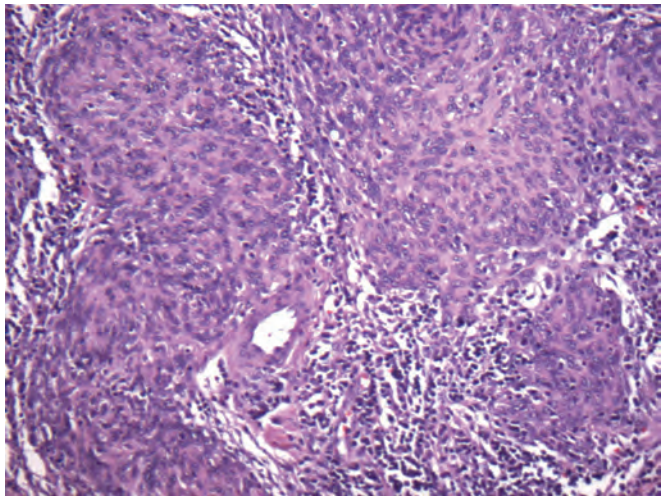
• **Figure 19.78** Rosai-Dorfman disease. Immunostaining with antibodies to S-100 highlights the macrophages in dark brown *(arrow)*, with negative staining of the "passing by" cells (emperipolesis) (immunoperoxidase reaction).

Binucleated plasma cells and intracytoplasmic eosinophilic inclusions (Russell bodies) are commonly observed. There is usually a dense fibrous proliferation in the background. Special stains for organisms are necessary to rule out an infectious etiology and are generally negative. The large histiocytes are positive for S-100 protein and CD68, but negative for CD1a. Examination of immunostained sections can facilitate the recognition of emperipolesis (Fig. 19.78).[127] Necrosis, well-formed granulomas, and eosinophilia are not present.

The etiology of RDD is not known, but it has been suggested that it is an autoimmune disease. There has been no definitive evidence of any infectious agent. It is currently considered a benign, idiopathic histiocytosis.[128]

Meningiomas With Dense Lymphoplasmacytic Response

Some meningiomas may be largely replaced by a pronounced chronic inflammatory reaction of lymphocytes and plasma cells



• **Figure 19.79** Meningioma with dense lymphoplasmacytic response. Although it can be confused with an infectious etiology, areas of meningothelial growth reveal the nature of the process.

(Fig. 19.79). However, classic areas of meningioma can be identified. Confirmatory immunostaining for epithelial membrane antigen (EMA) may be helpful in the diagnosis, to avoid confusion with an infectious process.[129]

Langerhans Cell Histiocytosis

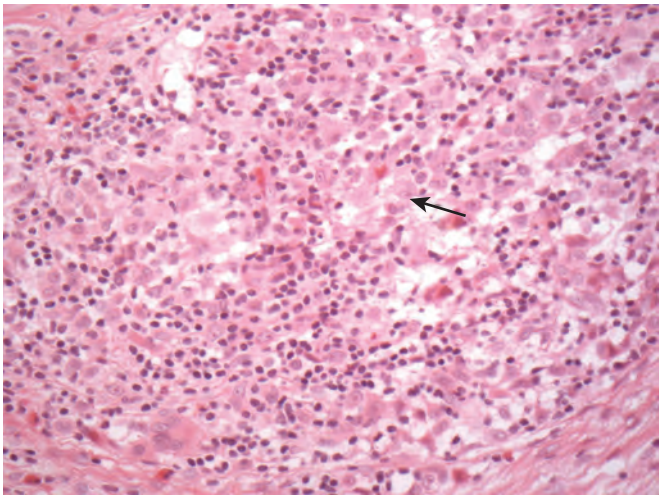
Langerhans cell histiocytosis primarily affects children. It can manifest as a single lesion (eosinophilic granuloma); as multiple lesions with disseminated disease and involvement of lymph nodes, skin, liver, and pancreas (Letterer-Siwe disease); or as multiple lytic lesions involving the calvarium and base of the skull with clinical diabetes insipidus or exophthalmos (Hand-Schüller-Christian disease).[130]

Langerhans histiocytosis can also manifest as discrete histiocytic nodules attached to the dura.

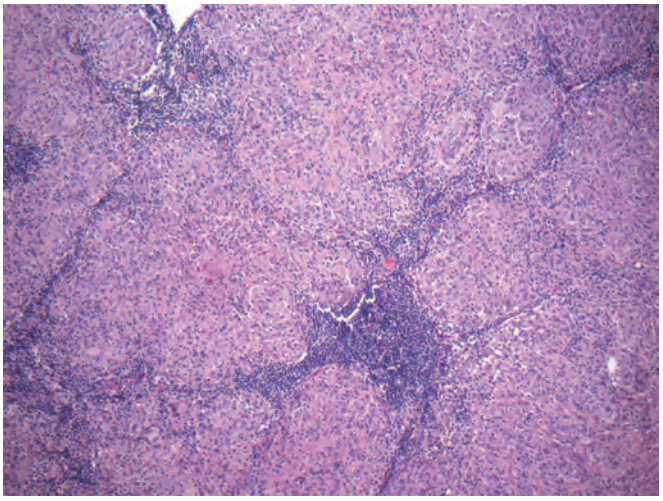
Histologically, Langerhans cells are histiocytes with characteristic cytologic features consistent with moderate to abundant clear cytoplasm and a reniform nucleus with longitudinal nuclear grooves and lobulations. Numerous eosinophils are often present and are helpful in the diagnosis. Other inflammatory cells, such as plasma cells and neutrophils, may be seen, although in a lower proportion than eosinophils. Langerhans histiocytes are CD1a and S-100 positive (Fig. 19.80). Ultrastructural studies demonstrate the classic Birbeck granules.[131,132]

Sarcoidosis

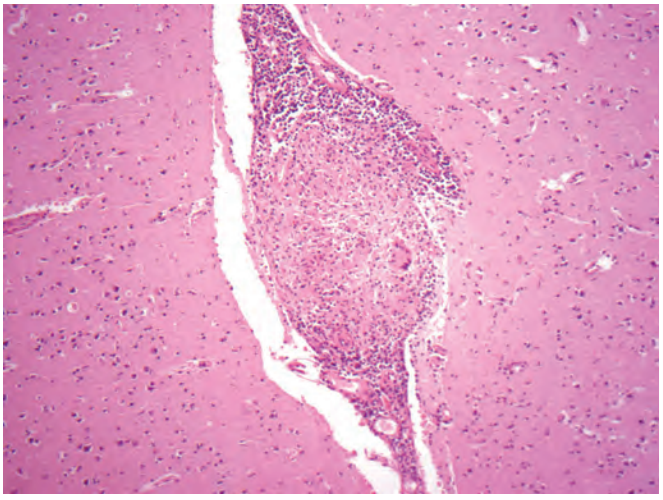
Sarcoidosis is a systemic granulomatous disorder of unknown etiology. The lungs are the usual site of disease, but the meninges, skeletal muscle, and peripheral nerves can also be involved. Sarcoidosis of the nervous system is usually a basilar meningitis manifesting as multiple cranial nerve or spinal root palsies. The CSF generally shows elevation of angiotensin-converting enzyme (ACE).[133,134] Occasionally, the hypothalamus is involved, leading to diabetes insipidus. Histologically the lesions are composed of compact, noncaseating, epithelioid granulomas surrounded by lymphocytes and monocytes. They may coalesce and form large masses simulating neoplasms (Fig. 19.81).[135] The granulomas tend to be located around blood vessels and follow the Virchow-Robin spaces (Fig. 19.82). Granulomas can occasionally be seen intraparenchymally around blood vessels, surrounded by a dense reactive



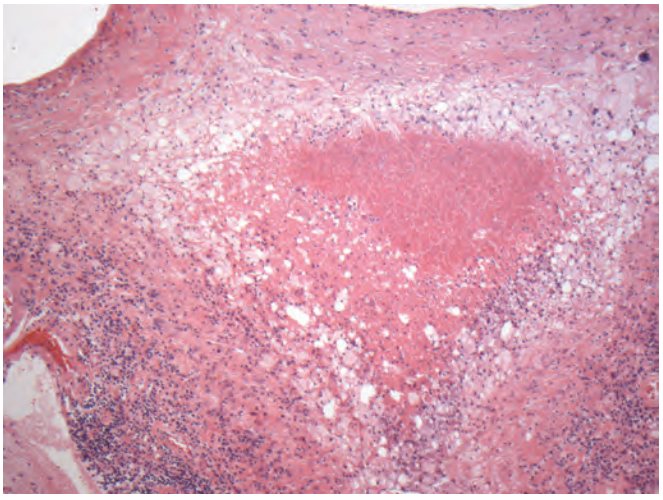
• **Figure 19.80** Langerhans histiocytosis. Dense chronic inflammation is present, with occasional eosinophils admixed with Langerhans cells, which have characteristic reniform or cleaved nuclei *(arrow)*.



• **Figure 19.81** Sarcoid. Multiple, tightly packed, noncaseating granulomas coalesce and mimic a mass. The granulomas are surrounded by lymphocytic infiltrate.



• **Figure 19.82** Sarcoid. A granuloma around a blood vessel is present in the subarachnoid space. The granulomas can also be found in the Virchow-Robin spaces.



• **Figure 19.83** Rheumatoid meningitis. Rheumatoid nodule in the subarachnoid space consists of a lymphoplasmacytic infiltrate with a necrotic center.

gliosis; they can also be found around the third ventricle or in the choroid plexus.[136,137] This is a diagnosis of exclusion, and special stains to rule out an infectious etiology (e.g., fungal infection, tuberculosis) must be done.

Rheumatoid Meningitis

Another cause of chronic pachymeningitis or leptomeningitis is rheumatoid arthritis. Rheumatoid nodules in the meninges manifest as localized masses in the dura or the subarachnoid space. They consist of a lymphoplasmacytic infiltrate with a necrotic center and the typical radial arrangement of fibroblasts and inflammatory cells seen in peripheral rheumatoid nodules, but they are not usually as well defined as those in the periphery (Fig. 19.83).[138,139] This is a diagnosis of exclusion because a gumma or tuberculous granuloma has to be ruled out. Special stains for bacteria, fungi, and other infectious organisms are negative.

Granulomatous Hypophysitis

Granulomatous hypophysitis is a rare condition of unknown etiology that usually causes hormonal imbalance, hyperprolactinemia, diabetes insipidus, headache, and nausea. Imaging reveals a contrast-enhancing mass in the pituitary, and the diagnosis is made by biopsy. Although the biopsy is performed to rule out an adenoma, it is very rare for a pituitary adenoma to manifest with diabetes insipidus. Microscopically, large collections of histiocytes admixed with lymphocytes and plasma cells, with occasional multinucleated giant cells, almost obliterate the normal anterior pituitary architecture. Recognizing the presence of embedded anterior pituitary cells is the first clue to the diagnosis (Fig. 19.84). Lymphoplasmacytic infiltrates can be prominent and may obliterate normal gland architecture. Special stains for microorganisms are required to eliminate an infectious etiology, and tests for lymphocytic markers are needed to rule out lymphoma. Stains for the pituitary hormones reveal the scattered remaining anterior pituitary cells in the background of the inflammatory reaction.[140,141] Lymphocytic hypophysitis is thought to be an autoimmune disorder because it can be accompanied by other endocrine autoimmune disorders, such as Hashimoto thyroiditis or adrenalitis.[142]

Foreign Body

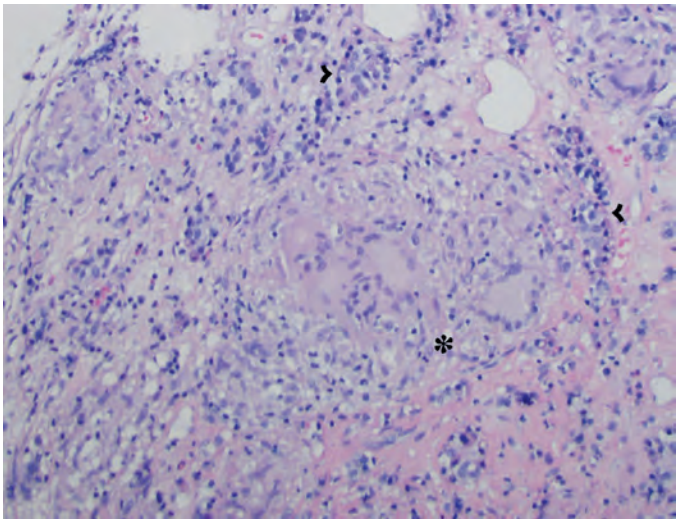
Patients with prior surgery, trauma, or embolization treatments may develop a focal, dense, chronic inflammatory response with a mixed lymphoid and plasma cell infiltrate and foreign body giant cell reaction. The foreign material can usually be identified by light microscopy or by polarization.

Minimal or No Inflammatory Response

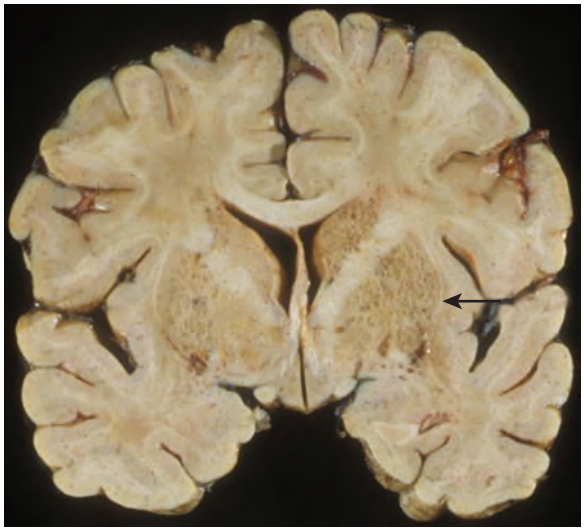
Fungi

Cryptococcus

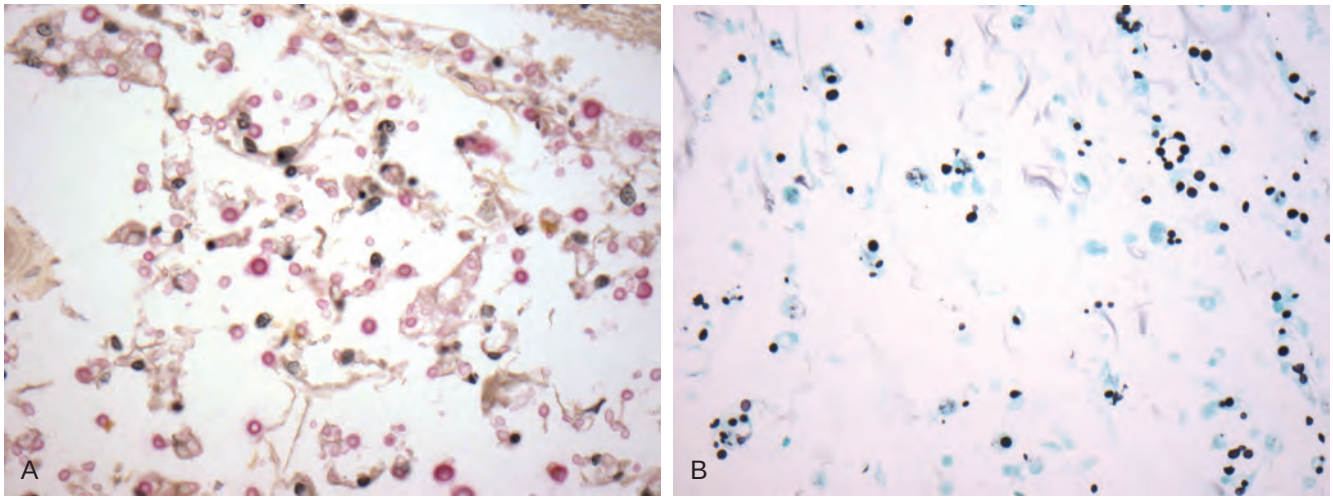
Cryptococcus is the most common mycosis of the CNS. It often affects immunocompromised patients. *Cryptococcus* usually causes minimal inflammatory response or mild chronic meningitis in both immunocompetent and immunocompromised individuals. The symptoms are severe headache with or without neck stiffness. The pattern of inflammation varies depending on the host response. Because of the mucoid capsule that protects the organism from being recognized by the immune system, dissemination through the meninges is fast, producing a gelatinous or mucoid surface to the brain and a honeycomb-like appearance with multiple cystlike spaces where the organism floats in a mucin background in the brain parenchyma (Fig. 19.85). On the other hand, patients who elicit a strong immune response may have sclerotic and granulomatous masses containing only a few yeast forms. *Cryptococcus* is a round budding yeast that varies in size from 2 to 15 μm in diameter. The bud has a narrow neck, in contrast to the broad-base budding yeast of blastomycosis (Fig. 19.86).[143,144] The microorganism is highlighted by PAS and GMS staining. The yeast is surrounded by a polysaccharide capsule that can be identified by mucicarmine or alcian blue stains.



• **Figure 19.84** Granulomatous hypophysitis. Large collection of histiocytes, some of them multinucleated *(asterisk)* admixed with normal anterior pituitary acini *(arrowheads)*.



• **Figure 19.85** *Cryptococcus.* Coronal section of the brain demonstrates the honeycomb-like appearance, with multiple cystlike spaces where the organism floats in a mucinous background *(arrow)*.



• **Figure 19.86** *Cryptococcus.* **A,** Mucicarmine staining highlights the polysaccharide capsule around the yeasts in the subarachnoid space. **B,** Grocott methenamine silver staining shows small yeasts (2 μm) in the subarachnoid space with narrow budding.

Caution should be taken not to confuse corpora amylacea in the brain with *Cryptococcus*. Corpora amylacea are the same size as *Cryptococcus* and also stain positively with PAS and GMS. The distinction can be made by examination of the CSF using India ink, which allows one to see the cryptococcal budding yeasts surrounded by clear halos. Although the sensitivity of this test is only 25%, it increases to 53% when multiple samples are used.[145] A combination of capsular antigen detection in CSF (sensitivity, 60% to 95%) and culture is currently used for the diagnosis.

Hyphal Organisms in the Immunocompromised Host

This category includes such pathogens as *Aspergillus*, *Mucor*, *Pseudallescheria*, *Fusarium*, and *Penicillium* spp. These organisms usually affect persons who are at risk of systemic mycosis, such as chronic alcoholics, intravenous drug abusers, and patients who are immunosuppressed therapeutically. The organisms often spread by direct intracranial extension from adjacent structures. Although in immunocompetent hosts they may cause a strong inflammatory response, as addressed earlier, in the immunocompromised host these organisms may be primarily intraparenchymal with minimal host inflammatory response.[146,147]

Viruses

Zika Virus

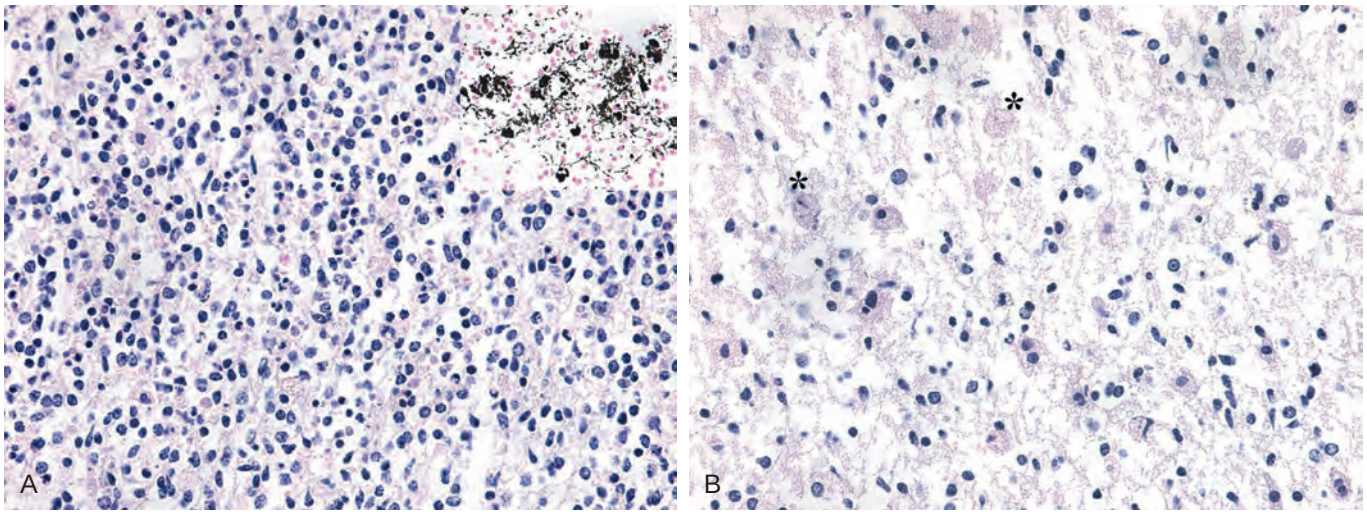
Zika virus (ZKV) is a mosquito-borne flavivirus originally confined to Africa, Southeast Asia, and some islands of the Pacific. Recently (2015) ZKV has been reported in South and Central America and the Caribbean. In addition to the usual maculopapular rash, fever, ocular pain, headache, arthralgias and myalgias, ZKV has been recently associated with increased risk of congenital microcephaly. High ZKV RNA viral loads have now been isolated from fetal brain, placenta, and umbilical cord, indicating a vertical transmission. Neuropathologic alterations reported to date include microcephaly, intraventricular hemorrhages, microcalcifications, agyria or thinning of the cortical mantle, and partial or complete absence of corpus callosum. Histologically, there is apoptosis of the intermediately postmigratory neurons in the neocortex with relative preservation of the well-differentiated neurons of the basal ganglia, limbic system, and germinal matrix (Fig. 19.87), with very little inflammation. No evidence of encephalitis or meningitis has been reported. Electron microscopic examination has revealed spherical viral particles (42 to 54 nm).[148,149] In experimental studies of infected mice, ZKV targets the radial glial cells, important progenitor cells in cortical development.[150] The diagnostic test is ZKV RNA detection in serum or blood during the first week of symptomatic infection; after that, immunoglobulin G (IgG) and IgM serology is the recommended test. ZKV RNA can be detected in urine for more than a week after infection and in semen for currently unknown lengths of time.

Rabies

Most human rabies infections result from introduction of the virus via saliva from a rabid animal bite. Rabies is endemic particularly in raccoons and bats in the United States, as well as in other mammals in the Americas, Europe, Africa, and Asia.

The virus replicates within the skeletal muscle, then reaches the neuromuscular junction and is transported via the axon centrally to the CNS, where it spreads rapidly between neurons in the spinal cord, brainstem, cerebrum, and cerebellum.[151,152] After the infection has reached the CNS, the virus spreads peripherally through axons to salivary and lacrimal glands, skin, heart, lungs, gastrointestinal tract, adrenal glands, kidneys, and bladder. The incubation period ranges from 1 to 3 months but can be as long as 1 year. Rare cases have followed an even longer incubation period.[153] Early postexposure prophylaxis virtually eliminates the disease. The disease course is inexorable once clinical signs appear.

In general, inflammation is minimal and there is usually a discrepancy between the viral load and the degree of inflammation. Leptomeningeal inflammation with perivascular lymphocytic infiltration and neuronophagia can be seen. Rabies is an RNA virus that replicates in the cytoplasm, forming what corresponds to Negri bodies.[154] Negri bodies are sharply delineated, round to



• **Figure 19.87** Zika virus infection in a 21-week fetal brain. **A,** Cerebral cortical plate at this age should be filled with small blue neurons; in this microphotograph, there are severe neuronal loss and apoptosis affecting the intermediately differentiated postmigratory neurons. The *inset* highlights areas of early mineralization (von Kossa staining). **B,** White matter with axonal disruption, rarefaction, and foamy macrophages *(asterisks)*. (Photographs courtesy Dr. Cheng-Ying Ho, Neuropathologist, Children's National Health System).

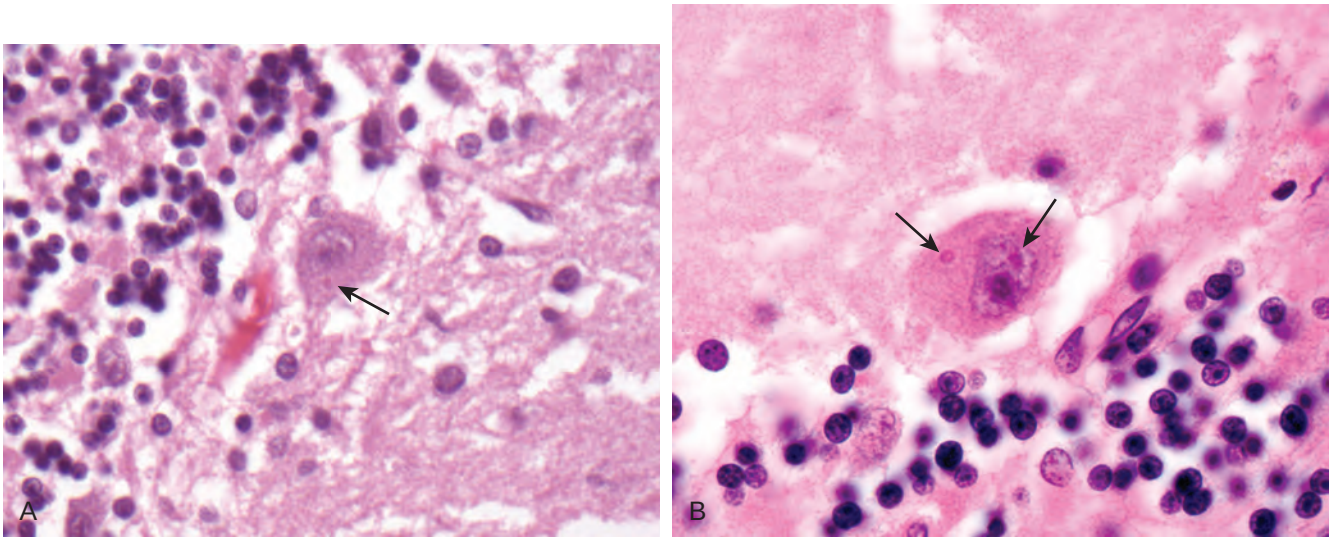
oval, eosinophilic cytoplasmic inclusions found in most parts of the CNS, although they are easiest to identify in Purkinje cells (Fig. 19.88), hippocampal pyramidal cells, and brainstem nuclei. Ultrastructurally, rabies virus has a characteristic bullet shape, 70 to 85 nm in diameter and 180 nm in length (see Chapter 5).

Prions

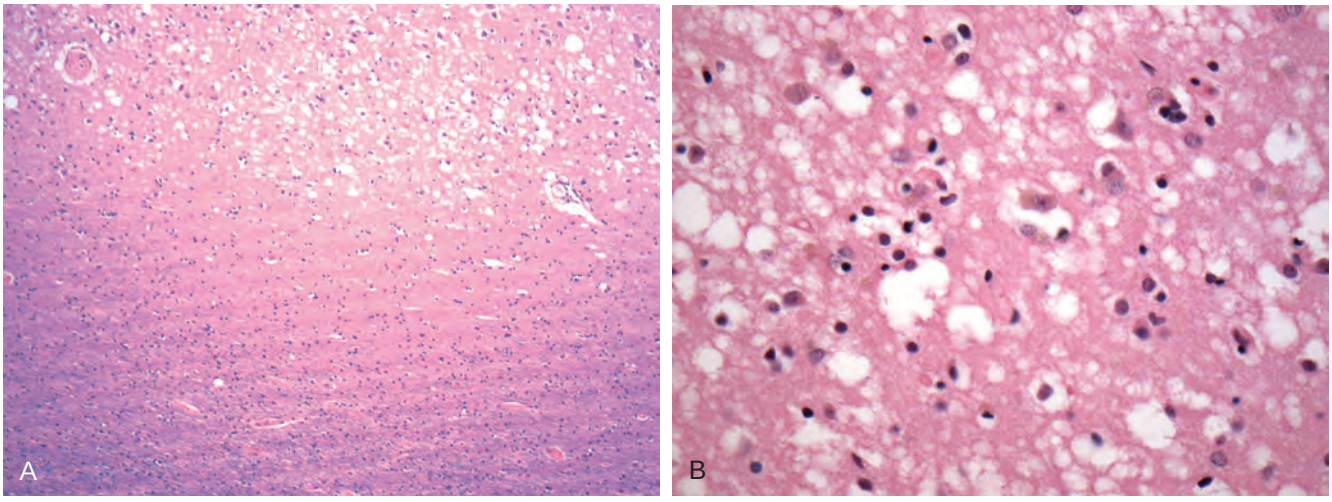
Creutzfeldt-Jakob Disease

Creutzfeldt-Jakob disease (CJD) is a rare transmissible spongiform encephalopathy that is associated with the accumulation of an abnormal prion protein in neuronal processes and neuropil, which leads to destruction of the neurons with a striking reactive astrocytosis. Most cases have no obvious inflammatory reaction. The patients present with a rapidly progressive dementia associated with neurologic findings, such as ataxia, visual abnormalities, myoclonus, and extrapyramidal signs.[155,156] Magnetic resonance imaging often shows an increased signal in the basal ganglia, with variable degrees of signal intensity changes in the cerebral cortex.[157,158]

The characteristic histologic feature is the development of a spongiform appearance of the tissue, with multiple tiny vacuoles confined to the gray matter, accompanied by neuronal loss and reactive gliosis without an inflammatory infiltrate (Fig. 19.89). The severity of the changes varies within different regions of the brain.[159,160] The abnormal prion protein can be demonstrated by immunohistochemistry and/or by Western blot analysis, which in the United States is performed at the National Prion Disease Surveillance Center at Case Western Reserve University.[161,162] The definitive diagnosis can be made only from brain tissue, but the characteristic clinical course and the presence of protein 14-3-3 in CSF leads to a high suspicion. The presence of protein 14-3-3 in the CSF can also be found in other neuronal destructive diseases and in stroke.[163,164] If the diagnosis is suspected and surgery is



• **Figure 19.88** Rabies. Purkinje cells show well-circumscribed, eosinophilic intracytoplasmic inclusions (Negri bodies) in **A** *(arrow)* and **B** (*arrow on the left)*; **B** also demonstrates an intranuclear inclusion *(arrow on the right)*.



• **Figure 19.89** Creutzfeldt-Jakob disease. **A,** Brain with spongiform changes, composed of multiple vacuoles of varying size, confined to the gray matter. Some of the vacuoles are contained within the thickness of the section. **B,** Higher magnification reveals neuronal loss and reactive gliosis without any inflammatory response.

necessary, disposable instruments should be used because the disease has been transmitted by the use of contaminated surgical instruments and by dural and corneal transplants.[165] There is little or no justification for brain biopsy. The abnormal prion protein is highly resistant to most decontaminating agents, and tissue can remain infectious unless it is treated according to a precise protocol.[166] Any tissue block coming from a patient with suspected CJD should be fixed in formalin for 24 hours and then soaked in 90% formic acid for 1 hour before returning it to formalin for routine histologic processing.[167]

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20
Skin Infections

CARLOS N. PRIETO-GRANADA, ALICE Z.C. LOBO, MARTIN C. MIHM JR.

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Cutaneous infections are very common and can be caused by a wide variety of different organisms. In addition, it is not uncommon to find involvement of more than one infectious agent affecting the skin, particularly in immunocompromised individuals. Following the onset of both the widespread practice of allogeneic bone marrow and solid organ transplantation, as well as the human immunodeficiency virus (HIV) epidemic, more and more patients are becoming profoundly immunocompromised and thus afflicted by many novel and long-forgotten resurgent infection agents. In addition, the nefarious combination of environmental degradation of native forests with proliferation of disease vectors together with a sharp increase of global travel have produced rapid dissemination of infectious agents, particularly viral. These phenomena often pose major challenges both in the diagnostic and therapeutic aspects of infectious disease management.

Clearly, a book chapter on cutaneous infectious diseases cannot be simply a descriptive iteration of a disease, its clinical features, and pathogenic agents but must also illuminate the significance of the infectious agents in the pathogenic hierarchy of the host organism, as well as the host's response to assault by microbes.

Diagnostic Approach to Cutaneous Infections

The approach to the patient with a suspected infection, no matter how trivial it may seem, must be a multifaceted one and requires a complete history (including travel history) and physical examination. The possibility of an underlying condition and/or medication that may exacerbate or lead to increased susceptibility to infections must be investigated; one example being a patient with undiscovered diabetes presenting with persistent folliculitis. Much less obvious are individuals taking one of the newer antipsoriasis biologic medications that suppress cellular immunity and predispose to infections by intracellular and fungal organisms. After this background work has been performed, one can begin to make a clinical diagnosis and go on to work up the infection.[1]

After an appropriate inquiry and examination has taken place and an infection is established, the next step is organism identification. Histopathologically, pathogens can by identified by their morphologic features on routine hematoxylin and eosin (H&E) stain, and one can always resort to a variety of histochemical and immunohistochemical stains that can help to highlight the organisms. Among the histochemical stains most

heavily used in the diagnosis of infectious agents of the skin are the modified Brown-Hopps tissue Gram stain for bacteria, the periodic acid–Schiff (PAS) as well as the silver impregnation-based stains (Grocott-Gomori methenamine silver [GMS]) for fungi, and finally the Ziehl-Nielsen and Fite preparations, used for mycobacteria. As far as the immunohistochemical stains are concerned, the majority of the available antibodies are directed towards viral agents, such as the ones in the herpesvirus group. However, antibodies have been developed for other infectious agents, such as the ones in the *Leishmania* spp. group.

It is important to remember that when one evaluates the skin for infectious agents, the resident normal flora of the skin should be taken into account. For example, in the pilosebaceous unit, one can find not only *Demodex folliculorum* mites but also yeast forms of *Pityrosporum ovale*, gram-positive cocci and coccobacilli (*Staphylococcus epidermidis* and *Micrococcus* spp.), and corynebacterial organisms (*Corynebacterium* spp.). In contrast, the presence of gram-positive cocci outside the follicles in the dermis or fat is always abnormal and can be evidence of a very serious infection, such as cellulitis or even fasciitis.

When evaluating for bacterial infections, one should remember that these could be secondary to another underlying infection or to a noninfectious condition of the skin. As the clinician must also think of underlying causes or other infections, so must the pathologist be aware of how widespread secondary bacterial infections are. For example, a Majocchi granuloma, caused principally by dermatophyte fungi, can become secondarily infected and be mistaken for a bacterial folliculitis. In the scalp, persons with seborrheic dermatitis can develop secondary bacterial infections that render the dermatitis more difficult to treat.

Although histopathology by H&E and histochemical and immunohistochemical stains can be very helpful, the most reliable way to make a definitive diagnosis of a skin infection is by culture. However, culture, by itself, is not always reliable. As stated by Rapini, less than 10% of cultures are positive in infectious cellulitis.[1] Swab cultures should be used only for the most superficial types of bacterial infections, such as impetigo or ecthyma. They are useless in deep infections, such as cellulitis or panniculitis. In these instances the best approach is to culture the tissue directly. Optimally, a biopsy should be taken, with at least half of the specimen saved for routine processing and diagnostic microscopy. The other half should be transported immediately to the laboratory, where it will be appropriately dissected into small pieces and placed on the appropriate culture medium. Mishandling and subsequent desiccation of the tissue ensures a negative culture. If there is a chance of delay in getting tissue to the laboratory, the tissue may be transported on sterile, normal saline–soaked gauze. Touch preparations may be used in special cases, such as in chancroid or granuloma inguinale, entities in which organisms are very infrequently found in tissue-stained samples. In addition, touch preparations are extremely useful in infections with acid-fast organisms. In expert hands, touch preparations of traumatized ear lobe from patients with lepromatous or borderline lepromatous leprosy can yield a very good estimate of the number of mycobacterial organisms. The Fite stain is particularly useful in identifying the Hansen bacillus in tissue sections. When liquid specimens are available, auramine-rhodamine stain preparations under fluorescence microscopy are also useful for mycobacterial identification due to its sensitivity, with the caveat of a lack of specificity. Finally, more recent methods using molecular biologic techniques, such as evaluation of tissue by polymerase chain reaction (PCR)-based assays, 16S ribosomal RNA (rRNA) sequencing, and in situ hybridization (ISH), have proved to be extremely useful, particularly if speciation is needed. Their use is not yet widespread but can be obtained in many instances by contacting the US Centers for Disease Control and Prevention (CDC). Many of the academic centers in the United States are also developing at least the immunohistochemical techniques for identifying organisms.[1a,1b]

In this chapter we will attempt to address the main infectious conditions affecting the skin, starting with the most commonly encountered ones, bacterial, viral, and fungal infections, and then moving on to the more unusual entities caused by complex organisms, such as protozoa, helminths, arthropods, and algae.

Bacterial Infections of the Skin

Reaction Patterns

The overwhelming pattern seen in cutaneous bacterial infections is that of an exuberant neutrophilic infiltrate, often accompanied by debris and a variable amount of microorganisms (Fig. 20.1). According to the location of the neutrophilic collection in relationship to the skin's microanatomy, the lesions are given different names. If the neutrophils are predominantly subcorneal, they are designated as subcorneal abscesses, the most common cause of which is impetigo. When they coalesce in abscesses that replace the epidermis, the lesion is called ecthyma. Dermal neutrophilic infiltrates and abscess are called cellulitis; in the panniculus, a suppurative reaction or panniculitis; and in the fascia, neutrophilic, suppurative, or necrotizing fasciitis, depending on the extent and the clinical presentation.

As neutrophilic infections become more chronic, they exhibit plasma cells. Very rarely do infections, except for syphilis and other treponemal infections, cause a predominantly plasma cell infiltrate. Infiltrates may be superficial and deep, involving vessels with prominent endothelial swelling and showing perineural inflammation. Only a few bacteria are associated with lymphocytes, the principal one being the agent of Lyme disease. There are admixed plasma cells with this infiltrate, which is also superficial and deep and can show involvement of nerves. In addition, one must always remember that 40% of early secondary lues is associated with lymphocytes.

Some bacteria induce the formation of granulomas, *Mycobacterium tuberculosis* being an excellent example. However, *M. tuberculosis* infection begins as a neutrophilic cellulitis that goes on gradually to be infiltrated by lymphocytes and finally by monocyte-macrophages that form a granuloma, resulting from a type IV hypersensitivity response.

The following sections will be devoted to describing cutaneous infections produced by specific group of bacteria. We will start with the diseases produced by gram-positive organisms, followed by the ones caused by gram-negative, mycobacteria, treponemal, and the bartonelloses. We will then finalize the bacterial infection segment with the intracellular rickettsial and chlamydial organisms as a transition to the next section, the viral infections.

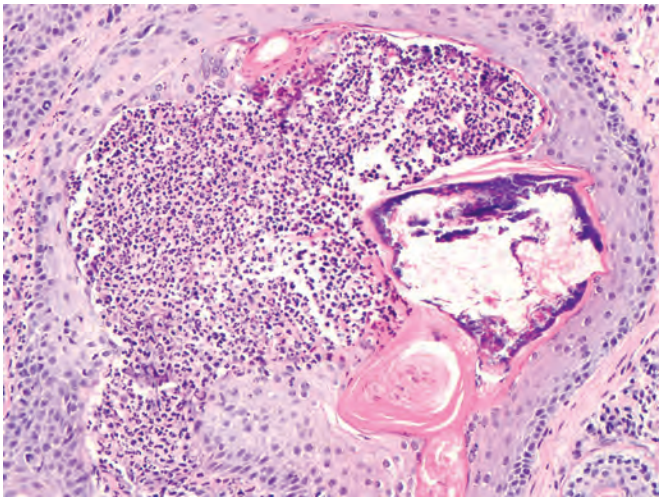
Infections Caused by Gram-Positive Bacteria

Impetigo

Clinical Features. Impetigo affects mainly children, commonly around the mouth, nose, and groin, often after trauma. The lesions occur acutely. They are most commonly crusted and have been described as exhibiting a honey-colored crust.[2] There is

a bullous form in which lesions 1 to 2 cm in size appear, especially below the nose and in the groin and upper thighs.[3] These lesions are not pustular but are clearly bullous. Impetigo frequently occurs in a setting of other dermatoses. For example, eczematous dermatitis commonly becomes infected, especially when weeping begins. The previously mentioned honey-colored crusts are the hallmark of this complication.

Histopathology. The acute lesion consists of a subcorneal blister in which there are varying numbers of neutrophils (see Fig. 20.1). Independently of the presence or absence of a bulla, lesions of impetigo tend to harbor a variable amount of acantholytic cells. The diagnosis of pemphigus foliaceus must be excluded in cases in which these acantholytic cells are particularly abundant in the superficial layers of the epidermis (Fig. 20.2A). A tissue Gram stain can demonstrate the presence of organisms, confirming the diagnosis of impetigo (see Fig. 20.2B). However, if doubt persists, immunofluorescence studies may be necessary to rule out pemphigus foliaceus. If there has been a preceding eczematous response, the eczematized tissue will reveal a prominent scale crust. Spongiform pustules are very infrequent in lesions of impetigo lesions. Rather, the neutrophilic infiltrate is disposed in a scattered fashion below the crusted area. This feature helps to distinguish it from pustular psoriasis.

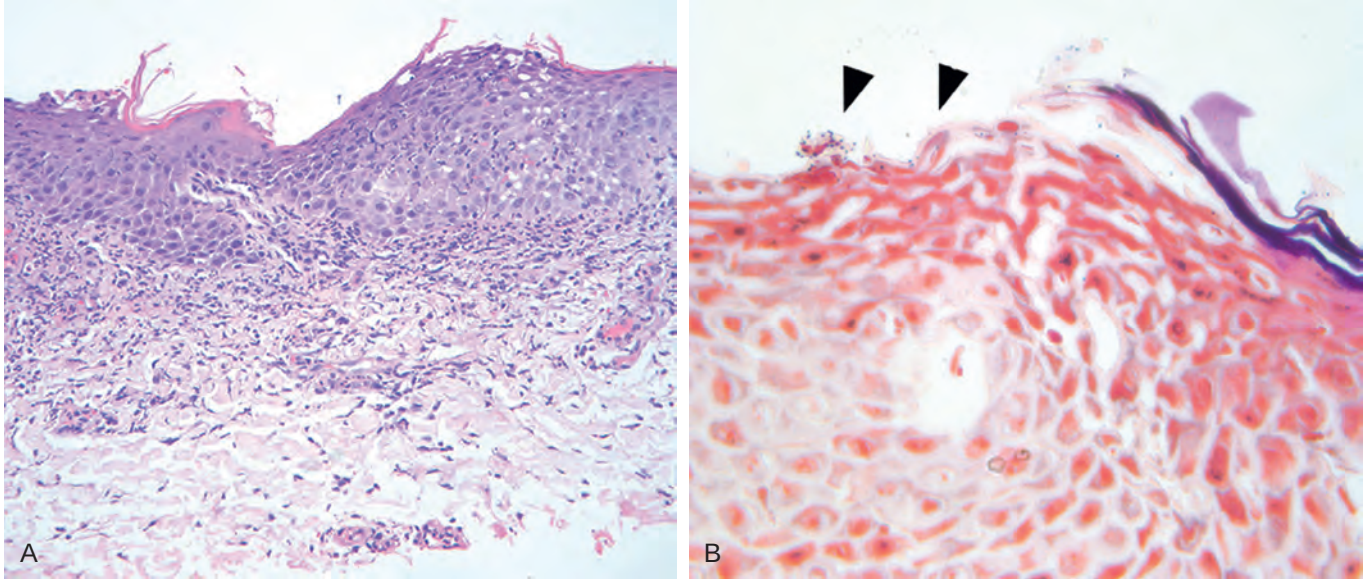


• **Figure 20.1** Bacterial folliculitis. There is a marked infiltration of neutrophils in response to proliferation of gram-positive bacteria in the follicular infundibulum.

Etiology and Pathogenesis. The etiology of impetigo includes, in many instances, prior trauma. Poor hygiene in children, hot and humid weather, and occlusive clothing with sweating all appear to contribute to the process. In adults, it can very often be seen arising associated with a disruption of the epidermis due to nonmelanoma skin cancers (NMSCs), such as squamous and basal cell carcinomas. The organisms most commonly involved are *Staphylococcus aureus* and *Streptococcus pyogenes*. Either of these agents can cause the lesion,[4] but the overwhelming majority of the bullous form of impetigo is related with *S. aureus*, particularly with the strains belonging to the group II and phage type 71.[5] Strains that have been proven to elaborate the A and B types of exfoliative toxins often result in lesions with prominent acantholysis and subcorneal bulla formation. These toxins act on desmoglein 1, which is the target of antibodies in pemphigus foliaceus, rather than desmoglein 3.[5,6] The streptococcal-induced forms of impetigo have been associated with glomerulonephritis in rare instances, approximately 5%, particularly the serotypes 1, 4, 12, 25, and 49.[7] For this reason, many clinicians treat impetigo with oral antibiotics in addition to topical care.

Staphylococcal Scalded Skin Syndrome

Clinical Features. The staphylococcal scalded skin syndrome (SSSS), also known as Ritter disease (not to be confused with Ritter syndrome), is a potentially serious complication of staphylococcal infections leading to generalized blistering and desquamation accompanied by systemic symptoms. Small children, mainly younger than 5 years of age and even neonates, are among the most often afflicted by SSSS. This disorder usually begins with the appearance of bright red skin around the head and neck, which most commonly goes on to blister.[8] The bright erythema



• **Figure 20.2** Impetigo. There is superficial acantholysis along with a prominent neutrophilic infiltrate with exocytosis **(A)**. A tissue Gram (Brown-Hopps) stain reveals gram-positive cocci *(arrowheads)* **(B)**.

of the head and neck gradually spreads to the trunk, axillae, groin, and then extremities. The source of infection is usually occult; conjunctivitis, groin or nostril infection, or even gastrointestinal or urinary tract infection may be the origin. Patients develop constitutional symptoms as the lesions progress. Mortality in children is usually low, except in neonates. In adults, mortality can be as high as 60%, and the disorder is often associated with kidney failure or other debilitating illness.[9] Adults with SSSS present clinically with desquamation of the skin, not blistering.[9] SSSS differs from a disease called staphylococcal scarlet fever, in which there is severe desquamation without blistering. The latter disorder also has circulating enterotoxins and may represent a form of toxic shock syndrome (TSS), discussed later.[10] The major differential diagnostic consideration is toxic epidermal necrolysis (TEN). In this disorder, there is sloughing of the skin due to full-thickness necrosis of the epidermis involving the entire skin and the oral, conjunctival, and genital mucosal surfaces.

Histopathology. The pathologic changes of SSSS are minimal but distinctive. The cleft is seen between the granular layer and the stratum corneum; at the floor of the blister, there is a very regular, smooth surface of keratinocytes that is associated with variable acantholysis. There is no hint of dyskeratosis to suggest pemphigus foliaceus. Because toxins secreted at a distant site mediate SSSS, inflammation is scant to nonexistent and bacterial forms are not seen, in contrast to bullous impetigo, in which the same toxins are secreted and act locally ("localized form of SSSS"). As mentioned before, TEN is the major differential diagnosis. Rapid diagnosis using frozen section is usually helpful to distinguish SSSS from TEN. One need only take a Q-tip and roll up the sloughed skin. In SSSS, one sees only stratum corneum with a rare keratinocyte, as opposed to the full-thickness necrotic skin observed in TEN. The presence of lymphoid inflammation and a subepidermal bulla favor TEN.

Etiology and Pathogenesis. This disorder is caused by the production of the epidermolytic exfoliative toxins A or B and is frequently and mainly associated with a specific strain of *S. aureus*, namely the phage type II group, especially type 71.[11] As mentioned previously in the bullous impetigo section, these toxins target desmoglein 1, the same antigen targeted by autoantibodies in bullous impetigo and in pemphigus foliaceus.[6] Because the kidneys in children do not appropriately excrete SSSS toxins until they mature, the damage done by them is greatly potentiated. In adults, there must be at least some impairment of kidney function or other debilitating disease that impedes release of the toxin.

Toxic Shock Syndrome

Clinical Features. TSS is clinically characterized by prominent high fever, systemic symptoms, and a very diffuse eruption composed of brightly erythematous patches resembling sunburn. This generalized erythrodermic rash subsequently exfoliates often with marked desquamation of the palms and soles. The patient also experiences nausea with vomiting and diarrhea, headache, and possibly vertigo. Marked hypotension is usually present as another indication of multiple system involvement. Toxins secreted by *S. aureus* are implicated in the overwhelming majority of TSS cases. However, toxins derived from *S. pyogenes* may cause a small fraction of TSS cases, which are denominated streptococcal TSS (STSS) or toxic shock–like syndrome (TSLS). The CDC has defined a set of clinical criteria for staphylococcal TSS that include the following: (1) body temperature greater than 38.9°C (102.02 F), (2) hypotension (systolic blood pressure <90 mm Hg), (3) diffuse macular erythroderma, (4) desquamation of skin (including palms and soles) 1 to 2 weeks after onset, and (5) involvement of three or more organ systems. In addition, a laboratory criterion (6) represented by a lack of positive cultures for other bacteria/organisms other than *S. aureus* is required. A confirmed case will have all six criteria (including the laboratory criterion), whereas a probable case will show the laboratory criterion together with the five or four of the clinical criteria.[12]

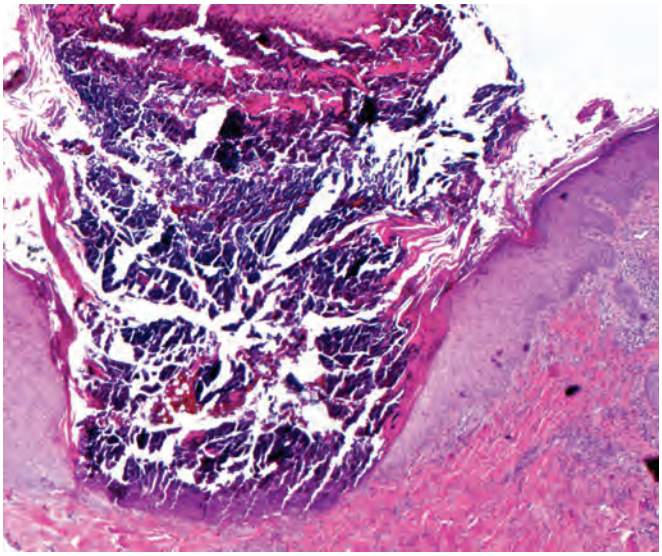
Histopathology. Microscopic skin changes found in TSS vary and include, at first, a mild, spongiotic reaction, with intraepidermal lymphocytes and neutrophils overlying dermal edema, and a perivenular lymphoid infiltrate with scattered neutrophils and red blood cells. The presence of a few aggregates of neutrophils in the epidermis, with dyskeratotic cells, may indicate TSS as the correct diagnosis. In the most florid reaction, there is a pustular vasculitis, with a neutrophilic abscess in the subcorneal space, in the epidermis, beneath the epidermis, or in the follicular orifice of the sweat glands. In addition, in the dermis, either necrotizing vasculitis with focal fibrinoid necrosis of vessels or a Sweet-like reaction may take place. The latter exhibits endothelial cell swelling and a perivascular lymphocytic infiltrate admixed with occasional neutrophils and eosinophils. There is no evidence of fibrinoid necrosis, but there is definite extravasation of red blood cells.[13] These findings are not entirely specific for TSS because pustular vasculitis may be seen in other septic conditions, including deep fungal infections, connective tissue diseases, and Behçet disease. Drug eruptions can also manifest with similar histologic changes.

This syndrome was first described in association with superabsorbent tampon use and was related to the causative organisms growing in the vagina in the medium created by the tampon and proteinaceous menstruum.[14,15] Fortunately, after discontinuation of these types of tampons by the manufacturers, a sharp decrease in tampon-related TSS cases was observed. However, to date, a significant number of TSS cases have been associated with other infections, including abscesses, wound infections, and other systemic infections.[16]

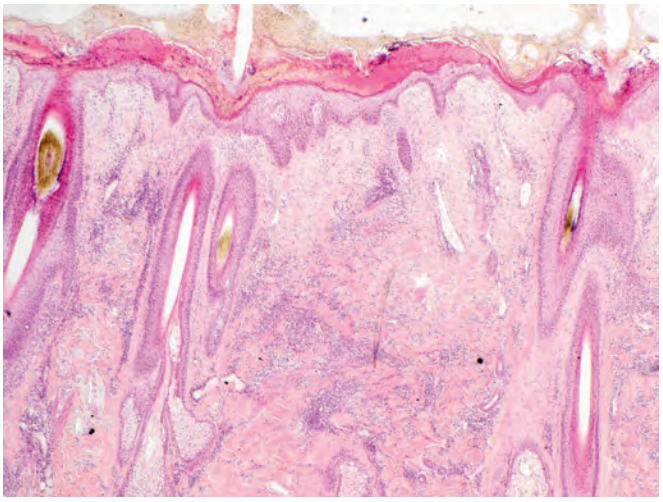
Etiology and Pathogenesis. The staphylococcal-derived exotoxins that can cause TSS are definitely different from the one that causes SSSS and are represented by type B enterotoxin and toxic shock syndrome toxin 1 (TSST-1). In cases of STSS/TSLS the toxins secreted by *S. pyogenes* organisms are the phage-encoded Streptococcal pyrogenic exotoxins SpeA and SpeC. These toxins are clear examples of the so-called superantigens. These protein-based antigens strongly interact with T lymphocytes expressing the variable portion of the β chain (Vβ) in their T-cell receptor (TCR) molecules.[17,18] This persistent stimulation results in a marked expansion of this particular subset of T cells that trigger a massive cytokine release, mainly from macrophages. Particularly noteworthy is the abundant secretion of interleukin-1 (IL-1) and IL-2 and tumor necrosis factor (TNF), two well-known mediators of shock.[18] This uncontrolled "cytokine storm" results in TSS and also explains why the therapeutic approach using intravenous immunoglobulin G (IgG) therapy is quite successful in some cases.[19]

Ecthyma

Clinical Features. Ecthyma is a localized bacterial cutaneous infection that, like impetigo, favors children and can similarly affect the occlusive areas of the body. It often appears as a well-demarcated crusted ulcer filled with purulent material in the lower limbs. Ecthyma resembles any type of inflamed, infected skin lesion with crusting. Culture is the best manner of diagnosing the exact cause of the lesion.



• **Figure 20.3** Ecthyma. This sharply demarcated ulcer, characteristic of ecthyma, contains purulent material and bacterial colonies.



• **Figure 20.4** Erysipelas. Note the diffuse edema of the papillary and reticular dermis with extensive diffuse infiltration by neutrophils.

Histopathology. The lesion usually has a sharply demarcated border in which aggregates of neutrophils are present in the center of the lesion and diffusely throughout the superficial dermis. There is frequently a neutrophil-impregnated crust (Fig. 20.3). Bacteria can often be visualized using tissue Gram stain.

Etiology and Pathogenesis. Ecthyma has features similar to those of impetigo as far as the organisms and pathogenesis are concerned. However, one of the causative organisms is usually *S. pyogenes*. Often, culture reveals mixed staphylococcal and streptococcal infections. It is important to mention that the term "ecthyma," from the Greek εκθύω "break out," which literally means "break out in a rush" and in this case will be of purulent material, is used in several other types of lesions produced from a variety of organisms, such as parapoxvirus in ecthyma contagiosum (also known as orf). Among bacterial-related ecthymas, there is significant overlap between ecthyma and ecthyma gangrenosum (discussed previously), both likely representing a cutaneous infection reaction pattern. Some authors propose unifying both concepts under the "ecthyma gangrenosum" rubric.[20] Nevertheless, the lesions referred to as ecthyma gangrenosum often reflect a more serious condition (see later).

Ecthyma Gangrenosum

Clinical Features. This lesion begins with a red macule or papule and progresses rapidly to a pustule or bulla in a very sick, often immunosuppressed patient. It then progresses to become a bulla and/or directly ulcerates, with formation of a black eschar. In some patients, multiple lesions develop. These patients usually have an underlying mild coagulopathy that sets the stage for the thrombotic septic changes that occur. The lesional history is characteristic, with lesions appearing in 12 hours or less. The eschar or crust has been classically referred to as "sphacelus."[21] The history and physical features are classic and are considered to portend a serious prognosis unless treated adequately and promptly.

Histopathology. The histologic changes are quite characteristic in the classic case. They include an ulcerated, necrotic epidermis with a fibrin-laden base filled with debris overlying a pauci-inflammatory vasculopathy or vasculitis with minimal perivascular neutrophils and sometimes lymphocytes. In addition, one may see clusters of gram-negative rods in the vessel wall and surrounding the vessels. The principal differential is indicated by the presence of a fungal organism, such as *Zygomycetes* spp., that can also cause necrosis and vasculitis. Appropriate stains aid in diagnosis.

Etiology and Pathogenesis. The lesion usually appears in severely debilitated or immunosuppressed patients who are septic. Although the offending organism in the classic case is *Pseudomonas aeruginosa*,[22] various other organisms have been implicated.[20]

Erysipelas

Clinical Features. The infection known as erysipelas (from the Greek ἐρυσίπελας "red skin") was already recognized as a clinical entity back in the times of the ancient Greeks. Characteristically, erysipelas often affects the face and manifests rapidly as an area of swelling that is hot and bright red and shows well-demarcated advancing borders.[23,24] Lymphangitis and lymphadenitis can often be found, and in some lesions the swelling is so marked that blisters or bullae appear. It is occasionally associated with marked systemic symptoms of malaise, fever, vomiting, and high fevers. Cultures are only rarely positive.[24] Detection of elevated antistreptolysin O or anti-DNAase B in the patient's serum is useful; however, the clinical symptomatology is so striking and characteristic. Prompt treatment is necessary because a small percentage of patients develop bacteremia. Erysipelas can be recurrent and associated with diabetes. Other diseases, such as a severe contact dermatitis, photoallergy, or even lupus erythematosus, may resemble this infection, but the lesions of erysipelas have a more specific distribution pattern; in contrast to contact dermatitis or photoallergy, patients usually lack severe fever and constitutional symptoms.

Histopathology. This lesion is a classic example of severe cellulitis. There is marked edema in all compartments of the skin (Fig. 20.4). At the least, there is epidermal intercellular edema, but, in more severe cases, subepidermal bullae can appear. The papillary and reticular dermal fibers are markedly separated and, due to fibrin exudation, may have a more bluish, less refractory character. Lymphatics are dilated. There is also edema of the subcutis, with small zones of fat necrosis caused by a relative ischemia. Scattered throughout the edematous areas are neutrophils and neutrophilic debris. There is no particular perivascular localization. Bacteria are difficult to observe in this lesion. In other

types of cellulitis, bacteria may be easily demonstrable with Gram staining, especially in the presence of immunosuppression.

Etiology and Pathogenesis. The classic implicated organism is *S. pyogenes* (group A streptococcus [GAS]). Less frequently, other streptococci and even *S. aureus* can develop a similar lesion. Several toxins produced by the streptococci, possibly including hyaluronidase, streptolysin, and other exotoxins, are likely implicated in the production of this lesion.

Erysipeloid

Clinical Features. Erysipeloid, also known as Rosenbach disease, was so named because it clinically resembles erysipelas, with prominent edema and a well-demarcated border. However, it is less severe and affects mainly the fingers by inoculation via finger microtrauma. Erysipeloid is an occupational disease and primarily affects fishermen and persons who handle fish or other animal tissues, such as raw meat.[25] Two main clinical presentations related are described: a cutaneous infection, which can be localized or generalized, and a septicemic one. The localized cutaneous infection (erysipeloid) is the overwhelmingly more common one seen in humans.[25,26] The first manifestation of erysipeloid is usually a bluish or erythematous macule at the site of inoculation. As the lesion gradually and slowly increases in size, it develops well-defined and raised borders and there is a tendency to develop a central area of clearing. The affected area, usually the fingers, tends to develop notorious edema ("bubble finger"). In contrast to erysipelas, constitutional symptoms are seldom encountered in the localized cutaneous form. However, in the extremely rare generalized cutaneous form constitutional symptoms are the rule.[25,26] Other types of cellulitis may resemble this infection on the hands, especially those caused by staphylococcal and streptococcal organisms. Brucellosis also affects the hands of persons who are in contact with animal tissues, but it is clinically different, with multiple papules and pustules. Anthrax and tularemia both ulcerate. Because other organisms can cause hand infections, culture of the tissue is imperative.

Histopathology. The changes of the erysipeloid lesion include marked papillary dermal edema with scattered neutrophils infiltrating between the collagen bundles. In this disorder, lymphocytes and plasma cells can be frequently observed. Occasional eosinophils are visualized. Lymphatics are dilated. In most cases, there is no vasculitis and no perivascular prominence of the infiltrate.[26,27] While the overall changes are much less severe than in erysipelas, lesions exhibiting marker changes, including epidermal necrosis and involvement of the subcutaneous adipose tissue can be seen in immunocompromised patients.[26] The organisms are very difficult, if not impossible, to recognize in tissue with Gram stain.[27]

Etiology and Pathogenesis. The causative organism *Erysipelothrix rhusiopathiae* can be found as a commensal or pathogenic organism in a variety of animals but principally in swine and fish.[25] Cultures of *E. rhusiopathiae* can resemble other gram-positive infections, so it is important to communicate the suspected diagnosis to the laboratory personnel. Although culture can lead to identification of the organism, PCR assays have been successfully used to identify this pathogen.[28]

Cellulitis, Including Perianal Streptococcal Dermatitis

Clinical Features. Cellulitis is a general term that refers to an inflammation of the skin that manifests as a red, brightly erythematous macule or plaque or as an indurated nodule or plaque that is warm and usually tender. Thus it qualifies for the ancient definition of infection: *tumor*, *dolor*, *calor*, and *rubor*. As the lesions become more chronic, scaling, bullae, and even ulceration may appear. Lymphangitis and even lymphadenopathy are sometimes present. The most common causative organisms in the immunocompetent patient are *S. aureus* and GAS. In the immunosuppressed patient, there are many other possible causative organisms. A distinctive type of perianal cellulitis that exhibits the characteristic features of cellulitis is termed perianal streptococcal "dermatitis" or "cellulitis."[29]

Histopathology. The previous description of cellulitis summarizes the findings. There is diffuse dermal and subcuticular edema with a diffuse infiltrate of neutrophils. Depending on the degree of edema, there can be vascular ectasia with extravasation of red blood cells. Dilated lymphatics are also observed and are a clue to the nature of the process. Organisms are almost impossible to observe, even with appropriate stains. In the more chronic lesions, the epidermis may show spongiosis with intraepidermal vesicles and even ulceration and necrosis. Likewise, intraepidermal pustules may be noted. In extensive disease, neutrophil-rich areas may give the appearance of vasculitis; such vascular involvement is considered secondary to the cellulitis. In very chronic lesions, fibrosis may also be noted in reaction to the zones of necrosis. Culture of tissue is necessary to identify the organisms. In cases of marked cellulitis with neutrophils, the question of neutrophilic dermatoses is raised. The latter lesions differ by their clinical presentation, often by the patterns of neutrophilic infiltration, and by negativity of tissue cultures.

Etiology and Pathogenesis. As indicated earlier, the most common causes of cellulitis are *S. aureus* and GAS. However, in the immunosuppressed patient, one may find acid-fast organisms, fungi, and a variety of bacteria, including *Klebsiella* spp., *Proteus* spp., *Clostridium* spp., as well as fungi, such as cryptococcal organisms and other yeasts, and even amebas. The pathologist must be aware of the possibility of finding these many diverse organisms. Immunosuppressed patients may have multiple infections, presenting a challenge to pathologist and clinician. One type of cellulitis that should be emphasized is the facial or periorbital cellulitis that is caused by encapsulated forms of *Haemophilus influenzae* type B. This infection occurs in the first years of life.[30]

Necrotizing Fasciitis

Clinical Features. Necrotizing fasciitis is an often extremely destructive infection that occurs acutely in the fascia and the soft tissues, with a very rapid course, resulting in extensive necrosis of the tissue. It usually occurs in a background of peripheral vascular disease, diabetes, chronic alcoholism, or drug abuse[31] but can occur spontaneously in otherwise healthy young patients. There is often a history of injury.

Three types of necrotizing fasciitis have been described. Type I is a mixed infection.[31,32] Type II is caused by either *S. aureus* or GAS.[31-33] Type III is the most destructive type and often occurs after an injury or puncture wound. Its cause is the *Vibrio* group of organisms, such as *Vibrio vulnificans*.[34] Because these infections are all so virulent, intense antibiotic therapy and radical surgical débridement are often necessary to save the life of the patient.[35] The lesions manifest clinically as rapidly progressive, painful areas of marked induration and erythema. Blisters and ulceration develop quickly with the extensive necrosis. The mortality rate is approximately 50%.

Histopathology. In contrast to cellulitis, this process involves the dermis and subcutis, but the areas exhibiting the most severe changes are the fascia and deep soft tissues, where extensive

hemorrhage, necrosis, thrombosis, and secondary vasculitis are observed. Evaluation of the extent of disease using frozen section analysis has been found to be useful by some groups.[36]

Etiology and Pathogenesis. As stated previously, numerous organisms in addition to *S. aureus* and GAS can cause necrotizing fasciitis. These include *Proteus* spp. and, very importantly, anaerobes such as the clostridial organisms, *Bacteroides* spp., *Peptostreptococcus* spp., and the marine group of *Vibrio* spp.[32,34]

Pyoderma Vegetans (Blastomycosis-like Pyoderma)

Clinical Features. Pyoderma vegetans is basically a reaction pattern to a variety of bacterial organisms that mimics fungal infection presenting in immunocompromised individuals. However, it can occasionally present in immunocompetent persons.[37,38] This disorder manifests clinically as one or more verrucous plaques that are studded with pustules and exhibit sinus tracts. The most common sites of involvement are the axillae and groin and the lower extremities. There is a facial variant that can be markedly disfiguring. To make a definitive diagnosis of pyoderma vegetans, one should rule out a fungal infection (negative culture and serology) and exposure to bromide or iodide and have a positive culture for one of the known bacteria causing the disorder.[37]

Histopathology. Marked pseudoepitheliomatous hyperplasia associated with hyperkeratosis and parakeratosis and multiple intraepidermal abscesses characterize this disorder. There are also dermal abscesses. A perivenular infiltrate of lymphocytes, histiocytes, neutrophils, and occasional eosinophils is present. Scattered granulomas may also be observed.

There are two important differential diagnoses: deep fungal infection and pemphigus vegetans. A deep fungal infection will usually demonstrate readily identifiable fungal structures, either by H&E and/or by fungal histochemical stains. Pemphigus vegetans exhibits histologically unequivocal acantholysis and suprabasilar clefting, along with intraepidermal and papillary dermal collections of eosinophils.

Etiology and Pathogenesis. The most common infectious cause of this disorder is considered to be *S. aureus*, although a few other causative bacteria have been described.[39,40] It also occurs as a variant of pyoderma gangrenosum associated with inflammatory bowel disease.[41]

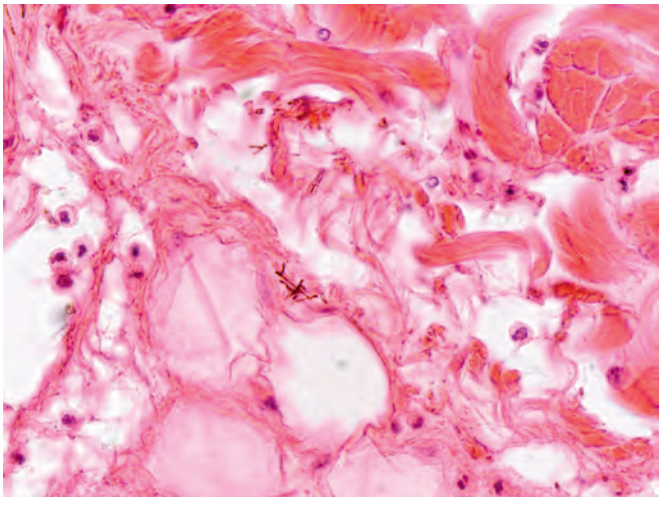
Anthrax

The causative organism of anthrax is the gram-positive, spore-forming rod *Bacillus anthracis.* The incidence of anthrax is low in the United States, but in some parts of the world the disease is much more common. Although infections by this agent are more prevalent in animals and human infection has been long considered an occupational disease, in the first decade of the 21st century this agent has been the focus of interest due to its status as a category A bioterrorism weapon and prior use as such in the early 2000s.[42] There are three essential forms of infection: cutaneous, inhalational, and gastrointestinal, with the cutaneous form being the most common and less severe variant. The inhalational form leads to the most serious form of infection and is the one exploited by bioterrorism. The gastrointestinal form can occur after ingestion of contaminated meat. Following the sharp increase in intravenous drug abusers (IVDAs) that occurred from 1999 to 2011, a fourth form of infection, injectional, has been described related to contaminated heroin.[43]

Clinical Features. Because the spread is carried out by direct contact of the spores with skin abrasions, the exposed skin is characteristically involved. In adults the lesion is more common on the hands due to direct contact with spores carried by infected animals, such as rabbits, goats, and sheep. In children the head and neck area is the most frequently involved site, likely from environmental contamination of prior wounds. The initial lesion arises after an incubation period of 1 to 10 days as a red macule that rapidly goes through a papular, vesicular, and ulcerative phase with the formation of a black adherent eschar, also known as a sphacelus, usually around day 5 or 7. Although the initial lesion may be pruritic and closely resemble an arthropod or spider bite, the well-formed ulcer is often painless, a distinct feature.[1,42] As the lesion evolves, there can be severe, striking edema with blisters. Tender regional lymphadenopathy can be found ("ulceroglandular form"), sometimes in association with systemic symptoms, such as fever and chills. Lesions heal in 1 to 2 weeks in most cases, and the mortality rate of the cutaneous form with antibiotic treatment is less than 1%. However, death has been reported in 20% of untreated cutaneous cases.[1]

Inhalational anthrax is characterized by hemorrhagic mediastinal lymphadenitis accompanied by pleural effusions, whereas gastrointestinal anthrax is associated with vomiting, bloody diarrhea, acute abdominal pain, and hemorrhagic ulcers in the ileum and cecum. Both of these forms show much higher mortality than the cutaneous form, particularly if not treated appropriately with antibiotics.[1,44]

Histopathology. Biopsy of an early cutaneous anthrax lesion exhibits marked, severe dermal edema with necrosis and ulceration of the epidermis, hemorrhage, and a diffuse lymphocytic and neutrophilic infiltrate. After an eschar develops, the biopsy will show a dense necrotic zone of tissue beneath which there is striking neutrophilic infiltration that can be associated with vasculitis. In addition to the necrotic debris, one can see marked foci of chronic inflammation.[1,44] The organism is usually easily demonstrated as gram-positive rods that range up to 10 µm in length. They are especially present in the necrotic debris and beneath in the inflamed dermis (Fig. 20.5). Thus, to maximize the yield of obtaining organisms, one should biopsy the center and periphery of the eschar.[44] If there is lymph node affectation, it will be represented by necrotic and hemorrhagic lymphadenitis. A newly



• **Figure 20.5** Cutaneous anthrax. There is a prominent inflammatory infiltrate composed mostly of polymorphonuclear leukocytes. The elongated bacilli are readily identified.

developed immunohistochemical stain can be helpful in identifying the organisms.[44]

The differential diagnosis includes other infectious disorders, such as staphylococcal pyoderma, sporotrichosis, tularemia, and orf. At times, insect bites with secondary infection may resemble this lesion. The differential diagnosis of sphacelus includes the membrane of diphtheria, the eschar of ecthyma gangrenosum, tularemia, the dense crust of diabetic ulcers or ischemic ulcers associated with an arterial vasculitis, and the dark-crusted center of ulcerated lymphoma nodules.

Corynebacterial Infections

Diphtheria

Clinical Features. Caused by *Corynebacterium diphteriae*, diphtheria was once common in the United States before being virtually eradicated by an effective immunization program that is required of almost all children and adults. Because this disease is still prevalent in some parts of the world (particularly Russia and the ex-Soviet satellites nations), the antivaccination movement gaining momentum in recent years (2009–2017) poses a tremendous threat of reemergence.[45] Cases of cutaneous diphtheria occur in older adults and the immunosuppressed and are related to colonization of previously traumatized skin from wounds, surgery, impetigo, pyoderma, or insect bites.[46] These lesions often present in lower limbs and hands and start as a vesicle or pustule filled with straw-colored fluid that gives rise to single or multiple punched-out ulcers (ecthyma diphtericorum), single or multiple with elevated borders. The ulcers are painful initially and then become anesthetized due to bacterial exotoxins. Similar to the lesions encountered in the pharynx and larynx, cutaneous diphtheria shows an adhering eschar (dark pseudomembrane) during the first 1 to 2 weeks.[46] The differential diagnosis includes other lesions with a membrane (e.g., anthrax), some ischemic ulcers, and ulcers caused by vasculitis or nodular lymphomatous infiltrates, as well as the primary lesion of typhus.

Histopathology. The principal histopathologic change noted in cutaneous diphtheria is ulceration covered by a dense fibrin mesh containing foreign material and debris. Neutrophils may be present on the surface of the ulcer. If the diphtheria occurs secondary to another infection or lesion, evidence of the prior dermatosis will also be seen. The diagnosis is histologically confirmed by the identification of gram-positive filamentous rods of *C. diphtheriae.* Similar changes occur in areas of eczema or scabietic nodules that have been superinfected by the bacteria.

Etiology and Pathogenesis. The virulence of *C. diphteriae* responds to the active secretion of the phage-induced, polypeptide-based diphtheria exotoxin. Appropriate cultures can be done only if the laboratory is alerted to the possible diagnosis and uses the appropriate medium (i.e., Löffler medium).

Erythrasma

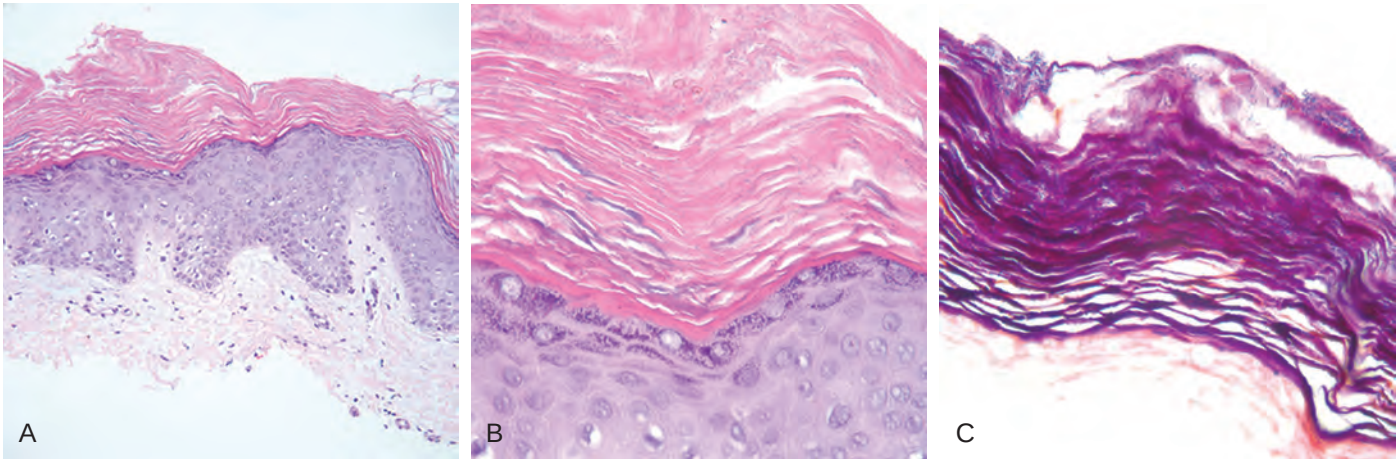
Clinical Features. This disorder is a superficial infection by corynebacteria. These organisms require moist areas of the body, where they infect the stratum corneum. Thus this interesting entity is associated with the presence of characteristic patches of brown, dusky discoloration in the groin, axillae, and inframammary regions.[47] Sometimes they also are found between the toes. There may be scaling. Other disorders that should be considered include superficial fungal infections, yeast infections, and intertrigo. A diagnostic test involving a Wood light examination reveals a characteristic coral-red fluorescence and helps to differentiate erythrasma from these other infections.

Histopathology. A very characteristic picture is seen in which there is virtually no host response but the changes are limited to the stratum corneum ("invisible dermatosis"). One can often see the coccobacillary and filamentous bacterial structures on H&E examination in the midst of an area of parakeratosis with a "grungy" low-power appearance (Fig. 20.6A and B). The bacteria are gram positive (see Fig. 20.6C) and also stain with PAS, GMS, and Giemsa stains.[47,48]

Etiology and Pathogenesis. Erythrasma is associated with a variety of corynebacteria, the most common being *Corynebacterium minutissimum*, with rare cases being caused by *Corynebacterium afermentans*.[49] These organisms are known to produce keratolytic agents that facilitate the colonization of the stratum corneum.[48]

Trichobacteriosis (Trichomycosis)

Clinical Features. The appearance of yellow to white or even black and red nodules on axillary and pubic hairs is the



• **Figure 20.6** Erythrasma. The process is centered in the stratum corneum, which shows "grangy" parakeratosis and hyperkeratosis; note the complete lack of changes in both the epidermis and remaining dermis **(A)**. Upon closer examination, one discerns the presence of cocci and elongated bacilli **(B)**, which are highlighted by a tissue Gram (Brown-Hopps) stain **(C)**.

characteristic finding of this entitiy.[47,50] These concretions can be malodorous and appear to be caused by a combination of substances secreted by the organism (biofilm) and aggregation and drying of apocrine sweat. The diagnosis of this lesion is made clinically, and one can resort to a Wood lamp to show a pale yellowish fluorescence.[47] Other concretions on the hairs include the so-called white and black piedra. Black piedra occurs on the scalp, and it is caused by *Piedraia hortae*. White piedra, caused by *Trichosporum cutaneum*, occurs on the scalp, mustache, and groin hair. These entities will be described elsewhere in this chapter.

Histopathology. Filamentous and coccobacillary corynebacteria are present within the nodules on the hair. They are visible with H&E but are best seen with Giemsa stain. The organisms surround the hair shaft without penetrating it, forming a sheet of organisms.[47]

Etiology and Pathogenesis. Trichobacteriosis (Trichomycosis) is caused by a variety of bacteria with the most commonly implicated organism is *Corynebacterium tenuis*. Production of biofilm by the organisms contributes to the formation of the lesions.

Pitted Keratolysis

Clinical Features. This disorder is most prevalent in young men and adolescents and particularly in individuals who are frequently barefoot. The plantar surfaces appear macerated and exhibit small (1 to 2 mm) pits, which are malodorous. It is often mistaken for a fungal infection and unsuccessfully treated as such for long periods of time.[47] Because of the location on the feet, the maceration, and the odoriferous quality of the lesion, pitted keratolysis can usually be distinguished from pits on the palms and soles caused by Darier disease or possibly basal cell nevus syndrome.

Histopathology. Numerous coccobacillary and filamentous forms are found in the stratum corneum. They can be seen with H&E staining (Fig. 20.7A) but are best observed with Gram (see Fig. 20.7B), PAS, GMS, or Giemsa stain. There is no host response.

Etiology and Pathogenesis. Species of *Corynebacterium* and other organisms, including *Actinomyces* spp., can result in the superficial disorder of the skin known as pitted keratolysis.[47,51,52] Prolonged occlusion of the feet associated with heat and subsequent moisture accumulation is the background necessary for this disorder to occur. The organisms produce an enzyme that is responsible for the formation of the pits by protein digestion.

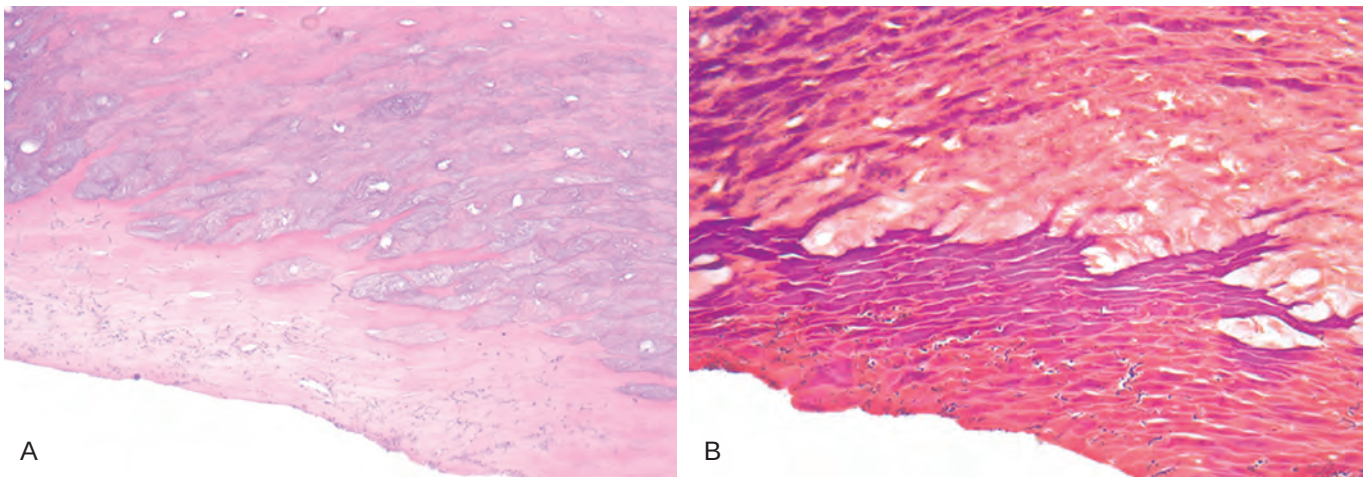
Cutaneous Nocardiosis and Actinomycosis

Nocardiosis

The *Nocardia* group, formerly classified in the Fungi kingdom, are aerobic, branching, gram-positive, weakly acid-fast bacteria that are ubiquitous and can be isolated from soil, water, and organic matter. The two most common species associated with skin involvement are *Nocardia asteroides*[53] and *Nocardia brasiliensis*.[54,55] Other clinically relevant species are *Nocardia farcinica* and *Nocardia nova*.[55]

Clinical Features. Human infection by microorganisms of the *Nocardia* genus is usually related to an immunosuppressed status, such as in patients with HIV/acquired immunodeficiency syndrome (AIDS),[56] transplant recepients,[57,58] or in those receiving immunosuppressive medications, such as corticosteroids or TNF inhibitors (infliximab).[59] The classic route of systemic infection is via the respiratory tract (inhalation), with pulmonary infection and subsequent systemic dissemination, mainly to the central nervous system (CNS) and skin. This mechanism is usually seen in immunocompromised patients.[58-60] On the other hand, immunocompetent individuals acquire cutaneous lesions through traumatic percutaneous inoculation, usually through contaminated thorns,[53,61] with rare cases of infection following esthetic procedures.[62] It is important to point out that secondary hematogenous nocardiosis skin lesions may be indistinguishable to the primary cutaneous form, so it is always important to evaluate all affected patients for pulmonary lesions.

Cutaneous involvement by *Nocardia* may appear clinically either as a lymphocutaneous lesion[63] or showing chancriform[64] or sporotrichoid[65] morphology. In children the lymphocutaneous presentation often presents as a cervicofacial lesion.[66] Nocardial infections can also appear forming a mycetoma,[61,67] as well as other common superficial cutaneous infections, such as abscesses, ulcers, or cellulitis, the latter particularly in patients who are immunocompromised.[56,57] Clear-appearing granules can be expressed from actinomycosis-like lesions. The granules' characteristics differ according to the different *Nocardia* and *Actinomyces* species (Table 20.1).



• **Figure 20.7** Pitted keratolysis. This superficial portion of acral skin shows cocci and elongated bacilli **(A)** that are highlighted with a tissue Gram (Brown-Hopps) stain **(B)**.

Histopathology. Microscopically, lesions can appear as abscesses surrounded by a dense neutrophilic infiltrate, which can extend from the dermis to the subcutis and can exhibit necrosis, hemorrhage, and ulceration.[67,68] A fibrous capsule surrounded by chronic inflammatory lesions is seen in chronic cases.[69] One can also find sulfur granules, which can be indistinguishable from the ones found in actinomycosis (see later discussion). However, the granules contain bacterial forms that are positive for acid-fast staining techniques. These organisms are among the ones capable of producing eosinophilic crystalline structures in a starburst pattern, the so-called Splendore-Hoeppli phenomenon.[70] Other less frequent presentations include pseudoepitheliomatous hyperplasia overlying an extensive mixed neutrophilic/lymphohistiocytic infiltrate (Fig. 20.8A) containing the filamentous organisms and an infective eccrine hidradenitis.[71]

The bacteria cannot be visualized on routine H&E-stained sections. One must rely on Gram stain or acid-fast stains, which reveal fine, branched, filamentous bacteria that break up into coccobacillary forms. The silver methenamine method (GMS) is also useful to highlight the organisms (see Fig. 20.8B). In addition, acid-fast stains are weakly positive, whereas a Fite stain usually shows stronger positivity. If organisms are not visualized morphologically, molecular methods, such as rRNA 16S sequencing[72] or PCR-based assays, or culture can be used to identify the agent.

TABLE 20.1 Morphologic Features of Grains Associated With Microorganisms Causing Mycetomas in Humans

Agent	Color	Diameter (mm)	Consistency
Actinomadura madurae	White	1.5–2	Soft
Actinomadura pelletieri	Red	0.2–0.5	Hard
Nocardia brasiliensis	White or orange	<0.5	Soft
Nocardia asteroides	White	<0.5	Soft
Streptomyces somaliensis	Yellow	0.5–2	Hard
Madurella mycetomatis	Black	1–2	Hard

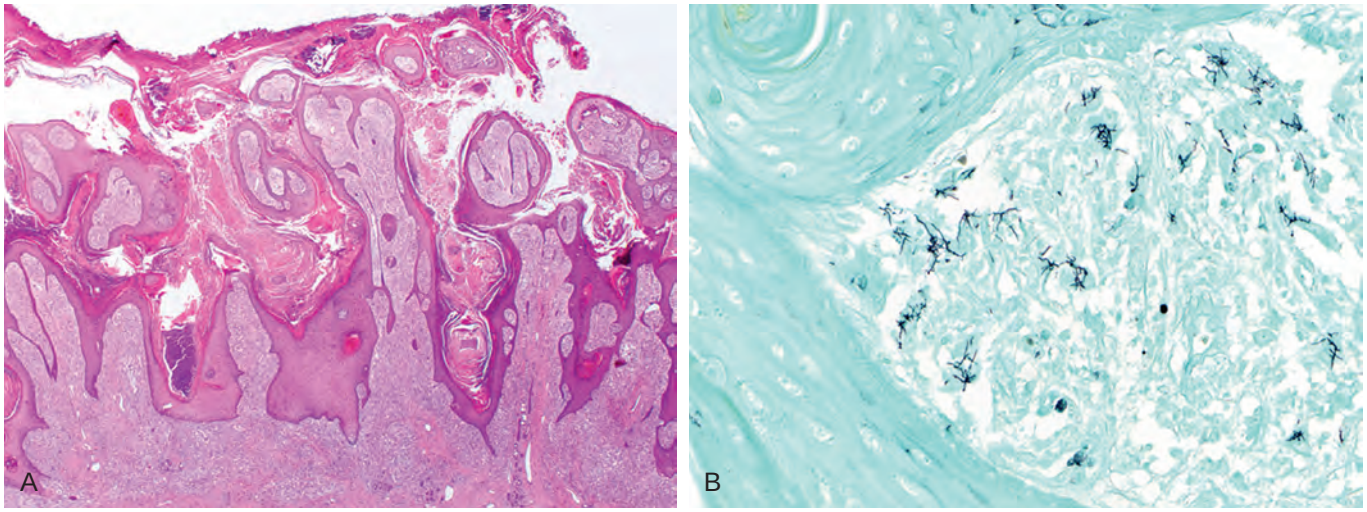
Data from Lupi O, Tyring SK, McGinnis MR. Tropical dermatology: fungal tropical diseases. *J Am Acad Dermatol.* 2005;53:931-951.

Actinomycosis

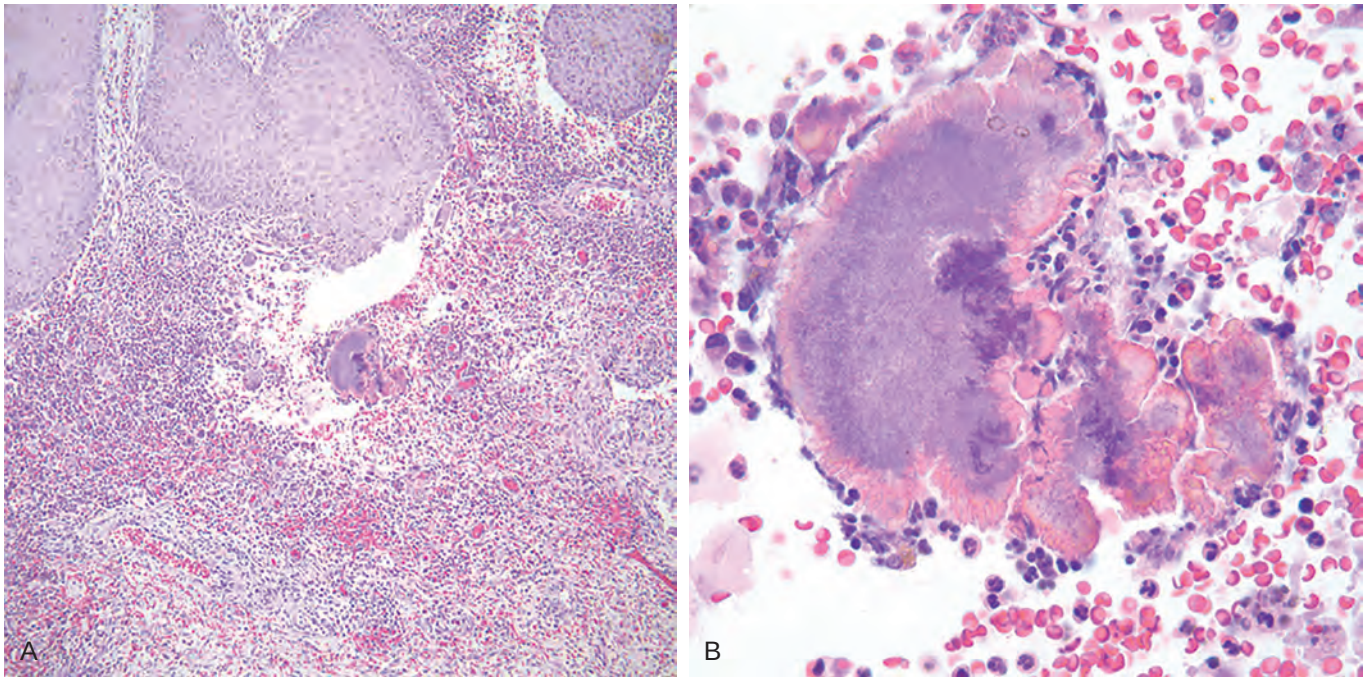
Organisms from the *Actinomyces* genus are anaerobic, nonvirulent organisms that are usually present in the oropharynx, gastrointestinal tract, and female genitalia as part of the resident microbial flora. The four clinically important species are *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus*, and *Actinomyces odontolyticus*.[73] Infection by these organisms requires either a disruption of the natural barriers, from trauma or iatrogenic instrumentation, or an alteration of the normal microbial flora.[73] The classic clinical forms are cervicofacial (55%), abdominopelvic (20%), thoracic (15%), and disseminated (10%). The disseminated form can involve multiple organs, such as the CNS, skin, pericardium, and the extremities.[73] Infection can be found in immunocompromised individuals, such as individuals with HIV/AIDS[74] and chronic granulomatous disease (CGD).[75] However, the risk of actinomycosis infections is also increased in immunocompetent persons who suffer from osteonecrosis,[73] either from bisphosphonates or radiation, as well as in women who use intrauterine devices as a method of contraception.[76]

Clinical Features. Cutaneous actinomycosis is a rare event and can be the product of direct inoculation, sinus tract formation from head and neck lesions, and via hematogenous seeding.[76,77] Clinically the lesions show soft, painless nodules usually with multiple sinus tracts and fistulas with discharge of purulent material and so-called sulfur granules, which are represented macroscopically by firm, yellowish grains.[76-78] See Table 20.1 for the characteristics of the granules produced by several *Actinomyces* species.

Histopathology. The histologic picture is dominated by the presence of abscesses and sinus tracts surrounded by granulation tissue and an outer fibrous capsule with a diffuse, mixed



• **Figure 20.8** Cutaneous nocardiosis. **A,** Prominent epidermal hyperplasia and a diffuse lymphohistiocytic infiltrate with scattered giant cells can be found, especially in immunocompromised individuals. This patient was a renal transplant recipient. **B,** Grocott-Gomori methenamine silver stain highlights the bacteria, which grow by forming branching filaments.



• **Figure 20.9** Actinomycosis. **A,** There is prominent epidermal hyperplasia accompanied by a dermal mixed inflammatory infiltrate and occasional basophilic granules that contain tightly packed filamentous bacteria. **B,** Note the eosinophilic radiating spicules at the periphery of the granule as part of the Splendore-Hoeppli phenomenon.

inflammatory infiltrate composed of foamy histiocytes, lymphocytes, and plasma cells (Fig. 20.9A). The sulfur granules are readily identified as discrete multilobulated granules of basophilic material, which are tangled bacterial colonies (see Fig. 20.9B). These bodies can measure from 300 µm up to 1 to 2 mm in diameter and are surrounded by a crown of closely attached neutrophils.[76-78] Sometimes, one can also see an eosinophilic, loose rim around the granules, which is known as the Splendore effect (see Fig. 20.9B), part of the Splendore-Hoeppli phenomenon.[70] The bacteria are highlighted by Gram stain and PAS stain with diastase, and they are negative for acid-fast stains. With the specific stains, the microorganisms are visualized as filamentous, branching mycelia that sometimes adopt a "V" shape.[76]

Neisserial Infections

Meningococcal Infection

Clinical Features. This serious infection may occur as isolated cases or in sporadic epidemics, particularly in confined spaces, such as dormitories and barracks, because the respiratory route spreads the disease. Patients who develop meningococcal septicemia are usually severely ill with high fever and exhibit a variety of skin lesions, including papules, nodules, and ecchymoses. The earliest lesions may be petechial and can lead to a correct diagnosis clinically.[79,80] A devastating complication of the disease, known as the Waterhouse-Friderichsen syndrome, is associated with adrenocortical insufficiency and shock. Chronic meningococcemia is associated with recurrent episodes of fever and arthritis. Especially noteworthy is the presence of petechiae and papulopustules that occur on the lower extremities during these episodes.[81,82] The acute phase of meningococcemia can resemble the effects of other infective agents, such as viruses, gonococcemia, and other gram-negative organisms. The chronic form requires ruling out other causes of necrotizing vasculitis, such as Henoch-Schönlein syndrome, Still disease, and connective tissue diseases. Evaluation with appropriate cultures is indicated.

Histopathology. The histopathology varies according to the stage of the disease. In patients with the fulminant form, microthrombi are almost always present in dermal vessels. At times, one can also find necrotizing vasculitis with or without thrombi and pustular thrombi. Pustular vasculitis includes an intraepidermal or intrafollicular or subepidermal abscess with extensive vasculopathy and vasculitis of vessels throughout the dermis. The picture of microthrombi with necrotizing vasculitis must always raise the possibility of a septic process as well. The chronic form of meningococcemia exhibits a relatively paucicellular type of vasculitis associated with perivascular neutrophils and lymphocytes and debris. There is also necrotizing fibrinoid necrosis. The organisms may be found in the cytoplasm of infected endothelial cells, in the neutrophils, and in extracellular locations.

Etiology and Pathogenesis. *Neisseria meningitides*, the causative agent, can be found in the throats of a small number of asymptomatic persons who are carriers.[80] The organism can be identified easily in patients with meningitis from the blood and spinal fluid, as well as from the skin. Bacterial wall components, such as lipopolysaccharide (LPS), are powerful endotoxins, triggering shock.[83] The shocklike events appear to be a result of specific thrombosis of the pulmonary microvasculature. The thrombi appear to be due to the effect of toxins elaborated by the bacteria, which cause aggregation of fibrin and neutrophils. The pulmonary infection and vascular compromise result in cor pulmonale. This event does not respond to heparinization. On the other hand, another meningococcal endotoxin may result in disseminated intravascular coagulation in which one finds pure fibrin thrombi. This form of the disease is treatable by heparin. The pathogenesis of the Waterhouse-Friderichsen syndrome is

usually associated with disseminated intravascular coagulation leading to vasomotor collapse. Other virulence agents include cell wall polysaccharides and proteins that evade the immune system, as well as an IgA protease.[84-86]

Gonococcal Infection

Clinical Features. The venereal disease called gonorrhea manifests differently in men than in women. Women most commonly have endocervicitis, whereas men develop an acute urethritis with definite painful micturition. They also can develop proctitis or oral pharyngitis.[87] Another classic presentation of gonococcal disease includes joint involvement, exhibiting arthritis and tendonitis. After becoming septicemic, a common presentation of gonococcal septicemia includes the presence of scattered hemorrhagic pustules that are usually few in number but sometimes manifest as a folliculitis or even with bullae formation. Because the *Neisseria* species organisms are incapable of invading intact skin, the presence of pustules and vasculitis implies disseminated disease. Because arthritis is often present, this presentation is one of the dermatitis-arthritis syndromes. The classic gonococcal picture in the skin is a pustule surrounded by an erythematous ring associated with arthritis and tendonitis.[88,89] Thus the differential diagnosis must include, importantly, Reiter syndrome because of the urethritis, as well as meningococcemia and other bacteremias.

Histopathology. The histopathology of the characteristic gonococcal cutaneous lesion is that of pustular vasculitis with intraepidermal subcorneal intrafollicular or subepidermal abscesses associated with diffuse vasculitis and vasculopathy throughout the dermis. Lesions that resemble cellulitis have a much more diffuse picture without vascular prominence. A simple test to confirm the diagnosis involves performing a Gram stain on a smear of one of the pustular lesions.

Etiology and Pathogenesis. The causative agent, *Neisseria gonorrhoeae*, is known to produce agents that facilitate immune-evasion, such as the Opa and pilli type IV via antigenic variation.[84,85] The pilli type IV also serves a "grappling hook" that allows the organisms to be attached to epidermal structures.

Miscellaneous Infections Caused by Gram-Negative Bacteria

Brucellosis

Clinical Features. Brucellosis occurs after ingestion of contaminated food or following direct inhalation of organisms. The incubation period is usually 30 to 60 days but can be as short as 5 days. A small percentage of patients have a polymorphous rash that may include macules, eczema, a measleslike appearance, erythema multiforme (EM), or even blistering disorder and petechiae. Other symptoms include severe headache, nausea, vomiting, and possibly associated arthritis or arthropathy. There is an inoculation type of brucellosis that results in redness, papules, and pustules in workers who deal with animals, especially veterinarians.[90]

Histopathology. The eczematous rash is associated with dyskeratotic cells and some extravasation of red blood cells. The EM-like rash is associated with a superficial and deep lymphohistiocytic infiltration. Inoculation brucellosis may be associated with eosinophils. Both septal and lobular panniculitis may be observed, and sometimes they are associated with necrosis.[1,91]

Etiology and Pathogenesis. *Brucella melitensis*, *Brucella suis*, *Brucella abortus*, and *Brucella canis* may all cause the cutaneous lesions of brucellosis. These gram-negative coccobacilli are usually found in milk or animal products. Due to their increased infectivity, these organisms are considered potential type B biologic warfare agents.[44] Serologic diagnosis is preferred because of the long delay in culture (fastidious organism).[90,91] An immunohistochemical stain specific to these bacteria is available at the CDC.[44]

Yersiniosis and Plague

The *Yersinia* genus is composed of gram-negative bacilli; the medically relevant species, which are zoonotic, include *Yersinia pestis*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*. Although *Y. enterocolitica* and *Y. pseudotuberculosis* lead to yersiniosis, often showing reactive polyarthritis associated with fever, gastrointestinal symptoms and signs of erythema nodosum,[92] *Y. pestis* gives rise to a frequently lethal infection known as plague. We will devote this section to the severest form of yersiniosis, plague. The normal hosts of *Y. pestis* are wild rodents, and their fleas act as vectors. Humans are sporadically involved when bitten by infected fleas or by contact with infected material through abrasions in the skin. A third, more severe form of involvement is the so-called pneumonic plague, which is acquired through aerosolized droplets. The latter is also the manner by which human-to-human spread of the disease is carried out.[93] Untreated, or even with appropriate treatment, the pneumonic and septicemic forms plague are almost 100% lethal. The bubonic form, with appropriate rapid treatment, will show approximately 40% to 95% survival. Due to its tremendous virulence, this agent is considered a category A bioweapon with potential use in bioterrorism.[42,44]

Clinical Features. There are essentially three forms of plague: bubonic, pneumonic, and septicemic. All of these variants can show cutaneous involvement. The site of inoculation (flea bite) may show a pustule with subsequent ulceration, sometimes showing an ecthyma-gangrenosum–like picture. This is often followed by the development of pronounced lymphadenitis (bubo or buboes), which can sometimes fistulize to the skin, draining purulent material. An important clinical sign is that pustules or carbuncles may exhibit centrifugal vesicles around the central inflammation, usually at the site of the bite or bubo. Fever and generalized malaise are characteristic. The terminal stage of both pneumonic and cutaneous plague is the septicemic form. A primary septicemic form is also known when the port of entry is the oropharynx. Septicemic plague is characterized by generalized infection and septicemia with shock and death. Diffuse erythema is common, but one may also find petechiae and purpura with large areas of ecchymosis, changes that likely gave the denomination of "Black Plague" during the Middle Ages.[42,44,94]

Histopathology. The histopathology of the various lesions depends on their clinical presentation. The site of inoculation will show nonspecific arthropod assault-related changes, sometimes with large areas of necrosis is ulceration ensues. The characteristic buboes exhibit a lymph node with edema, hemorrhage, necrosis, and clumps of bacilli represented by ground-glass amphophilic material. The bacilli will show the classic bipolar, "safety pin" gram-negative morphology by tissue Gram stain. Both Giemsa and silver impregnation histochemical stains can also be used. An immunohistochemical stain is available to highlight the bacilli.[44] The morbilliform rash is associated with a perivenular lymphoid infiltrate. In lesions with petechiae and purpura, and especially in those associated with shock, there is usually evidence of disseminated intravascular coagulation (intravascular fibrin thrombi).[42,44]

Appropriate cultures of blood and stools, throat, sputum, or aspirated fluid from buboes lead to the correct diagnosis and a Gram stain of tissue or fluid from infected sites may reveal the organisms. Serologic and molecular PCR-based assays tests are also available, predominantly at the CDC.

Tularemia

Like plague, tularemia is a zoonotic infection caused by a gram-negative pleomorphic coccobacillus, *Francisella tularensis*. This organism is one of the most virulent organisms known to humans, with fewer than 10 organisms being enough to cause an infection, in addition to being quite resistant to environmental factors. This combination of features, in a similar way to anthrax and plague, makes *F. tularensis* a quite dangerous agent classified as a potential category A bioweapon.[42,44,95] The bacteria and their reservoirs, which include wild rabbits, beavers, and other rodents, are present in wilderness areas of the United States, Japan, and Russia. Vectors, such as deerflies (*Chrysops* genus), ticks, and mosquitoes, as well as through contact with infected animals or their feces, transmit the disease.[42,96]

Clinical Features. Three to 5 days after inoculation, all forms of tularemia show initial symptoms of abrupt fever, rigors, and chills with accompanying headaches, generalized malaise, coryza, and sore throat. The most common clinical form of this disease is the so-called ulceroglandular presentation and is the one most frequently related with cutaneous lesions. When infection was produced from handling wild animal carcasses, the finger or hand is the first site of the inoculation, whereas in case of vector-related inoculation, it usually occurs in the lower extremities. The area of inoculation shows a rapidly growing papulonodule that later ulcerates.[95,97] The ulcer tends to show "heaped-up," irregular, overlapping borders and a necrotic/hemorrhagic base.[42] A black eschar or sphacelus eventually develops within 3 to 5 days after contact. Other important forms of tularemia include oculoglandular, glandular, pharyngeal, typhoidal (septicemic), and pulmonary types, all with high mortality rates, especially the typhoidal and pneumonic types.[42] In almost all forms, one tends to encounter prominent regional lymphadenitis. Culture of the organism is problematic and could result in infection of laboratory personnel; serologic diagnosis is safer and more effective. PCR-based assays are likewise diagnostic.[98]

Histopathology. Ulcers will show dense fibrin necrotic debris–laden exudate overlying dermal necrosis with palisading neutrophils and suppurative granulomatous response. Likewise, the draining lymph nodes exhibit geographic areas of necrosis surrounded by palisading granulomatous inflammation with abundant neutrophils, resembling a picture seen in cat-scratch disease.[44] The organisms are gram-negative and difficult to observe, but the Dieterle silver impregnation histochemical stain may be helpful. A specific immunohistochemical stain can highlight the organisms.[44]

Lesions that can resemble tularemia include atypical acid-fast infections and sporotrichosis. Cat-scratch disease also must be considered in the differential diagnosis; however, the skin lesion with eschar is quite different from these other infections.[99] The clinical history of prior contact with wild animals or their products in endemic areas is usually very helpful in the differential diagnosis.

Granuloma Inguinale

This sexually transmitted infection, also known as donovanosis, is caused by the gram-negative coccobacillus *Calymmatobacterium granulomatis*, an intracellular organism closely related to the *Klebsiella* genus. The relationship between these two organisms is so close that some authors use the name *Klebsiella granulomatis* to refer to this agent. Granuloma inguinale affects communities of low socioeconomic level in tropical and subtropical areas and frequently affects individuals of African descent.[100,101]

Clinical Features. The lesions manifest as painless, often-malodorous ulcers affecting the genitalia or the anal and perianal areas that develop marked granulation tissue. These lesions spread in an unusual serpiginous pattern. Accompanying lymphadenopathy is common. Large unattended ulcers can result in prominent destruction of tissue and possible abdominal dissemination. Involvement of extragenital regions in the head and neck and trunk has been described.[101]

The clinical differential diagnosis includes chancroid, syphilis, and lymphogranuloma venereum. Verrucous or exuberant forms of herpes simplex virus (HSV) infection can at times resemble this disorder. Lesions of granuloma inguinale can also be confused with squamous cell carcinoma (SCC).

Histopathology. Lesions show exuberant granulation tissue with florid reactive epidermal hyperplasia adjacent to the ulcers. Away from ulcerated areas, one may see lymphocytes and macrophages, as well as small abscesses. The abscesses are associated with macrophages up to 20 µm in diameter that contain the intracellular organisms. The organisms are 1 to 2 µm in size and exhibit a characteristic "safety pin" appearance in sections stained with Giemsa or Warthin-Starry. These intracellular structures represent the so-called Donovan bodies, originally described by Charles Donovan in 1905.[102] There are similarities between these cells and the so-called *Mikulicz cells* found in rhinoscleroma, another infection caused by an organism related to the Klebsiella genus (see later).

The diagnosis can also be made via smears of tissue stained with either Giemsa or Warthin-Starry reagents. Likewise, smears made from biopsy samples may be crushed or cut into 1-µm sections and prepared for transmission electron microscopy to allow for identification of the organisms.[101] Culture can be performed, although appropriate techniques are not widely available.[103,104]

Rhinoscleroma

Rhinoscleroma is a rare granulomatous disorder that was described originally in central Europe, but, in the 20th and 21st centuries, most cases occur in poor areas of South and Central America, Africa, the Middle East, and Eastern Europe. The gram-negative, encapsulated, and nonmotile *Klebsiella pneumoniae* subsp. *rhinoscleromatis* is the agent causing this disease. The organisms are transmitted upon inhalation of contaminated droplets.[105,106]

Clinical Features. An overlapping sequence of three phases has been described: (1) an atrophic (rhinitic, catarrhal) stage, (2) a proliferative (hypertrophic, granulomatous) stage, and finally (3) a cicatricial (fibrous, sclerotic scarring) stage. The infection begins in the subepithelium of the nasal mucosa but can spread to the pharynx and the upper respiratory passages, including larynx and trachea. In some patients, it extends onto the lip, which is then markedly indurated with granulomatous inflammation. Involvement and destruction of other head and neck structures, such as the orbit, have been described.[106]

Histopathology. The histopathologic picture of the proliferative phase of the lesion includes a prominent plasmacellular infiltrate with scattered lymphocytes and occasional neutrophils in a setting of proliferated vessels. The plasma cells characteristically bear prominent Russell bodies. Careful inspection reveals

large histiocytes, measuring 10 to 100 μm in diameter, with markedly vacuolated cytoplasm, the so-called Mikulicz cells. The gram-negative *K. pneumoniae* subsp. *rhinoscleromatis* organisms populate these vacuoles and are best seen on PAS, Giemsa, or Warthin-Starry histochemical stains. They are faintly visible on H&E staining. This bacterium measures 2 to 3 μm in size, and they can be arranged in clusters within the vacuoles. The best clue to the Mikulicz cell is that it has a foamy appearance, and the bacteria can be appreciated on high-power microscopy. The cicatricial phase of the disease will show dense fibrosis and far less numerous Mikulicz cells and plasma cells. Electron microscopy likewise reveals bacilli with a filamentous coat of mucopolysaccharides, explaining the PAS positivity. On a purely histologic basis, this lesion can resemble granuloma annulare and must be differentiated from other infections and disorders that contain vacuolated macrophages, including the mycoses, Leishmania, and Hansen disease.[106,107]

Chancroid

Chancroid is a rather common sexually transmitted disorder that may occur alone or as part of mixed infection with other venereal organisms. Mixed infections are less common in the United States but may be found in travelers returning from some developing countries. The cause of this infection is the gram-negative rod *Haemophilus ducreyi*.[104] This infection is known to synergize with HSV type 2 and HIV viral infections.

Clinical Features. The lesion of chancroid exhibits a very shallow, minimally indurated ulcer with a necrotic and dirty bed. Sometimes several ulcers are observed. The ulcers have a yellow to grayish exudate on their surface and can be exquisitely tender. Secondary tender lymphadenitis can occur unilaterally or bilaterally and sometimes can drain purulent material through fistula formation.[108] When multiple small ulcers are present, the differential diagnosis includes infection by HSV type 2. Smears can help to differentiate these two lesions: in HSV (Tzanck test) one may find multinucleated cells, whereas in chancroid one often might see gram-negative rods in a "school of fish" pattern.[108] The ulcer of granuloma inguinale may resemble chancroid but is nontender and does not show undermining at the margin.

Histopathology. The histologic picture is distinctive and essentially includes three zones of alteration. At the surface of the lesion, the exudate contains fibrin, neutrophils, neutrophilic debris, and red blood cells. The middle zone is broad and exhibits a generous granulation tissue. At times, thrombi are observed in association with focal necrosis. The third layer at the base exhibits a dense, prominent infiltrate of lymphocytes and plasma cells. However, typical zonation is not present in all cases.[109] The bacillus is a small gram-negative coccobacillus that is approximately 1.5 μm in length and approximately 0.2 μm in width and can be seen arranged in chains amongst the cells of the surface-most portion of the lesion with Gram or Giemsa histochemical stains. Confirmation of the diagnosis by culture requires blood-enriched agar.

Malacoplakia

Malacoplakia is a rare inflammatory/infectious process that is most commonly found in the urinary tract and occasionally in the gynecologic and gastrointestinal tracts, as well as in other organs.[110,111] Involvement of the skin, although rare, has been well documented in the literature.[112-114] The lesion tends to arise in debilitated and immunocompromised individuals and it is thought to result from an inability of the macrophages to process bacteria. Myriad organisms, including *Escherichia coli*, *S. aureus*, *Pseudomonas* spp., and *Proteus* spp., to name a few, have been described affecting patients with malakoplakia.[110]

Clinical Features. The clinical presentation of malakoplakia in the skin is widely variable. Very nondescript red nodules or papules are present in the lower abdomen and the area of the groin, often around follicles. In some cases, abscesses and sinus tracks, as well as cystic or polypoid masses, may develop.[112]

Histopathology. Lesions show numerous ovoid macrophages with small eccentric nuclei containing discrete eosinophilic and periodic acid–Schiff with diastase (PAS-D)-positive cytoplasmic granules that are called von Hansemann histiocytes or Hansemann cells. Some of these eosinophilic cytoplasmic granules are larger (5 to 15 μm), show a concentrically laminated pattern, and stain slightly basophilic in comparison with the other granules. They are strongly highlighted with a von Kossa histochemical stain and also stain positive with a Perls stain. These structures are the so-called Michaelis-Gutmann bodies. These bodies are composed of iron and bacterial products; they are focally calcified. Because there are portions of bacteria in the cytoplasm of the macrophages, the Gram stain can be positive focally. In addition, there is a quite striking infiltrate of lymphocytes and plasma cells amid the infiltrative macrophages.[110,112] Cutaneous malakoplakia must be differentiated from fungal infections, particularly *Histoplasma capsulatum*, and other granulomatous inflammatory processes.

Botryomycosis

Botryomycosis is a chronic bacterial infection with deep fungal infection-like features that often affects immunosuppressed patients with diabetes mellitus,[115] HIV/AIDS,[116] cystic fibrosis,[117] and other immune-dysregulating conditions.[118] However, botryomycosis can rarely occur in an otherwise healthy individual.[119]

Clinical Features. This disease manifests clinically as tumors or plaques that involve the head and neck region and the extremities The lesions are often ulcerated and have discharging sinuses draining small whitish granules; hence they closely mimic mycetoma or other fungal infection.[120] The granules are also found within the abscesses, and they can be disposed in clusters shaped as a bunch of grapes (*botrys* is the Greek term for "cluster of grapes"). The causative agents are usually pyogenic, gram-positive bacteria, such as *S. aureus*. However, gram-negative organisms, such as *P. aeruginosa*,[121] *Proteus* spp., and *Actinobacillus* spp., have also been implicated.[120,122] Culture is determinant, not only to definitely identify the agent but also to provide appropriate antibiotic therapy.

Histopathology. The histologic picture closely resembles that of mycetoma lesions. It is composed of an abscess cavity filled with purulent material, which contains the variably sized, irregularly shaped basophilic granules. The granules are composed of massive colonies of bacteria, which can be gram-positive or gram-negative.[120] One can occasionally find a surrounding eosinophilic rim as part of the Splendore-Hoeppli phenomenon.[70] Transepidermal elimination of granules can occasionally be found.

Mycobacterial Infections

The mycobacteria can be divided into two major groups: tuberculous and nontuberculous. They can also be separated in two groups based on their culture growth (slow vs rapid growers) and biochemical characteristics. The slow growers include *M. tuberculosis*, *Mycobacterium avium* and related organisms, *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*. The rapid growers include *Mycobacterium fortuitum* and

Mycobacterium chelonei. Another slow grower, *Mycobacterium haemophilum*, has special culture requirements. Totally separate from this classification is *Mycobacterium leprae* because it cannot be grown in culture. The bacilli of tuberculosis and leprosy are intracellular parasites. Because they are limited to the tissues of humans and animals, they are transmitted by exposure to afflicted hosts.

The other mycobacteria are present in soil and water. The exposure of persons to these bacteria is widespread but usually is not associated with significant clinical disease. As far as staining characteristics are concerned, these organisms are weakly gram positive and are best identified by techniques that show that they are acid-fast (Fite and Ziehl-Neelsen).[1]

Tuberculosis

M. tuberculosis infection had been well controlled for most of the second half of the 20th century in North America and Europe, but it has remained a prominent problem in resource-poor countries. During the first portion of the 21st century, there has been a surge in incidence in developed countries, mainly due to increase in migration of persons from Asia and Africa, where tuberculosis is endemic, as well as increased poverty, increased numbers of refugees from developing countries and the Middle East, and the HIV pandemic.[123]

Although the pulmonary route of infection is the most common, *M. tuberculosis* can also infect the skin. There are three ways in which the skin can be affected by *M. tuberculosis*.[124] First is direct inoculation, which results in a primary nodule or chancrelike lesion, a verrucous response (so-called *tuberculosis verrucosa cutis*), or inoculation of the mucosae, resulting in *tuberculosis cutis orificialis*. Second, the infection can be spread from an internal focus, such as the lung, to the skin by a hematogenous route, which results in lesions, such as lupus vulgaris or gumma. Finally, infection can occur by direct extension from an underlying infected lymph node that has broken down and caused cutaneous ulceration. This form of tuberculosis is relatively rare in the skin and can occur (e.g., as a result of a needlestick injury)[125] by accidental inoculation during an autopsy[126] or a surgical procedure or even during mouth-to-mouth resuscitation.[127]

Primary Inoculation, Tuberculosis Verrucosa Cutis, and Milliary Tuberculosis

Clinical Features. In primary inoculation, a nodule usually appears within 2 to 4 weeks and then becomes ulcerated with an overlying crust. The draining lymph nodes become affected and may suppurate. This is the classic chancriform complex that includes an ulcerated nodule with draining lymph nodes. Often, pus can be expressed from the surface. The most common sites of involvement are the hands in adults, but in children the lesions may occur especially in the lower extremities.[124] *Tuberculosis verrucosa cutis*, which occurs frequently due to needle inoculation during an autopsy, results in an isolated lesion that is verrucous and surrounded by an inflammatory border. Miliary tuberculosis describes infection of the skin by the hematogenous route; it consists of a generalized cutaneous eruption including papules and pustules a few millimeters in diameter.

Histopathology. Inoculated human tissues show a specific series of reactions. First, there is an acute, neutrophil-mediated infiltrate produced while the bacilli are rapidly multiplying. Next, macrophages migrate to the area and phagocytize the bacteria. As the T cells become sensitized, the tuberculous antigens result in cytokine release, which causes activation of macrophages to become epithelioid cells and giant cells. As the host delayed hypersensitivity reaction becomes stronger, necrosis of the aggregates of macrophages occurs and inactivates or indeed kills the offending organisms. A necrotic granuloma is the final picture.[124,126]

The course after primary inoculation depends on a variety of factors, including the quantity of bacteria, the manner in which the infection occurred, and the patient's immune response. HIV infection or the AIDS, debilitating therapy, and immune dysregulatory drugs, particularly the new TNF inhibitors, can all affect the outcome.[1] Histology of the primary lesion exhibits epidermal ulceration, in the base of which is fibrin and neutrophilic debris. In the dermis, depending on the age of the lesion, there may be only diffuse neutrophils, but as the delayed hypersensitivity response becomes stronger, granulomas form randomly in the dermis. Acid-fast bacilli are usually present and easily demonstrable by special stains.[126]

In tuberculosis verrucosa cutis, there is marked epidermal hyperplasia with prominent hyperkeratosis and papillomatosis. In early lesions, dermal abscesses may be observed, but as the infection progresses, well-formed granulomas with variable necrosis are present. Miliary tuberculosis regularly exhibits a central area of abscess formation in the dermis containing neutrophils, debris, and easily visible tuberculosis bacilli.[1,128]

Cutis Orificialis

Clinical Features. The lesions of cutis orificialis can occur by direct inoculation of the skin or mucosa or secondarily, in patients who have extensive tuberculosis (e.g., in the bowel) and are actively excreting bacilli. Patients with disseminated tuberculosis (e.g., in the colon with lesions that are pouring bacilli into the feces) can eventually develop perianal or perineal ulcers.[129,130]

Histopathology. A superficial ulcer is often present in association with well-formed granulomas; the lesions are profoundly necrotic and extend deep into the skin. Tubercle bacilli are easily demonstrable with appropriate stains.[1]

Lupus Vulgaris, Scrofuloderma, and Tuberculous Gumma

Clinical Features. The lesions of lupus vulgaris commonly occur on or adjacent to the nose[1,131] and almost always in the head and neck region. The lesions consist of well-formed patches or plaques that on palpation reveal a prominent nodule. The individual lesions may vary from 1.0 mm to a few centimeters. As the lesions progress, areas of atrophy appear, while at the same time other areas may become raised or verrucous. Ulceration occurs in approximately 20% of the lesions and rarely may be associated with SCC.[132] Scrofuloderma refers to an ulceration of the skin overlying a tuberculous lymph node or bone. The lesion, at first a nodule, becomes fistulized and ulcerated and exudes purulent material at times. Tuberculous gumma results from hematogenous spread of organisms to the skin. A large dermal and subcutaneous nodule that is partially necrotic gradually appears and can often ulcerate the skin.

Histopathology. Lupus vulgaris characteristically produces numerous granulomas in the upper third to upper half of the dermis, where they abut the dermal epidermal interface. Caseation necrosis is focal and sometimes absent.[131] The granulomas contain Langhans-type giant cells. The inflammation can also extend along hair follicles into the subcutis. The overlying epidermis exhibits atrophy, alternating often with hyperplasia and papillomatosis. Occasionally, SCC can supervene in areas of hyperplasia.[132] Tuberculous bacilli are present in very small numbers and

are rarely demonstrable. Patients with this manifestation usually have very intense delayed hypersensitivity reactions to the purified protein derivative (PPD) test.[1,124] Scrofuloderma produces a large cutaneous ulcer with abscesses and areas of small, well-formed but edematous granulomas with prominent necrosis. Tubercle bacilli are easily found. Tuberculous gumma exhibits massive caseation necrosis with a rim of tubercles and epithelioid cells and giant cells. There are also often lymphocytes and plasma cells admixed with the giant cells.

Etiology and Pathogenesis of Tuberculosis. Pathogenesis of these lesions directly relates to the degree of immunity of the host. In the naive host, the first sign of infection is the presence of neutrophils[1,124] and, as the delayed hypersensitivity reaction progresses, granulomas occur. The clinical features also reflect hypersensitivity, so that, for example, a person with a fairly established immune response will exhibit a papillary epidermal hyperplasia with a very prominent lymphogranulomatous and neutrophilic infiltrate.

There are many disorders that must be considered in the differential diagnosis because of the variety of different reactions that tuberculosis may cause. One of the most important is sarcoidosis. This disorder usually manifests with so-called naked tubercles without inflammation. However, inflammation can occur in sarcoidosis, so one must be certain of the etiology of the granuloma. Necrosis can also be seen in sarcoidosis, but it is usually fibrinoid rather than the caseating granular type seen in tuberculosis. Silica granulomas and zirconium granulomas can look very much like sarcoidosis. A polarizing light examination of the granulomatous infiltrate should always be performed to rule out a foreign body as the cause of the inflammation. Special stains, including acid-fast stain, PAS, and GMS, must also be performed to rule out other infections.[1]

Traditional mycobacterial culture methods are frequently used to identify these agents, with the caveat of them being laborious and time consuming. Fortunately, several PCR-based assays have now been shown to be very useful in demonstrating evidence of tuberculosis bacilli in tissue with improved sensitivity and specificity.[133,134]

Atypical Mycobacterial Infections

Atypical mycobacteria are acquired by direct inoculation or by blood-borne spread from another infected site.[1,135-137] Because these organisms are present in soil, water, and the natural environment, they are found throughout the world.[137] In addition, the immunosuppressed states induced by HIV/AIDS,[138] as well as by iatrogenic causes, lead to more frequent infection by these agents. In general, the histopathology of these lesions shares virtually the same spectrum as that of the tuberculosis organism.[138-140] Only a few of these atypical mycobacteria are highlighted here.

Mycobacterium kansasii

Clinical Features. The most common presentation after infection by *M. kansasii* is with pulmonary and nodal infections.[141] Skin lesions are rare but can be inoculated.[142] A resultant ulcerated nodule appears, and multiple lesions may spread along the extremity, similar to sporotrichosis.[143]

Histopathology. Histologically the lesions show acute inflammation with abundant neutrophils that is associated with some granulomatous response and numerous acid-fast bacilli being highlighted with Ziehl-Neelsen or Fite.[1] The bacilli of *M. kansasii* tend to be arranged haphazardly within the epithelioid cells, and they are often long and beaded, showing folds or even bended into themselves.[144] This is in contrast to the findings usually seen in *M. avium-intracellulare* (MAI) complex (see next section).

Mycobacterium avium-intracellulare Complex

Clinical Features. MAI infection can occur in children, manifesting as a swollen cervical lymph node or as a cause of pulmonary infection in lungs that have been previously injured or damaged.[145] The onset of the HIV/AIDS pandemic has led to rather common infections with this organism, manifesting as a variety of cutaneous papules and nodules.[146] Generalized systemic infections with MAI (and potentially with any mycobacteria or intracellular pathogen) with involvement of the bone marrow and skin can be found in conditions that lead to a debilitated cellular immunity, such as in the X-linked inherited syndrome characterized by mutations in the *IKBKG* gene (Xq28) which codes the NF-kappa B essential modulator (NEMO) kinase.[147]

Histopathology. The histology varies according to the immune competence of the patient. There may be an acute inflammatory response, a mixed acute and chronic inflammatory response, or a histiocytic infiltrate suggestive of lepromatous leprosy (Fig. 20.10A). There are also sometimes lesions that have virtually no inflammation but are teeming with organisms.[148] The index of suspicion of possible infection with these organisms should be very high in patients with HIV/AIDS. As mentioned previously, the bacilli morphology in MAI differs from *M. kansasii* by the fact that the intracellular organisms in MAI are arranged orderly and somewhat parallel to each other (see Fig. 20.10B). The bacilli themselves appear less beaded and not folded as *M. kansassi* bacilli.[144] Importantly, both *M. kansasii* and MAI are positive with PAS, in contrast with other mycobacteria, which tend to be negative. MAI organisms tend to show a stronger reaction with PAS.[144]

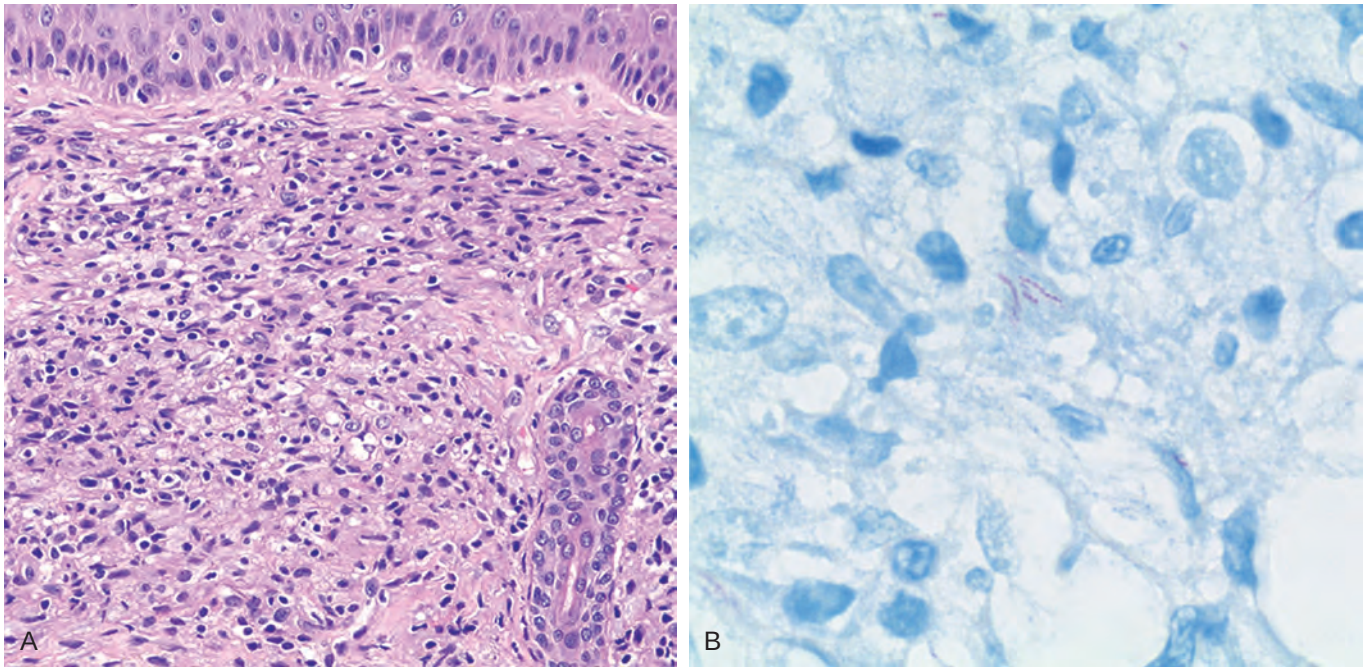
Mycobacterium marinum

Clinical Features. The most common cause of infection by *M. marinum* is trauma, typically a minor abrasion incurred while bathing in a swimming pool, lake, or the ocean. However, documented cases have occurred in patients after they cleaned an aquarium in their home.[149] Epidemics can occur if a swimming pool is affected.[150] Approximately 3 weeks after inoculation, reddish, hyperkeratotic, papillomatous papulonodules and plaques can occur. Ulceration is occasionally noted. Prominences of the extremities, hands, and feet are the most affected sites. Similarly to infections by other atypical mycobacteria, sporotrichosis-like spread can occur.[151]

Histopathology. The changes resemble the evolution of tuberculosis infection. In the early lesions, neutrophils and macrophages are found, but, as the lesions mature, multinucleated giant cells with small granulomas are noted. In the well-formed lesion, verrucous hyperplasia can be associated with tubercle formation.[152] Acid-fast bacilli are too difficult to observe after the very early infection. The late lesions show virtually no bacilli and must be cultured or detected by molecular biology means.[133]

Mycobacterium ulcerans

Clinical Features. Infections by this agent result in the so-called Buruli ulcer, which is now considered a reemergent and neglected tropical infection. The lesion occurs after direct inoculation or a bite by a wide array of insects, including mosquitoes and aquatic insects, as well as by contact with infected fish.[153] The organism is found near inland lakes and rivers, predominantly in West and Central Africa, Central America, and South Australia.[153-155]



• **Figure 20.10** Cutaneous infection by *Mycobacterium avium-intracellulare* complex. Note the prominent dermal histiocytic infiltrate **(A)**. A Wade-Fite histochemical stain reveals long, predominantly straight mycobcateria, visible here on high magnification **(B)**.

Interestingly, it appears that transmission of *M. ulcerans* can also occur when the organisms "hitchhike" within the cytoplasm of amebas from the *Acanthamoeba* species.[156] The lesion begins as a papulonodule that relatively rapidly progresses to ulceration of the skin, with resultant large subcutaneous effects that undermine the epidermis and extend into fascia and bone. The lesions are usually painless. More rarely, ulcers occur on the trunk or even on the face.[153,154]

Histopathology. The first change results in an ischemic alteration of fat and collagen with prominent collagen necrobiosis. There are large aggregates of mycobacteria in the necrotic fat zones. Eventually, there is some neutrophilic infiltration at sites of necrosis, and the vessels exhibit thrombosis. Granulation tissue can be observed in more chronic lesions in the borders of the necrotic fat. If treatment is successful, healing results in scar formation with reepithelialization. Clumps of bacteria are present in the areas of necrosis.[157,158] This unique organism has a pathogenesis related to a toxin that is a polyketide mycolactone and causes direct extensive necrosis. Healing is associated with the presence of a lymphocytic host response associated with a delayed hypersensitivity reaction. A study showed a correlation among the presence of granulomas, a high expression of interferon-γ, and prognosis.[155,159,160] Because these organisms live best in nonoxygenated tissue, hyperbaric chamber therapy has been successful.

Mycobacterium fortuitum-chelonei

Clinical Features. Cutaneous infections with unusual features caused by these rapidly growing mycobacteria have been reported.[161] These lesions manifest as nodules in the dermis and subcutaneous fat and may have a sporotrichoid spread. They may be mistaken for erythema nodosum when on the extremities. The patients usually have some immunosuppressive feature, such as prednisone therapy or another immunodeficient state.

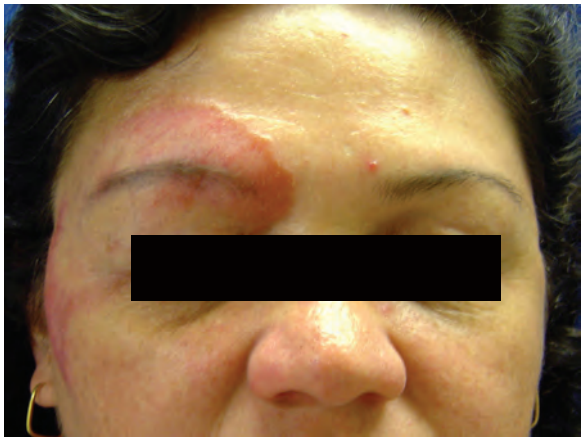
Histopathology. The dermis and subcutis exhibit mainly a suppurative inflammatory response with focal necrosis and marked karyorrhexis. Pseudocyst formation has also been noted with numerous organisms within these cystic spaces that are lined by neutrophils. There is usually little, if any, granulomatous formation. The lesions can be mistaken for a Sweet-like reaction pattern. Organisms are easily visible after staining with acid-fast stains. The Fite stain is sometimes more consistently positive. Reportedly, the organisms are characteristically focally gram positive. Culture must be performed to rule out other causes of the infection.[1,161]

Leprosy or Hansen Disease

Leprosy, more commonly referred to now as Hansen disease, is worldwide in distribution and affects millions of people.[1] Because the causative organism, *M. leprae* does not tolerate well the human core temperature of 37°C, this disease is principally one of the skin and the peripheral nerves. The disease is endemic mainly in the sub-Saharan countries in Africa, Brazil, the Indian subcontinent, and Southeast Asia.[162] In the United States, most cases are seen in immigrants. However, native cases have been detected in Louisiana[163] and Texas.[164] These cases are likely related to contact with armadillos *(Dasypus novemcinctus)*, a known reservoir of *M. leprae*.[165]

The mode of transmission in leprosy is unclear. It is hypothesized that the leprosy bacillus enters the environment contained in droplets from the nasal passages, sebum, and saliva of an infected person with limited immunity in whom it has proliferated, and then it is transmitted to other immunosuppressed individuals. Another source may be the soil. There is no evidence that skin-to-skin contact produces infection. Inhalation of the bacteria discharged into the atmosphere from an infected patient results in spread to various organs, particularly the skin and nerves, where the organism then induces a reaction. The extent of the reaction determines the course of leprosy in the patient.[162,166]

Clinical Features. The classic Ridley-Jopling classification[167] divides leprosy in a spectrum ranging from two "poles,"



• **Figure 20.11** Tuberculoid leprosy. There is a plaque with raised, irregular, erythematous borders and a flat, hypopigmented center. These lesions cause sensory impairment due to the neurotropism of the mycobacteria and the host response. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

tuberculoid to lepromatous, with several intermediate or "borderline/intermediate" stages. This classification is directly related with the host's immune status, with the tuberculoid form occurring in persons who have strong immunity, whereas individuals with limited/anergic immunity will present with the lepromatous variant. There is also an indeterminate form.

In the tuberculoid form, patients have intact immunity and usually exhibit a single lesion or a small cluster of lesions (Fig. 20.11). Borderline leprosy is subdivided into borderline lepromatous, borderline tuberculoid, and pure borderline forms, in direct relation to the degree of possible immunity. As immunity improves, the lesions become less widespread, so that, for example, a borderline or borderline tuberculoid lesion would occur. The anergic patient has diffuse involvement of the skin, which can lead to hypopigmented or erythematous papules, macules, and plaques. The so-called leonine facies, in which there is edema of the face with loss of eyebrows and eyelashes, is an example of severe lepromatous leprosy.[166,167] A variant of lepromatous leprosy is the so-called histoid leprosy, characterized by firm nodules, sometimes resembling a dermatofibroma.[168]

Indeterminate leprosy refers to the very earliest manifestation of the disease. It consists of a few hypopigmented macules with varying degrees of loss of sensation. Indeterminate leprosy can be associated with healing and eradication of the disease, or it can evolve into one of the subtypes of leprosy.[166,167]

Types of Reactional Leprosy. There are basically three types of reactions that occur in leprosy. They affect 30% to 50% of all leprosy patients and can occur both with and without treatment and are directly related to the immune status. They can be quite severe and represent medical emergencies.[166,169]

In the first of the so-called type I reactions, also known as the lepra or reversal reactions, the patient has shifted during therapy from a lepromatous pattern toward a tuberculoid pattern. This change is directly related to an increase in the immune response of the patient. There is also a downgrading reaction that occurs when a patient who is not undergoing therapy progressively becomes more anergic and begins to show changes of lepromatous leprosy. These are the two manifestations of the so-called type I reaction, which can result in systemic symptoms with a change in the size of the lesions and the peripheral nerves.[166]

The type II reactions, which are known as erythema nodosum leprosum, occur principally in borderline lepromatous patients. This type of reaction is also associated with changes in immune response and may occur with treatment or spontaneously. It is characterized by the formation of immune complexes resulting in a vasculitis or vasculopathy. The lesions evolve as very tender, red papules, plaques, and nodules that occur in normal skin.[166]

The third type of reaction, also called Lucio phenomenon, is associated only with lepromatous leprosy.[169] The patient develops a striking, diffuse skin edema, which tends to efface the age-related wrinkles on the face and gave rise to the historic term, "*la belle femme*." However, hemorrhagic plaques can occur suddenly on the arms, legs, and buttocks, and these eventually lead to widespread severe ulcerations.

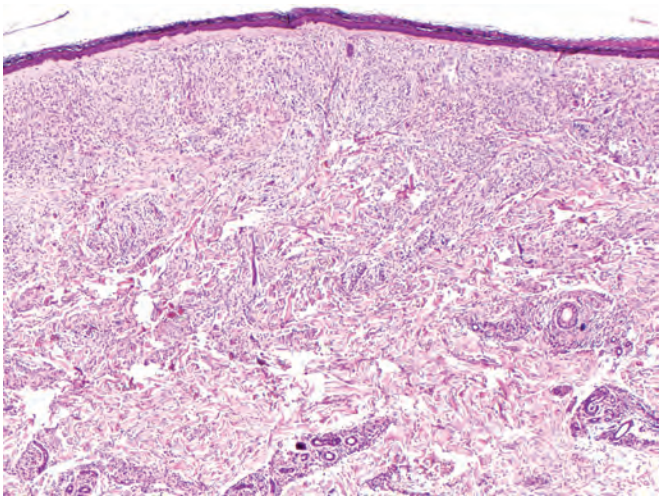
Clinical Pathologic Correlation

Early Indeterminate Leprosy. In early indeterminate leprosy, clinically sparse lesions that are hypopigmented macules can be associated with hypoesthesia. These sometimes resemble an eczematous patch. They may disappear completely if there is eradication of the disease by the host response, or they may persist with development of one of the types of leprosy. The Mitsuda test is a delayed hypersensitivity reaction test to the lepromatous bacilli; it can help to predict the evolution of the indeterminate lesion. If the Mitsuda test is negative, the patient will usually go on to lepromatous or borderline lepromatous leprosy. If the Mitsuda test is positive, the patient will more likely develop tuberculoid leprosy or have a spontaneous remission of the lesion.[170]

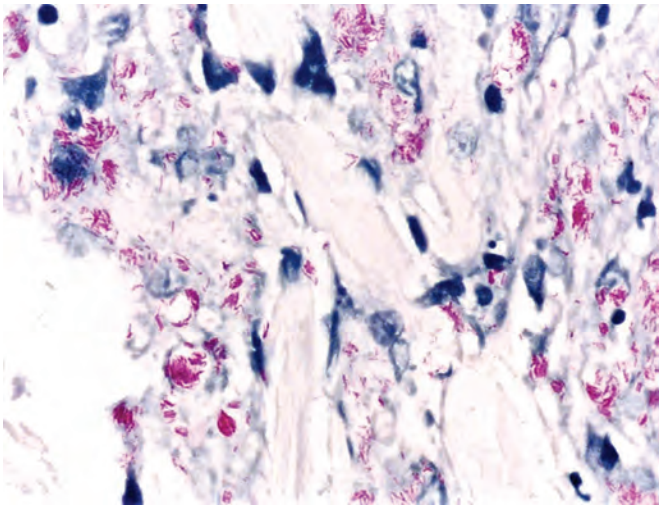
The changes can be very subtle and include a very slight lymphohistiocytic infiltrate. These cells are present particularly around neurovascular bundles and affect both superficial and deep venular plexuses, as well as the sweat glands and the arrector pili muscle. One may observe infiltration into the epidermis and may actually see lymphocytic inflammation of dermal nerves. Careful observation reveals a few acid-fast bacilli, and the diagnosis is made if the bacilli are found in the nerve or in the arrector pili muscle, just under the dermis, or in a perivascular monocyte-macrophage cell. If bacilli are not demonstrated, then the diagnosis is considered presumptive.[1,170,171]

Lepromatous Leprosy. In lepromatous leprosy, there are diffuse lesions throughout the body. These may be macular, poorly defined, and either slightly hypopigmented or erythematous. They are frequently infiltrative, so that they become plaquelike. The most common type, the infiltrative nodular type, usually develops from a macule. These lesions are often dull red; they affect the forehead and cheeks, resulting in the leonine facies, and also affect hair follicles, resulting in loss of eyebrows and eyelashes. As the lesions progress, the nerves become involved, swollen, and very prominent. These changes are associated with some dysesthesias and nerve paralyses. The most commonly involved nerves are the ulnar, radial, and common perineal nerves.[166]

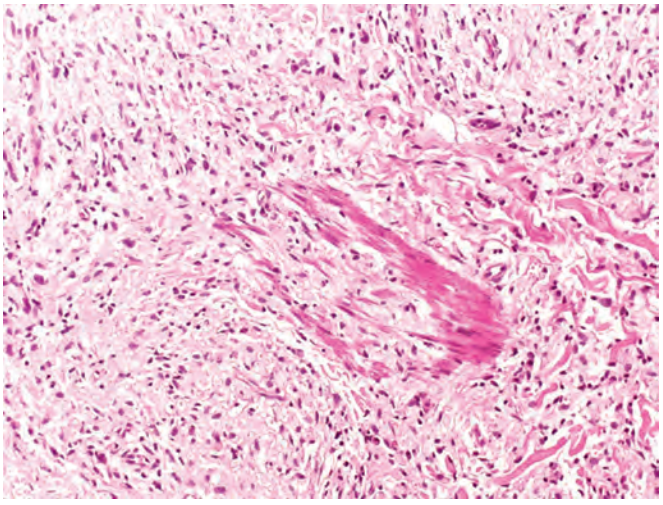
The histopathology of the lepromatous lesion includes an extensive, diffuse cellular infiltrate. This infiltrate involves the entire dermis but is clearly separated from the epidermis by a small grenz zone of collagen (Fig. 20.12). The infiltrate causes destruction of the appendages and also extends into the subcutaneous fat (Fig. 20.13). The involved monocyte-macrophages exhibit a rather eosinophilic cytoplasm in which one can observe bacilli, many of which are fragmented. In these chronically persistent lesions, one can observe clumps of bacteria and bacterial fragments that are called globi.[170]



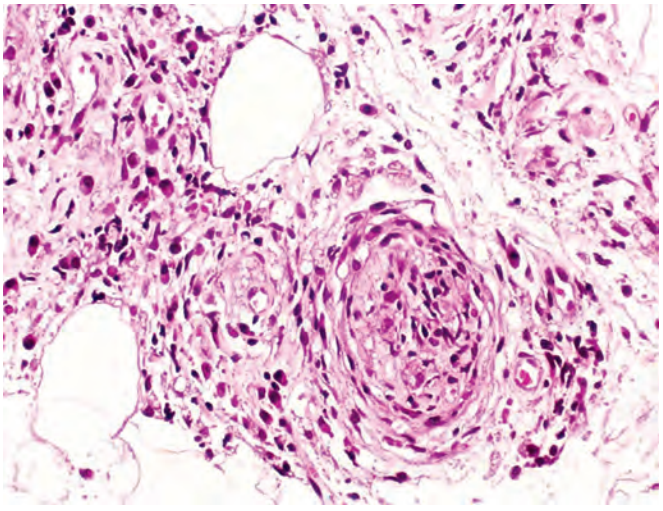
• **Figure 20.12** Lepromatous leprosy. At low magnification, one sees a diffuse dermal infiltrate composed of histiocytes with a foamy appearance and a scant lymphoid infiltrate. Granulomas are absent. Note the characteristic grenz zone. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.14** Lepromatous leprosy (Wade-Fite stain). The foamy histiocytes contain thick aggregates of mycobacteria, known as globi. Note the relationship between the bacilli and the vessels. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.13** Lepromatous leprosy. In this example, diffuse infiltration of the arrector pili muscles is seen. (Courtesyof Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



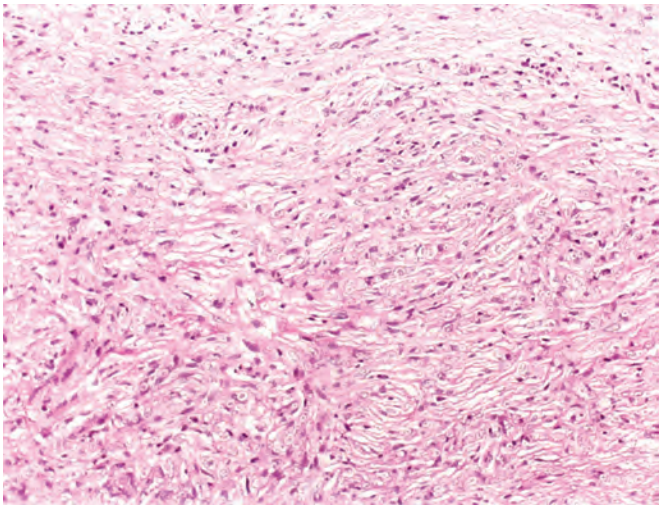
• **Figure 20.15** Lepromatous leprosy—subpolar lepromatous leprosy. In the transition between these to variants of the leprosy spectrum, one can find plasma cells and the classic "onionskin" pattern of perineural fibroblastic proliferation. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

There is no evidence of any formation of epithelioid cells, and there are few lymphocytes, which are found percolating amidst the infiltrate. As the lesions persist, one can observe vacuoles in the macrophages that contain quite prominent lipid associated with fragmentation of the bacteria. The Fite stain is useful in identifying numerous mycobacteria under these circumstances (Fig. 20.14). Nerves can exhibit very prominent bacilli in the lepromatous lesion.[170] Plasma cells are frequently found in and peripheral to the infiltrate, especially in lesions in the transition to borderline lepromatous form (subpolar lepromatous leprosy) (Fig. 20.15).

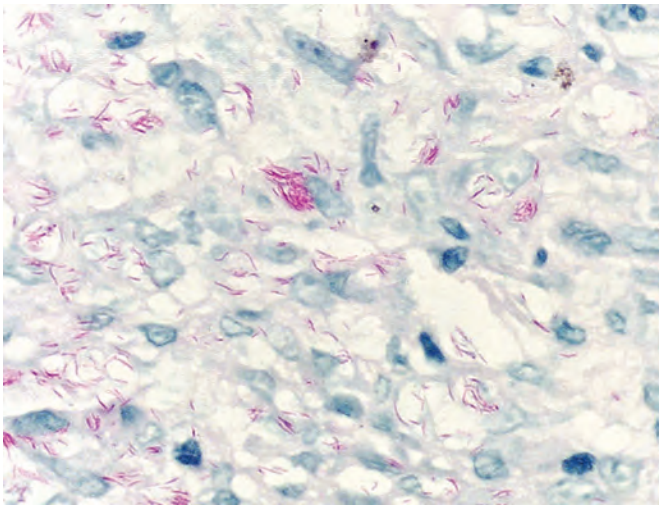
The histiod variant of lepromatous leprosy is associated with cells that have a fusiform shape and may be arranged in a storiform pattern (Figs. 20.16 and 20.17). These fibrohistiocytic cells usually have a basophilic or amphophilic cytoplasm that is laden with mycobacteria, often arranged in stacks and aligning with the cell's axis (Fig. 20.18).[168]

Borderline Lepromatous Leprosy. The lesions in borderline lepromatous leprosy are fewer in number and are not symmetrically disposed. They often have a central delve or dimple. The lesions vary in number according to the degree of host response. In borderline lesions with poor host response, they are more numerous. As the host response becomes stronger, fewer lesions appear and form plaques, designated as borderline tuberculoid leprosy.

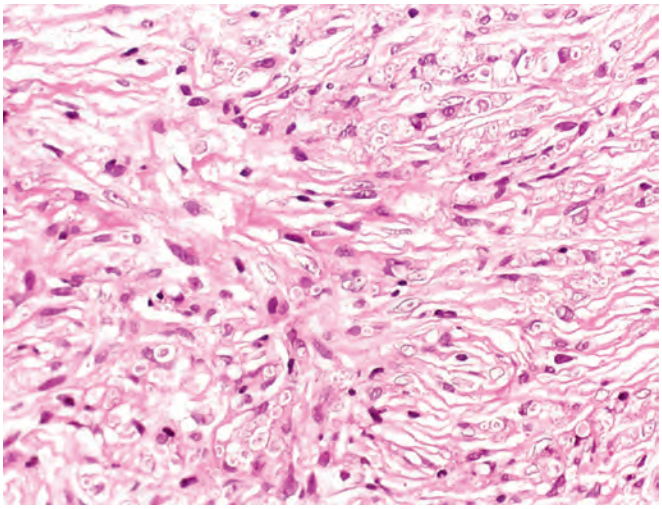
In the histopathology of this lesion, there are lymphocytes and some areas of possibly very early, poor granuloma formation (Fig. 20.19). Interestingly, there can be an "onionskin" appearance to



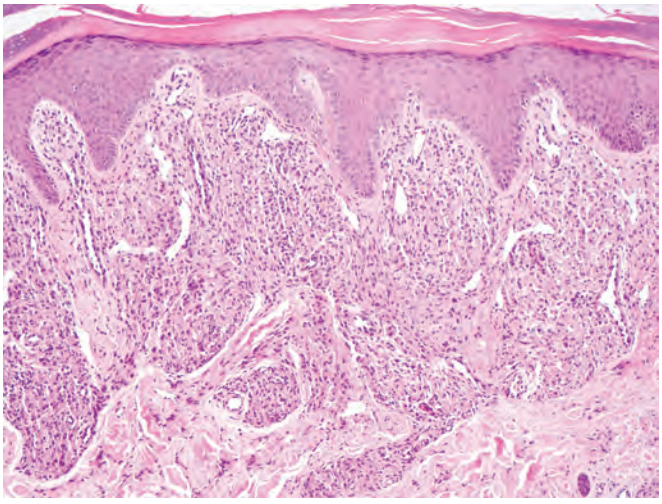
• **Figure 20.16** Histoid leprosy. This variant manifests as a prominent proliferation of spindle cells that can be confused with a fibrohistiocytic neoplasm or a sarcoma. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.18** Histoid leprosy (Wade-Fite stain). Large numbers of mycobacteria, arranged in bundles that are oriented parallel to the long axis of the cell, are seen within the histoid cells. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.17** Histoid leprosy The spindle cells show an amphophilic cytoplasm that is filled with mycobacteria. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.19** Borderline lepromatous leprosy. The infiltrate is somewhat more circumscribed, and there are more lymphocytes and a tendency to form granulomas. Note the accentuation of the neurovascular bundles by the infiltrate. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

some of the nerves because of perineural fibroblastic proliferation and plasma cells are present (Fig. 20.20). Foamy cells are much less prominent, and one does not see globi in this form.[170,171]

Mid-Borderline Leprosy. The lesions of mid-borderline leprosy are very irregularly dispersed and plaquelike but have what appear to be prominent central depressions. These lesions are also quite edematous and have been referred to as saucerlike lesions.[162,166]

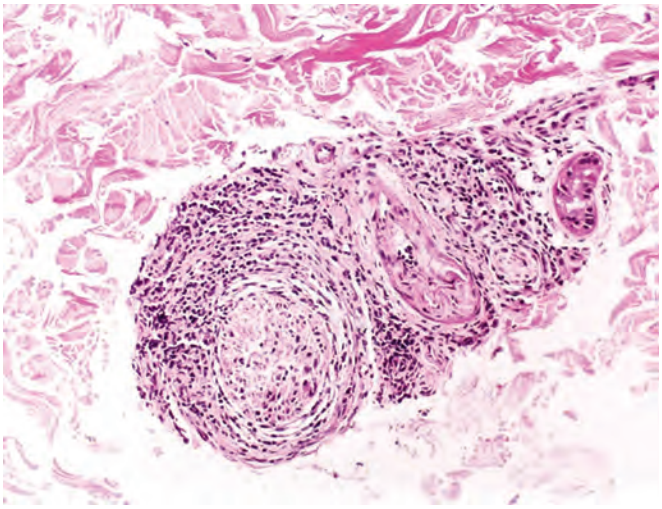
Histopathologically the monocyte-macrophages now begin to form epithelioid cells that are scattered but display no true granuloma formation (Fig. 20.21). There are also scanty lymphocytes and scattered Langhans giant cells.[170]

Borderline Tuberculoid Leprosy. Borderline tuberculoid leprosy is associated clinically with a few scattered plaques with central hypopigmentation. These plaques are also hairless. Clinically, nerve enlargement is very marked.

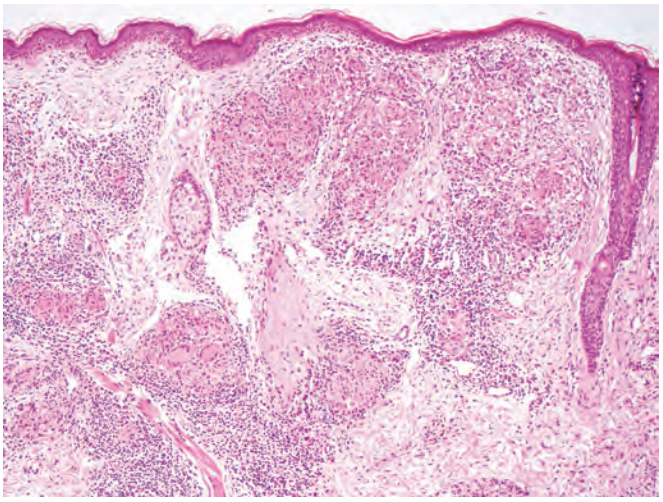
The histopathology of these lesions includes granulomas associated with peripheral lymphocytes that follow along the neurovascular bundles. Occasional Langhans giant cells are noted (Fig. 20.22). Accumulation of granulomas along the superficial vascular plexuses is often observed (Fig. 20.23), but there is no evidence of infiltration of the epidermis itself. Bacteria are scant in number.[170,171]

Tuberculoid Leprosy. Tuberculoid leprosy is associated with the most striking immune response and manifests as very dry, scaly, hypopigmented patches or plaques with very sharply defined edges. The plaques are usually anesthetic except those on the face. The number of lesions ranges from one to five. Peripheral nerves can be very prominent.[162,166]

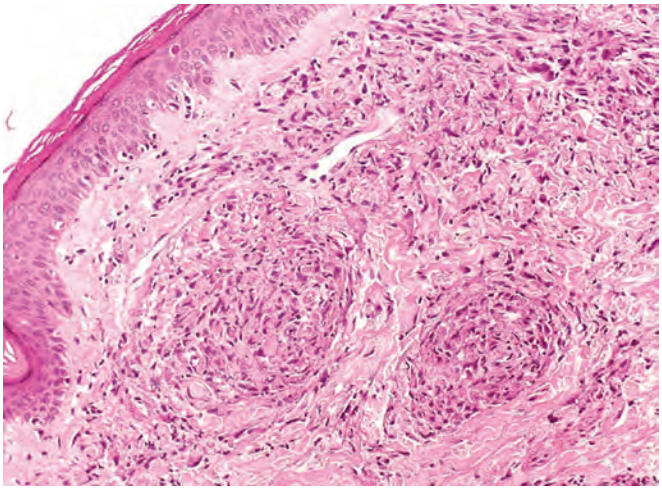
The histopathology of this lesion exhibits true epithelioid cell granulomas following along the neurovascular bundles. Langhans



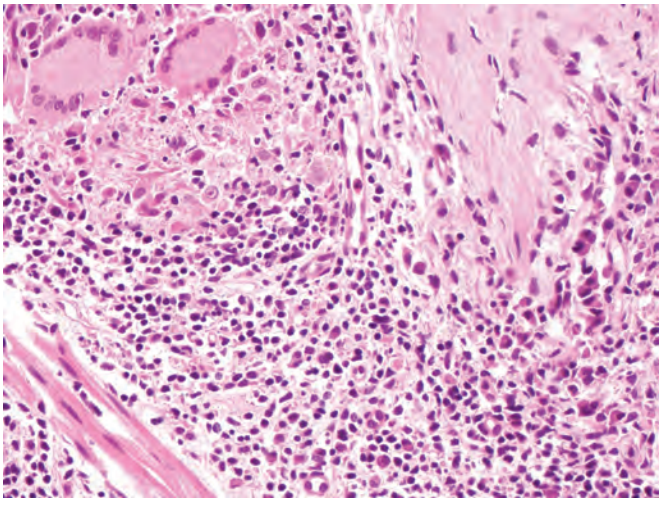
• **Figure 20.20** Borderline lepromatous leprosy. Another example of perineural "onionskin" fibroblastic proliferation, which can often be seen in this variant. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.22** Borderline tuberculoid leprosy. The granulomas are better formed and exhibit occasional Langhans giant cells and a lymphocytic cuff. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.21** Mid-borderline leprosy. Poorly formed granulomas are seen scattered in the dermis. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



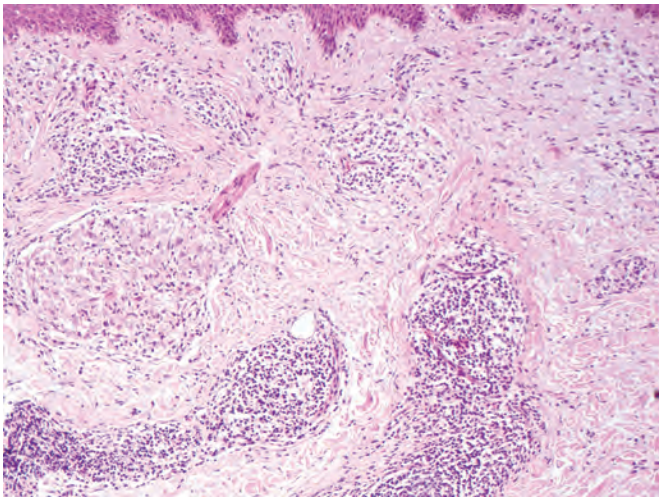
• **Figure 20.23** Borderline tuberculoid leprosy. The Langhans giant cells are surrounded by an exuberant lymphocytic infiltrate. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

giant cells can be prominent in some cases and the granulomas are associated with a dense lymphocytic cuff (Figs. 20.24 and 20.25). Lymphocytic infiltration of the nerves is frequently seen (Fig. 20.26). Acid-fast bacilli are rarely, if ever, found. The lesion is very similar to sarcoidosis or silicosis, except that the nerves show a very marked destructive appearance.[170]

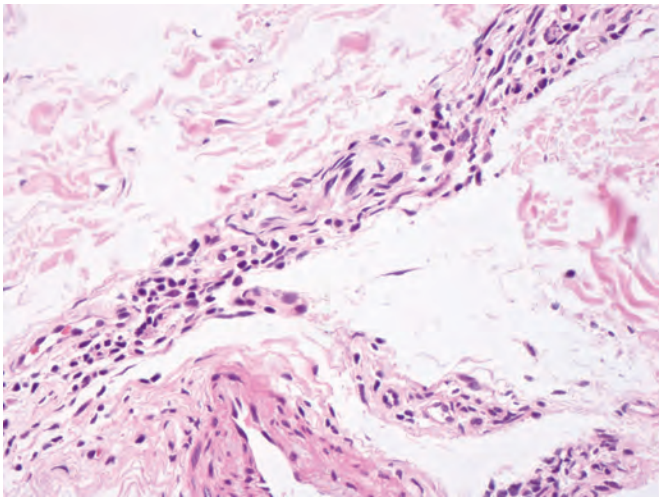
Reactions in Leprosy. The type I reactions in leprosy have not been very well characterized. The type II reaction, erythema nodosum leprosum, clinically has some features that are more akin to EM in the reaction state; this reaction occurs only in lepromatous or borderline lepromatous patients. One can observe rather tender plaques and nodules scattered over the skin surface. There are usually marked constitutional symptoms, especially malaise and arthralgias. There are numerous neutrophils scattered in the sea of infected monocyte-macrophages, and even dermal abscesses may be noted (Figs. 20.27 and 20.28). There is a perivascular infiltrate that may vary from neutrophils to neutrophils and lymphocytes with evidence of vasculitis (Fig. 20.29).[170]

The type III or Lucio reaction is again confined to patients with lepromatous leprosy who have had no treatment or inadequate treatment. The systemic symptoms are not present, but the lesions show striking, irregularly shaped, hemorrhagic plaques that can ulcerate. The histopathology differs in that there is striking luminal obliteration of medium-sized vessels by endothelial cell proliferation, with variable thrombosis (Fig. 20.30). There is a sparse lymphohistiocytic infiltrate, but acid-fast bacilli are very prominent in the walls and endothelium of normal vessels, as well as in those vessels showing the proliferative changes (Fig. 20.31). The resultant ischemic necrosis leads to ulceration in many patients.

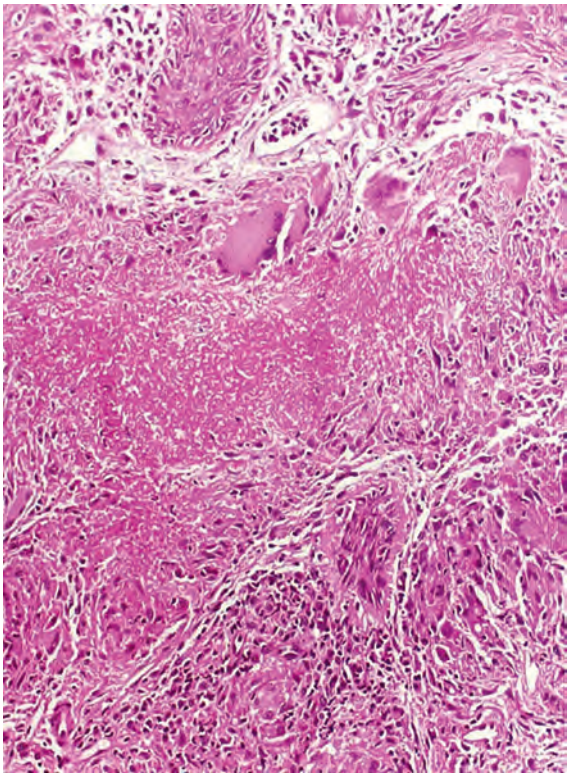
The basic pathogenetic mechanism of leprosy is a defect in T-cell activation against the mycobacteria.[162,166] This failure of reaction leads to an inability of macrophages to destroy the bacilli.



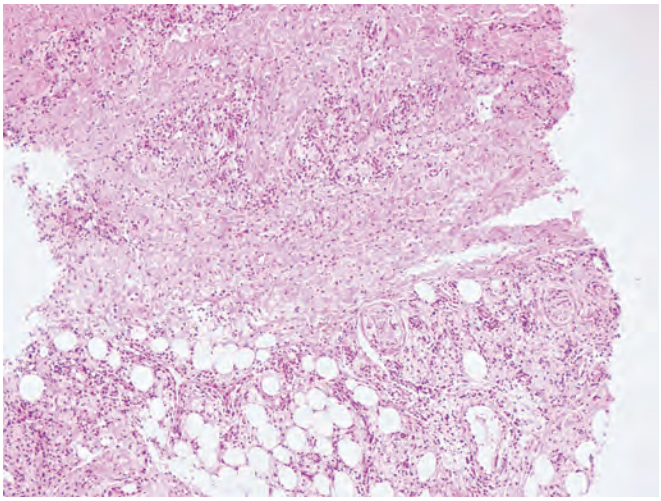
• **Figure 20.24** Tuberculoid leprosy. Well-formed granulomas are seen throughout the dermis. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.26** Tuberculoid leprosy. A perineural lymphoid infiltrate is more often seen in this variant instead of destructive granulomatous infiltration of the nerves. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.25** Tuberculoid leprosy. Depending on the immune status of the patient, central necrosis may be found in the granulomas. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

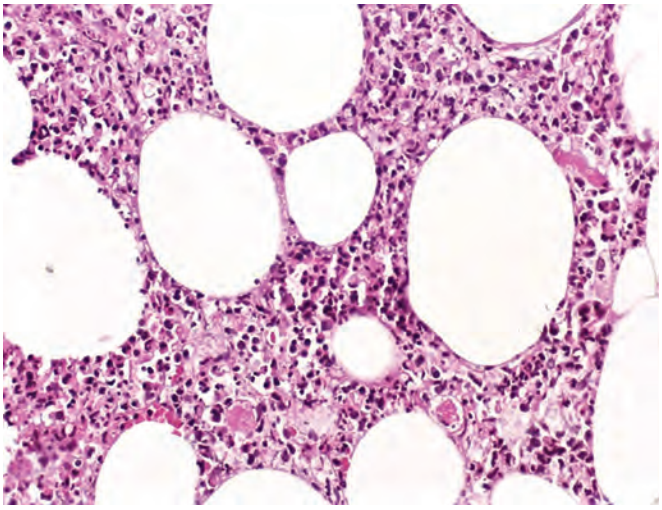


• **Figure 20.27** Erythema nodosum leprosum. Low-power view shows striking resemblance to ordinary erythema nodosum. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

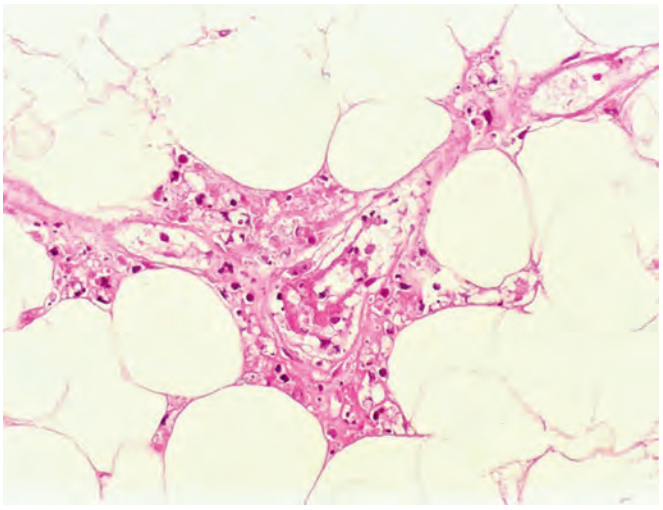
The specificity of the defect is demonstrated by patients with lepromatous leprosy reacting to other immunologic stimuli. Lymphocytes from lepromatous patients do not produce macrophage migration-inhibiting factor (MIF) after incubation with lepromin. On the other hand, patients with tuberculoid leprosy produce MIF after such exposure. Patients with tuberculoid leprosy have the ability to produce Th1 reactions, whereas those with lepromatous leprosy produce Th2 reactions. The Th2 reactions give rise to humoral immunity, which is ineffective against leprosy.[166,172,173] In those patients who have either erythema nodosum leprosum or Lucio reaction, one can find IgG and C3 in the vessel walls, indicating the probable role of immune complexes in the pathogenesis of these reactions (i.e., they are apparently type III reactions).[174]

Treponemal Diseases

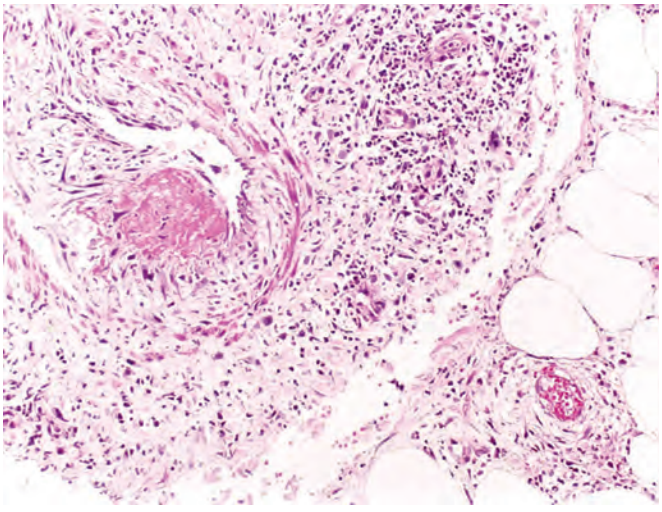
The treponemal group consists of disorders that have been classified as venereal and nonvenereal, as reviewed in the treatise by A.N. Crowson et al.[175] The etiologic agents are elongated, motile bacteria that belong to the Spirochaetaceae family, among which are the *Treponema* and *Borrelia* genera and the Leptospiraceae family, to which the *Leptospira* genus belongs.



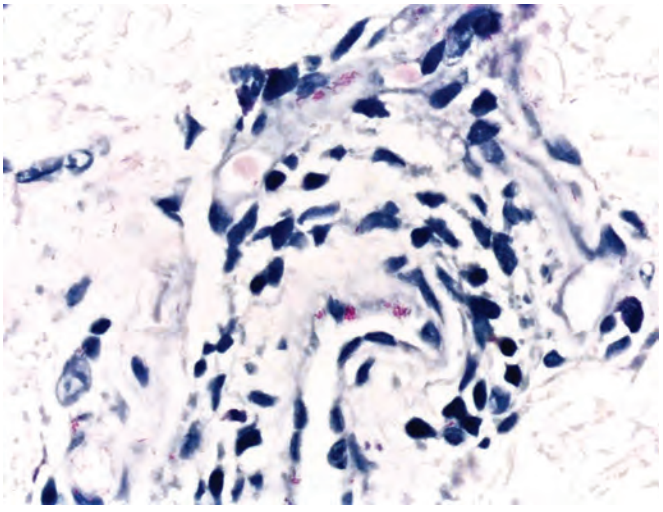
• **Figure 20.28** Erythema nodosum leprosum. Note the abundant neutrophilic infiltrate and the foci of fibrinoid material. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.30** Lucio phenomenon. Severe vascular involvement is seen in this example that exhibits thrombogenic vasculitis. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.29** Erythema nodosum leprosum. Striking vasculopathy with thrombosis and perivascular inflammatory infiltrate are seen. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.31** Lucio phenomenon (Wade-Fite stain). Colonization of the endothelial cells by the mycobacteria is characteristic. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

Venereal Syphilis

The first recorded case of venereal treponemal infection, namely acquired syphilis (also known as lues and historically "the French disease" and the "great pox"), occurred in the 15th century, and syphilis became a dreaded disease with high mortality.[176] High rates of mortality and morbidity continued into the 20th century, when increased public health awareness and the advent of antibiotics, especially penicillin, greatly reduced the incidence.[177] Recently, especially with the spread of HIV, the incidence has been increasing. Transmission usually occurs by contact of an infected site with an abrasion or minor injury. *Treponema pallidum*, the causative agent, has a transmission rate of 10% to 60%. Yaws, pinta, and endemic syphilis are the nonvenereal treponematoses.

Primary and Secondary Syphilis

Clinical Features. The primary lesion of venereal syphilis, known as chancre, appears within 21 days after exposure and manifests as a red-brown, indurated nodule or plaque 1 to 2 cm in size. It is frequently ulcerated and is painless. With the appearance of a swollen, draining lymph node, the so-called chancriform complex is complete. Lesions may appear on the tongue and be mistaken for SCC. The primary lesion may disappear spontaneously, but it always responds to appropriate therapy.

After a few weeks, a generalized eruption of reddish-brown, scaly, often oval plaques—the secondary eruption—disseminates all over the body. This eruption may resemble pityriasis rosea or psoriasis, or it can rarely be pustular.[178] Perinasal and perioral lesions in a "split-papule" pattern are characteristic. The palms and

soles are characteristically affected. Involvement of the scalp may lead to "moth-eaten" alopecia. Involvement of the anogenital or axillary areas can result in a confluence of papillomatous papules (e.g., in perianal array). These lesions are known as condyloma lata. In late secondary lues the eruption may have a serpiginous appearance and can appear as annular lesions. These lesions, if left untreated, may persist for some time or recur, as may partially treated syphilis. A particular clinical form of secondary syphilis is the so-called malignant, ulcernodular, or rupioid syphilis, which is characterized by multiple pleomorphic round to oval papules, papulopustules, or nodules with ulceration, without central clearing, and additionally exhibiting a "rupioid," oysterlike lamellated brown to black crust (Fig. 20.32). These lesions have a propensity to arise in the head and neck region, and they are often accompanied by constitutional symptoms.[179-183] This particular presentation of lues was vanishingly rare until the onset of the HIV/AIDS epidemic, after which it has been reported with more frequency.[184,185] In keeping with the "great imitator" moniker, this form of syphilis can easily mimic cutaneous lymphoma, sometimes even showing positive TCR rearrangement studies.[179] Treponemal fluorescent serologic studies are positive in almost 100% of cases.

The tertiary phase appears after the primary and secondary phases go into a latency period, or latent phase, which has been defined by the CDC to be early if present for less than 1 year or late if present for more than 1 year. The World Health Organization (WHO) uses 2 years as the cutoff between early and late latent phase.[175] Meningovascular syphilis can occur during the late secondary phase or in the tertiary phase.[186] The other manifestations include skin nodules and cardiovascular and neurologic disorders.[187,188] The skin and mucosal lesions are nodules referred to as gummas.

Congenital syphilis should be suspected in the child born of a mother with active or inadequately treated syphilis, or in the event of a reactive serologic test for syphilis, a positive test of the cerebrospinal fluid, elevated cerebrospinal fluid protein, or an elevated white blood cell count of unknown cause.[189] The clinical features of the early form include the so-called snuffles or rhinitis, hepatosplenomegaly, or a widely disseminated rash of unknown cause.[190] The late phase exhibits, among other features, the classic Hutchinson triad: notched incisors teeth (Hutchinson teeth), keratitis, and deafness. Transplacental infection occurs and is more common in mothers with secondary syphilis (approximately 50%) and less common in mothers with early (40%) or late (10%) latent syphilis.[189]

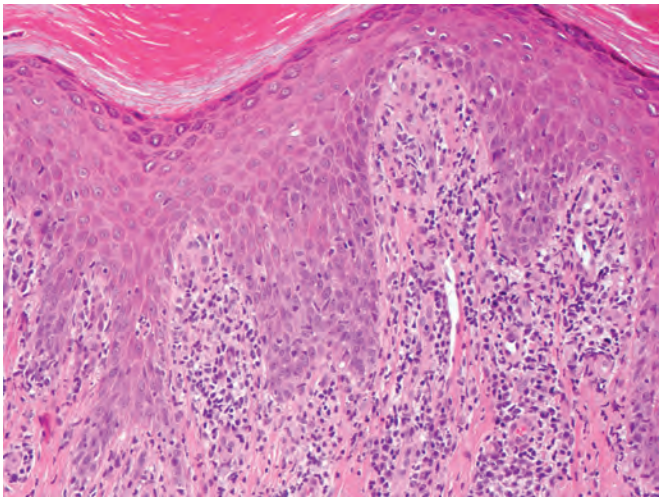
• **Figure 20.32** Secondary syphilis, malignant or rupioid variant. Note the "oyster-like" crusted lesion on this human immunodeficiency virus/acquired immunodeficiency syndrome patient's neck. (Courtesy Thomas Hagele, MD, Department of Dermatology, University of South Florida, Tampa.)

Histopathology. Two very important histologic observations, common to all forms of syphilis, are: (1) the presence of endothelial swelling, often with obliteration of the vessel lumen, and (2) the presence of a perivascular lymphocytic and plasmacellular infiltrate.

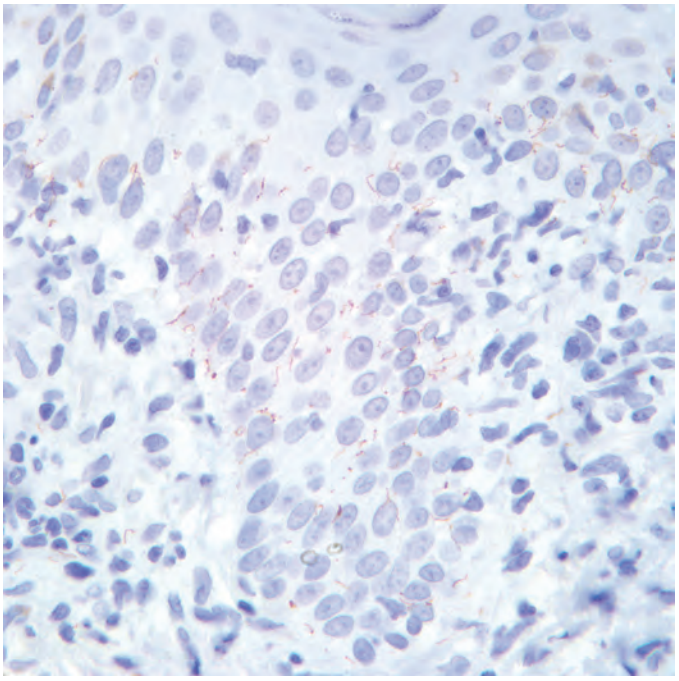
The hallmark of the primary phase of the disease, the syphilitic chancre, often exhibits a markedly hyperplastic epidermis that is frequently ulcerated. Beneath the epidermis is a dense infiltrate of mainly lymphocytes and plasma cells with scattered histiocytic mononuclear cells. A striking endarteritis obliterans is present, with the lymphoid cells and plasma cells infiltrating the walls of the vessels. Spirochetes can be identified with appropriate silver stains (Warthin-Starry or Steiner) or by electron microscopy.[191-193] When fresh material is obtained from the chancre, organisms can be seen by darkfield microscopic examination. *T. pallidum*, the causative organism, is 8 to 16 µm in length and 0.10 to 0.18 µm in width and is seen between the keratinocytes, at the basement membrane zone, and in the dermis in perivascular array.[175,191,192] The infiltrate is so dense and activated that lymphoma is sometimes considered in differential diagnosis. The regional lymph nodes draining the chancre area can exhibit reactive changes, with numerous plasma cells associated with follicular hyperplasia. Prominent endothelial cell changes can be seen, which are similar to the ones seen in the skin, discussed previously. Poorly formed granulomas may occasionally be observed. Spirochetes are plentiful and can be seen with proper staining.[194] Because silver impregnation histochemical stains are notorious for being difficult to interpret, the development of much more sensitive and specific immunohistochemical stains has been more than welcomed.[195]

The main differential diagnosis of cutaneous primary syphilis is chancroid, caused by *H. ducreyi*. However, in that disorder, the ulcer shows characteristic zonation: a superficial area with acute inflammation, a middle layer with numerous proliferating capillaries, and a lymphoplasmacellular infiltrate at the base. Giemsa staining reveals the numerous coccobacillary forms between keratinocytes and along the basement membrane zone (see later for an at length discussion of this entity).

Cutaneous lesions in secondary syphilis often show epidermal hyperplasia, which is often psoriasiform, overlying a bandlike infiltrate of lymphocytes and plasma cells (Fig. 20.33). There may be rare neutrophils with debris. Lichenoid/interface change is often seen with apoptotic keratinocytes present at the basal layer. Organisms can be quite numerous and are easily identified in the epidermis with the help of immunohistochemical stains (Fig. 20.34). Often, there are characteristically thin rete ridges in an "icepick" configuration. Beneath is a superficial and deep perivascular infiltrate with mononuclear cells that form almost granuloma-like aggregates. Endothelial swelling is prominent and may in part lead to the granulomatous appearance.[193,196,197] However, in some cases the infiltrate is focal and slight, and the endothelial swelling is the clue to the diagnosis in the presence of the plasma cells. In other cases the infiltrate may be so dense and



• **Figure 20.33** Secondary syphilis. There is a combination of psoriasiform epidermal hyperplasia with pointy rete ridges with interface dermatitis, as well as a prominent lymphoplasmacytic dermal infiltrate.



• **Figure 20.34** Secondary syphilis. Immunohistochemical stains for spirochetes can be extremely useful in identifying the organisms, which are seen in great numbers here in the epidermis.

associated with activated lymphoid cells as to suggest mycosis fungoides.[198]

In late stages of secondary syphilis, granulomas are invariably present[199] and may even suggest sarcoidosis. In 25% to 40% of early secondary syphilis, lymphocytes predominate, with the plasma cells entering later. As far as vascular changes are concerned, one may find fibrinoid necrosis in some cases of syphilis, especially in so-called lues maligna, in which thrombotic vasculitic changes maybe present.[182,183] Other features seen in malignant syphilis include pseudoepitheliomatous epidermal hyperplasia, ill-formed granulomas, and interface change.[179]

With regard to luetic alopecia, the hair follicles tend to show plugging, bulbar infiltration by lymphocytes and plasma cells, and perifollicular fibrosis. Both a lichenoid and perivascular lymphoplasmacellular infiltrate are also noted.[200]

Nodular tertiary syphilis exhibits small nodules with scattered giant cells, rare granulomas, and scattered lymphocytes and plasma cells. In gummatous tertiary syphilis, there is quite prominent necrosis that is acellular. It is associated with striking plasmacellular vascular changes that extend into the dermis.[201]

As the host's immune response becomes more and more exuberant with the evolution of the disease, in both late secondary syphilis and in the tertiary form, organisms become increasingly difficult to identify. In late secondary lues, appropriate silver stains demonstrate the spirochete in approximately one-third of cases, best visualized in the epidermis.[175] Even with the help of *T. pallidum* immunohistochemistry, the chances of identifying the organisms in tissue are markedly reduced in this phase of the disease, often requiring examination of multiple tissue levels.[202]

Yaws

Yaws, also known as *frambesia tropica*, thymosis, or *polypapilloma tropicum*, is a tropical infection caused by *T. pallidum* subsp. *pertenue*, a subspecies of *T. pallidum*. *T. pallidum pertenue* is differentiated from *T. pallidum* by substitution of a single nucleotide in its DNA.[203] Yaws is spread by skin-to-skin contact in a nonvenereal fashion. It is found predominantly in warm climates, such as the hot, humid areas of South America, Africa, Southeast Asia, and Oceania.[204,205]

Clinical Features. The site of inoculation often shows an area of erythema and involves usually the lower limbs. Approximately 15 to 21 days after inoculation, a nodule appears at the site; it enlarges centrifugally to a lesion 1 to 5 cm in size, with a reddish, crusted appearance, surrounded by pustules. The lesion is said to resemble a raspberry and therefore has been dubbed "frambesiform." Lesions can spontaneously disappear and leave a scar after 3 to 6 months.[205] Fever and constitutional symptoms may be present. Weeks or months later, the secondary phase of the disease ensues with affectation of skin and bones. Constitutional symptoms and generalized lymphadenopathy are seen. Cutaneous lesions are represented by the onset of a widespread eruption of smaller lesions that have been designated "daughter yaws"[206] due to their resemblance to the primary lesion. The lesions can be annular and thereby resemble syphilis, and they can, in the same way as syphilis, affect the axillae and groin areas. These annular lesions can appear in the orofacial area. Pustular lesions may be noted. Hyperkeratotic lesions also can occur on both hands and feet; when present on the feet, they result in an awkward crablike waddling gait known as "yaws gait" or "crab yaws."[205] Lesions can recur for up to 5 years and affect mainly the perioral areas and the axillae. Secondary bone lesions may result in periosteal nodules and thickening.

Tertiary yaws occurs in approximately 10% of cases and in the skin produces nodules, ulcers, and serpiginous tracts, as well as scars and keloids.[207] In some cases, there is keratoderma and even palmar-plantar hyperkeratosis. Involvement of other organs is characteristic. The bone and joint lesions can include osteomyelitis and sometimes hypertrophic periostitis; chronic tibial disease can give rise to "saber shin." The facial bones can be involved with bilateral hypertrophy of the nasal component of the maxilla, an obstructive lesion known as "goundou." The tertiary phase of yaws can also give rise to rhinopharyngitis mutilans, also called "gangosa," which is characterized by ulceration and destruction of the nasal septum, palate, and surrounding bone and soft tissue,

often leaving disfiguring, mutilating scars.[205,207] These complications can all be averted by proper antibiotic therapy.

Histopathology. The primary lesion exhibits hyperplasia of the epidermis with intercellular edema and intraepidermal abscesses with focal papillomatosis. In the dermis, there is a striking infiltrate of lymphoid cells, with histiocytes and plasma cells and scattered neutrophils and eosinophils. The prominent vascular change seen in syphilis is not present.[206] The secondary form is associated with epidermal hyperplasia and papillomatosis, and the infiltrate is again diffuse without vasculopathy. The tertiary yaws cutaneous lesions resemble the ulcerative granulomatous lesions of syphilis.

One can demonstrate the organisms by darkfield microscopy from material obtained from lesions in the first two phases of the disease. On histologic samples, silver stains, such as Warthin-Starry, will reveal the organisms to be present between keratinocytes, but they do not appear in the dermis.[206,208] Developments of molecular PCR-based assays appear to be promising new tools that will aid the diagnosis of yaws and the other nonvenereal treponemal diseases.[209]

Pinta

Pinta is the only treponemal condition that affects only the skin. The disease occurs endemically in hot, humid areas of Central and South America and is not found outside the Western Hemisphere. It is caused by *T. pallidum* subsp. *carateum*. Transmission is also by skin-to-skin contact, often among family members.[209,210]

Clinical Features. This disease is limited to the skin, and its manifestations are predominantly associated with abnormalities of pigmentation. The primary lesion often appears in the lower limbs and is represented by a small, scaly, reddish to reddish-brown papule that is very pruritic and is surrounded by a halo. The lesion gradually spreads and may reach a size of 10 to 12 cm with a psoriasiform or eczematous appearance. Ulceration is not a feature of primary pinta.[209]

The secondary lesions, called pintids, are small, scaly papules that coalesce into psoriasiform plaques. Both the primary and secondary lesions are highly infectious. The third stage consists of hypopigmentation over the ankles, wrists, knees, and elbows. These areas are mixed with areas of hyperpigmented or hypopigmented skin. The hypopigmentation is often symmetric and, in late achromic stages, has a stockinglike or glovelike appearance. Both atrophic and hyperkeratotic areas may be present in a given lesion.[211]

Histopathology. Histologically, irregular epidermal hyperplasia with a sparse infiltrate of lymphocytes, plasma cells, and neutrophils around dilated vessels is characteristic of both the first and second stages,[207] with melanophages more prominent in the second stage. In the third stage, there is either marked hyperpigmentation with numerous melanophages or profound depigmentation with a scattered lymphocytic and plasma cellular infiltration. The organisms are present in all but the very late tertiary-stage hypopigmented lesions.[206,207,211]

Endemic Syphilis

Endemic syphilis, also known as *bejel*, *belesh*, or *bishel* in Arab countries and as *dichuchwa* and *njovera* in African countries, is another disease caused by a *T. pallidum* subspecies, namely *T. pallidum* subsp. *endemicum*. This particular organism is also limited in its geographic location, to the southern border of the Sahara Desert and the Arabian Peninsula.[205,211] Transmission is produced via skin-to-skin contact and affects mainly children up to 15 years of age. It is also known to spread by common use of pipes or drinking cups.

Clinical Features. The primary lesions are rarely identified and sometimes manifest as papules or ulcerated lesions of the oropharynx. Rarely, an infected infant can give the disease to a nursing mother, with a resultant nipple lesion occurring.[211]

On the other hand, secondary lesions are common and may be the first sign of the disease. Small, painless papules and shallow ulcers appear on the lips, mouth, pharynx, and even larynx, with resultant hoarseness. Regional adenopathy is common. Less commonly, one can find condyloma lata–like lesions in the axillae and the groin.

The tertiary phase can manifest as gummatous lesions that form ulcers that scar and affect skin, nasopharynx, larynx, and bone. The bony lesions can greatly resemble those of yaws, in which one can find marked periosteal thickening. In addition, there can be perforation of the nasopharynx or the palate.[205,211]

Histopathology. The histology of the lesions appears to be similar to what one would find in venereal syphilis. Early lesions show a dermal perivascular inflammatory infiltrate composed of lymphocytes and plasma cells. Granulomas may be present. Lesions in the late, ulcerated stage show epidermal and epithelial hyperplasia with a loosely arrayed lymphohistiocytic and plasmacellular infiltrate. Spirochetes are present in the early lesions, with prominent intraepidermal involvement.[207,211,212]

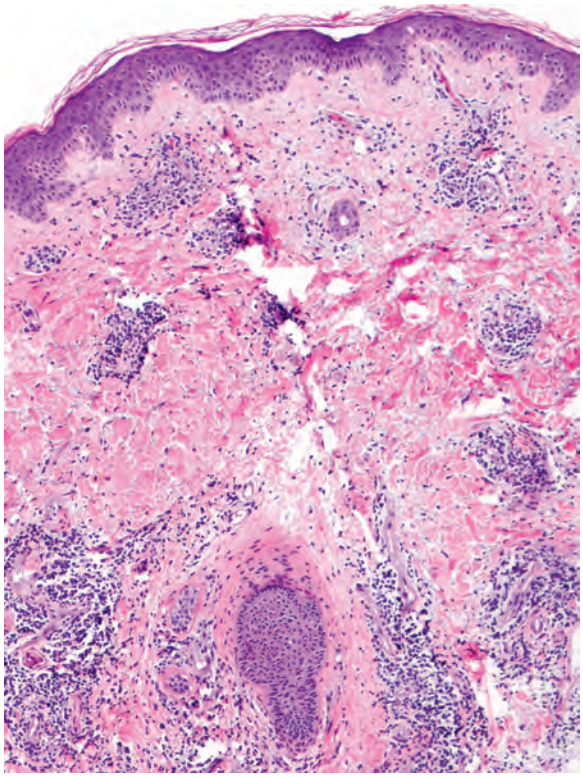
Borrelioses

Lyme Disease

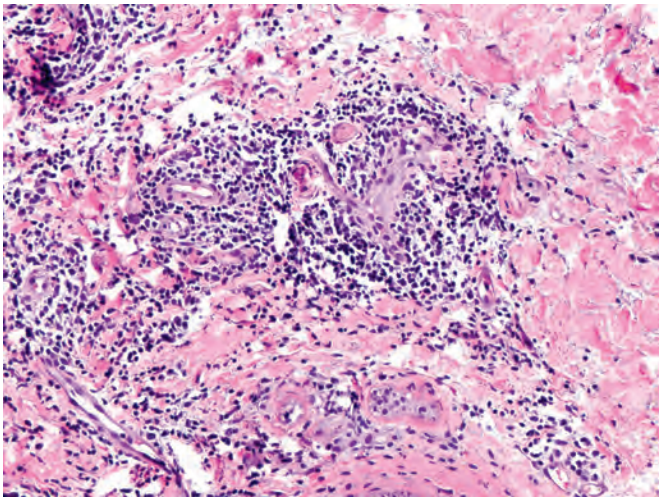
Lyme disease derives its name from the city of Lyme, Connecticut, where the first epidemic of the disease was described.[213] The lesion is caused by the spirochete *Borrelia burgdorferi*, which is primarily transmitted by the tick *Ixodes dammini* (deer tick). Other soft ticks of the *Ornithodoros* genus have also been implicated.[214] The disease has been identified in up to 32 states in the United States, as well as elsewhere in the world, including in Europe, Africa, and Asia.[215]

Clinical Features. There are three phases of the disease. The first is associated with a characteristic, albeit nonpathognomonic, lesion at the site of inoculation, the so-called *erythema chronicum migrans* (from new Latin "chronic migrating redness"), also known as *erythema migrans*. This lesion appears 3 to 30 days after the tick bite as a red papule that then spreads centrifugally with central clearing. The lesion can reach a size of 25 cm in diameter.[216] Some variants of the presentation include hemorrhagic vesicles and linear lesions. This component lasts for approximately 1 month in the American variant and 2 to 3 months in the European variant. The lesion may be associated with hematogenous spread and can result in multiple cutaneous lesions, orchitis, pneumonia, and arthritis, all associated with fever. The second phase of the lesion involves mainly the cardiac and neural systems. The nervous system changes can include meningitis, neuritis, radiculoneuritis, and even cerebritis. Heart involvement includes heart block and arrhythmias associated with myocarditis. The third phase of the disease shows predominantly chronic neural symptoms in European patients and musculoskeletal symptoms in patients in the United States.[217]

Histopathology. The primary lesion is associated with full-thickness involvement of the dermis by a prominent lymphoplasmacellular infiltrate with rare eosinophils that is superficial and deep. The lesion is angiocentric with a vasculopathy, neurotropic, and eccrinotropic (Fig. 20.35). The vessel involvement includes



• **Figure 20.35** Lyme disease (erythema chronicum migrans). Note the superficial and deep perivascular lymphocytic and plasmacellular infiltrate.



• **Figure 20.36** Lyme disease (erythema chronicum migrans). The infiltrate is composed predominantly of lymphocytes and plasma cells. Note infiltration of the vascular walls.

transmural infiltration of lymphocytes and plasma cells, with a dissecting effect on the vessel architecture (Figs. 20.36). There is often a granulomatous component to the vasculitis and neuritis and, at times, a thrombogenic vasculopathy. An interstitial infiltrate is associated with a sclerosing reaction. Plasma cells are more intensely seen in the periphery of the lesions, with eosinophils more in the center.[214,218] A reactive psoriasiform hyperplasia with spongiosis is commonly observed. An exuberant lymphoid reaction displaying a pseudolymphomatous pattern ("lymphocytoma cutis") has also been described as associated with *B. burgdorferi* infection.[219] The lesion can resemble a prominent delayed hypersensitivity reaction, as seen especially in association with an arthropod bite or drug reaction. One must also consider a connective tissue disease. Other figurate erythemas can also give a similar picture.

The spirochetes can be seen in almost half of the lesions in the advancing border, where they manifest as scattered organisms measuring 10 to 25 μm by 0.2 μm. Myocardial biopsy specimens show an interstitial lymphoplasmacellular infiltrate with a band-like infiltration beneath the endocardium. Acute myocarditis can also be seen. However, the diagnosis of Lyme disease is most often done through serologic and molecular (PCR-based assays) means.[219,220]

Infections Caused by Organisms From the *Bartonella* Genus

These organisms are gram-negative bacilli and coccobacilli that are part of the Alphaproteobacteria class, which also includes organisms of the *Rickettsia* genus. They produce a variety of disorders, including cat-scratch disease, bacillary angiomatosis, Carrión disease (Oroya fever and verruga peruana), as well as trench fever. The first three entities will be described because they tend to show cutaneous manifestations.

Cat-Scratch Disease

After much investigation, *Bartonella henselae*, a gram-negative bacillus, is now thought to be the cause of this disease.[99,221]

Clinical Features. A small papule or macule resembling an insect bite is the first sign of the disorder and usually occurs at the site of a cat scratch or bite. The lesions usually occur in children, often on the hand or forearm, and are nonpruritic.[222] After 10 to 14 days, the small (5.0 mm wide) maculopapules resolve spontaneously. However, some patients develop fever or other constitutional symptoms, as well as other skin lesions, such as morbilliform and even EM-like eruptions.[223] The chief problem in the patients who develop a reaction to the bite is regional lymphadenopathy, which appears several weeks after the original trauma. The node may suppurate and break down. The conjunctiva is often the site of entry in Parinaud oculoglandular syndrome, with patients exhibiting conjunctivitis, retrotarsal granulation tissue, and preauricular lymphadenopathy.[1]

Histopathology. The characteristic picture of the site of inoculation includes a palisading granulomatous response resembling granuloma annulare around zones of necrobiosis. Included in the palisading inflammation are lymphocytes, eosinophils, and multinucleated giant cells. The morbilliform rashes that can occur show interface dermatitis with a perivascular lymphoid and plasmacellular infiltrate both superficial and deep and may even include a lymphocytic panniculitis. The small (0.2 to 0.5 μm), gram-negative rods can also be identified with the Warthin-Starry silver impregnation histochemical stain. The lymph node histology includes the distinctive stellate microabscesses with granulomatous inflammation surrounding foci of necrosis. As the lesions become more chronic, a more granulomatous response appears. Organisms can be identified in this area but in early stages of the lesion.

Because the lesion exhibits a palisaded appearance histologically, one must consider other lesions in the differential diagnosis. The clinical presentation and history should be helpful. The stellate abscesses of lymph nodes can be seen also in tularemia,

lymphogranuloma venereum, sporotrichosis, and yersiniosis. Likewise, some acid-fast infections, as well as Kawasaki disease and Kikuchi disease, must also be considered.[1]

Because of problems in culture of the organism, intradermal skin tests (Hanger-Rose and Mollaret tests) have been used historically to aid in the diagnosis. However, new PCR assays, as well as serology using immunofluorescence antibody (IFA) and enzyme-linked immunoabsorbent assay (ELISA) methods, are currently used for diagnosis.[221,224]

Bacillary Angiomatosis

Bacillary angiomatosis has been described mainly in immunocompromised patients, many of whom were individuals with HIV/AIDS[225-227] with low $CD4^+$ counts, but has also been seen in patients who have undergone organ transplantation or are immunosuppressed due to chemotherapy.[228,229] Bacillary angiomatosis has also rarely been described in patients with no known immunosuppressive disorder.[230] Both *B. henselae* and *B. quintana* have been described as the main organisms that cause this disorder.[224] The angiomatosis variant refers to papulonodules of the skin that have a granulation tissue–like appearance. If the liver or spleen is affected, the disorder is called bacillary peliosis. It has been known to occur also in the gastrointestinal tract, lungs, soft tissues, and even bone.

Clinical Features. Clinically, pyogenic granuloma-like nodules appear in the skin. In some instances, they are related to a cat scratch. Rarely, the lesions manifest as subcutaneous nodules or even fungating ulcerated lesions.[229] In patients with AIDS, the disorder appears when $CD4^+$ T-lymphocyte counts are low (<100 cells/mm^3) and can go on to systemic disease with death. The disease course can be fulminant and intractable in some AIDS patients, leading to death.[227]

Histopathology. In the dermis, single or multiple vascular proliferative nodules are associated with an inflammatory infiltrate in which there are both neutrophils and mononuclear cells. On low power, the changes resemble granulation tissue.[229] On higher-power examination, one can appreciate extracellular smudgy areas that are bluish to amphophilic but actually represent masses of bacilli. There are scattered neutrophils with debris around these pale, amphophilic, amorphous, and smudged areas. The organisms can be highlighted by tissue Gram, Wharthin-Starry, GMS, and Giemsa histochemical stains as gram-negative cocci and coccobacilli. Electron microscopy can be used in difficult cases.[228] Immunohistochemistry has been developed to identify the organisms.[224,231]

As far as differential diagnosis is concerned, the most important consideration is to rule out Kaposi sarcoma (KS), which can be easily done using human herpesvirus 8 (HHV-8) immunohistochemistry. In addition, one must also consider benign vascular tumors, such as pyogenic granuloma, epithelioid hemangioma, and the so-called cutaneous epithelioid angiomatous nodule (CEAN).[232] The most useful and helpful clue is an appreciation of the pale blue or amphophilic granular smudged areas that abut vessels.

Carrión Disease

This tropical disease, mainly confined to Peru, Ecuador, and Colombia, is caused by *Bartonella bacilliformis* and is transmitted by the phlebotomus *Lutzomyia verrucarum* ("sand fly"). Both heavy deforestation and climate change, including more pronounced El Niño phenomenon, have contributed to the spread of this disease.[224] The clinical picture consists of two phases: (1) the so-called Oroya fever, an initial acute phase characterized by systemic manifestations, such as fever, hemolytic anemia, headache, pallor, myalgia, and arthralgia, followed by a (2) widespread eruption of verrucous lesions, predominantly on the head and extremities, called verruga peruana (literal translation: "Peruvian wart").[224] Diagnostic methodology using IFA and ELISA is available for diagnosis.[231]

Clinical Features. The clinical lesions of verruga peruana can appear 2 weeks to up to years after the initial phase. Lesions somewhat resemble those of bacillary angiomatosis. Patients develop pleomorphic, red to purple ("ferruginous") nodules and papules, some arranged in plaques and some fungating with a tendency for bleeding. There are three described forms of verruga peruana: (1) a milliary form, showing widespread small reddish papules, some showing a collar (penumbra), (2) a mular form, characterized by larger (>5 mm) erythematous sessile or pedunculated nodules, and finally (3) a diffuse form that shows widespread subcutaneous lesions.[224] The milliary form appears to be the most common presentation.

Histopathology. Although the overall histopathologic features are somewhat similar to those of bacillary angiomatosis, namely an exuberant angioblastic-appearing vascular proliferation, cutaneous lesions of verruga peruana tend to be much more cellular, containing dermal dendrocytes and inflammatory cells. In addition, the lesional cells can show considerable atypia, mimicking sarcomatous or other malignant tumors.[233] The organisms can be visualized in the extracellular space in clumps or intracellularly. They can be highlighted by Warthin-Starry, Giemsa or GMS histochemical stains or by recently developed immunohistochemistry.[224,231]

Rickettsia and Rickettsia-Like Organisms

Much like organisms in the Chlamydiae phylum (discussed later), these unusual bacteria are obligate intracellular parasites and are somewhat midway between bacteria and viruses. Because their habitat is the nutrient-rich cytosol of parasitized cells, these organisms have a unique genome that lacks portions that encode diverse enzymes related to sugar, amino acid, and lipid metabolism.[234] Confusion regarding classification had plagued this group of organism for some time. In the past, a great number of organisms, including *Coxiella* spp., were classified as part of the *Rickettsia* genus. This well-known taxonomic conundrum has since been partially resolved through phylogenetic studies giving rise to a group of *Rickettsia*-like organisms.[234] We currently know that such organisms as *Coxiella burnetii* are part of the Legionellales order.[235] In addition, the agent of the scrub typhus, formerly designated *Rickettsia tsutsugamushi*, is now called *Orientia tsutsugamushi* and is part of the Orientia genus. New species, as well as new strains of existing species, are being characterized, and there is also reemergence of "older" organisms.[234] The Rickettsiales order contains organisms that are broadly divided into the spotted fever and typhus groups.

Spotted Fever Group

Rocky Mountain Spotted Fever. The first organism to be considered in the spotted fever group is *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever (RMSF), which is the most common tick-borne disease in the United States.[236] The disease is distributed mainly in countries of the Western Hemisphere.[237] RMSF is considered to be one of the most virulent infections in humans. This bacterium is a small (0.2 to 0.5 μm),

fastidious, gram-negative, pleomorphic coccobacillus that infects vascular endothelial cells, causing severe systemic manifestations. Ticks from the Ixodidae family (hard ticks), particularly the ones from the *Dermacentor* genus, act as both reservoirs and vectors of this organism.[238] Other mechanisms of infection include blood transfusion and inhalation of contaminated aerosol, the latter occurring almost exclusively in laboratories.

Clinical Features. The mean incubation period of RMSF is 7 days, and multiple systems and organs are characteristically affected. The early manifestations include sudden onset of fever, malaise, and severe headache. A rash is present in only 3% of the cases in this phase. Rather, the cutaneous lesions typically appear 2 to 5 days after the onset of the fever, thereby completing the classic triad of fever, headache, and rash.[237,239,240] The rash is composed initially of 1- to 5-mm, blanching erythematous macules on the wrists and ankles. These lesions extend first centrifugally to the palms and soles and then centripetally to the trunk.[239,240] In severe infections, widespread skin necrosis and gangrene may even require amputation.[241] The rash is absent in 9% to 12% of cases, and this phenomenon is related to fatal cases, older patients, and occurrence of the infection in African-American patients.[237]

The clinical diagnosis of RMSF is always troublesome due to the wide array of manifestations that result from affectation of multiple organs and systems. Serologic tests can be helpful, with the caveat that specific antibodies do not appear until 7 to 10 days after disease onset.[242] The classic Weil-Felix agglutination test has become somewhat obsolete and is falling into disuse, with ELISA-based assays being currently favored.[237] As far as direct detection of the organisms, IFA-based assays are considered as the gold standard, albeit with a lack of specificity and overlapping among species. There are now available more specific methods, such as newer quantitative PCR-based molecular assays, that solve this problem.[243]

Histopathology. Because the infection is targeted toward endothelial cells, it is not surprising to find prominent lymphohistiocytic and sometimes-neutrophilic small-vessel vasculitis. There is a spectrum of histologic findings according to the stage of the disease and the severity of the infection. In the acute phase, maculopapular lesions exhibit a tight perivascular infiltrate that extends into the vessel walls, causing endothelial cell swelling, extravasation of red blood cells, and edema. More advanced lesions show a clinical presentation with hemorrhage and petechiae caused by a leukocytoclastic vasculitis with endothelial necrosis. Focal fibrin thrombi and an interface dermatitis also appear at this stage. Rare findings represent an eccrine hidradenitis and acute lobular panniculitis.[244]

The differential diagnosis is quite broad and includes conditions exhibiting small vessel vasculitis, such as insect bites or drug reactions, and diseases associated with leukocytoclastic vasculitis.[244] Rickettsial antigens can be demonstrated in paraffin-embedded sections with immunohistochemical stains. This can be useful in making the diagnosis, especially when a rash is biopsied in the acute phase.[245]

Other *Rickettsia* Species From the Spotted Fever Group. The other rickettsial organisms that form the spotted fever group cause conditions that clinically are similar to RMSF but with the main difference that an eschar is formed at the site of the tick bite (inoculation eschar).

One of the principal members of this group is *Rickettsia conorii*, which has numerous strains and is distributed in the Mediterranean area. The various strains of the organism cause the disease known as Mediterranean spotted fever, which has some regional characteristics.[246]

Another related disease that is part of the spotted fever group is African tick bite fever. This condition, caused by *Rickettsia africae*, has been found predominantly in sub-Saharan Africa, and in the Caribbean.[247]

Typhus Group

The name typhus is derived from *tuphos*, the Greek word for "smoke," because the disease characteristically affects the sensorium. Typhus, also known as epidemic typhus, is related to poor sanitary conditions (e.g., wars, famines) and was probably first identified in as early as 1083 in Spain. However, there is considerable clinical overlap and nomenclature confusion with typhoid fever (caused by the enterobacterium *Salmonella typhi*), which sometimes impedes correct classification.[248] This group is composed of two organisms: *Rickettsia typhi* and *Rickettsia prowazekii*. *R. typhi* is transmitted by fleas and causes the murine typhus and is more common in warm climates, whereas *R. prowazekii* is transmitted by body lice *(Pediculus humanus corporis)* and has a peak in transmission during cold months.[249]

Clinical Features. The incubation period is usually 10 to 14 days, and the ulterior clinical course is dominated by high fever, as well as debilitating headaches and myalgias.[249] Dermatologic manifestations are quite common, and they appear first as a nonconfluent, blanching, erythematous rash on the trunk, which evolves into a nonblanching petechial and even purpuric rash with centrifugal spread to the extremities.[249,250] Similarly to RMSF, severe cases may exhibit symmetric gangrene of fingers and toes.

The so-called Brill-Zinsser clinical form represents a reactivation of the disease that can occur years after the primary episode. This reactivation phenomenon is unique to this group of rickettsial diseases and produces as a milder and shorter episode in comparison with the original infection. However, it can be fatal in some cases.[249,251]

As is characteristic of this type of organisms, the clinical diagnosis can be quite troublesome. Several methods can aid the clinician, including culture and PCR-based assays.[249]

Histopathology. Common to all rickettsial infections, biopsies will show massive injury of endothelial cells. This results in a widespread vasculitis with concomitant edema, extravasation of red blood cells, and activation of the coagulation cascade with formation of thrombi. These thrombi are accompanied by inflammatory infiltrates composed of macrophages, lymphocytes, and plasma cells.[249]

Rickettsia-*Like Organisms*

Scrub Typhus. Caused by the reclassified organism *O. tsutsugamushi*, scrub typhus is a systemic disease that occurs predominantly in Asian countries and is transmitted by mites of the *Leptotrombidium* genus ("chiggers").[252]

Clinical Features. Early in the disease, one finds the triad of a cutaneous eschar at the site of inoculation, regional lymphadenectomy, and a maculopapular rash. The presence of these eschars is one of the most useful tools to make the diagnosis clinically, and they are most frequently found where clothes and skin meet, namely in the axillae, groin, neck, waist, and inguinal areas.[253] Eschars usually begin as a small papule, which evolves into a necrotic lesion with a black crust and an erythematous halo. The disease then becomes systemic and affects multiple organs.[252]

Histopathology. Histologically, there is dermal vasculitis with a lymphohistiocytic perivascular infiltrate and necrosis.[252]

Immunohistochemistry can be extremely helpful to identify the organisms in biopsy samples taken from the eschars.[254] Molecular and serologic tests are also available to aid in the diagnosis.[252]

Q Fever. Q fever is a zoonosis caused by *C. burnetii* that has a worldwide distribution with the exception of New Zealand.[235] It can be acquired through inhalation of contaminated aerosols, which makes this organism highly infective and a potential category B biologic weapon.[44,255] Another possible means of contamination is consumption of unpasteurized dairy products.[256] Known reservoirs of the organism include cattle, sheep, and goats.[235] This intracellular bacterium parasite infects human macrophages and interestingly also can infect free-living amebas, such as *Acanthamoeba* spp., which serve as "microscopic reservoirs."[257]

The clinical course can vary from an asymptomatic infection to a systemic disease with prominent involvement of the myocardium and the lungs. The clinical manifestations in the acute phase depend on host factors (e.g., gender, age, immune status), inoculum size, strain specificity, and route of infection. The cutaneous manifestations of Q fever are not specific and are usually represented by transient punctiform rashes, maculopapular eruptions, and, rarely, erythema nodosum.[258,259]

Chlamydial Infections

There are at least two clinically significant disorders that belong in this group. One is an infection by *Chlamydia psittaci* (now known as *Chlamydophila psittaci*) that is transmitted by birds (psittacosis), whereas the other is a venereal disease caused by *Chlamydia trachomatis*, lymphogranuloma venereum (not to be confused with granuloma inguinale, another venereal condition caused by *Calymmatobacterium—Klebsiella granulomatis*). Psittacosis is predominantly a disease that primarily affects the lungs, resulting in atypical pneumonia with only indirect and rarely seen cutaneous manifestations (Horder spots and sometimes erythema nodosum-like rash). Therefore we will devote this section to lymphogranuloma venereum.

Lymphogranuloma Venereum

This sexually transmitted disease is more common in African Americans and occurs more frequently in areas of warm climate, in a similar distribution to granuloma inguinale (discussed previously). However, outbreaks in Europe and the United States occurring in he first decade of the 21st century, particularly in homosexual males, indicate that this infection appears to be reemerging.[260] The causative organism is the intracellular obligate organism *C. trachomatis*, specifically the L1, L2, and L3 serotypes.[260,261]

Clinical Features. The genital lesions appear an average of 7 days after contact and manifest as small (2 to 3 mm) papules or ulcers that are usually nontender. This lesion heals rapidly and may be associated with a nonspecific urethritis. Within 10 days to 2 weeks after the initial lesion appears, inguinal adenopathy appears in men and is associated with the so-called groove sign, which results from enlargement of lymph nodes surrounding the Poupart ligament. Inguinal involvement can also occur in women, but, at times, because of infection of the lower vagina, the affected nodes are the iliac and anal rectal. Such nodal involvement can lead to rectal stricture. As late complications, massive edema of the penis and scrotum can occur in men and marked vulvar edema in women.[260,262]

In the differential diagnosis the lesions of chancroid are larger than those of lymphogranuloma venereum and very painful, but swollen nodes can be found in both disorders. The cultures of *Chlamydia* and serologic studies, as well as smears for *H. ducreyi*, are very helpful in differentiating these lesions. Granuloma inguinale has much larger ulcers with prominent granulation tissue. The classical Frei test, similar to the tuberculid test, has fallen into disuse in favor of more sensitive and specific serologic and PCR-based assays.[260,261]

Histopathology. The cutaneous lesions have a granulomatous inflammation associated with endothelial cell swelling, histiocytes, activated lymphocytes, and plasma cells. Only rarely are the organisms visible by Giemsa stain. On the other hand, the lymph nodes have stellate microabscesses in which necrotic debris is surrounded by granulomatous inflammation.[261]

Viral Infections of the Skin

Diseases caused by viruses are common, and many virus types can affect the skin. With the onset of widespread transplantation of solid organs and bone marrow, as well as with the HIV/AIDS epidemic, even more viruses have emerged and reemerged, often producing serious sequelae in these immunocompromised patients. Viruses are intracellular obligates with a genome composed of either DNA or RNA and no replication machinery. Thus they replicate within the host cells by taking over their ribosomes and mitochondria. The infection process is similar throughout viral organisms and includes, first, attachment of the infecting agent to specific cell surface receptors present in the host cell membrane. The virus then enters the cell cytoplasm via endocytosis, during which the virus becomes covered with part of the plasmatic membrane. This mode of entry is associated with coating of the virus by glycolipoproteins. Digestion of the outer coat of membrane takes place in the cytoplasm, whereupon the viral material or nucleoids lose their structural framework. At this point the viruses are considered to be in an "eclipse phase," virtually disappearing within the cell. They reappear only after sufficient replication of new virions has occurred. The proteins that are produced after viral infection of a cell are more characteristic of the virus rather than the host cell. Finally, the release of new virions to the exterior occurs by lysis of the cell or by budding from the cell surface.

Viruses can only be observed by light microscopy when they are gathered to form an inclusion body. Although the size of the individual virus ranges from 10 nm in the case of echoviruses to 300 nm for viruses of the pox family, 7 μm is the average size of the viral inclusion body. Most of them occur in the nucleus, particularly in DNA viruses. The inclusion bodies are found by light microscopy in only three groups of viruses: herpesviruses, papillomaviruses, and poxviruses. However, we can indirectly identify the presence of certain viral organisms via cytopathic changes like is the case in the human papillomavirus (HPV) group (koilocytes).

One the most common viral organisms affecting the skin are members of the Herpesviridae family, which includes the following subfamilies: *Alphaherpesvirinae* (herpes simplex 1 and 2 and varicella-zoster), *Betaherpesvirinae* (cytomegalovirus [CMV], HHV-6, and HHV-7), and *Gammaherpesvirinae* (Epstein-Barr virus [EBV] and HHV-8 or KS-associated herpesvirus). These are DNA viruses and thus proliferate in the nucleus of an infected cell. The second most common viral family that involves the skin is the Poxviridae. These are also DNA viruses, but they replicate in the cytoplasm of the host cell. Notable members of this family include the agents of smallpox (vaccine virus, *Orthopoxviridae*

genus), milker's nodules/orf (*Parapoxvirus* genus), and molluscum contagiosum. The third family of dermotropic DNA viruses that affect the skin is the Papillomaviridae. These viruses replicate in the host's cell nucleus and include the various genotypes of HPV. Among the RNA virus affecting the skin the main ones are the ones belonging to the Picornavirdae and Retroviridae families. The so-called hand-foot-and-mouth disease is caused by coxsackievirus A16 and belongs to the Picornaviridae family, whereas the most important member of the Retroviridae family is HIV, the cause of the AIDS.

Many other virus families can infect the skin, such as Polyomaviridae, Adenoviridae, Togaviridae, Reoviridae, Parvoviridae, Paramyxoviridae, Flaviviridae, and Hepadnaviridae. The lesions from the first three DNA virus families discussed in this section (i.e., Papillomaviridae, Herpesviridae, and Poxviridae) contain diagnostic viral inclusions. Importantly, there are specific viral agents that are associated with the development of malignancies, among which we have the HPV-driven SCCs, KS (HHV-8), and the great majority of Merkel cell carcinoma (MCC) tumors (Merkel cell polyomavirus).[263]

As far as confirming histologic changes by other methods, one can perform viral cultures. Other routes are immunohistochemistry and electron microscopy. With the use of the PCR amplification technique, one can detect the presence of the specific viral DNA. In addition, there are now probes that can be used for this detection. As far as the serologic approaches are concerned, one examines acute and convalescent sera to detect whether there has been a greater than fourfold rise in antiviral IgG. The detection of virus-specific IgM in a single acute or early recovery phase sample is very useful. This technique is currently used for certain types of infection, such as infectious mononucleosis, viral hepatitis, and parvovirus B19 infection. In any case the specimen must be obtained directly at the site of the infection during early eruption. Specimens must be kept at 4°C in the refrigerator or on wet ice. If the material must be stored for later use, one must use a freezer at −7°C.

In the following sections, we will discuss the most important virus groups affecting the skin, starting with the DNA viruses and then the RNA viruses.

Papillomaviridae

The HPV family consists of nonenveloped, double-stranded DNA viruses exhibiting extreme molecular heterogeneity with more than 150 genotypes described to date.[264] The clinical manifestations usually arise 1 to 8 months after infection by the virus. The HPV viruses target cutaneous or mucosal squamous epithelial cells mainly from the oral, cervical, or anogenital area. Initially and thanks to the pioneering work of Professor Harald zur Hausen, HPV was implicated in the carcinogenesis of carcinoma of the uterine cervix.[265,266] Now we know that different serotypes of HPV (mainly HPV-16, -18, -31, -33, and -51) play pivotal roles in carcinomas (mainly SCCs) of the external genitalia,[267,268] head and neck,[269] esophagus[270] and skin.[271] The introduction of the polyvalent HPV vaccine will hopefully produce a steep decline of infection with the most deleterious HPV genotypes with a subsequent reduction of the related malignancies.

The high-risk HPV oncogenic potential stems from integration of viral genomic material into the host's infected cells DNA resulting in production of E6 and/or E7 viral proteins, which disrupts both the Rb and p53 pathways.[272] The resultant accumulation of p16 protein that can be detected immunohistochemically acts as a surrogate marker of high-risk HPV infection. Another way to directly detect HPV genome in lesions is through ISH assays for both high- and low-risk HPV that are available to perform in formalin-fixed, paraffin-embedded (FFPE) tissue. The low-risk positive staining will show a diffuse nuclear positivity (nonintegrated pattern), whereas the high-risk stain will show pinpoint intranuclear positivity, reflecting that the viral genome is integrated within the host's DNA. Newly developed immunohistochemical stains directed to HPV L1 capsid antigen can help to highlight HPV infection, albeit not being helpful in subtyping the virus.

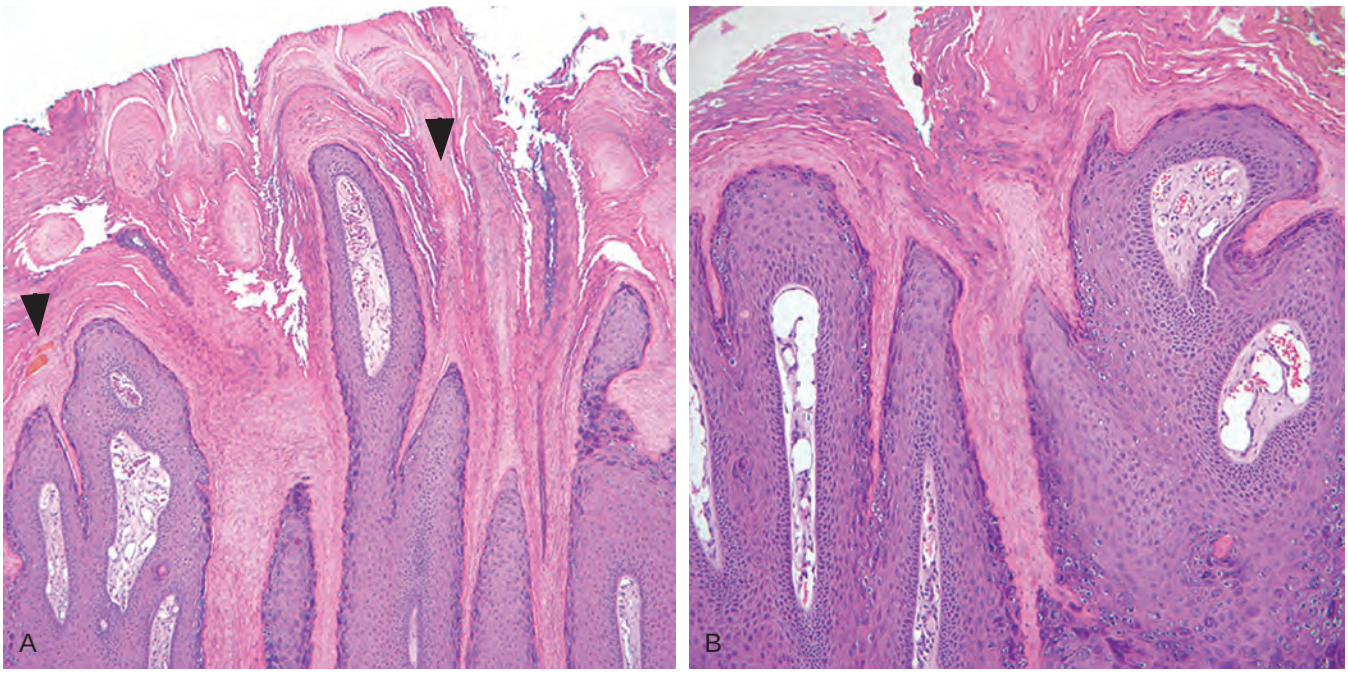
Close follow-up of patients known to harbor these HPV genotypes is recommended, particularly in the setting of immunosuppression.[273] Molecular pathology has contributed greatly to identification of this group of patients with the help of DNA hybridization or amplification by PCR of HPV genomic material.

Verruca Vulgaris and Palmoplantar Warts

Verruca vulgaris (common wart) and palmoplantar warts are related to the 1, 2, 4, 7, and 49 genotypes of HPV, which have been associated with defective cell-mediated immunity and immunosuppression of the host. The viral particles are 50-nm spherical bodies and their presence can be easily inferred in histologic sections stained with H&E by looking for cytopathic effect (koilocytes) and prominent keratohyaline cytoplasmic granules. ISH and PCR-based techniques can be used for definitive identification of the virus' genetic material, but it is rarely used because this lesion is quite characteristic, both clinically and histopathologically. Multiple lesions with features of verruca vulgaris are known to arise in up to 49% of high-stage melanoma patients undergoing systemic therapy with inhibitors of BRAF.[274] However, these lesions lack features associated with HPV infection (koilocytes among others).

Clinical Features. Verruca vulgaris commonly manifests on the dorsal aspect of the hands and fingers of children and adults, with a great predilection in butchers and slaughterhouse workers. The face and scalp are less commonly affected, whereas the oral mucosa is an exceptional site of development. The verrucae occur usually as painless, sometimes tender, whitish or pink, single lesions 1 to 10 mm in diameter. They may also occur in groups. They are well circumscribed, firm, and elevated, with a papillomatous hyperkeratotic surface. Spontaneous regression has been reported. Recurrences and appearance of new warts at sites of trauma have been documented. Palmoplantar warts can present either as a painful nodule with a keratotic plug (often related with HPV-1) or in a superficial cobblestone-like lesion that is usually painless (related with HPV-4). Both lesions usually appear in pressure points and show dark dots that are thrombosed capillaries.[275]

Histopathology. Verruca vulgaris is easily identified on histologic sections. Acanthosis, papillomatosis, hyperkeratosis, tiers of parakeratotic cells, and foci of clumped keratohyaline granules are features of verruca vulgaris, combined with a characteristic architecture of elongated peripheral rete ridges oriented toward the center of the lesion. Slender papillomatous projections and intense hyperkeratosis are characteristic of the fliform type of verruca vulgaris, which is often seen in the face and scalp area (Fig. 20.37A). The parakeratotic scale that overlies the tips of the papillomatous projections often show foci of hemorrhage (see Fig. 20.37A, *arrowheads*) and the cells from the granular layer show heavy, irregular clumps of keratohyaline are located in the intervening valleys (see Fig. 20.37B). Foci of koilocytosis in the upper



• **Figure 20.37** Verruca vulgaris. **A,** There is prominent papillomatosis along with hyperkeratosis with foci of intracorneal hemorrhage *(arrowheads)* that correspond to the areas where there are dilated papillary dermal vessels. **B,** Close examination reveals areas showing keratinocytes with prominent and coarse basophilic keratohyaline granules and perinuclear halos.

portion of the hyperplastic epidermis, together with small, round, intensely basophilic nuclei and pale-staining cytoplasm surrounded by a clear halo, are characteristic and prominent in young lesions but can be absent in old ones.

Palmoplantar warts are characteristically endophytic with ant colony–like rete configuration (also known as "myrmecia," after the name of an ant genus) (Fig. 20.38A). The keratohyaline granules are larger and appear as eosinophilic or basophilic cytoplasmic globules (see Fig. 20.38B).

In regressing lesions, lymphocytes are present in the hyperplastic epithelium in conjunction with basal cell liquefaction, epidermal degeneration, and vessel thrombosis in the upper dermis. A cutaneous horn may be present. Verruca vulgaris has only exceptionally been associated with in situ or infiltrative SCC, specifically when related with prior radiation to the site.[276] Plantar warts can very rarely show malignant degeneration into a particular entophytic SCC variant called "carcinoma cuniculatum" (rabbit burrow–like).[272]

Verruca Plana

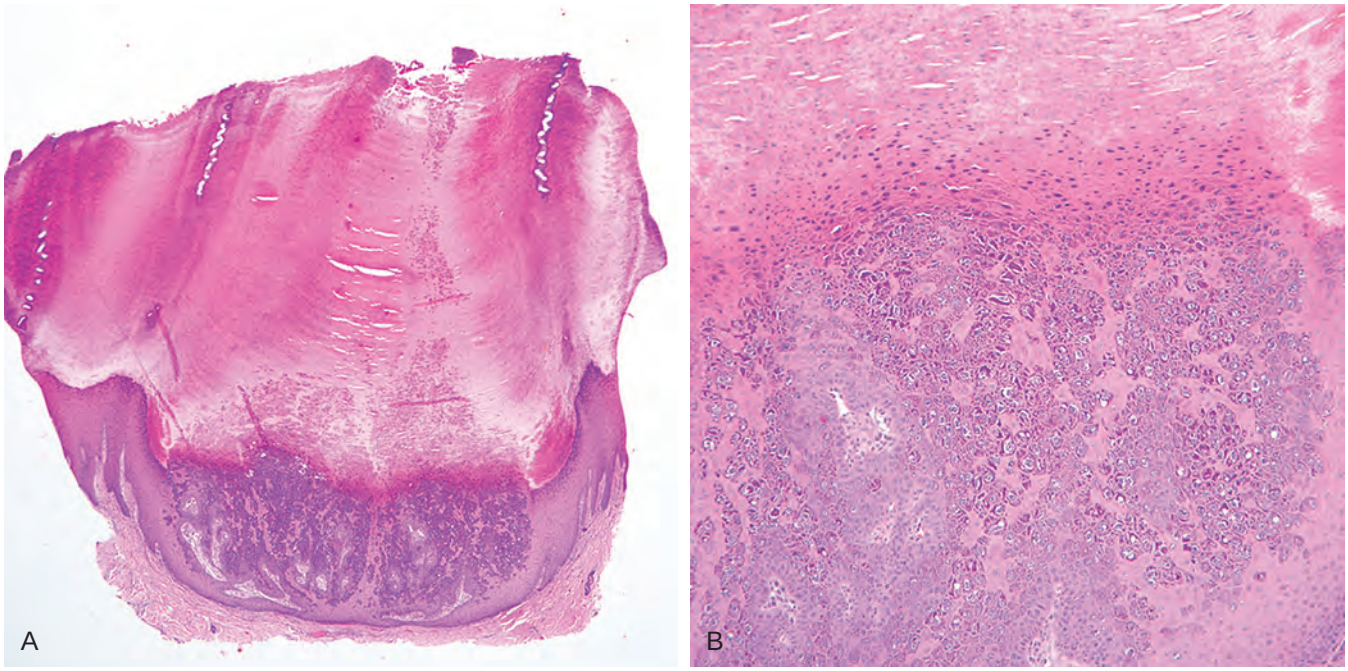
Clinical Features. Verruca plana, usually related to the low-risk HPV-2, -3, or -10 genotypes, appears as flat, grayish-yellow, possibly hyperpigmented, and slightly elevated papules of a few millimeters in diameter, with a smooth surface. Lesions are usually located on the face, shins, and dorsal area of the hands. Verruca plana is common in childhood. In adult life, it can be found in women and men with HIV-related immunodeficiency. The lesions are usually few in number, but, in children who have a coexistent familial incidence, they may be multiple, distributed on the extremities and the trunk. Regression can spontaneously take place, and multiple lesions may appear in areas of scratching. Multiple verrucae planae in young patients with a familial distribution can bring up problems in differentiating this disease from epidermodysplasia verruciformis (EV; discussed in the next section). The absence of red, tinea versicolor–like lesions is helpful for the correct diagnosis. In progressively regressed lesions, the presence of cells with koilocytic appearance is helpful for making the correct diagnosis.

Histopathology. Verruca plana shares with verruca vulgaris the presence of acanthosis, hyperkeratosis, and koilocytic-appearing cells, but there is no parakeratosis or papillomatosis. Hypergranulosis is prominent throughout the lesion, and a basket weave–like appearance of hyperkeratotic material is considered to be characteristic (Fig. 20.39). Regressed lesions have apoptotic cells and foci of exocytosis in the epidermis and a lymphocytic infiltrate with extravasation of red blood cells in the upper dermis.

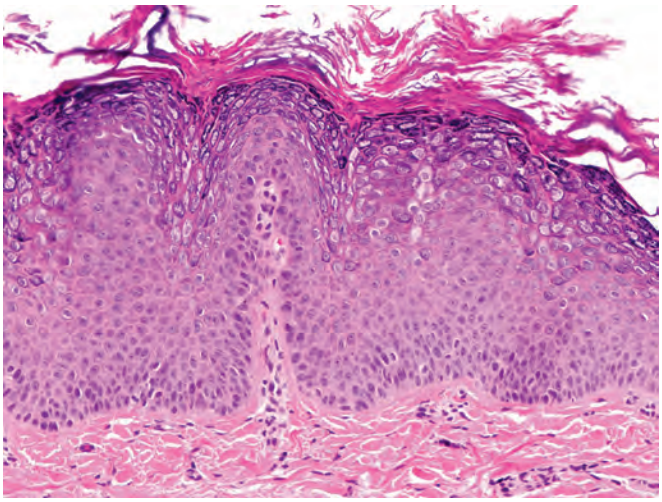
Epidermodysplasia Verruciformis

EV is a rare autosomal recessive genetic disease that is related to a range of HPV genotypes, specific for the lesion (EV-HPV types 5, 8, 9, 12, 14, 15, 17, 19, 25, 36, 38, 47, and 50). These HPV genotypes commonly do not cause problems in otherwise healthy individuals. EV appears to be related with mutations of the *EVER1* and *EVER2* genes in the EV1 region of chromosome 17 and with alterations of the EV2 region of chromosome 2.[277] In approximately one-fourth of the patients, it is associated with the development of squamous cutaneous carcinoma, mostly of the intraepidermal Bowen type and occasionally with an invasive pattern. The EV-HPV types most commonly related with development of SCC are HPV-5 and -8.[263] An EV-like type of picture can be seen in individuals with immunosuppression related to HIV infection[278] or other causes,[279] the so-called acquired EV.

Clinical Features. The disease commonly appears early in life (6 to 7 years of age), but cases of adult-onset EV have been documented. The first lesions to appear are flat and verrucous (verruca plana–like) and arise on the extremities. These lesions are followed



• **Figure 20.38** Plantar verruca. This example of a plantar verruca exhibits the classic endophyic growth pattern **(A),** as well as the characteristic viral cytopathic effect with koilocytosis and prominent basophilic inclusions **(B)**.



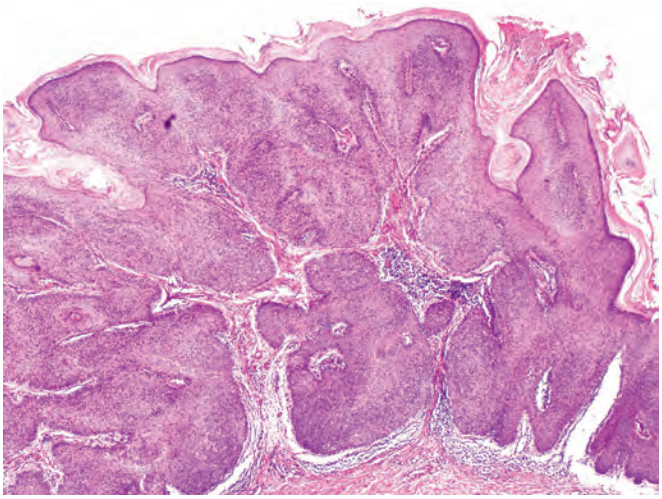
• **Figure 20.39** Verruca plana. There is prominent hyperkeratosis and acanthosis with numerous vacuolated keratinocytes in the granular layer.

by the appearance of pityriasis versicolor (PV)-like lesions that show up a few years afterwards. This common clinical course goes on and progresses to either a so-called benign form (usually related to HPV-3 and/or -10) or a malignant form (mainly related with HPV-5 and -8). The benign form shows flat wartlike lesions on the trunk and extremities that become large hyperpigmented plaques. The malignant form has also flat verrucous lesions, along with a PV-like polymorphous eruption, in addition to actinic keratosis and seborrheic keratosis-like lesions. SCC often develops in these patients, particularly in the sun-exposed areas.[280] By far the most dramatic and well-publicized form associated with this disease is the so-called tree-man clinical presentation, although these type of cases are quite unusual.[281]

Histopathology. EV in nonimmunocompromised patients exhibits extensive and prominent epidermal hyperplasia that is reminiscent of a verruca plana. One sees hyperkeratosis, relatively mild acanthosis, and swollen squamous cells with large clear or pyknotic nuclei and with abundant intracytoplasmic presence of coarse basophilic keratohyaline granules present. These basophilic cytoplasmic granules together with the swollen appearance of the keratinocytes result in a blue-gray areas of pallor, usually in the superficial layer of the epidermis.[282] In immunosuppressed patients the histology differs, with focal thickening of the granular layer being the most characteristic finding in a slightly hyperplastic epidermis. Squamous dysplasia is a common finding in this variant.[283] EV can be misdiagnosed as a verruca plana if the clinician does not focus on the more extensive and prominent character of the epidermal hyperplasia in EV. The abundance of coarse keratohyaline granules and the clear appearance of the nuclei of the keratinocytes also point to the correct diagnosis.

Condyloma Acuminatum and Giant Condyloma Acuminatum of Buschke-Löwenstein

Condyloma acuminatum (CA) is a mostly asymptomatic anogenital warty lesion, related to infection with low-risk HPV genotypes (HPV-6 and HPV-11), affecting the anal and genital area. Condiloma acuminata is considered one of the most common sexually transmitted diseases, affecting 1% to 2% of all sexually active individuals in the United States, and often present in young adults of both genders. When present in the genital area of children, sexual abuse must be strongly considered. The presence of such lesions in the birth canal can result in laryngeal papillomatosis in the baby, a benign but devastating condition. Although the great majority of CA will resolve with time, some lesions have concomitant infection with high-risk HPV genotypes (HPV types 16, 18, 31 to 33, 35, 39, 42, and 51 to 54). These types of lesions with both low- and high-risk HPV strains, if present in the female



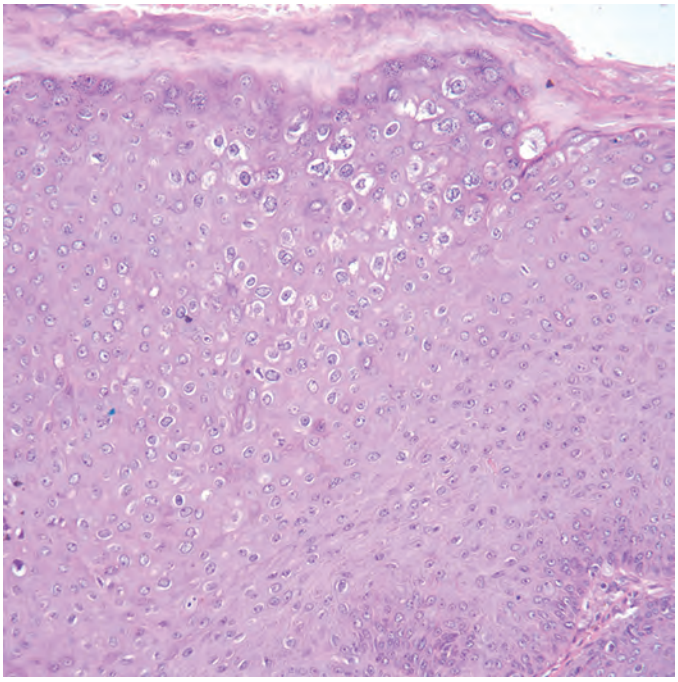
• **Figure 20.40** Condyloma acuminatum. Arborescent keratinocytic proliferation with pronounced acanthosis.

external genitalia, warrant investigation of the lower anogenital tract because it is in these sites where malignant transformation will likely occur. Rare cases of malignant transformation of CA can be found in the literature.[284] Giant CA of Buschke and Löwenstein is extremely rare and consists of a large, cauliflower-like lesion, frequently located in the glans penis and foreskin of men. This lesion is considered an intermediate between CA and SCC and is locally destructive, with a tendency for ulceration and recurrence. Up to 50% of cases can show malignant transformation. It is related to HPV genotypes 6, 11, and 16 and can undergo malignant transformation with low metastatic potential.

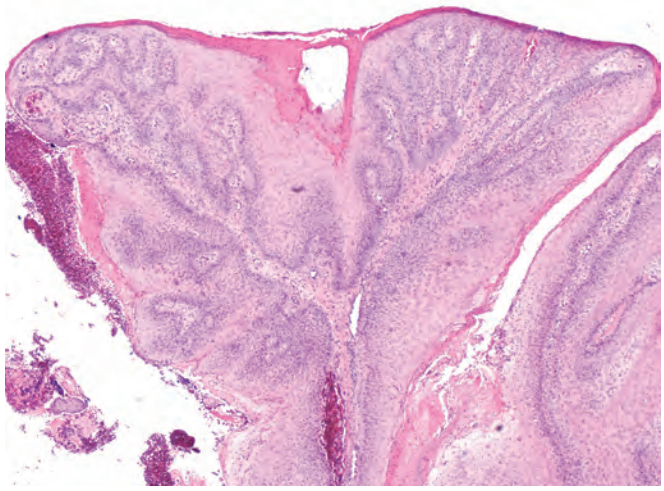
Clinical Features. The clinical presentation of CA is that of 2- to 5-mm papular or pedunculated lesions with granular papillations in the surface that arise in genital and perineal areas prone to microtrauma. The giant form of CA (Buschke-Löwenstein) appears as an often foul-smelling fungating mass in the genital area, and it particularly affects immunosuppressed individuals.[285]

Histopathology. CA is a papillomatous epidermal lesion exhibiting acanthosis, hyperkeratosis, and parakeratosis with an "arborescent" appearance (Fig. 20.40). Coarse keratohyaline granules can be seen in the superficial part of the lesion. Vacuolated keratinocytes with morphologic characteristics of koilocytosis are diagnostic when found in both upper and lower portions of the hyperplastic epidermis (Fig. 20.41). Numerous mitoses and necrotic keratinocytes at the bottom of the epithelium may be seen in lesions previously treated with podophyllin. In the absence of koilocytosis, CA can mimic seborrheic keratosis. ISH or PCR-based techniques for low-risk HPV can be helpful in detection of the virus. Indeed, a great percentage of vulvar seborrheic keratoses may represent senescent condylomas.[286]

In giant CA of Buschke and Löwenstein, the overall histopathologic picture is quite similar to CA, but the lesions are exoendophytic and much more extensive. The vacuolated keratinocytes may be few or absent, and the endophytic deep part of the lesion has a well-developed basal cell layer (Figs. 20.42 and 20.43). These lesions need to be thoroughly examined to exclude an SCC of the verrucous subtype. Detailed examination of morphology, especially in the lower portion of the lesion, is helpful in the establishment of the correct diagnosis. Increased mitoses,



• **Figure 20.41** Condyloma acuminatum. High-power view of the lesions reveals the koilocytic viral change of the cells.

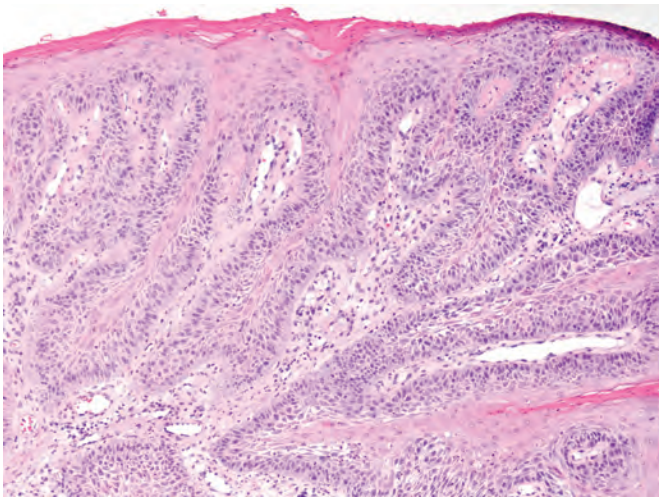


• **Figure 20.42** Giant condyloma (Buschke-Löwenstein). Prominent papillomatous growth with marked epithelial hyperplasia and hyperkeratosis.

nuclear atypia, and foci of dermal microinvasion in conjunction with immunohistochemical overexpression of Ki-67 proliferation index and p53 oncoprotein overexpression throughout the hyperplastic epithelium are evidence of malignant transformation.[287]

Bowenoid Papulosis

Clinical Features. Bowenoid papulosis (BP) is a clinical term that refers to a mostly papular and exceptionally verrucous, small, red or red-brown, discrete lesion of approximately 4 mm in diameter that can be related to HPV, mainly the HPV-16 and HPV-18 genotypes. It develops in the genitalia of sexually active young men or women (glans and shaft of penis, vulva) and in the perineal or the perianal area in the form of multiple lesions, partially forming a plaque. Exceptionally, it may be found in extraanogenital sites such as the neck,[288] toes[289] and other sites.[290]



• **Figure 20.43** Giant condyloma (Buschke-Löwenstein). Medium-power view exhibiting a well-developed basal cell layer.

Although spontaneous regression has been often related with this entity,[291] rare development of carcinoma has been documented.[292] BP can be differentiated from Bowen disease (SCC in situ) on the basis of circumscription, slight elevation, and size of the lesion, as well as the multiplicity and the young age of the patients.

Histopathology. BP has microscopic features that are indistinguishable from SCC in situ of the basaloid/warty type. There is acanthosis with full-thickness atypia of keratinocytes and nuclei partially dyskeratotic, pleomorphic, or multinucleated. There are multiple mitoses, some of them atypical. Hyperkeratosis and vacuolated keratinocytes can be seen in the superficial portion of the lesion, and true koilocytosis is uncommon. Nevertheless, lesions in BP tend to span only the length of three to four rete ridges, translating the clinical appearance of small papules. An important distinction is to be made from the so-called undifferentiated form of SCC in situ of the anogenital zone, which usually has a higher incidence of malignant transformation. A strong clinicopathologic correlation would make this distinction possible and spare the often-young patient affected by BP a potentially mutilating procedure.

Herpesviridae

These are double-stranded DNA viruses that are further classified into subfamilies with three main subfamilies that harbor clinically significant organisms. The first subgroup is Alphaherpesvirinae containing the *Simplexvirus* (type 1 and 2 HSV) and *Varicellovirus* (varicella zoster virus [VZV]) genera. The second subfamily, Betaherpesvirinae includes the CMV (also known as HHV-4 and HHV-5) and *Roseolovirus* (HHV-6 and 7) genera. Finally, the subfamily Gammaherpesvirinae contains the *Lymphocryptovirus* (EBV, also known as HHV-4) and *Rhadinovirus* (HHV-8) genera.

Herpes Simplex

Herpes simplex infection can be caused by HSV-1 (orofacial type) or HSV-2 (genital type). HSV-1 infection is very common, particularly in communities from developing countries, where in adolescence 70% to 80% of the population has been exposed.[293] Infection with HSV-1 is usually asymptomatic, and it is often first acquired during childhood with a subsequent lifelong infection. The virus can also be asymptomatically acquired at any time in life. However, in more than 10% of cases an acute gingivostomatitis will be present, more commonly in children than in adults. Some very unusual and severe presentations related to HSV-1 infection in infants and immunosuppressed adults include a fulminant respiratory infection, Kaposi varicelliform eruption (eczema herpeticum) and keratoconjunctivitis. These severe forms of HSV-1 infection can result in fatal widespread disease affecting most organs in the body.[294] As far as recurrent herpes is concerned, the most common manifestation is that of cold sores on the vermilion border of the lips.[293] However, any part of the body can be affected by HSV-1. For example, *herpes gladiatorum* may occur in such athletes as wrestlers when infected material is transferred from the lips to the mat and from there to other parts of the body.[295] The HSV-2 infection usually occurs after the onset of sexual activity.[296] HSV-2 seroprevalence rises from approximately 20% to 30% at age 15 to 29 years to 35% to 60% by age 60 years. From the late 1970s to late 1980s a dramatic increase in prevalence was observed.[293] Perinatally acquired HSV-2 is transmitted during birth, when the child comes into contact with an herpetic infection of the birth canal[297] Although HSV-1 and HSV-2 are regarded as nongenital and genital forms, respectively, HSV-1 can affect the genital area in some cases and vice versa.

Clinical Features. Primary and recurrent herpes simplex in the earliest stages causes slight erythema with discomfort, followed by the rapid appearance of groups of tiny, clear vesicles. These lesions occur most commonly around the lips but can occur anywhere on the body, as described earlier. Triggering factors include emotional or physical stress, the onset of menses, hot or cold temperature changes, and sunlight. In some patients the lesions are triggered by fever and are called "fever blisters."[293] It is important to mention that HSV infection is one of the most common causes of EM, particularly the recurrent variant of EM (61% to 100% of cases).[298] The primary infection of herpes usually manifests with a sense of dysesthesia and the appearance of very clear, small vesicles grouped on an edematous, erythematous base. The lesions may break down rapidly or become pustular; they eventually heal within a few days without scarring, unless there is a superimposed bacterial infection.[293] More unusual forms of HSV infection will be discussed in the following section.

Eczema Herpeticum. Also known as Kaposi varicelliform eruption, eczema herpeticum represents a complication in patients with atopic eczema but also occurs sometimes in association with seborrheic dermatitis, pityriasis rubra pilaris, pemphigus foliaceus, or Darier disease.[299,300] A report published in 2012 describes a case of eczema herpeticum related with side effects of BRAF inhibitor therapy for advanced-stage melanoma.[301] It is usually caused by HSV-1, and rarely by HSV-2, but also can be caused by Coxsackie A16 virus and vaccinia. The primary type occurs mainly in infants and children and may result from external infection or from reactivation of the virus. Primary eczema herpeticum is a serious disease, especially in children, and can spread to the entire system. Secondary (recurrent) infection is less severe and is not associated with internal organ disease unless the patient is immunocompromised. Death sometimes ensues because of bacterial superinfection or in cases of impaired immunity. Ten percent of immunocompromised patients succumb.[302] The characteristic rash is an extensive vesiculation of the skin with small pustules associated with high fever. The vesiculopustules are scattered especially on skin affected by the dermatitic disorder.

Herpetic Folliculitis. This is an unusual variant of herpes simplex that manifests as a small area of grouped, painful papules that have a follicular orifice in the center, which may or may not

be associated with a vesicle. These usually heal within a few weeks.[303]

Herpetic Whitlow. Deep infection of the nail results in painful paronychia or pain on the volar surface of the fingers and an abscesslike lesion. This disorder, so-called herpetic whitlow, is very common in physicians, as well as in dentists and their assistants.[304]

Hyperplastic (Verrucous) Herpes. This unusual form of herpes preferentially arises in immunosuppressed individuals and manifests as a nodule, usually in the genital area, and can be mistaken for SCC or condyloma acuminata.[305]

Congenital Herpes Simplex Infection. This unfortunate manner of infection can be transmitted to the fetus perinatally during delivery or transplacentally. Perinatal transmission is usually made from a mother with an active infection in the vagina or vulva and hence is usually caused by recurrent HSV-2 viral infection. The neonate often will only show skin lesions without systemic involvement, but involvement of the CNS has been described.[306] As part of the TORCH (**T**oxoplasmosis, **O**ther, **R**ubella, **C**MV, **H**erpes simplex) group of organisms, HSV can produce significant fetal damage in cases of primary HSV-2 infection during pregnancy; the timing of the exposure is very important. Very rare cases of intrauterine transmission related with HSV-1 infection have been reported.[307] Marked congenital malformations of the fetus can result if the infection occurs during the first 8 weeks of gestation. If the infection is produced later in pregnancy, the effects on the fetus are less severe and can include even a generalized vesicular eruption.[308]

Disseminated Herpes Simplex Virus Infection of the Immunocompromised. These presentations include a chronic ulcerative disorder that begins on the face or perineum and gradually spreads.[309] A second type of infection is acute mucocutaneous herpes simplex that becomes generalized after a localized vesicular eruption (Fig. 20.44).[310] Generalized systemic HSV infection can result in infection of many organs and can cause marked hepatorenal necrosis, pancreas necrosis, encephalitis, and brain necrosis.

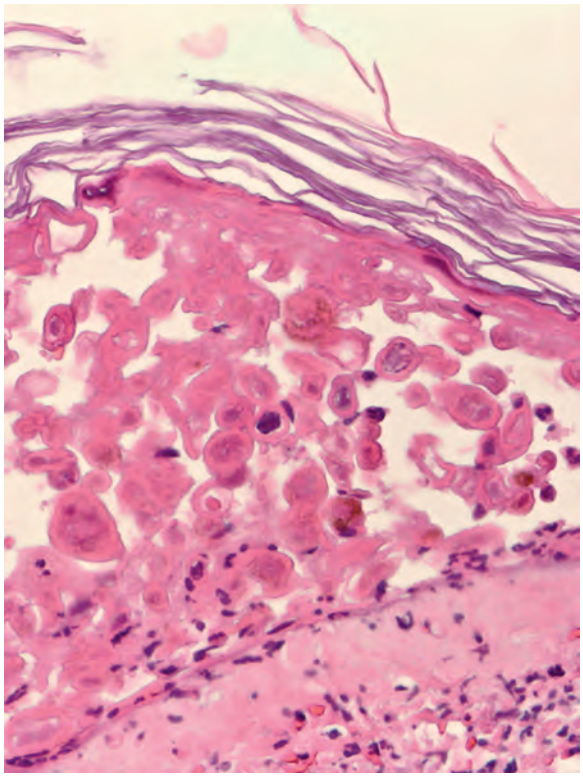
• **Figure 20.44** Disseminated herpes simplex infection in a patient with untreated human immunodeficiency virus/acquired immunodeficiency syndrome. Note the generalized distribution of the crusted lesions.

Histopathology. Both type 1 and type 2 HSV infections will give the exact histopathologic picture. On low-power microscopic view the most frequently found is primarily characterized by the presence of an intradermal or subepidermal blister or a shallow ulcer that is accompanied by acantholysis, ballooning keratinocytes, and necrosis. These lesions are commonly quite inflamed with a prominent blandlike mixed inflammatory infiltrate. A leukocytoclastic vasculitis can be seen in up to 67% of the times.[311]

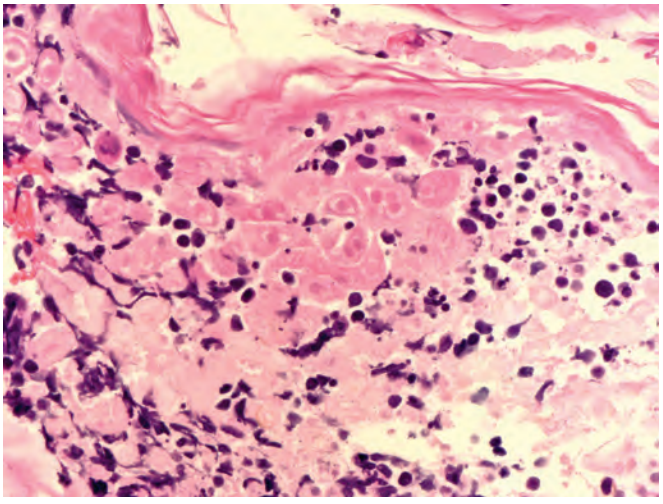
When considering the overall epidermal changes, the first features of HSV infection to appear affect the basilar layer and result in cytoplasmic swelling. These changes gradually involve the entire epidermis, exhibiting two patterns of injury of the keratinocytes: ballooning degeneration (swelling of the keratinocytes) and reticular degeneration (intercellular edema with cell rupture). As the cells become more edematous, they separate from one another and exhibit acantholysis and subsequent necrosis. The reticular degeneration occurs because of rupture of ballooning cells and resembles vesicles in eczema, but often one can see the cell membranes intact focally, the cytoplasmic contents having been evacuated from the cell due to focal rupture of the membranes.

After the virus involves the nuclei, there is cell fusion resulting in multinucleated giant cells. The multiple nuclei appear to be in close contact to each other and showing nuclear molding. However, one should not rely solely on their presence to arrive at the diagnosis because other dermatoses can show similar giant cells.[312] There is also margination of the nuclear chromatin with change of its coloration to a gunmetal-gray or blue-gray hue. As the virus passes into the nucleus, it leaves behind a prominent 3- to 8-μm eosinophilic body that is surrounded by a halo or clear space, the so-called Cowdry A or Cowdry type I body (Figs. 20.45 to 20.47) (The Cowdry B or type II body was related to poliovirus infection and has fallen in disuse). These nuclear changes are seen predominantly in the basal cells, but multinucleate cells can be seen extensively in the acantholytic areas, as can cells with the characteristic inclusions. Similar nuclear changes can also be observed in endothelial cells, sebaceous gland cells, and cells of the hair follicle and eccrine ducts. These inclusion bodies can be seen in infections with other herpesviruses, such as EBV. Importantly, one of the first changes that can be observed in herpetic infections is necrosis of sebaceous glands (Figs. 20.48).

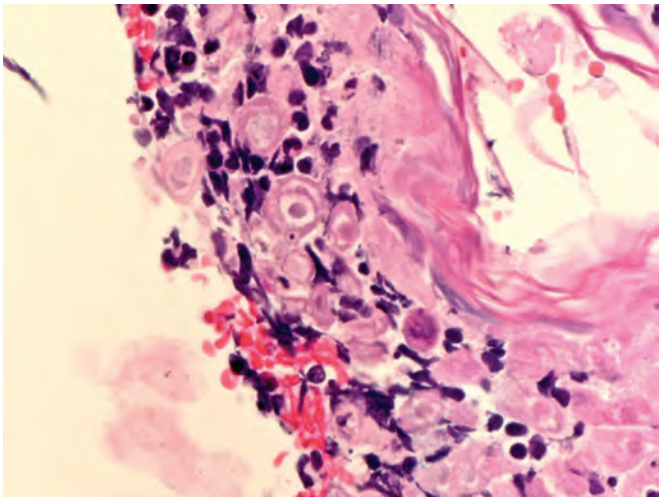
Several histologic variants related to HSV can be seen, such as the verrucous form in which one will find prominent pseudoepitheliomatous epidermal hyperplasia, simulating SCC with only focal areas with the previously mentioned changes.[305] Another particular type of HSV infection pattern is the one denominated "herpes incognito" in which one sees a variety of inflammatory features ranging from a lichenoid infiltrate to perivascular infiltrate and even a pseudolymphoma-like picture. As the moniker "incognito" suggests, no features of HSV cytopathic effect are initially seen, and it is only after deeper levels that one sees these features, usually involving a follicular structure. Thus any time one observes interface dermatitis along the epidermis and hair follicle that is associated with eosinophilic necrosis of a sebaceous gland, the first diagnostic consideration should be HSV infection.[303,313] Some



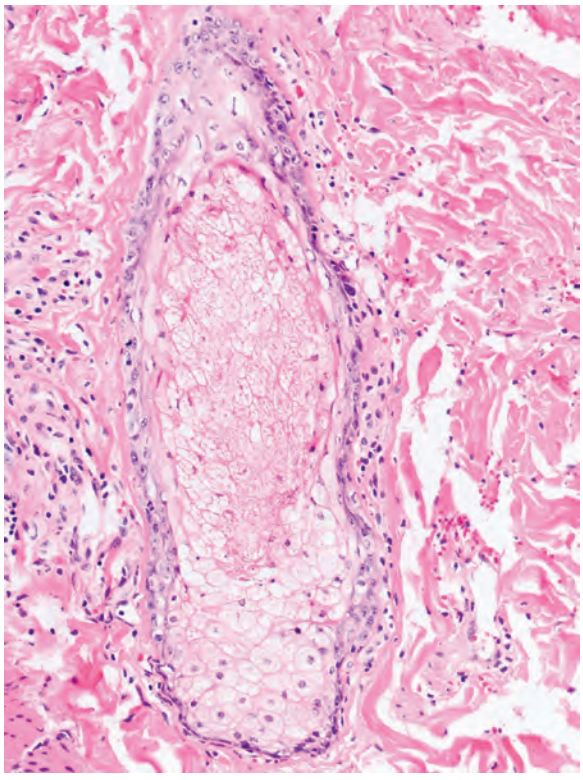
• **Figure 20.45** Herpes simplex infection. Note the intraepidermal vesicle, in which the keratinocytes exhibit acantholysis and prominent ballooning degeneration. In addition, there are multinucleated cells with nuclear molding.



• **Figure 20.46** Herpes simplex infection. Numerous keratinocytes exhibit eosinophilic intranuclear inclusions also known as Cowdry A bodies.



• **Figure 20.47** Herpes simplex infection. Formation of multinucleated cells is also characteristic of this process.



• **Figure 20.48** Herpes simplex infection Necrosis of the sebaceous glands is often observed in herpes simplex virus infection and can be a useful clue for the diagnosis.

authors argue that this form is most commonly seen with VZV reactivation (herpes zoster/shingles).[314]

The differential diagnosis of HSV infections includes lymphoproliferative disorders, such as natural killer (NK)/T cell and $CD30^+$ processes (approximately 83% of HSV lesions will show $CD30^+$ cells!), among others. A variety of inflammatory processes can be also mimicked by HSV infection.[311] Fortunately, if one confronts a difficult case, immunohistochemical stains are available either as a cocktail (type 1 and 2) or targeting individual subtypes.[315]

Varicella and Herpes Zoster

Both varicella and herpes zoster are caused by the same agent, VZV, with varicella being the manifestation of the initial infection with the virus, whereas herpes zoster occurs in persons who previously experienced varicella (reactivation). Ganglia of major

sensitive nerves, such as the spinal cord sensory ganglia or the ganglia of the fifth cranial nerve, are the sites where VZV remains latent after the initial (varicella) infection. When reactivation occurs, VZV spreads along the sensory nerve tracts into the skin, manifesting clinically as herpes zoster, or "shingles."

Varicella. Also known as chickenpox, varicella is a highly contagious disease affecting predominantly children and transmitted through airborne droplets. Because VZV is a highly lymphotropic virus, the first cells to be affected are T lymphocytes in the Waldeyer ring; dissemination later ensues through infected mononuclear cells with subsequent transmission of the virus to the epithelial cells, resulting in the clinical features of varicella. The virus appears to affect first the follicular structures. After the varicella lesions have cleared, the late phase of the process occurs, with the virus entering the ganglia of sensory nerves.[316] Reactivation of latent virus, which causes herpes zoster, may be triggered by fever, trauma, or immunosuppression.

Most cases of primary varicella occur in children of 14 years of age or younger, and when varicella occurs as a primary event in adults, serious complications may ensue, including pneumonia and death.[317] Three specific complications associated with varicella must be mentioned: primary varicella pneumonia, Reye syndrome (which is also associated with other viral diseases), and varicella affecting the fetus and the newborn. Approximately one-fifth of adults with this condition develop varicella pneumonia, which is associated with increased mortality. Reye syndrome is an acute, severe, and usually fatal encephalopathy associated with hepatic fatty degeneration that occurs predominantly in children and young adults related with concomitant ingestion of aspirin.[318] Varicella occurring during the first 20 weeks of pregnancy carries a small (<5%) but real risk of congenital malformations.[319] Furthermore, varicella occurring in late pregnancy can be transmitted to the fetus during passage through the birth canal.

Fortunately, both hospitalizations and death due to this infection have been reduced dramatically in all age groups following the onset of widespread vaccinations against VZV in 1995.[320]

Clinical Features. After the incubation period of 2 weeks, the patient suffers with the symptoms of a viremia with fever and malaise. This is followed by a cutaneous eruption that consists of erythematous macules that become vesicular; in some of the more severe cases, there are also pustules. The picture of vesicles on an erythematous base has been poetically described as a "nude drop on a rose petal." Over the course of several days, more lesions arise, resulting in numerous lesions at various stages of eruption and healing. Usually, these lesions do not leave scars unless they become superinfected or are traumatized in some manner.

If varicella occurs in immunosuppressed persons, there is a more prolonged course, and there are more severe complications, which can include a usually fatal viral pneumonia. Cutaneous lesions in immunosuppressed patients consist of large, vegetating, and usually necrotic lesions with ulceration.[321]

Herpes Zoster. As indicated earlier, herpes zoster (shingles) occurs as a result of reactivation of VZV previously acquired as varicella that remains latent in the sensory ganglia of sensitive nerves. This reactivation may occur many years after the original varicella eruption and involves spread of the virus along sensitive cutaneous nerves into the skin, usually demarcating the affected nerve's dermatome. The mechanisms that lead to the interruption of the dormant state include trauma, stress, older age, underlying immunosuppressive conditions (such as lymphoma), and iatrogenic-induced immunosuppression caused by various types of therapy. The incidence of herpes zoster increases with age.[322] A very small percentage of zoster cases occur in children younger than 15 years of age.

Clinical Features. The typical lesions of zoster are clustered vesicles, often on a hemorrhagic base and following along a dermatome. In addition, there may be scattered lesions on the skin and even occasionally in the oral cavity. The most commonly involved sensitive ganglia are the ones from the thoracolumbar and facial regions. The result of the viral reactivation is usually severe pain due to neuritis. This occurs along with dysesthesia and prominent hyperesthesia.

These symptoms appear to be worse in people older than 50 years and in the immunosuppressed. The incidence of herpes zoster is higher in immunocompromised hosts, especially in patients with advanced Hodgkin disease and HIV/AIDS, on which one will see lesions can become chronic and painful, exhibiting a vegetating, hyperplastic appearance with ulceration. A fatal outcome is much more common in these immunocompromised individuals.[323]

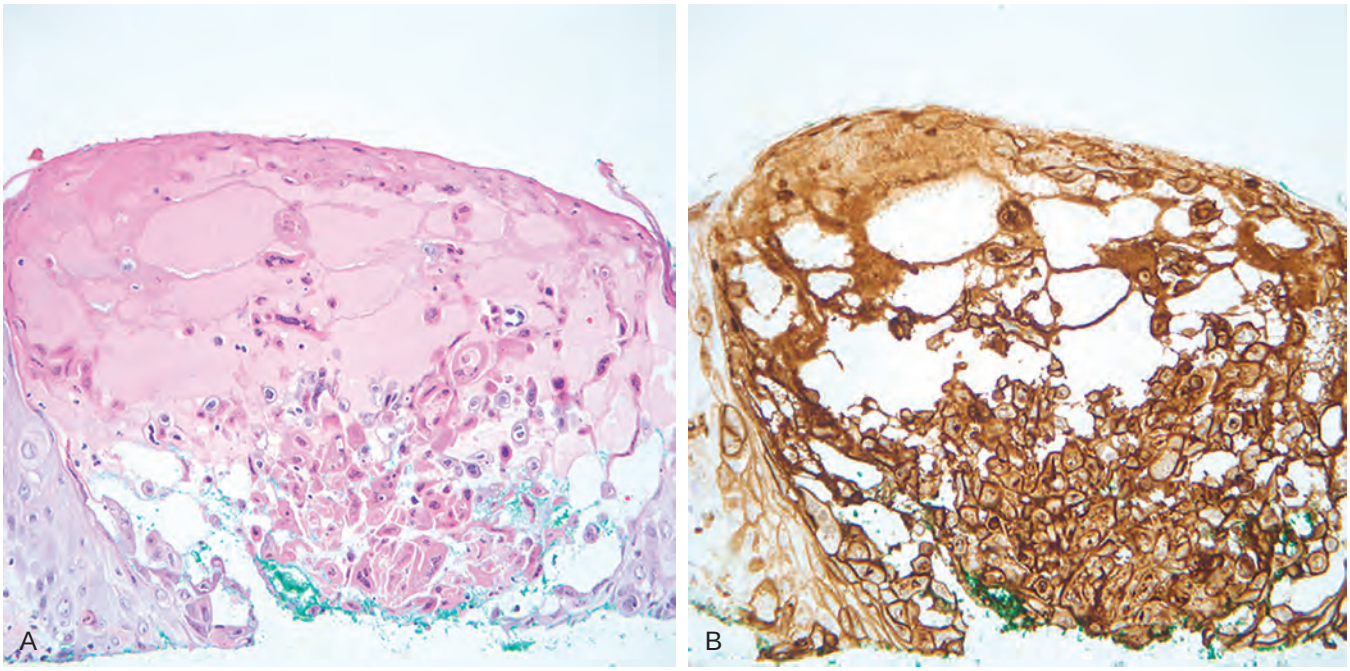
Histopathology. The histologic pictures of herpes zoster and herpes simplex, as far as the viral lesions are concerned, are virtually indistinguishable by routine microscopy (Fig. 20.49A). However, in herpes zoster, one usually finds more evidence of vasculopathy, including vasculitis in some instances, and a thrombogenic vasculopathy as well. Although this can occur rarely in herpes simplex, it is more common in herpes zoster.[324] Another feature that can be found more frequently in varicella and herpes zoster is the involvement of the follicular structures.[314] In very severe cases of either herpes zoster or varicella, usually seen in immunocompromised hosts, nuclear inclusions can be observed in endothelial cells and even in nerves. Lesions of herpes zoster might heal leaving scars, which can occasionally show sarcoidal-type granulomas.[325] There are monoclonal antibodies that allow for a separation between VZV and HSV with immunohistochemistry (see Fig. 20.49B).[326]

HSV will grow in ordinary tissue cultures, whereas VZV grows only in human fetal diploid kidney cells or fibroblasts of human foreskin. Culture is the gold standard for identification. Electron microscopic examination of VZV lesions shows viruses in the capillary endothelium and sometimes in the dermal nerves. HSV and VZV cannot be distinguished on the basis of ultrastructure examination.

Cytomegalovirus

Infection with CMV is very common throughout the world. It is considered that approximately 1% of newborns are infected transplacentally, and as many as 5% acquire the infection during passage through the birth canal. Antibodies to the virus continue to increase throughout life but especially during adolescence. CMV, also known as HHV-4, can be disseminated either via saliva or through sexual contact. Furthermore, because the virus is lymphotropic and remains latent in peripheral blood leukocytes, it can be transmitted through blood transfusions.[327] A similar virus from the same genus, HHV-5, is known to produce a mononucleosis-like condition.

Clinical Features. The initial infection is generally asymptomatic, but it can be associated with a mononucleosis-like syndrome with mild malaise, flulike symptoms, and mild hepatitis but without prominent lymphadenopathy. Subsequently, the virus becomes latent, persisting in a variety of tissues, preferentially in peripheral blood leukocytes. For this reason, patients with HIV/AIDS or another immunosuppressive state can exhibit a reactivation of the virus with involvement of almost any organ.[328]



• **Figure 20.49** Varicella zoster virus (VZV) infection (herpes zoster). Note the presence of an intraepidermal vesicle with ballooning degeneration and other features encountered with herpes simplex virus (HSV) infections: multinucleation, chromatin margination, and steel gray chromatin **(A)**. An immunohistochemical stain for VZV highlights the infected cells; in this case, both HSV-1 and HSV-2 immunohistochemical stains were negative **(B)**.

Cutaneous manifestations of CMV infection are usually only seen in immunocompromised patients and consist primarily of a maculopapular eruption. Ulcerations of the genitalia or other areas of the skin as well as hyperkeratotic, urticarial, or even bullous lesions have also been described.

As part of the TORCH group of organisms (discussed previously in the herpes virus section), intrauterine CMV infection also results in tremendous damage to the fetus' brain along with chorioretinitis and pneumonitis. Neonatal/congenital CMV infection can also result in a variety of lesions, including petechiae and purpura, papules, plaques, and nodules with bluish discoloration, a pattern denominated "blueberry muffin baby." These lesions are composed of foci of extramedullary hematopoiesis due to massive involvement of the bone marrow and can be seen in a variety of other neoplastic and infectious processes.[329]

Histopathology. Cutaneous lesions of CMV will often show ulceration with nonspecific inflammation. However, on careful inspection, one can identify the first clue to CMV infection—the presence of dilated dermal vessels with endothelial cells exhibiting large (approximately 10 μm), very eosinophilic (cherry red), and hyperchromatic intranuclear inclusions ("owl eyes–like"). There are also smaller, intracytoplasmic, basophilic inclusions that are approximately 3 μm in size. Although these inclusions are characteristic, they are often quite focal, requiring multiple tissue levels. Importantly, one will find these cytopathic viral changes predominantly in stromal cells, namely fibroblasts and endothelial cells. Vasculitis (leukocytoclastic or necrotizing lymphocytic) associated with a mixed inflammatory infiltrate or a vasculopathy can also be seen. An immunohistochemical stain is available to help to identify CMV in tissue, yielding a nuclear signal in early lesions and a nuclear and cytoplasmic signal in late stages.[315]

Serologic studies are frequently done to assess for anti-CMV IgM antibodies in active disease. If necessary, CMV culture can be successfully accomplished by the use of human fibroblasts. As far as molecular techniques are concerned, PCR-based assays can be useful in diagnosing CMV. Finally, electron microscopic examination will reveal large intranuclear particles that are approximately 100 nm in diameter and resemble herpes simplex virions.

Human Herpesviruses 6 and 7

These viruses are quite widespread and have been known to cause primary infections, as well as chronic persistent infections. They may remain in a state of latency for many years, until some change in the immune status of the patient leads to reactivation.

Human Herpesvirus 6. This virus has been subclassified into two variants, HHV-6A and HHV-6B, and attaches to the host's cells by the widely expressed CD46 receptor. The B variant of HHV-6 is the causative agent of the childhood eruption known as exanthema subitum. This is a quite ubiquitous virus with nearly all children showing antibodies against HHV-6B in their blood. HHV-6 is present in the salivary glands and actively shed in saliva.

The primary infection may go unnoticed, or it may manifest with the classic clinical picture of exanthema subitum (roseola infantum), namely a prodrome of high fever for 3 to 5 days being followed by a diffuse erythematous maculopapular blanchable eruption. Reactivation of the virus is often triggered after allogeneic bone marrow transplantation, particularly in the first month, manifested as a skin rash or even a generalized vesiculobullous disorder.[330] Interestingly, fulminant reactivation of HHV-6B in the context of drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome has been described.[331] In addition, a purpuric variant of the "gloves-and-socks" syndrome,[332] papular

acrodermatitis of childhood (Gianotti-Crosti syndrome),[333] pityriasis rosea,[334] among several other entities,[335] have all been linked to HHV-6 infection.

Human Herpesvirus 7. Since it uses the CD4 molecule to attach to T cells, HHV-7 is considered to be a lymphotropic herpesvirus that remains latent in peripheral lymphocytes. Similarly to HHV-6 and other viruses from this family, it is also shed in the saliva. Children are infected at a young age, and HHV-7 is capable of originating a similar picture to exanthema subitum. Other conditions, including pityriasis rosea, have also been linked to HHV-7.[335] Similarly to HHV-6, cases of reactivation in the context of DRESS have been reported.[336]

Epstein-Barr Virus

A wide variety of entities have been associated with EBV, from benign processes like infectious mononucleosis and oral hairy leukoplakia (OHL) to myriad lymphoproliferative and epithelial malignancies, including Burkitt lymphoma (tumor from which the virus was originally described), nasopharyngeal carcinoma, and many others.[337,338] Infections with EBV often begins in the mucosal surfaces, and the virus is profusely present in body fluids, such as saliva; for this reason, spread from person to person is quite easy ("kissing disease"). Venereal transmission is also described. After the initial infection, the virus remains latent, apparently for life, in the host's mononuclear cells, particularly in germinal center-based and memory-type B lymphocytes.[338]

This viral infection is one of the most ubiquitous and widespread known to man, with more than 95% of the world's population harboring the virus. Early infection is most often seen in developing countries, showing a prevalence of 60% to 80% in children. When infection occurs in childhood, it is usually asymptomatic. However, if it is acquired in adolescence or adult life, which is the case in developed countries, there is a high possibility of developing infectious mononucleosis.[339] We will describe the findings in OHL and infectious mononucleosis and briefly review the other cutaneous manifestations and EBV-related lymphoproliferative disorders.

Oral Hairy Leukoplakia

Clinical Features. OHL is a benign process that, although it is commonly seen in the setting of HIV/AIDS (present in up to 53% of these patients), it has also been described as associated with a variety of immunosuppressive states. In HIV-positive individuals, appearance of OHL precedes and predicts the onset of AIDS. The lesions are white patches that typically occur on the lateral surfaces of the tongue and may be unilateral or bilateral.[339] There is usually a verrucous filiform surface that can be scraped off. Patients may complain of dysesthesia and burning, but this is probably associated with coinfection with *Candida* spp.

Histopathology. The histopathology of OHL shows an irregular epithelial hyperplasia with filiform projections ("hairy") and striking associated parakeratosis. The keratinocytes show ballooning degeneration and very small pyknotic nuclei with intranuclear inclusions (Cowdry A–like); these intranuclear inclusions are deeply basophilic and have halos around them, in a koilocyte-like pattern. The cytoplasm may have a glass-like appearance. Hyphae of *Candida* species can be easily observed in the superficial epithelium and in the keratin layer in up to 80% of cases.[339] There is a mixed lymphohistiocytic infiltrate beneath the epithelium. If necessary, one can resort to ISH studies for the Epstein-Barr–encoded RNA (EBER) (preferred method) or to available EBV-specific immunohistochemical stains.[315]

Infectious Mononucleosis

Clinical Features. The classic clinical features of nonexudative pharyngitis, fever, general malaise, and cervical and submandibular lymphadenopathy is classic. Splenomegaly is also often seen. Skin manifestations are present in approximately 15% of cases and typically consist of a transient maculopapular/morbilliform eruption, usually located on the trunk and upper extremities with occasional involvement of palms and soles. The oral mucosa can show petechial lesions similar to the ones seen in rubella. Patients treated with ampicillin and other antibiotics will develop a pruritic, copper-colored macula eruption.[339,340]

Histopathology. The histology of the eruption in infectious mononucleosis is usually that of an "id-like" reaction, which includes interface dermatitis with a perivascular lymphoid infiltrate that extends into the deep dermis. A rare reported case of a patient with chronic EBV infection exhibiting palisading infiltrate resembling granuloma annulare can be found in the literature.[341]

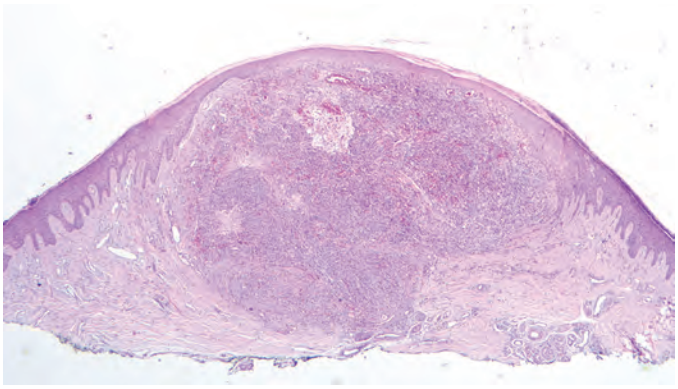
Other EBV-Related Dermatoses. The virus has been implicated in myriad other cutaneous disorders, including nonsexually related acute genital ulcers occurring in young females (Lipschütz ulcers), pediatric conditions such as the papular acrodermatitis of childhood (Gianotti-Crosti syndrome), and hydroa vacciniforme, as well as hypersensitivity to arthropod bites and the lupuslike condition known as Kikuchi-Fujimoto disease (histiocytic necrotizing lymphadenitis).[339]

EBV-Related Lymphoproliferative Disorders Affecting the Skin. In addition to the wide spectrum of EBV-related dermatosis described previously, this quite "prolific" virus is also related to several lymphoproliferative disorders that can manifest in the skin. These include Burkitt lymphoma (rarely affects the skin), lymphomatoid granulomatosis, EBV-positive diffuse large B-cell lymphoma of older adults, extranodal NK/T-cell lymphoma, and angioimmunoblastic lymphoma, as well as the mixed cellularity and lymphocyte-depleted subtypes of classic Hodgkin lymphoma. In addition, a good percentage of posttransplant lymphoproliferative disorders are EBV-related; however, these rarely have skin manifestations.[342]

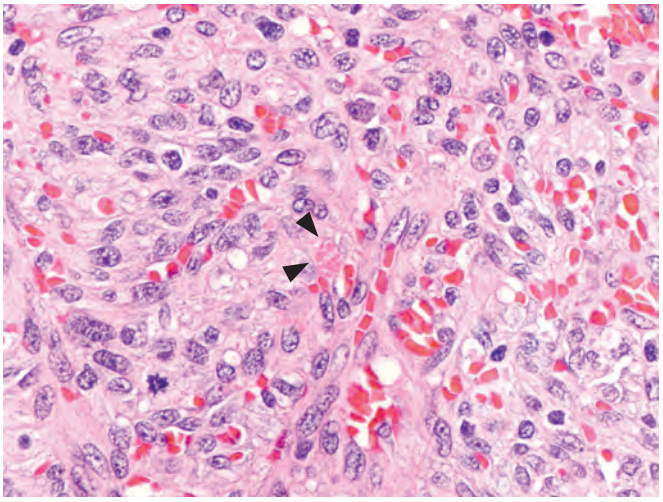
Human Herpesviruses 8, Kaposi Sarcoma-Associated Herpes Virus

This particular herpesvirus has multiple subtypes, appears to be lymphotropic, and appears to be restricted to where KS appears. Although HHV-8 is detected in multiple bodily fluids and secretions, the manner of transmission of this virus is still unknown and presumably involves sexual contact. Viral transmission through solid organ transplantation and blood transfusions has been documented.[343] The cutaneous manifestations of HHV-8 include both KS and an exanthematous eruption following the primary infection.[335] In addition to the several variants of KS, HHV-8 has also been associated with multicentric Castleman disease, the so-called primary infusion lymphoma and HHV8-positive plasmablastic lymphoma.[344] A very interesting feature of KS is the production of a viral homologue of the cytokine IL-6 (vIL-6), which has a strong vascular endothelial growth factor (VEGF)-like effect, as well as having a strong influence in the production of plasma cells form B cells, with both of these effects being evident in the histopathologic picture of KS (vascular tumor with plasma cells). This finding is also prominent in the plasma cell–rich, multicentric Castleman disease.[344] We will discuss the features of KS in the following section.

Clinical Features. There are four clinical types of KS: (1) the classic form, which arises in Mediterranean or Ashkenazi Jewish



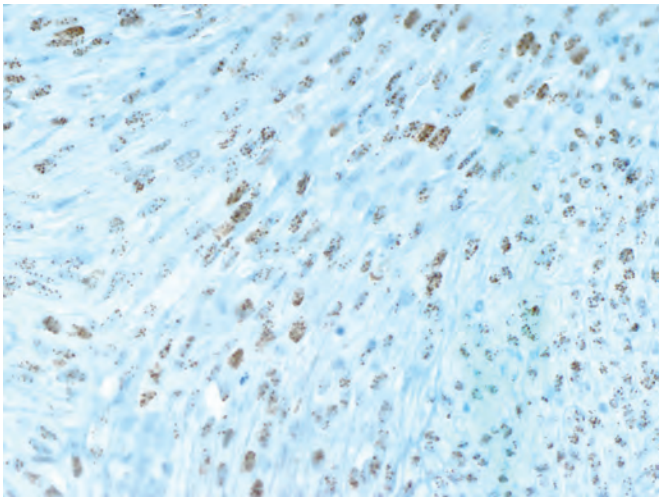
• **Figure 20.50** Kaposi sarcoma (nodular stage). Low-power view of this viral-induced neoplasm shows a dermal nodule composed of vascular channels surrounded by spindle cells and flanked by an epidermal collarette.



• **Figure 20.51** Kaposi sarcoma (nodular stage). High-power view of the tumor exhibiting the classic features of Kaposi sarcoma including the"boxcar" disposition of the red blood cells and the eosinophilic bodies *(arrowheads)*.

men, (2) the endemic form, occurring in young individuals predominantly in sub-Saharan Africa, (3) the iatrogenic or post-transplant form, and finally the most frequently seen (4) HIV/AIDS-related variant.[344] Lesions of KS can present both in skin and mucosal surfaces as purplish/reddish blue or brown/black areas of discoloration in the patch and plaque forms and as nodules or frank tumors with similar coloration in the nodular form. The classic form of KS usually presents restricted to the skin in lower extremities as broad, coalescing plaques, particularly on the feet, and are much more indolent than the other types. The endemic, immunosuppression, and HIV/AIDS variants usually show diffuse involvement of skin, lymph nodes, and visceral organs.[343,344]

Histopathology. There are three main components to look for in KS: (1) abnormal vessels, (2) spindle cell cuff around vessels, and (3) plasma cell infiltrate. There are also three stages of disease, starting from patch stage, through plaque stage, and finally nodular stage. The patch stage of KS can be extremely subtle and show only a very mild vascular proliferation with a small cuff of spindle cells. Plasma cells are very useful as a diagnostic clue. The plaque stage will show a quite noticeable increase in abnormal vessels, spindle cells, and plasma cells. Both patch and plaque stages can show the so-called promontory sign, which translates to the presence of a dilated lymphatic lumen containing a peninsula-shaped invagination containing a small capillary. Although useful, this feature can also be seen in benign vascular tumors, such as the targetoid hemosiderotic hemangioma (hobnail hemangioma). The nodular stage is easily recognizable because it exhibits a large aggregate of spindle cells and vessels (Fig. 20.50) with red blood cells arranged in a boxcar disposition, along with a plasmacytic infiltrate (Fig. 20.51). Scattered hyaline globules (degenerated red blood cells) can be seen throughout the tumor, which also represent a useful diagnostic clue (see Fig. 20.51, *arrowhead*). Although no distinct viral inclusion is seen on H&E with KS, the presence of the virus can be easily demonstrate with the use of immunohistochemistry directed to the C-terminus of the latent nuclear antigen-1 (LNA-1), which produces a very characteristic stippled nuclear signal (Fig. 20.52).[315]



• **Figure 20.52** Kaposi sarcoma (nodular stage). A human herpes virus 8 immunoperoxidase stain showing the characteristic stippled nuclear pattern.

Poxviridae

The poxvirus family is composed of relatively large (300 nanometers), brick-shaped to oval, enveloped DNA viruses. The medically important members are part of the Chordopoxvirinae subfamily, which includes the *Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus* genera. These viruses are closely related genetically, with a genetic homology that ranges from 90% between smallpox, cowpox, and vaccinia to 70% homology among smallpox and molluscum contagiosum virus.[345] Most of the conditions produced by these viruses are zoonoses, with only the smallpox and molluscum contagiosum viruses being obligate human pathogens.[346] Although eradicated in 1977, the smallpox virus remains a latent danger for humans, and the CDC considers it a high-priority bioterrorism agent (class A).[42] Potential biowarfare use of other virulent poxviruses (e.g., monkeypox, camelpox) is also possible.[347] In addition, the rising popularity of exotic pets, which are usually wild animals imported directly from their original habitats, can potentially give rise to outbreaks of virulent imported poxvirus zoonoses. The generic term "pox" relates to the formation of pocks in the skin, with subsequent scarring and formation of pockmarks, a classic finding among smallpox survivors.

Orthopoxvirus

Variola (Smallpox). The WHO officially declared smallpox eradicated from the world in 1979, with the last recorded case occurring in 1977 in Somalia. This condition, historically denominated as "smallpox" (with syphilis being "the Great Pox"), has been known to affect humans since the time of the ancient Egyptians (3000 BC). Retrospective studies revealed that the epidemics followed a seasonal pattern and were usually associated with dry weather conditions.[348]

Both the CDC and the Institute of Virus Preparations in Moscow, Russia stockpile strains of variola virus. Thus, because vaccination against this virus has not been performed worldwide since 1982 and only 20% of vaccinated individuals retain immunity against smallpox, a potential reintroduction of the virus in the form of a class A biological weapon would be catastrophic.[42] Therefore constant reminder of the characteristics of the illness and continuing efforts in obtaining better vaccines and treatment are thoroughly justified.[349] Transmission of the virus occurs usually through respiratory droplets, with subsequent invasion of the mucous membranes and the lymphatic system.[349] Percutaneous inoculation has also been described, as well as rare cases of conjunctival and transplacental transmission.

Clinical Features. The classic WHO classification of smallpox encompasses five variants: (1) variola major (90% of cases), (2) a "modified," milder form (variola minor), (3) a flat or malignant type, (4) the hemorrhagic variant, and finally (5) the *variola sine eruptione* variant.[349] A simplified classification designates a binary variola major—minor system with four of the subvariants, except the minor variant, being classified under the major form.[42]

Variola major. After an incubation period of 7 to 17 days, during which the virus massively multiplies in the reticuloendothelial system, a prodromal period begins abruptly with sudden high fever (40°C), vomiting, and body aches, lasting for 2 to 3 days. After the prodromal phase subsides and the individual feels much better, enanthematous lesions on the mouth and oropharynx appear, usually 24 hours before the cutaneous eruption is established. The skin eruption, which is caused by viral invasion of the capillary endothelium in the dermis, is quite characteristic, with lesions beginning as small, reddish macules that become 2- to 3-mm papules and then 4- to 6-mm pustules, all in a period of 3 or 4 days. The rash occurs first on the face and extremities, and then spreads generally throughout the body in a centrifugal manner. Usually, all of the lesions are in the same state of evolution; they last for a total of 5 to 8 days with umbilication and crusting.[42,349] The less frequent hemorrhagic and flat or malignant subvariants are characterized by the lack of pustular lesions and often show severe systemic complications. The hemorrhagic variant exhibits sloughing of skin lesions and mucosal surfaces, similar to Stevens-Johnson syndrome. The *variola sine eruptione* appears to be a milder form and usually shows influenza-like symptoms. Although the ordinary variola major form has a mortality of 30%, the hemorrhagic and flat subvariants are 100% deadly.[349]

Variola minor ("modified major"). This milder variant appears in previously vaccinated individuals and shows similar lesions as the major form but much smaller in size and rapidly developing, with crusting occurring by day 7.[42] This variant is not characterized by a lethal outcome.

Death from smallpox results mainly from the cytopathic effects of the virus in the target organs[350]—contrary to the classic literature, which attributed the demise of smallpox patients mainly to sepsis with massive immune complex formation and hypotension. Studies using molecular engineering revealed the presence of a protein complex termed **s**mall**p**ox **i**nhibitor of **c**omplement **e**nzymes (SPICE), which specifically inhibits the human C3b and C4b components of complement. This virulence factor is absent in the other less virulent orthopoxviruses, such as vaccinia and molluscum contagiosum virus.[351]

Vaccinia Virus. Because the vaccinia virus (VACV) is antigenically very similar to smallpox virus, with both viruses presumably being derived from a common ancestor, VACV has been used as a vaccine against smallpox. Vaccination with VACV also confers immunity against several viruses from the *Orthopoxvirus* genus. In addition, VACV has been used experimentally as a viral vector in anticancer gene therapy. Adverse reactions do occur following VACV vaccination, either against smallpox[352] or when the virus was used as part of cancer vaccines,[353] especially in immunocompromised or atopic patients.

Clinical Features. Inoculation with VACV leads to cutaneous lesions similar to those seen in smallpox. The inoculation site evolves to a vesicle that becomes pustular, crusts, and finally heals, leaving a scar. Systemic manifestations, such as fever, can also be seen.[354] The postvaccination adverse reactions can still be seen in people still receiving the smallpox vaccine (e.g., military personnel) and is divided into four types: (1) direct dissemination from the vaccination site lesion into mucosal surfaces; (2) generalized vaccinia, which is self limited; (3) progressive vaccinia, slow and progressive disease in immunosuppressed individuals; and finally (4) eczema vaccinatum, generalized eruption in atopic and immunosuppressed persons.[355]

Since 1999, there have been reports of outbreaks involving strains of VACV, particularly in rural areas of Brazil.[354] In these cases the clinical picture and transmission mechanisms bear a striking resemblance to cowpox infection (discussed later), including acquisition of the infection from cows through udder lesions and the presence of umbilicated papular lesions with lymphangitis and regional lymphadenopathy. However, several molecular studies have characterized the causative virus as VACV.[354]

The possible origin, mechanisms of circulation, and reservoirs of these zoonotic VACV infections are largely unknown. Laboratory-acquired infection is also possible because VACV is extensively used in laboratory-based research in the study of virology, immunology, and vaccine development. Because VACV remains pathogenic to unvaccinated individuals, laboratory workers who handle VACV strains that are not highly attenuated must receive the appropriate vaccine.[356]

Cowpox Virus. The primary reservoirs of cowpox virus are wild small rodents, especially bank voles *(Clethrionomys glareolus)* and wood mice *(Apodemus sylvaticus)*. The vectors that spread the virus are the rodents themselves[357] or their predators, which are usually domestic cats.[358] The disease is known as cowpox because it can infect humans through lesions in the teats and udders of infected cows The pioneering observations made by Edward Anthony Jenner in 1798 determined that cowpox infection, when induced by inoculation in healthy persons, would reduce the severity of subsequent smallpox disease. This groundbreaking discovery gave rise to one of the most successful interventions in the history of medicine.

Human cowpox infection has been seen almost exclusively in European and former Soviet countries,[357,359,360] and the disease was almost forgotten after the eradication of smallpox in the late 1970s. However, due to the loss of immunity against orthopoxviruses after the interruption of smallpox vaccination, cowpox is

now regarded as a reemergent disease that poses a considerable threat, especially to immunocompromised and atopic patients.[361,362] The route of infection is almost always a skin lesion, but some cases of mucosal transmission have been reported.[360]

Clinical Features. After inoculation, there is an incubation period that varies from 7 to 12 days, after which an inflamed macule appears that successively changes to a papule, a vesicle, and a pustule, which is usually hemorrhagic. Finally, this pustule ulcerates and gives rise to the characteristic black eschar surrounded by erythema and edema.[359,360] The eschars are usually painless, can show adherence to the underlying tissues, and heal in approximately 2 weeks, sometimes leaving a scar. A regional tender lymphadenopathy is usually present.[357] Systemic manifestations, such as malaise and flulike symptoms, are often seen.[359,360] In immunosuppressed patients and in atopic individuals, the infection tends to have a much more severe course. In these patients, one can see a generalized, partly confluent poxvirus exanthema comprising hemorrhagic pustules with central necrosis that evolve to deep-seated, black eschars. This occurs along with severe systemic manifestations that can lead to a lethal outcome.[362]

The important differential diagnostic considerations include herpesvirus infection, orf, and anthrax. If the port of entry is a mucous membrane, such as the conjunctiva or the nasal mucosa, the infection can mimic an *H. influenzae* phlegmon or erysipelas due to the massive edema that is usually seen in these cases.[363]

The suspicion of cowpox virus is usually raised after failure of empirical antibiotic treatment of a skin lesion. Various methods are available to confirm the diagnosis, and they usually require extraction of material from the pustules. Electron microscopy will reveal the brick-shaped viral particles.[364] The more accessible PCR-based molecular methods are also useful in characterizing this agent.[357,364] Culture is another option, and a variety of cell culture lines can be used.[359]

Monkeypox Virus. Monkeypox virus (MPV) infection is a rare zoonosis that occurs mainly in west and central Africa in the Congo River basin.[365,366] Because this virus is capable of producing a severe smallpox-like systemic disease, the WHO considers MPV to be the most medically important *orthopoxvirus* in the post-smallpox era. MPV has one great advantage in comparison to variola: the existence of a wide range of hosts and reservoirs,[367] which would preclude worldwide eradication through a massive vaccination program. Fortunately, it appears that, in contrast to smallpox, human-to-human transmission of MPV is quite difficult.[368] A 2003 outbreak of MPV in the United States from imported infected prairie dogs to be commercialized as pets[366] raised concern and awareness regarding possible widespread spread of this agent. This could potentially be accomplished either through the practice of importing wild animals as exotic pets, as a consequence of the ever-increasing volume of international travel, or as a component of biological weapons in terrorist attacks.[367] Studies published in 2008 have revealed the presence of mechanisms of immune evasion that allow the virus to spread systemically and sometimes to remain dormant for long periods.[366]

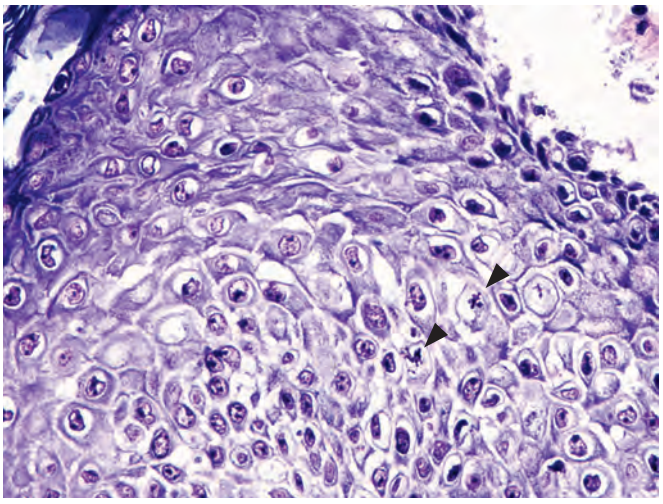
The route of infection can be percutaneous, through bites or scratches of infected animals or during manipulation of infected material, or via respiratory or mucosal routes. In African endemic regions, spread of the infection is related to the hunting, skinning, preparing, and eating of infected rodents and monkeys.[367]

Clinical Features. The clinical manifestations of monkeypox infection are strikingly similar to those found in smallpox, including an incubation period of approximately 14 days and a 2-day prodrome with fever and malaise. However, during the prodrome phase, there is a feature that is present only in monkeypox infection: a severe lymphadenopathy that can become generalized. The eruption usually has a centrifugal distribution, lasts for 14 to 21 days, and shows the typical maculopapular-papular-vesicular-pustular progression before sloughing and leaving dyspigmented scars.[367,369] The clinical differential diagnostic considerations are chickenpox, drug eruption, eczema herpeticum, dermatitis herpetiformis, rickettsial infection, and molluscum contagiosum. In addition, smallpox would be an important diagnostic consideration in the unfortunate case of a reemergence of this terrible illness.[367]

Histopathology of Cutaneous Orthopoxvirus Infections. The microscopic characteristics of cutaneous lesions caused by the different members of the *Orthopoxvirus* genus are quite similar. Changes bearing some resemblance to those seen in herpes simplex, herpes zoster, and varicella infections are seen and include massive intraepidermal vesiculation with ballooning degeneration and apoptosis of keratinocytes, and serous exudation (vesicular stage), followed by exudate influx with acute inflammation and formation of multinucleated cells (pustular phase).[370,371] The distinctive feature is that, instead of replicating in the nucleus and cytoplasm like the viruses in the Herpesvirus family, orthopoxviruses replicate in the cytoplasm of the infected cells, thus creating intracytoplasmic inclusion bodies. These intracytoplasmic inclusions, also known as Guarnieri bodies, have been classified into two types. Type A inclusions, represented by cytoplasmic eosinophilic corpuscles that are usually located near the nuclei and have a halo, are formed late in the viral replication cycle. In contrast, the type B inclusions are present in the early stages of viral replication and consist of smaller, rather basophilic particles (Fig. 20.53).[370,372] An immunohistochemical stain directed towards VACV is available, which will likely cross-react with other species of the genus and decorates the cytoplasm of the infected cells highlighting the Guarnieri bodies.[370]

Parapoxvirus

Paravaccinia (Milker's Nodule). Milker's nodule is a zoonosis that characteristically affects ungulates, such as cows, and



• **Figure 20.53** Variola (smallpox). The infected keratinocytes show severe intracellular edema and cytoplasmic inclusion bodies known as Guarnieri bodies *(arrowheads)*. Note that the inclusion bodies are located in close relation to the nuclei and are surrounded by a clear halo.

it is known as "pseudocowpox." It is transmitted to humans through direct contact during manipulation of an infected cow's udders.[373] Indirect transmission through fomites has also been reported.[374]

Clinical Features. The usual clinical presentation involves a solitary lesion on the hands, although other areas of the body can be affected, and sometimes multiple lesions are seen. The lesions usually are 0.5- to 2-cm, red-violaceous nodules that sometimes show crusting or ulceration.[371,375]

Orf (Ecthyma Contagiosum). Orf affects primarily young sheep and goats in the form of perioral lesions, and it is transmitted to humans who have contact with these lesions.[375] Transmission through trauma inflicted by contaminated fomites has been described.[376]

Clinical Features. Orf lesions are usually on the hands and forearms, but they can appear on the scalp, on the face, and in other locations and are represented by 1- to 3-cm nodules that usually show umbilication and an erythematous halo. The lesions last for 7 days until regression occurs.[375,377] They can be complicated by painful regional lymphadenitis, impetiginization, and even a widespread varicelliform eruption.[377] Other cutaneous disorders that can be associated with orf are bullous pemphigoid (BP),[378] EM,[379] and toxic erythema.[380]

Histopathology of Cutaneous Parapoxvirus Infections. Paravaccinia and orf lesions are considered to be microscopically indistinguishable[375] and similar to other poxviridae infections. Their histopathologic features depend on the stage of the lesion. Early lesions (vesicular phase) show prominent moderate acanthosis and severe vacuolization with ballooning degeneration of keratinocytes in the upper third of the epidermis.[375,381] This ballooning degeneration, especially when it occurs in follicular structures, has been termed "spongiform degeneration" and can lead to vesicle formation.[375] As seen in other poxvirus infections, intracytoplasmic viral inclusions are usually readily identified in some cases.[382]

As the lesions progress, epidermal necrosis can be found, and it can be quite extensive, especially in orf lesions (pustular phase). Extensive epidermal necrosis is usually associated with ulceration and crusting. In the papillary dermis, one sees prominent edema with ectatic vessels, which can become quite prominent and, when seen combined with the rapid growth of the lesion, can simulate malignant vascular proliferation.[383] A prominent inflammatory infiltrate, composed of lymphocytes, histiocytes, plasma cells, and occasional eosinophils, is seen in the papillary and mid-dermal regions. Neutrophils can be observed in the epidermis when epidermal necrosis occurs. Regressing and more advanced lesions exhibit prominent reduction of the acanthosis and of the inflammatory infiltrate.[375,377,381]

As with all poxviruses, electron microscopy is quite useful for rapid diagnosis when the crusts of early lesions are examined, revealing the classic brick-shaped viral particles.[384] Immunoperoxidase techniques are useful if it is necessary to discern between the two species.[381,385]

Molluscipoxvirus

Molluscum Contagiosum. Molluscum contagiosum virus infection is a relatively common skin condition presenting in both immunocompetent and immunocompromised patients. Transmission of the infection can be made through direct skin-to-skin contact, through fomites, or during sexual activity.[386,387] Cases involving vertical transmission have been also described.[388] The disease is more commonly found among adolescents and young adults. Persons with an immunocompromised status are frequently affected by this virus.[386,389]

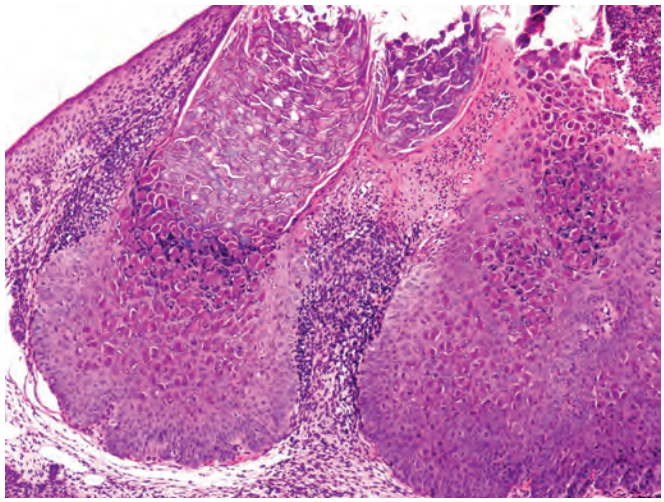
Clinical Features. The lesions of molluscum contagiosum have a predilection for the head and neck region, flexural areas, and genitalia. They may be solitary or may appear in crops, sometimes forming plaques. They consist of dome-shaped papules and nodules that are flesh-colored, have a waxy consistency, and exhibit a dell or umbilication in the center.[386] These lesions measure 2 to 8 mm on average, although solitary lesions can be larger.

Although molluscum contagiosum is often a self-limited infection that spontaneously regresses in approximately 1 year, lesions in atopic individuals tend to become chronic and sometimes manifest with complications such as pitted scarring.[390] In immunosuppressed individuals the clinical course is typically complicated with large and extensive lesions that usually require treatment with cryotherapy and topical antivirals, such as cidofovir.[391]

Although molluscum contagiosum is usually readily identified clinically, the clinical presentation can be quite atypical in patients with HIV/AIDS.[389]

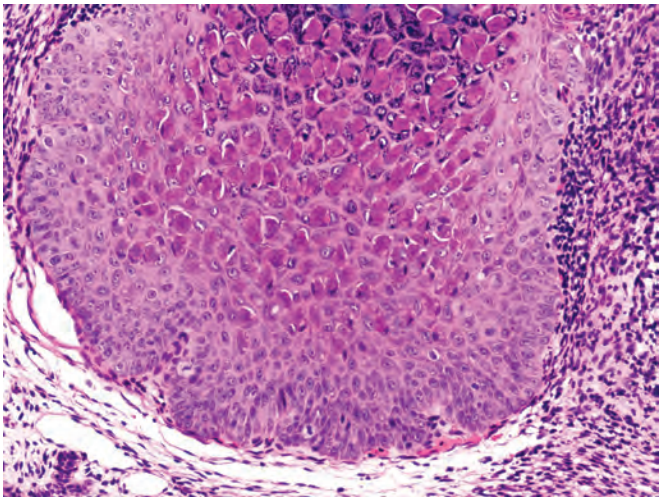
Histopathology. The microscopic features of molluscum contagiosum infection usually involve an inverted expansile growth of lobules composed of enlarged basal cells and keratinocytes. These nodules compress the intermingled dermis to form fine fibrovascular septa (Fig. 20.54). The infected epithelial cells contain the characteristic intracytoplasmic viral inclusions, which are called molluscum bodies or Henderson-Patterson bodies. These viral particles appear in the keratinocytes forming the Malpighi layer. As these granules ascend, they increase in size, up to 35 μm in diameter, and they become intensely basophilic (Fig. 20.55). As they reach to the stratum corneum, these infected cells disintegrate into a mixture of keratinaceous debris and molluscum bodies that are eliminated through the central pore of the lesion (Fig. 20.56). This central crater with its extruding material represents the umbilication seen in clinical lesions.[392]

The dermis surrounding the lesions can show a variety of inflammatory patterns. In early processes, there is usually no or minimal inflammatory infiltrate; a variable chronic inflammatory response is found in regressing lesions (Fig. 20.57). Foreign body



• **Figure 20.54** Molluscum contagiosum. The distinctive histologic picture of molluscum contagiosum, with its well-circumscribed, lobulated endophytic hyperplasia, makes the lesion instantly recognizable. Note that this lesion is arising in the midst of a nevus.

giant cell response, as well as eosinophils with even flame figures,[393] can be seen in response to intrusion of infected keratinocytes or molluscum bodies. Rarely, one can find a pseudolymphomatous $CD8^+$ lymphoid infiltrate with scattered $CD30^+$ cells.[394] Molluscum contagiosum lesions can be found associated with a variety of cutaneous processes, such as melanocytic lesions and epidermal cysts.[392] Occasionally, cutaneous involvement by *Cryptococcus neoformans* can show a molluscum-like histopathologic pattern.[395]



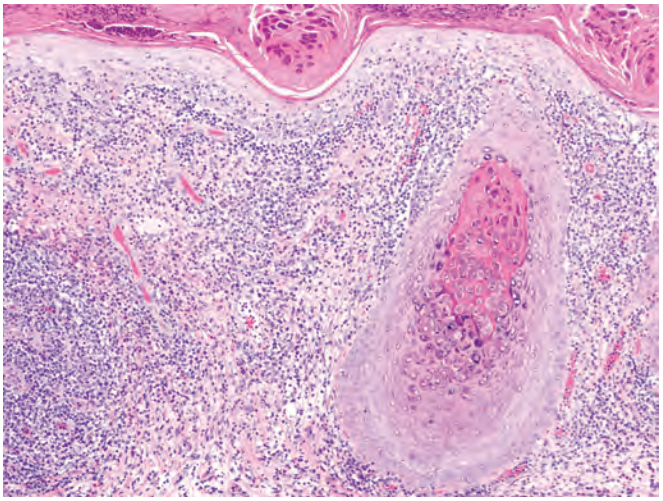
• **Figure 20.55** Molluscum contagiosum. The intracytoplasmic viral inclusion bodies arise near the basal layer and displace the nuclei of the keratinocytes; they later acquire strong basophilic tinctorial properties as they ascend toward the stratum corneum to their final elimination through the central pore of the lesion. The basophilic staining is due to DNA that has been uncovered by an enzyme that digests the RNA, which stains pink.

Parvoviridae

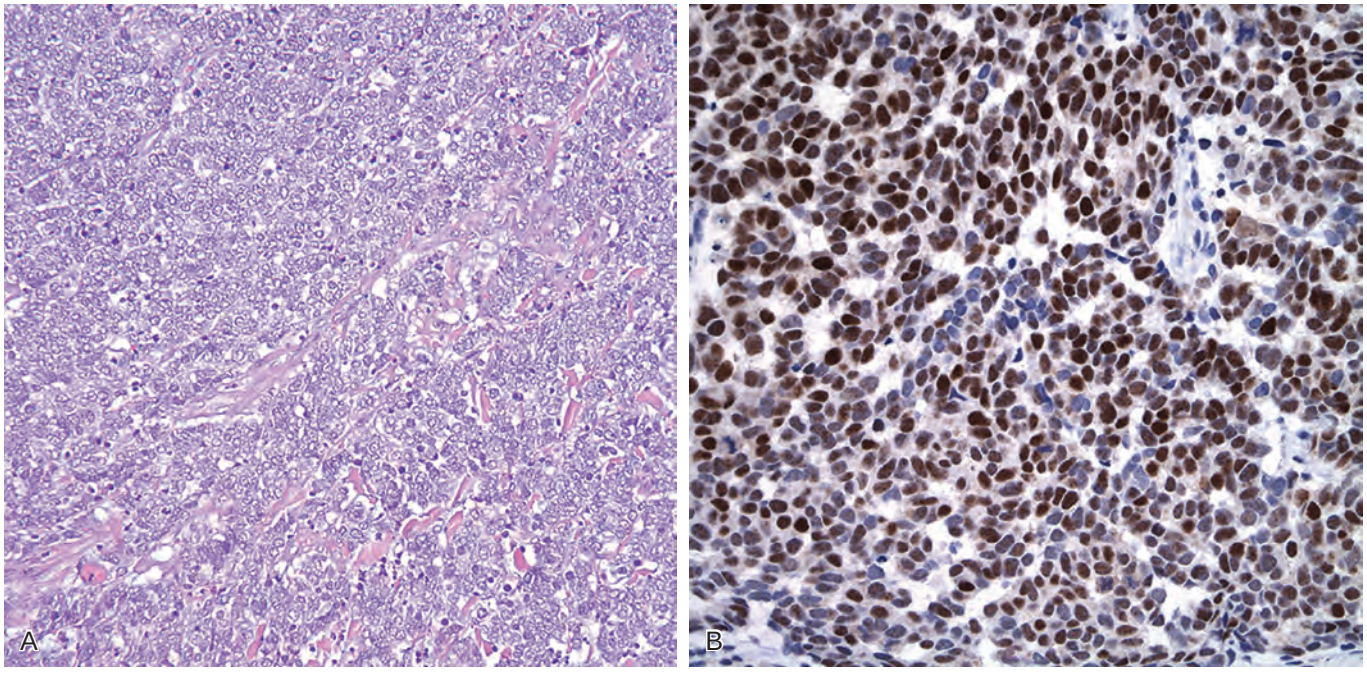
This virus family is composed of small (18 to 23 nm) (*parvum*/*parvus*, Latin for small), nonenveloped DNA viruses, the most famous of which is the parvovirus B19 (*Erythroparvovirus* genus).

Parvovirus B19

Human infection with Parvovirus B19 is quite ubiquitous, with a reported seropositivity of up to 90% in older persons.[396] The infection is acquired mainly through the respiratory route but can be transmitted through blood products, particularly factor VIII and factor IX concentrates.[397] Transplacental transmission of



• **Figure 20.56** Molluscum contagiosum. Some cases can show an exuberant, almost pseudolymphomatous host response, as is seen in this regressing lesion. Characteristic changes of molluscum may only be found after extensive leveling of the tissue.



• **Figure 20.57** Merkel cell carcinoma (MCC). Classic morphology of MCC with sheets of "small round blue cells" with "washed-out" or neuroendocrine chromatin pattern **(A)**. Immunohistochemical stain (CM2B4 clone) for the MCPyV T antigen showing the nuclear positivity in the tumor cells **(B)**.

parvovirus B19 is an important cause of fetal demise during the second trimester in women infected during pregnancy.[398]

The pathogenic action of this virus is targeted toward the erythroid precursors, erythrocytes, and endothelial cells. The distinct tropism for erythroid cells is caused by the presence of the P antigen, a globoside (neutral glycosphingolipid) that is also referred to as the blood group P antigen, which acts as the virus receptor.[399] Consequentially, it has been found that individuals lacking this antigen are resistant to parvovirus B19 infection.[400]

At the endothelial level, parvovirus B19 produces an immune-based microvascular injury through upregulation of the TNF-α pathway, which leads to accelerated apoptosis of endothelial cells with subsequent release of numerous nuclear and cytoplasmic antigens (e.g., Ro, La, RNP) that later trigger several autoimmune phenomena.[401] This mechanism suggests a possible relationship between B19 infection and the development of several autoimmune conditions, including scleroderma[402] and dermatomyositis[403].

Clinical Features. Infections with parvovirus B19 in immunocompetent individuals tend to be asymptomatic or be manifested by polyarthritis and polyarthralgias, sometimes accompanied by a mild transient anemia.[404] The presence of an underlying hemoglobinopathy (e.g., sickle cell disease) or an immunosuppressed state can give rise to a severe pure aplastic anemia.[404] In the skin, two main clinical presentations of parvovirus B19 are recognized: the "gloves and socks" papular-purpuric syndrome and erythema infectiosum (fifth disease), the latter seen mostly in children.

The Gloves-and-Socks Syndrome. This pattern, also seen in other viral infections (see the previous HHV-6 and HHV-7 section), usually features erythematous, pruritic papules involving the hands and feet with some cases showing petechiae[405] and even purpuric lesions.[406] Systemic symptoms, such as fever and arthralgias, may be present. Although the disease usually has a self-limited course in immunocompetent individuals, immunosuppressed patients tend to have a protracted and complicated course.[404]

Erythema Infectiosum of Infancy. Also known as fifth disease, this manifests initially with the classic "slapped cheek" sign, which is usually unilateral. Perioral pallor and a subsequent diffuse reticular erythema of the extremities follow this localized rash. A petechial rash may also be seen in acral surfaces. A prodrome of fever, sore throat, myalgia, and arthralgias usually precedes these symptoms.[404]

Histopathology. The microscopic features of parvovirus B19 infection are somewhat nonspecific and can show a wide variety of presentations. Slightly dilated and irregular papillary dermal vessels flanked by a tight perivascular lymphocytic infiltrate located in postcapillary venules usually comprise the histologic features of cutaneous parvovirus B19 lesions, regardless of the clinical presentation. An epidermis showing mild vacuolar change, some exocytosis of lymphocytes, and a rare apoptotic keratinocyte overlies this perivascular inflammatory infiltrate[407,408] Cases exhibiting petechiae and purpuric lesions show the corresponding extravasated red blood cells and chronic lesions can exhibit a prominent granuloma annulare–like picture along with a hypercellular dermis with mucin deposition.[407] Immunohistochemical stains directed to the viral protein VP2 will highlight the cytoplasm of the infected endothelial cells.[385]

Polyomaviridae

Composed of small (40 to 50 nm), nonenveloped DNA viruses, this rapidly expanding family was once part of the now defunct papovavirus family that was divided into polyoma and papillomavirus. These viruses commonly manifest themselves in an immunosuppressed milieu, with most well-known members being both the JC and BK viruses, as well as the recently discovered Merkel cell carcinoma polyomavirus (MCPyV), the trichodysplasia spinulosa–associated polyomavirus (TSPyV), and the human polyomavirus 7 (HPyV-7). All of these viruses share the same pathogenic pattern of reactivation in immunosuppressed patients.

Merkel Cell Carcinoma–Associated Polyomavirus

The discovery of the MCPyV[409] has indeed completely changed our understanding of MCC pathogenesis and behavior. MCC is a rare and aggressive form of NMSC that affects preferentially older adults and the immunosuppressed. MCPyV is the only polyomavirus that so far has an associated oncogenic potential. Several studies have demonstrated that MCPyV is quite prevalent in the healthy adult population, and its presence is detected in peripheral blood either directly by molecular DNA amplification techniques[410] or indirectly by serology.[411,412] Evidence of the virus has also demonstrated from cutaneous swabs.[413] A prospective study has correlated high serum MCPyV antibody titers in healthy individuals with an increased risk of subsequent MCC development in older age.[414] Transmission of MCPyV is likely to occur in early childhood, especially between family members[415] and the virus remains dormant in a wide variety of tissues[416] until immunosuppression-triggered reactivation, as is characteristic of this viral group. The importance of MCPyV resides in that it is part of the two proposed models of MCC pathogenesis, the other being an ultraviolet (UV)-damage induced pathway, which involves *TP53* mutations and is unrelated to MCPyV.

Although no assembled virions are seen in MCC cells, the causative oncogenic role of MCPyV in MCC is currently widely accepted because the viral genetic material is integrated in clonally expanded MCC tumor cells.[409]

Clinical Features. An aggressive and rare nonmelanoma skin tumor of older adults, MCC frequently arises on chronically sun-damaged skin of the head and neck and extremities, with rare cases arising from the trunk. It usually presents as a rapidly growing, painless, and often firm nodule or mass with erythematous, pink, violaceous or even bruise-like overlying skin that is often nonulcerated.[417,418]

Histopathology. MCC is most often a dermal-based tumor, centered in the mid-dermis and exhibiting a grenz zone, and it is composed of "small, round-blue cells," often with "washed-out chromatin" (see Fig. 20.57A). There is often lymphovascular invasion and an aggressive growth pattern. One can see an association with an SCC, either invasive or in situ, and such cases have been regarded by some authors to be unrelated with MCPyV.[419] Both the CM2B4 (see Fig. 20.57B) and Ab3 monoclonal antibodies, directed to the MCPyV T antigen peptide, allows for immunohistochemical detection of MCPyV genome integration in tumor cells, with the Ab3 being much more sensitive and showing the best correlation with molecular studies.[385]

Trichodysplasia Spinulosa

This follicle-centered condition has been most frequently seen in immunocompromised individuals, particularly in solid organ allograft transplant recipients. Trichodysplasia spinulosa (TS) has been reported in the past under a wide variety of names, including "viral associated trichodisplasia," "trichodisplasia of

immunosuppression," and "spiny hyperkeratosis (hairlike hyperkeratosis)."[420] Although the relationship between TS and a viral cause was known or suspected, in 2010 the causative agent was described as a polyomavirus, the TSPyV.[421] This virus is highly prevalent in humans, with positive serology present in up to 88% of the adult population.[412]

Clinical Features. This infection often manifests itself by the presence of multiple keratotic spiny and sometimes friable papules on the ears and central face and less commonly involving trunk and extremities. Some cases will go on to develop alopecia of eyebrows and eyelashes.[420] Lesions of TS tend to persist in individuals while they sustain immunosuppressive therapy and eventually resolve after this therapy ceases.

Histopathology. Lesions of TS have a distinctive microscopic picture that consists of dilated and distorted anagen follicular structures with a disorganized inner root sheath that are filled by sheets of eosinophilic cells containing exaggerated and large trichohyaline granules.[420,421] These cells abruptly cornify into a blue-gray aggregate of keratinaceous debris that emerges through the follicular ostium and results in the spiny papules. The presence of the virus can be confirmed by either PCR-based assays and electron microscopy, and immunohistochemical stains are currently being developed.[385]

Infection With Human Polyomavirus 7

This virus was found to be present incidentally in cutaneous swabs from asymptomatic individuals and has a prevalence of up to 66% in adults.[412,422] HPyV-7 was implicated in the development of pruritic, brown plaques on the trunk and extremities in patients receiving immunosuppressed therapy who also developed viremia.

Clinical Features. Lesions associated with HPyV-7 reactivation appear very distinctive and consist of intensely pruritic, finely scaled, and velvety-appearing plaques predominantly involving the lower back, immediately above the gluteal cleft.[422]

Histopathology. Microscopically, HPyV-7 lesions show slight acanthosis with an expanded stratum corneum containing clusters of nucleated keratinocytes along with plump, eosinophilic keratinocytes containing viral inclusions at different levels of the epidermis arranged in a "peacock tail–like" pattern.[422] A mild, superficial perivascular lymphocytic infiltrate is seen in dermis. The presence of the virus can be demonstrated either by immunohistochemistry and/or by PCR-based molecular assays.[422]

Picornaviridae

This very large family is composed of small, RNA viruses ("PicoRNA") and includes the equally extensive *Enterovirus* genus (polio and nonpoliovirus), as well as the hepatitis A virus *(Hepatovirus)*. The main cutaneous infection caused by this family of virus is hand-foot-and-mouth disease.

Hand-Foot-and-Mouth Disease

The condition known as hand-foot-and-mouth disease, caused by the Coxsackie A16 virus and enterovirus 71, usually affects children and rarely affects healthy adults.[423] The disease is transmitted through direct contact with nasal secretions, feces, and blood, and it typically manifests as small community outbreaks, but severe epidemics have been reported both in Taiwan[424] and in continental China.[425]

Clinical Features. The incubation period ranges from 3 to 7 days and is followed by a prodrome of fever, malaise, headache, abdominal pain, and diarrhea. After this prodromal period, painful blisters and ulcers appear on the buccal mucosa, tongue, and lips. A self-limited eruption follows, first as small, erythematous papules that erupt on the buttocks, as well as in palms and soles, fingers, and toes.[423] These papules progress to gray-colored vesicles with an erythematous halo.

Although complications of hand-foot-and-mouth disease caused by Coxsackie A16 virus are rarely seen, severe sequelae have been described in enterovirus 71–related cases, including myocarditis, encephalitis, aseptic meningitis, and a poliomyelitis-like disease.[426] The mortality rate with enterovirus 71–induced disease is approximately 8%, and deaths are usually related to cardiorespiratory failure with pulmonary edema.[424]

Histopathology. Histologically the lesions show intraepidermal vesiculation with prominent reticular degeneration and ballooned cells. Epidermal necrosis and dyskeratosis can be observed, along with prominent edema of the papillary dermis and a mild perivascular infiltrate.[427] The differential diagnosis includes HSV infection, but no giant cells or steel-gray nuclei are seen. Immunohistochemical stains directed to the enterovirus-related VP1 peptide can help to diagnose difficult cases.[385]

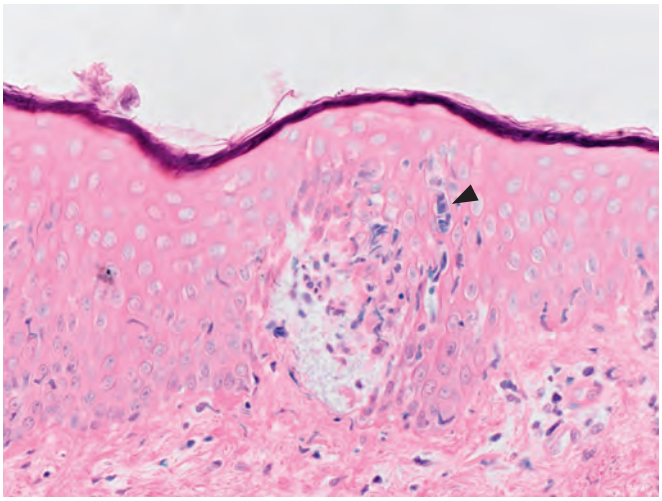
Paramyxoviridae

Measles Virus

The measles virus, an enveloped, single-strand RNA virus that is part of the Paramyxovirus family and *Morbillivirus* genus, causes a potentially serious systemic infection with a characteristic generalized cutaneous maculopapular erythematous eruption. This illness has been known as "morbilli," "rubeola," and "red measles," with humans being the only known host and reservoir. Measles spreads via the respiratory route and remain in lymph nodes from which it can spread to almost any organ. Unfortunately, this disease is currently experiencing a "comeback," a product of the very dangerous and misinformed parental decision to not vaccinate their children.[428]

Clinical Features. Measles is characterized by a prodrome stage that shows a pathognomonic enanthem that consists of white papules in the oral mucosa: the Koplik spots. After this prodromal phase, the full-blown clinical features appear, including fever, malaise, cough, nasal congestion (coryza), and conjunctivitis. The erythematous maculopapular skin eruption appears 3 or 4 days after the onset of fever and spreads in a craniocaudal direction, becoming confluent as the disease progresses. Although the disease is often self-limiting in immunocompetent and immunized individuals, it may be severe and even deadly in immunocompromised patients. Serious complications may develop, including the devastating but fortunately rare subacute sclerosing panencephalitis, which can develop up to 15 years after the primary infection.

Histopathology. The histologic examination of measles lesions reveals a nonspecific pattern that includes a spongiotic epidermis with keratinocytes showing degenerative changes, such as cytoplasmic swelling and dyskeratosis.[429] Epidermal vesiculation can also be seen. The dermis shows a superficial perivascular lymphocytic infiltrate.[385] Multinucleated cells may occasionally be seen in the upper epidermis (Fig. 20.58, *arrowhead*), in hair follicles, and in the acrosyringium.[429,430] Immunohistochemistry can highlight the presence of the virus in the epidermis and folliculosebaceous units, as well as in endothelial cells and inflammatory cells.[385]



• **Figure 20.58** Measles. The cutaneous histologic features of this systemic viral infection are composed mainly of spongiosis and vesiculation of the epidermis. Note the multinucleated cells *(arrowhead)*.

Retroviridae

This family is composed of RNA virus with the unique capability of converting RNA to DNA through the reverse transcriptase enzyme. The main components of clinical significant are the human T-cell lymphotropic virus type 1 (HTLV-I), also known as adult T-cell lymphoma virus type 1, and the HIV from the *Deltaretrovirus* and *Lentivirus* genera, respectively. Both of these viruses are associated with profound immunosuppression of the hosts.

Human T-cell Lymphotropic Virus Type 1

Endemic in Japan and in the Caribbean, HTLV-1 is transmitted through sexual intercourse, blood transfusions, needle sharing among intravenous drug abusers, and breastfeeding. This virus manifests itself in four different forms, according to the WHO classification of tumors: acute and chronic leukemic, lymphomatous, and smoldering types. Cutaneous manifestations are seen predominantly in the smoldering variant. Two cutaneous disorders are associated with HTLV-1 infection: an exudative and eczematous dermatitis from the scalp, axillae, groin, and small folds of the face that is denominated infective dermatitis[431] and cutaneous manifestations of acute T-cell leukemia/lymphoma (ATLL). Lesions of ATLL are clinically and microscopically indistinguishable from mycosis fungoides and its variants. Immunohistochemistry can highlight the infected lymphoid cells.[385]

Human Immunodeficiency Virus

Infection with HIV and subsequent development of AIDS is accompanied by a myriad cutaneous processes of an infectious, inflammatory, or neoplastic nature. The HIV/AIDS-related dermatologic infectious conditions are mostly caused by atypical and opportunistic agents, including viruses, bacteria, fungi, and protozoa. Ectoparasitic infestations (e.g., scabies, demodicosis) are also seen in these patients and are reviewed later in this chapter.

HIV-Related Dermatoses. Dermatologic conditions that are commonly found in the general population manifest in HIV/AIDS patients with atypical and aggressive features. An explosive episode of psoriasis or seborrheic dermatitis may be the first manifestation of HIV infection. Such manifestations are thought to be a product of the profound HIV-induced immune dysregulation and immune suppression. However, some of the lesions may be related solely to the direct action of HIV as a pathogen. The presence of these dermatoses in a patient who presents with a suspicious clinical picture provides a useful clue to the correct diagnosis. In the following section we will describe some of these HIV/AIDS-related inflammatory manifestations.

HIV Seroconversion-Related Rash. The maculopapular rash seen in the acute phase of HIV infection, also known as acute exanthem of HIV infection,[432] develops in up to 67% of cases.[433] The histopathologic picture is similar to that seen in most viral exanthems, consisting of a tight, superficial perivascular, lymphohistiocytic infiltrate accompanied by a variety of mild epidermal changes, including spongiosis, vacuolar alteration, scattered dyskeratotic cells, and sometimes even epidermal necrosis.[434]

Immune Reconstitution Inflammatory Syndrome. Presenting in a subset of patients undergoing treatment with highly active antiretroviral therapy (HAART), immune reconstitution inflammatory syndrome (IRIS) is a paradoxical worsening of opportunistic infections and inflammatory conditions that can appear days or months after the initiation of HAART. IRIS can show multiple types of skin manifestations including sarcoidosis and a lupuslike syndrome, as well as infections with atypical mycobacteria, viruses, and many other agents.[435] IRIS is possibly a product of immune dysregulation and redistribution of various populations of T cells.[436]

Pruritic Papular Eruption of HIV. One of the most common cutaneous conditions seen in HIV patients, pruritic papular eruption (PPE) affects up to 46% of these patients.[323] Some authors consider PPE a manifestation of primary HIV infection of the skin. However, it can also be found in non-HIV patients with $CD4^+$ lymphocytopenia. Clinically, PPE is characterized by multiple pruritic, discrete, skin-colored papules on the extremities, face, and trunk. The condition tends to be chronic and waxes and wanes.[437] Microscopic examination of PPE lesions reveals basically a dense, superficial and deep, perivascular, lymphoeosinophilic infiltrate associated with epidermal hyperplasia with hyperkeratosis, acanthosis, and focal dyskeratosis. A pattern showing dermal fibrosis with a proliferation of factor XIIIa+ cells and a dermal infiltrate composed of lymphocytes, plasma cells, eosinophils, and mast cells have also been described.[437]

Eosinophilic Folliculitis. This condition can appears both in advanced HIV cases and also as part of IRIS. Clinically, it manifests as intensely pruritic, erythematous, and folliculocentric papules that can evolve to pustules. The lesions appear most commonly on the forehead, neck, shoulders, trunk, and upper arms.[438] Microscopically the lesions are composed of folliculocentric aggregates of eosinophils and lymphocytes, often with necrosis of sebaceous glands.

Seborrheic Dermatitis. This dermatosis is rather common in these individuals, affecting up to 85% of the HIV/AIDS patient population.[323] The clinical picture is similar to that seen in the general population, but the lesions tend to be much more exuberant, sometimes with an erythroderma-like presentation.[439] Likewise, AIDS-related seborrheic dermatitis biopsy specimens show an atypical histopathologic picture, including marked hyperkeratosis with confluent parakeratosis, follicular plugging, acanthosis, and slight spongiosis. Numerous yeast forms of *Malassezia furfur*, as well as exocytosis of lymphocytes and neutrophils, along with dyskeratosis, are also seen.[439] Other inflammatory dermatoses that present more exuberantly in HIV/AIDS patients include xerosis, atopic dermatitis, pruritus, Reiter syndrome, and psoriasis.[323]

Fungal Skin Infections

The most important fungal infections of the skin will be discussed in the next section, divided into superficial and deep mycoses.

Superficial Mycoses

Fungi that are only capable to invade fully keratinized tissues, including the stratum corneum, hair, and nails are agents causing superficial mycoses. They are usually divided into two groups: noninflammatory and inflammatory superficial mycoses, according to the degree of the host response (Table 20.2).

Pityriasis Versicolor

PV, or tinea versicolor, is caused by lipophilic yeasts grouped in the genus *Malassezia* (formerly known as *Pityrosporum*).[440] This condition has worldwide distribution but is significantly more common in humid and tropical climates.[441] The genus *Malassezia* includes 10 anthropophilic and obligatory lipophilic species (*M. globosa*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. furfur*, *M. sympodialis*, *M. japonica*, *M. yamatoensis*, *M. dermatis*, and *M. nana*) and three zoophilic species (*M. pachydermatis*, *M. caprae*, and *M. equina*).[442] Although *M. furfur* was historically considered as the main culprit, it is now known that *M. globosa* seems to be the predominant species found in the lesions of PV.[440,443,444] *Malassezia* yeasts are considered part of the normal flora of the skin, being particularly common on the scalp, face, and trunk.[445] Under certain predisposing conditions, the yeast form undergoes a dynamic change to a pathogenic mycelial form associated with clinical disease. Heat and humidity, oily skin, hyperhidrosis, hereditary factors, and immunosuppression (including AIDS) are thought to be predisposing factors.[441]

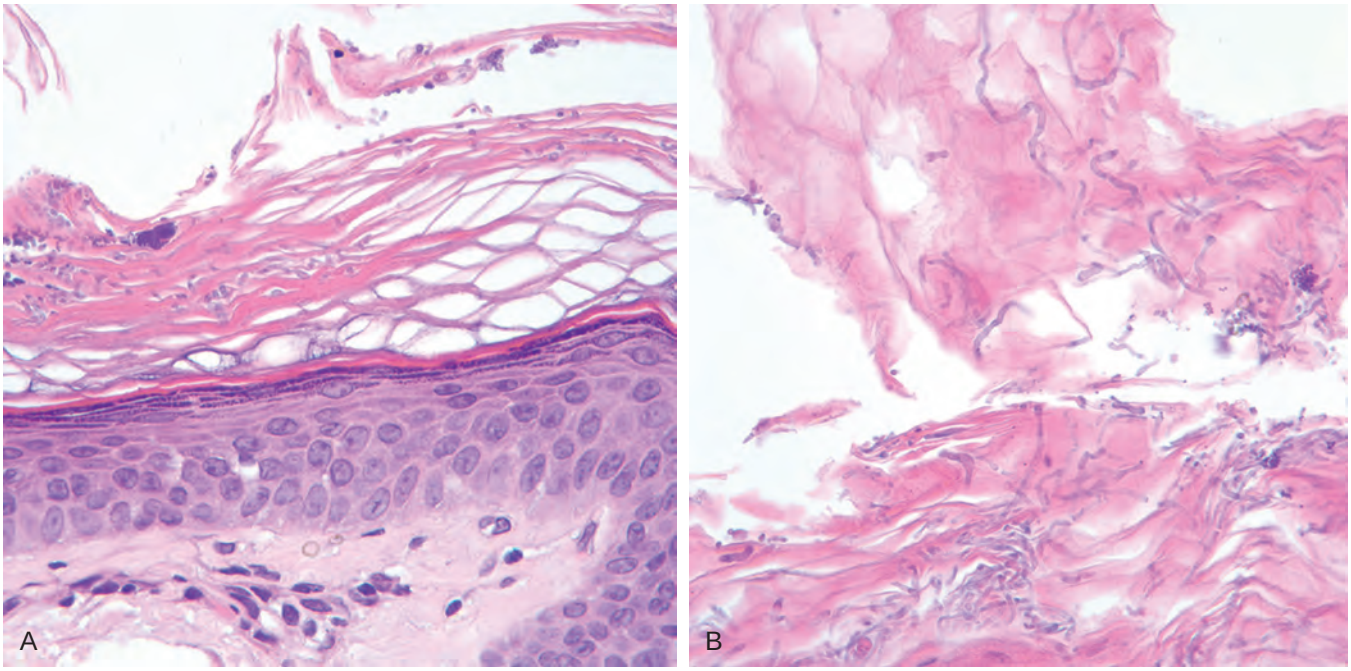
TABLE 20.2 Superficial Skin Mycoses

Clinical Presentations	Etiologic Agents
Noninflammatory (Producing Little or No Tissue Reaction)	
Pityriasis versicolor	*Malassezia* spp.
Tinea nigra	*Hortaea werneckii*
White piedra	*Trichosporon* spp.
Black piedra	*Piedraia hortae*
Inflammatory (Commonly Leading to Inflammatory Response)	
Dermatophytoses	*Trichophyton*, *Microsporum*, *Epidermophyton* spp.
Cutaneous candidiasis	*Candida* spp.

Clinical Features. Most cases occur during adolescence and young adulthood, with no clear gender predilection and the infection, which is not considered contagious, follows a chronic and recurrent course.[441]

Skin lesions manifest as macules and patches, which tend to become confluent, involving large areas of the chest, neck, upper arms, and back.[446] Involvement of the face is unusual in temperate climates but seems to be common in tropical and subtropical regions.[440] The macules and patches are yellowish-brown, pale yellow, or dark brown, sometimes reddish or pinkish, appearing hypopigmented or hyperpigmented and will typically show fine scaling when scratched.[446]

Histopathology. Histologically, there is mild hyperkeratosis and acanthosis (Fig. 20.59A). Short, septate hyphae and round budding yeasts are present in the stratum corneum, in a pattern resembling "spaghetti and meatballs" (see Fig. 20.59B). The organisms can be highlighted with special stains, such as PAS, methenamine silver nitrate, or Giemsa stains.[441] A mild, chronic, superficial perivascular inflammatory reaction is occasionally



• **Figure 20.59** Tinea (pityriasis) versicolor. The fungal elements are located in the stratum corneum with almost no alteration of the rest of the epidermis **(A)**. Note the classic "spaghetti and meatballs" morphology seen with *Malassezia furfur* and *Malassezia globosa* **(B)**.

observed in the dermis. Hyperpigmented lesions can show pigment incontinence, and, when compared with hypopigmented lesions, they display a thicker keratin layer containing more organisms, along with a more intense perivascular infiltrate.[441,447,448]

Although the pathogenesis behind the hypopigmentation observed in PV has not been fully elucidated,[449] it appears that dicarboxylic acids produced by the fungi (e.g., azelaic acid) might inhibit the dopa-tyrosine reaction to induce hypopigmentation.[447,450] In addition, the size and distribution of melanosomes within melanocytes and keratinocytes might also be modified, producing hypopigmentation or hyperpigmentation, respectively, when the melanosomes are abnormally small or abnormally large.[448,451]

Pityrosporum Folliculitis

Pityrosporum folliculitis is a form of chronic folliculitis caused by *Malassezia* yeasts that preserves the former name of the genus in its name. In a similar way to PV, it has a worldwide distribution and is more commonly found in tropical climates.

Clinical Features. The condition is seen in adults, who present with erythematous follicular papules and pustules located mainly on the trunk, neck, and upper arms. These lesions may be asymptomatic or pruritic.[446] Predisposing factors for *Pityrosporum* folliculitis include exposure to heat and humidity, use of systemic and topical steroids, and immunosuppression.[446,452] Follicular occlusion is thought to be the primary event in the development of this condition, with yeast overgrowth being a secondary occurrence. Increased conversion of triglycerides in sebum to free fatty acids by the fungi may promote a free fatty acid–mediated inflammation within the follicle.[453]

Histopathology. Histopathologic examination shows a chronic inflammatory infiltrate around the infundibular region of the pilosebaceous follicles.[441] These follicles are usually plugged and dilated, containing keratinous debris and clusters of round to oval budding yeast forms, which are observed within the infundibulum.[446,452] The organisms are GMS- and PAS-positive and measure 2 to 4 µm, and, unlike PV, hyphae are rarely seen.[441] Furthermore, a lymphohistiocytic perivascular inflammatory infiltrate is observed in the superficial dermis; it is composed primarily of $CD4^+$ T cells associated with an increased number of Langerhans cells.[441,454] Intradermal rupture of the follicular epithelium can lead to an intense folliculocentric, neutrophilic inflammatory response with a perifollicular foreign body giant cell reaction. Intrafollicular deposits of mucin are sometimes present.[455]

Tinea Nigra

Tinea nigra is caused by the dematiaceous fungus *Hortaea werneckii* (formerly known as *Phaeoannellomyces werneckii*, *Exophiala werneckii*, and *Cladosporium werneckii*).[456] Another dematiaceous fungus, *Stenella araguata*, which occurs in Venezuela, produces a similar clinical picture.[441,457] Tinea nigra typically occurs in children and young adults, with female predominance, and it is observed in patients living in warm countries and in those who have lived in or visited the tropics or subtropics.[456] The organism attaches to the skin surface, probably aided by minor trauma and moisture.[458] Although *H. werneckii* can be found in soil, compost, and sewage; on wood; in house dust, on salted dried fish; and even on clinically normal-appearing skin,[458] *S. araguata* has not been isolated from the environment to date.[457]

Clinical Features. Tinea nigra is clinically characterized by a sharply marginated, light-brown to black, nonscaly, oval-shaped macule. In some patients, this macule is mottled and might be irregular in shape. Although lesions present usually as single and asymptomatic and they are mainly observed on the palmar surface (Fig. 20.60), involvement of the plantar surface, neck, or trunk can also occur.[456] Hyperhidrosis could be a risk factor. Lesions of tinea nigra are quite characteristic, but they can be clinically misdiagnosed as junctional melanocytic nevus and even as malignant melanoma.[457]

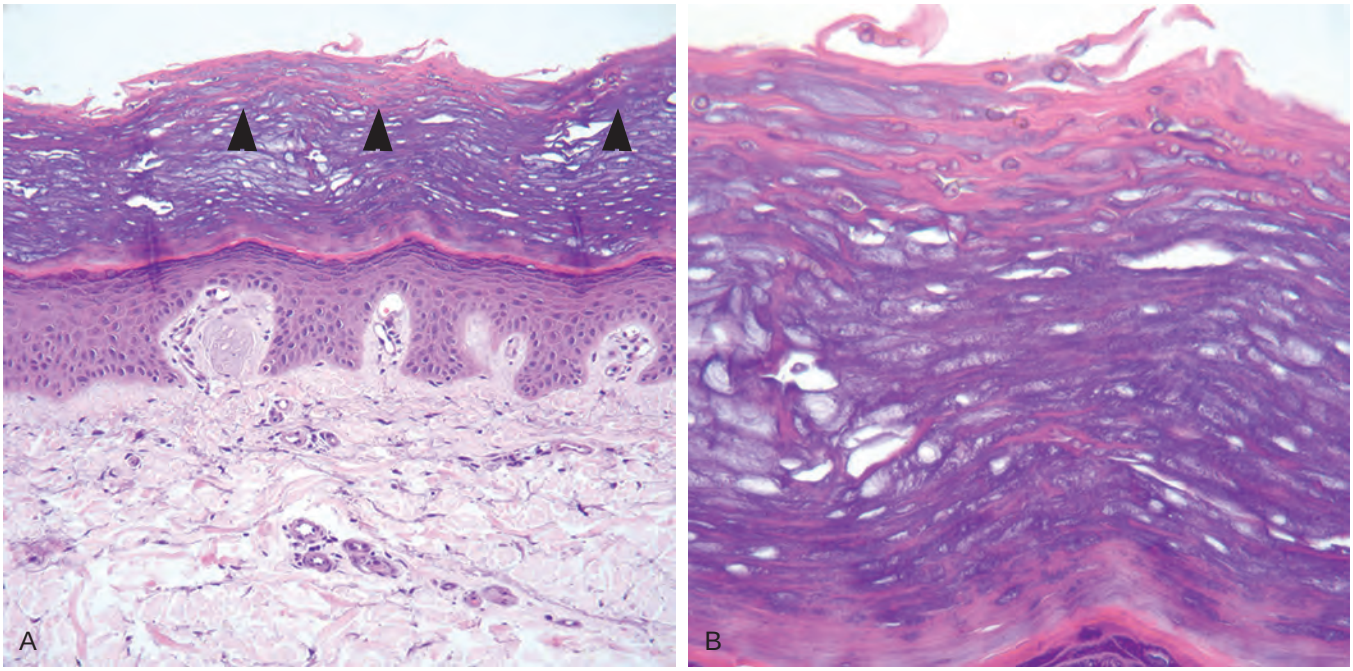
• **Figure 20.60** Tinea nigra. This well-demarcated hyperpigmented lesion on the palm of a young patient was believed to be a melanocytic lesion. (Courtesy John Hamill, MD, Gulf Coast Dermatology, Tampa and Paul Rodriguez-Waitkus MD, PhD, Department of Dermatology, University of South Florida, Tampa.)

Histopathology. Dark-colored, branching, septate hyphae and round to oval yeast forms are present in the superficial layers of the stratum corneum. There is generally no inflammatory response (Fig. 20.61A). As a result of pigmented cell walls, these fungi are usually evident on routine staining with H&E (see Fig. 20.61B).

White Piedra and Trichosporonosis

White piedra is a fungal hair shaft infection caused by fungi from the *Trichosporon* genus class Basidiomycetes. This condition was previously thought to be caused by *Trichosporon beigelii* (now called *Trichosporon cutaneum*).[459] However, molecular studies have shown that the *Trichosporon* genus includes several pathogenic species, including *T. cutaneum*, *T. asahii*, *T. mucoides*, *T. ovoides*, *T. asteroides*, and *T. inkin*.[460] White piedra is most prevalent in temperate and subtropical climates.[441] The natural habitats of *Trichosporon* species are soil, lake water, and plants, and such fungi are occasionally seen as normal flora of the human skin and mouth.[456] White piedra is not considered contagious; instead, it is probably acquired from the environment by susceptible individuals under appropriate conditions.[441]

Clinical Features. White piedra is characterized by small, white to tan, irregular nodules that encircle the hair shaft.[459] These nodules are soft and can be easily detached from the hair



• **Figure 20.61** Tinea nigra. Pigmented hyphae are usually visible without special stains and are present in the stratum corneum; note the lack of any dermal or epidermal inflammation **(A)**. Pigmented hyphae of tinea nigra at high magnification **(B)**.

shaft; the affected hairs may be split or broken.[456] A variety of hairy areas may be affected, including the scalp, beard, moustache, genitals, axillae, eyebrows, and eyelashes. Typically, only one area is involved.[441] In genital white piedra, a synergistic coinfection, usually with coryneform bacteria, might occur.[461] *Trichosporon* species can also cause onychomycosis, visceral infection, and summer-type hypersensitivity pneumonitis. Disseminated trichosporonosis is seen primarily in immunosuppressed patients, especially those with hematologic malignancies and neutropenia.[462,463] Nevertheless, disseminated infection can also occur in relatively immunocompetent individuals, such as intravenous drug users, patients with prosthetic devices, and patients with severe burn injuries.[464] Cutaneous involvement occurs in approximately 30% of these patients and most frequently manifests as purpuric papules and nodules with central necrosis or ulceration.[464]

Histopathology. In white piedra, potassium hydroxide mounting of an affected hair reveals sleevelike concretions that are composed of loosely arranged hyphae, blastoconidia, and arthroconidia.[456] The use of a fungal stain, such as chlorazol black E, can highlight these elements.[465] In patients who show coinfection with corynebacteria, these can typically be seen at the periphery of the nodules of white piedra.[461] In systemic infections, hyphae and budding yeasts can be observed in the walls of blood vessels and in the dermis.[464] In neutropenic patients the inflammatory infiltrate is usually sparse.[466]

Black Piedra

Black piedra is an infection of the hair produced by the ascomycete *P. hortae*.[467] The condition is usually seen in the tropics worldwide, where it is hot and humid, and sporadically in other regions as a result of international travel.[468] *P. hortae* is present in soil; however, stagnant water and crops can also contain the fungus.

Clinical Features. Black piedra is characterized by stone-hard black nodules, measuring 1 to 2 mm, that are tightly adherent to the hair shafts.[441] This infection usually affects scalp hair, whereas both facial and pubic hairs are typically not affected. A single hair shaft may have one or multiple nodules scattered along its entire length.[441] This fungus has been shown to have keratinolytic activity, with the capacity to damage the cuticle and hair cortex.[468]

Histopathology. Direct microscopic examination of affected hair shafts shows tightly packed, darkly pigmented hyphae, as well as asci and ascospores within black nodules.[456] Each asci contains two to eight crescent-shaped ascospores.[441] The periphery of the nodule has regularly aligned hyphae and arthroconidia. Both sexual and asexual reproduction occur, with sexual reproduction giving rise to ascospores and asexual reproduction to arthrospores.[469]

Dermatophytoses

Dermatophytes are keratinophilic fungi associated with infections of the skin, hair, and nails. They belong to three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*.[470] The incidence of dermatophyte infections and their epidemiologic characteristics depend on social, geographic, and environmental factors and may change over time.[470-472]

Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites.[470] Zoophilic and geophilic dermatophytes generally tend to form lesions that are more inflammatory than those formed by anthropophilic dermatophytes, and those lesions are also more likely to resolve spontaneously.[470]

The clinical manifestations vary depending on the infecting fungus, the body site infected, and the keratinization at that site, as well as the immune status of the host.[473] The dermatophytoses are traditionally divided according to the site infected. Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical

lesions. The major etiologic agents (e.g., *Trichophyton rubrum*) are globally distributed, whereas the distribution of others varies geographically.[470]

Clinical Types

Tinea Barbae. Tinea barbae is a dermatophytic infection that is limited to the coarse hair–bearing beard and mustache areas in men.[474] Clinically, it ranges from mild and superficial to a severe inflammatory pustular folliculitis.[470] In inflammatory cases the zoophilic ectothrix dermatophytes *Trichophyton verrucosum* and *Trichophyton mentagrophytes* are most frequently responsible for the infection.[473,475]

Tinea Capitis (Scalp Ringworm). Tinea capitis can be located on the scalp, eyebrows, or eyelashes and is caused by members of the genera *Microsporum* and *Trichophyton*.[473] Both the skin surface and hairs are involved. The clinical presentation can vary, from scaly and erythematous inflammatory lesions with or without alopecia, to noninflamed lesions resembling seborrheic dermatitis, to large inflamed kerions (see below) that may resemble an abscess.[476] Since the 1950s *Trichophyton tonsurans* has been observed to be the most frequent cause of tinea capitis in the United States and the United Kingdom.[476,477]

Kerion Celsi. Kerion celsi is the inflammatory, suppurative type of tinea capitis often seen in young African American individuals. It produces a painful, inflammatory, crusted mass that can be associated with purulent discharge and regional lymphadenopathy.[478] Kerions are mainly caused by zoophilic dermatophytes.

Tinea Favosa. This clinical form, usually caused by *Trichophyton schoenleinii*, is characterized by the presence of yellow, cup-shaped crusts called scutula on the scalp and glabrous skin.[470,477] Direct microscopic examination of hairs in potassium hydroxide shows an endothrix invasion, with hyphae and air spaces in the hair shafts.[479] Although *T. schoenleinii* infection was widely distributed in Europe in the 18th century, it has almost disappeared from developed European countries, although it is found in areas of Africa.[480]

Tinea Corporis. The ringworm tinea corporis is the typical infection of the exposed glabrous skin and can be caused by any dermatophyte.[473] Patients typically present with an annular patch or plaque with an advancing, raised, scaling border and central clearing.[473]

Tinea Cruris. Tinea cruris is an infection of the groin most commonly seen in adult men, with *T. rubrum* and *Epidermophyton floccosum* being the most frequent etiologic agents.[470,473] Lesions are usually bilateral, erythematous to reddish-brown, and sharply demarcated. The periphery of these lesions shows fine scaling, with papules and pustules being seen in more inflammatory cases.[470,473]

Tinea Imbricata (Tokelau). Tinea imbricata is a chronic dermatophytosis that affects glabrous skin and is exclusively caused by the anthropophilic *Trichophyton concentricum*.[481] Skin lesions are characteristically concentric and lamellar plaques of scale resembling erythema gyratum repens.[473] The infection is geographically restricted to Southeast Asia, India, Central America, and southwest Polynesia.[473]

Tinea Manuum. In tinea manuum the palmar and interdigital areas of the hand are affected, most commonly showing unilateral diffuse hyperkeratosis with accentuation of the flexural creases.[470] *T. rubrum* is the most frequent implicated dermatophyte.[473]

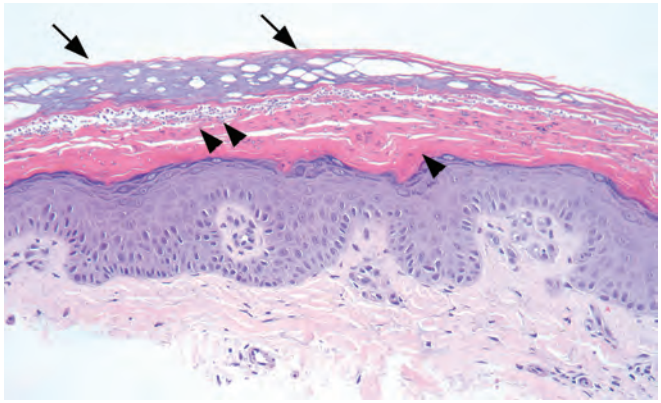
Tinea Pedis (Foot Ringworm, Athlete's Foot). Tinea pedis involves the feet, especially the soles and web spaces. In the interdigital form the web spaces of the feet show maceration, scaling, and fissuring. The spaces between the fourth and fifth toes are the most commonly affected.[470] Another common presentation is the chronic squamous, hyperkeratotic form, in which fine silvery scales cover the pinkish skin of the soles, heels, and sides of the foot.[470] In addition, there is an acute inflammatory form, in which vesicles, pustules, and sometimes bullae are observed. Infections due to *T. mentagrophytes* var. *interdigitale* often lead to inflammatory lesions.[473] *T. rubrum* is usually associated with chronic, noninflammatory lesions, whereas *E. floccosum* may produce either type of reaction.[473]

Tinea Unguium. This condition is caused by invasion of the nail plate by a dermatophyte. The term *onychomycosis* also includes infection of the nail by nondermatophytic fungi. There are three main types of nail involvement: distal-lateral subungual onychomycosis, superficial white onychomycosis, and proximal white subungual onychomycosis.[482] Proximal white subungual onychomycosis occurs almost exclusively in immunodeficient patients.[483] The most commonly detected dermatophyte in tinea unguium is *T. rubrum*, followed by *T. mentagrophytes*.[484]

Majocchi Granuloma. Majocchi granuloma, or nodular granulomatous perifolliculitis, is a dermatophytic infection in which the lesions clinically manifest as nodules or plaques and histologically show granulomatous perifolliculitis. This presentation is commonly seen in immunocompromised individuals.

Immunology. Cell-mediated immunity seems to be the major immunologic defense in clearing dermatophyte infections.[470] The majority of chronic and recurrent dermatophyte infections are caused by *T. rubrum*; these patients often fail to express a delayed-type hypersensitivity reaction to trichophytin when it is injected intradermally.[485]

Histopathology. In tinea corporis, cruris, manuum, and pedis, the epidermis usually shows parakeratosis with neutrophils and variable acanthosis and spongiosis. In the acute forms, there is more dermal edema. Chronic forms usually display epidermal hyperplasia and hyperkeratosis. Subcorneal pustules are sometimes present. The underlying dermis shows mild perivascular inflammation, with lymphocytes and occasionally neutrophils or eosinophils. If there is follicular involvement, the dermal inflammation is heavier. The fungal elements are often found in between a zone of parakeratotic or hyperkeratotic scale at the bottom and normal basket weave keratin on top, constituting the so-called sandwich sign (Fig. 20.62). These fungal elements consist of septate hyphae

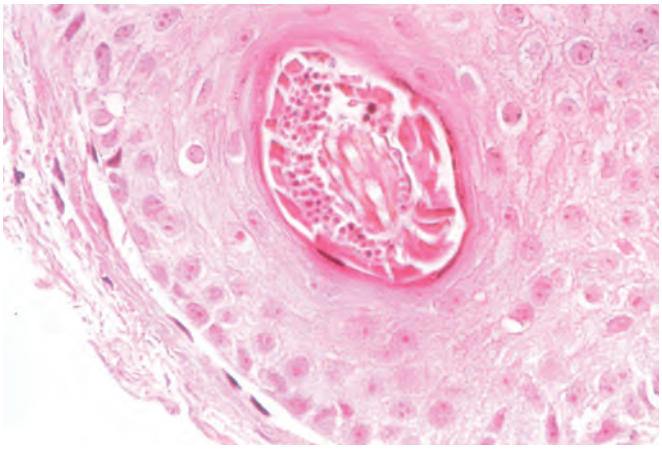


• **Figure 20.62** Dermatophytosis (tinea corporis). This image illustrates the so-called sandwich sign with the compact parakeratosis and hyperkeratosis at the bottom *(arrowheads)*, the hyphal forms in the middle, and the basket weave keratin on top *(arrows)*.

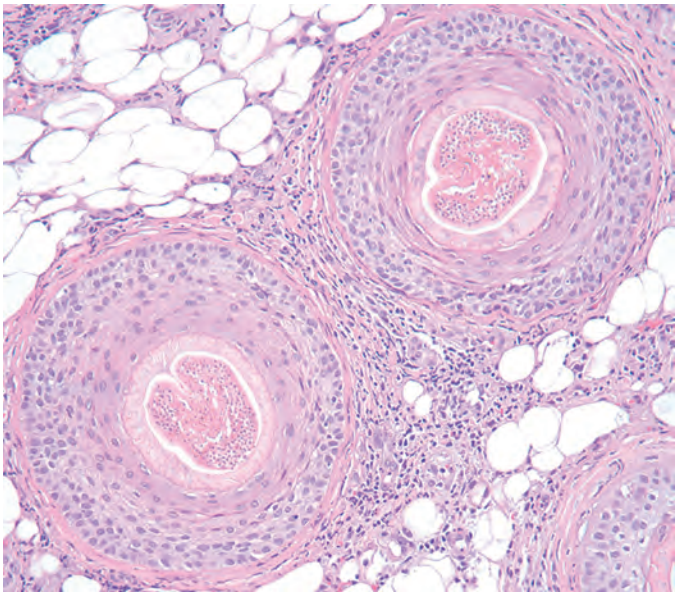
that are arranged parallel to the long axis of the epidermis and are highlighted by the PAS stain.

Folliculitis caused by dermatophytes is characterized by the presence of fungal elements in follicles and signs of a foreign body reaction, with foreign body giant cells in the dermal inflammatory infiltrate.[470] Granuloma formation may occur.

Infection of hairs may be ectothrix (sheath of arthroconidia formed on the outside of the hair shaft) (Fig. 20.63) or endothrix (arthroconidia formed within the hair shaft, preferentially caused by *T. tonsurans* and *T. violaceum*) (Fig. 20.64). In kerion, parakeratosis, intraepidermal collections of neutrophils, acanthosis, and spongiosis are present. The tissue reaction is suppurative with formation of abscesses, which may discharge via sinuses. A chronic inflammatory infiltrate with multinucleated giant cells may be present in the vicinity of disrupted hair follicles.[478] Ultimately, the process may resolve with fibrosis.



• **Figure 20.63** Tinea capitis. An example of the ectothrix pattern of infection, which results in fungal proliferation outside the hair shaft in addition to the hyphal forms in the hair itself. This pattern is mostly seen with *Microsporum audouinii*, *Microsporum canis*, *Trichophyton verrucosum*, and *Trichophyton mentagrophytes* infection, among others.



• **Figure 20.64** Tinea capitis. The endothrix pattern of infection is shown here, which is seen mainly in infection with *Trichophyton tonsurans* and *Trichophyton violaceum*.

In Majocchi granuloma, there is a dense perifollicular mixed inflammatory infiltrate, with granulomatous features. Hyphae and arthrospores are present within hair follicles and in the dermis.

In tinea favosa of the scalp, hyphae (often empty or vacuolated) are observed within infected hairs, whereas the scalp shows cup-shaped areas of densely interwoven mycelium, scales, and debris referred to as scutula.[470] A chronic inflammatory infiltrate is seen in the underlying dermis. Affected hair follicles tend to atrophy.

In onychomycosis, fungal hyphae and rounded arthroconidia are present between the cells of the nail plate which can easily be identified in a PAS histochemical stain.[486]

Candidosis

Candidioses, or candidiases, are infections of the skin, mucous membranes, or internal organs caused by yeasts of the genus *Candida*. This genus includes many species, with *Candida albicans* being the major pathogenic species.[487] *C. albicans* is a human commensal inhabiting the gastrointestinal tract, oral cavity, vagina, and skin in a significant proportion of healthy individuals; therefore the source of infection is usually endogenous.[487] Candidiases are considered opportunistic infections.[488] Disease occurs in the presence of local or systemic predisposing factors.[489] Local predisposing factors include occlusion, increased skin moisture, maceration, and topical corticosteroid use.[488] Systemic predisposing factors include immunosuppression (including AIDS), age (infancy, old age), pregnancy, endocrinopathies (diabetes mellitus, Cushing syndrome), drugs (corticosteroids, broad-spectrum antibiotics, immunosuppressors), and indwelling catheters.[488-490]

The candidiases range from relatively trivial superficial infections to life-threatening disseminated disease. The superficial infections mainly include cutaneous, oral, and genital candidiasis. *Candida* species may also enter the bloodstream (candidemia), and they can penetrate almost any organ of the body, causing systemic or disseminated candidosis.[490]

Clinical Types

Oral Infections. Oral mucous membrane infection can have various clinical presentations with the three major clinical variants being the pseudomembranous, erythematous, and hyperplastic types.[491] Pseudomembranous candidiasis (thrush) is characterized by curdlike white plaques that can be removed by scraping, leaving an erythematous base.[491,492] The white plaque consists of a tangled mass of fungal hyphae, blastospores, bacteria, inflammatory cells, fibrin, and desquamated epithelial cells.[491] Erythematous candidiasis is the most common variant seen in HIV infection.[491] The clinical presentation consists of a red patch, often on the midposterior dorsum of the tongue, palate, or buccal mucosa.[491,492] Lesions on the tongue manifest as depapillated areas.[491] Hyperplastic candidiasis, or candidal leukoplakia, manifests as palpable whitish plaques or nodules that cannot be removed by scraping.[493] These lesions usually occur on the inside surface of one or both cheeks at the commissural areas, and less frequently on the lateral surfaces of the tongue.[493] Candidal leukoplakia is associated with an increased risk of malignant transformation.

In addition to these clinical variants, there are several other oral lesions associated with *Candida* in which the fungus is not considered the sole etiologic agent including denture stomatitis, angular cheilitis, median rhomboid glossitis, and linear gingival erythema.[491]

Genital Infections. Vulvovaginal candidiasis is one of the most common vaginal infections, representing 40% to 50% of all cases of infectious vulvovaginitis. Between 70% and 75% of sexually

active women will experience at least one episode of vulvovaginal candidiasis in their lifetime, and 5% to 8% of adult women have recurrent disease, defined as four or more episodes per year.[494]

Infection with *Candida* species, usually *C. albicans*, is the most common cause of balanitis.[495] Candidal balanitis manifests with erythema, papules, and pustules that are easily ruptured on the glans.

Cutaneous Infections. Cutaneous infection with *Candida* species is commonly seen in intertriginous areas, such as the submammary region, inguinal folds, intergluteal crease, and finger spaces. The affected sites present shiny, erosive erythema that is typically accompanied by satellite pustules. This infection is also common in the diaper area of infants (napkin or diaper candidiasis).

Decubital candidiasis is a particular form of cutaneous candidiasis that occurs on the dorsal skin of chronically bedridden patients who show lesions clinically similar to the ones observed in intertriginous and napkin candidosis.[488]

Candida Onychomycosis. *Candida* onychomycosis is a rare condition that exclusively affects immunocompromised individuals, including patients with chronic mucocutaneous candidiasis, patients with HIV infection, and patients receiving immunosuppressive drugs.[496] Nail invasion by *Candida* produces primary total onychomycosis associated with periungual swelling.[496]

Congenital Cutaneous Candidiasis. Congenital cutaneous candidiasis appears to be acquired in utero by the ascension of organisms from an infected vagina into the uterine cavity and it manifests within the first 6 days of life.[497] Clinically, there are generalized erythematous macules, papules, vesicles, pustules, or some combination of these. Although all infants are at risk for disseminated disease, this risk seems to be higher in preterm infants.[498]

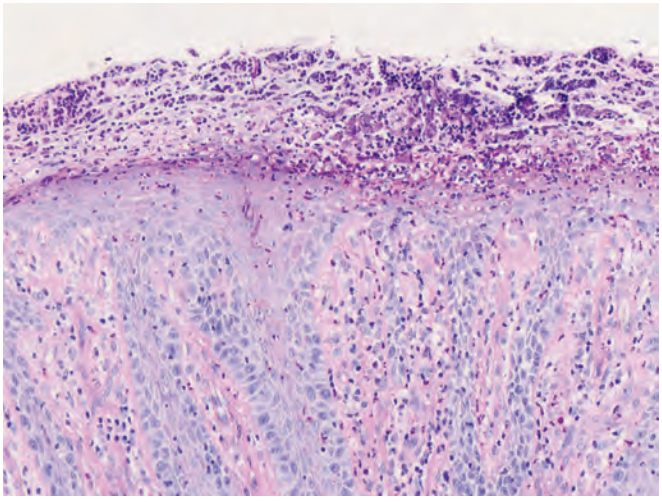
Neonatal Candidiasis. Neonatal candidiasis is acquired during passage through the birth canal during birth or later from nursery contacts, and it usually appears after the fifth day of life and commonly manifests as oral thrush. Associated diaper dermatitis can also be seen.[499] The incidence of neonatal candidemia or disseminated candidiasis in extremely low birth weight infants (<1000 g) is higher than in neonates with a birth weight greater than 1500 g.[499]

Infantile Gluteal Granuloma. This form of cutaneous candidiasis is seen in the anogenital region of infants as erythematous to violaceous nodules and plaques, usually oval in shape that are observed on the buttocks, vulva, perianal region, and scrotum, often as a complication of diaper dermatitis. Predisposing factors include prolonged topical corticosteroid use, long-term diarrhea, occlusive plastic pants, detergents, and *C. albicans* infection.[500]

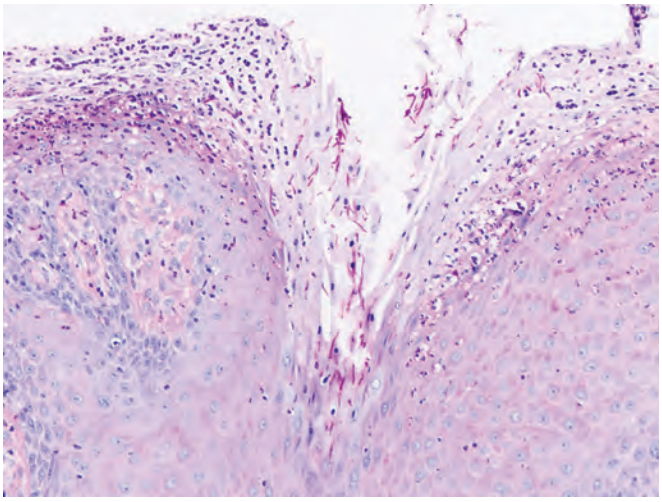
Chronic Mucocutaneous Candidiasis. Chronic mucocutaneous candidiasis is not a single entity but a heterogeneous group of immunologic disorders characterized by persistent or recurrent infections of the skin, nails, and mucous membranes by organisms of the genus *Candida*, usually *C. albicans*.[501] Impaired cellular immunity to *Candida* species has been reported in patients with chronic mucocutaneous candidiasis.[502] In some individuals the defect is specific to *Candida*, but in others the immunologic response to other organisms is also defective.[501] The associated findings include autoimmune disorders, polyendocrinopathies, thymoma, interstitial keratitis, alopecia, and vitiligo.[503] The age at onset and the clinical spectrum of candidal infections are variable. Affected nails are dystrophic and can be associated with paronychia. In some patients the skin lesions are markedly thick and keratotic, forming cutaneous horns, usually on the face and scalp.[501] Esophageal stricture formation has been described in some patients.[504]

Disseminated or Systemic Candidiasis. *Candida* organisms are among the 10 leading causes of bloodstream infections in developed countries, with approximately 33% to 55% of all episodes of candidemia occurring in intensive care units (ICUs). *Candida* fungemia may have an endogenous or an exogenous origin and has an associated mortality rates from 5% to up to 71%.[505] Predisposing factors to candidemia and systemic candidiasis include intravascular catheters, neutropenia, acute renal failure, parenteral nutrition, broad-spectrum antibiotics, a prolonged ICU stay, use of corticosteroids, mucosal colonization with *Candida*, chemotherapy, bone marrow transplantation, underlying cancer, and immunosuppression.[505,506] Involvement of multiple organs can occur, with cutaneous lesions being observed in 13% of patients with systemic candidosis.[506] Cutaneous lesions include erythematous macules, papules, pustules, nodules, and petechiae on the trunk and extremities.[506] Lesions resembling ecthyma gangrenosum have been reported.[507] Systemic candidiasis has also been described in heroin addicts, and these patients present with cutaneous, ocular, and osteoarticular involvement.[508] The cutaneous lesions in these patients are painful nodules and pustules that occur mainly on the scalp and beard area.[508]

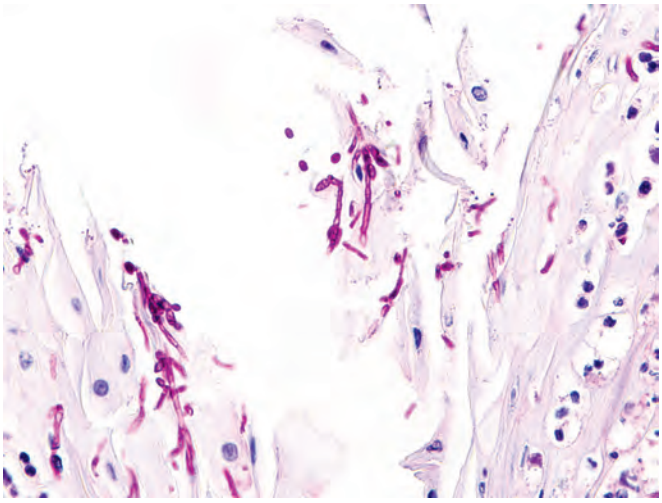
Histopathology. The histopathologic features are similar in cutaneous and mucous membrane infections and typically show epidermal (or epithelial) infiltration by large numbers of neutrophils (Fig. 20.65).[492] Occasionally, subcorneal or spongiform pustules can be seen, a pattern that should be differentiated from impetigo and the latter from psoriasis.[488] The epidermis displays acanthosis and focal spongiosis. A chronic inflammatory infiltrate is usually seen in the underlying connective tissue.[488,492] Yeast forms and pseudohyphae can be found in the stratum corneum with the use of standard H&E preparations, but visualization is easier with a fungal stain, such as PAS (Fig. 20.66). The organisms may show budding, particularly if the implicated organism is *C. glabrata*.[509] Importantly, the candida pseudohyphae appear oriented perpendicular to the great axis of the epidermis or epithelium, in contrast to dermatophytes, which are often arranged parallel to such axis (Fig. 20.67). Candida yeast forms can be seen



• **Figure 20.65** Cutaneous candidiasis (periodic acid–Schiff stain). Note the prominent acanthotic and spongiotic epidermis associated with a heavy infiltration by neutrophils, especially in the superficial layers.



• **Figure 20.66** Cutaneous candidiasis (periodic acid–Schiff [PAS] stain). Yeast and pseudohyphal forms are readily identified with a PAS stain.



• **Figure 20.67** Cutaneous candidiasis (periodic acid–Schiff stain). High-power view of the organism exhibits both yeast and pseudohyphal forms, resulting from germ tube formation.

as commensal organisms in the oral mucosa and does not necessarily indicate pathogenicity.

In chronic mucocutaneous candidiasis, the histologic features resemble the ones described earlier, but the lesions are usually more acanthotic and hyperkeratotic.[501] Yeast forms and pseudohyphae are often numerous and easy to visualize with the PAS stain.

In systemic candidiasis, the fungi are located predominantly within the dermis and around damaged vascular structures, indicating hematogenous spread.[506] Features of leukocytoclastic vasculitis have been described to occur in this variant.[509] Focal dermal necrosis, hemorrhage, and perivascular lymphocytic infiltration are also seen.[506] In lesions that resemble ecthyma gangrenosum, ulceration with papillary dermis displays edema and a large number of pseudohyphae, which may extend into vessels.[507]

In heroin addicts, lesions show dermal abscesses with abundant neutrophils, surrounded by lymphocytes, histiocytes, and giant cells, as well as yeasts and pseudohyphae, with invasion of the hair shafts in some cases.[508]

Etiology and Pathogenesis. Although the majority of the previously cited infections are caused by *C. albicans*, a polymorphic microorganism that exists in three basic morphologic entities (namely, yeasts, pseudohyphal, and hyphal forms),[487] in the early 2000s, a shift in the relative prevalence of species has occurred. A relative decrease in prevalence of *C. albicans* was noted with a corresponding increase in non-*albicans Candida*, such as *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*.[487] By means of various molecular mechanisms, which comprise (1) overexpression ATP-binding cassette (ABC) proteins, which pump the drug out of the organisms, and (2) modification of the drug targets (*ERG11* gene mutations), an increase resistance to azole-based antifungals has been seen in almost all *Candida* species but particularly in *C. glabrata* and *C. krusei*.[510] Other attributes that contribute to *C. albicans* virulence include adhesion, hyphal formation, phenotypic switching, and extracellular hydrolytic enzyme production.[511]

Deep or Systemic Mycoses

Systemic mycoses are caused by truly pathogenic fungi, which are capable of causing infection in otherwise healthy individuals. The fungi traditionally included in this group, and the first ones to be reviewed in this section, are *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *H. capsulatum* (var. *capsulatum* and var. *duboisii)*, *and Cryptococcus* spp. These organisms are thermally dimorphic, growing as mycelia in their natural state and converting to yeast form in tissues. The infection is usually acquired through the respiratory tract, and the organisms can disseminate hematogenously to reach the skin and other sites. The severity of infection is determined both by the extent of the exposure to the organism and by the immune status of the patient. The systemic mycoses have always been a significant health problem, but they have gained importance due to the growing immunosuppressed population. Following the discussion of the previously mentioned organisms, we will also touch upon deep fungal infections caused by a variety of other agents, such as *Penicillium marneffei*, *Sporothrix schenckii*, and *Lacazia (Loboa) loboi*, as well as infections caused by groups of fungi, such as Hyalohyphomycosis, Chromoblastomycosis, Mycetoma (Eumycetoma), Phaeohyphomycosis, and Zygomycosis.

Blastomycosis

Blastomycosis, or North American blastomycosis, is caused by *B. dermatitidis*. This fungus is present in decaying wood and soil near lakes and rivers, with most cases of human infection occurring in North America,[512] but cases have also been documented in India, Africa, and Central and South America.[513] There are three forms of blastomycosis: pulmonary, systemic, and primary cutaneous. The disease is more common in adult men, and almost all cases of blastomycosis begin as a primary pulmonary infection resulting from inhalation of conidia. The primary infection may progress or resolve spontaneously.[514] Hematogenous spread to other organs may occur, with the skin being the most common extrapulmonary site, present in 40% to 80% of the cases.[515]

Clinical Features. Cutaneous blastomycosis causes a variety of skin lesions, which are typically divided into ulcerative or verrucous.[516] The verrucous type has large lesions with a serpiginous, advancing, raised border and an atrophic crusted center that may reach many centimeters in diameter.[514,516] Ulcerative-type skin lesions begin as a small pustule that progresses to an ulcer with heaped borders.[517] The cutaneous lesions tend to occur on the face

and other exposed areas; mucosal membranes may also be affected. Unusual clinical presentations of blastomycosis include widespread pustular eruption[518] and Sweet syndrome–like lesions.[519]

The primary cutaneous form is rare and results from direct inoculation of organisms into the skin. It usually is reported after autopsy, laboratory accidents, dog bites, infection of open wounds, or outdoor trauma.[516] Lymphangitic lesions, similar to those of sporotrichosis, can occur.

Although immunologically compromised patients, such as diabetics, individuals receiving long-term corticosteroid therapy, or undergoing cytotoxic therapy for malignancy, are often affected by the fungus, the infection is relatively uncommon in HIV/AIDS patients.[520]

Histopathology. Initial lesions of blastomycosis show a dermal inflammatory infiltrate of polymorphonuclear leukocytes with numerous organisms. After a few weeks, occasional giant cells may be seen.[520] In more chronic and verrucous lesions, the advancing edge of these lesions displays downward proliferation of the epidermis, often progressing to pseudoepitheliomatous hyperplasia with numerous intraepidermal aggregates of neutrophils forming microabscesses.[520] There is a polymorphous dermal inflammatory cell infiltrate, usually with numerous neutrophils and abscesses, as well as scattered multinucleate giant cells.[515,520] Occasionally, tuberculoid granulomas are seen but without caseation necrosis.

The blastomycosis yeasts are usually found in the center of the abscesses and within giant cells. The yeast fungal forms measure 8 to 15 μm in diameter, have thick, refractile cell walls, and show the characteristic broad-based budding forms, with a single bud attachment base measuring 4 to 5 μm, a far broader base in comparison to *P. brasiliensis*, *C. neoformans*, or other spherical yeasts.[521] The daughter cell is often as large as the mother cell before detachment, and in well-fixed tissue the multiple nuclei of the yeast forms can be seen and both PAS and GMS histochemical stains can highlight the fungal forms.[521]

Some variation in size, shape, staining, and wall thickness of yeast forms of *B. dermatitidis* can occur in different fields of the same specimen in immunocompetent hosts.[521,522] However, the greatest variation in yeast forms is found in immunosuppressed patients, in whom even hyphal forms may also be observed.[523]

The diagnosis of blastomycosis can often be made by morphologic examination of cytologic or tissue samples.[520] In this context, two other organisms should be distinguished from *B. dermatitidis*: *P. brasiliensis* and *Cryptococcus* spp. The former exhibits more polymorphic shapes, thinner cell walls, and multiple small buds surrounding the large parent yeasts. *Cryptococcus* spp. organisms have a thick, mucicarmine-positive, mucoid capsule, as opposed to the thin, incomplete, mucicarmine-positive layer occasionally seen in *Blastomyces* yeast forms.[520] Giant yeast forms (>40 μm in diameter) are very rare but have been reported and can cause confusion with *C. immitis*.[521,524]

Other Diagnostic Techniques. A positive culture can also render a definite diagnosis but requires a 2- to 4-week incubation period. Being a dimorphic fungus, culture at body temperature yields a slow growing, white or light brown yeast colony, whereas tissue culture on Sabouraud dextrose agar at room temperature (25°C) produces a fluffy white colony (micelial form).[512]

Coccidioidomycosis

Coccidioidomycosis, caused by *C. immitis*, is found in the southwestern United States, in northern Mexico, and in some arid and desertic areas of South America (mainly in Argentina and Paraguay).[525] This infection usually presents as an acute, self-limited pulmonary process resulting from inhalation of airborne arthrospores from the soil. Systemic dissemination of the infection is rare, occurring in approximately 1% of the cases[526] and particularly in HIV-infected persons, organ transplant recipients, and other immunocompromised patients, as well as in pregnant women.[526] For some unknown reason, both African Americans and Filipinos have a greater risk of severe and disseminated disease.[527]

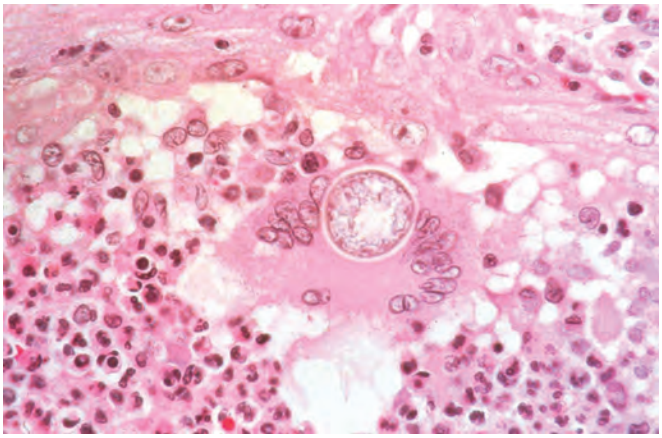
C. immitis is a dimorphic fungus that exists as a mold in the soil. As the soil dries or nutrients become limiting, the fungus reproduces asexually by disarticulating the hyphae into small, environmentally resistant arthroconidia, which are easily dispersed into the air when the soil is disturbed by wind or human activities.[526] After inhalation by the host, the arthroconidia lodge in the tissue and become spherules, which have thick walls and measure 10 to 80 μm in diameter. The spherules then grow larger and contain hundreds to thousands of endospores. After rupture into the tissues, the endospores grow to form new spherules, thereby repeating the growth cycle in the host.[525] Given the combination of high infectivity and ease of dissemination, *C. immitis* is often considered as a potential bioterrorism agent.[528]

A second species, *Coccidioides posadasii*, has been identified as the predominant organism outside California.[526,529] This related species was named after Alejandro Posadas, an Argentinian physician who first described the infection in 1892. Although *C. immitis* and *C. podasasii* are genetically different, they currently show the same phenotype, immune response, and clinical features.[526]

Clinical Features. Cutaneous involvement in most cases results from dissemination from a primary pulmonary focus. Although joints, bones, and the CNS are frequently involved in disseminated disease, the skin is the most common extrapulmonary site affected in coccidioidomycosis.[525,526,530] Cutaneous manifestations can be quite variable, consisting of verrucous papules, nodules, or plaques or of subcutaneous abscesses, which may form draining sinuses.[526,531] Multiple papulopustular lesions can occur and probably indicate acute hematogenous dissemination.[531] Being one of the "great simulators" in dermatology, cutaneous coccidiomycosis lesions can resemble mycosis fungoides[532] and lepromatous leprosy,[533] among other skin conditions.[526]

Primary cutaneous coccidioidomycosis is very rare and results from inoculation of the organisms at sites of minor trauma. It usually consists of an ulcerated nodule on an extremity or other exposed area. Secondary nodules in a linear distribution along the lymphatic pathways, similar to sporotrichosis, can be seen.[534] Regional lymphadenopathy may be associated, and the affected lymph nodes may ulcerate.[526] Reactive skin eruptions, which contain no viable organisms, are common and include erythema nodosum, an acute generalized exanthem, EM, Sweet syndrome, and reactive interstitial granulomatous dermatitis.[526,535]

Histopathology. The histopathologic features in the skin are similar in disseminated infection and in primary cutaneous infection,[526] with established cutaneous lesions showing pseudoepitheliomatous hyperplasia with intraepidermal microabscesses. A diffuse infiltrate of neutrophils, lymphocytes, eosinophils, plasma cells, histiocytes, and giant cells is present in the dermis. Eosinophils are usually prominent and can be present either in the perivascular inflammation or form eosinophilic abscesses. Small flame figures have been described.[531] There may be abscess



• **Figure 20.68** Coccidiomycosis. A multinucleated giant cell exhibiting a *C. immitis* spherule. Note the background mixed inflammatory infiltrate.

formation, with surrounding granulomatous response. These histologic features resemble those of blastomycosis, but, in coccidioidomycosis, caseation necrosis may occur.[533]

Exuberant vascular changes can be seen, including plump endothelial cells proliferated in capillary masses with an admixture of eosinophils, lymphocytes and plasma cells. However, no evidence of vasculitis (e.g., fibrinoid change, vessel necrosis) is present.[531]

The fungal spherules are observed in the dermis, both free and within giant cells. Endospores (sporangiospores) are seen within the spherules (sporangia) (Fig. 20.68). In some cases the organisms are few, and meticulous examination is required to see them.[531] Transepidermal elimination (perforation) of inflammatory tissue together with the organisms has been described.[531] They can be identified in H&E-stained slides but are demonstrated best by methenamine silver stain. The endospores are PAS-positive, but the spherules are negative. The so-called subcutaneous myoespherulosis[536] structures (aggregates of degenerated red blood cells) can, albeit rarely, mimic the appearance of an endosporulating fungus, such as *C. immitis*.

The spherules found in tissue are much larger than the yeast forms of other fungi, such as *Blastomyces* or *Cryptococcus*. On the other hand, the spherules and endospores of coccidioidomycosis are much smaller than those of rhinosporidiosis. However, on rare occasions, atypical morphologic forms of *C. immitis* in human tissues have been described, including morula-like, broad-based yeastlike, moniliform, and true hyphae types.[537] Because this could lead to confusion with the tissue forms of other pathogenic fungi, culture remains the most reliable means to establish a definite diagnosis in these atypical cases.[537]

Skin biopsies of the acute generalized exanthem or "toxic erythema" associated with coccidioidomycosis show a nonspecific pattern of spongiotic dermatitis and/or interface dermatitis with a mild perivascular inflammatory infiltrate including lymphocytes, neutrophils, eosinophils, and karyorrhectic debris. Necrotic keratinocytes are rare.[526,535]

Paracoccidioidomycosis

Paracoccidioidomycosis, also known as South American blastomycosis or Lutz-Splendore-Almeida disease, is caused by the dimorphic fungus *P. brasiliensis*. The disease is confined to Latin America, with endemic areas in Brazil, Argentina, Ecuador, Colombia, and Venezuela.[538,539] The incidence of the disease before 12 years of age is similar in boys and girls. However, after this age, the incidence increases progressively in males. Up to 90% of affected individuals are men between 29 and 40 years old, predominantly rural workers.[538,539] The presence of β-estradiol receptors in the cytoplasm of the mycelium and yeast forms of *P. brasiliensis* suggests that this hormone is capable of inhibiting the transformation of the mycelium to the yeast form with changes in gene expression.[540] This could be one reason for the lower incidence of cases in adult women.

In a similar way to Hansen disease, a possible reservoir of the fungus appears to be the nine-banded armadillos *(D. novemcinctus)*.[541]

Clinical Features. The respiratory tract is the usual portal of entry; from there, hematogenous dissemination to other parts of the body occurs. Paracoccidioidomycosis is usually classified as acute/subacute or chronic.[538,539] The acute/subacute form usually affects children, teenagers, and young adults and is also called "juvenile type." This form mainly involves the spleen, adrenals, liver, lymph nodes, intestinal tract, and bones.[539,542] On the other hand, lung and mucosal lesions are rare.

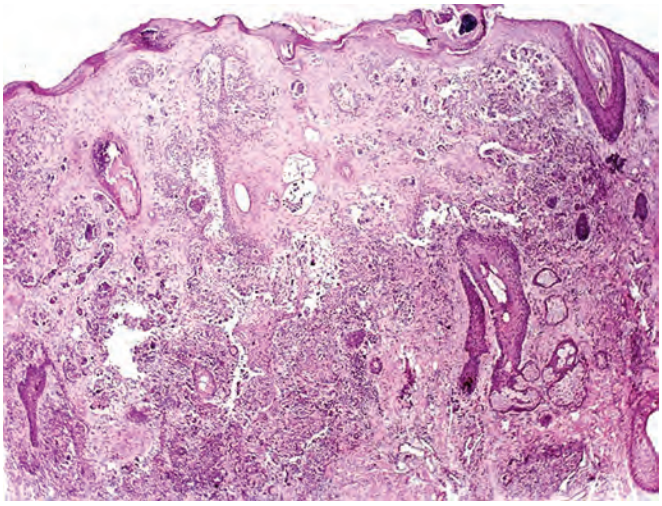
The chronic form, or adult type, is much more common and generally affects individuals older than 30 years of age. This form is characterized by involvement of the lungs, mucosa, and skin.[539,542] Oropharyngeal mucous membrane ulcerations are frequent; they have a characteristic appearance, showing hemorrhagic dots over a granulomatous base, and are known as Aguiar-Pupo stomatitis.[539,543] Ulcerated mucosal lesions can be often quite chronic and therefore mimicking SCC.[544] Skin lesions can manifest as crusted papules and nodules, ulcers, or verrucous plaques. They can also exhibit hemorrhagic dots, similar to the lesions in mucous membranes.[539] Cases of primary cutaneous infections appear to be rare.[545,546]

Paracoccidioidomycosis in AIDS patients usually manifests in a form similar to severe acute/subacute cases; however, the incidence of coinfected individuals is low.[538,546]

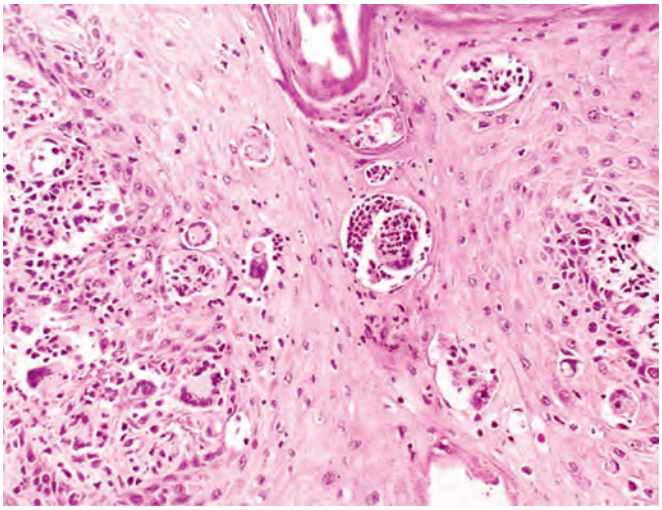
Histopathology. The lesions of paracoccidioidomycosis are characterized by a granulomatous inflammatory response with formation of epithelioid granulomas with giant cells.[542] These granulomas range from well-organized to poorly organized, probably reflecting the degree of the host's cellular immune response.[547] Epidermal changes include hyperkeratosis, parakeratosis, acanthosis, and pseudocarcinomatous hyperplasia,[547] which can be exuberant and mimic SCC (Figs. 20.69 and 20.70).[544] Epidermal microabscesses can also be seen, sometimes with fungi among the polymorphonuclear cells (Fig. 20.71).[547] In addition to the granulomatous reaction, the dermis also shows a perivascular inflammatory infiltrate with lymphocytes, plasma cells, and macrophages.[544,547] Some cases may show vascular alterations characterized by swelling of endothelial cells.[547]

P. brasiliensis is a dimorphic fungus that appears in tissues as small and large budding yeasts that measure 2 to 30 μm in diameter.[539] They are round to oval and have a birefringent capsule.[526] The diagnostic forms of the organisms have multiple buds resembling a "mariner's wheel" or a "Mickey Mouse head," with narrow points of attachment of buds to mother cells.[526,539,543] The outlines of the fungi can be seen in the cytoplasm of macrophages and giant cells in routine H&E-stained sections (Fig. 20.72), and they are highlighted by the PAS or GMS stains (Figs. 20.73 and 20.74).[539,543,544]

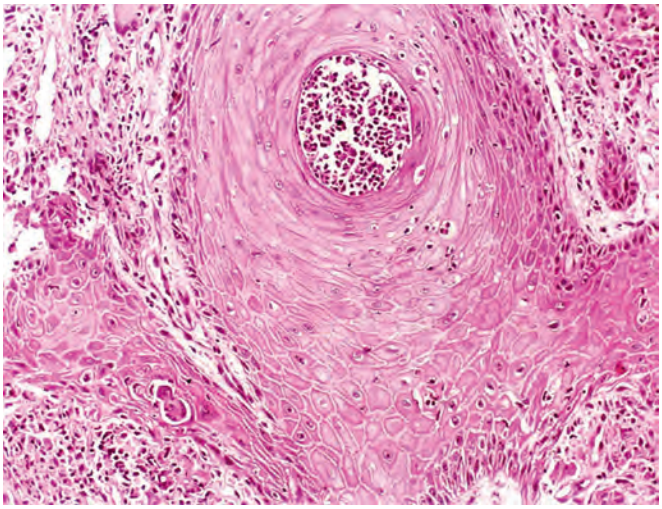
P. brasiliensis organisms can be confused with *B. dermatitidis* if only single budding is observed; however, the former organism has thinner cell walls and narrow-based buds. *Cryptococcus* can



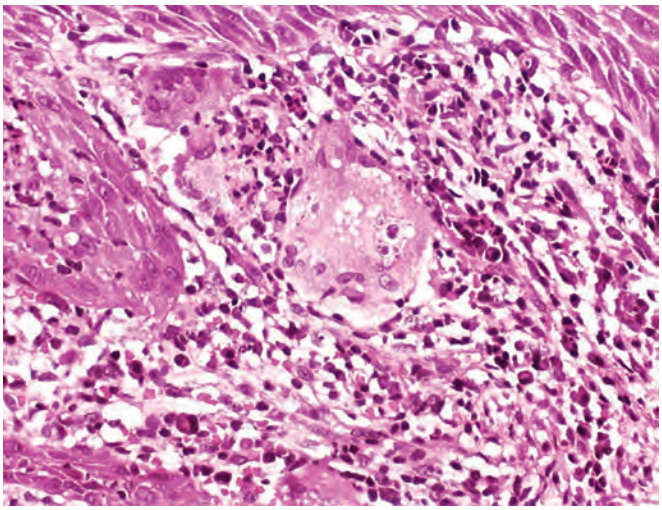
• **Figure 20.69** Paracoccidioidomycosis. A low-power view of the lesion shows an exuberant pseudoepitheliomatous hyperplasia with intraepidermal neutrophilic abscesses overlying a dermal granulomatous and suppurative inflammatory infiltrate. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.70** Paracoccidioidomycosis. The intraepidermal inflammatory infiltrate is quite prominent in this example. Organisms are frequently found in the intraepidermal abscesses. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.71** Paracoccidioidomycosis. In some cases the pseudoepitheliomatous epidermal hyperplasia is striking and can be confused with squamous cell carcinoma. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.72** Paracoccidioidomycosis. The budding yeast form of the fungus, with the classic "steering wheel" morphology, can be recognized even without the use of special stains. In this excellent example, the organisms are outlined by the cytoplasm of the giant cell. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

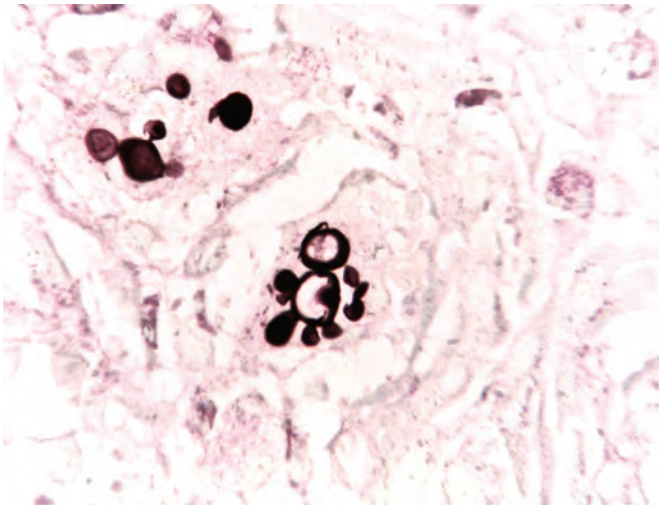
show similar variation in yeast size but has the characteristic mucicarmine-positive mucinous capsule.

Histoplasmosis

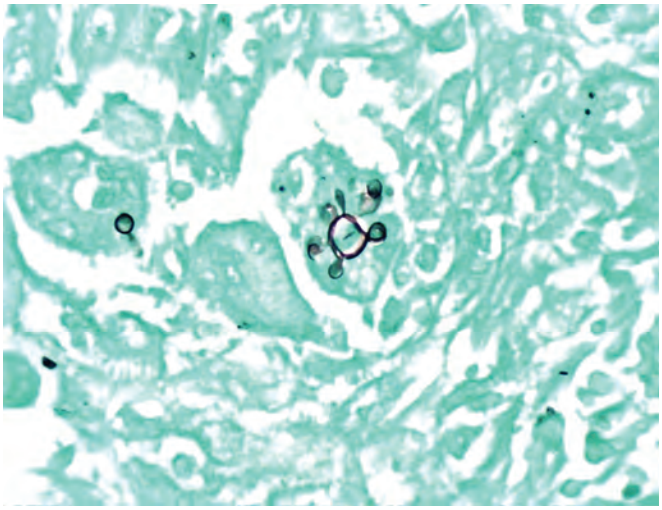
Histoplasmosis, or Darling disease, results from infection with *H. capsulatum* var. *capsulatum*. The disease has a worldwide distribution, but the largest endemic focus is in the central eastern United States, especially the Ohio, Mississippi, and Missouri River valleys.[548]

The mycelial form of *H. capsulatum* (var. *capsulatum*) is found in the soil, especially in areas contaminated with bird or bat droppings, which provide added nutrients for growth.[548] Thus cave exploration ("spelunking") represents a high-risk activity to acquire this infection.[549] The microconidia (mold form) are inhaled and deposited in the alveoli of the infected individual. At body temperature, they convert into the yeast form and are ingested by macrophages. The yeasts multiply within these cells and gain access to the blood circulation, disseminating throughout the body.[548] If adequate cellular immunity ensues, macrophages become fungicidal and control the organisms, resulting, in 99% of cases, in a pulmonary infection that is self-limited and asymptomatic.[548] Symptomatic disseminated disease is usually seen in infants and immunosuppressed patients; particularly those with HIV/AIDS.[550] In addition, acute life-threatening histoplasmosis may complicate immunotherapy with TNF-α antagonists, particularly infliximab.[551]

Clinical Features. Mucous membrane lesions are common in disseminated histoplasmosis, usually manifesting as oral



• **Figure 20.73** Paracoccidioidomycosis (Grocott methenamine silver stain). The budding yeast form is highlighted by this special stain. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

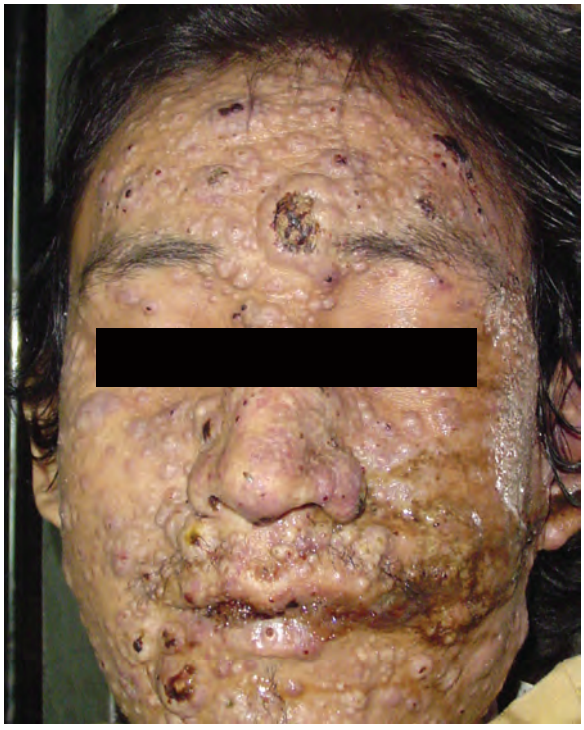


• **Figure 20.74** Paracoccidioidomycosis (Grocott methenamine silver stain). The classic "steering wheel" configuration of *Paracoccidioides brasiliensis*. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

ulcerations.[548,552] Cutaneous involvement occurs in approximately 6% of patients with disseminated disease.[553] Skin lesions are variable and are not considered characteristic, manifesting as papules, nodules, pustules, ulcers, cellulitis-like areas, acneiform lesions, or molluscum-like papules.[513,548,552] Patients with severe and untreated HIV/AIDS may show florid infections with severe cutaneous involvement (Fig. 20.75).

The primary cutaneous form is rare and usually manifests as a solitary, self-limited, ulcerated nodule at the site of fungal inoculation.[554] Erythema nodosum and EM can occur as secondary hypersensitivity manifestations.[513]

Histopathology. Cutaneous lesions show a granulomatous infiltrate in the dermis, sometimes extending into the subcutaneous tissue, with numerous macrophages (Figs. 20.76A). Tiny oval yeast forms (2 to 4 μm), surrounded by a clear halo (pseudocapsule), are seen in the cytoplasm of both macrophages and multinucleated giant cells (see Fig. 20.76B).[548] An infiltrate containing



• **Figure 20.75** Histoplasmosis. This untreated human immunodeficiency virus/acquired immunodeficiency syndrome patient presented with disseminated disease with extensive cutaneous involvement. Note the disfiguring lesions ranging from papules to ulcerated plaques.

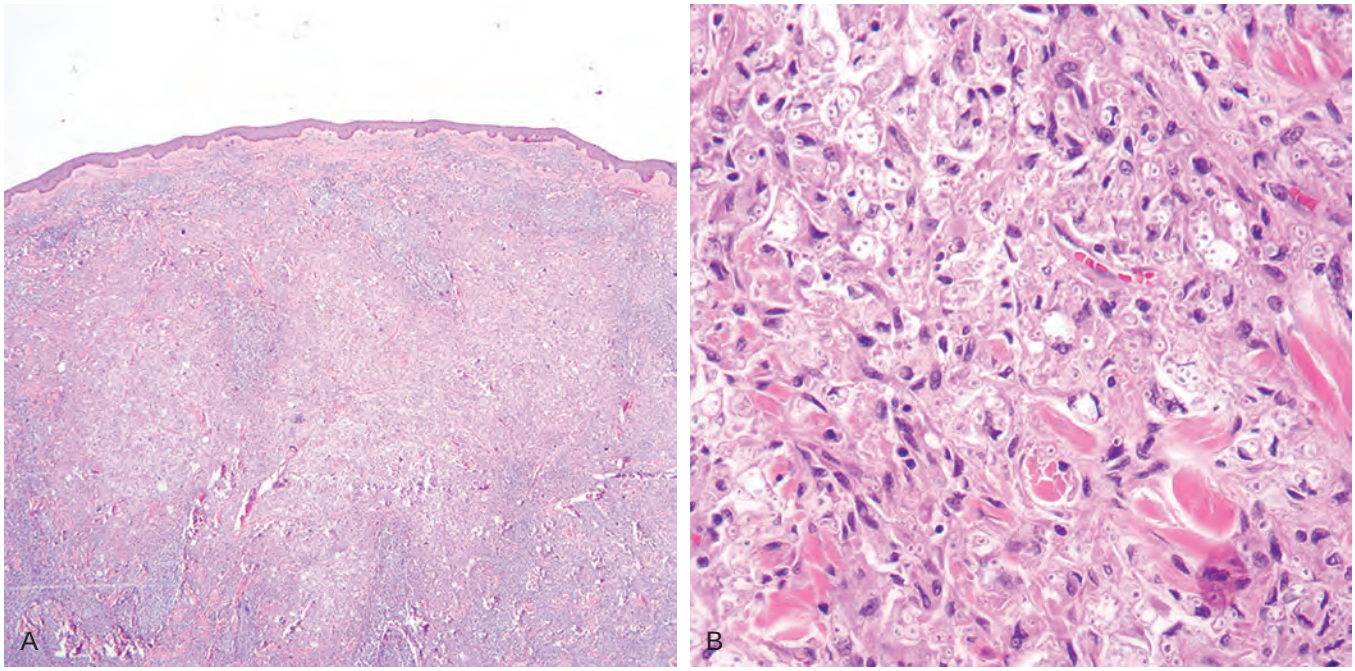
lymphocytes, plasma cells, and giant cells is usually present. However, in acute disseminated cases, parasitized macrophages predominate.[553] The intracellular organisms are haphazardly disposed within intracellular vacuoles with no "marquee sign" seen in *Leishmania* spp. organisms (see later; see Fig. 20.76B). Extracellular organisms are occasionally seen, particularly in immunosuppressed individuals.[548,552] The organism is not encapsulated, but in tissues it appears to be surrounded by a clear halo, which was misinterpreted by S.T. Darling as being a capsule in his original description of *H. capsulatum*.[548,555]

In patients with HIV/AIDS, there is a very sparse to absent inflammatory cell infiltrate (Fig. 20.77).[513] Numerous organisms, both intracellular and extracellular, are seen (Figs. 20.78 and 20.79). Leukocytoclasia and dermal necrosis can also be observed.[556] *H. capsulatum* within cutaneous nerves has been described in disseminated histoplasmosis in patients with HIV/AIDS.[557] Although the fungi are readily visible on H&E-stained sections, PAS or the GMS histochemical stains can be used to better visualize the fungi (Fig. 20.80; see also Fig. 20.19).

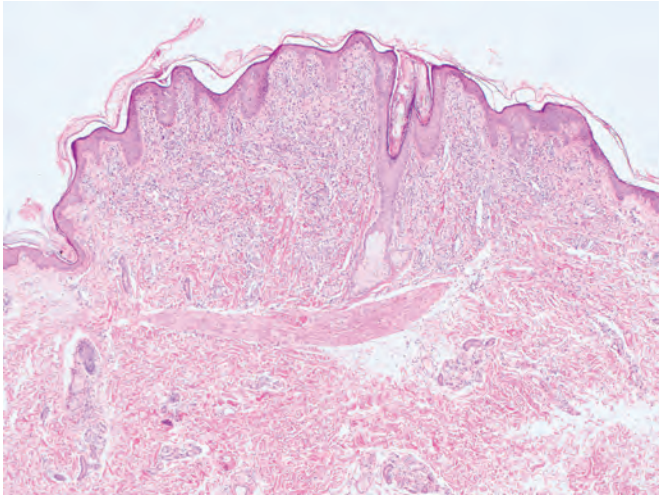
The differential diagnosis includes other conditions in which parasitized macrophages are seen, especially leishmaniasis and *P. marneffei* infections. The parasites in leishmaniasis lack the clear halo seen in *H. capsulatum* and have a tendency to aggregate at the periphery of the intracytoplasmic vacuoles of giant cells and macrophage (marquee sign). The *P. marneffei* yeast forms replicate by binary division and have a septate appearance, whereas *H. capsulatum* divides by budding.[552]

African Histoplasmosis

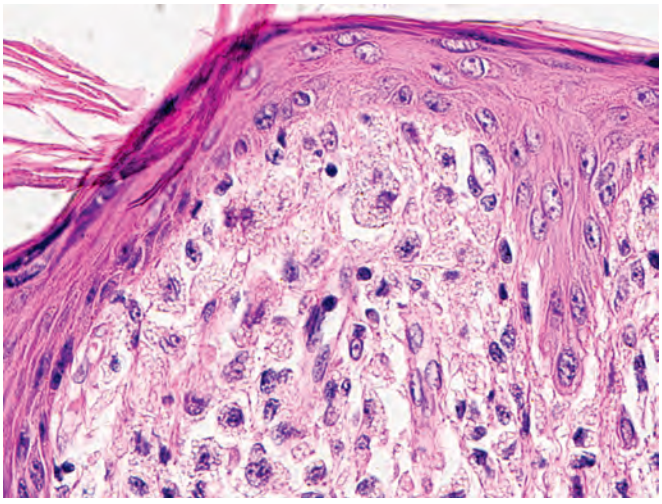
African histoplasmosis is caused by *H. capsulatum* var. *duboisii*. It is endemic in central and west Africa and on the island of



• **Figure 20.76** Histoplasmosis. This lesion presented in an immunocompetent patient and showed a central granulomatous area composed of parasitized histiocytes surrounded by a cuff of lymphocytic infiltrate **(A)**. Higher-power examination reveals histiocytes containing numerous intracytoplasmic yeast forms of *Histoplasma capsulatum.* Note the rather haphazard distribution of the organisms within the vacuoles **(B)**.



• **Figure 20.77** Histoplasmosis. There is a diffuse infiltration of the papillary and reticular dermis by histiocytes filled with organisms, with little or absent inflammatory response. This skin biopsy specimen is from the untreated human immunodeficiency virus/acquired immunodeficiency syndrome patient depicted in Fig. 20.75.

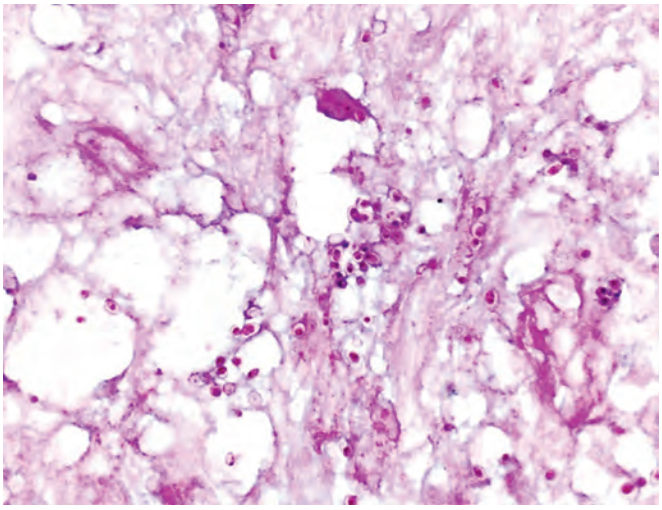


• **Figure 20.78** Histoplasmosis. In this high-power view, one can identify the fungi-laden histiocytic population. This biopsy was also taken from the human immunodeficiency virus/acquired immunodeficiency syndrome patient depicted in Fig. 20.75.

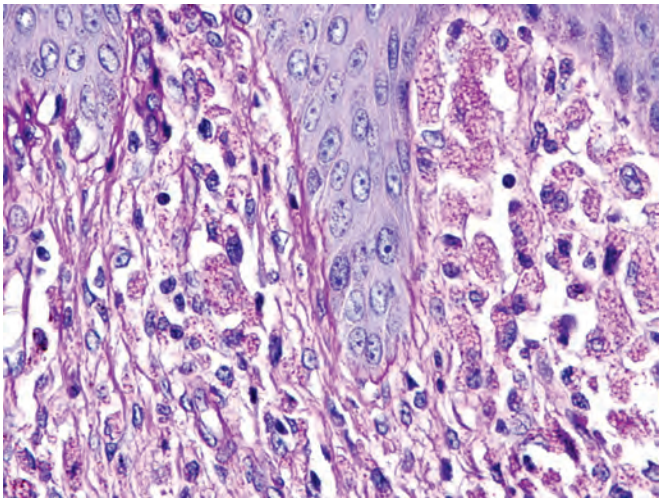
Madagascar.[558] Rare cases have been reported in Europe but were all imported.[548,559] The pathogenesis of African histoplasmosis remains unclear. The exact portal of entry into the body is unknown, but inhalation into lungs and direct inoculation in the skin have been incriminated.[558]

Clinical Features. This form of histoplasmosis is characterized by the presence of granulomatous and suppurative lesions on the skin, subcutaneous tissues, lymph nodes, and bones.[558] In contrast to *H. capsulatum* var. *capsulatum*, pulmonary disease is rare.[560] Although HIV infection and *H. capsulatum* var. *duboisii* coexist in Africa, this coinfection seems to be rare.[548] As expected, HIV-infected patients have substantially more disseminated disease than do HIV-negative individuals.[559]

Histopathology. The histopathology is different from that of classic histoplasmosis, particularly regarding the organism's morphology. Characteristic lesions show a granulomatous inflammatory reaction with numerous giant cells of foreign body and Langhans type, most of them containing numerous oval yeast cells



• **Figure 20.79** Histoplasmosis (periodic acid–Schiff stain). In immunosuppressed patients, "free" fungal forms can be found in the midst of the inflammatory infiltrate. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.80** Histoplasmosis (periodic acid–Schiff [PAS] stain). *Histoplasma capsulatum* is strongly PAS-positive and can be differentiated from other intracellular parasites, such as *Leishmania* spp., by the absence of a kinetoplast and the presence of a "capsulelike" clearing.

(8 to 15 μm in diameter).[561] The yeast cells may sometimes occur in chains of four or five cells.[558] Necrosis and suppuration are commonly present.[559] The organisms can also be observed extracellularly in clusters.[561] The fungus is visible in H&E-stained sections, but is better demonstrated by silver methenamine or PAS stain.[558]

H. capsulatum var. *duboisii* has much larger yeasts forms than *H. capsulatum* var. *capsulatum*; in addition, they have thicker walls and are lemon-shaped, with narrow budding.[559] In cultures grown at 25°C, the mycelia from the two *H. capsulatum* varieties cannot be distinguished, either macroscopically or microscopically.[560]

Cryptococcosis

Cryptococcosis is caused by the yeast forms of *C. neoformans* and *Cryptococcus gattii*, which have worldwide and a subtropical distribution, respectively.[562,563] *C. neoformans* is found predominantly in the excreta of pigeons and other birds, as well as decaying wood in soil and in fruits, whereas *C. gattii* is often related to Eucalyptus trees.[562] In general, the nonencapsulated forms of the organisms enter the body through inhalation into the respiratory tract. Once in the human host, a thick mucinous capsule develops, which helps its hematogenous dissemination to other sites.[564] Although this infection is observed mainly in immunosuppressed individuals, such as HIV/AIDS patients,[564,565] cryptococcosis can also occur in apparently immunocompetent hosts, particularly with *C. gattii*.[562,566]

The CNS is the most common site of extrapulmonary disease, resulting in a meningoencephalitis that has a high mortality if untreated.[564,565] Other sites commonly involved in disseminated disease include the skin, bones, joints, and urinary tract.[564,565] Secondary skin lesions are observed in 10% to 15% of patients with disseminated cryptococcosis.[564] Cutaneous manifestations can precede and may be the only clue to disseminated disease. Primary cutaneous infection, although rare, can be seen with both *C. neoformans*[563,567] and *C. gattii*.[568]

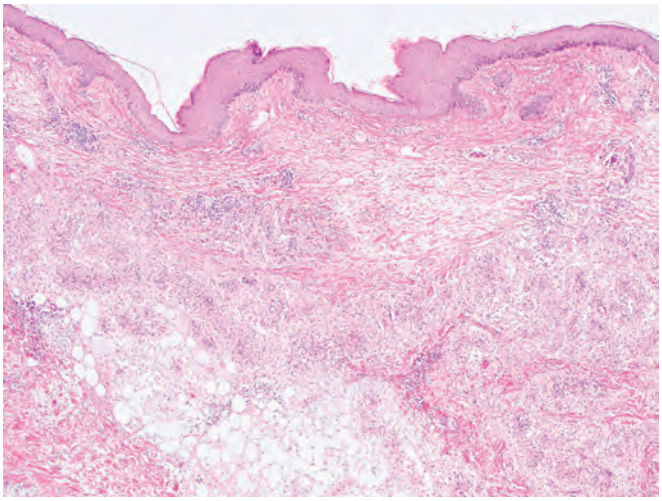
Cell-mediated immunity plays an important role in susceptibility to infection and in dissemination of the organisms, providing an explanation for the increased incidence of infection in immunosuppressed patients.[562,564] Important virulence factors of *Cryptococcus* include the thick mucinous capsule, which impairs phagocytosis, as well as the melanin-containing cell wall of the fungus.[564,569] Interestingly, the fungus appears to use catecholamines in the brain to make melanin, thereby protecting the organism from oxidative damage by scavenging free radicals, which explains the notable tropism for the CNS of this agent.[570]

Clinical Features. Skin involvement in cryptococcosis is often nonspecific, producing a wide variety of lesions, including papules, nodules, pustules, vesicles, plaques, subcutaneous abscesses, ecchymoses, and ulcers.[564,571] Lesions resembling those of molluscum contagiosum,[395,572] KS,[572] or acne have been described in patients with HIV/AIDS and in solid organ transplant recepients.[573] Cryptococcal cellulitis has also been reported, mostly in renal transplant recipients receiving corticosteroids.[565,573] Although lesions can be widespread, they are most commonly observed on the head and neck.[564,565]

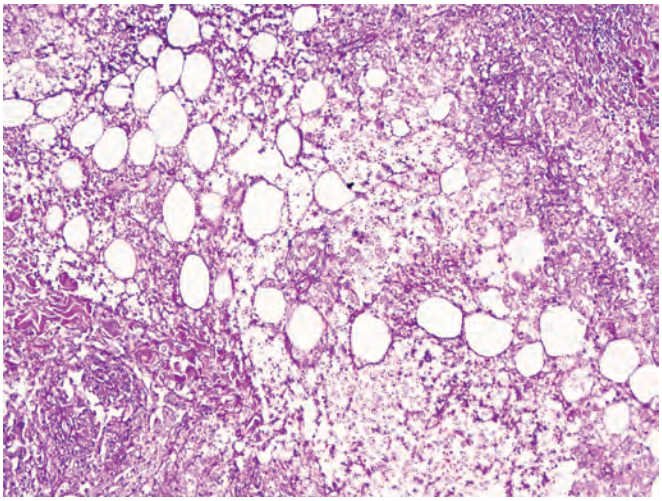
Because primary cutaneous cryptococcosis, via direct inoculation, is such a rare occurrence, it is reasonable to consider any patient presenting with cutaneous lesions as potentially having disseminated cryptococcosis.[565]

Histopathology. There are two types of histologic reaction to cryptococcal organisms: granulomatous and gelatinous (paucireactive), with the possibility of both types coexisting in the same cutaneous lesion.[564,574,575] In the granulomatous reaction, which is related to more preserved cellular immunity, skin lesions display a dense infiltrate of histiocytes, giant cells, lymphoid cells, and plasma cells.[575] Necrosis can occur and, in some cases, suppuration. A small number of yeast forms are present, usually within the cytoplasm of giant cells and histiocytes, but also free in the tissue. A molluscum contagiosum-like pattern can be seen.[395] In contrast, the gelatinous reaction, found primarily in immunocompromised individuals, has skin lesions showing sheets of numerous aggregated organisms with thick mucinous capsules, forming mucoid masses, and almost no inflammatory infiltrate (Figs. 20.81 and 20.82).[575]

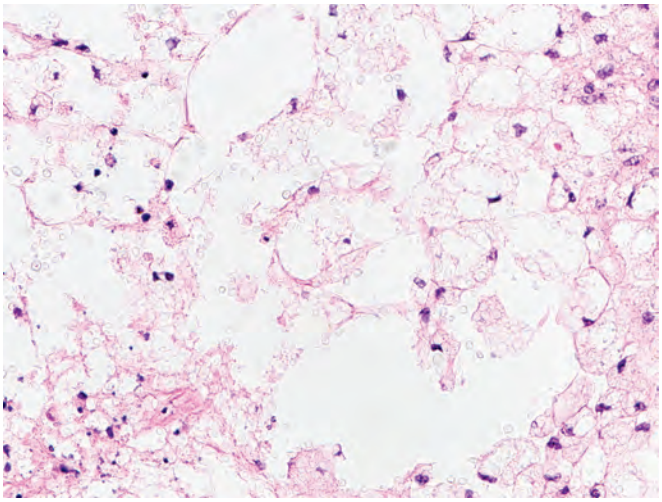
C. neoformans is seen in tissues as a round to oval yeast form and may exhibit pronounced size variability (range: 2 to 20 μm), and occasional organisms may show the "crushed ping-pong ball" appearance with folded edges. The cell wall of *C. neoformans* stains



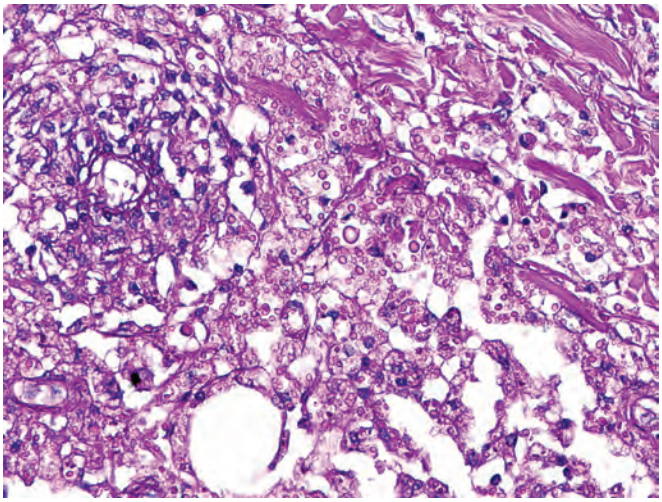
• **Figure 20.81** Cryptococcosis. This example originated from an untreated human immunodeficiency virus/acquired immunodeficiency syndrome individual and illustrates the gelatinous form of the infection, which is represented by a diffuse mucoid infiltrate with a scant inflammatory infiltrate.



• **Figure 20.83** Cryptococcosis (periodic acid–Schiff stain). Sheets of fungal organisms are present in the gelatinous form of the infection.



• **Figure 20.82** Cryptococcosis. Numerous yeast forms of *Cryptococcus neoformans* are seen here exhibiting different sizes as well as the characteristic thick capsule.



• **Figure 20.84** Cryptococcosis (periodic acid–Schiff stain). Numerous organisms are seen here; note the different sizes as well as the scattered collapsed, "ping-pong ball"–like appearance of some of the fungi.

with PAS (Figs. 20.83 and 20.84), methenamine silver, and Fontana-Masson histochemical stains.[576] The mucinous capsule can be highlighted red with mucicarmine and blue with alcian blue.[576] Narrow-based fungal budding and fragmentation of the mucoid capsules can sometimes be observed.

Penicilliosis

Penicilliosis, caused by *P. marneffei*, is endemic in tropical Asia, especially Thailand, northeastern India, China, Hong Kong, Vietnam, and Taiwan.[577] The organism was discovered in 1956 as an infection of bamboo rats[578] and has been isolated from the internal organs of four species of rodents (*Rhizomys sinensis*, *Rhizomys pruinosus*, *Rhizomys sumatrensis*, and *Cannomys badius*), as well as from soil samples that have been collected from bamboo rat burrows.[577] The importance of *P. marneffei* as a human pathogen was recognized only after the HIV pandemic arrived in Asia and the prevalence of infection increased in local populations among visitors from areas where the infection is not endemic.[577,579,580] After tuberculosis and cryptococcosis, penicilliosis is the third most common opportunistic infection in patients with AIDS in northern Thailand.[580]

Similar to other fungi of this group of deep mycoses, *P. marneffei* exhibits temperature-dependent dimorphic growth, being the only known component of the *Penicillium* genus that shows such a feature. At temperatures lower than 37°C, the fungus grows as mycelia with the formation of septate hyphae, bearing conidiophores and conidia typical of the genus *Penicillium*. At 37°C, the fungus grows in a yeast-like form with the formation of fission arthroconidium cells.[577]

Infection presumably results from inhalation of conidia from the environment. Upon inhalation, conidia are believed to be phagocytized by alveolar macrophages, where they grow in the yeast phase. From there, the fungus can spread to other parts of the body.[577]

Clinical Features. Severity of the disease depends on the immunologic status of the host. Clinical features of disseminated *P. marneffei* infection in HIV/AIDS patients are fever, anemia, weight loss, lymphadenopathy, hepatosplenomegaly, respiratory signs, and skin lesions.[577] Approximately 60% to 70% of patients will have skin eruptions, which are usually represented by skin-colored or pigmented papules or nodules, some of which have central umbilication or necrosis.[581] Lesions of *P. marneffei* can clinically resemble tuberculosis, molluscum contagiosum, cryptococcosis, or histoplasmosis.[582]

Histopathology. *P. marneffei* infection can lead to various tissue responses, probably reflecting the degree of cell-mediated immunity.[583] The formation of granulomas helps to localize and prevent the dissemination of the infection and is seen in immunocompetent patients. The inflammatory infiltrate usually consists of multinucleated giant cells, lymphocytes, and neutrophils.[584] On the other hand, in immunocompromised patients, the granulomatous response is usually minimal or absent.[584] Instead, there is necrosis and a diffuse infiltration of macrophages engorged with proliferating yeast cells.[584] *P. marneffei* yeastlike cells can be seen in H&E-, GMS-, or PAS- stained tissue sections both inside the cytoplasm of histiocytes and extracellularly.[577] The intracellular yeasts are oval or round cells, 2 to 3 µm in diameter, that multiply by binary fission, whereas the extracellular *P. marneffei* cells are elongated (up to 13 µm) and have one or two septa.[577]

Being one of the organisms that can be found intracellularly inside macrophages and giant cells, *P. marneffei* has to be differentiated from *H. capsulatum* and *Leishmania* spp. This can be accomplished by looking at the type of division of the yeasts: budding in *H. capsulatum* and binary septation in *P. marneffei*.[577,584] Furthermore, *H. capsulatum* lack large extracellular forms. On the other hand, *Leishmania* spp. organisms have a particular arrangement within intracellular vacuoles, the so-called marquee sign. One could resort to PCR-based assays in difficult cases.[581]

Sporotrichosis

Sporotrichosis is caused by the dimorphic fungus *S. schenckii* and has worldwide distribution, especially in tropical and warm temperate climates.[585,586] Most commonly, the infection is acquired through traumatic implantation of *S. schenckii* present in organic material, such as wood splinters, thorns ("rose handler's disease"), hay, or sphagnum moss.[585,587] Pulmonary infection may result from inhalation of conidia into the lungs.[588] A pet-transmitted (cat and dogs) epidemic occurred in Rio de Janeiro, Brazil.[586]

Clinical Features. Sporotrichosis is usually classified into four forms: lymphocutaneous, fixed cutaneous, disseminated, and extracutaneous.[586,587] The lymphocutaneous (or sporotrichoid) form is the most common and begins as a nontender papule on an exposed area of the skin, such as the face, hand, or arm. This papule grows into a nodule and usually evolves with ulceration. Subsequently, other nodules appear along the lymphatic distribution draining the area in the classic "sporotrichoid" type of spread (Fig. 20.85)[585-587] that can be seen in other types of infections. These lymphatic nodules may undergo suppuration and ulceration. In addition, lymphadenopathy and lymphangitis may develop.[565]

In the fixed (localized) cutaneous form of sporotrichosis, there is no lymphatic spread. A solitary plaque or a group of lesions is present on an exposed area of the skin. These lesions can be scaly, acneiform, verrucous, or ulcerative.[539,587]

• **Figure 20.85** Sporotrichosis. Clinical picture illustrating the classic presentation of the lymphocutaneous form, with nodules arising along the lymphatic drainage of an ulcerated lesion, the classic so-called sporotrichoid dissemation pattern.

Disseminated sporotrichosis is uncommon and results from hematogenous spread or autoinoculation.[565] Extracutaneous disease is also rare and can involve one or more sites, such as the lungs, joints, bones, genitourinary tract, meninges, and eyes.[565] Pulmonary sporotrichosis occurs after inhalation of the organism rather than by cutaneous inoculation.[588] Disseminated and extracutaneous sporotrichosis have been described in patients with chronic alcoholism, hematologic malignancies, chronic obstructive pulmonary disease, diabetes, use of corticosteroids, or HIV/AIDS.[565,589] Disseminated skin lesions are usually nodules or subcutaneous abscesses that later develop punched-out ulcerations.

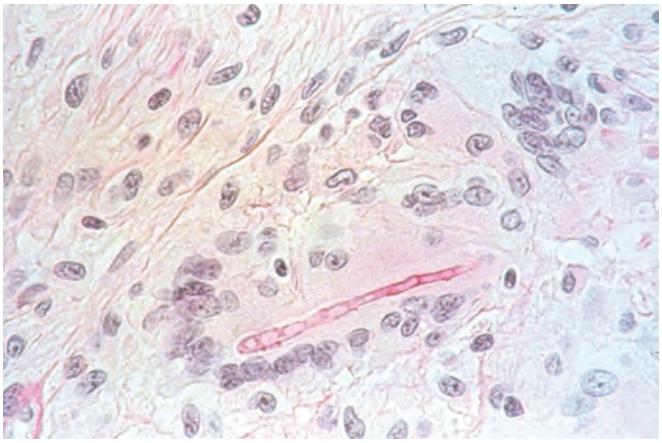
Histopathology. The histopathologic features include epithelial hyperplasia, with marked pseudoepitheliomatous hyperplasia and overlying hyperkeratosis and parakeratosis. Intraepidermal neutrophilic microabscesses are common.[590] In the dermis, a characteristic arrangement of the infiltrate in three zones may occur.[591] There is a central suppurative zone composed of polymorphonuclear leukocytes, which is surrounded by a granulomatous process with a crown of lymphoplasmacytic inflammation.[590,591]

In the lymphangitic form, the inflammatory nodules are located in the deep dermis or subcutaneous tissues, and there are usually no epidermal changes. Similar to the primary lesions, they consist of a central necrotic zone with neutrophils, a surrounding zone of epithelioid cells and giant cells, and an outer zone of plasma cells and lymphocytes with fibrosis.

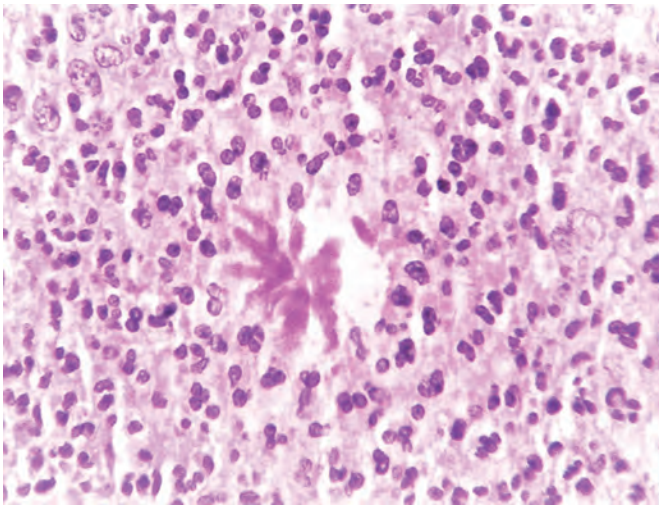
In tissues, *S. schenckii* may be rarely identified as yeast cells that measure 3 to 5 µm in diameter, are round to cigar-shaped, and can show single or multiple buds (Fig. 20.86). Although they are rare, nonseptate hyphae have also been observed.[592]

The asteroid bodies of sporotrichosis are 15- to 35-µm extracellular eosinophilic structures that are found in the center of intraepidermal or dermal abscesses. One to three asteroid bodies may be found in a section.[591] They consist of a central yeast form surrounded by eosinophilic spicules (Splendore-Hoeppli phenomenon).[70,591] The central yeast form is positive on both PAS with diastase and GMS staining, whereas the spicules are negative for these two stains (Fig. 20.87). In addition, the yeast stains with the anti-*Sporothrix* antibody, whereas the spicules do not.[591]

Histopathologic examination is not considered the best diagnostic method for the diagnosis of sporotrichosis because it can be difficult to identify *S. schenckii* in histologic sections stained with routine histochemical stains such as H&E, PAS, and GMS.[590] However, careful examination of multiple serial sections may show fungal elements, according to some authors.[592]



• **Figure 20.86** Sporotrichosis (periodic acid–Schiff stain). Usually difficult to find in lesions from immunocompetent individuals, the fungal spores can manifest as elongated, cigar-shaped structures within giant cells and can even exhibit hyphal forms in immunosuppressed patients.



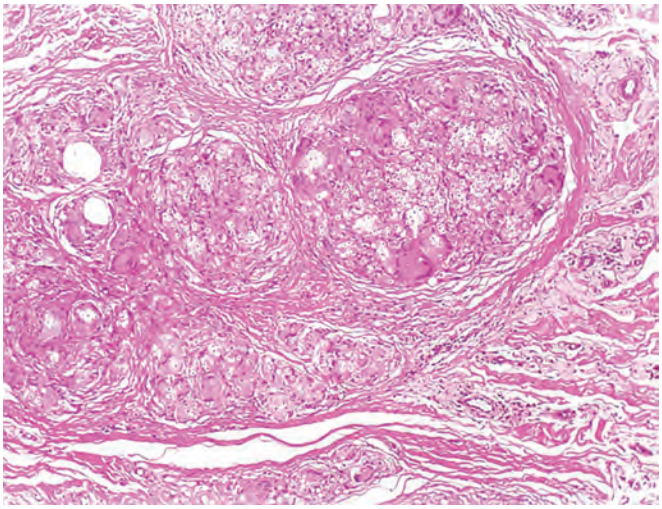
• **Figure 20.87** Sporotrichosis (periodic acid–Schiff stain). Asteroid body in sporotrichosis; yeast forms of the organism can be found surrounded by spicules of eosinophilic material, as part of the Splendore-Hoeppli phenomenon. These structures are often found in the midst of a dermal abscess. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

Immunohistochemical staining using primary antibodies directed against *S. schenckii* may increase the percentage of cases in which the organism can be demonstrated to as much as 83%, more than double what is achieved with ordinary histochemical methods.[590]

Other Diagnostic Techniques. If clinical suspicion is high, fungal cultures are recommended because *S. schenckii* can easily be grown on routine mycologic plates (e.g., Sabouraud dextrose agar, Mycosel) at 25°C, even if fungi were not observed in the tissue. Growth is commonly seen after 3 to 5 days, but cultures should be held for 4 to 5 weeks.[586] Molecular methods can also reliably identify these organisms both from cultures and from tissue.[593]

Lobomycosis

The fungus *Lacazia (Loboa) loboi* causes lobomycosis, also known as keloidal blastomycosis or Jorge Lobo disease. The infection is restricted to tropical areas of Central and South America and is



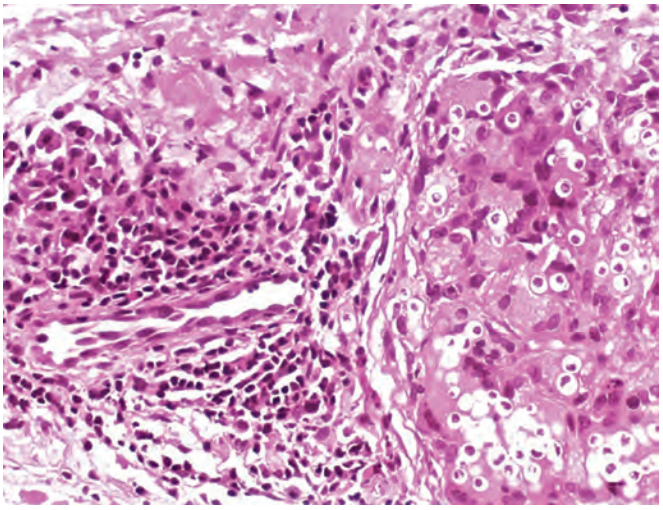
• **Figure 20.88** Lobomycosis. Early lesions exhibit a prominent granulomatous response, whereas chronic ones are associated with extensive collagenous keloidal response. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

hyperendemic in areas of Brazil, and the organism is known to cause disease in humans and dolphins.[594] However, the natural reservoir of *L. loboi* is unknown. The infection occurs mainly in men from rural areas who work in close contact with vegetation and aquatic environments.[594]

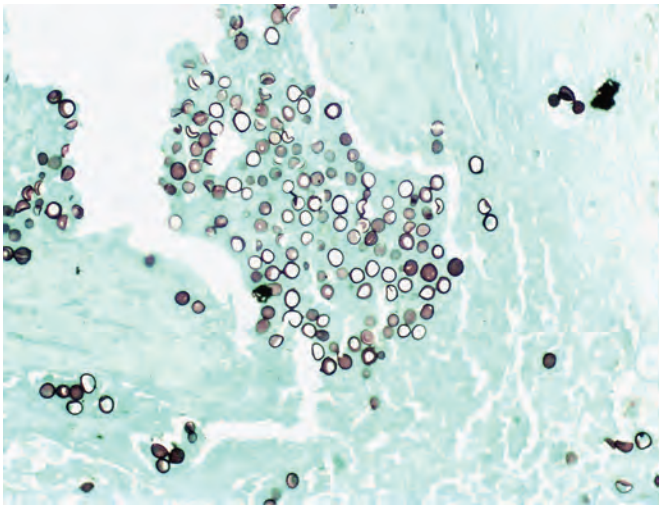
Clinical Features. The disease is limited to the skin and subcutaneous tissues, except for the possibility of lymph node involvement.[595] Because infection probably occurs by inoculation of the organism into the skin during minor trauma,[594,596] lesions are mainly observed on exposed skin, such as the face, ears, and extremities.[594] These lesions have slow growth and are usually asymptomatic but can be pruritic. They consist of papules, nodules, or plaques with a smooth surface that resemble keloids.[594] Some authors divide them into five subtypes: infiltrated, keloidal, gummatous, ulcerative, and verrucoid.[594] Although cutaneous lesions are often restricted to distinct anatomic areas, cases of disseminated lobomycosis have been reported, probably reflecting the host's degree of immunity against the fungus.[597] SCC can occur in long-standing lesions.[598]

Histopathology. On histopathologic examination, the epidermis is commonly atrophic, with flattened rete ridges. However, hypertrophic epidermis, with hyperkeratosis or parakeratosis, is seen in some cases. There is a diffuse, granulomatous dermal inflammatory infiltrate composed of numerous histiocytes and multinucleate giant cells (both foreign body and Langhans), which contain numerous fungi (Figs. 20.88 and 20.89).[594,599] A dermal mixed inflammatory infiltrate composed of lymphocytes, plasma cells, and, less frequently, neutrophils can be present.[599] Some cases exhibit asteroid bodies similar to the ones seen in sporotricosis.[600] The infiltrate is occasionally separated from the epidermis by a narrow grenz zone. These inflammatory lesions eventually evolve into pauci-inflammatory keloidal-appearing areas of scarring, seen as brightly eosinophilic thick collagen bundles. Both the notorious keloidal-type reaction and the paucity of inflammatory cells are due to the increased secretion of TGF-β, a known fibrosing and immunosuppressive factor.[601]

The fungi are identified in tissue as thick-walled, rounded, or lemon-shaped, yeast-like cells, which measure 6 to 12 μm, occurring either singly or in "sleeve-like" chains of budding cells



• **Figure 20.89** Lobomycosis. The organisms are represented by thick-walled, yeastlike cells that have a tendency to form chainlike structures. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



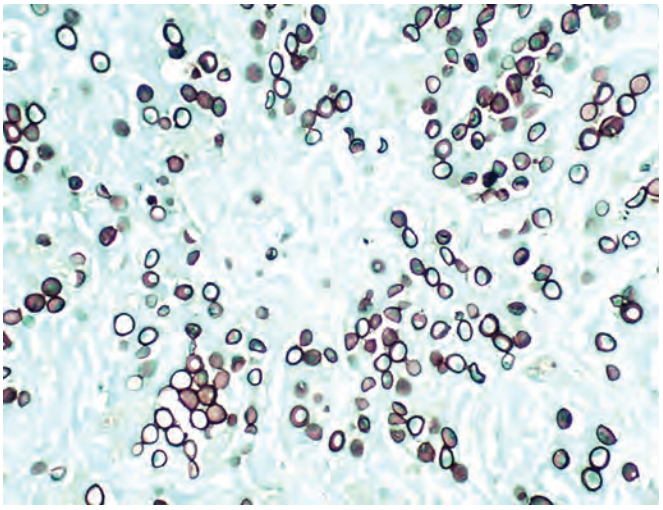
• **Figure 20.90** Lobomycosis (Grocott methenamine silver stain). Fungal structures exhibiting tubular interconnections. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

interconnected by thin tube-like structures (2 to 10 cells) (Figs. 20.90 and 20.91).[594,599] The fungi are beautifully highlighted by PAS and methenamine silver histochemical stains and are mucicarmine negative.

L. loboi has never been cultivated in vitro, but it has been successfully transmitted to mice, tortoises, and armadillos under experimental conditions.[594,596,602]

Phaeohyphomycosis

Phaeohyphomycosis is a term used to describe subcutaneous and systemic diseases caused by a variety of dematiaceous fungi (black molds) that develop in infected tissue as darkly pigmented yeast-like cells, hyphae, pseudohyphae, or a combination of these forms.[603-605] Dematiaceous fungi can produce three different types of infection: phaeohyphomycosis, chromoblastomycosis, and mycetoma.



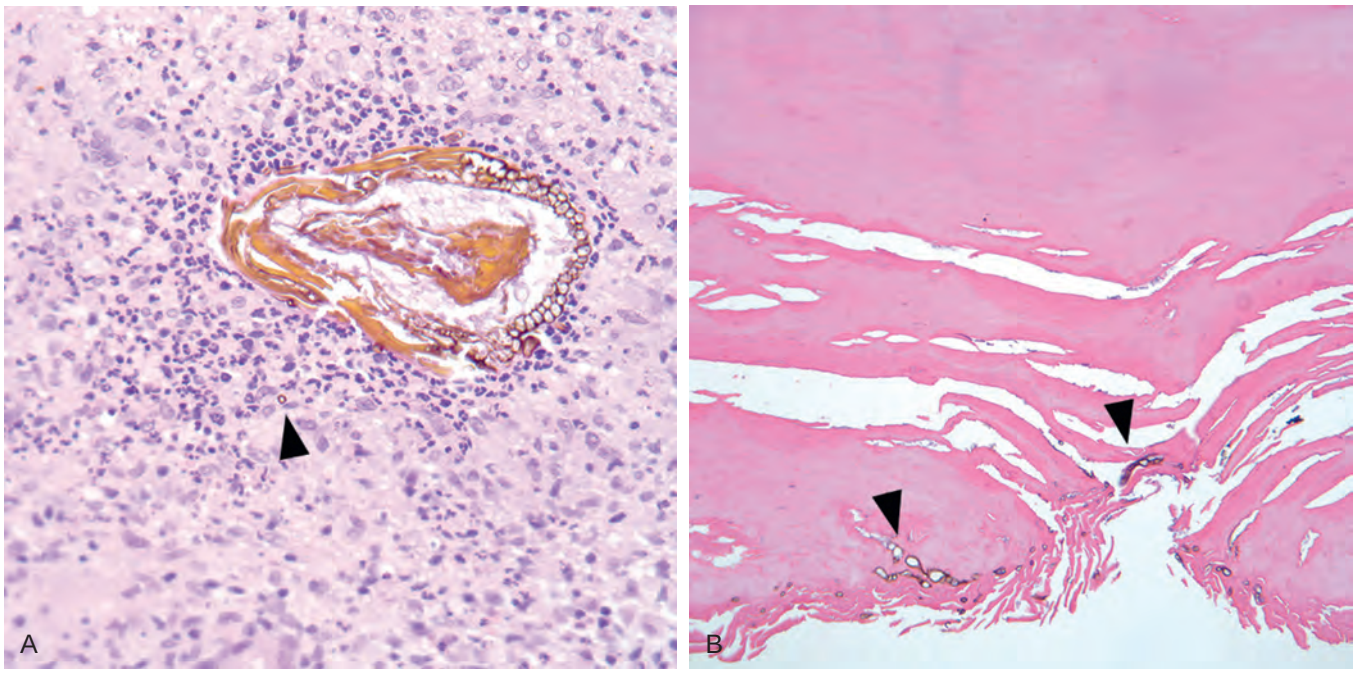
• **Figure 20.91** Lobomycosis (Grocott methenamine silver stain). High-power view of the organisms shows the thick-walled, rounded or lemon-shaped, yeast-like cells that occur singly or in chains of budding cells. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

More than 130 fungal species belonging to 70 diverse genera have been reported as causative agents in human and animal phaeohyphomycoses.[604,605] *Exophiala jeanselmei* and *Wangiella dermatitidis* are the most commonly isolated fungi in cutaneous and subcutaneous phaeohyphomycoses.[603] These fungi grow on soil, rotten wood and other plant materials, and decaying organic matter, and the infection is usually acquired by inoculation of the organism into the skin and subcutaneous tissues through trauma.[603,605] However, other possible portals of entry have been suggested, including inhalation of the fungus with lung or sinus invasion, ingestion of contaminated food or water followed by penetration through the gastrointestinal tract, and contamination of vascular catheters.[605]

Clinical Features. Phaeohyphomycosis can be divided into at least nine clinical syndromic groups: (1) superficial (including black piedra and tinea nigra, discussed previously in the superficial mycoses section); (2) onychomycosis; (3) subcutaneous; (4) corneal or mycotic keratitis; (5) allergic fungal sinusitis; (6) allergic fungal bronchopulmonary mycosis; (7) pneumonia; (8) brain abscess; and (9) disseminated disease.[604] Disseminated infection is uncommon, but its incidence is increasing, particularly among immunocompromised patients.[606] The most important predisposing factor for cutaneous and subcutaneous infection is exposure to contaminated material present in the environment (i.e., decaying wood, plants).[605] Subcutaneous phaeohyphomycosis usually manifests as a single cyst or abscess on an extremity or another exposed area of the skin.[607]

Histopathology. Unlike chromoblastomycosis or mycetoma, there are neither sclerotic bodies nor grains in phaeohyphomycosis. Three different tissue patterns have been described in phaeohyphomycosis.[608]

Keratotic plaques represent the first pattern, and nodules usually show parakeratosis and irregular epidermal hyperplasia with microabscesses, while a suppurative and granulomatous inflammatory process occupies the dermis. Solid and necrotizing granulomas and microabscesses are observed. Brown yeast-like cells, pseudohyphae, and true hyphae are seen among epithelioid cells and neutrophils (Fig. 20.92A). Some organisms are found



• **Figure 20.92** Pheaohyphomycosis. Components from this group of fungi are usually inoculated into the dermis with vegetable material, such as splinters, and produce a vigorous mixed inflammatory infiltrate. Note the invading pigmented fungal elements *(arrowhead)* **(A)**. Onychomyosis produced by dematiaceous fungus; note the bulbous, pigmented hyphae *(arrowheads)* **(B)**.

within multinucleated giant cells. The PAS stain highlights the fungi. The yeast-like cells can be observed singly or arranged in chains of varying lengths.[608]

The second tissue pattern shows an intradermal, multiloculated cystic structure with a dense fibrous capsule. Broad bands of connective tissue separate individual cystic structures. The epidermis shows no abnormalities. The larger cysts can be empty and lined by granulomas and neutrophils. The smaller cysts are usually filled with neutrophils and lined by granulomas. Many dematiaceous yeast-like cells, true hyphae, and pseudohyphae are seen within the inflammatory infiltrate, as well as within giant cells. Particles of wood splinter can also be seen.[608]

The third tissue pattern is that of a well-defined, dermal unilocular cyst surrounded by dense fibrous tissue. The epidermis is uninvolved. The center of the cyst contains necrotic debris and neutrophils. The inner lining of the cyst is composed of granulomas, which contain dematiaceous organisms. Similarly to the second pattern, fragments of vegetable material can be seen in the cystic lumen.[608]

The histopathology in the onymocycotic presentation is similar to the one seen onycomycosis caused by dermatophytes but with the specimen showing pigmented hyphae in the midst of the nail clipping material (see Fig. 20.92B).

Chromoblastomycosis is characterized in tissues by sclerotic bodies, which are not seen in phaeohyphomycosis (see later). The yeastlike cells in phaeohyphomycosis may resemble the sclerotic bodies of chromoblastomycosis; however, the former are lighter, have thinner walls, show septation in only one plane, and have a chain morphology.[608]

The etiologic agents of phaeohyphomycosis are characterized by yellow to brown pigmentation of their cell walls and distinctive brown to black colony morphology. In most cases the pigment is melanin, specifically dihydroxynaphthalene melanin.[609]

Some dematiaceous fungal species cause both phaeohyphomycosis and chromoblastomycosis. The different pathologic features produced in tissues by the same fungus are believed to be a result of undefined host factors.[606,610]

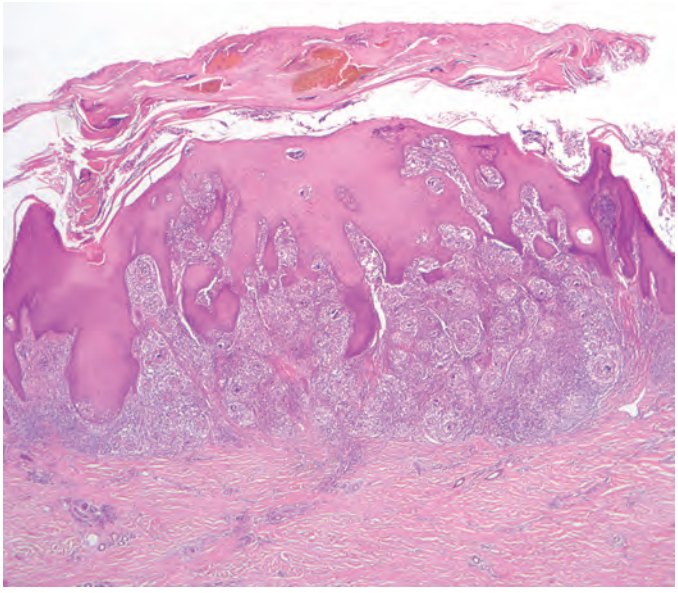
Chromoblastomycosis

Chromoblastomycosis (chromomycosis) is a chronic, suppurative, granulomatous mycosis of the skin and subcutaneous tissues that is caused by various pigmented (dematiaceous) fungi, which are observed in the tissues as sclerotic pigmented bodies.[611] The most common fungal species associated with chromoblastomycosis are *Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Phialophora verrucosa*, *Rhinocladiella aquaspera*, and *Cladosporium carrionii*.[611] *F. pedrosoi* has been isolated from approximately 95% of patients in some series.[565,612] The infection is more common in the tropics, especially in the humid areas, and, although it often affects individuals who are healthy and without any predisposing factors,[611] immunocompromised persons can also acquire the infection.[565] The fungi are present in soil, wood, and vegetable debris and probably infect patients after trauma. When considering immunocompetent individuals, the most often affected are mainly male rural workers.[611]

Clinical Features. Initial lesions of chromoblastomycosis are usually erythematous papules, which slowly enlarge to display varying morphologies, such as verrucous nodules, cauliflower-like tumors, and psoriasis-like plaques.[613] The cutaneous lesions are found on the feet and legs or on other exposed areas of the body.[611] Central scarring and ulceration can occur.[611] As the infection progresses, dissemination may occur by extension of the lesions as satellites along the lymphatic vessels or by autoinoculation through scratching.[614] Secondary bacterial infection is a frequent complication and can lead to lymphedema, resulting in further limb deformity.[612] Involvement of underlying tissues

usually does not occur, but hematogenous spread to the CNS has been described. The development of SCC is a possibility in long-standing disease.[611]

Histopathology. There are usually exuberant epidermal changes, including hyperkeratosis with parakeratosis, acanthosis, marked pseudoepitheliomatous hyperplasia, and papillomatosis.[615] The pseudoepitheliomatous epidermal hyperplasia can be so prominent that it can easily mimic SCC (Fig. 20.93).[616] Fungal bodies can occasionally be seen between keratinocytes and in the overlying scale undergoing transepithelial elimination (Fig. 20.94A).[617] Intraepidermal abscesses are frequently observed, with or without fungal cells (see Fig. 20.94B).[615] Dematiaceous hyphae can rarely be seen in the stratum corneum. The dermis shows a dense inflammatory infiltrate composed predominantly of neutrophils, which occasionally form dermal abscesses, and macrophages. Eosinophils, lymphocytes, plasma cells, and mast cells can also be seen. Both epithelioid granulomas and poorly organized granulomas formed by aggregates of macrophages and epithelioid cells with giant cells (Langhans or foreign body type) can be observed.[618] These granulomas frequently have a suppurative center. Many cases show marked cicatricial fibrosis, which can extend from the superficial dermis to the subcutaneous fat, obliterating skin adnexa.



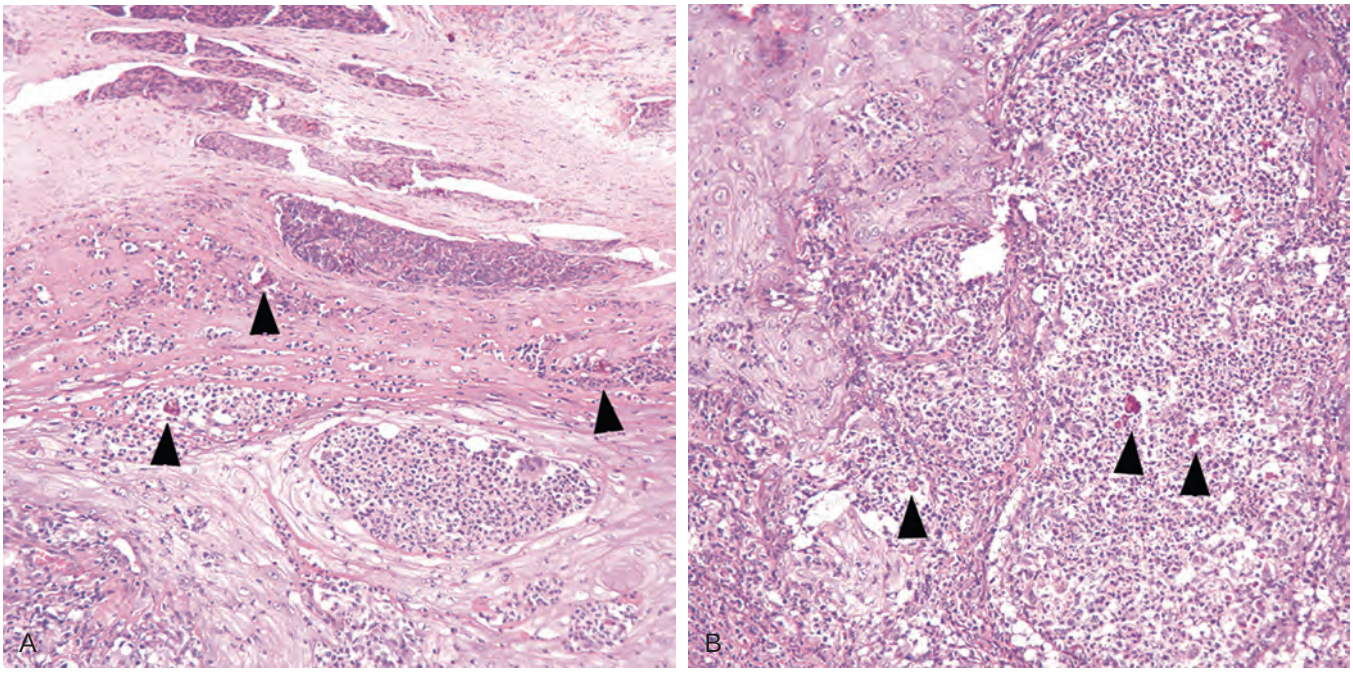
• **Figure 20.93** Chromoblastomycosis. There is often a pseudoepitheliomatous epidermal hyperplasia with prominent acanthosis and hyperkeratosis overlying a granulomatous infiltrate.

Because of their color, the fungi can be easily identified without special stains. The characteristic sclerotic bodies are round or polyhedral, pigmented, thick-walled fungal cells that measure 5 to 12 μm in diameter.[603] They can be detected in the center of granulomas and within giant cells. They are dark brown and round, resembling "copper pennies," and may be seen singly or in chains or clusters (Fig. 20.95).[615] They represent an intermediate vegetative form, arrested between yeast and hyphal morphology.[613] Chromoblastomycosis was previously believed to be related to blastomycosis; however, cellular division occurs by internal septation and not by budding as in blastomycosis.[611]

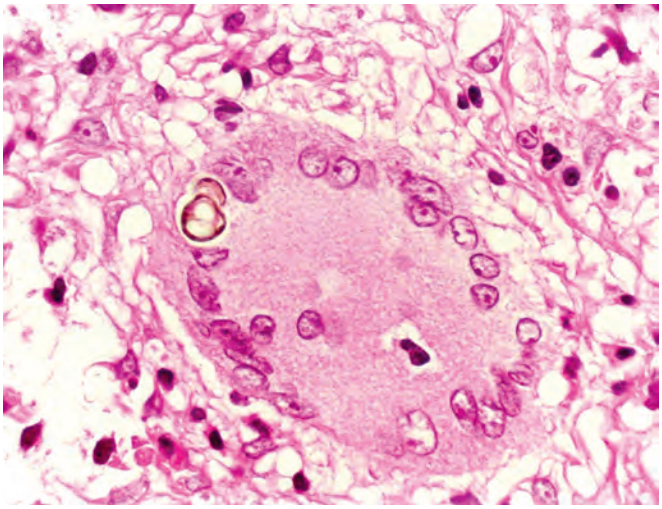
The sclerotic bodies are easily identified by microscopic examination of crusts and exudates from the skin lesions under potassium hydroxide.[613] However, fungal culture is necessary for classification of the causative agent.

Eumycetoma

Mycetoma is a chronic granulomatous infection of the skin and subcutaneous tissues, characterized by the triad of tumefaction, draining sinuses, and the presence of grains in the exudate.[619] They



• **Figure 20.94** Chromoblastomycosis. Transepidermal elimination of fungal elements is occasionally seen *(arrowheads)* **(A)**. Prominent papillary dermal and intradermal microabscesses are the places to hunt for fungal forms, easily recognizable due to their pigmentation *(arrowheads)* **(B)**.



• **Figure 20.95** Chromoblastomycosis. High-power view of the pigmented fungal forms with the classic "copper pennies" appearance within a Langhans-type multinucleated giant cell. Note the septation characteristic of this organism and evidence of cell division. This feature distinguishes this organism from those that bud. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

can be caused either by filamentous bacteria (actinomycetomas), discussed previously in the bacteria section, or true fungi (eumycetomas). These infections are most common in tropical and subtropical areas and are endemic to India and several countries in Africa and South America.[585] The organisms are present in the soil and are implanted into the host tissue through trauma.[539] The infection is most frequent in adult men, usually rural workers.[585]

More than 20 species of fungi and bacteria have been implicated as etiologic agents of mycetoma and the predominant causative organism varies by region.[620] Eumycetomas predominate in Africa and southern Asia, whereas actinomycetomas predominate in Latin America.[619]

Madurella mycetomatis is the most prevalent fungal etiologic agent worldwide,[619,621] and *Pseudallescheria boydii* is the most common cause of eumycetoma in the United States.[611,620] Other important agents include *Leptosphaeria senegalensis*, *Madurella grisea*, *Acremonium recifei*, and *Pyrenochaeta romeroi*.[621]

Worldwide, approximately 60% of all mycetomas are caused by actinomycetes, the commonest agent being *N. brasiliensis*.[539,619] Other causative organisms include *Streptomyces somaliensis*, *Actinomadura madurae*, and *Actinomadura pelletierii*.[611]

The grains are aggregates of fungal hyphae or bacterial filaments, and their size, consistency, and color provide the initial clue to the causative species (see Table 20.1).[619] The black granules are seen only in eumycetomas, such as those produced by *M. mycetomatis*.[620] The consistency of most grains is soft, but those of *M. mycetomatis* and *S. somaliensis*, for example, can be hard.[539] Culture is required for accurate identification of the causal agent.

Clinical Features. The foot is the most common site of infection (Madura foot), but any exposed area is susceptible.[619,621] The lesion starts as a painless papule, which enlarges to become a discharging nodule. The disease progresses with extension to the adjacent tissues, forming multiple draining sinuses. The drainage contains grains, which can sometimes be seen with the naked eye.[539] The sinus tracts are rarely observed before 3 months but are usually present by the end of the first year.[585] The affected area becomes distorted by inflammation, edema, and fibrosis. The infection can extend to involve the underlying fascia, muscle, and bone. Eumycetomas are usually less inflammatory and less invasive and have a slower progression, compared with actinomycetomas.[539]

Histopathology. Similar to actinomycetomas, the characteristic granules are present, which represent colonies of organisms. Three somewhat defined types of tissue reaction have been described.[620] In the type I reaction, the eumycetoma grains are surrounded by an abscess-like structure composed predominantly of polymorphonuclear leukocytes, with the innermost neutrophils closely attached to the surface of the grain. Outside the suppurative zone, there is granulation tissue with macrophages, lymphocytes, plasma cells, and a few neutrophils. Macrophages can have large vacuolated cytoplasms. The capillary vessels surrounding the inflammation may exhibit concentric layers of fibrin. The dermis surrounding the inflammatory area will exhibit fibrosis. The type II reaction, related to more chronic lesions, shows that most of the neutrophils are replaced by macrophages and multinucleated giant cells that are engulfing grain material. Other inflammatory cells and histologic changes are similar to those observed in type I reactions. Finally, the type III reaction is characterized by well-organized epithelioid granulomas that occasionally contain Langhans giant cells; remnants of fungal material can be seen in the center of the granuloma. There are remaining foci of active inflammation, and histologic changes are similar to those seen in type I and type II reactions.[620]

The fungal hyphae in eumycotic grains are thicker than the thin filaments seen in actinomycotic grains.[620] Histochemical stains, such as Gram, can help to distinguish the subtype of mycetoma. In actinomycetomas, the grains have gram-negative centers with gram-positive, fine, radiating fringes, whereas in eumycetomas, the grains show gram-negative septate hyphae embedded in intercellular cement.[620] The Splendore-Hoeppli phenomenon can occasionally be seen surrounding the granules.[70]

Hyalohyphomycoses

Hyalohyphomycosis is a term used to designate a group of invasive fungal infections caused by organisms exhibiting hyaline (nonpigmented) septated hyphae in tissue.[622] The genus *Aspergillus* and *Fusarium*, as well as species from the *Penicillium*, *Scopulariopsis*, *Scedosporium*, *Acremonium*, and *Paecilomyces* genera, can be included in this group.[623] Being the two most important pathogens from this group, we will discuss infections caused by organisms from the *Aspergillus* and *Fusarium* genera.

Aspergillus. Similar to other fungi, organisms from this genus are present in decaying plant material and stored grains. From the more than 300 species contained in the *Aspergillus genus*, *Aspergillus fumigatus* appears to cause up to 90% of overall infections in humans.[624,625] However, primary cutaneous infections are almost exclusively produced by *Aspergillus flavus* with isolated cases caused by *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus utus*.[624,625]

Invasive aspergillosis (IA) is one of the most common infections in immunocompromised individuals, particularly in patients with hematologic malignancies, and it usually starts in the lungs after inhalation of infective material. Other frequently involved organs are the sinuses and the CNS. The mortality rate of IA in immunosuppressed individuals has been reported to be between 35% and 55%.[624] Early diagnosis and intervention is very important and can improve survival in this patient population.[626]

Cutaneous involvement by IA can be produced either by inoculation (primary cutaneous IA [PCIA]) or from hematogenous dissemination (secondary cutaneous IA [SCIA]). A much worse prognosis is seen with SCIA.[624]

Clinical Features. Because it is a product of percutaneous inoculation of the agent, PCIA often presents as an isolated infiltrative eschar-like lesion forming a plaque or a nodule. Other presentations include a black-crusted plaque (the black color being due to necrosis not fungal pigment), erythematous papules and hemorrhagic bullae. On the other hand, SCIA usually presents as multiple necrotic nodules, plaques, and papules that are products of septic emboli.[624,625]

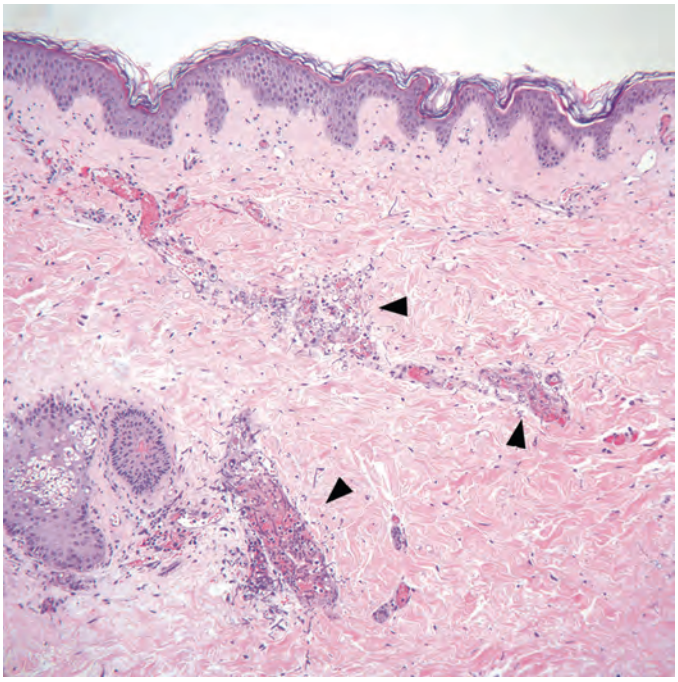
Histopathology. Being an angioinvasive fungus, *Aspergillus* spp. hyphae are usually found involving thrombosed small- and medium-sized vessels and accompanied by a mixed inflammatory infiltrate. The infiltrate can be attenuated or absent in profoundly neutropenic individuals. The fungal elements show septate hyphae measuring 2.5 to 6 µm with the characteristic acute angle branching and often show an "arborizing" or "hierarchical" branching pattern. The hyphae can be highlighted with PAS and GMS histochemical stains and with immunohistochemical stains.[624,625] Cases involving the adipose panniculus can potentially mimic pancreatic and gouty panniculitis because aspergillus organisms are capable of producing several enzymes, including lipase.[627]

Fusarium. *Fusarium* is a plant and human pathogen readily recovered from soil and water and is part of water biofilms.[622] The species most frequently causing infection in humans are *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium moniliforme*.[628] The principal portal of entry for *Fusarium* spp. is the airways, followed by the skin at sites of tissue breakdown, and, possibly, the mucosal membranes.[622]

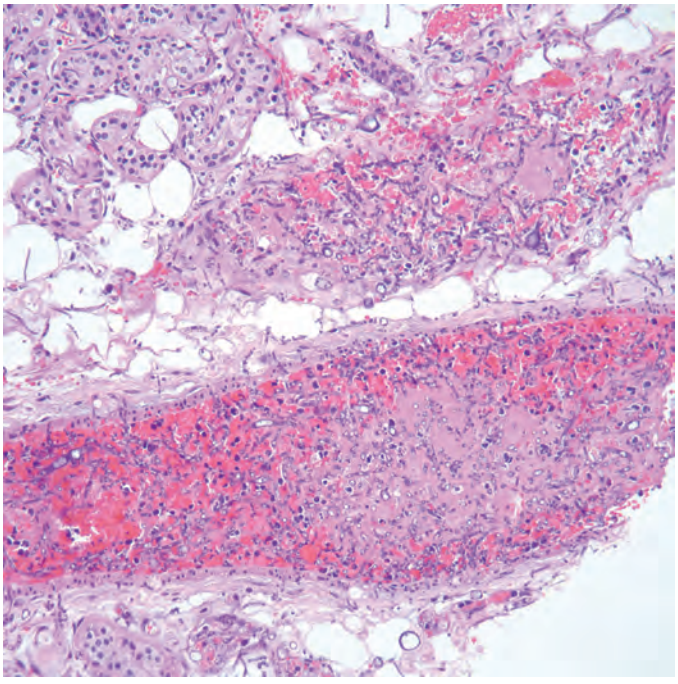
Fungi from the *Fusarium* genus cause a broad spectrum of disease in humans, including superficial, locally invasive, and disseminated infections, with the last occurring almost exclusively in severely immunocompromised patients.[622,629] The most common infections in immunocompetent hosts are keratitis and onychomycosis.[622,623,629] The infection may also occur as a result of skin breakdown (e.g., burns, wounds) or from the presence of foreign bodies, such as contact lenses (keratitis),[630] continuous ambulatory peritoneal dialysis (peritonitis),[631] and catheters (catheter-associated fungemia).[632] Immunocompromised patients at high risk for fusariosis are those who have prolonged neutropenia and T-cell immunodeficiency.

Clinical Features. Disseminated fusarial infection leads to cutaneous involvement in more than 70% of patients. The usual course includes fever, multiple skin lesions, myalgias, and fungemia.[633] Three forms of skin lesions can be observed: multiple subcutaneous nodules, ecthyma gangrenosum–like lesions, and target lesions consisting of ecthymiform lesions surrounded by a thin erythematous rim.[634] These lesions can involve any site, with a predilection for the extremities, and evolve rapidly, usually over a few days.[573,622] Fusariosis in immunocompromised patients usually responds poorly to antifungal therapy and is associated with a high mortality rate.

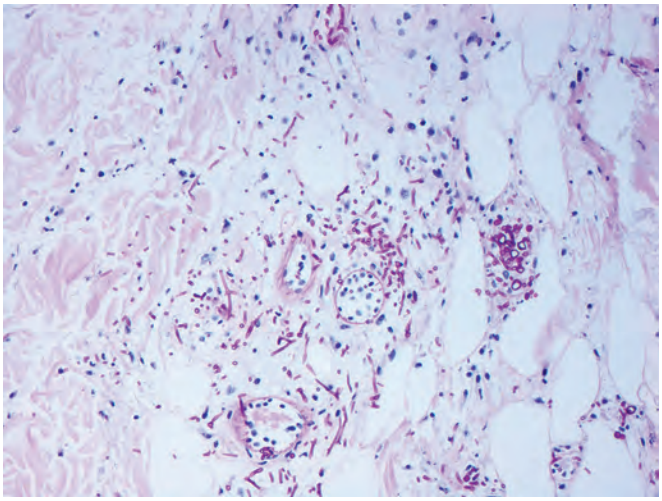
Histopathology. In tissue, the hyphae are similar to those of *Aspergillus* species, with angioinvasive hyaline and septate hyphal forms that typically dichotomize at acute and right angles (Figs. 20.96 and 20.97).[622] However, adventitious sporulation may be present in tissue, and the finding of hyphae and yeast-like structures together (fusoid macroconidia and unicellular ovoid microconidia) is highly suggestive of fusariosis in the high-risk population.[625] Another feature that should raise the suspicion of *Fusarium* is the presence of dilated hyphae with so-called varicosities and balloon-like structures (see Fig. 20.97). This fungus is also quite angioinvasive, producing vascular damage and thrombosis (see Fig. 20.97), along with an exuberant mixed inflammatory infiltrate if the host is able to mount one. Cases from profoundly suppressed individuals are pauci-inflammatory (see Figs. 20.96



• **Figure 20.96** Hyalohyphomycosis (*Fusarium* sp.). This skin biopsy from a profoundly immunosuppressed individual shows the prominent angioinvasive nature of the fungus with multiple hyphal forms arising from the mid-dermal vessels *(arrowheads)*.



• **Figure 20.97** Hyalohyphomycosis (*Fusarium* sp.). Subcutaneous thrombosed veins teeming with fungal elements. Note the varicosities and dilatations of the hyphae, which suggest *Fusarium*.



• **Figure 20.98** Hyalohyphomycosis (*Fusarium* sp.) (periodic acid–Schiff stain). The presence of septate hyphae branching in acute or right angles is characteristic. Note the presence of yeastlike structures, a helpful feature for differentiating this organism from *Aspergillus* sp. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

and 20.97). Both PAS (Fig. 20.98) and GMS histochemical stains can highlight the fungi. In the absence of microbial growth in cultures, the differential diagnosis between fusariosis and other hyalohyphomycoses is difficult and requires the use of ISH in paraffin-embedded tissue specimens.[635]

Zygomycosis. The term *zygomycosis* includes any infection caused by a member of the Zygomycota phylum (Zygomycetes). Two orders belonging to this phylum contain organisms that cause human disease: the Mucorales and Entomophthorales.[636] Members of these two orders produce different disease manifestations: Mucorales typically cause acute, angioinvasive infections called mucormycoses, whereas Entomophthorales cause a group of infections referred to as entomophthoramycoses, which are more indolent and chronically progressive.[637]

Mucormycosis. Mucormycosis is the third most frequent invasive fungal infection, after aspergillosis and candidiasis, with the most commonly recovered genera are *Mucor*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Cunninghamella*, and *Saksenaea*.[637] These fungi have worldwide distribution and can be found in soil and decaying organic matter.[638] The spores from these molds are transmitted by inhalation, percutaneous inoculation, or ingestion. Human infection typically occurs in immunocompromised hosts as an opportunistic infection.[637]

Risk factors for infection include diabetes mellitus, neutropenia, sustained immunosuppressive therapy, chronic prednisone use, iron chelation therapy, broad-spectrum antibiotic use, severe malnutrition, and primary breakdown in the integrity of the cutaneous barrier (e.g., trauma, surgical wounds, needlesticks, burns).[637,638] Mucormycosis is rare in immunocompetent individuals and usually occurs product of contamination of wounds, burns, and trauma sites.[638]

Clinical Features. The clinical manifestations include rhino-orbito-cerebral, pulmonary, cutaneous, and gastrointestinal effects. If the diagnosis is not made early, dissemination often occurs.[637] Rhino-orbital-cerebral zygomycosis originates in the paranasal sinuses and extends into the brain.[638] The overall mortality rate is approximately 62% in rhinocerebral zygomycosis and 16% in sinus infection without cerebral involvement.[639] A black necrotic eschar on the palate or nasal mucosa is a classic diagnostic clue, although this finding is not seen in all cases.[637] As the disease progresses, periorbital swelling, orbital cellulitis, blurred vision or visual loss, proptosis, eyelid gangrene, cranial neuropathies, and altered consciousness, among others symptoms and signs, can be observed.[637,638]

Cutaneous mucormycosis may be primary, resulting from local inoculation, or secondary, occurring after hematogenous dissemination.[640] Cutaneous mucormycoses range from superficial skin involvement, such as plaques, skin swelling, pustules, cellulitis, blisters, nodules, ulcerations, and ecthyma gangrenosum–like lesions, to deeper infections, such as necrotizing fasciitis, osteomyelitis, and disseminated infection. Secondary cutaneous disease usually manifests as nodular subcutaneous lesions which may ulcerate.[637]

Histopathology. In mucormycosis, the fungal hyphae are broad (7 to 15 mm in diameter), pleomorphic, ribbonlike, hyaline, and pauciseptate, exhibiting a branching pattern of 45 to 90 degrees. The hyphae invade blood vessels, leading to vasculitis, hemorrhage, thrombosis, infarction, and necrosis.[625] The inflammatory response is variable and can be neutrophilic (most common), pyogranulomatous, absent, or granulomatous (rare).[641] Perineural invasion may be seen.[641] The organisms are usually visualized even on H&E preparations. In addition, they can be seen with special stains for fungi. The hyphae of the zygomycetes have thinner walls compared with other fungal hyphae, which possibly accounts for their weaker staining with GMS and PAS techniques compared with fungi with thicker cell walls. In addition, the hyphae of the zygomycetes appear more prone to twisting and folding than those of other fungi.[641]

An unusual pattern resembling gouty or pancreatic panniculitis has been described in cases in which the fungus invades the subcutaneous tissue. This peculiar pattern, also observed in cutaneous aspergillosis (see previous discussion),[627] is probably related to secretion of lipases by the fungus.[642]

Entomophthoramycosis. Human disease caused by Entomophthorales is seen predominantly in tropical regions, with transmission occurring by implantation of spores via minor trauma, such as insect bites, or by inhalation of spores into the sinuses.[637] Agents of the Entomophthorales comprise two pathogenic genera, *Conidiobolus* and *Basidiobolus*, which produce the main diseases called conidiobolomycosis and basidiobolomycosis, respectively.[638] In contrast to *Aspergillus*, *Fusarium*, and *Mucor*, all are opportunistic agents; the Entomophthorales fungi are true pathogens, infecting primarily immunocompetent hosts and generally do not invade blood vessels and rarely disseminate.[637]

Clinical Features. Basidiobolomycosis is caused by *Basidiobolus ranarum.* This organism resides in decaying plant material, soil, leaves from deciduous trees, and the intestines of fish, frogs, toads, insects, reptiles, and insectivorous bats.[637] The disease is more common in children.[643] Clinically, there is involvement of the skin and subcutaneous tissues, characterized by the formation of fluctuant, firm, and nontender swellings.[643] The overlying skin is usually normal, and there is no bone involvement. The most frequently affected parts of the body are the buttocks, thighs, and trunk.[643] Lymph node or muscle involvement can rarely occur.[643] Cases of both gastrointestinal infection[644] and disseminated basidiobolomycosis[645] affecting immunocompetent hosts have been reported.

Entomophthoromycosis due to *Conidiobolus coronatus* is characterized by lesions that originate in the inferior turbinate and

spread through ostia and foramina to involve the facial and subcutaneous tissues and paranasal sinuses.[646] Most cases have been described from areas of tropical rainforest in West Africa, with agricultural and outdoor workers (aged 20 to 60 years) being the ones most commonly affected.[646] The fungus is found in soil and decaying vegetation. Patients usually present with unilateral nasal obstruction, nasal discharge, epistaxis, sinus tenderness, and swelling of the nose, upper lip, and face.[637,638] The disease usually involves the subcutaneous tissue either as a granulomatous infection without bone destruction or as an ulcerated skin lesion, and the clinical course is slowly progressive.[638] Disseminated infections, including those with fatal outcomes, have been reported with *C. coronatus* in both immunocompetent and immunocompromised hosts.[637] *Conidiobolus incongruus* is a rare agent of the disease.[646]

Histopathology. Similarly to mucormycosis, entomophthoromycosis shows fungi that are also seen as broad, pauciseptate hyphae, with right-angled branching. However, the hyphae usually do not invade blood vessels.[636] There is granulomatous inflammation in the dermis and subcutis. The inflammatory infiltrate contains foreign-body giant cells, histiocytes, lymphocytes, epithelioid cells, and, frequently, eosinophils and plasma cells. The eosinophils may be numerous, forming microabscesses.[646] Areas of necrosis are infrequently seen in the center of lesions.[646] The hyphae are generally visible in H&E-stained sections, and they can be seen surrounded by eosinophilic material (Splendore-Hoeppli phenomenon).[70] Hyphal fragments can be found within giant cells.[643]

Infections Caused by Algae

Protothecosis

Human protothecosis is a rare infection caused by members of the alga genus *Prototheca*. Algae belonging to this genus are achlorophyllic and ubiquitous in nature, with protothecosis cases being reported worldwide.[647] Trauma and inoculation with contaminated water is the most common mechanism of infection in humans.[647] Infections can occur in both immunocompetent and immunosuppressed patients, although more severe and disseminated infections tend to occur in the immunocompromised.[647,648]

Currently, the two species reported to cause infections in humans are *Prototheca wickerhamii* and *Prototheca zopfii*, with *P. wickerhamii* being more common.[647-650]

Clinical Features

The great majority of patients who develop protothecosis are immunocompromised and older than 30 years of age, although cases have also been reported in children[650] and neonates.[651] The clinical manifestations have been classified into three types: (1) cutaneous lesions, (2) olecranon bursitis, and (3) disseminated or systemic disease.[647,648]

The cutaneous lesions are located mainly on exposed areas, such as the extremities and face.[647] They usually have a slowly progressive and persistent course with no tendency to self-heal.[652] The clinical presentation can be quite variable, with lesions presenting as erythematous plaques, pustules, papules, nodules, vesicles, verrucous lesions, ulcers, or hypopigmented or atrophic lesions.[647,649,650,653] In addition, lesions resembling herpes zoster,[654] eczema,[655] subcutaneous mycoses,[653] and cellulitis/pyoderma[653,656] have also been described.

Histopathology

The host tissue responses to *Prototheca* are variable, ranging from severe granulomatous necrosis to an absence of inflammatory changes.[625,657] There is usually a chronic granulomatous reaction throughout the dermis, which may also involve the subcutaneous tissue, associated with a variable admixture of lymphocytes, neutrophils, plasma cells, and occasional eosinophils.[625,657] The granulomas can exhibit central necrosis.[657] The epidermis can exhibit hyperkeratosis, focal parakeratosis, and pseudoepitheliomatous hyperplasia.

The organisms are observed in the cytoplasm of macrophages and multinucleated giant cells but also extracellularly. They manifest as thick-walled, spherical bodies with no budding.[625,657] *Prototheca* species may not be readily apparent in H&E-stained slides, but they stain with the silver methenamine and PAS stains.

Prototheca organisms are spherical, unicellular organisms ranging from 3 to 30 µm in diameter.[647] Reproduction is asexual; during cell maturation, the cytoplasm undergoes a process of cleavage to form endospores. Approximately 2 to 20 endospores are present in each mother cell. These endospores grow, causing the mother cells (sporangia) to rupture. The sporangia of *P. wickerhamii* (3 to 10 µm in diameter) are usually smaller than those of *P. zopfii* (7 to 30 µm in diameter).[625,647] Another distinguishing feature of *P. wickerhamii* is the morula form of its sporangia, in which endospores are arranged symmetrically in a daisy-like or "soccer ball" appearance; other species, including *P. zopfii*, do not form these multiple septated structures.[625,647]

The differential diagnosis might include *Coccidioides* and *Rhinosporidium.* Sporangia of *Coccidioides* are much larger than those of *Prototheca* species, whereas the individual endospores of *Coccidioides* are smaller. Rhinosporidium has one of the largest sporangia, measuring up to 5 mm.[625]

Protozoal Infections

Rhinosporidiosis

The causative organism of this infection, *Rhinosporidium seeberi*, which in terms of taxonomy is still on the fence between the Fungi and Protista kingdoms,[658,659] appears to be present in lake and lagoon stagnant waters, as well as in dust in the form of spores. Although still mysterious, the parasite's life cycle would include fish. Acquisition of the infection by humans appears to occur by contact.[659] Rhinosporidiosis is endemic in Sri Lanka and certain regions of India, but cases have been reported elsewhere in the world.[659] Most affected patients appear to be immunocompetent.

Clinical Features

The most common presentation of the disease is by involvement of the mucosal surfaces of the nose and oropharynx, although several other sites in the head and neck area, as well as elsewhere in the body, can be affected.[660] In addition, dissemination of the disease to internal organs can be rarely seen.[660,661] Lesions present as chronic, painless, large, and friable fungating tumors that often protrude outside the nostrils.

Cutaneous rhinosporidiosis can present in three patterns: (1) satellite lesions adjacent to nasal mucosal involvement; (2) generalized disseminated infection, usually from a head and neck focus; and (3) primary cutaneous involvement.[662] These skin lesions can show similar features to the ones from the oropharynx, presenting as polypoid and friable "strawberry-like" tumors,[663,664] whereas in

other cases they present as verrucous plaques, subcutaneous nodules, and even forunculoid lesions. Multiple lesions favor disseminated spread of the disease.[662,665,666]

Histopathology

Rhinosporiosis has a very distinct microscopic picture with marked epidermal hyperplasia and papillomatosis and a low-power "Swiss cheese" appearance due to the presence of the massive *R. seeberi* rounded sporangia (measuring up to 500 µm). These structures possess a thick wall and contain innumerable sporangiospores, which are much smaller (6 to 7 µm) and can occasionally leak into the surrounding tissue provoking a prominent inflammatory response with foreign body giant cells.[625,660]

The differential diagnosis includes coccidioidomycosis, which shows much smaller sporangia (60 µm) also containing smaller sporangiospores. Protothecosis can also be considered but will also show much smaller (3 to 10 µm) sporangia with the classic "soccer ball" appearance. Finally, myospherulosis, which is basically an encysted collection of red blood cells, can possibly mimic the parasite.[625]

Amebic Infections

Amebas are primitive, unicellular organisms that belong to the Protista kingdom. Human amebic infections are caused by either the pathogenic species, which are strictly parasitic, or the so-called free-living amebas, which are opportunistic agents and accidentally infect human beings. The majority of amebic infections in humans are caused by parasitic-pathogenic species, such as *Entamoeba histolytica.* The group of free-living amebas includes *Acanthamoeba* spp., *Naegleria fowleri*, and, more recently, *Balamuthia mandrillaris* and *Sappinia diploidea*.[667] We will discuss the three main species of amebas that can cause skin disease: *E. histolytica*, *Acanthamoeba* spp., and *B. mandrillaris.*

Cutaneous Amebiasis

Entamoeba histiolytica. *E. histolytica* has a fecal-oral life cycle that involves ingestion of cysts and the subsequent development of dysenteric intestinal disease. Then, the infection can spread elsewhere, usually to the liver or lungs. This type of amebic infection occurs mainly in the tropics and most frequently manifests with a dysenteric diarrhea and liver abscesses.[667] Cutaneous involvement by *E. histolytica* is a rare event and can be either primary or secondary. The most frequently found scenario is the secondary spread of the parasite from the gastrointestinal tract to the anus and then to the perianal, perineal, or genital skin.[668-670] Secondary involvement of the skin surrounding colostomies and abdominal wounds produced by drainage of hepatic abscesses has been reported.[669,671] Another mode of transmission is through sexual contact, resulting in penile lesions.[668]

Primary cutaneous amebiasis caused by *E. histolytica*, which occurs without gastrointestinal involvement, is extremely rare with very few cases reported in the literature.[668,672,673] It is thought to occur due to poor hygiene via direct inoculation of contaminated material by scratching or trauma.

Although the host develops humoral and cellular immune responses to the parasite, these organisms are capable of evading the immune system through several mechanisms.[668] These include expression of *N*-acetyl-D-galactosamine (Gal/GalNCc), a specific lectin that has a similar sequence and thus an antigenic cross-reactivity with CD59. In humans, CD59 prevents assembly of the complement C5b-C9 membrane attack complex.[674] In addition, the parasite produces serine proteases capable of cleaving IgA and IgG molecules.[667]

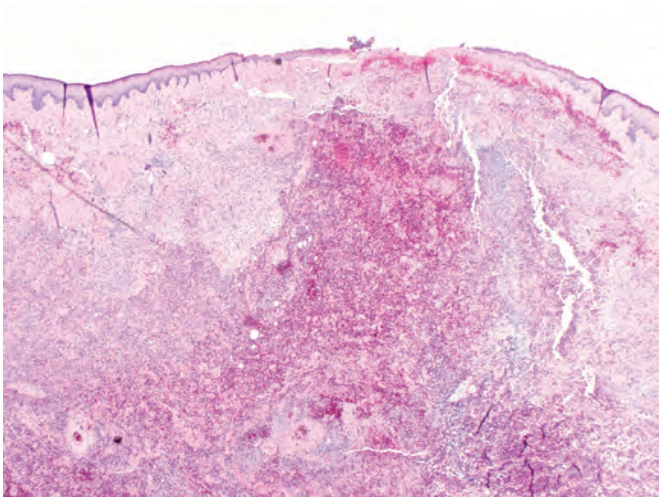
Clinical Features. Cutaneous lesions of *E. histolytica* infection appear as painful, foul-smelling ulcers covered by gray slough. There are often rolled or undermined borders, as well as superinfection by common bacteria.[668,673] In genital and perineal areas, large exophytic ulcers can develop, sometimes mimicking carcinoma.[670,675]

Histopathology. The main features are usually the presence of extensive ulceration with extensive necrosis and exudate being flanked by prominent pseudoepitheliomatous epidermal hyperplasia.[668,672,676] Both the invasiveness of the organism and the massive necrosis produced by it relate to active secretion of several lytic enzymes, as well as the capability of phagocytizing and lysing immune cells.[668] *E. histolytica* trophozoites usually measure 12 to 20 µm, are strongly PAS positive, and can be found in the exudate singly or in clusters. They differ from macrophages by the presence of or a fine rim of eccentrically placed nuclear chromatin with a small but prominent karyosome.[668,672,676] Another feature, present in all cases, is the presence of phagocytized red blood cells within the cytoplasm of *E. histolytic* trophozoites. This feature is useful to differentiate *E. histolytica* trophozoites from macrophages and from other species of amebas, particularly from the innocuous and *Entamoeba dispar*, which is otherwise almost identical to *E. histolytica*.[668] The trophozoites of *Acanthamoeba* spp. will not also exhibit the characteristic marginally disposed nuclear chromatin and the show a much larger nucleolus. One could perform a CD59 immunohistochemical stain, which can help to distinguish between *E. histolytica* trophozoites that will be positive with this marker (see previous discussion), versus *Acanthamoeba* spp. trophozoites that will be completely negative.[677]

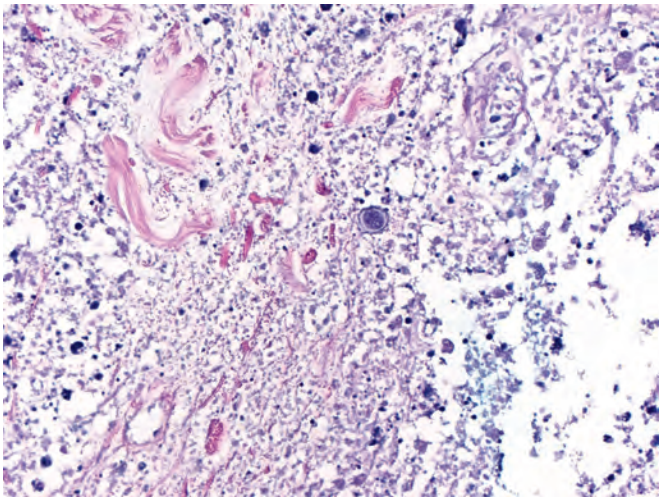
The main differential diagnosis, both clinically and microscopically (particularly at low power), includes verrucous SCC, especially with lesions from the genital areas.[668,670] The microscopic picture can also be suggestive of a deep fungal infection, but this is promptly ruled out with visualization of the trophozoites.[668]

***Acanthamoeba* spp.** Part of the free-living ameba group, the organisms belonging to the *Acanthamoeba* genus are ubiquitous. They can be isolated from soil, dust, air, natural and treated water, seawater, air-conditioning units, and several other environments, and they have a worldwide distribution.[675] These organisms can also be found in nasal and pharyngeal mucosa of healthy individuals.[678] These organisms affect mainly immunocompromised individuals, but they can also cause infections in immunocompetent persons. The most frequent human infection with *Acanthamoeba* spp. is the amebic keratitis that is related to contact lens use.[679] A chronic, protracted, slowly progressive CNS infection, granulomatous amebic encephalitis, has also been described.[675] The rare event of cutaneous involvement by *Acanthamoeba* spp. is found to occur in immunosuppressed individuals, such as HIV/AIDS and transplant patients, as well as those using immunosuppressive drugs, and may or may not be associated with CNS involvement.[675,680-682]

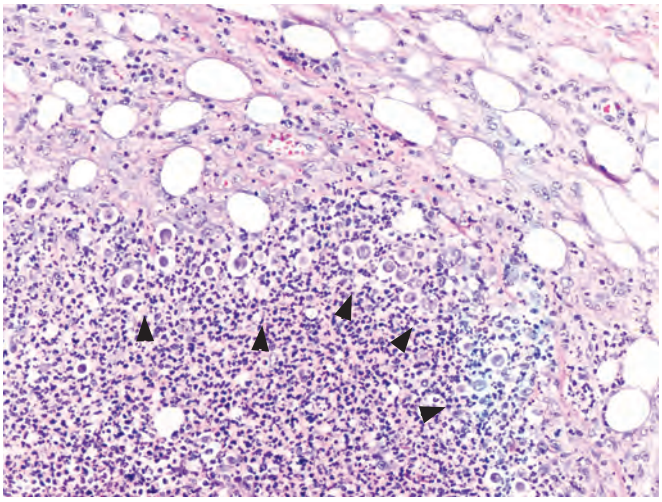
Clinical Features. Clinically, cutaneous lesions are described as erythematous, violaceous nodules or indurated papules that become suppurative and eventually form nonhealing ulcers. The lesions appear usually on the face, trunk, and extremities and can occur in crops.[675,680-683] Whether these cutaneous lesions represent a primary focus or the result of hematogenous spread from infectious foci in the respiratory tract, the maxillofacial sinuses, or the CNS is not known.



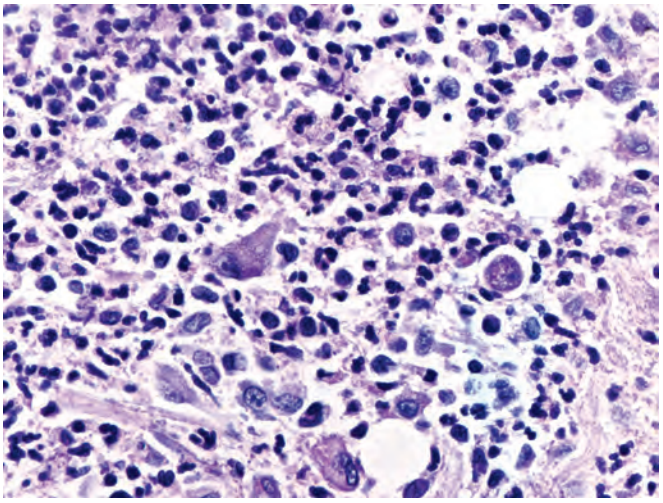
• **Figure 20.99** Cutaneous acanthamebiasis. Lesions tend to be deep and very destructive. This extensive, purulent, and ulcerated, predominantly subcutaneous lesion was present in an individual undergoing posttransplantation immunosuppressive therapy.



• **Figure 20.101** Cutaneous acanthamebiasis. Under adverse conditions, such as the predominantly necrotic area of this lesion, the organisms change to a cystic form that exhibits a prominent thick capsule, which is periodic acid–Schiff positive.



• **Figure 20.100** Cutaneous acanthamebiasis. At the edges of the lesion, an "advancing front" of macrophage-like organisms is identified *(arrowheads)*.



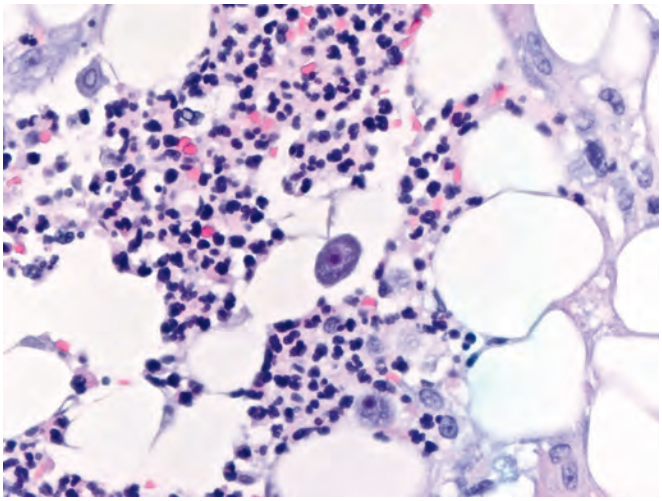
• **Figure 20.102** Cutaneous acanthamebiasis (periodic acid–Schiff [PAS] stain). The PAS stain weakly decorates the *Acanthamoeba* sp. trophozoites, in contrast to *Entamoeba histolytica* trophozoites, which are strongly PAS positive.

Histopathology. The histologic sections often will show granulomatous reaction with a mixed inflammatory infiltrate (Fig. 20.99). In patients with HIV/AIDS, granulomas are not always present. Abscess formation,[681] panniculitis,[684] and leukocytoclastic vasculitis[685] can also be observed. Both trophozoites and cysts can be observed with the trophozoites disposed in an "advancing front" type of pattern right at the interface between the abscess area and the uninvolved tissue (Fig. 20.100). On the other hand, cyst forms are often found in areas where conditions are more adverse, such as in the center of the abscess (Fig. 20.101). Both structures are PAS and GMS positive (Fig. 20.102).

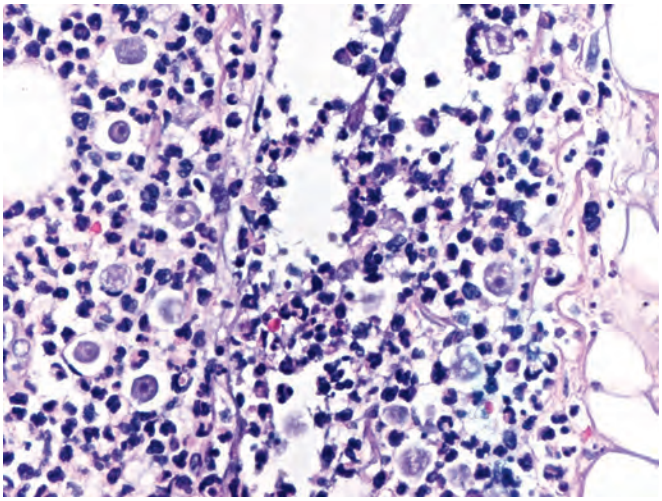
Trophozoites of *Acanthamoeba* spp. are pleomorphic and measure approximately 15 to 45 µm and have a large nucleus with a large, centrally located karyosome but no peripheral chromatin rimming (Fig. 20.103 and 20.104). *E. histolytica* trophozoites stain well with PAS, but their cysts tend to be PAS negative.[683] In addition, *E. histolytica* trophozoites usually contain red blood cells within their cytoplasm.[668] Nevertheless, *Acanthamoeba* spp. trophozoites will occasionally show intracytoplasmic red blood cells. As mentioned previously, *Acanthamoeba* trophozoites are negative on CD59 staining (Fig. 20.105), whereas both *N. fowleri*[686] and *E. histolytica*[677] express a CD59-like molecule as part of their escape from the immune system.

The histologic lesions can mimic fungal and viral infections or a foreign-body granulomatous response.[675] Organisms in tissue sections have been confused for yeast forms of *B. dermatitidis*, sporangia of *R. seeberi*, *C. neoformans*, or *Protheca wickerhamii*. Cases of acanthamebiasis have been mistaken for cat-scratch fever, KS, or even cells with CMV inclusions.[675,683]

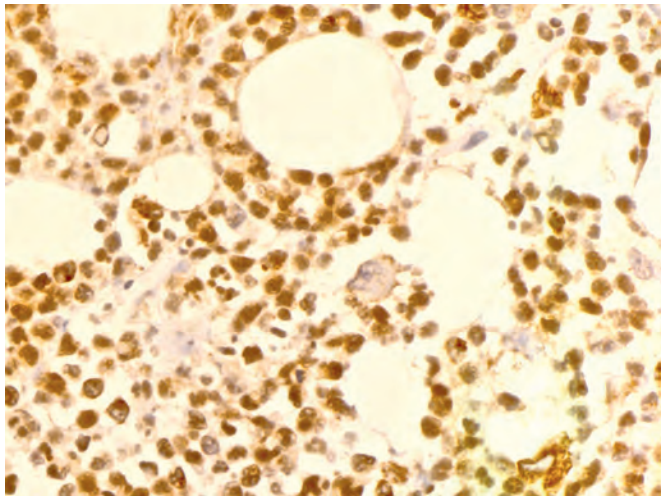
Similarly to *E. histolytica*, *Acanthamoeba* trophozoites are known to secrete several lytic enzymes, such as proteinases, elastases, and phospholipases. Some of these proteases, the serine-proteases, are capable of degrading human IgA and IgG antibodies.[675,683] Inhibition of the classic pathway of complement,



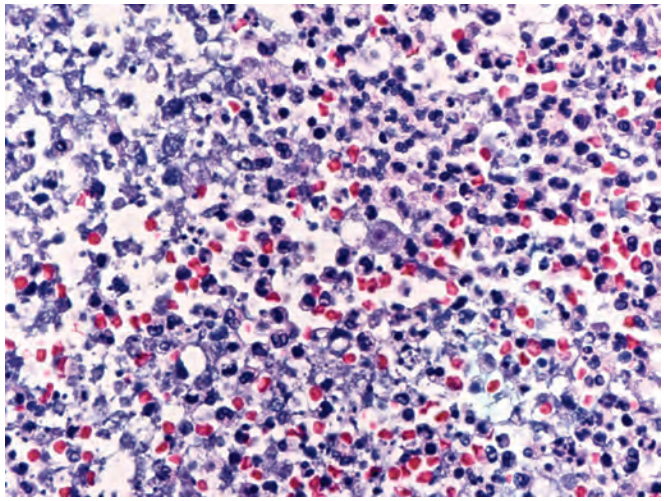
• **Figure 20.103** Cutaneous acanthamebiasis. High-power view of *Acanthamoeba* sp. with its prominent nucleus and nucleoli.



• **Figure 20.104** Cutaneous acanthamebiasis. Numerous trophozoites are surrounded by a dense inflammatory response. Some of the parasites appear to have ingested red blood cells, which is most often seen with *E. histolytica* but can be occasionally seen with *Acanthamoeba* sp. organisms.



• **Figure 20.105** Cutaneous acanthamebiasis (CD59 immunohistochemical stain). The negativity of the trophozoites with this marker (CD59, a complement inhibitor) is contrasted by the marked positivity of the inflammatory cells being ingested by the parasite and the ones in the background.



• **Figure 20.106** Cutaneous acanthamebiasis. A trophozoite form actively ingesting inflammatory cells through pseudopods.

by blockade of C1 and by active binding of C1q, is also part of these parasites' immune-evading repertoire.[687] Active phagocytosis and destruction of leukocytes is another mechanism by which these organisms combat the immune response (Fig. 20.106; see also Fig. 20.105).

Also noteworthy is the probable role of *Acanthamoeba* spp. as reservoirs and even vectors of several bacterial species, such as *Legionella pneumophila*,[688] *M. ulcerans*,[156] and *Chlamydia pneumoniae*,[689] among others.[675,690] Interestingly, these bacteria that can survive inside *Acanthamoeba* organisms are also mainly human intracellular pathogens that infect macrophages.

Infections Caused by Flagellates

The Trypanosomiases

The trypanosome group is composed of several species of protozoan flagellates that belong to the Kinetoplastida class. They infect most species of vertebrates. The species that are known to affect humans are *Trypanosoma brucei* and subspecies in sub-Saharan Africa and *Trypanosoma cruzi* in the Americas.[691] Cases of human infection with *Trypanosoma evansi*[692] and *Trypanosoma lewisi*,[693] which affect mainly large ungulates and rodents, respectively, have been reported in the Indian subcontinent.

African Trypanosomiasis. African trypanosomiasis is caused by the *T. brucei* and two of its subspecies, *Trypanosoma brucei gambiense*, which affects western and central Africa, and *Trypanosoma brucei rhodesiense*, which is prevalent in eastern and southern Africa.[691] They are transmitted by flies from the *Glossina* genus (tsetse or tik-tik flies), blood transfusions,[694] contaminated needles,[695] or congenital infection.[696] Their known reservoirs or natural hosts comprise a large range of ungulate species, including cattle and camels. Humans also can act as reservoirs.[691]

Clinical Features. The disease is divided into two stages, hemolymphatic and meningoencephalitic. Cutaneous involvement is

present mostly in the first stage at the site of inoculation, forming an inflammatory nodule or ulcer known as the "trypanosomal chancre."[691,697] This lesion produces a 2- to 5-cm, erythematous, indurated, painful plaque, which appears within approximately 2 to 4 weeks of inoculation and heals after 3 to 4 weeks. It usually heals leaving an overlying desquamation, sometimes with altered pigmentation. The trypanosomal chancre develops in approximately 50% of the cases with *T. brucei rhodesiense* but rarely in the ones with *T. brucei* gambiense.[698-700] Another dermatologic manifestation is a pruritic circinate maculopapular eruption that arises usually on the trunk several weeks after inoculation and is known as "trypanids."[698,699]

Histopathology. Microscopic examination of the trypanosomal chancre reveals a superficial and deep, perivascular lymphoplasmacellular infiltrate, somewhat resembling secondary syphilis.[699] Organisms are usually very difficult to identify in routine stained tissue sections. Trypomastigotes can be identified in Giemsa-stained smears of the secretions found in the chancre. The organisms in the trypomastigote stage have the classic trypanosomal morphology, measure 1.5 to 3.5 μm in width, and contain the characteristic kinetoplast with a central nucleus.[691,698,699]

Microscopically the trypanid lesions exhibit mild spongiosis with exocytosis of lymphocytes, along with a superficial perivascular lymphoid infiltrate and a mild diffuse neutrophilic infiltrate with leukocytoclasis.[699]

The cutaneous manifestations of African trypanosomiasis could be confused with bacterial pyoderma caused by secondary infection of a minor wound or an insect bite.[700] In the rare event that intracellular parasites are identified as amastigotes in tissue sections, they can be differentiated from other intracellular parasites, such as *H. capsulatum* and *T. gondii*, by the presence of the kinetoplast, which is found in both *Leishmania* and trypanosome amastigote forms.

American Trypanosomiasis. *T. cruzi*, the agent of Chagas disease, is transmitted by infected insects of the Triatominae subfamily (kissing bugs). The reservoirs are usually mammals, such as armadillos or opossums. The disease is prevalent in 18 countries in North, Central, and South America. It is a disease strongly associated with low socioeconomic conditions.[691,701]

The trypomastigote forms of the parasite are highly concentrated in the vector's feces, which are deposited while the vector feeds and enter the host through microabrasions caused by scratching.[691] Similarly to the African version, this disease can be transmitted also through blood transfusions,[702] by contaminated needles,[703] and via vertical placental infection.[704] Rare forms of transmission include contaminated food,[705] solid organ transplant[706] and even breastfeeding.[707]

Clinical Features. The disease has an acute and a chronic stage. The acute stage begins 6 to 10 days after the inoculation and lasts for 1 to 2 months. It is in this stage that most of the cutaneous manifestations occur. An edematous lesion, known as a chagoma, appears at the site of inoculation. If this site is the conjunctiva, unialpebral or bipalpebral swelling is noticed (the Romaña sign).[691] The chronic phase of the disease appears usually 10 to 15 years after inoculation; visceral effects characterize it, such as dilated cardiomyopathy and megaviscera.[691] If an infected individual becomes immunocompromised (e.g., AIDS, transplantation), the disease can reactivate. This reactivation results in painful erythematous papules and nodules, as well as plaques, named reactivation chagomas, that when disseminated are known as metastatic chagomas.[708-710]

Histopathology. Reactivated disease, seen in immunocompromised patients, can exhibit cutaneous lesions that usually show an infiltrate of macrophages in the deep dermis and subcutaneous fat. These macrophages contain intracellular amastigote forms. In addition, *T. cruzi* amastigotes can be found intracellularly in stratum corneum keratinocytes, in endothelial cells from surrounding blood vessel walls and arrector pili muscle myocytes.[708-710]

In tissue sections, the amastigote forms are best visualized with Giemsa stain and can be distinguished from other intracellular protozoa by the presence of the kinetoplast. They are PAS negative, which helps to differentiate them from the PAS-positive intracellular fungus, *H. capsulatum.*

Leishmaniasis

Leishmaniasis is a condition caused by protozoa with an ample geographic distribution, being endemic in several countries of southern Europe, North Africa, the Middle East, Central and South America, and the Indian subcontinent. It is estimated that 1.5 to 2 million children and adults develop symptomatic disease annually; 1 to 1.5 million have cutaneous effects, and in 0.5 million the viscera are involved.[711] Both the reported cases and affected geographic areas have increased, in concert with increasing rates of deforestation and global climate change.[712,713] As with most parasitic conditions, leishmaniasis is a disease of the poor, undeveloped, and neglected areas of the world, with the probable exception of southern Europe.[714]

The parasite can be transmitted by almost 70 known species of sandflies. The species most commonly involved are from the *Lutzomyia* genus, found mainly in the Americas, and the *Phlebotomus* genus, identified in the remainder endemic regions.[715] The disease is also spread via blood transfusions,[716] vertically to the fetus through the placenta,[717] via the use of contaminated needles,[718] and via organ transplants.[715] There are a multitude of known reservoirs, including canids, such as dogs and foxes, rodents, and large mammals.[715]

Depending upon what clinical manifestations predominated, the disease is divided into three types: cutaneous, mucocutaneous, and visceral. However, significant overlap among these forms can be seen, sometimes forming a clinical spectrum. The clinical presentation results from the balance between a variety of factors, such as host cell-mediated immunity, delayed-type hypersensitivity idiosyncrasy, and the parasite burden.[715]

Cutaneous Leishmaniasis. Cutaneous leishmaniasis is caused by multiple *Leishmania* species and subspecies from different parts of the globe. There are two great subgenera *Leishmania (L.)* and *Viannia (V.)* that correspond to Old and New World variants, respectively. The Old World agents include *Leishmania major*, *Leishmania tropica*, and *Leishmania (L.) aethiopica*, as well as *Leishmania infantum* and *Leishmania chagasi* in the Mediterranean and Caspian Sea regions.[715] Lesions resulting from these agents received such names as "oriental sore" and "Jericho buttons." New World organisms, such as *Leishmania mexicana*, *Leishmania amazonensis*, *Leishmania (V.) braziliensis*, *Leishmania (V.) panamensis*, *Leishmania (V.) peruviana*, and *Leishmania (V.) guyanensis*, are prevalent in South and Central America and parts of North America.[715] Disease caused by *L. mexicana* gets called "chiclero's ulcer," referring to the gum workers who usually perform their job in forested areas. Importantly, New World organisms can occur concomitantly in one patient.[719]

Clinical Features. There are four recognized forms: acute, chronic, recidivous, and disseminated.[715] The lesion in the acute form usually begins at the site of the sandfly bite and evolves into

a nodule that ulcerates after 1 to 3 months.[720,721] Affected individuals usually exhibit several nonhealing skin lesions on the exposed skin of the limbs. There are some differences according to the causative parasite. The Old World disease usually manifests as papules and nodules, as well as flat plaques, hyperkeratotic, and wart-like lesions that can occasionally show ulceration.[719,721] Some lesion patterns tend to be species dependent: *L. tropica* often cause fewer than three ulcers on the head and neck area that resolve in approximately 2 years, whereas *L. major* infection usually develops more than three ulcers that are self-limited and heal within 1 year.[719]

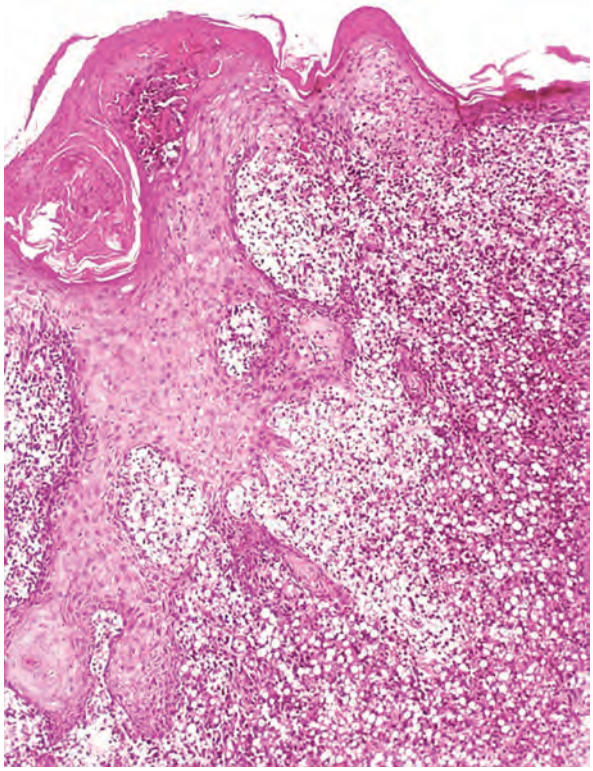
On the other hand, ulcerative lesions are more characteristic in New World disease.[719,721] The medallion-shaped ulcers show rolled borders and an ulcer bed with prominent granulation tissue and can be quite persistent (Fig. 20.107).[719] *L. braziliensis* tend to produce severe disease. Unusual presentations, such as paronychial, palmoplantar, chancriform, annular, zosteriform, sporotrichoid and erysipeloid forms, have been described.[719] These lesions can closely simulate numerous skin conditions, such as pyoderma gangrenosum and fungal and mycobacterial infections, to name a few.[722]

The recidivous type, part of the chronic manifestations, is also known as "lupoid"[723] or "recidivans."[724] These lesions are represented by single or multiple, raised, nonulcerated verrucous plaques that persist for 1 to 2 years. These long-standing chronic lesions can easily mimic neoplastic processes, such as nonmelanoma skin cancers.[722] Erythematous, often circinated papules can develop in the proximity to scars of healed lesions. This type is mostly seen in Old World disease *(L. tropica)* but has also been reported in Latin America (*L. [V.] panamensis*).[725]

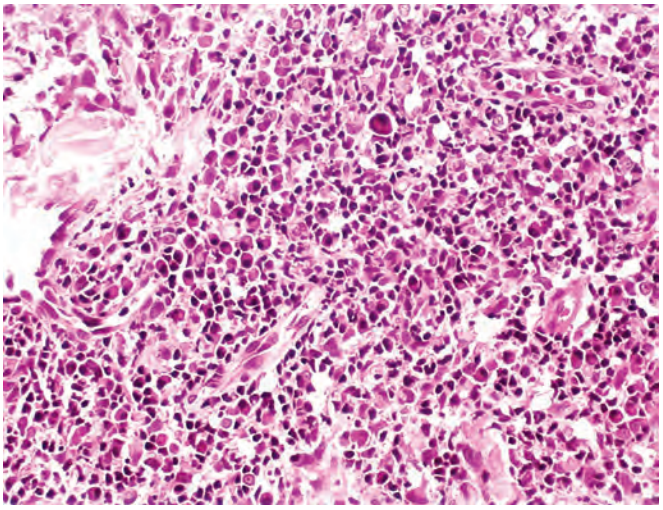
The disseminated form can be found in both groups of agents, but it is usually related to Old World parasites. It develops in anergic individuals and consists of macules and nodules without ulceration that have a widespread and diffuse distribution.[725,726]

Histopathology. A skin biopsy from the ulcer border has more possibilities to be diagnostic. Acute lesions show hyperkeratosis and acanthosis or rarely atrophy. Ulceration and intraepidermal abscesses are often present.[722,727] As lesions become chronic, a nonspecific pseudoepitheliomatous epidermal hyperplasia type of pattern can be found (Fig. 20.108). There is usually a prominent dermal inflammatory infiltrate composed of $CD4^+$ and $CD8^+$ lymphocytes, plasma cells, and eosinophils, as well as of parasitized macrophages, epithelioid cells, and giant cells (Fig. 20.109).[728] Although identification of intracellular amastigotes (the so-called Leishman-Donovan bodies) within affected macrophages and/or giant cells is of crucial importance, subspeciation is not possible without the help of molecular methods.[722] The amastigote forms are represented as uncapsulated 2- to 4-μm, round to oval, basophilic structures (Fig. 20.110) that are usually located at the periphery of the intracytoplasmic vacuole that contains them (marquee sign) (Fig. 20.111). Sometimes it is difficult to distinguish them from karyorrhectic debris (Fig. 20.112). The salient feature that can help in the differential diagnosis is the presence of the kinetoplast, which can be visible only under great magnification and/or with the help of transmission electron microscopy. The morphologic features of these parasites can be seen in both

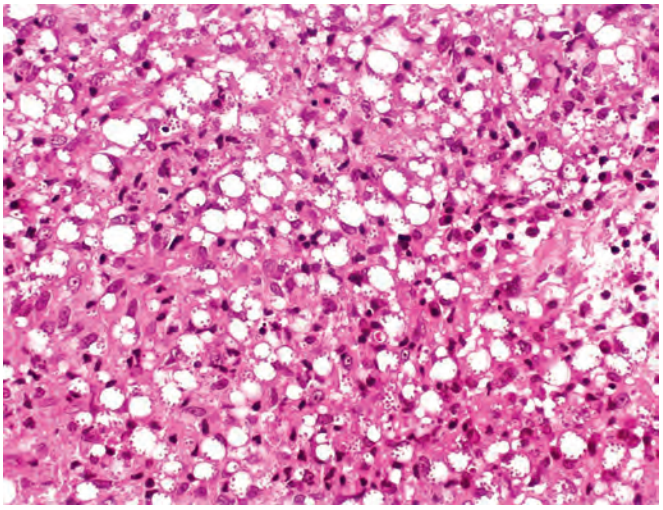
• **Figure 20.107** Cutaneous leishmaniasis. New World cutaneous leishmaniasis caused by *Leishmania (V) panamensis*; note the well-demarcated nature of the ulcers and the sporotrichoid distribution. This patient had been to forested areas of Costa Rica before development of these lesions. (Courtesy Nishit Patel, MD and Jennifer Divine, MD, Department of Dermatology, University of South Florida, Tampa.)



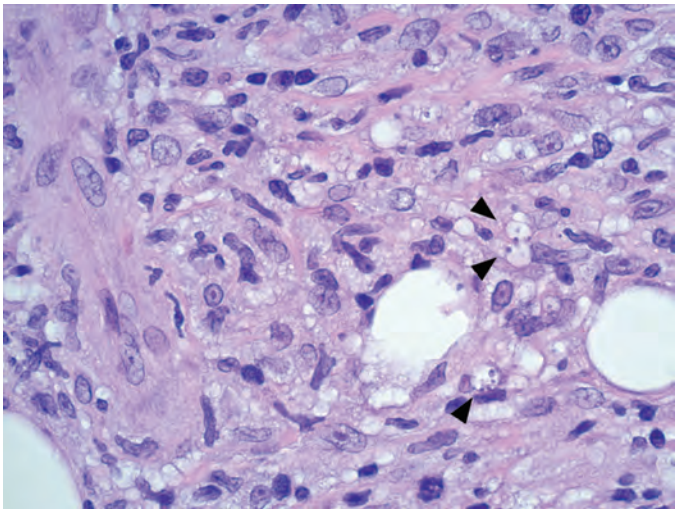
• **Figure 20.108** Cutaneous leishmaniasis. Pseudoepitheliomatous epidermal hyperplasia with a diffuse granulomatous inflammatory response. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



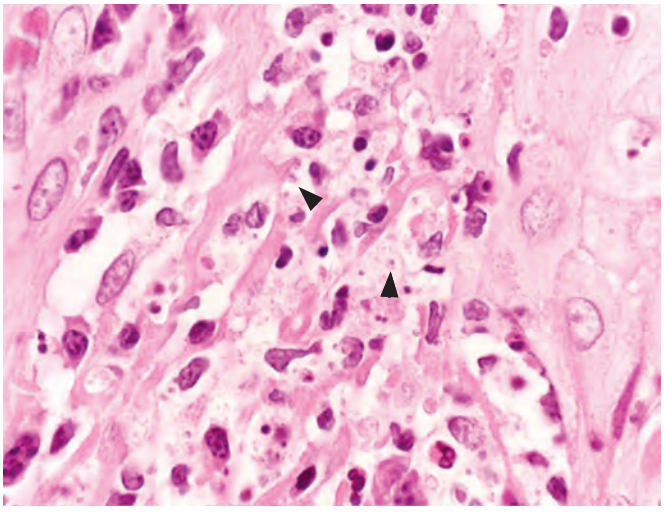
• **Figure 20.109** Cutaneous leishmaniasis. An exuberant inflammatory infiltrate with prominent plasma cells is found associated with the granulomatous response to the parasites. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.111** Cutaneous leishmaniasis. Very illustrative example of the classic "marquee" sign, which is the placement of the parasites at the periphery of the infected cells as the lightbulbs in a marquee sign. This feature is useful in distinguishing *Leishmania* spp. from other intracellular organisms. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)



• **Figure 20.110** Cutaneous leishmaniasis. Amastigote forms can be identified within giant cells and macrophages and sometimes in the interstitial spaces; note the darker area of the parasite representing the nucleus *(arrowheads)*. This biopsy was taken from the lesions depicted in Fig. 20.107.



• **Figure 20.112** Cutaneous leishmaniasis. Sometimes amastigotes are difficult to find among a background of inflammatory debris *(arrowhead)*. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

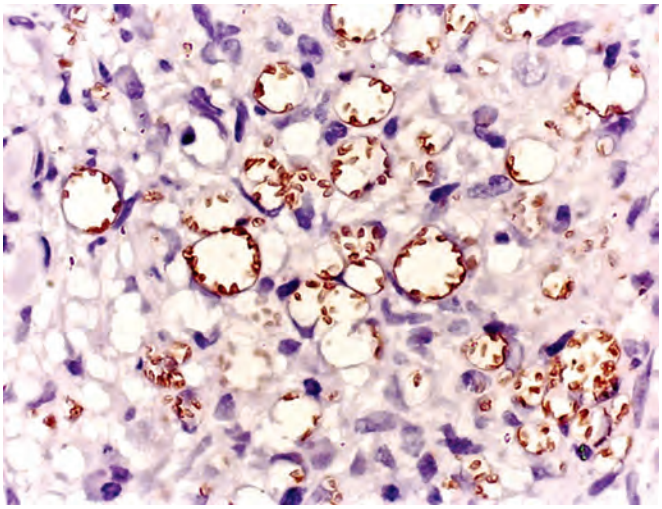
routine H&E, as well as in Giemsa-stained sections. Immunohistochemical stains using monoclonal antibodies directed to antigens present in several *Leishmania* species are of great utility in difficult cases (Fig. 20.113).

Chronic lesions will show dermal tuberculoid, noncaseating granulomas containing only rare parasitized macrophages.[722,729] Focal central caseous-type necrosis can be seen.[730] The recidivous and lupoid types have a histologic picture that resembles lupus vulgaris, with sarcoidal granulomas surrounded by histiocytes, lymphocytes, and an occasional giant cell; parasites are hard to find.[723,724]

Disseminated cutaneous leishmaniasis, commonly found in anergic and immunocompromised individuals, is composed almost exclusively of parasitized macrophages with only occasional lymphocytes and sometimes eosinophils.[722,731,732]

Mucocutaneous Leishmaniasis. Mucocutaneous leishmaniasis is seen mainly in South and Central America (caused by *L. braziliensis*, *L. panamensis*, and *L. guyanensis*)[733] but has also been reported in patients with *L. infantum*,[734] *L. tropica*,[735] and *L. major*[736] infection. Mucosal dissemination has reportedly developed in 1% to 10% of the cases of cutaneous disease caused by New World agents, most commonly *L. braziliensis*. It can occur either 1 to 5 years after the cutaneous lesions have healed or concomitantly with active skin lesions.[733,737]

Clinical Features. Mucosal lesions usually begin with erythema and ulceration of the nares, which can evolve to highly destructive lesions that can perforate the nasal septum and can involve the nasopharynx and tongue, causing significant deformities.[719,738] This destructing and mutilating lesion is known as espundia and can develop up to 25 years after the primary clinical lesion.[719]



• **Figure 20.113** Cutaneous leishmaniasis (immunohistochemical stain). Parasites nicely disposed in the "marquee" sign pattern and being highlighted by a monoclonal immunohistochemical stain. (Courtesy Mirian Sotto, MD, PhD, Department of Dermatology, Hospital das Clinicas, University of Sao Paulo.)

Histopathology. Four stages of evolution are usually described for these lesions: edematous, granulomatous, proliferative, and granulomatous necrotizing.[722] Microscopic examination of mucosal lesions usually reveals extensive ulceration flanked by pseudoepitheliomatous epidermal hyperplasia. There is a nonspecific chronic inflammation with only a few parasite-laden macrophages.[739] Tuberculoid and suppurative granulomas are often seen. The presence of prominent necrosis with a reactive response is seen as a favorable prognostic feature.[740]

Visceral Leishmaniasis. The visceral variant of leishmaniasis is mainly caused by *L. donovani* in the Indian subcontinent, Asia, and Africa and affects both adults and children. *L. infantum* and *L. chagasi* affect predominantly young children in the Mediterranean region, southwest and central Asia, and South America.[715] The disease tends to affect solid organs, such as the liver and spleen, as well as the bone marrow, and the clinical course of the infection can either be subclinical or oligosymptomatic or can cause a full-blown disease (kala-azar).

Clinical Features. The clinical features are fever, weakness, night sweats, anorexia, and weight loss.[715] Darkening of the skin (kala-azar means "black fever" in Hindi) is rather infrequent. Further progression leads to hepatosplenomegaly and serious anemia, neutropenia, and thrombocytopenia that can be lethal.[715,741] Cutaneous involvement is seen in more than 5% of cases, usually 1 to 5 years after the primary infection, in the form of post–kala-azar dermal leishmaniasis (PKDL). PKDL is seen more frequently in the Indian subcontinent,[742] and it consists of erythematous lesions and nodules, usually on the face, as well as hypopigmented and hyperpigmented macules on the trunk.[719,742] The affected skin has normal sensation, which helps to distinguish the lesions from those of leprosy. Cutaneous involvement in visceral leishmaniasis, with florid manifestations including KS-like nodules,[743] is also seen in HIV/AIDS patients.[744]

Histopathology. Lesions in PKDL consist of an atrophic epidermis overlying a prominent inflammatory infiltrate in the upper dermis that is composed mainly of macrophages, epithelioid cells, lymphocytes, and plasma cells.[745] Tuberculoid granulomas can be seen. In some cases the infiltrate occupies the entire dermis in a nodular fashion.[745] In patients with neuropathy, perineural inflammation can be identified.[746] Lesions of the face can show follicular plugging. Organisms are often readily found, but their quantity varies. To better appreciate the parasite morphology, Weigert iron–stained sections are superior to Giemsa-stained sections.[745]

Helminth Infestations

This group contains a number of phyla and subclasses of organisms, including the Platyhelminthes phylum (flatworms), composed by the trematodes (flukes) and the cestodes (tapeworms), and Nematoda phylum, also known as nemathelminthes (roundworms).

Trematodes

Schistosomiasis

Parasitic trematode worms that have as definitive hosts several species of vertebrates, including humans, cause this infection. More than 200 million people in 74 countries have the disease, and 20 million of them have severe illness.[747] The life cycle of this organism is complex and involves several species of freshwater snails as intermediate hosts.[747] Four major species participate in human infections: *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, and *Schistosoma mekongi.* The first two are prevalent in sub-Saharan Africa, and *S. mansoni* is also endemic in certain regions of Brazil, Venezuela, and the Caribbean. *S. japonicum* can be found in China, Indonesia, and the Philippines. *S. mekongi*, a species related to *S. japonicum*, is found in Cambodia and Laos.[747,748] Cercarial forms of the bird schistosome *Trichobilharzia regenti* cause the more innocuous "swimmer's itch" eruption.[749]

Clinical Features. Several types of skin lesions have been described in schistosomiasis. The parasites penetrate the skin as cercariae forms. At the site of penetration, there is usually a pruritic erythematous and urticarial rash.[750,751] Similar lesions characterize the swimmer's itch, eruption, although in this condition the cutaneous manifestations tend to be relatively less severe and there is no systemic disease because the cercarial organisms die in the skin.[748,749]

As the disease progresses, urticarial lesions can also be seen in association with the dissemination of the cercariae and with the laying of eggs by adult flukes.[750-752] Deposition of ova in the dermis can give rise to papular, granulomatous, or even warty genital lesions[753,754] and several extragenital cutaneous manifestations.[750,752,755] The development of SCC from genital verrucous lesions has been reported[754] in a similar way to what is seen in the urinary bladder. Some patterns are associated with particular species, with infections with *S. japonicum* usually being associated with urticarial lesions[756] and *S. haematobium* and *S. japonicum* infections usually being complicated by extragenital cutaneous lesions,[752] but these are rarely are seen with *S. mansoni* infection.[757]

Histopathology. The lesions related to the penetration of cercarial forms are associated with exocytosis of eosinophils and neutrophils, sometimes with microabscess formation. The dermal reaction is usually mild and consists of edema, vascular dilatation, mild perivascular inflammatory infiltrate, and rare interstitial eosinophils. The larvae usually are not seen.[751]

In genital and perineal lesions, ova are easily identified in the dermis and are accompanied by a prominent granulomatous

reaction or eosinophil microabscesses.[751,753,754] There is usually an overlying hyperkeratotic and acanthotic epidermis, sometimes with prominent pseudoepitheliomatous hyperplasia with focal ulceration and draining sinuses. Adult parasites may be identified in cutaneous vessels. Acute extragenital lesions exhibit necrobiosis and palisading granulomas around numerous ova. These features are often accompanied by an infiltrate composed of eosinophils, neutrophils, and foreign body giant cells.[758] Chronic lesions show degenerating and calcified ova with plasma cells and variable fibrosis.[755,759]

The ova share an oval-shaped appearance but have specific species-related morphologic features. *S. haematobium* ova have a characteristic apical spine, whereas *S. mansoni* ova have a lateral spine, and those of *S. japonicum* have no spine.[748]

Cestodes

Cysticercosis

Humans are accidental hosts for *Taenia solium*, a pork tapeworm. The larvae lodge in the subcutaneous tissue and form 1- to 3-cm nodules.[760,761] There is a certain predilection for the chest wall, arms, and thighs,[762] and lesions can be multiple and grow rapidly, which can mimic metastatic malignancy.[763]

The nodules are composed of cystic structures containing a larva attached to one edge of a membrane and a fibrous host response surrounds the cyst.[761,764] Visualization of the scolex of the cysticercus larva is helpful to make the diagnosis. The host response around the cyst is composed of fibrosis, chronic inflammatory infiltrate with eosinophils and sometimes giant cells.[761,764]

Sparganosis

Sparganosis is an infection caused by a pseudophyllidean tapeworm larva of the genus *Spirometra*.[765] The infection is more frequent in the Orient and is sporadically reported from Australia, British Guiana, Africa, Madagascar, and South America.[765,766] Rare cases have been reported in the United States[767] and Europe,[768] mainly among travelers. The parasite has a complex life cycle with canids and felids as definitive hosts and humans are usually involved as second intermediate hosts. Transmission to humans usually occurs through ingestion of water contaminated with infected freshwater copepods of the *Cyclops* genus or consumption of contaminated meat.[765,766]

Clinical Features. A slow-growing, subcutaneous mass that is sometimes migratory is the most frequent presentation.[765,766] When the parasite migrates, there is often pain and pruritus. The infection mainly affects the subcutaneous tissue, but it can spread to internal organs, including brain, lungs, and bladder.[766] Examination and bisection of the subcutaneous nodules reveal a cyst containing a flat, threadlike worm, usually measuring 0.1 cm in thickness and several millimeters to 50 cm in length.[765,766,769]

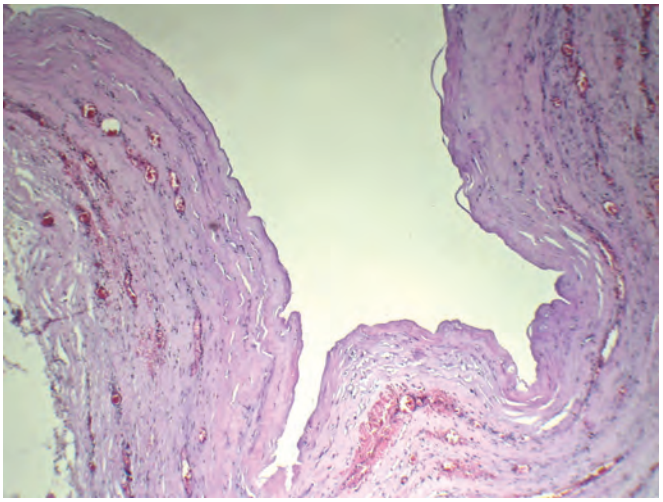
Histopathology. Microscopically the wall of the cyst is composed of granulation tissue and fibroadipose tissue with a variable number of inflammatory cells, including eosinophils, lymphocytes, histiocytes, plasma cells, neutrophils, and foreign body giant cells.[766,769] Portions of the larva are usually seen within the cavity.[767] The parasite structure is composed of a brush border of microvilli, eosinophilic tegument, two layers of smooth muscle, and a row of tegumental columnar cells. The smooth muscle bundles are often disposed in a "checkerboard" fashion.[765,766] The internal structure of the parasite is also composed of a loose matrix and basophilic calcareous bodies.[770] The microscopic differential diagnosis includes trichinosis, cysticercosis, and visceral larva migrans.[766]

Echinococcosis

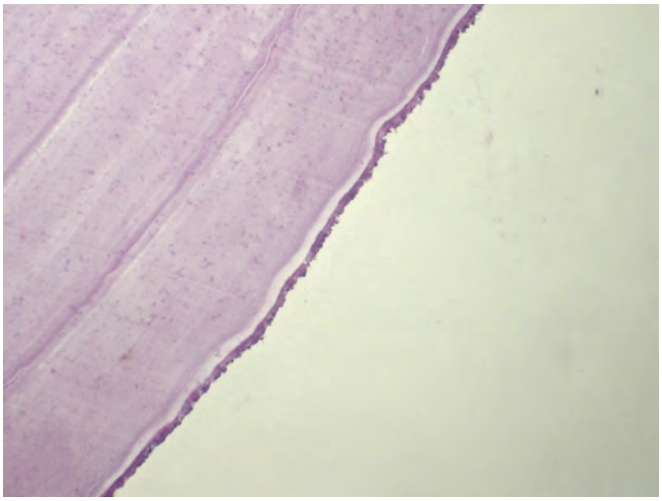
Echinococcosis is a zoonotic infection caused by tapeworms of the Taeniidae family that still represents a public health problem in New Zealand and Australia, as well as countries of the Mediterranean, the Middle East, southern Africa, and South America.[771] The life cycle of the parasites more frequently involves sheep and canids, and there are four species involved in human infection. The most important are *Echinococcus granulosus*, which causes cystic echinococcosis or hydatid disease, and *Echinococcus multilocularis*, which causes alveolar echinococcosis.[771,772] Two other species, *Echinococcus vogeli* and *Echinococcus oligarthus*, rarely affect humans but are responsible for polycystic echinococcosis in Central and South America.[771,773]

Clinical Features. Cutaneous involvement in echinococcosis is a rare event[771,772] that can be a result of contiguous direct extension from an infected muscle[774,775] or from the liver through the falciform ligament.[772] However, the far more frequently seen spread to the skin is via cutaneous fistulization of a ruptured infected organ (most commonly liver and lungs).[776-778] Clinically, cutaneous echinococcosis manifests as painless soft tissue tumors, usually diagnosed as lipomas.[775]

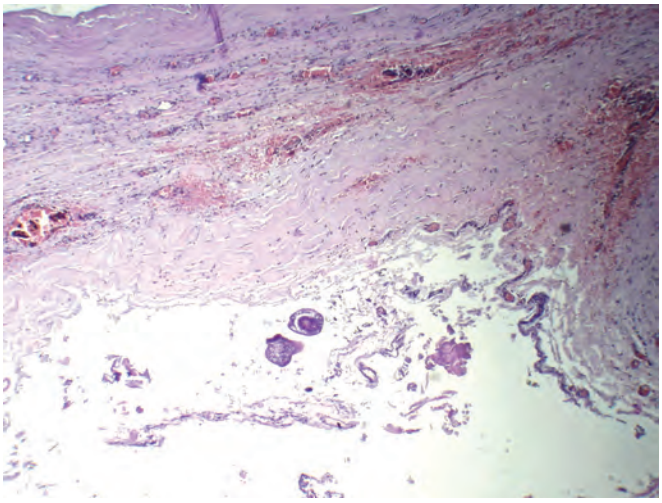
Histopathology. The histologic pattern seen with *Echinococcus* cysts displays three layers. The (1) outermost layer or pericyst produced by the host is grossly a fibrotic capsule and is composed of fibroblasts, giant cells, and eosinophils. (2) The mid chitinous, laminated membrane is an acellular, 2-mm-thick layer produced by the organism. Finally, (3) the inner germinal or germinative layer is grossly thin and translucent and composed microscopically of a mesothelial-like epithelium (Figs. 20.114 and 20.115). Scolices originate from the germinal membrane in the form of evaginations called brood capsules (Figs. 20.116 and 20.117).[772,779] In cutaneous lesions, one can find either the classic cyst with all its elements or a granulomatous palisading reaction with pseudocyst formation containing PAS-positive membrane remnants. The latter can be confused with several other palisading granulomatous reactions, such as granuloma annulare or even foreign body reactions to keratin.[780]



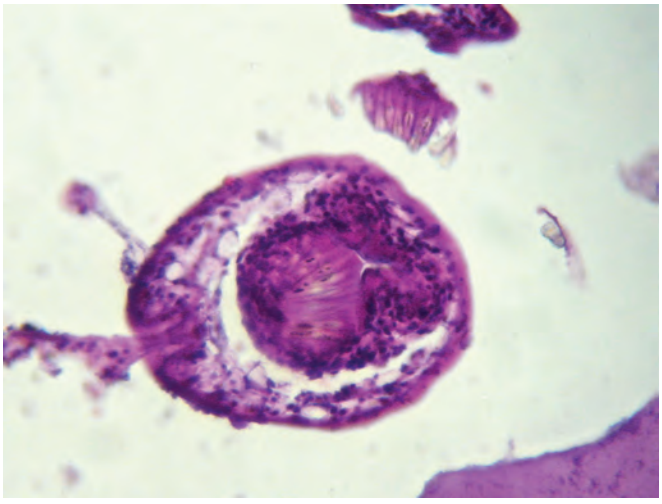
• **Figure 20.114** Echinococcosis. The cyst is mostly acellular and surrounded by fibrosis and lymphocytic infiltrate.



• **Figure 20.115** Echinococcosis. High-power view of the cyst wall with its various layers, including the inner germinal layer.



• **Figure 20.116** Echinococcosis. Scattered within the cyst's interior, the scolices originate from the germinal layer.



• **Figure 20.117** Echinococcosis. Close-up view of a scolex exhibiting its chitinous hooks.

Nematodes

Onchocerciasis

Human infection by *Onchocerca volvulus*, known as "river blindness," used to affect an estimated of 17.7 million people in more than 34 poor countries from Africa, the Middle East, South America, and Central America.[781] However, its prevalence has been steadily decreasing, particularly in Latin American[782] and African countries,[783] thanks to effective policies and the introduction of ivermectin. The parasite has a five-stage life cycle, with the blackfly (genus *Simulium*) as an intermediate host and vector. The shores of rapidly flowing streams are the habitat of these flies.[781]

Clinical Features. The most common clinical presentation involves a diffuse papular dermatitis, often with intense pruritus, which is usually seen in the acute stage of the infection. Patients with chronic infection present with pruritic lesions that are heavily lichenified or with asymptomatic hypopigmented lesions, producing the so-called leopard skin pattern.[784,785] Some chronically infected patients can also display a similar papular eruption to that seen in the acute phase.[786] There might be focal involvement of one limb by the dermatitis, which is known as "sowda" in Sudan and Yemen.[787]

Another characteristic dermatologic manifestation is the so-called onchocercoma. These are subcutaneous nodules identified over bony prominences of the torso and hips in the African cases and on the head and shoulders in the South American cases.[781,785,786,788] The most debilitating feature of this disease is the ocular involvement by the microfilaria, which leads to blindness.[781,786]

There appears to be a symbiotic relationship of the parasite with an obligate endosymbiont, a rickettsial-like bacteria of the genus *Wolbachia*. This organism seems to be necessary for reproduction of the parasites.[786,789] Furthermore, these organisms appear to be important because the release of these bacteria upon lysis of the parasite has also been associated with ocular manifestations in the human hosts.[786,789] This feature has also been described in dirofilariasis (see next section).[790] Efforts have been focused on combating the previously mentioned parasites by targeting these bacteria.[791]

Histopathology. The onchocercal dermatitis lesions are composed microscopically of an acanthotic and hyperkeratotic epidermis overlying a mild, superficial and deep, dermal inflammatory infiltrate composed of lymphocytes and eosinophils.[792]

The characteristic subcutaneous nodules have an outer wall of dense fibrous tissue and a center exhibiting coiled-up adult female parasites surrounded by granulation tissue and mixed inflammatory infiltrate. Degenerating parasites can be also seen, usually surrounded by degranulating eosinophils. When a viable parasite is present in the nodule, it releases smaller microfilarial forms that can be identified among collagen fibers in the upper dermis; particularly around lymphatic channels.[793,794] There appears to be increased angiogenesis and lymphangiogenesis, which are perhaps orchestrated by the parasites, with increased expression of several factors, including CXCL12, CXCR4, VEGF-C, angiopoietin-1, and angiopoietin-2. The microfilaria appear to spread through lymphatic channels.[793]

Chronic lesions tend to show progressive fibrosis of the dermis.[792,794] Parasites are not usually found, with calcification, chronic inflammatory infiltrate, and a foreign body giant cell response seen instead.[792,794] Although acute lesions elicit a systemic Th1-type lymphocytic response, in chronic lesions a predominance

of Th2 is found, leading to an increased intensity in depigmentation, papular dermatitis, atrophy, and lichenified dermatitis.[795]

Dirofilariasis

Dirofilaria is an important genus of parasites that mainly infect canids (dog heartworm) and felines. The endemic areas include the Mediterranean region and some Middle Eastern countries with cases of human infection being predominantly caused by *Dirofilaria repens* and *Dirofilaria immitis*.[796-800]

Clinical Features. Humans infection by *D. repens* and *D. immitis* usually causes solitary, erythematous, subcutaneous nodules.[797,801] These nodules are usually tender and sometimes are related to a sensation of "movement" under the skin.[799] *D. immitis* can asymptomatically involve the lungs.[797]

Histopathology. The nodules comprise a prominent inflammatory infiltrate composed of lymphocytes, plasma cells, histiocytes, eosinophils, and giant cells.[797] There is usually central suppuration with abundant neutrophils, and in the center of the nodule the degenerating filaria is usually identified.[797,801] The parasite has a thick, laminated chitinous cuticle, longitudinal ridges, and large lateral cords, as well as a characteristic disposition of smooth muscle fibers.[797,801,802]

Cutaneous Larva Migrans and Larva Currens

Cutaneous larva migrans is caused by nematode larvae that are not able to progress beyond the dermis, therefore wandering in the dermis and epidermis and producing the characteristic clinical sign known as creeping eruption.[803-805] Although *creeping eruption* and *cutaneous larva migrans* are usually used as synonyms, the former refers to a clinical sign and the latter to a syndrome.[803,804]

Creeping eruption is a linear, serpiginous, slightly elevated, erythematous track that moves forward in an irregular pattern.[803] This particular sign can be elicited not only by larvae from a hookworm or other nematodes[803,805] but also by other parasites, such as *Gnathostoma* spp.,[806] as well as by *Sarcoptes scabiei* and migratory myiasis.[807]

Hookworm-related cutaneous larva migrans, which is caused by animal-derived (dogs or cats) hookworms, such as *Ancylostoma braziliense*, *Ancylostoma caninum*, *Uncinaria stenocephala*, and *Bunostomum phlebotomum*, is quite common in tropical areas.[805,807] This infestation is endemic in poor communities from developing countries, such as in shantytowns and impoverished rural areas from Brazil,[808-810] India,[805] and the West Indies,[811] among others. The peak incidence usually coincides with the rainy season.[809,810]

Showing almost identical clinical presentation to larva migrans, *larva currens* is the term used when creeping eruption is related to *Strongyloides stercoralis* hyperinfection; the name refers to the very fast movement of the larvae.[812,813] The underlying severe form of *S. stercoralis* infection (hyperinfection) is seen in immunosuppressed individuals, has systemic involvement, and can be fatal.[814-816]

Clinical Features. The lesions are usually located on the feet, buttocks, and thighs, but they can occur wherever unprotected skin comes in contact with contaminated soil.[805,808] Larvae can occasionally be transmitted through contaminated clothes or other fomites. Clinically, an itchy reddish papule appears at the site of penetration of the larva.[805,807] There is usually a period of 1 to 5 days until the characteristic elevated tracks appear; this period can last longer, especially in infected returning travelers.[817] Pruritus is the main symptom of the disease and can be rather intense, often producing bacterial superinfection.[805,808] Vesiculobullous lesions were seen in 9% to 15% of the cases in one large series, with bullous lesions reaching several centimeters in diameter in some cases.[817] Papulopustular inflammation of the follicles[818] and EM[819] are rarely seen to be associated with this condition. In addition to the classic creeping eruption pattern, *larva currens* can present also as multiple purpuric lesions.[814-816]

The diagnosis of these conditions is usually based on the clinical history and visualization of the typical creeping eruption pattern. Biopsy is usually not useful, nor is epiluminescence microscopy.[805]

Histopathology. The parasite is rarely identified in histologic sections of the lesions. However, the tracks left by the larvae are seen as small cavities in the epidermis. There is usually a dermal mixed inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, and numerous eosinophils. In the rare event of finding a larva, there is usually no inflammation around the parasite. An eosinophilic folliculitis is sometimes seen.[820]

In larva currens, there is usually a prominent perivascular and interstitial lymphoeosinophilic infiltrate accompanied by abundant, 9- to 15-μm-thick larvae are visualized amid the dermal collagen and rarely within vessels.[814-816] Lesions that present in profoundly immunosuppressed individuals may lack inflammation, thus showing very subtle histopathologic findings.

Arthropod-Induced Diseases

Arachnids

Scorpions

Only a small fraction of the existing species of scorpions has sufficiently potent venom to pose a risk to humans. Almost all of the most poisonous species are within the Buthidae family, and they are mainly found in deserted areas of the Middle East, Africa, and Asia.[821] The scorpion of primary concern in the United States is small (1.3 to 7.6 cm), with a small spine, or tubercle, at the base of its stinger, *Centruroides exilicauda* (formerly *Centruroides sculpturatus*).[822]

Clinical and Histopathologic Features. Because the majority of the effects of the venom are neurotoxic, there is virtually no dermatologic development other than edema and erythema around the sting site, as well as mild lymphadenopathy and rarely, echymoses.[821,822] However, stings by *Hemiscorpion lepturus*, a species found in Iran and Iraq, cause tremendous dermatologic manifestations at the site of inoculation. Lesions caused by this scorpion species begin as erythematous, purpuric plaques and then evolve to bullae, extensive cellulitis, and, later, necrotic ulcers. The venom has a prominent cytotoxic effect, producing severe vasculitis at the site of the sting. Prominent systemic effects may also be seen.[823]

Spiders

Similarly to scorpions, only a small number of spider species are considered dangerous to humans. The pharmacology of spider venoms is highly variable among species, so identification of the involved spider is medically important.[821,822]

Black Widow Spider *(Latrodectus)*. Spiders from the genus *Latrodectus*, popularly known as black widow spiders, are small and shiny black and have hourglass-shaped red markings in the abdomen.[821,822] The venom produced by these spiders contains neurotoxic peptides (mainly α-latrotoxin), which cause systemic symptoms, such as contracture of the abdominal muscles (often misdiagnosed as acute abdomen), generalized muscle cramps, sweating, malaise, nausea, and hypertension.[824] At the site of

inoculation, there are usually two markings, corresponding to the spider's fangs, surrounded by erythema and some petechial branching lesions.[821,822,825]

Brown Recluse *(Loxosceles)*. The other commonly medically important spiders are the ones from the genus *Loxosceles.* Also known as recluse or violin spiders, these are medium size, uniformly brown spiders with fine hairs and a violin-shaped cephalothorax. A distinctive feature is the presence of six eyes grouped in three dyads.[821,822] Manifestations associated with the bites from these spiders range from localized mild lesions to massively necrotic, slow-healing lesions that can have life-threatening systemic manifestations, such as acute renal failure and disseminated intravascular coagulation.[824,826,827] The venom is overtly cytotoxic, with sphingomyelinase as its main active component, and causes disruption of the endothelial cells, massive activation of the neutrophils, and hemolysis.[824,827] Fully evolved lesions exhibit the classic bull's-eye lesion, with a central erythematous bleb separated from a peripheral cyanotic region. This lesion becomes necrotic usually 7 days after the assault, leaving a depressed ulcer.[826,828]

Other Important Spider Species. Other medically important spiders include the so-called hobo spiders *(Tegenaria agestis)*, which can cause dermonecrotic lesions similar to but somewhat less severe than those seen with *Loxosceles* spiders.[822,829] In addition, large spiders from the genera *Phoneutria* (wandering or banana spider)[830] and *Atrax* (Sydney funnel spider),[831] which are found in South America and Australia, respectively; have potent neurotoxic and potentially lethal venoms. However, their bites express few dermatologic manifestations.

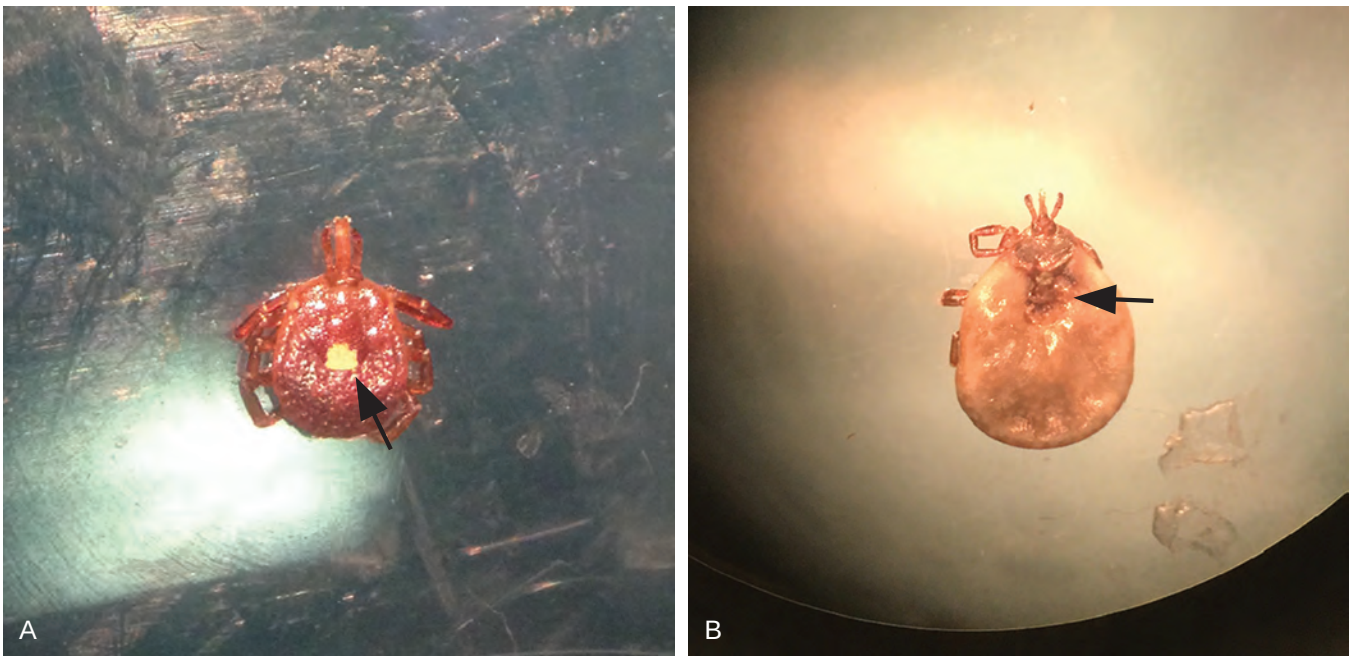
Histopathology. The term "necrotizing arachnidism" is used to designate necrotic lesions caused by spiders that produce predominantly cytotoxic venom.[832] Microscopically the lesions usually show a prominent polyarteritis nodosa–like neutrophilic vasculitis with abundant hemorrhage. This leads to arterial wall necrosis, eschar-covered ulceration, and even subcutaneous tissue necrosis. A prominent lymphoeosinophilic inflammatory infiltrate is usually found early in the evolution of the lesion, followed by massive areas of coagulative necrosis.[833] On the other hand, the neurotoxic venom causes little alteration at the site of the bite.

Ticks

These organisms, along with mites, are included in the Acari subclass with ticks that have dermatologic importance falling in the Ixodida suborder. The Argasidae family, or soft ticks (*Ornithodoros* spp. and others), are usually not perceived by the host and can cause nodulohemorrhagic local lesions and even anaphylactic shock.[834,835] In contrast, the more frequently seen organisms from the Ixodidae family, or hard ticks, which includes the *Ixodes* and *Dermacentor* species, are usually perceived by the host because they remain attached to the skin while they engorge with blood.[822,834]

The main tick species are included in the Ixoda (hard tick) family and are represented by the *Amblyomma* (including *A. americanum*, the "lone star" tick) (Fig. 20.118A and B), *Dermacentor* (*D. variabilis*, also known as "dog tick"), and *Ixodes* (*I. scapularis*, "deer tick"). When not engorged, these ticks are relatively easy to identify; however, when engorged with blood, one must rely on the *scutum* (shield) portion of the thorax (see Fig. 20.118B). The University of Rhode Island offers a fantastic online tick identification resource.[836] Ticks are known vectors of a whole host of infectious agents that cause a wide array of conditions, some of which are described elsewhere in this chapter and book.

The remarkable mechanism through which these arachnids feed involves the gnathosoma (capitulum), which is a cutting/sucking device located in the anterior portion of the tick. During feeding, a substance is secreted that acts as a "cement," fixing the mouthparts to the skin.[822,834] This process is aided by the biologic



• **Figure 20.118** Example of a resting **(A)** and engorged **(B)** female Lone star tick *(Amblyomma americanum)* with the characteristic yellow-gold spot (**A**, *arrow*), which is barely visible and only present in the scutum area when the tick is engorged (**B**, *arrow*). This tick species is widely distributed in the West Coast and Midwest areas of the United States and is known to be a vector of human erlichiosis and tularemia.

action of several chemicals contained in the regurgitated saliva. These include vasodilators, anticoagulants, immunosuppressants, antiinflammatory molecules, and enzymes, such as hyaluronidases and metalloproteinases.[837,838]

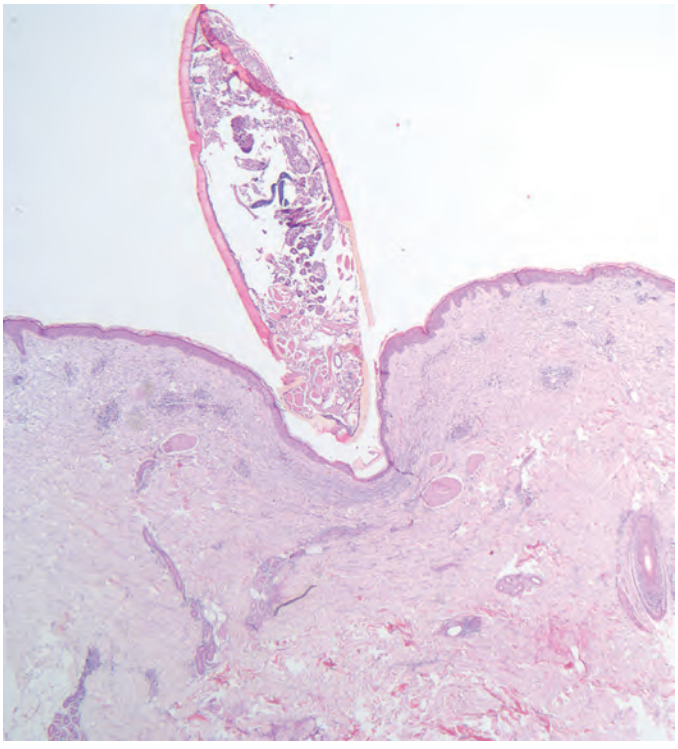
Clinical Features. The clinical presentation varies from local erythematous lesions to a persistent papular and nodular rash.[839] Tick bites can occasionally induce panniculitis, bullae, and hemorrhage.[822,840] The erythema (chronicum) migrans pattern caused by *I. scapularis* is described elsewhere in this chapter. Tick-related papular urticaria is another rather distinctive presentation of chronic nature that is usually found in children.[841]

Histopathology. Acute lesions show an intradermal cavity with prominent red blood cell extravasation and a rather dense inflammatory infiltrate composed predominantly of neutrophils together with lymphocytes and histiocytes. The characteristic eosinophilic infiltrate appears relatively late in the acute phase of the process.[834] The diagnosis can be difficult if the refractile tick mouthparts are not identified. More evolved lesions show a tract of necrosis and perivascular fibrin deposition.[834] Intravascular fibrin thrombi are sometimes seen. These can resemble the findings of monoclonal or mixed cryoglobulinemia.[842,843] A neutrophilic panniculitis may occur.[840] If the biopsy includes the organism, its internal structure can be appreciated on histologic sections (Fig. 20.119).

Chronic lesions are characteristically composed of a diffuse, superficial and deep, lymphoeosinophilic inflammatory infiltrate that can be wedge-shaped. This is overlaid by an acanthotic and pseudoepitheliomatous epidermis. Granulomas can be found and are usually a mixed immune and foreign body immune type. They arise in response to the strongly immunogenic, collagen-like proteins of the cement or the viable core and the sclerotized cuticle of the tick's mouthparts.[834] This persistent nodular arthropod-bite reaction appears as a T-cell or B-cell lymphoid hyperplasia, which can be differentiated from other types of pseudolymphomatous lesions by the vascular changes and the presence of numerous eosinophils.[834,844] These lymphoid aggregates can exhibit pseudoclonality in both T-cell receptor rearrangement and light chain molecular studies.[845] Therefore one must rely on the history and a correct clinical correlation. Given the presence of mixed inflammatory infiltrate, one of the lymphomas that is most often considered in the differential diagnosis of a chronic tick bite is the primary cutaneous $CD4^+$ pleomorphic/medium sized T-cell lymphoma, a WHO provisional entity that is extremely low grade, which raises suspicion that there might be overlap between these two entities.

The histopathologic details of erythema chronicum migrans of Lyme disease, often transmitted by ticks from the *Ixodes* group ticks, were reviewed earlier in this chapter. The papular urticaria pattern can appear chronically as a wedge-shaped lesion involving the dermis and even subcutaneous fat.[841]

A peculiar reaction to tick bites is the appearance of a patch of alopecia at the site of the bite. This unusual presentation is clinically similar to alopecia areata,[846,847] and it is believed to be caused by tick-derived epilating anticoagulants injected with saliva.[846] There are several histopathologic features that one can find helpful to distinguish alopecia areata from tick bite–induced alopecia. In alopecia areata the lymphocytic infiltrate is located mainly in the bulbs of the hair follicles. In alopecia secondary to a tick bite, the lymphoid reaction is located at the hair's isthmus.[834]



• **Figure 20.119** Histologic section of a tick still attached to the epidermis. Note the internal structure of the organism as well as the superficial perivascular inflammatory infiltrate in the superficial and mid dermis.

Mites

Demodicosis. Follicle mites live in hair follicles and sebaceous glands of mammals, such as cats, dogs, and horses.[848] These organisms colonize the pilosebaceous unit and use sebum as nourishment. Two species have been identified in humans: *D. folliculorum* and *Demodex brevis*.[849] *D. folliculorum* is longer than *D. brevis*, measuring up to 0.4 mm, and it is usually found aggregated in groups in the infundibular portion of the hair follicle, whereas *D. brevis* is usually solitary and inhabits the deeper portion of the sebaceous glands and ducts.[822,849] The prevalence approximates 100% in middle-aged and older adults, although mite density is low in healthy skin.[848] The mites can be found in up to 10% of skin biopsies from all sites and in 12% of all follicles of any given biopsy specimen.[850] The face is most often involved, but mites can be also seen in skin from the neck and chest.[822] Colonization is likely to occur during childhood by direct contact.[848] Although they seem to be not involved in the etiology of rosacea, with the possible exception of the granulomatous variant, the mites definitely can aggravate this disease.[851]

Clinical Features. There are three basic clinical forms of *Demodex* infestation: (1) pityriasis folliculorum, (2) rosacea-like demodicidosis, and (3) demodicidosis gravis. Pityriasis folliculorum manifests clinically as a diffuse but faint facial burning sensation with fine follicular plugs and scales in a "frosted" pattern. The condition is frequently seen in individuals using heavy facial creams and make-up.[852,853] The rosacea-like demodicidosis has a sudden, rapid onset with follicular scaling, superficial small papulovesicles, and vesicopustules.[854] It can manifest with an asymmetric distribution and can affect the eyelids (demodectic blepharitis).[855] It is not related to flushing, persistent erythema, or photosensitivity, which is helpful to distinguish it from rosacea.[848] The third clinical variant of *Demodex* infestation, demodicidosis gravis, involves a clinical picture very similar to that of granulomatous rosacea.[856]

Demodex infestation has also been related to other dermatologic conditions, such as pustular folliculitis,[857] papulopustular scalp eruptions,[858] perioral dermatitis,[859] and hyperpigmented patches of the face.[852]

Histopathology. Microscopically the usual picture of *Demodex* mite colonization includes follicular dilatation and the presence of dense eosinophilic material around the mites. Only when the mites are misplaced in the dermis, usually due to follicular rupture, are inflammation and granulomas enveloping extrafollicular mites seen, especially in granulomatous and pustular rosacea.[859,860]

In pityriasis folliculorum, one sees a diffuse dermal perivascular lymphocytic infiltrate with no granuloma formation.[853] The histologic picture of rosacea-like demodicosis exhibits a mononuclear, mainly perifollicular inflammatory infiltrate that can be granulomatous. Up to 10 to 15 *D. folliculorum* organisms can be seen in each follicle. Telangiectatic superficial vessels are also seen.[848,852,854]

Scabies. Both highly contagious and neglected, this cutaneous condition is an important cause of worldwide public health problems. It is usually linked to poor hygiene and overcrowded environments. The causative agent is the mite *S. scabiei* var. *hominis*, which involves the upper layers of the epidermis, burrowing in the stratum corneum of the skin. Transmission can occur during direct skin contact or through contaminated fomites.[861] Interestingly, a primary infection induces clinical symptoms only 4 to 8 weeks after the inoculation.[861,862] The reasons behind this delayed response are largely unknown. However, studies have shown that substances with immunomodulatory features are actively secreted by the mites and affect keratinocytes, fibroblasts, and endothelial cells.[863,864]

Scabies tends to have a cyclical character, recurring at intervals many apart years,[865] and shows increased incidence during winter in some communities.[866]

Clinical Features. There are three clinical presentations: (1) a papulovesicular variant, (2) a form presenting as persistent nodules, and (3) the so-called Norwegian (crusted) scabies. The first two presentations are the most commonly encountered.

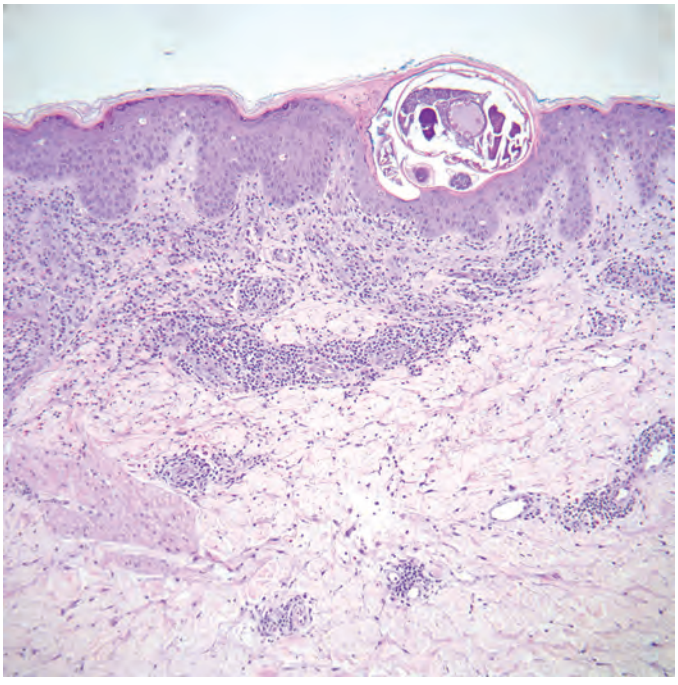
The classic scabies and by far more frequently encountered presentation is that of the papulovesicular variant, which includes, unsurprisingly, intensely pruritic papules and papulovesicles. The pruritus is usually worse at night. The vesicles are found at the end of fine, wavy, dark lines, which represent the excreta-soiled tunnels created by the female parasite in the horny layer while depositing eggs. These burrows are specific, but they may be absent.[861,867] In addition to the burrows, there is almost always a secondary rash of small papules related to autosensitization.[861,867,868] The most commonly involved sites are the interdigital skin folds, palmar aspect of the hands and fingers, wrists, nipples, inframammary regions, and male genitalia. Diagnosis of the disease relies heavily on the history and clinical features.[861,867] Detection of the mites can be enhanced by both epiluminesence[869] and dermoscopy.[870] Molecular PCR-based assays of the biopsied tissue, as well as serologic ELISA-based techniques, can be used in problematic cases.[871]

Rare clinical presentations of papulovesicular scabies include a bullous-predominant variant that strongly resembling BP.[872,873] This presentation can sometimes pose a true diagnostic dilemma. In some cases of bullous scabies, circulating antibodies against BP180 or BP230 or both have been detected in sera, suggesting that *S. scabiei* infection could trigger a bona fide bout of BP.[874]

The persistent nodular form appears in approximately 7% of the patients, particularly in young adults and children. It manifests as reddish-brown, pruritic nodules on the lower trunk, scrotum, and thighs.[875] The lesions persist for a year regardless of treatment, and they usually are devoid of organisms.

At the severe end of the spectrum, we find the disseminated form of scabies, namely the crusted or Norwegian form. Heavily linked with immunocompromised states, it can occur in patients infected with HIV[876] or HTLV-1,[877] as well as in individuals receiving immunosuppressive therapy.[878] In addition, it has been described in patients with previously treated leprosy, substance abuse, systemic lupus erythematosus, tuberculosis, diabetes mellitus, and hepatitis B.[878] Cases have also been reported in an otherwise healthy pregnant woman[879] and in patients with epidermolysis bullosa.[880,881] Norwegian scabies is notorious for exhibiting extensive thick, hyperkeratotic, and crusted lesions with abundant mites distributed all over the body, including the neck, the scalp, and under the nails. Secondary bacterial infection, accompanied by regional lymphadenopathy, is commonly present.

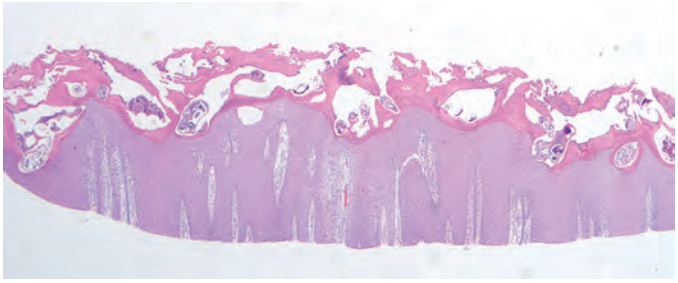
Histopathology. On biopsy, in the classic papulovesicular scabies infestation, one usually finds a spongiotic epidermis with exocytosis of eosinophils and neutrophils with occasional pustule formation. The spongiotic dermatitis overlies a superficial and deep perivascular infiltrate showing lymphocytes, histiocytes, mast cells, and eosinophils.[875,882,883] The superficial vessels can show fibrin thrombi in up to 40%.[883] Some cases will exhibit a predominance of Langerhans cells in the inflammatory infiltrate, which can potentially mimic Langerhans cell histiocytosis.[884] If a burrow is captured in the biopsy, eggs, larvae, feces, and mites (either whole or in fragments) may be visualized, sometimes requiring multiple tissue levels (Fig. 20.120).[883] The so-called pink pigtails, somewhat coiled acellular eosinophilic bodies appearing in the stratum corneum, are believed to be egg casings, which are diagnostic and present in up to 28% of cases.[883] Rarely, one can identify flame figures,[885] necrotizing vasculitis,[886] and subepidermal bullae in a BP-like pattern.[872]



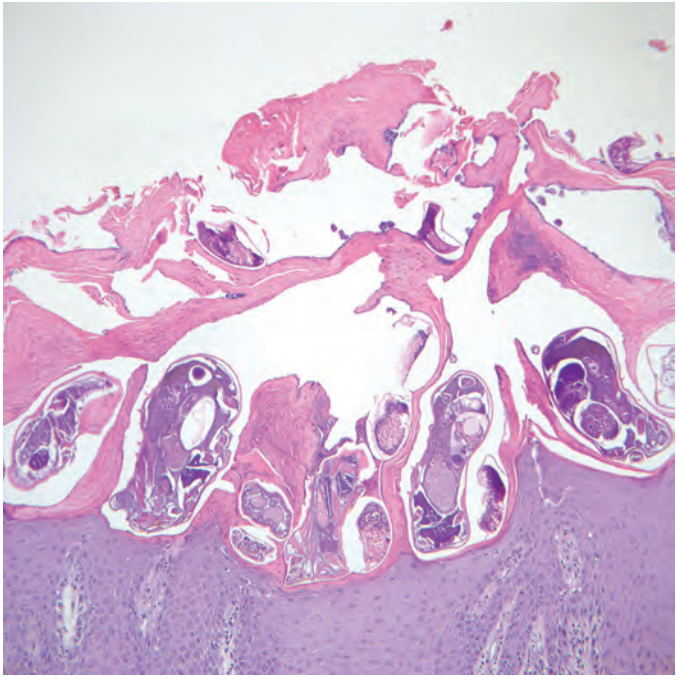
• **Figure 20.120** Scabies. A single *Sarcoptes scabiei* mite is seen here in an intracorneal burrow. Note the prominent dermal inflammatory infiltrate.

In persistent nodular scabies, the histologic picture includes superficial and deep lymphocytes (even atypical forms), macrophages, plasma cells, eosinophils, and Langerhans cells (intermediate cells) disposed in perivascular and periappendageal array.[887] This infiltrate, probably directed to mite antigens,[888] has often an overlying pseudolymphomatous pattern.[844] These pseudolymphomatous infiltrates can be quite exuberant and even extend into subcutaneous fat.[889] Viable mites are usually not observed in this variant, but if the specimen is serially sectioned, mite parts may be found in 20% of cases.[890]

Finally, the histologic picture of Norwegian scabies is that of a massive orthokeratotic and parakeratotic scale containing innumerable mites disposed at different levels of the scale[883] (Figs. 20.121 and 20.122). Below this massive scale, there is usually a psoriasiform hyperplasia with spongiosis and exocytosis of neutrophils and eosinophils; microabscess formation may occur. The underlying dermis shows lymphocytes and eosinophils as well as a chronic inflammatory infiltrate, which shows an abnormal $CD8^+$ predominance, resulting from a Th1/Th2 imbalance. This lymphoid anomaly is probably related to psoriasis-like dysregulation of the keratinocytes and the host's inability to control the infection.[891]



• **Figure 20.121** Scabies, Norwegian (crusted) variant. Multiple parasites are seen here in the midst of a prominent hyperparakeratotic scale. Note the prominent psoriasiform epidermis hyperplasia in the epidermis beneath.



• **Figure 20.122** Scabies, Norwegian (crusted) variant. Numerous mites, as well as mite fragments and ova are present.

Insects

Human Lice

Lice have been related to human activities since ancient times. Interestingly, the approximate historical period in which humans began to wear clothes has been determined by "dating" the mitochondrial DNA of the body louse.[892] These ectoparasites act as vectors of agents of such conditions as epidemic typhus *(R. prowazekii)*, discussed earlier.

There are three types of human lice. The species that affects the hairs of the scalp, *Pediculus humanus capitis*, is fairly common, affecting people from all socioeconomic backgrounds. This species represents a common and recurrent problem in schools.[893] The second type, *Phthirus pubis*, has venereal transmission pattern. Infestation with *P. pubis* (also known as "crabs") is strongly related to other concomitant sexually transmitted diseases.[894] This species usually infests the pubic hair, but it can be seen in the corporal hair of the trunk and limbs and even in the eyelashes.[895] Both the pubic louse and head louse cement eggs to the hairs, forming the so-called nit. Finally, the third type is the body louse, *P. humanus corporis*, which, in contrast to the first two variants, lives in the host's clothes as well as in body hairs.

Clinical Features. Scattered bluish spots of discoloration (maculae cerulea) can sometimes be found related to heavy body louse infestation.[896] The reaction to infestation of eyelashes by *P. pubis* can mimic conjunctival infections and blepharitis.[895,897] An erythema annulare centrifugum type of lesion may develop secondary to massive infestations with *P. pubis*.[898]

Histopathology. The saliva injected while the parasite feeds produces an allergic reaction, which can become superinfected, especially after heavy scratching. One can identify lice and eggs microscopically for confirmation.

Bed Bugs

These annoying insects, part of the Cimicidae family, are hematophagous, reddish-brown exoparasites with nocturnal feeding habits. They have a worldwide distribution but with a greater incidence in tropical and subtropical developing countries.[899] However, of late, a dramatic increase of these parasites has been seen, especially in large urban centers of the United Kingdom and the United States, as well as other industrialized nations.[900] Bed bugs are quite resistant to elimination using insecticides,[901] and adult forms can survive almost 1 year without feeding, usually hiding in crevices present in wooden bed frames and furniture.[902] Although transmission of diseases was not yet demonstrated to occur with bedbugs, these insects have the potential to transmit numerous pathogens.[902]

Clinical Features. *Cimex lectularius* and *Cimex hemipterus*, the two most common species, produce lesions that range from purpuric macules to urticarial erythematous papules. The classic presentation involves three bites in linear array, also known as "breakfast, lunch, and dinner" pattern. Lesions are commonly seen on exposed areas of the face, neck, hands, or arms.[902] In predisposed individuals, bullous lesions can be evident, which is due to a late-phase response to an IgE-mediated hypersensitivity to nitrophorin, an antigen present in the *C. lenticularis* saliva.[903,904]

Histopathology. In urticarial lesions, one can see edema of the papillary dermis and a perivascular lymphoeosinophilic infiltrate with scattered mast cells. Intraepidermal and subepidermal

edema is found in the vesiculobullous lesions.[905] Bullous lesions may show prominent hemorrhage.[903]

Myiasis

The infestation of human tissue by fly larvae of the Diptera order is known as myiasis. Although the majority of the cases are produced by *Dermatobia hominis*[906-908] and *Cordylobia anthropophaga*,[909,910] several other genera of flies can produce myiasis, including *Parasarcophaga*,[911] *Gasterophilus*,[912] and *Hypoderma.*[913] Rarely, larvae of flies from the *Cuterebra* species infest humans.[914] Larvae of the domestic fly, *Musca domestica*, can rarely infest open wounds in extremely neglected and debilitated persons.[915,916] Although *D. hominis*, the so-called human botfly, is prevalent in Central and South America,[906-908] the tumbu fly *(C. anthropophaga)* is found in certain parts of West and Central Africa.[909,910] However, all the other fly species have a more widespread geographic distribution.

The eggs are usually deposited directly by the flies in areas where there is damaged skin. However, ova can be transmitted and deposited by other insects, such as mosquitoes, as is the case with *D. hominis*.[906,908] In the case of *C. anthropophaga*, larvae hatch on the ground or on clothing and then invade the host's skin.[909,910] The larvae infest the skin for a variable period, usually from approximately 2 weeks to 3 months, depending on the species. After that, larvae exit the host to continue their life cycle as pupae.

Clinical Features. Myiasis infestations tend to occur during the summer and usually involve exposed skin surfaces, such as the feet and forearms. Other common sites are the face, scalp, neck, shoulders, and chest, with the lower body affected in fewer than 15% of the cases.[908-910] Rare cases of penile infestation have been reported, with the potential of being misdiagnosed as a manifestation of a sexually transmitted disease.[917,918]

Early myasis lesions often adopt a furuncle-like aspect or manifest as a single erythematous papule, usually with a punctum-like orifice where the larvae obtain oxygen.[906,908,919] The eruption can be migratory or nonmigratory, with migratory myiasis usually related to *Gasterophilus* spp. (horse botfly) or *Hypoderma* spp. (cattle grub). More evolved lesions manifest as ulcers or plaques with draining sinuses and fistulas with bacterial superinfection. Pulsating pain can be present in large and superinfected lesions.[906,908,910,919]

Histopathology. The developing larva is usually lodged in a cavity located in the dermis or subcutaneous fat. Around the larva, there is a prominent host response; this is a heavy, mixed inflammatory response with lymphocytes, histiocytes, foreign body giant cells, plasma cells, eosinophils, and neutrophils, as well as extensive fibrosis.[910,914,919] A sinus tract connecting the cavity to the exterior can be found. Fragmented larva parts, showing chitinous material with widely spaced spines and internal organs, can be seen inside the cavity. When botfly larvae are involved, one can identify them by the presence of prominent black spinous processes emanating from the chitinous cuticle which help the larva to attach itself to the host's tissues.[920,921] The different various species can be distinguished, especially during early development, by examining the location and patterns of their respiratory spiracles.

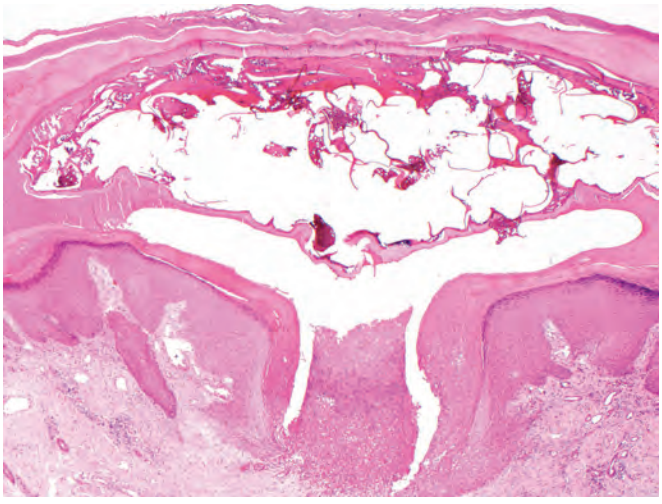
Tungiasis

Tungiasis is caused by *Tunga penetrans*, a variety of free-living flea that is found in tropical environments. Originally from South and Central America, the sandfleas spread first to tropical Africa in the 19th century and then to India and Pakistan in the beginning of the 20th century.[922,923] As is the case with most of the parasitic diseases, infection is related to poor hygiene and precarious conditions, which are often found in neglected communities.[922-924] The natural habitat of these fleas are sandy and warm terrains, such as beaches and arid regions, but they can also be found in stables and pigsties.[925] Infestation is commonly found in pigs, dogs, cats, and rats, and all of which can act as reservoirs of the insect.[925] In endemic regions, there is a seasonal pattern of infestations.[924]

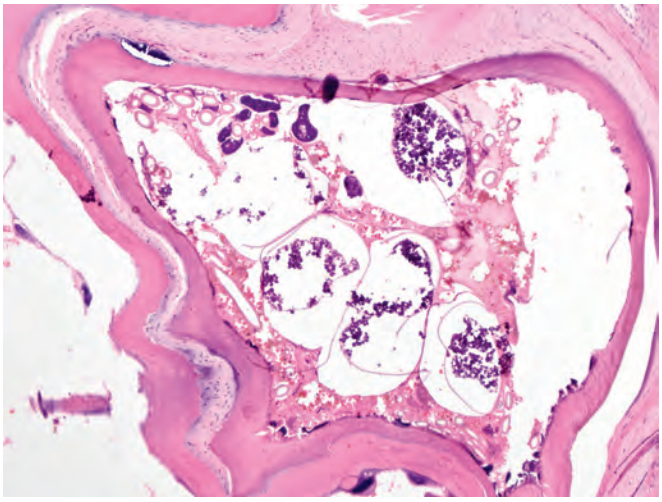
Clinical Features. The lesions are usually located on the feet because *T. penetrans* is a poor jumper, and they can produce severe impairment in walking due to pain.[923,926] Periungual affectation is not uncommon.[923,926] Lesions can also be found on the hands, especially in children.[927,928] Other sites that may be involved are the elbows, wrists, breast, back, buttocks, thighs, knees, and legs.[928]

The infestation, which usually lasts 4 to 6 weeks, begins with painless penetration of the parasite, probably with the help of keratinolytic enzymes. This is followed by several subsequent phases: a hypertrophic verrucous phase, a "white halo" phase, an involution phase, and finally the transepidermal elimination of the residues.[929] Overall, the lesions are usually composed of single or multiple, small, white papules that eventually develop into nodules. Lesions are often quite pruritic (earning the name "pique" which means "itch" in Spanish-speaking countries), and usually show a wart-like morphology,[923,926,929] as well as crusted,[923,926] pustular,[926,927] and/or ulcerative morphologies.[926] One can occasionally find bullous[930] lesions. The female flea measures approximately 0.1 cm at the beginning of the infection. The flea feeds and reaches a size of 0.6 cm or more, harboring up to 200 ova. At the end of the cycle, the adult flea dies, and the lesion ulcerates to release the eggs.[923,929] Bacterial superinfection is quite common, usually with *S. aureus* or enterobacteria.

Histopathology. Although the infestation is usually suspected from the history and clinical appearance, if a biopsy is available, one can identify the parasite by its characteristic exoskeleton and internal parts, including ova (Figs. 20.123 and 20.124). The parasite is usually lodged in the upper dermis, surrounded by a pseudocystic cavity. In the surrounding and overlying skin, there



• **Figure 20.123** Tungiasis. The flea is usually found intraepidermally, surrounded by extensive epidermal hyperplasia with hyperkeratosis. At the site where the proboscis is inserted into the dermis, there is a prominent inflammatory response composed of lymphocytes, plasma cells, and eosinophils.



• **Figure 20.124** Tungiasis. Note the parasite's exoskeleton and internal organs. Eggs are found within the flea's engorged abdomen.

is prominent hyperkeratosis, parakeratosis, and acanthosis.[923,929] The flea is surrounded with a prominent, mixed inflammatory infiltrate and fibrosis. A mass of eggs can sometimes be found within the stratum corneum. In advanced lesions, there is prominent ulceration and impetiginization.[923,929]

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21

Infections of the Eye and Its Adnexa

ANNA M. STAGNER, FREDERICK A. JAKOBIEC, RALPH C. EAGLE JR., NORMAN C. CHARLES

Masquerade Syndromes
- Chalazion
- Sebaceous Carcinoma
- Intraocular Lymphoma, Leukemia, and Behçet Disease With Hypopyon
- Necrotic Intraocular Tumors Simulating Panophthalmitis and Orbital Cellulitis

Conjunctival Infections
- Bacterial Infections
- Viral Infections
- Fungal Infections
- Parasitic Infections

Corneal Infections
- Bacterial Infections
- Viral Infections
- Fungal Infections
- Parasitic Infections

Intraocular Infections (Including Endophthalmitis and Infectious Retinitis and Choroiditis)
- Bacterial Infections
- Viral Chorioretinitis
- Fungal Infections
- Parasitic Infections

Eyelid and Orbital (Skin and Soft Tissue) Infections
- Bacterial Infections
- Viral Infections
- Fungal Infections
- Parasitic Infections

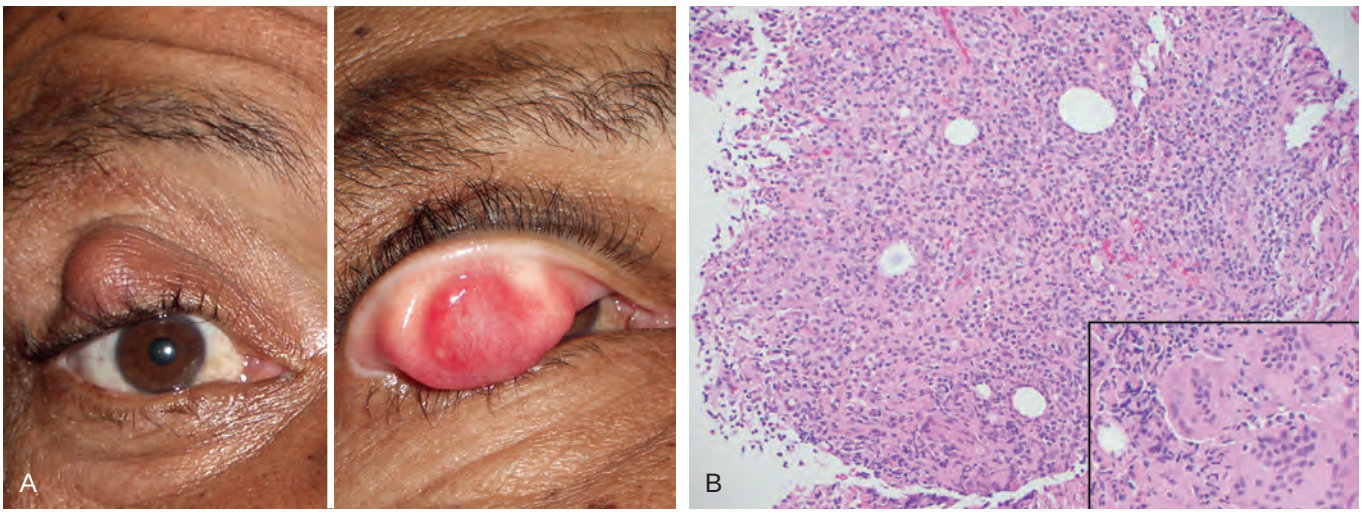
The histopathologic study of infections of the eye and ocular adnexa is a daunting undertaking given the variety of tissues comprising this proportionately small area of the body with extensive exposure to the external environment. Only one previous summary of infections of the eyelid and conjunctiva has been published,[1] but a broader conspectus combining extraocular and intraocular infections is not available. The term adnexa (plural noun, from the Latin *adnexus* meaning parts that bind together; adnexum is the singular noun) denotes all of the supporting and protecting structures around the eye. Infections may affect any adnexal structure, such as the mucous membrane (conjunctiva), the skin (eyelids), and the orbital contents (lacrimal gland, extraocular muscles, fibroadipose tissue, optic nerve, peripheral nerves and vessels). The cornea (infectious keratitis) shares many of the same susceptibilities as the conjunctiva. Infections can involve the interior of the globe and its layers (infectious retinitis, uveitis, scleritis, endophthalmitis of the vitreous and panophthalmitis of all layers, including the sclera and episclera) either from internal hematogenous spread (endogenous) or from external sources (exogenous), such as trauma or surgery. The clinical presentations and histopathologic findings are unique for each of these sites. They are discussed in the following chapter according to the region primarily harboring the infectious agent.

There are some peculiarities of each ocular structure that determine its response and resistance to infection. For example, the acellular, subepithelial collagenous Bowman layer of the cornea is able to be a temporary barrier against invasion of the corneal stroma, while Descemet membrane often attracts organisms, particularly fungi. The sclera is a poorly vascularized structure that generally does not become infected. The uvea (from the Latin noun uva, meaning grapelike tunic, due to its coloration grossly from resident melanocytes after removing the sclera) which subsumes the iris, ciliary body, and choroid, has a paucicellular fibroblastic component that tends not to proliferate or make scars in response to injury. For example, at the previous site of an infection of the choroid, one can see ophthalmoscopically a white focus that represents the inner aspect of the sclera in the wake of an absence of repair in the choroid. In contrast, the pigment epithelium is able to proliferate floridly and can produce pigmented scars at the edge of an infectious nidus and even undergo fibrous metaplasia with collagen synthesis, a true anomaly in view of the neuroectodermal origin of this tissue. The retina lacks fibroblasts throughout and therefore reaction and healing are glial, in the form of fibrous astrocytic proliferations.

A caveat should be offered: many biopsies for identification of offending pathogens, especially of fungi, may be altered in their morphology by ongoing topical and systemic therapy. These changes include shifts in tinctorial properties, width of fungal hyphae, and other morphologic features.

Masquerade Syndromes

In medicine we are accustomed to analyzing conditions that simulate one another. Among many such conditions in ophthalmology,



• **Figure 21.1** Chalazion. **A,** Clinical appearance of a chalazion *(left panel)*. Eversion of the eyelid *(right panel)* reveals focal, well-circumscribed erythema of the tarsal conjunctiva. **B,** A friable collection of epithelioid cells toward the left surrounds dissolved out lipid locules (lipogranuloma). The *inset* reveals numerous giant cells around extracellular lipid (×200). (**A,** Courtesy Michael Yoon, MD.)

there are four that deserve particular attention because they can mimic infectious diseases either clinically or histopathologically.

Chalazion

This inflammatory eyelid disorder, the most common of the eyelids (Fig. 21.1A, left panel), can easily mislead the general pathologist, because of its granulomatous features, into a diagnosis of a fungal or tuberculous infection (which are ruled out with negative Gomori methenamine silver [GMS] staining or acid-fast staining, respectively). The chalazion originates in the tarsus of the posterior part of the eyelid (almost never the primary site of infection) containing the meibomian glands that produce the lipid for the tear film (see Fig. 21.1A, right panel). Locules of dissolved extracellular fat are surrounded by the epithelioid and giant cells, along with acute and chronic inflammatory cells (a lipogranuloma) (see Fig. 21.1B). The chalazion represents a blockage in the ducts of the meibomian glands, which may be due to hyperparakeratosis near their orifices,[2] leading to rupture of the glands. Often commencing as an intratarsal bacterial infection, cytoplasmic lipid is eventually released extracellularly to elicit the granulomatous response.[3] Localized xanthogranulomas manifest lipid in the cytoplasm of histiocytes and in the eyelids are mostly xanthelasma, but systemic disorders, such as Erdheim Chester disease and necrobiotic xanthogranuloma, may have eyelid xanthomatous lesions.

Sebaceous Carcinoma

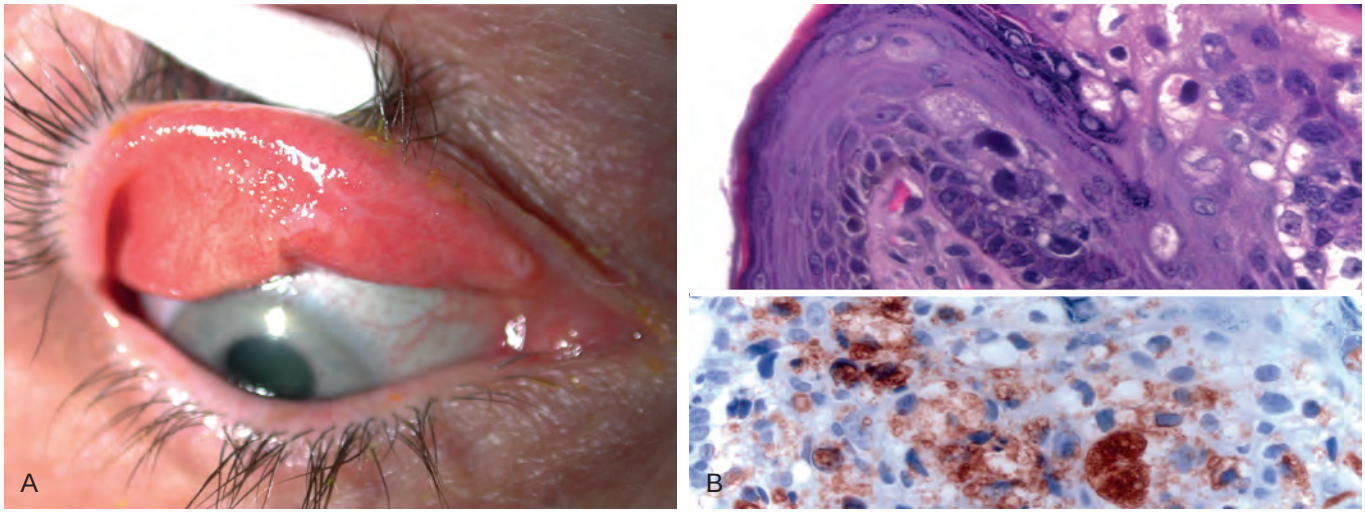
This tumor arises in the meibomian and Zeis glands, most often of the upper eyelids in older patients. Because it is capable of spreading within the conjunctival epithelium ("pagetoid extension"), it can create a unilateral red eye (Fig. 21.2A), mimicking infectious conjunctivitis. However, bacterial and viral infections are usually bilateral. A biopsy of reddened conjunctiva will reveal large atypical cells with frothy vacuolated cytoplasm (see Fig. 21.2A, top panel) that are androgen receptor and adipophilin positive (see Fig. 21.2B, bottom panel).[4]

Intraocular Lymphoma, Leukemia, and Behçet Disease With Hypopyon

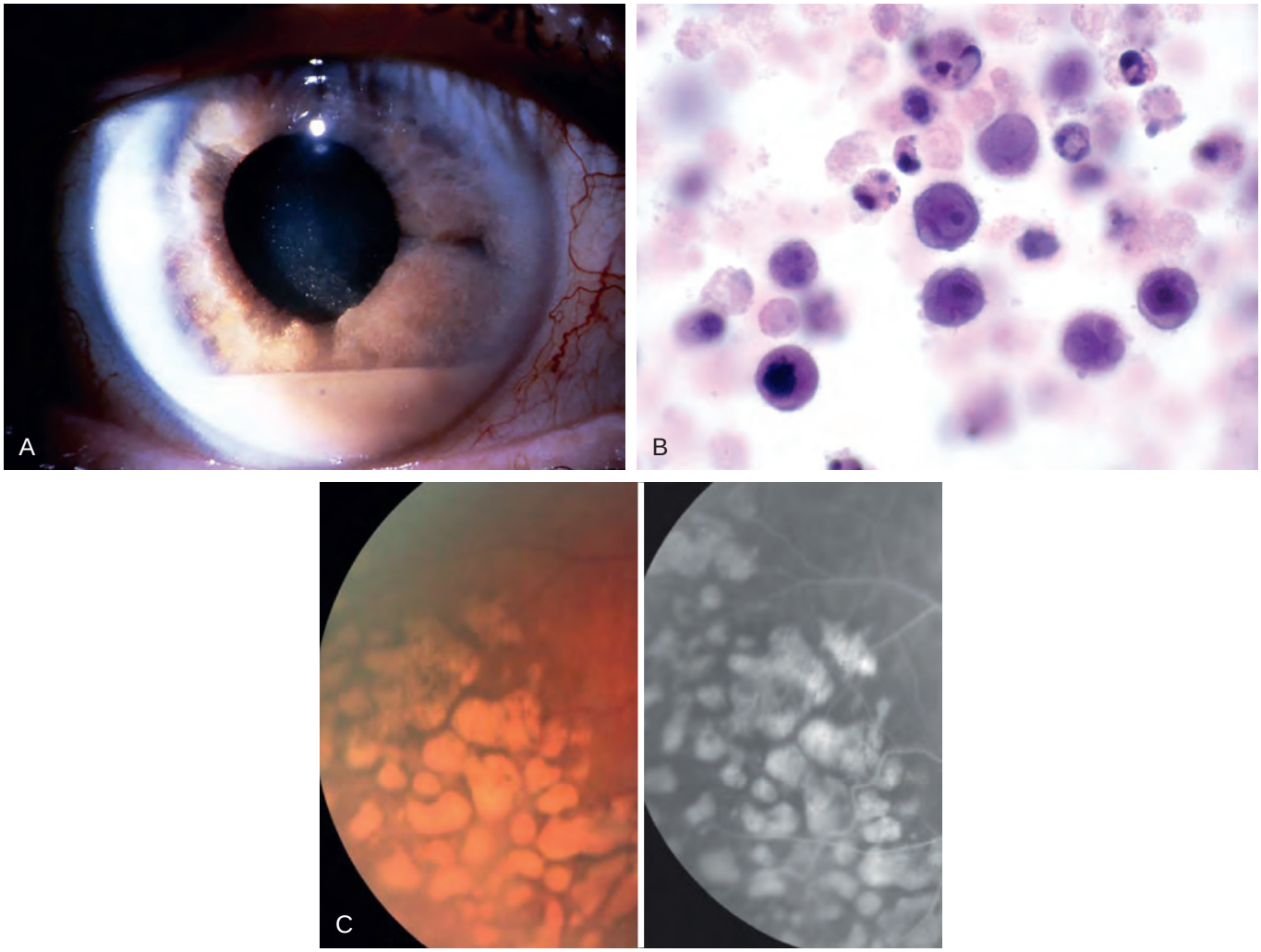
Lymphoma cells involving the retina, vitreous, or iris may seed aqueous of the anterior chamber and settle inferiorly to create a layered pseudohypopyon (Fig. 21.3A).[5] Vitreous and aqueous aspirations studied cytologically can reveal the presence of neoplastic cells or polymorphonuclear leukocytes (see Fig. 21.3B). In these cases the fundus exam may reveal subretinal white, placoid infiltrates (see Fig. 21.3C). Inflammatory disease (e.g., Behçet) or infections conditions (bacterial endophthalmitis) are often associated with hypopyon, but these typically cause an intensely injected and inflamed eye (Fig. 21.4A), whereas a lymphomatous pseudohypopyon is usually encountered in a noninflamed eye. Behçet disease additionally may display retinal vasculitis (see Fig. 21.4B). Retinoblastoma cells also cause pseudohypopyon in some cases, particularly the diffuse infiltrative form that tends to occur in older children. Imaging studies may reveal diagnostic intralesional calcifications.

Necrotic Intraocular Tumors Simulating Panophthalmitis and Orbital Cellulitis

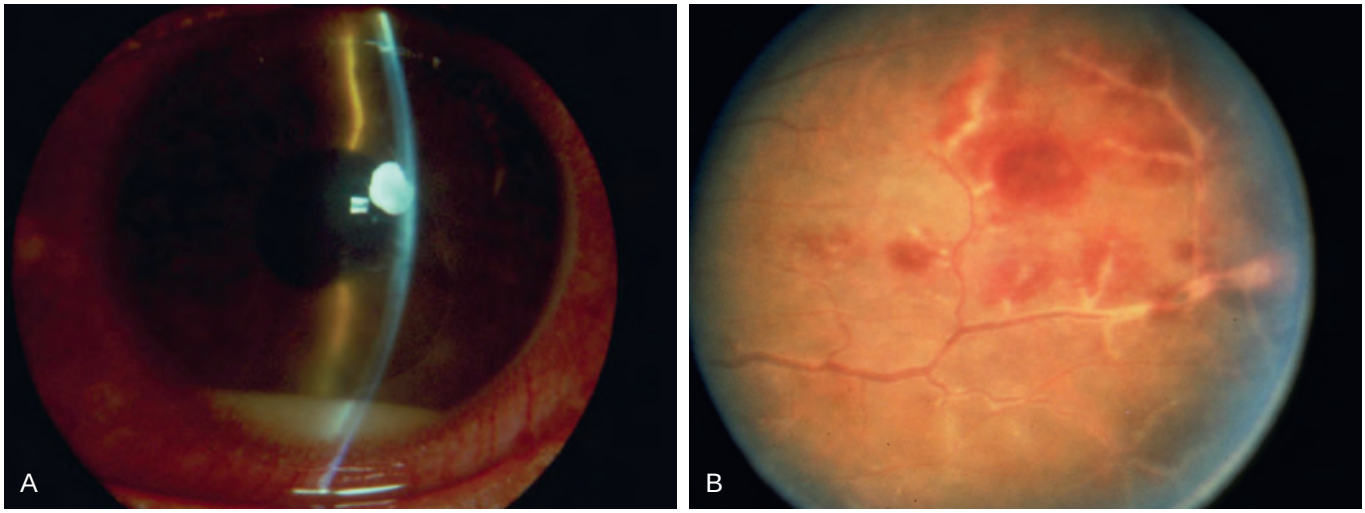
Panophthalmitis (inflammation of all tunics of the eye from retina through sclera into the surrounding orbit) can cause erythema of the eyelids and conjunctival edema with proptosis simulating an infectious orbital cellulitis. Rarely, massive necrosis of a retinoblastoma or uveal melanoma can produce intense periocular inflammation simulating a cellulitis (Fig. 21.5A).[6] Imaging studies, especially ultrasound, can help to differentiate these conditions. Histopathology reveals massive spontaneous tumor necrosis that typically involves other intraocular structures (see Fig. 21.5B and C).[7]



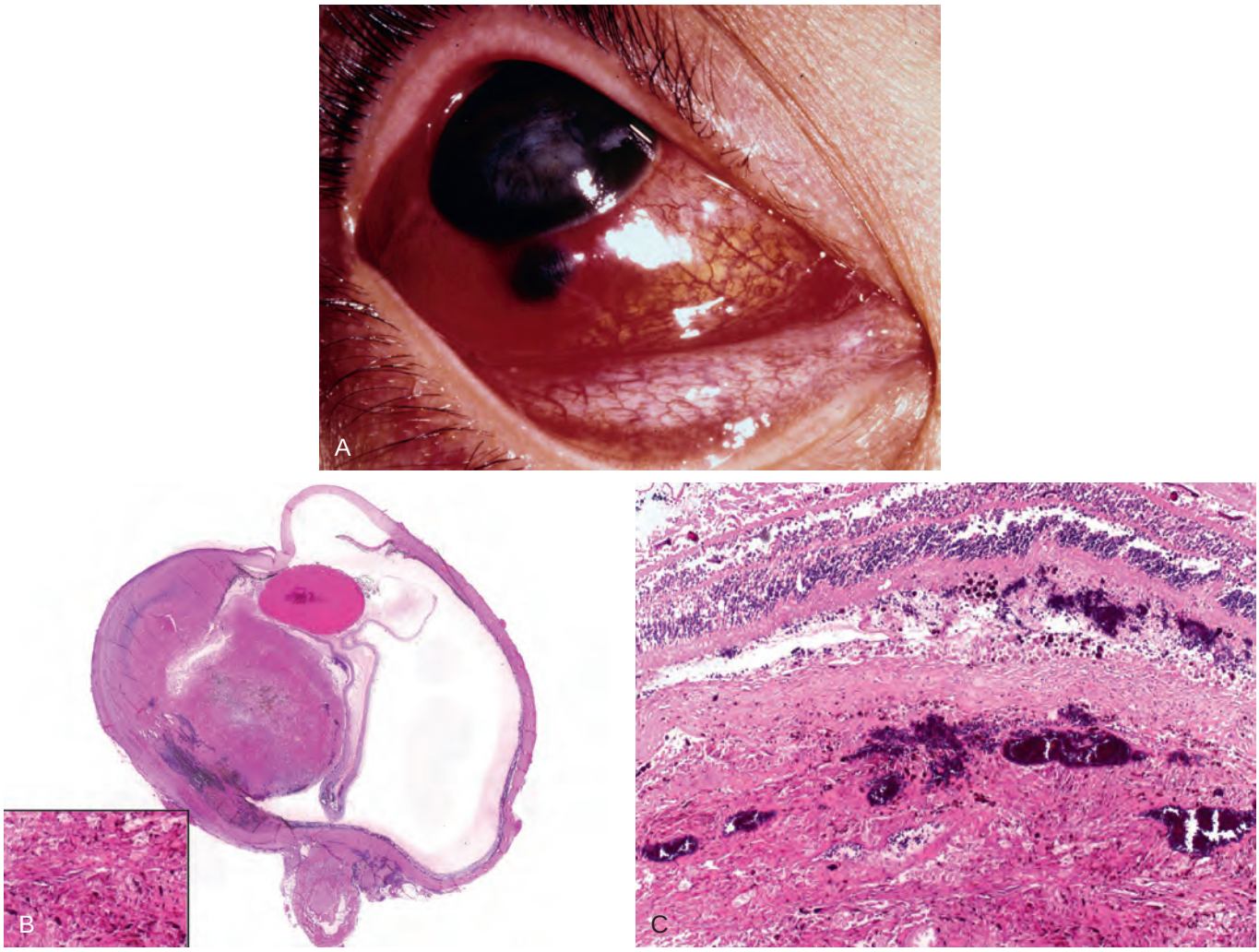
• **Figure 21.2** Intraepithelial sebaceous carcinoma mimicking unilateral infectious blepharoconjunctivitis. **A,** There is unilateral thickening and diffuse erythema of the superior tarsal conjunctiva. **B,** *Top panel*. Individual cells with large, hyperchromatic nuclei and vacuolated cytoplasm spread in a pagetoid fashion though the epithelium at the mucocutaneous junction of the eyelid (×400). *Bottom panel*. Positive immunostaining for adipophilin (×400). (**A,** Courtesy Suzanne Freitag, MD.)



• **Figure 21.3** Intraocular lymphoma mimicking an infectious hypopyon. **A,** A collection of white blood cells has settled in the anterior chamber of this uninflamed, white eye. The iris appears thickened with pinpoint white nodules at the pupil inferiorly. **B,** Aqueous cytology reveals large lymphocytes with irregular, hyperchromatic nuclei and prominent nucleoli, consistent with large cell lymphoma (×600). **C,** Deep, white subretinal plaques consistent with intraocular lymphoma *(left panel)*. The right panel shows an early to midphase fluorescein angiogram with early staining of the lymphoid infiltrates. (Courtesy Dean Eliott, MD.)



• **Figure 21.4** Behçet disease. **A,** A red eye with a sterile hypopyon in Behçet diease. **B,** Active retinal vasculitis (vascular sheathing) with retinal hemorrhage. (**A,** Courtesy Alan Friedman, MD; **B,** Courtesy Narsing Rao, MD.)



• **Figure 21.5** Necrotic choroidal melanoma simulating infectious panophthalmitis. **A,** There is diffuse conjunctival injection, eyelid erythema and a small epibulbar pigmented nodule. **B,** A mushroom-shaped ciliochoroidal tumor has undergone complete necrosis (×10). The *inset* (×200) shows amorphous eosinophilic debris; no cellular detail remains. **C,** A choroidal tumor beneath the retina has undergone complete coagulative necrosis; free melanin released from the pigment epithelium is also seen (×50). (**A** to **C,** Courtesy Hans Grossniklaus, MD.)

Conjunctival Infections

Conjunctivitis is a common ophthalmic condition that is largely infectious in nature. It is often diagnosed solely based on clinical examination. Bacterial conjunctivitis is the most frequent culprit in children; in adults, viral disease predominates. A follicular response (formation of lymphoid follicles histopathologically) is often seen in both cases. Impression or exfoliative cytology can occasionally be helpful in making a specific diagnosis. Biopsy with histopathologic examination is required for comparatively rare diseases, such as fungal conjunctivitis.

Bacterial Infections

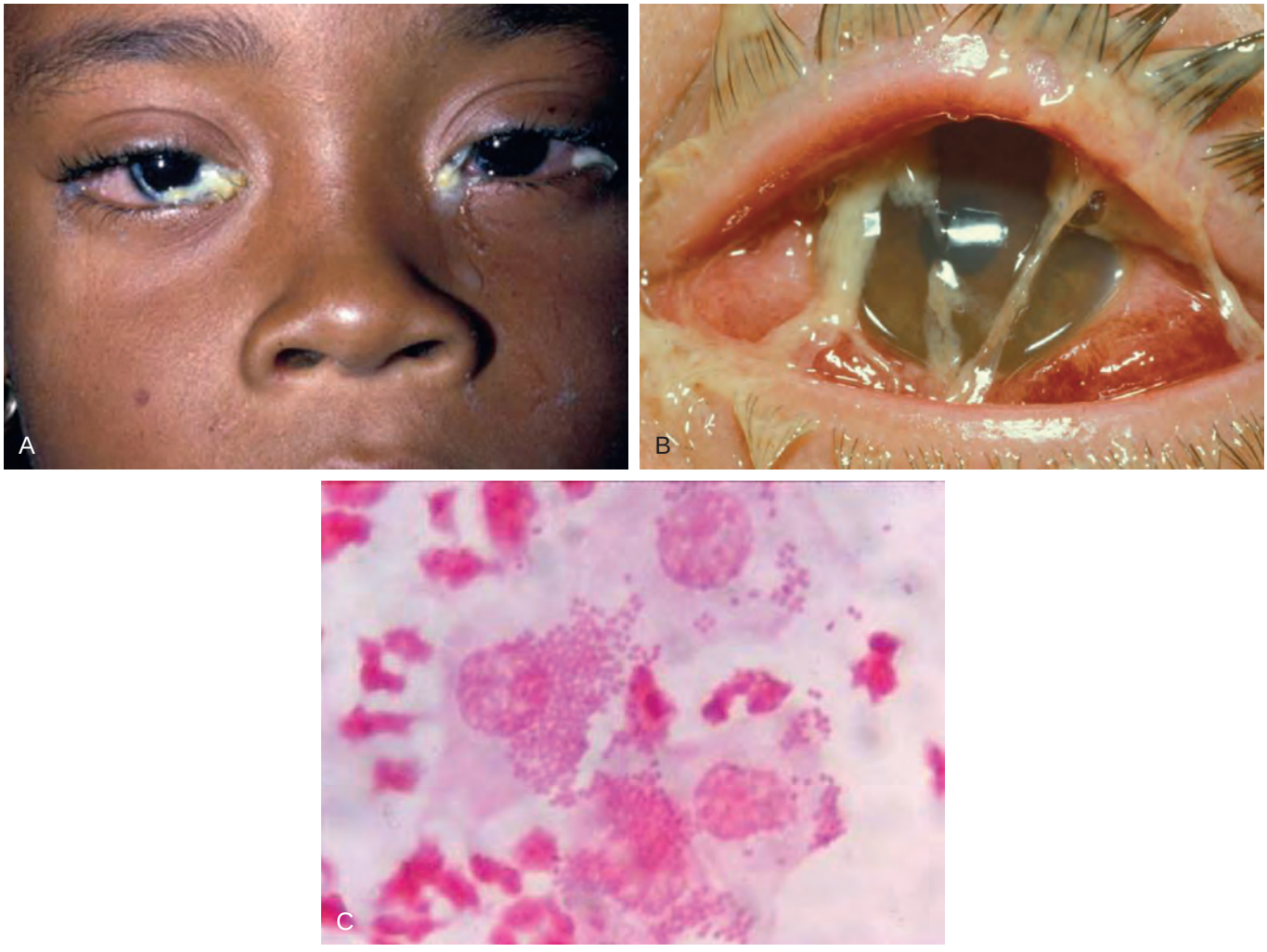
Common Bacterial Conjunctivitis

Bacterial conjunctivitis shows a predilection for children (Fig. 21.6A) and presents with purulent discharge and conjunctival injection, often bilaterally. The most common causative organisms are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.[8] In adults, various staphylococcal species predominate as etiologic agents, followed by streptococci and *H. influenzae*.

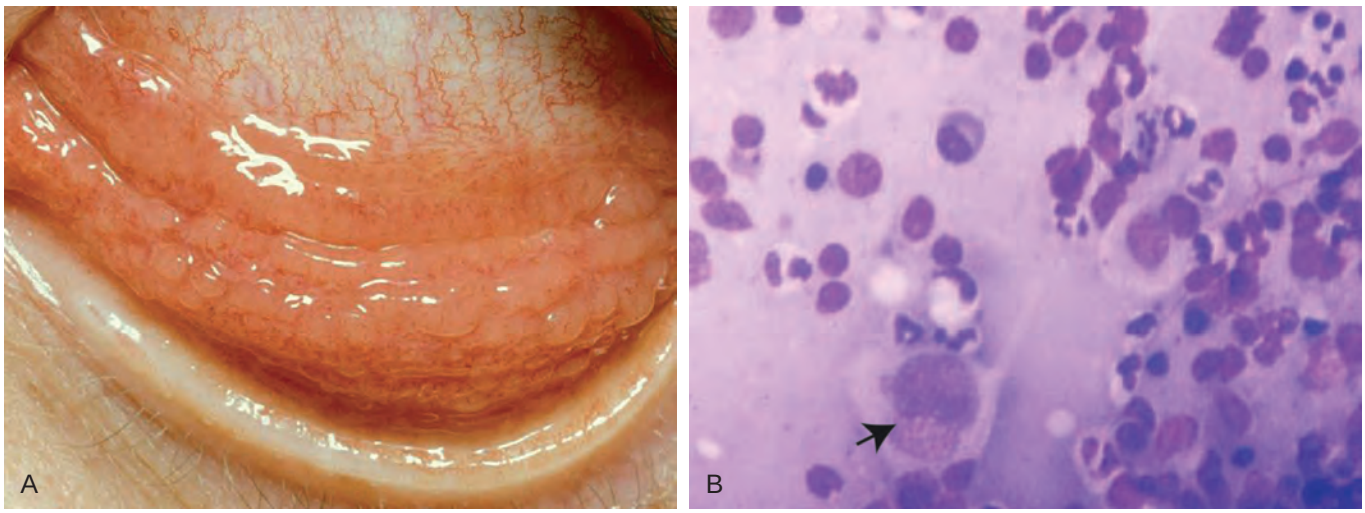
Of special interest is *Neisseria gonorrhoeae*,[9] an important cause of conjunctivitis in sexually active adults or neonates with direct genital-eye or genital-hand-eye contact. An infected maternal birth canal can cause a dramatic discharge in conjunctivitis neonatorum. Adult patients display a hyperacute presentation with intense purulence (see Fig. 21.6B). Infected infants and adults should receive oral or intravenous therapy because of the risk of corneal perforation. Conjunctival smears may reveal intracellular gram-negative cocci (see Fig. 21.6C); swabs sent for culture and polymerase chain reaction (PCR) are confirmatory.

Infections of the sclera are rare (inflammatory scleritis is much more likely to be autoimmune in nature), but such organisms as *Pseudomonas* may result in necrotizing scleritis with overlying conjunctivitis, especially after surgery; an abscess may form leading to ocular perforation.[10,11]

Chlamydia trachomatis causes a variety of conjunctivitis syndromes, depending on the serotype. Trachoma (serotypes A to C) is a chronic keratoconjunctivitis that results in severe conjunctival and corneal scarring and is a leading cause of blindness worldwide. More often seen in the Western world is acute chlamydial inclusion conjunctivitis from direct or indirect contact with genital secretions (serotypes D to K which cause genital infections).



• **Figure 21.6** Bacterial conjunctivitis. **A,** *S. aureus* conjunctivitis. This child developed a sequential, bilateral purulent conjunctivitis. **B,** *N. gonorrhoeae* conjunctivitis. Hyperacute, intensely purulent conjunctivitis with conjunctival chemosis in a sexually active adult. **C,** Conjunctival cytology demonstrates intracellular, gram-negative cocci (×600). (**A** and **B,** Courtesy Dan Jones, MD; **C,** Courtesy Susan Stenson, MD.)



• **Figure 21.7** Chlamydial conjunctivitis **A,** Diffuse lymphoid follicle formation of the inferior fornix (follicular conjunctivitis) in a patient with inclusion conjunctivitis. **B,** Conjunctival smear reveals plasma cells, polymorphonuclear leukocytes, and conjunctival epithelial cells with cytoplasmic inclusions (Halberstaedter and Prowazek bodies, *arrow*) (×600). (**A,** Courtesy Dan Jones, MD; **B,** Courtesy Susan Stenson, MD.)

Patients exhibit a follicular conjunctivitis (Fig. 21.7A) but with scant mucopurulent discharge and a relatively chronic course. Giemsa-stained conjunctival smears characteristically reveal many polymorphonuclear cells, with admixed mononuclear and plasma cells. Diagnostic and characteristic cytoplasmic inclusion bodies (so-called Halberstaedter and Prowazek bodies; see Fig. 21.7B) will be found along with gram-negative intracellular diplococci.

Cat-Scratch Disease and Granulomatous Conjunctivitis

Granulomatous conjunctivitis (Fig. 21.8A) with follicle formation and regional lymphadenopathy is known as *Parinaud oculoglandular syndrome.* The clinical syndrome is most commonly caused by severe non-granulomatous viral conjunctivitis while its granulomatous counterpart is most often a manifestation of cat-scratch disease due to *Barontella henselae* (and less commonly to tularemia, sporotrichosis, or other *Bartonella* species). Approximately 5% to 10% of patients developing cat-scratch disease, usually transmitted by a flea-infested kitten, have granulomatous conjunctival involvement. Serologic testing for *B. hensalae* antibodies or PCR can confirm the diagnosis.[12] Biopsy of conjunctival granulomas (of either the tarsal or bulbar conjunctiva) reveals clumps of slender gram-negative rods, which are also highlighted with the Warthin-Starry silver stain. The infectious agents are often discovered surrounding blood vessel walls (see Fig. 21.8B). Necrotizing granulomatous inflammation with microabscess formation is a common finding, along with an endothelial cell proliferation (see Fig. 21.8C).[13] Syphilitic conjunctivitis as a manifestation of secondary syphilis can also rarely cause a granulomatous conjunctivitis (Fig. 21.9A).[14] Spirochetes may also be visualized with silver staining of conjunctival tissue (see Fig. 21.9B).

Viral Infections

Adenoviral Conjunctivitis

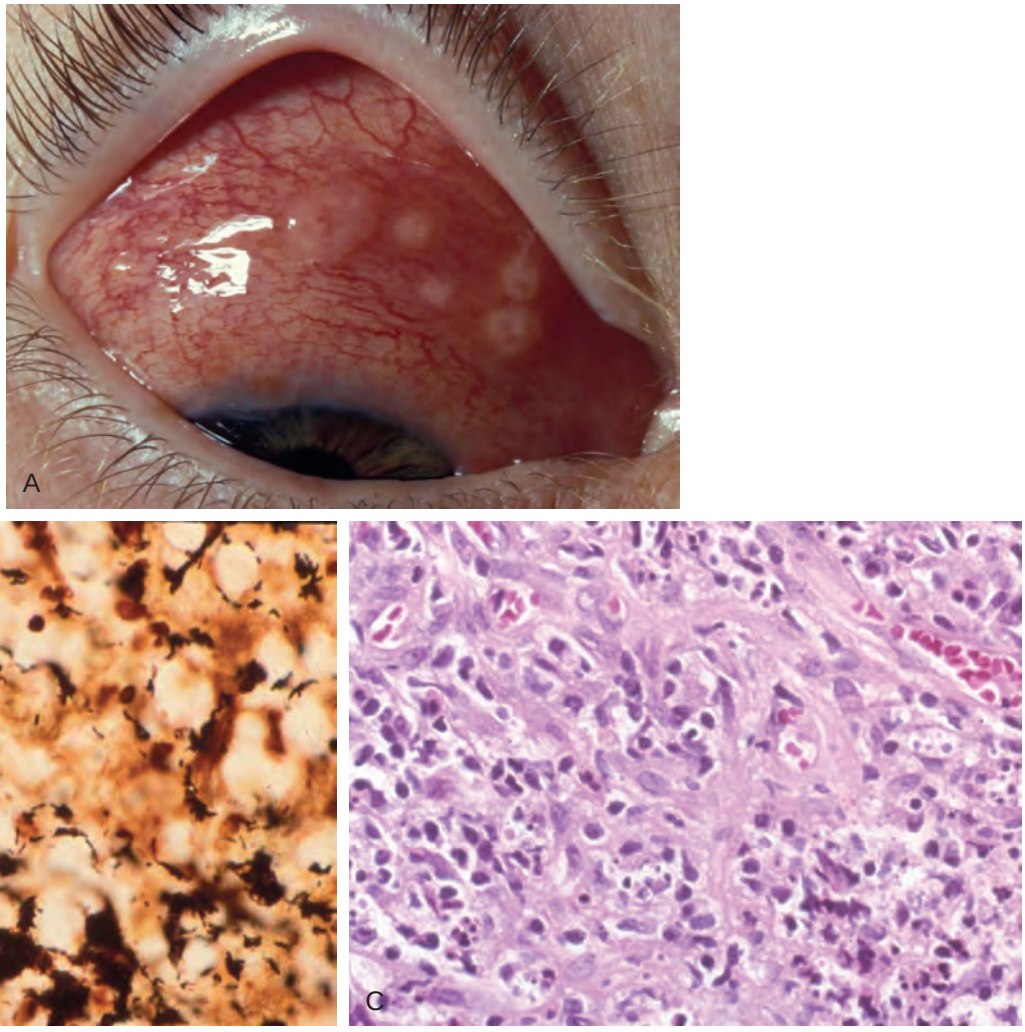
Various serotypes of adenovirus, a double-stranded DNA virus, can cause a simple, self-limited follicular conjunctivitis for which microbiologic or histopathologic examination is rarely performed. Adenoviral keratoconjunctivitis, especially serotype D, is responsible for so-called endemic keratoconjunctivitis or pink-eye infections that spread rapidly through contaminated ocular secretions in populations living or working together. Serotypes 8, 19, and 37 cause bilateral severe follicular conjunctivitis and can coexist with epithelial keratitis. There is prominent accompanying conjunctiva edema (chemosis) and prominent preauricular adenopathy.[15] Later, immunologically mediated multifocal stromal corneal infiltrates often develop. Enterovirus and coxsackievirus infections may cause a contagious hemorrhagic conjunctivitis.

A rapid immunodetection assay is often used to confirm the diagnosis, and PCR can be used to determine the serotype. Conjunctival pseudomembranes can form in cases of endemic conjunctivitis and may need to be peeled from the ocular surface for comfort. Histopathologically the pseudomembranes are composed of fibrin and many polymorphonuclear leukocytes without identifiable organisms.[16] Cytologic preparations of adenoviral conjunctivitis reveal many lymphocytes along with two types of conjunctival epithelial nuclear alterations: intranuclear inclusions and a so-called ground-glass nuclear appearance.[17]

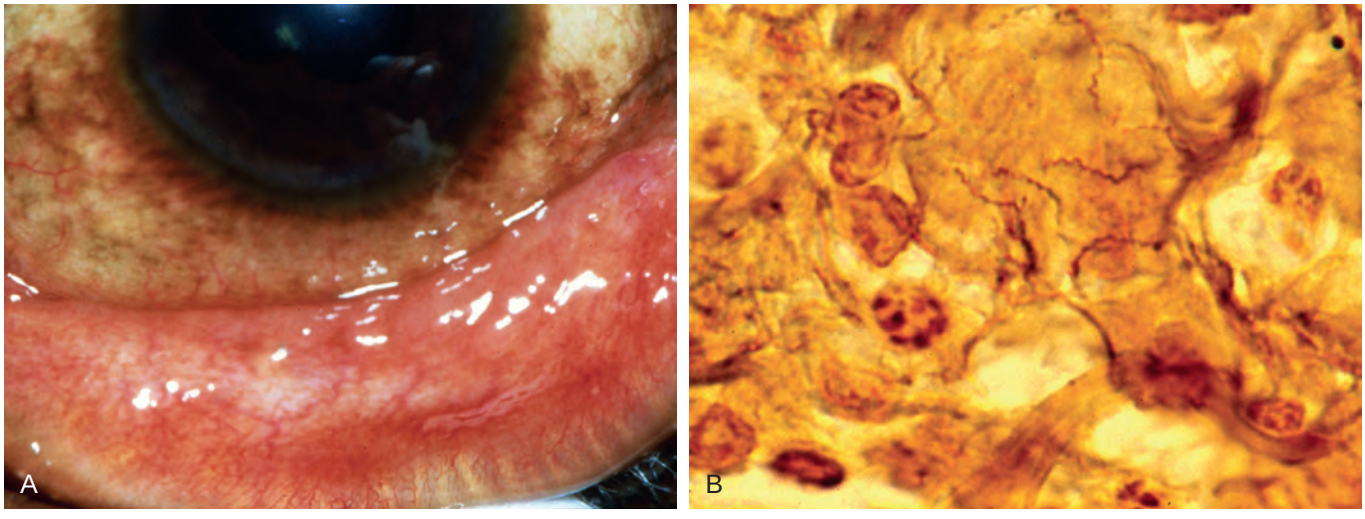
Herpesvirus Conjunctivitis

Because herpes simplex virus (HSV) infection is ubiquitous in humans and can infect the trigeminal ganglion, ocular herpetic disease can ensue and is an important cause of ocular morbidity and vision loss due to involvement of various ocular and adnexal sites (these manifestations are discussed later under viral keratitis and viral chorioretinitis). However, primary infection with HSV usually manifests as a unilateral blepharoconjunctivitis. The conjunctivitis is follicular in nature and often associated with a palpable preauricular lymph node and vesicles on the eyelid skin or at the eyelid margin. Ulcers can also develop on the bulbar conjunctiva, which is mildly injected.

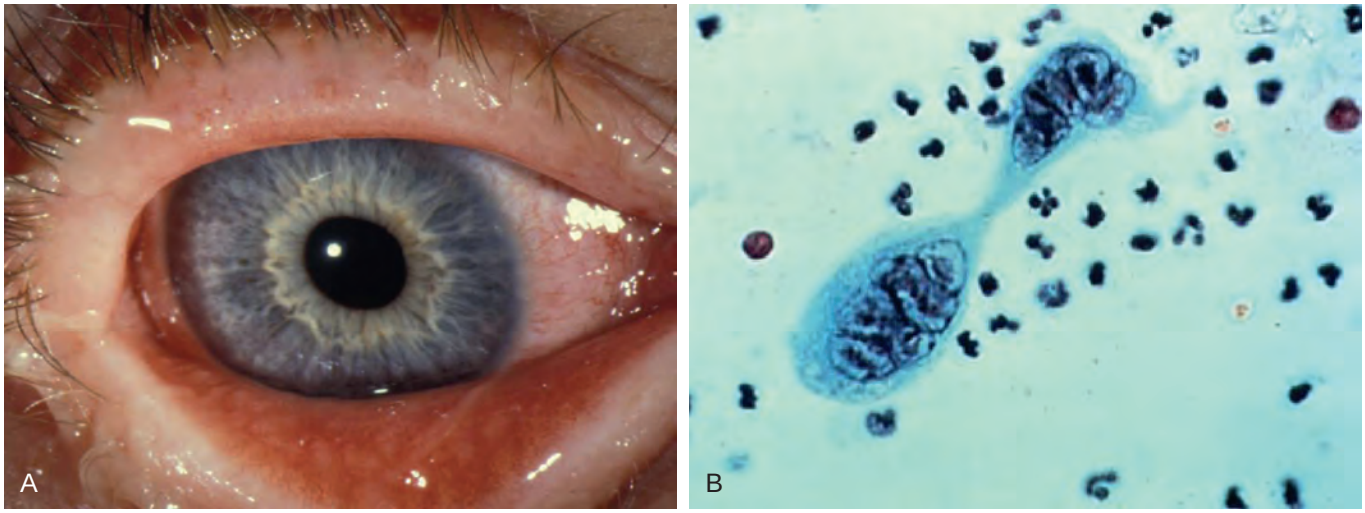
HSV conjunctivitis is usually unilateral, as opposed to adenoviral or bacterial conjunctivitis, and, in contrast to the latter's purulent discharge, a watery or slightly mucoid discharge is observed (Fig. 21.10A). Conjunctival scrapings and impression



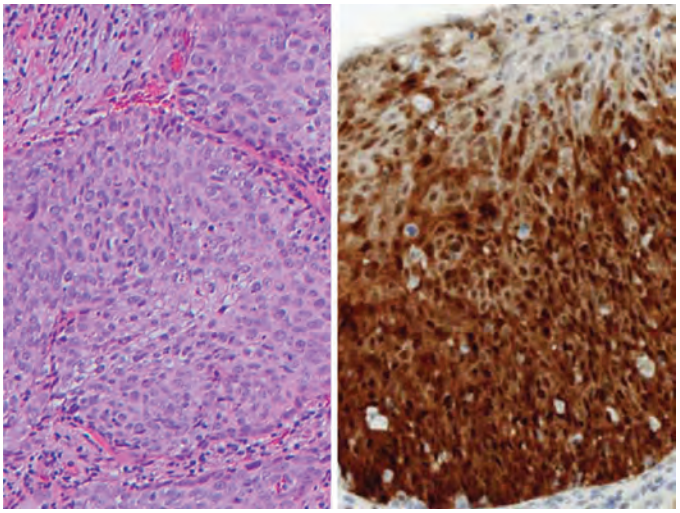
• **Figure 21.8** Cat-scratch disease. **A,** *Bartonella henselae* can manifest as Parinaud oculoglandular syndrome (conjunctivitis and swelling of the parotid gland from involved lymph nodes). There are multiple epibulbar granulomas. **B,** Warthin-Starry stain demonstrates slender rods in aggregates around blood vessels (×400). **C,** An endothelial cell proliferation with admixed histiocytes constitutes a conjunctival granuloma (×200). (**A** and **B,** Courtesy Dan Jones, MD.)



• **Figure 21.9** Syphilitic conjunctivitis. **A,** Diffuse and nodular thickening of the lower palpebral conjunctiva (granulomatous conjunctivitis). **B,** Warthin-Starry silver stain allows visualization of spirochetes in the conjunctival biopsy (×600).



• **Figure 21.10** Herpes simplex conjunctivitis. **A,** Blepharoconjunctivitis in a child with primary herpes simplex virus infection has caused mild conjunctival injection and a watery, slightly mucoid discharge. **B,** Conjunctival smear reveals a syncytium of multinucleated epithelial cells showing nuclear molding characteristic of herpes virus infection; polymorphonuclear cells are also seen (×400). (**A,** Courtesy Dan Jones, MD; **B,** Courtesy Susan Stenson, MD.)



• **Figure 21.11** Human papillomavirus related squamous cell carcinoma of the conjunctiva. **A,** Conjunctival squamous cell carcinoma in situ. **B,** Diffuse positive nuclear and cytoplasmic immunoreactivity for p16.

cytology of the conjunctiva may reveal characteristic multinucleated epithelial cells (see Fig. 21.10B).[18] Herpes antigens may be detected from impression cytology specimens.[19] Herpes viruses may also cause chronic scleritis, with nonspecific chronic inflammation of the sclera that is sometimes granulomatous. Immunoperoxidase staining is helpful in identifying the culprit in these unusual cases.[20]

Human Papillomavirus

Human papillomavirus (HPV) has been implicated in the development of conjunctival squamous cell carcinoma (Fig. 21.11) in approximately 30% of cases.[21-23] HPV type 16, as in oropharyngeal and cervical carcinoma, is the most common strain isolated with PCR.

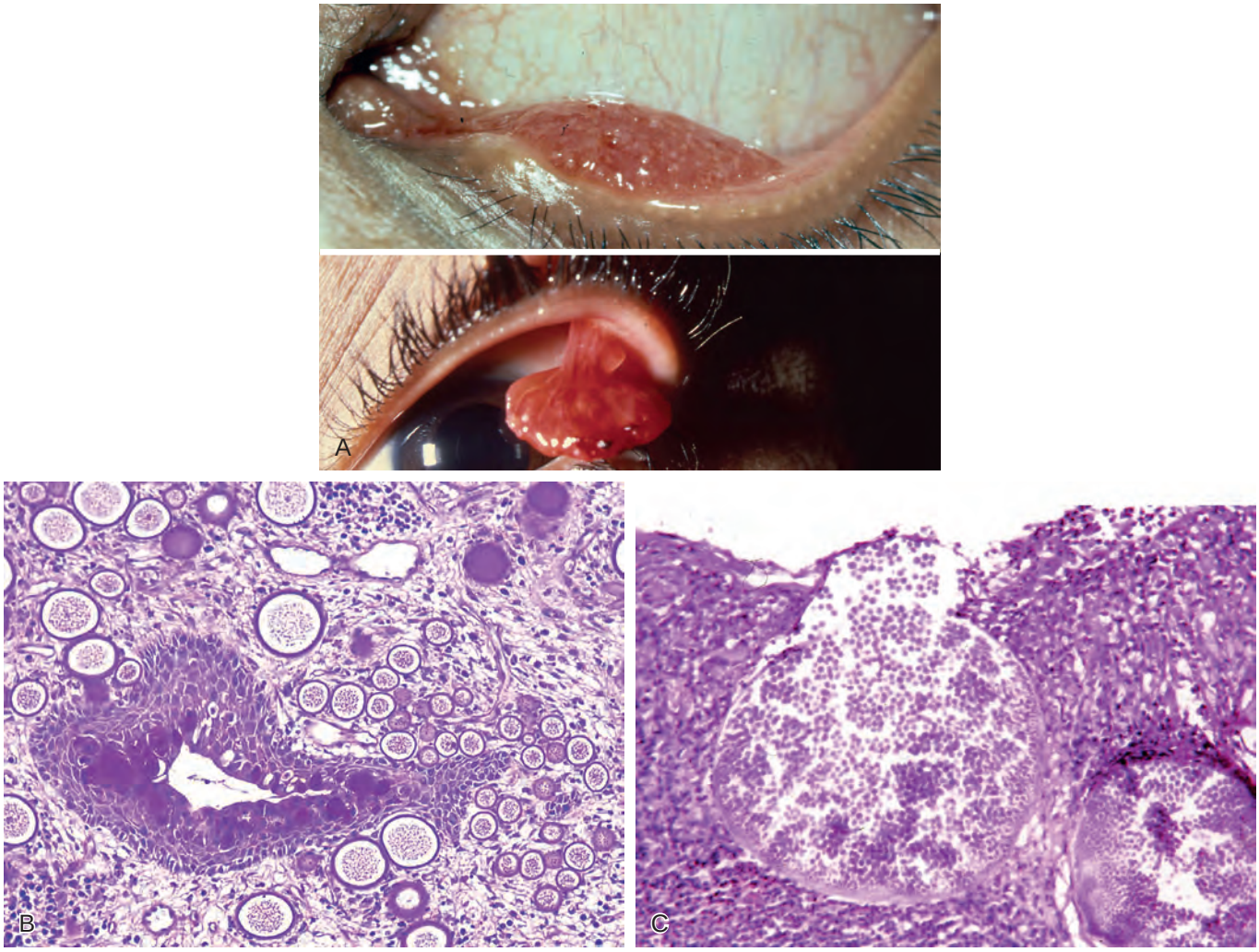
Fungal Infections

Rhinosporidiosis

Rhinosporidiosis is caused by infection with the fungus *Rhinosporidium seeberi*, which leads to the formation of a distinctive highly vascular, polypoidal, or pedunculated mass of the mucous membranes studded with white, subepithelial sporangia (Fig. 21.12A).[24] By far the most commonly infected site is the nasal mucosa. Infections are acquired by patients in warm, humid climates; 90% of cases occur in India and Pakistan. Infection probably develops as a result of direct exposure to stagnant water, although inhalation of spores may also play a role. Although rare, the bulbar conjunctiva is the second most commonly involved site of infection. Histopathologically, unique-appearing periodic acid–Schiff (PAS)-positive spherical sporangia of various sizes can be seen within a granulomatous inflammatory background (see Fig. 21.12B). As the organism matures, eventually reaching approximately 300 μm, a mature sporangium forms a pore through which mature spores (approximately 7 to 10 μm) are discharged (see Fig. 21.12C). As the life cycle repeats, the characteristic vascular mass grows. Complete excision of the sessile or pedunculated red mass is curative.[25]

Other Rare Fungal Conjunctivitis

Ocular cryptococcosis, an infection of predominantly patients with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), is rare and most commonly manifests in the posterior segment (discussed in further detail under intraocular fungal infections). Atypical cases of anterior segment involvement including conjunctival granulomas simulating squamous cell carcinoma have been reported.[26] Coccidiomycosis, which can cause a fulminant endophthalmitis (also see intraocular fungal infections later), has rarely been described to cause conjunctival or eyelid granulomas.[27] Microsporidiosis of the conjunctiva, initially thought to be a protozoal infection but has been reclassified as a fungal disease, was described in HIV/AIDS patients in the late 1980s and early 1990s as a bilateral, recalcitrant watery



• **Figure 21.12** Conjunctival rhinosporidiosis. **A,** Sessile *(top panel)* and pedunculated *(bottom panel)* vascular conjunctival masses with pinpoint, white subepithelial nodules. **B,** Multiple periodic acid–Schiff (PAS)-positive sporangia in the conjunctival substantia propria; also note the PAS-positive goblet cells within an invagination of surface epithelium constituting a pseudogland of Henle (×100). **C,** A mature sporangium has formed a surface pore through which spores are released (×200).

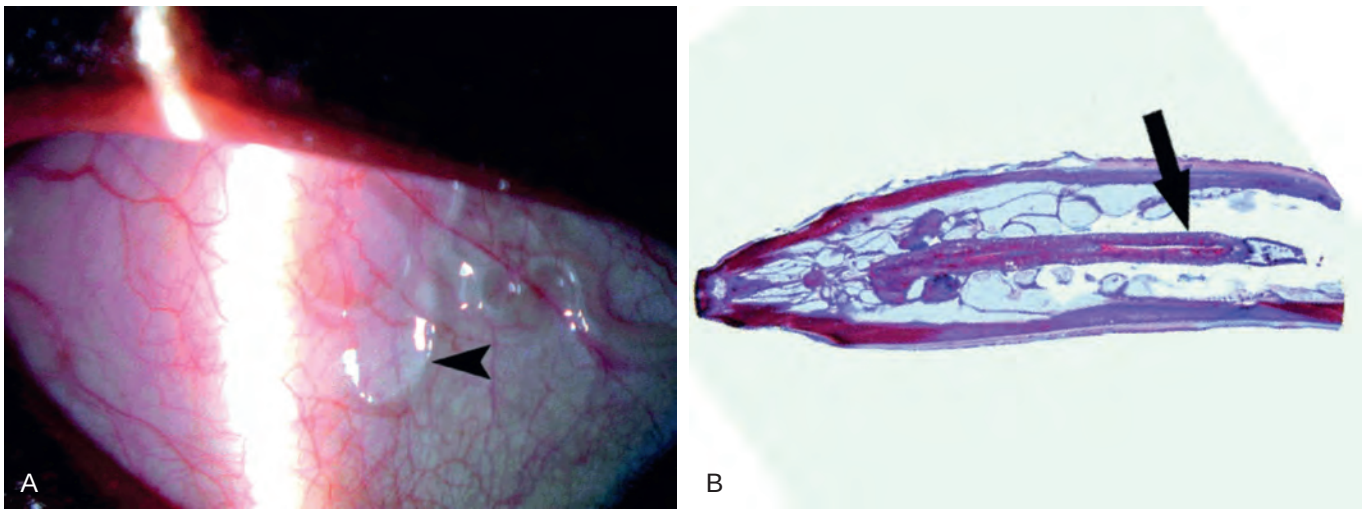
conjunctivitis with an associated coarse, epithelial keratitis (see also fungal keratitis later).[28] Conjunctival scrapings for cytology in conjunctival infections reveal numerous large (larger than bacteria) gram-positive, acid fast–positive, Giemsa-positive ovoid organisms within conjunctival epithelial cells and extracellularly.[29] The spores have ultrastructurally been found to measure 2×1 μm.[30]

Parasitic Infections

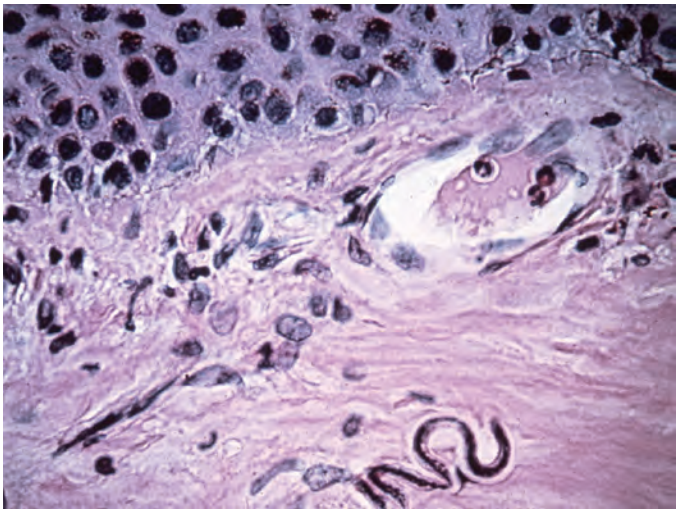
Loiasis and Onchocerciasis

Ocular loiasis is caused by *Loa loa*, a nematode for which the human is the definitive host. *L. loa* is endemic in West Africa and is transmitted to humans by infected mangrove *(Chrysops)* flies that are intermediate hosts for microfilaria.[31] Adult worms migrate through subcutaneous tissues, causing transient pruritic edema, especially in the periocular region; intense peripheral blood eosinophilia and microfilaremia are also seen. The adult worm can be visualized moving through the subconjunctival space (Fig. 21.13A), and patients nearly always present with the complaint of a sensation of something moving in or around the eye, mimicking common myokymia. Worms may also rarely be found in the periocular subcutaneous tissues or in the anterior chamber of the eye. The mature worm usually appears many years after initial inoculation and can be surgically removed[32] and examined histopathologically, revealing a tapered head and central esophagus (see Fig. 21.13B). Systemic treatment is also initiated for microfilaremia.

Another nematode, *Onchocerca volvulus*, also has a tropism for ocular tissues and can rarely be found crawling in the subconjunctival space. Posterior segment chorioretinitis and scarring also contribute to blindness from *O. volvulus*. This process occurs not due to the migration of live organisms through the cornea but instead to microfilarial death that provokes an intense inflammatory response. Larvae may also be encountered swimming in the anterior chamber. Histopathologically, again,



• **Figure 21.13** Ocular loiasis. **A,** Slit lamp examination reveals a motile subconjunctival worm *(arrowhead)* approximately 0.5 mm in diameter. **B,** The surgically excised female worm has tapered head and central esophagus *(arrow)* (×10) (**A** and **B,** Modified from Nam JN, Reddy S, Charles NC. Surgical management of conjunctival loiasis. *Ophthal Plast Reconstr Surg*. 2008;24:316-317).



• **Figure 21.14** Onchocerciasis. An intact microfilaria is seen in the substantia propria at the corneal limbus with mild surrounding inflammatory reaction (×400).

minimal inflammatory response is seen to live microfilariae (Fig. 21.14), but a chronic, nongranulomatous response forms around degenerating organisms. This leads to corneal stromal scarring and vascularization (so-called interstitial keratitis).[33]

Corneal Infections

Despite numerous mechanisms in place to prevent infection, including the antibacterial components of the tear film and the mechanical barrier of the rapidly cycling corneal epithelium, a variety of infectious diseases can take up residence within the cornea, especially in susceptible situations with epithelial breakdown. These include chronic exposure, accidental trauma, surgery, trichiasis, and increasingly contact lens wear.[34]

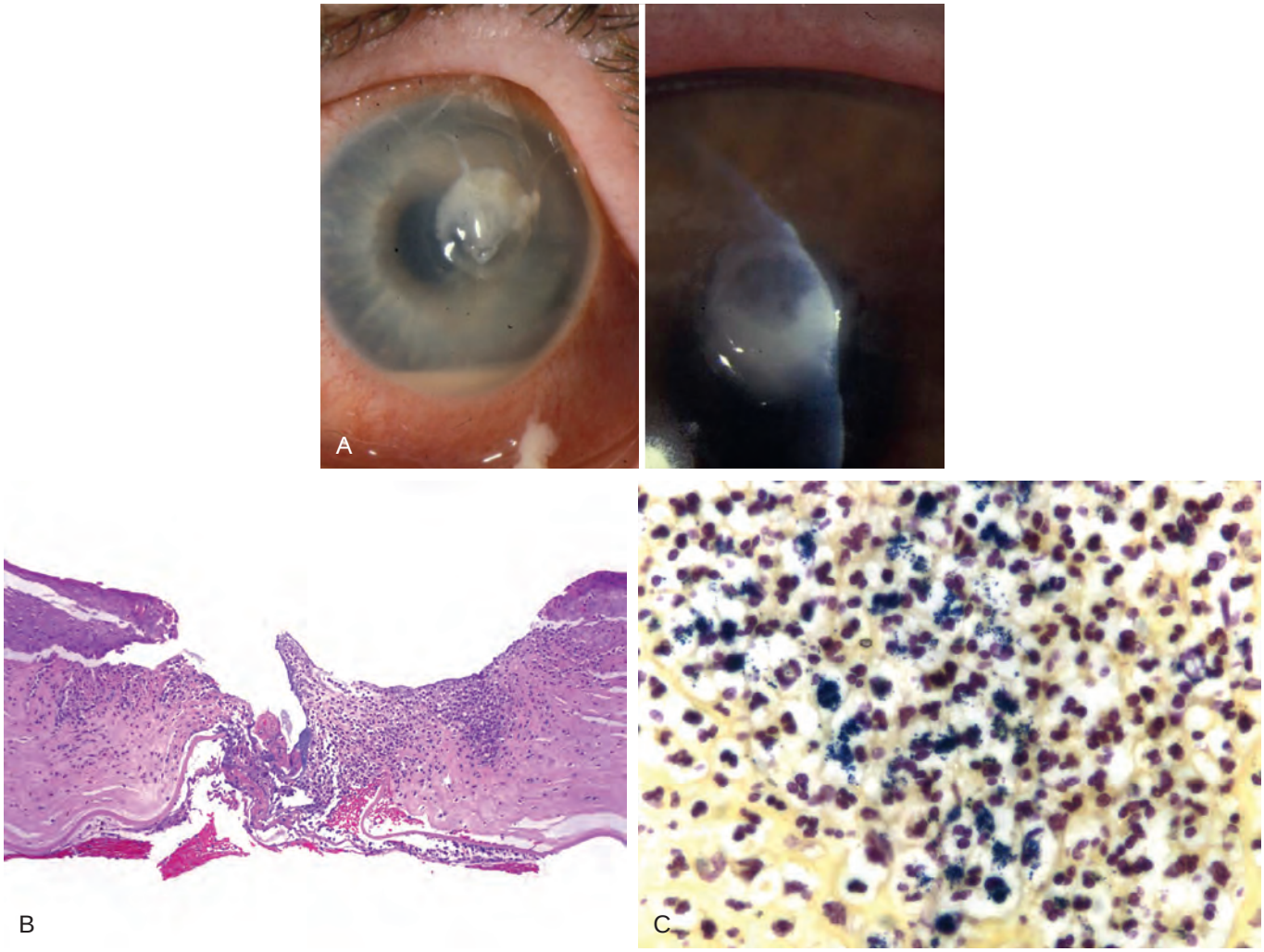
Bacterial Infections

Classic Bacterial Keratitis With Corneal Ulcer

This condition presents acutely, with severe pain, decreased vision, and photophobia. On examination, an epithelial defect is present with a white, purulent infiltrate of the corneal stroma (Fig. 21.15A).[35] Although most bacteria rely on a disruption of the corneal epithelium to cause disease, exceptions include *Neisseria*, *Corynebacterium*, and *Shigella* species. The epithelial abrasion allows species normally inhabiting the skin, mucous membranes, upper respiratory, and digestive tracts, such as gram-positive staphylococci, streptococci, and enterococci, to become pathogenic. Histopathologically, the classic infectious bacterial corneal ulcer exhibits loss of the corneal epithelium and destruction of Bowman layer with dense acute keratitis and a variable amount of stromal loss and necrosis. Acute stromal keratitis with accompanying collagenolysis may extend deep, culminating in a perforation of Descemet membrane (see Fig. 21.15B and C), necessitating emergency surgery. Gram-negative infections are usually more fulminant, especially those with the gram-negative rod *Pseudomonas aeruginosa*, a common pathogen isolated from corneal ulcers in contact lens wearers that may result in a densely suppurative and accelerating keratitis with early corneal melting and perforation.[36]

Infectious Crystalline Keratopathy

A unique clinical and histopathologic variant of bacterial keratitis known as infectious crystalline keratopathy is caused by fastidious organisms (often *Steptococcus viridans*) in patients with compromised corneal immunity (i.e., corneal transplant patients on chronic topic immunosuppression).[37] No true crystals form, but the opacity has a crystalloid appearance clinically. Histopathologically, crystalline keratopathy is characterized by dense gram-positive bacterial colonies in the corneal interlamellar spaces with a pronounced absence of surrounding inflammation (Fig. 21.16A and B). It is thought that the bacteria are sequestered by a polysaccharide-rich glycocalyx consistent with a biofilm. The overlying epithelium is often intact, rendering the precise



• **Figure 21.15** Bacterial corneal ulcer. **A,** *Left panel*. A well-delineated, white, purulent paracentral corneal infiltrate is present in an infected eye; a small hypopyon is also seen. *Right panel*. The ulcer is seen at higher power with a slit beam. **B,** Corneal ulcer with central loss of the epithelium and Bowman layer; there is acute keratitis and stromal collagenolysis leading to perforation of Descemet membrane (×100). **C,** Gram-positive cocci in clusters within the acutely inflamed corneal stroma (×400). (**A,** Courtesy Dan Jones, MD.)

organismal diagnosis of the infectious process difficult. A repeat corneal transplantation together with long-term postoperative antibiotic therapy may be necessary for cure.

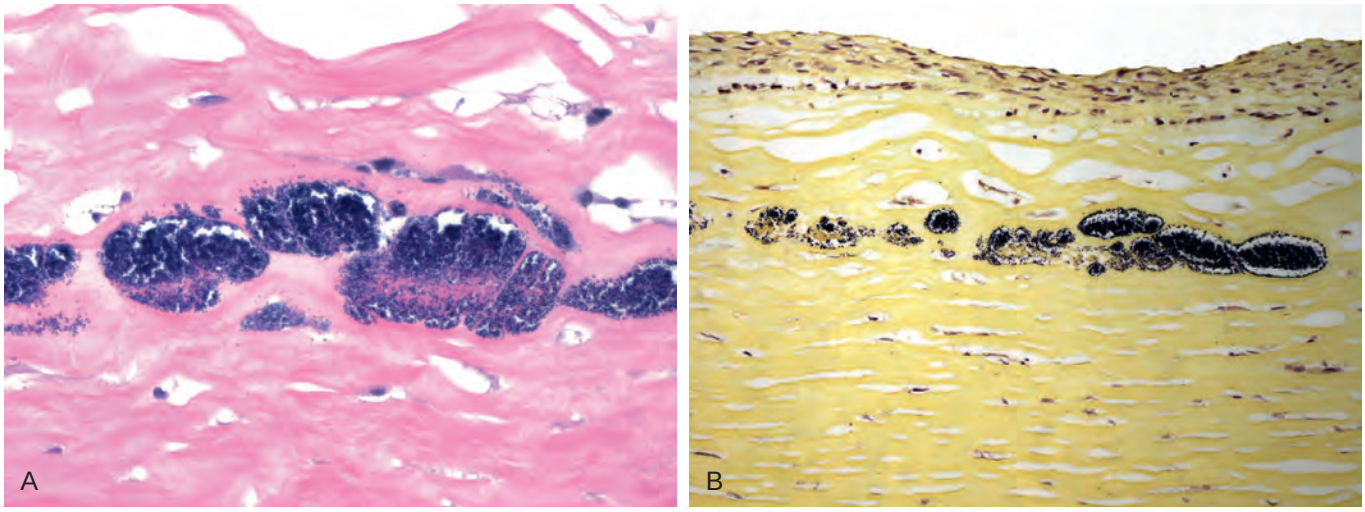
Mycobacterial Keratitis

Isolated mycobacterium tuberculosis infections of the cornea are uncommon (the uvea is the most common site of ocular tuberculosis infection). However, atypical mycobacterial infections (most commonly *Mycobacterium chelonae*, followed by *Mycobacterium abscessus*)[38] have been reported in a number of post-refractive surgery eyes (especially after laser-assisted in situ keratomileusis [LASIK]) and are the most common cause of infectious keratitis following LASIK in some series.[39] The clinical appearance may resemble crystalline keratopathy (discussed previously). Acid-fast bacteria are seen in small clusters within the stroma (Fig. 21.17A). Infectious involvement of the cornea by *Mycobacterium leprae* can manifest with nonspecific findings, such as fibrovascular pannus formation and interstitial keratitis. However, specific to the disease is the appearance of enlarged and opacified corneal nerves, resulting in corneal anesthesia and neurotrophic keratopathy, as also seen in herpetic disease.[40] Finally, limbal masses can form, resembling fibrous histiocytoma. Termed "corneal lepromas," they are composed of foamy histiocytes known as Virchow cells containing clusters of numerous acid-fast organisms (see Fig. 21.17B).[41,42]

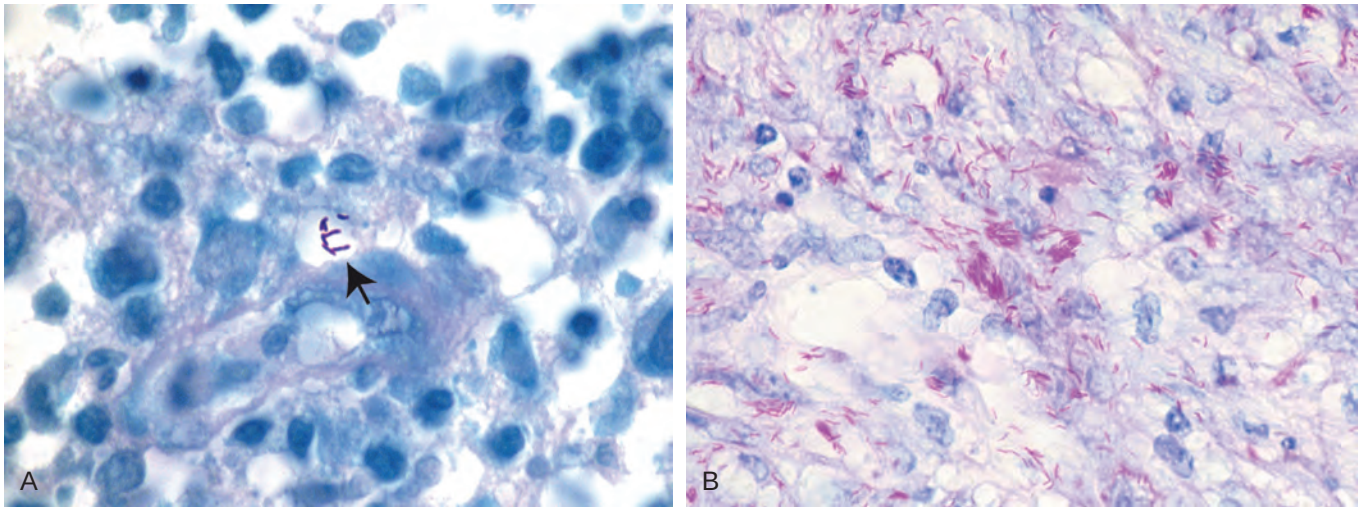
Viral Infections

Herpetic Keratitis

HSV manifests in myriad ways within the eye and in its surrounding tissues. In the cornea, most patients manifest with acute hypesthetic epithelial keratitis in a clinically classic arborizing pattern termed a dendrite (Fig. 21.18A, right panel, and B). Histopathologically, the epithelial lesion is composed of swollen, rounded epithelial cells forming vesicles due to their loss of intercellular attachments and syncytia of cells producing shallow ulceration and underlying destruction of Bowman membrane. Chronic stromal keratitis (see Fig. 21.18A, left panel) leads to



• **Figure 21.16** Infectious crystalline keratopathy. **A,** Dense aggregates of bacteria in the interlamellar corneal spaces with lack of surrounding inflammatory response (×400). **B,** Gram-positive organisms, usually *S. viridans*, are typically isolated (×200).



• **Figure 21.17** Mycobacterial keratitis. **A,** The *arrow* indicates clustered acid-fast bacilli (atypical mycobacteria) in a keratoplasty specimen (×400). **B,** Virchow cells (foamy histiocytes) containing acid-fast bacilli in a corneal leproma (×400). (Courtesy Narsing Rao, MD.)

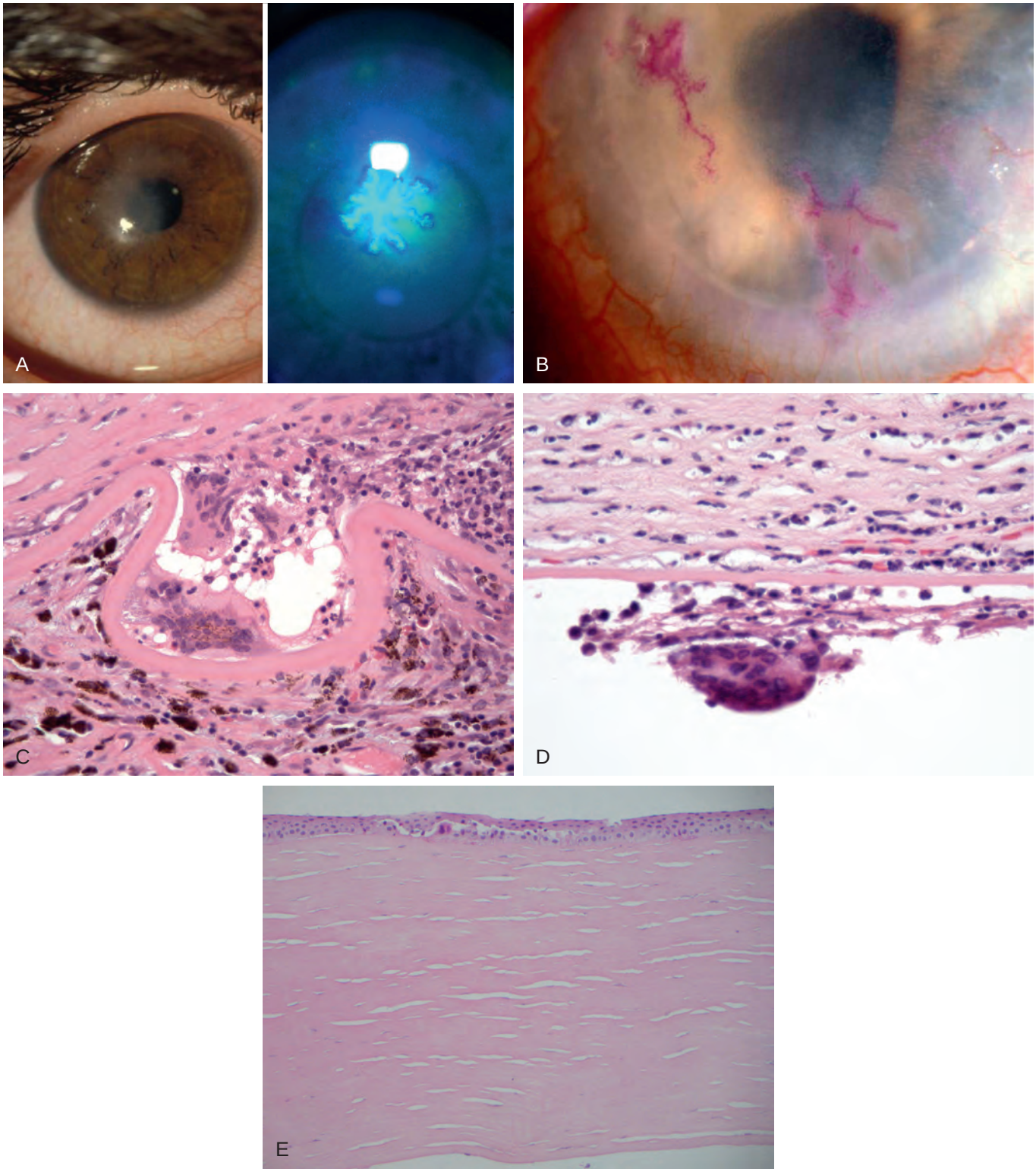
stromal scarring and vascularization—the most common cause of corneal transplantation related to infectious disease.

Stromal keratitis and so-called disciform keratitis are thought to represent an immune reaction to nonreplicating viral components.[43] Histopathologic findings are not specific but can include retrocorneal fibrous membranes, a granulomatous reaction to Descemet membrane, and stromal necrosis (see Fig. 21.18C and D). Chronic herpetic disease can lead to persistently nonhealing geographic areas of corneal epithelial ulceration eventuating in postherpetic neurotrophic keratopathy—histopathologically represented by a featureless corneal stroma with few remaining keratocytes (see Fig. 21.18E). Keratitis from varicella zoster virus (VZV) as a manifestation of herpes zoster ophthalmicus can also be epithelial, immune-stromal, or endothelial with similar histologic findings as in simplex conditions. Immunoperoxidase staining may differentiate between herpes zoster and herpes simplex. In zoster infections, pseudodendrites may occur, usually in the corneal periphery, and consist of swollen, poorly adherent epithelial cells. Unlike HSV dendrites, they lack terminal bulbs and dichotomous branching.

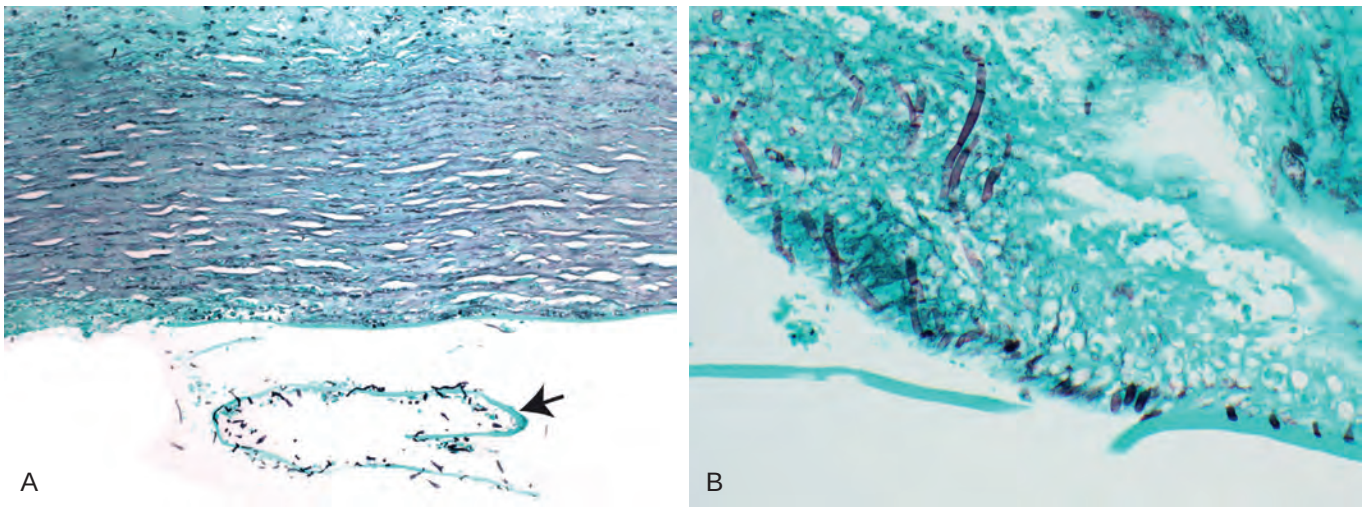
Fungal Infections

Classic Fungal Keratitis With Corneal Ulcer

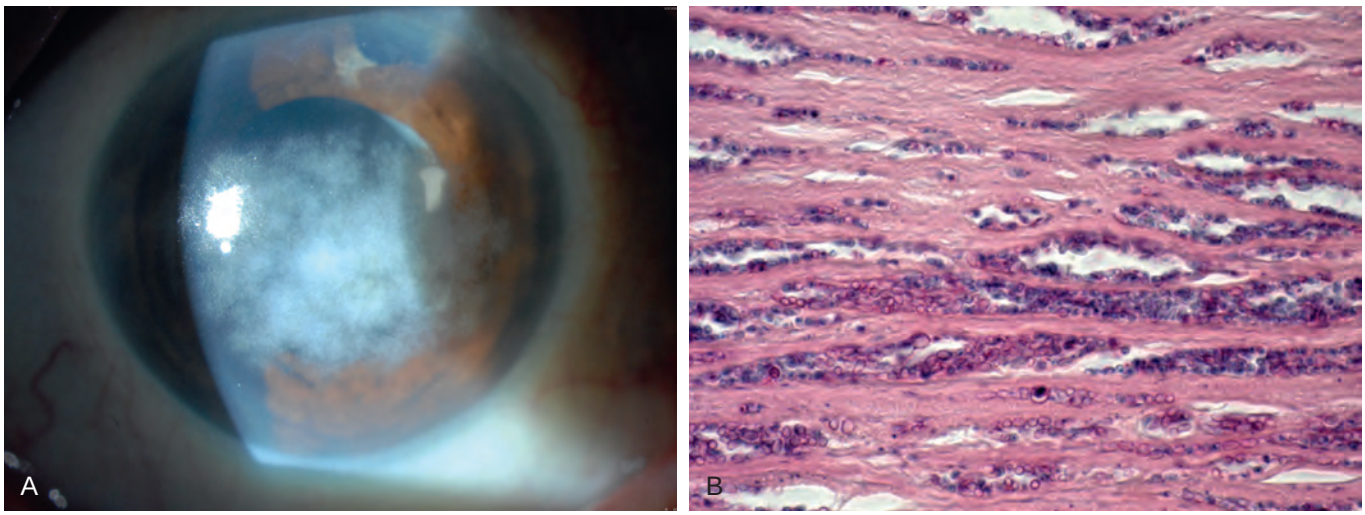
Fungal keratitis is almost always associated with local immunosuppression, often from chronic topical steroid therapy. In addition, it often occurs after trauma involving vegetable matter. It is most often seen in warm, humid climates. Fungal keratitis presents more indolently that bacterial keratitis and clinically displays a nonsuppurative corneal infiltrate with indistinct "feathery" borders and satellite lesions surrounding the main infiltrate.[44] Histopathologically, fungal hyphae are often located deep in the stroma, surrounding Descemet membrane (Fig. 21.19A), and are therefore more difficult to identify and culture without corneal biopsy. A polymorphous acute and chronic inflammatory cell infiltrate of the stroma is present. Fusarium, in the form of acutely



• **Figure 21.18** Herpetic keratitis. **A,** *Left panel*, Herpes stromal keratitis with central stromal haze and an intact epithelium. *Right panel*, Epithelial keratitis with fluorescein staining of an arborizing "dendritic" epithelial ulcer with terminal bulbs. **B,** Rose bengal staining highlights multiple herpetic dendrites. **C,** Chronic herpetic keratitis with a granulomatous reaction to Descemet membrane and a pigmented retrocorneal fibrous membrane (×400). **D,** Mononuclear cell infiltrate of the posterior corneal stroma and a "mutton fat" granulomatous keratic precipitate adherent to the posterior aspect of Descemet membrane (×400). **E,** Neurotrophic keratitis is characterized by a featureless corneal stroma devoid of keratocytes (×100). (**A,** *Left panel*, Courtesy Dan Jones, MD; *Right panel,* Courtesy Elisabeth Cohen, MD; **B,** Courtesy Dan Jones, MD.)



• **Figure 21.19** Fusarium keratitis. **A,** Gomori methenamine silver–positive hyphae surround a detached Descemet membrane *(arrow)* (×100). **B,** The filamentous fungal elements are concentrated deep in the corneal stroma and at the level of Descemet membrane (×400).



• **Figure 21.20** Microsporidal stromal keratitis. **A,** A coarse-appearing stromal keratitis. **B,** Numerous oval-shaped organisms are packed within the stromal lamellae with minimal surrounding inflammation (×200).

branching septate hyphae, is usually observed (see Fig. 21.19B). Other common pathogens are *Aspergillus* and *Candida* species. Such organisms as *Paecilomyces* may cause a fungal keratitis that is refractory to conventional treatment and requires penetrating keratoplasty and oral antifungal therapy. Again, histopathologic evaluation reveals the fungal elements to be concentrated in the deep corneal stroma; in the case of *Paecilomyces*, spores may also be seen.[45]

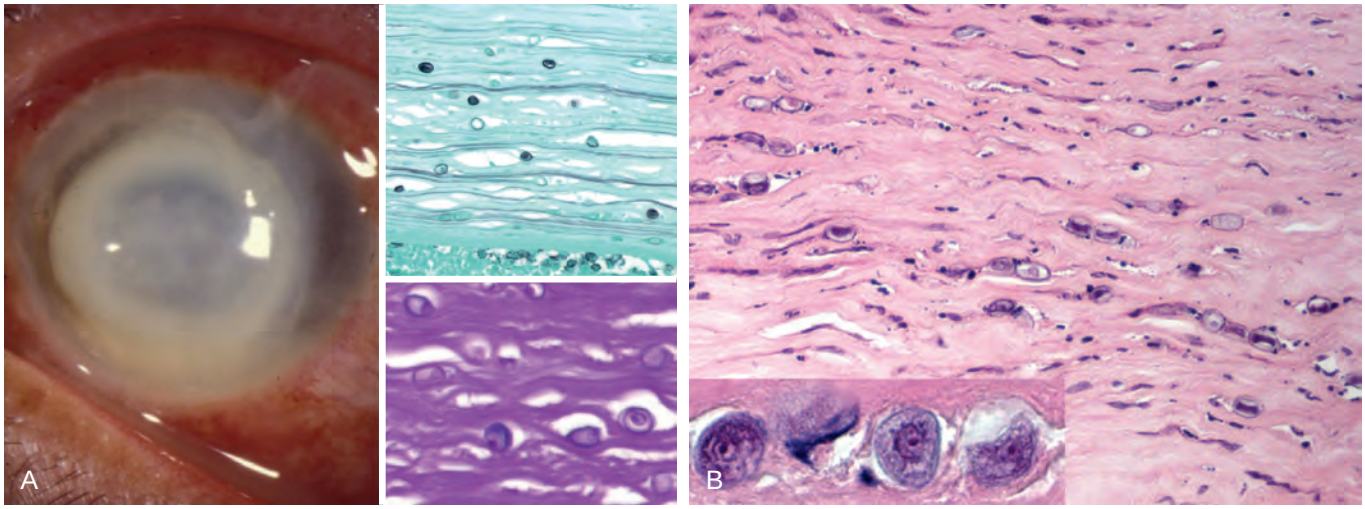
Microsporidial Keratitis

Microsporidia are obligate intracellular, spore-forming organisms previously thought to be protozoans but now classified as fungi. Microsporidial keratoconjunctivitis was initially described in immunocompromised patients with HIV/AIDS in the early 1990s. However, many cases have now been reported in contact lens wearers and overtly healthy individuals.[46] Clinically, coarse punctate corneal epithelial erosions are detected on examination, but many cases can resemble adenoviral or herpetic keratitis with deep stromal infiltration. This stromal keratitis (Fig. 21.20A), as opposed to conjunctivitis, occurs more commonly in immunocompetent individuals. Histopathologic examination of the cornea discloses multiple oval bodies with a polar nucleus packed between the stromal lamellae (see Fig. 21.20B), which stain positively with acid-fast methods. Polarization microscopy often discloses small birefringent granules in the microsporidia that can aid in diagnosis.

Parasitic Keratitis

Acanthamoeba Keratitis

Acanthamoeba keratitis is the classic example of corneal protozoal infection in contact lens wearers who are exposed to stagnant water and/or do not take appropriate sterilization precautions with their lenses. Patients complain of severe pain, often out of proportion to the initial clinical examination findings (due to a perineuritis). The corneal infiltrate is classically ring shaped or



• **Figure 21.21** Acanthamoeba keratitis. **A,** *Left panel*, A ring-shaped infiltrate in an intensely hyperemic eye. *Right panel*, Gomori methenamine silver (*top*, ×200) and periodic acid–Schiff (*bottom*, ×400) stain the acanthamoeba double-walled cysts. **B,** Multiple cysts (seen at higher power in the *inset*) are seen throughout the stroma with a minimal surrounding acute inflammatory cell infiltrate (×200, *inset* ×600). (**A,** *Left panel*, Courtesy Dan Jones, MD.)

circular and a hypopyon is often seen (Fig. 21.21A, left panel).[47] Early histopathologic examination reveals minimal stromal keratitis with abundant double-walled cysts and less common trophozoite forms; the organisms usually are apparent in hematoxylin and eosin (H&E)-stained sections, but trichrome, PAS, and GMS staining can augment detection in some cases (see Figs. 21.19B and 21.21A, right panel). Calcofluor white with fluorescent microscopy is another rapid, reliable mode of diagnosis.[48] As the disease progresses, marked corneal stromal necrosis can occur.[49] Large keratoplasty specimens are often received because the organisms spread toward the limbus and pathologists are asked to comment upon the presence of organisms at the graft-host junction, which serves to guide postoperative antimicrobial therapy.

Intraocular Infections (Including Endophthalmitis and Infectious Retinitis and Choroiditis)

A variety of pathogens can affect the uveal tract, often posteriorly, causing inflammation of the retina and choroid. Chorioretinitis refers to primary infection of the choroid with adjacent spread of the inflammatory process into the retina; retinochoroiditis denotes the opposite. The term endophthalmitis is used to describe inflammation of both the aqueous and vitreous cavities due to an infectious etiology, generally spreading from the choroid, retina, or a defect in the eye wall. It is categorized as exogenous (usually postoperative or traumatic) or endogenous (i.e., hematogenous dissemination from a distant source, such as the lung).

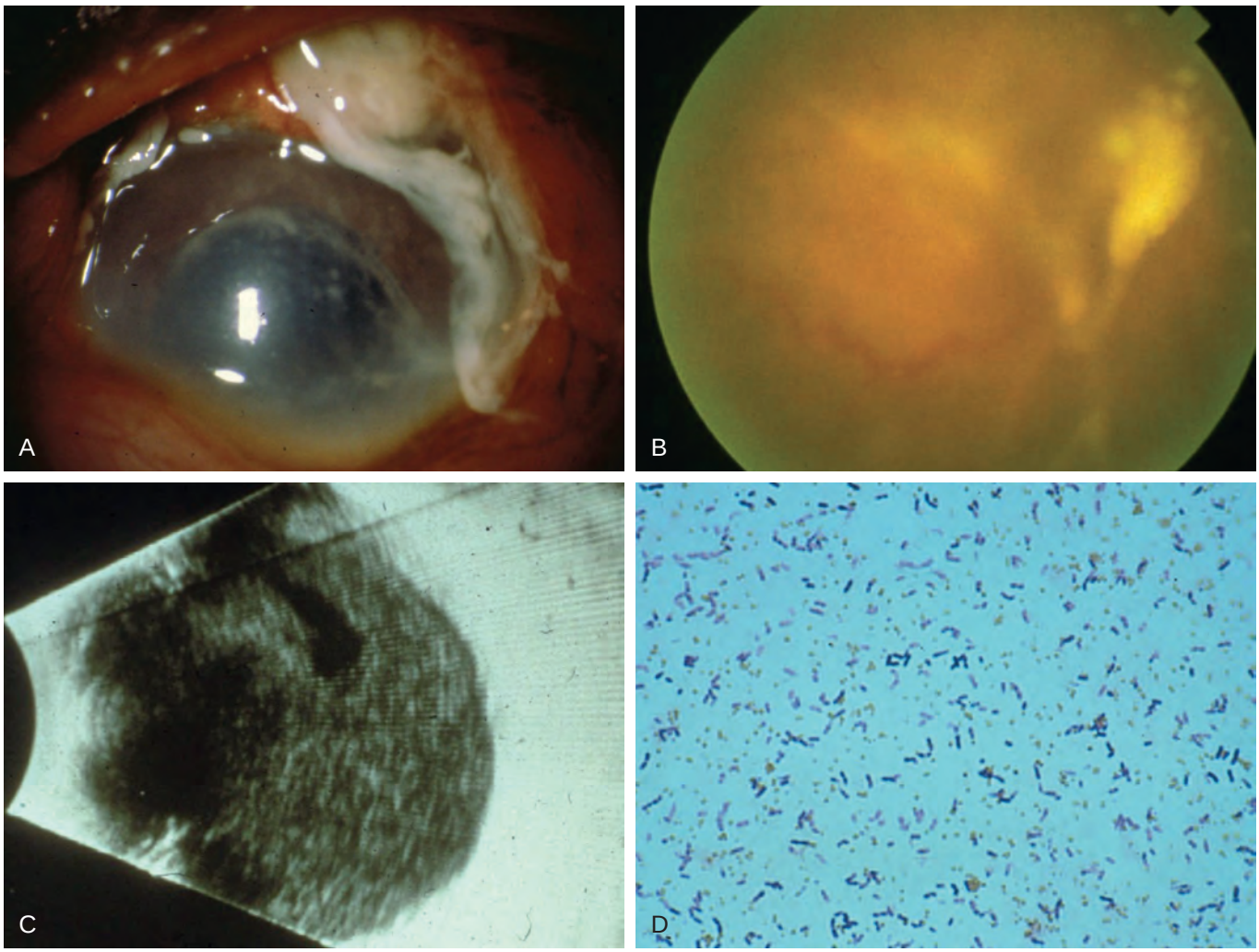
Bacterial Infections

Acute Bacterial Endophthalmitis

Bacterial endophthalmitis occurs acutely (several days) after intraocular surgery or after traumatic rupture of the eye wall. It is rare and one of the most dreaded complications of cataract or glaucoma-filtering surgery (bleb-related endophthalmitis, Fig. 21.22A). Patients develop pain, markedly decreased vision, and a hypopyon several days after surgery. The view of the fundus is commonly hazy due to the inflammatory response (see Fig. 21.22B) and ultrasonography reveals debris in the vitreous cavity (see Fig. 21.22C). Diagnosis and treatment are often provided concurrently with sampling of the vitreous cavity by aspirating a sample of the vitreous for Gram stain and culture (see Fig. 21.22D) followed by injection of intravitreal antibiotics on an empiric basis.

Tissue biopsy is uncommon, although enucleation specimens are occasionally obtained in fulminant, end-stage cases. One finds necrosis of the uveal tract (iris, ciliary body, choroid) and the retina with a dense polymorphonuclear leukocytic infiltration with vitreous body abscess formation (Fig. 21.23A and B). Coagulase-negative staphylococci (mostly *Staphylococcus epidermidis*) are most commonly cultured.[50] Worse outcomes are associated with more virulent organisms including *Staphylococcus aureus* and *Bacillus cereus*, which is a common culprit after penetrating trauma.[51] Gram-negative organisms (see Fig. 21.23C) are more commonly isolated in endogenous bacterial endophthalmitis,[52] especially *Klebsiella pneumoniae* (*P. aeruginosa* and *Neisseria meningitides* can also been seen). The most common gram-positive bacteria isolated from endogenous cases are *S. aureus*, group B streptococci, *S. pneumoniae*, and *Nocardia* species.

Nocardia in particular has an aggressive course, ultimately resulting in poor visual outcomes.[53] *Nocardia*, a weakly staining gram-positive rod, forms beaded branching filaments (Fig. 21.24A) along Bruch membrane, as in fungal endophthalmitis. Overall, common sources for hematologic dissemination are the liver, lung, and heart valves. Subretinal abscesses are known to occur with bacterial endophthalmitis, especially in endogenous *Klebsiella* infections.[54,55] These are treated by unroofing the overlying retina, which histopathologically reveals polymorphonuclear infiltration of the retinal layers with mixed inflammatory cells in the subretinal space (see Fig. 21.24B). A handful of cases of aggressive endophthalmitis have been reported due to *Listeria monocytogenes*, with a characteristic dark or black



• **Figure 21.22** Bacterial endophthalmitis. **A,** Bleb-related endophthalmitis. Purulent discharge from a glaucoma-filtering bleb with keratic precipitates on the corneal endothelium. **B,** Postoperative endophthalmitis. A hazy view of the fundus due to dispersed inflammatory cells in the vitreous cavity. There is retinal whitening and a vitreous abscess. **C,** Inflammatory debris is seen filling the vitreous cavity on B scan ultrasonogram. **D,** Vitreous aspirate reveals gram-positive rods, consistent with posttraumatic bacillus endophthalmitis (×400). (**A, C,** and **D,** Courtesy Donald D'Amico, MD; **B,** Courtesy Lucy Young, MD.)

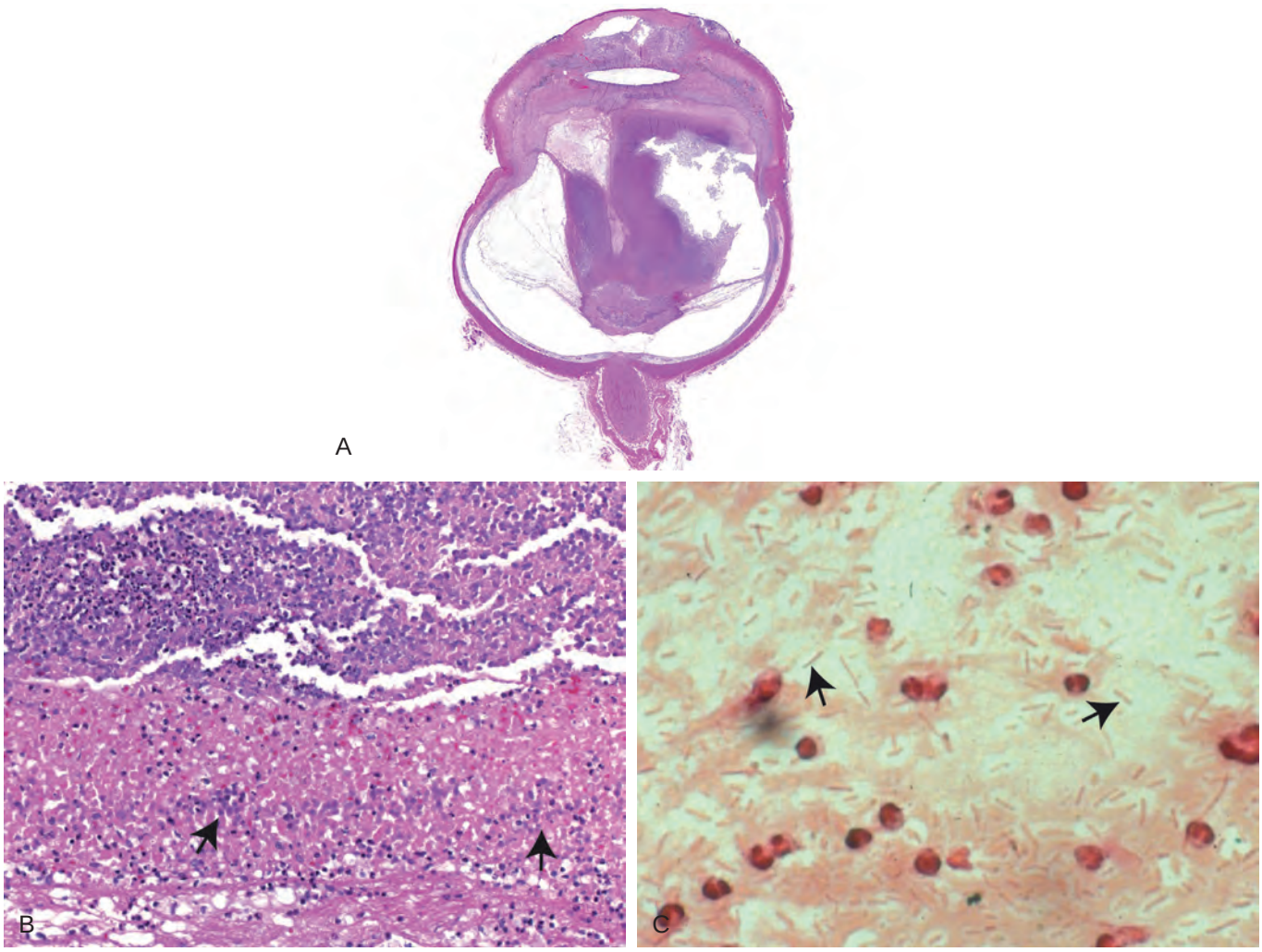
hypopyon from necrosis of the pigmented elements of the iris (see Fig. 21.24C).[56]

Chronic Postoperative Endophthalmitis

In contrast to the rapidly progressive, acute presentation of purulent postoperative endophthalmitis (after cataract surgery), chronic postoperative endophthalmitis has an indolent onset with multiple, recurrent bouts of inflammation beginning weeks to months after surgery. Less virulent organisms are causative, most commonly *Propionibacterium acnes*, a commensal organism normally found on the eyelid skin and conjunctiva. The organism is thought to be sequestered in the lens capsular bag between the implanted intraocular plastic lens and the posterior lens capsule. Clinically patients are often misdiagnosed with chronic, noninfectious iridiocyclitis and treated with topical steroid therapy until a whitish plaque develops on the intraocular lens (Fig. 21.25A and B). Treatment often requires removal of the lens and/or capsular bag.[57] At surgery, clusters of gram-positive rods can be aspirated (see Fig. 21.25C, right panel) or found adjacent to the lens capsule, admixed with residual lens fibers (see Fig. 21.25C, left panel).[58]

Whipple Disease

Intraocular Whipple disease (WD)[59-61] is believed to be a neurologic manifestation of WD. Although rare, it most commonly appears as a chronic uveitis or optic disc swelling (in addition to a variety of other neuro-ophthalmologic findings). Aspirated vitreous cytologic specimens may demonstrate PAS-positive macrophages, but molecular diagnosis performed on vitreous or cerebrospinal specimens is often required for accurate diagnosis. Enucleation specimens have revealed PAS-positive macrophages within the vitreous cavity and inner retinal layers (Fig. 21.26A). Organisms can also be seen free within the retina (see Fig. 21.26B). PAS-positive macrophages are not uncommon in eyes with necrosis and/or exudate and can closely simulate WD but lack discrete cytoplasmic organisms. Small intestinal biopsies are



• **Figure 21.23** Fulminant bacterial endophthalmitis. **A,** Enucleated globe manifests fulminant endophthalmitis with an abscess of the vitreous body and necrosis of the uvea (×10). **B,** Near-complete retinal necrosis with the remnant of a laminated retinal nuclear layer *(arrows)* (×200). **C,** Gram stain from endogenous *Klebsiella* endophthalmitis in a patient with a liver abscess reveals many gram-negative rods *(arrows)* (×400). (Courtesy Donald D'Amico, MD.)

performed in many cases to establish the diagnosis from myriad infiltrating PAS-positive macrophages in the villi.[62]

Viral Chorioretinitis

Herpes Family Chorioretinitis

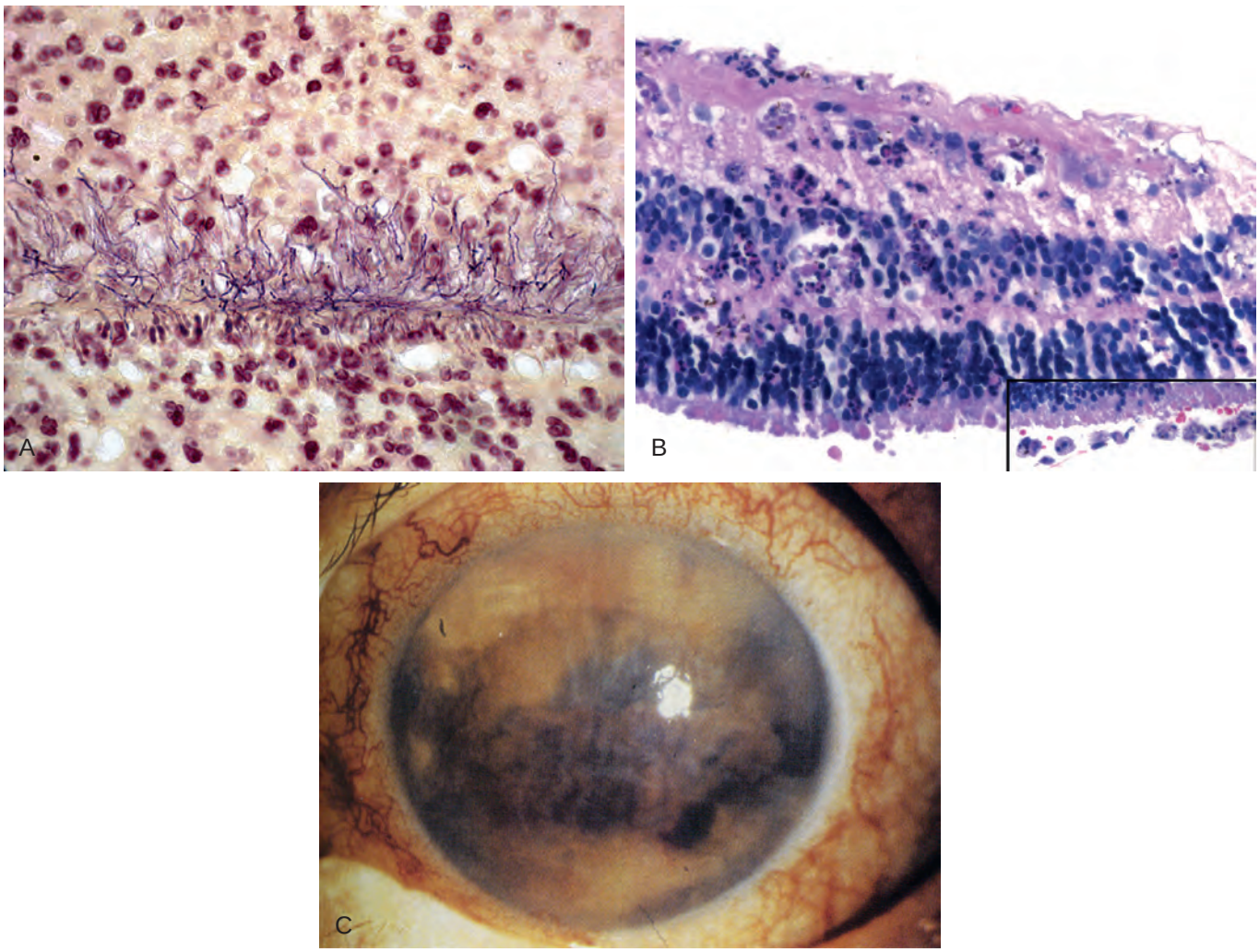
As discussed previously, herpetic disease can affect all aspects of the eye and ocular adnexa. The most catastrophic manifestation of these herpetic infections is posterior segment involvement in the form of acute retinal necrosis (ARN). Patients with HSV retinitis are typically otherwise healthy adults presenting with acute unilateral loss of vision, photophobia, floaters, and pain with significant anterior and posterior segment inflammation (keratic precipitates on the corneal endothelium and a dense vitritis) together with the evolution of peripheral retinal whitening and occlusive vasculitis.[63] Cytomegalovirus (CMV) can cause a very similar clinical picture in immunocompetent patients. A morphologic variant of ARN is seen in immunosuppressed patients (classically advanced HIV/AIDS), often attributable to VZV rather than HSV. Retinal necrosis and whitening begin more posteriorly, and there is a marked lack of inflammatory response within the eye because of the patient's severe immunodeficient and lymphopenic status.

In all cases of ARN due to the herpesviruses, histopathologic examination of microsurgical retinal biopsies or enucleation specimens reveals profound necrosis with occlusive retinal arteritis. Large eosinophilic intranuclear and intracytoplasmic inclusions are noted within the retina, along with a mononuclear cell infiltration and sloughing of the inner retina (Fig. 21.27A and B).[64-66] CMV infection occurs more commonly in immunosuppressed patients (especially HIV/AIDS patients with low CD4 lymphocyte counts) and can cause retinal nerve fiber layer infarcts, (cotton wool spots clinically), retinal hemorrhages, and perivasculitis without full-blown retinal necrosis if recognized and treated early.

Fungal Infections

Classic Fungal Endophthalmitis

As opposed to bacterial endophthalmitis, which is largely exogenous in nature, many cases of fungal endophthalmitis are



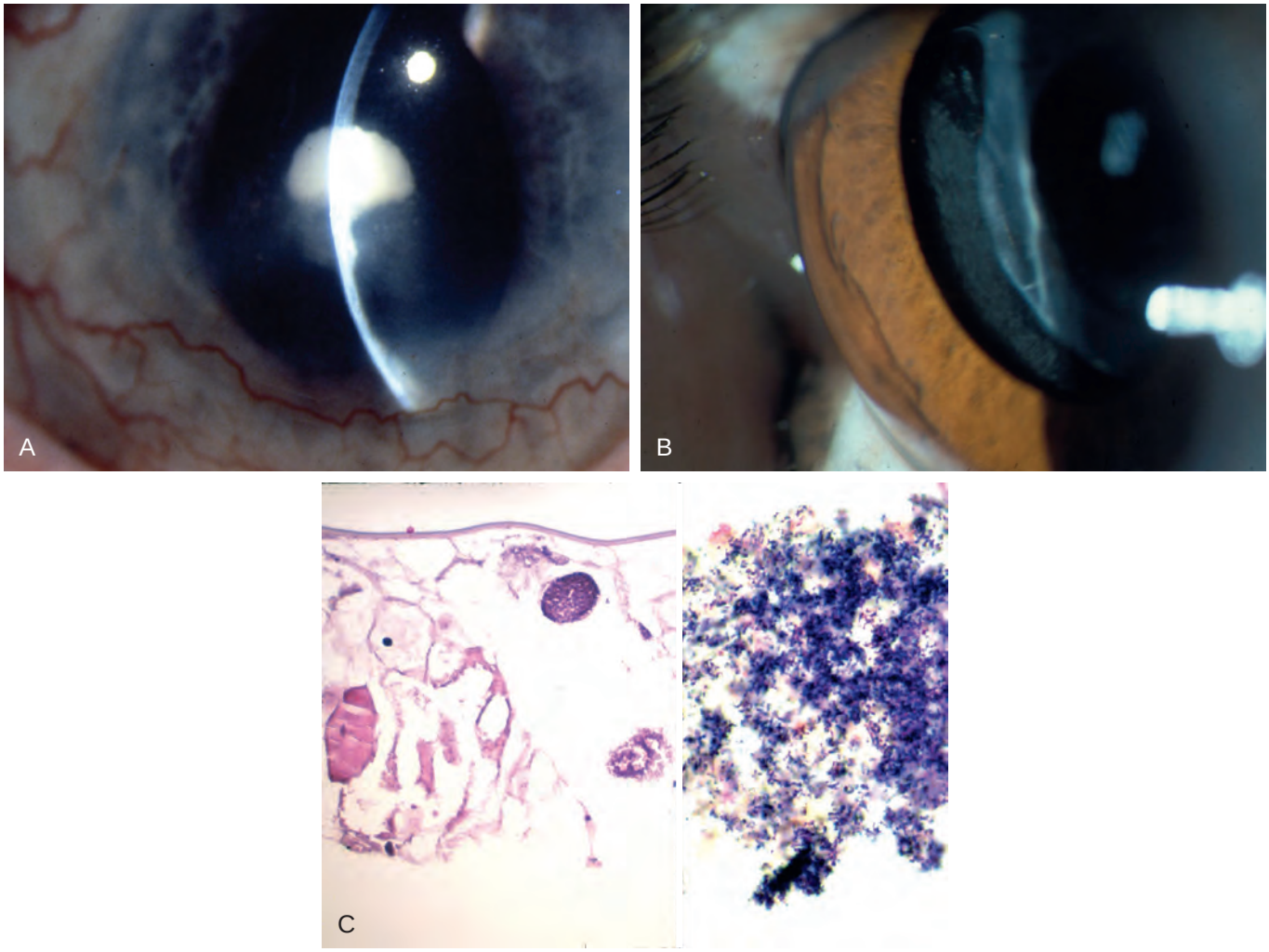
• **Figure 21.24** Nocardia and other unusual causes of endophthalmitis. **A,** Nocardial bacilli form beaded branching filaments at the level of Bruch membrane (×200). **B,** Subretinal abscess due to *Klebsiella* pneumonia. Polymorphonuclear leukocytes infiltrate all layers of the retina (×200). The *inset* shows a mixed inflammatory infiltrate in the subretinal space. **C,** *Listeria* endophthalmitis resulting in iris necrosis and a black hypopyon. (Modified from Eliott D, O'Brien TP, Green WR, Jampel HD, Goldberg MF. Elevated intraocular pressure, pigment dispersion and dark hypopyon in endogenous endophthalmitis from Listeria monocytogenes. *Surv Ophthalmol*. 1992;37:117-124.)

endogenous.[67] Such cases occur in chronically immunosuppressed patients on long-term steroid therapy, patients receiving parenteral nutrition or with central venous catheters, intravenous drug users, and solid organ transplant patients (especially of the liver). The most common organisms isolated are *Candida albicans* and other *Candida* species, *Aspergillus* species,[68] and *Cryptococcus* (discussed separately later). Primary infections often arise in the liver or lung with secondary dissemination to the eye. Classically, fundoscopic examination reveals a fluffy white, circumscribed vitreous "fungus ball" or "string of pearls" (Fig. 21.28A and B). Whole eye specimens may be received due to the high mortality of fungemia. In other cases, enucleation may have to be performed due to the fulminant intraocular infection. Histopathologic examination discloses necrotizing granulomas of the choroid and retina and within the vitreous cavity. Small budding yeast forms (3 to 5 µm) and pseudohyphae (in the case of *Candida*) can be seen within choroidal vessels (see Fig. 21.28C).

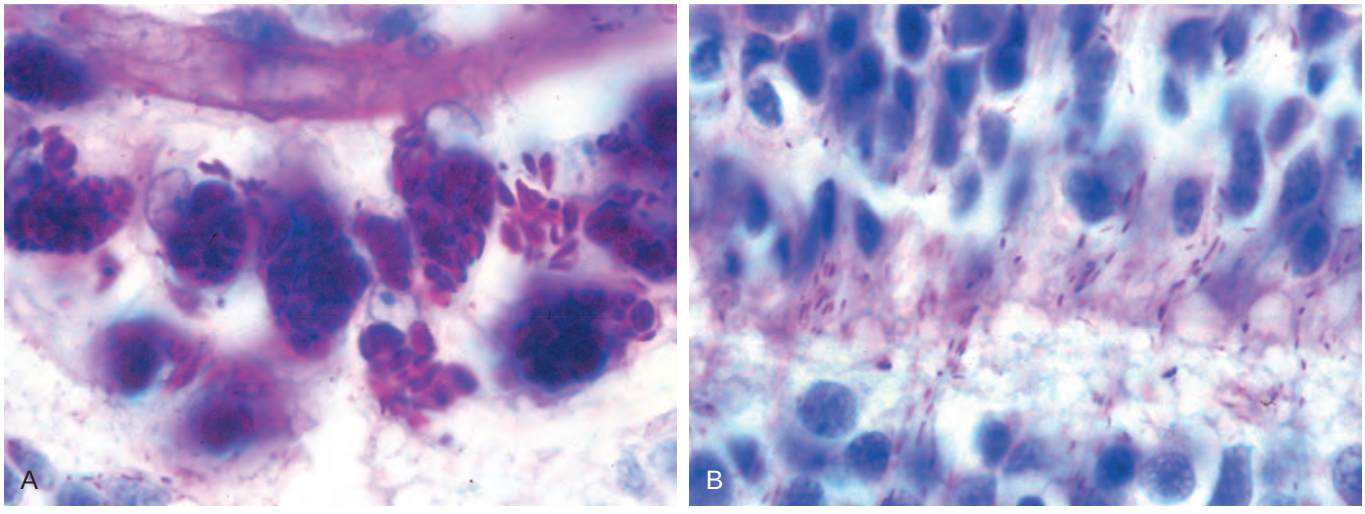
Aspergilli appear as intraretinal septate hyphae with acute angle branching, especially along Bruch membrane or within the vitreous (Fig. 21.29A and B). Because of the current use of injected intravitreal corticosteroids to treat a variety of common ophthalmic conditions (e.g., diabetic macular edema and vein occlusion), several unusual exogenous fungal infections have been introduced intraocularly, including *Bipolaris hawaiiensis*, due to medication contamination (see Fig. 21.29C).[69]

Cryptococcal Endophthalmitis and Other Unusual Causes of Fungal Endophthalmitis

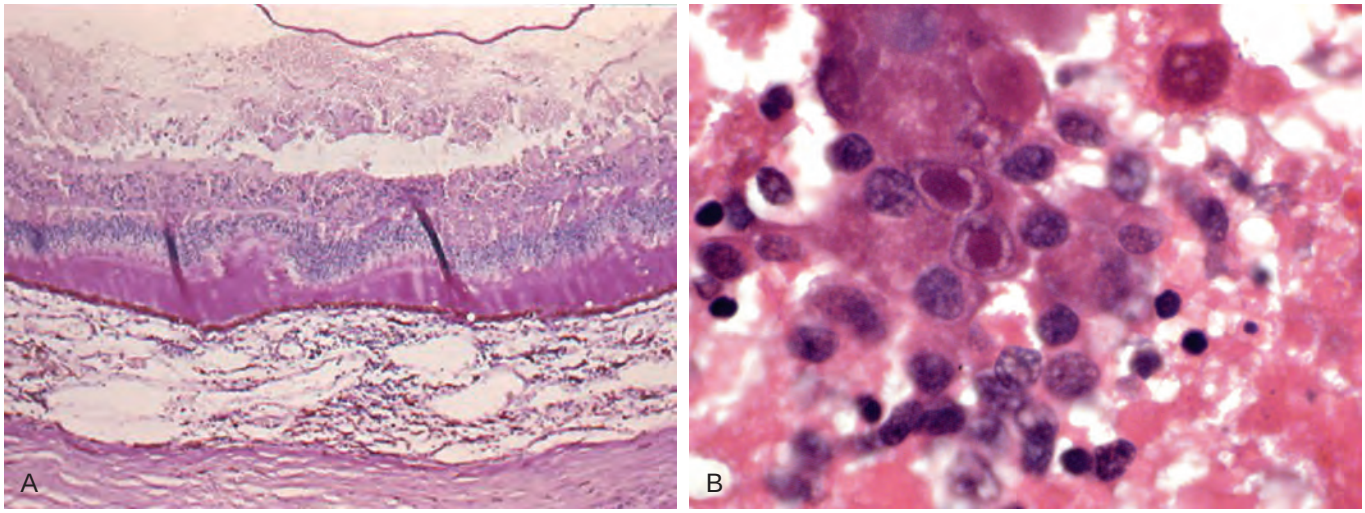
Cryptococcus neoformans is a thickly encapsulated, narrow-based budding yeast (4 to 10 µm) preferentially affecting the lungs and central nervous system in immunocompromised adults.[70,71] Intraocular infection, largely in the form of multifocal choroiditis, is due to either hematogenous dissemination or direct extension through the leptomeninges via the optic nerve sheath.[72]



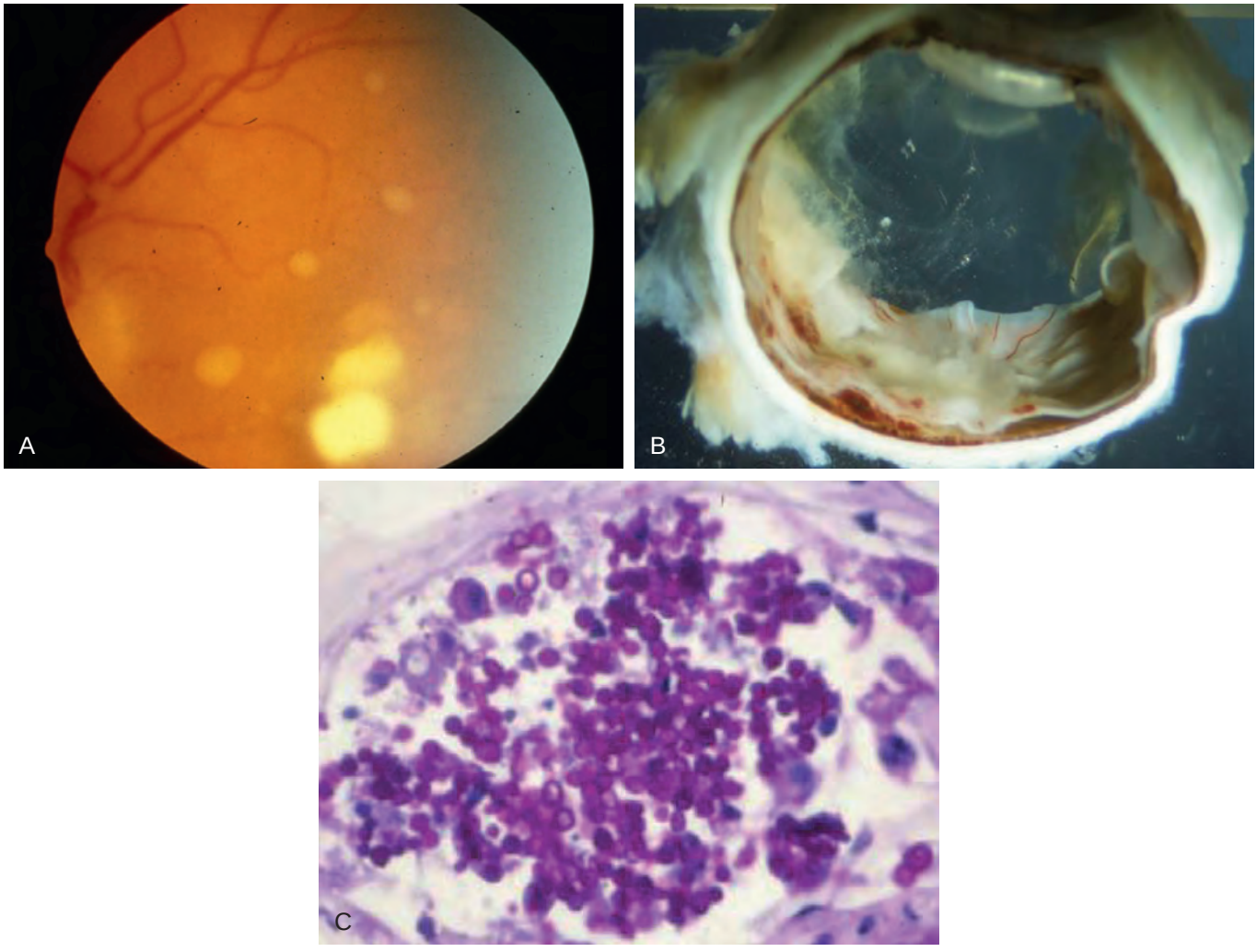
• **Figure 21.25** Chronic postoperative endophthalmitis. **A,** A central white plaque has developed on the plastic intraocular lens in a patient with chronic uveitis following cataract surgery. **B,** A whitish film is subtly forming across the peripheral intraocular lens. **C,** Gram-positive *Propionibacterium acnes* rods in clusters *(left panel)* (×200) under the lens capsule and aspirated from the lens capsular bag *(right panel)* (×400). (**B,** Courtesy Donald D'Amico, MD; **C,** *Left panel*. Courtesy Narsing Rao, MD.)



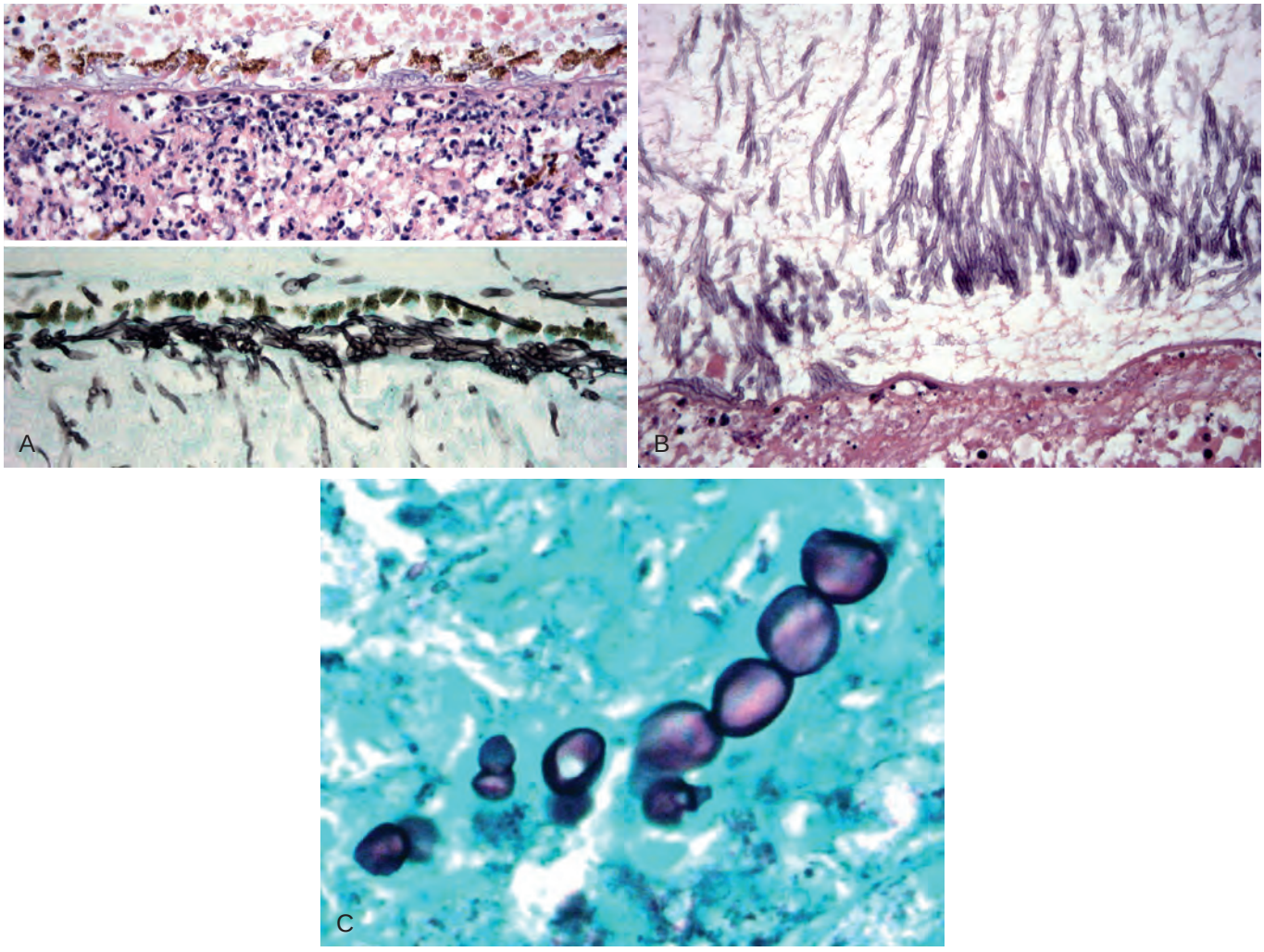
• **Figure 21.26** Intraocular Whipple disease. **A,** Periodic acid–Schiff–positive macrophages within the retina (×600). **B,** Free rod-shaped organisms are seen throughout the retinal layers (×400).



• **Figure 21.27** Acute retinal necrosis. **A,** Herpes simplex virus–associated retinal necrosis with sloughing of the inner, necrotic retina; subretinal exudate is also seen (×100). **B,** Cytomegalovirus acute retinal necrosis displaying eosinophilic intranuclear inclusions in the retina (×600). (**A,** Courtesy Donald Minckler, MD.)



• **Figure 21.28** Fungal endophthalmitis. **A,** A "string of pearls" of fungal balls within the vitreous cavity, anterior to the retina. **B,** Gross photograph of fungal endophthalmitis with fluffy preretinal and vitreal infiltrates. **C,** Periodic acid–Schiff–positive small yeast forms within a choroidal vessel in candida endophthalmitis (×150). (**A,** Courtesy Donald D'Amico, MD; **B** and **C,** Courtesy Hans Grossniklaus, MD.)



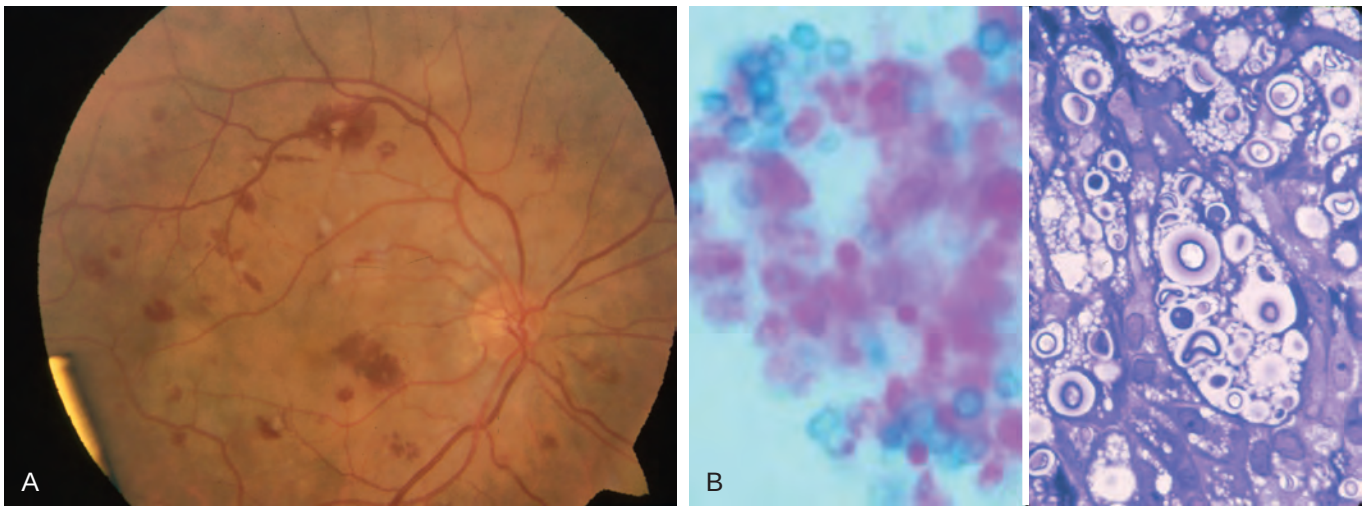
• **Figure 21.29** Fungal endophthalmitis. **A,** Fungal hyphae are congregating along Bruch membrane with disruption of the overlying retinal pigment epithelium *(top panel).* The organisms are better seen with silver staining *(bottom panel)* (×100). **B,** The vitreous cavity is filled with vertically oriented acutely branching, septate hyphae, consistent with aspergillus infection. The inner retina and its inner limiting membrane are identified below (×100). **C,** Spore chains from an eye infected exogenously with *Bipolaris hawaiiensis* after injection of contaminated compounded medication (×400). (Courtesy Donald Minckler, MD.)

Chorioretinitis, endophthalmitis, papilledema, and cranial nerve palsies are other ophthalmic findings associated with the disease. Multiple yellow-white choroidal lesions are discovered on fundoscopic examination, along with white-centered intraretinal hemorrhages (Fig. 21.30A). Histopathologic evaluation demonstrates small, rounded organisms within the choroid and/or retina, sometimes in macrophages and sometimes free. The accompanying chronic inflammatory infiltrate is notably sparse. Vitreous aspirates may contain the encapsulated yeast forms.[73] Mucicarmine or alcian blue staining highlights the cryptococcal mucinous capsule (see Fig. 21.30B). Subretinal cryptococcal mass lesions, so-called cryptococcomas, can rarely form and must be treated surgically.[74]

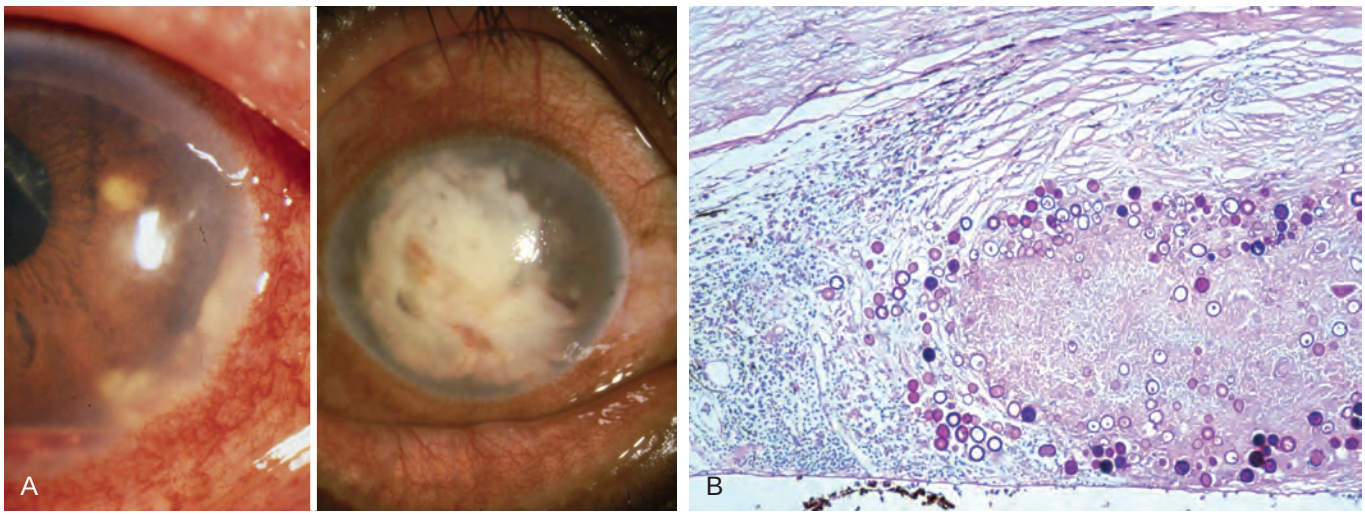
Coccidiodes, although often a self-limited pulmonary disease, can occasionally affect the eye, causing anterior symptoms (including eyelid, conjunctival, and iris granulomas [Fig. 21.31A] and chronic iridocyclitis), focal chorioretinitis without vitreous involvement, or a fulminant granulomatous endophthalmitis, usually requiring an enucleation.[75] Vitreous aspirational biopsy is often necessary for diagnosis. Endophthalmitis is rare but catastrophic and usually is associated with disseminated disease, although cases are being reported without concomitant systemic involvement.[76] Histopathologic examination of enucleated eyes demonstrates intraocular granulomas on the iris, in the retina, and within the vitreous cavity, which can appear sarcoidal but contain the fungal organisms (mature fungal spherules, see Fig. 21.31B). Such chronic granulomatous endophthalmitis leads to tissue destruction and disorganization of the intraocular contents. Chronic, necrotizing granulomatous retinochoroiditis has rarely also been attributed to *Sporotrichum schenkii*, a cigar-shaped, yeastlike organism that can be found within the necrotic retina and subretinally in enucleated eyes.[77]

Ocular Histoplasmosis

The most common manifestation of histoplasmosis is known as the "presumed ocular histoplasmosis syndrome," a multifocal chorioretinitis that is often asymptomatic (as is the primary pulmonary infection) and occurs in immune competent patients



• **Figure 21.30** Intraocular cryptococcosis. **A,** Fundus photo of a human immunodeficiency virus/acquired immune deficiency syndrome patient with multiple intraretinal hemorrhages, many white-centered hemorrhages, and scattered cotton wool spots. **B,** *Left panel*. Vitreous aspirate reveals alcian blue–positive spores (×400). *Right panel*. Giemsa stain highlights the bluish-purple capsules of the organism (×400). (**A,** Courtesy Narsing Rao, MD; **B,** *Right panel*, Courtesy Dean Eliott, MD; *Left panel,* Courtesy of Narsing Rao, MD.)



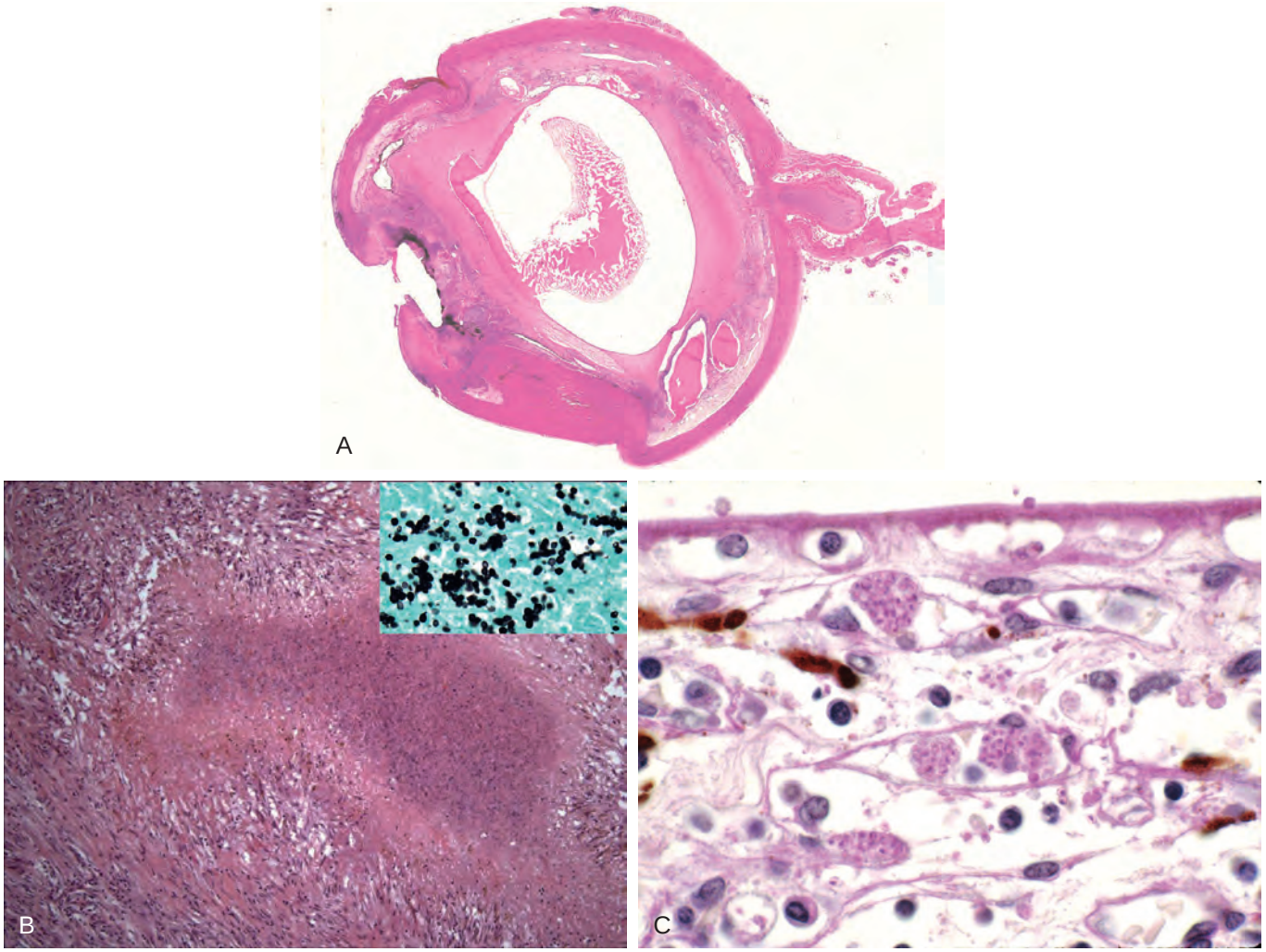
• **Figure 21.31** Intraocular coccidiomycosis. **A,** Granulomas on the surface of the iris *(left panel)* and filling the anterior chamber *(right panel)*. **B,** The choroid is expanded by a granuloma containing mature fungal spherules (×150). (Courtesy Narsing Rao, MD.)

in the Ohio and Mississippi River valleys. The choroiditis is thought to be a hematogenous dissemination following initial pulmonary infection. Patients have peripapillary (surrounding the optic nerve) atrophy and pigment changes and multiple atrophic choroidal scars ("histo spots") without vitreous inflammation. Rarely studied microscopically, eyes with the "presumed ocular histoplasmosis syndrome" have been found to have a chronic granulomatous and nongranulomatous choroiditis with positive immune staining for *Histoplasma* antigens but without identification the actual organisms.[78,79] Partial destruction of the retinal pigment epithelium with loss of outer retinal layers and focal chorioretinal scars are also exhibited. More recently *Histoplasma capsulatum* DNA has been isolated from the atrophic choroidal lesions.[33]

Histoplasmosis can cause an unusual focal retinochoroiditis or a fulminant endophthalmitis in either immunosuppressed (Fig. 21.32A) or immunocompetent patients who have disseminated disease.[80] Necrotizing granulomas within the retina characterize the fulminant intraocular infections (see Fig. 21.32B) that contain oval 2- to 4-µm yeast forms. Clusters of oval yeast forms within macrophages can be seen histopathologically within the choroid (see Fig. 21.32C) in focal choroiditis. The organisms can also appear in the vitreous cavity.

Pneumocystitis Choroiditis

Prior to the development of highly active antiretroviral therapy, *Pneumocystis jirovecii* manifested frequently in HIV/AIDS patients as a bilateral multifocal choroiditis, sometimes similar in



• **Figure 21.32** Intraocular histoplasmosis. **A,** Fulminant endophthalmitis from disseminated histoplasmosis with necrotizing granulomatous keratitis and chorioretinitis (×10). **B,** Higher power of a necrotizing choroidal granuloma (×200). The small oval yeast forms are highlighted with Gomori methenamine silver in the *inset*. **C**, Focal choroiditis in disseminated histoplasmosis. The yeast forms are seen within macrophages (×400). (**A** and **B,** Courtesy Danny Milner, MD; **C,** Courtesy Narsing Rao, MD.)

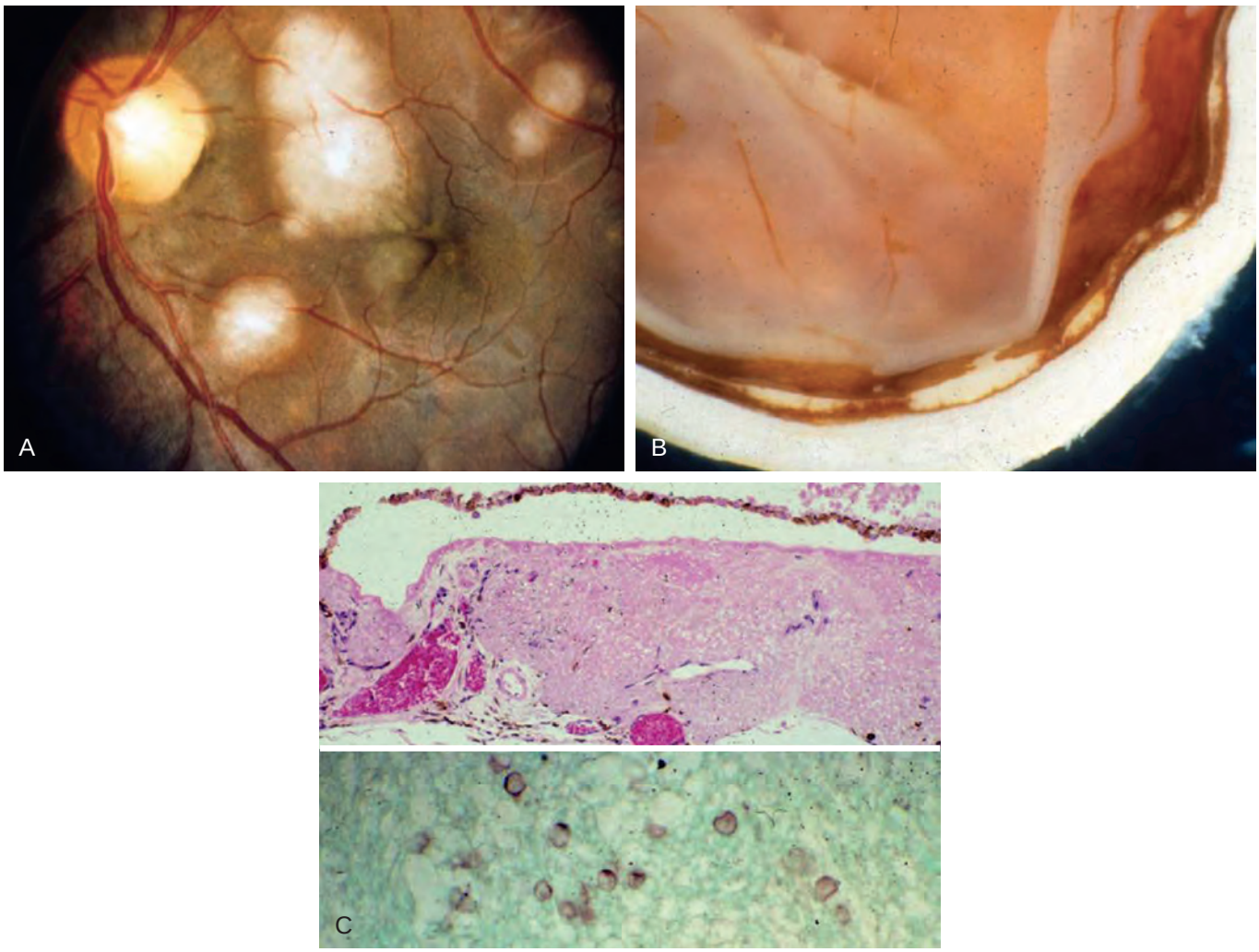
appearance to cryptococcal disease. Multiple round, pale yellow-white choroidal lesions of various sizes are seen in both eyes (Fig. 21.33A). Gross examination (see Fig. 21.33B) reveals focal thickening of the choroid, which harbors the organism's cysts in an eosinophilic exudate (see Fig. 21.33C). The organism has also been seen histopathologically in the ganglionic and plexiform layers of the retina. The choroidal necrosis displays striking lack of inflammation.[81]

Parasitic Infections

Toxoplasma gondii

Toxoplasmosis can be acquired congenitally and encountered in the neonate as characteristic bilateral, pigmented macular scars (Fig. 21.34A) (along with brain lesions). It is the most common cause of infectious posterior uveitis in adults and children, with a very characteristic fundus appearance. Immunocompetent patients, usually in their second to fourth decades of life, exhibit a focal, white retinitis with overlying vitreous inflammation (see Fig. 21.34B, so-called headlight in a fog) often adjacent to a pigmented, healed chorioretinal scar from prior disease episodes (Fig. 21.35A; see also Fig. 21.34C). The diagnosis is usually made clinically from the classic appearance, although antibody titers and PCR of the aqueous or vitreous fluid aspirates can be useful. Rarely, in immunosuppressed patients, diffuse retinal necrosis can be seen, as in viral retinitis. White retinal lesions similar in appearance to intraocular lymphoma have also been reported in immunocompromised patients.[82] Diagnostic vitrectomy specimens have revealed toxoplasma cysts.[83]

Most histopathologic studies have described ocular toxoplasmosis in advanced, atypical cases in immunosuppressed patients. *T. gondii* causes a retinochoroiditis, a term that emphasizes that the primary infestation with the obligate intracellular protozoan parasite occurs in the retina, and choroiditis (usually granulomatous) ensues. The infected retina shows partial- or full-thickness coagulative necrosis with loss of its normal lamellar architecture (see Fig. 21.35B and C). Tachyzoites, the rapidly multiplying free form of the organism whose crescentic morphology (see Fig.



• **Figure 21.33** *Pneumocystis jirovecii* choroiditis **A,** Multifocal placoid choroiditis in a human immunodeficiency virus/acquired immune deficiency syndrome patient. **B,** Gross photograph of an enucleation specimen reveals multifocal thickening of the choroid. **C,** The organisms are seen in the *top panel* in a lake of amporphous eosinophilic exudate and degenerating material within the choroid (×60). Gomori methenamine silver assists in the detection of the organisms *(bottom panel)* (×160). (**A** to **C,** Courtesy Hans Grossniklaus, MD.)

21.35B, left inset) is responsible for the disorder's name (toxo means arc or bow in Latin), and toxoplasma cysts containing bradyzoites can be observed in the retina (see Fig. 21.35B, right inset, and C, inset).[84]

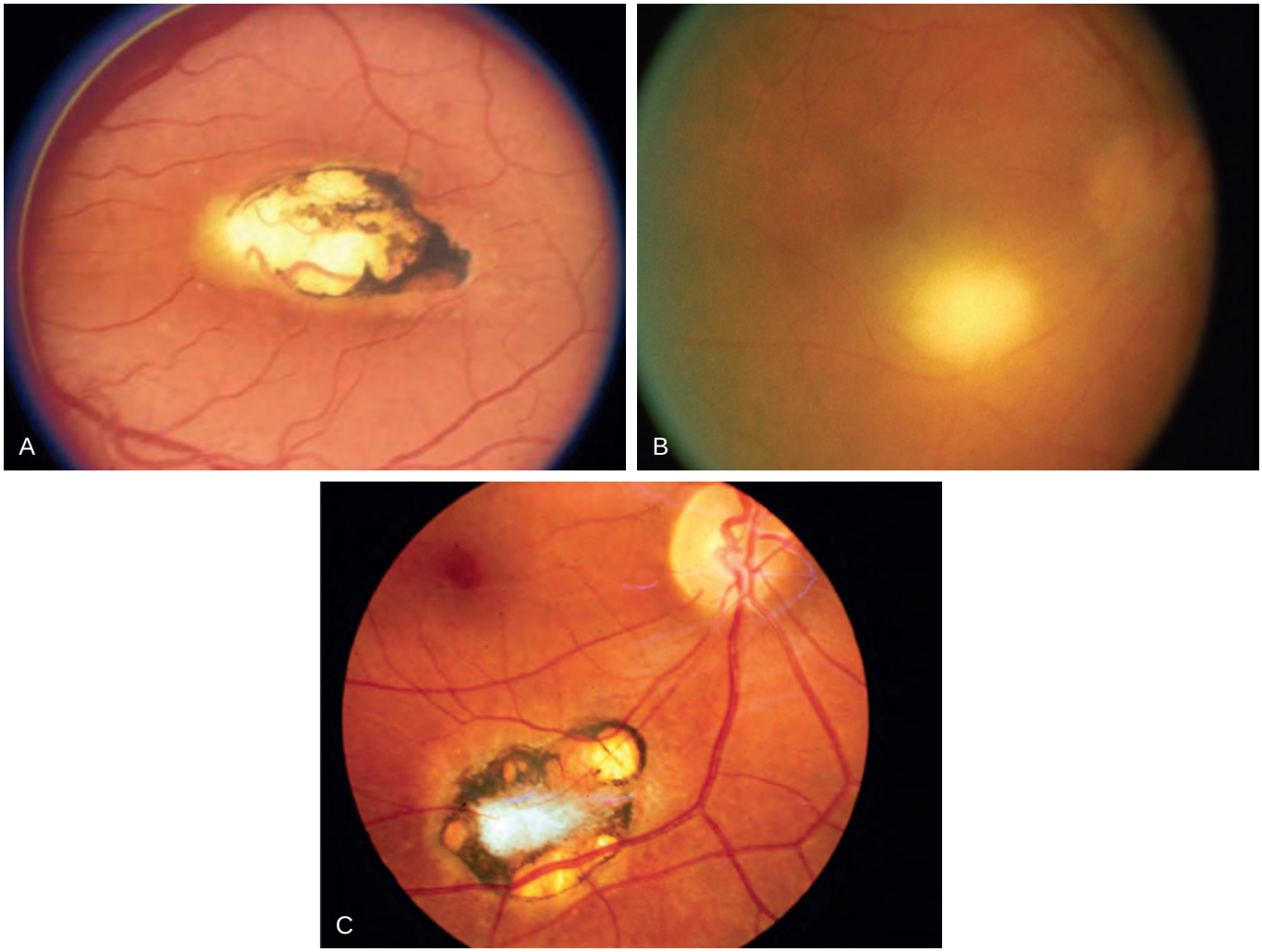
Diffuse Unilateral Subacute Neuroretinitis and Other Nematode Infections

Diffuse unilateral subacute neuroretinitis (DUSN) is caused by migration of a nematode (thought to be either *Ancylostoma caninum*, the dog hookworm, or *Baylisascaris procyonis*, the raccoon roundworm) through the subretinal space often in adolescent and otherwise healthy patients.[85] Recurrent inflammatory episodes of the retina, retinal pigment epithelium, and optic nerve have been seen due to migration of the worm, which can be visualized on fundoscopy in the subretinal space and treated with photocoagulation. Eventually, optic nerve atrophy and degeneration of the pigment epithelium occur (Fig. 21.36A and B).[86] Histopathologically, the outer retinal layers are particularly disrupted and a subretinal larva is seen (see Fig. 21.36C). The retinal pigment epithelium is hyperplastic, and there is an eosinophilic periarteriolar choroiditis.[87]

Ocular toxocariasis can also affect young patients, causing a posterior pole granuloma and leukocoria, simulating retinoblastoma. In such cases, serial sectioning may disclose fragments of toxocara larvae in a vitreous abscess composed of eosinophils. Cysticercosis, caused by the larval stage of *Tanenia solium*, can occupy the subretinal space, vitreous cavity, or anterior chamber (Fig. 21.37). The protoscolex or head undulates in response to light from the examiner's ophthalmoscope.

Eyelid and Orbital (Skin and Soft Tissue) Infections

Infection of the periocular skin and soft tissue (eyelids and orbital contents) is in many cases the consequence of spread from adjacent sinuses. The eyelids are complex motile shields that protect the cornea and eyeball and possess multiple layers: epidermis and



• **Figure 21.34** Clinical toxoplasmosis **A,** Congenital toxoplasmosis with a central macular scar revealing the underlying bare sclera. (Courtesy Lucy Young, MD.) **B,** "Headlight in a fog" phenomenon with a white area of retinitis and overlying vitreous haze. **C,** Inactive pigmented toxoplasmosis scar in an adult.

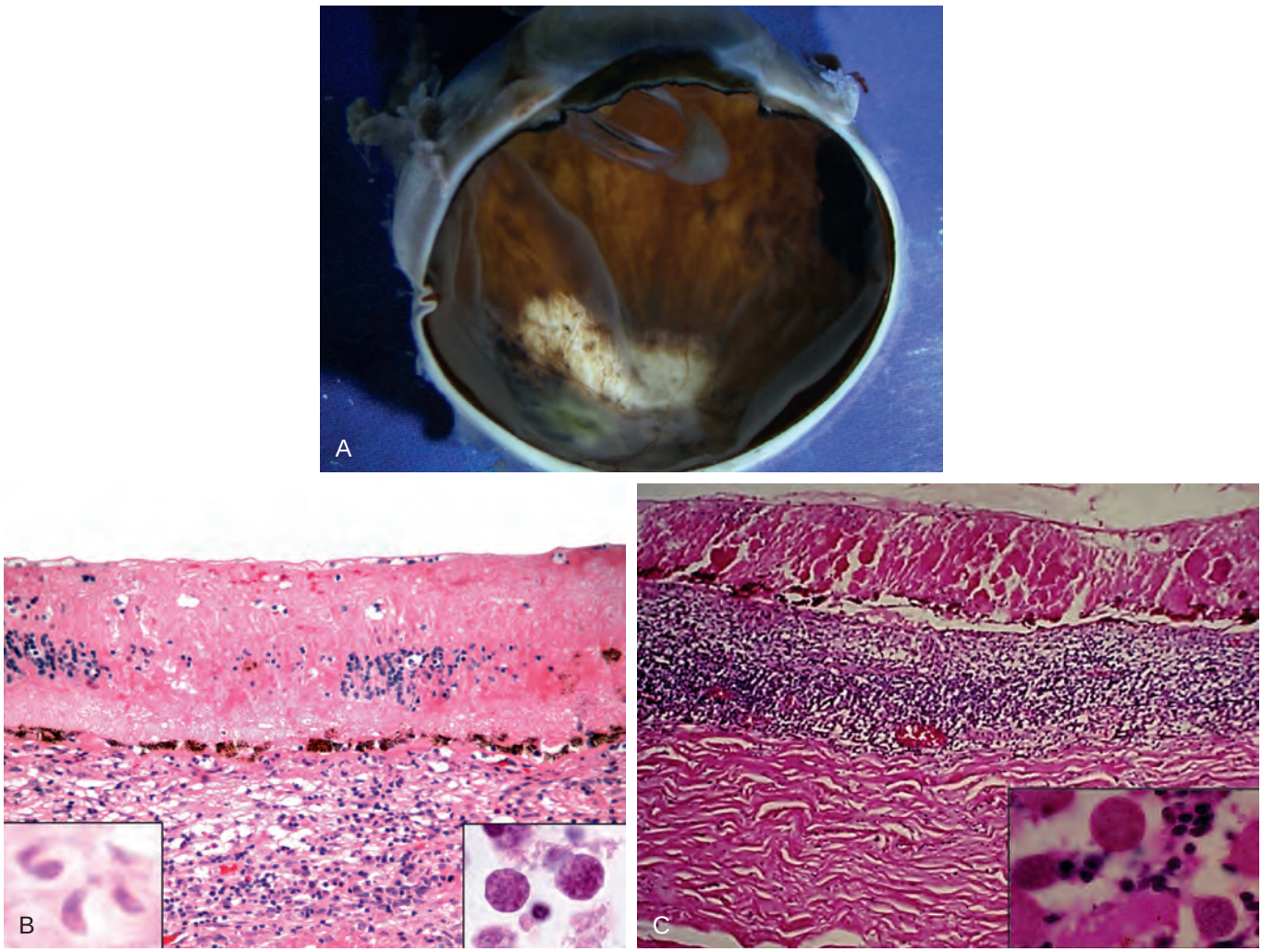
dermis, no significant hypodermis (fat), a tough fibrous plaque (tarsus) with embedded meibomian (sebaceous) glands, and a posterior layer of conjunctiva. The orbit (socket) commences at the anterior bony rim. It is separated from the eyelids by a reflection of the periosteal membrane in the form of the orbital septum, melding with the eyelid structures. The main lacrimal gland produces reflex tearing, whereas the accessory glands provide continuous tears that flow across the surface of the eyeball and lubricate the cornea. The tears are drained inferiorly from lacus lacrimalis near the caruncle, through the medial puncta of the eyelid margin, canaliculi, the lacrimal sac and nasolacrimal duct to finally enter the nose.

Cellulitis is defined as "preseptal cellulitis" if it is confined to the eyelid skin and its anterior lamellar tissues but does not extend posterior to the orbital septum (Fig. 21.38A). "Orbital cellulitis," on the other hand, involves tissues posterior to the orbital septum, a thin membranous structure originating from the bony orbital rim that delimits the deepest portion of the eyelids from the most anterior orbital fibroadipose tissue and muscles. In true orbital cellulitis, the globe itself is affected, leading to conjunctival edema (chemosis), extraocular motility deficits (see Fig. 21.38B), painful extraocular movements, and an afferent pupillary defect signifying impingement on the optic nerve.

Bacterial Infections

Bacterial Orbital Cellulitis

Bacterial infections of the eyelid skin and orbit originate from an adjacent sinus, especially in children (see Fig. 21.38C). Direct inoculation through the skin (e.g., after minor trauma) or spread from an infected tooth or the lacrimal sac are other causes of infection in adults. Bacteremic spread from a distant focus is rare.[88] Microbiologic causes of bacterial orbital cellulitis are constantly emerging. The introduction of prophylaxis using vaccines has been helpful for *H. influenzae* and *S. pneumoniae* infections. *Staphylococcus* species are now considered the most common organism isolated from orbital specimens, the majority of which are represented by methicillin-resistant *S. aureus* (MRSA). Streptococcus species (*S. anginosus* and group A β-streptococci) are the next most common pathogens.[89] Adults are more likely to have polymicrobial infections including anaerobes.



• **Figure 21.35** Histopathology of toxoplasmosis retinochoroiditis. **A,** Gross appearance of an inactive scar. **B,** Partial coagulative necrosis of the retina with some remaining lamination. Tachyzoites *(left inset)* and bradyzoites *(right inset)* can be found (×100; *inset*, ×400). **C,** Complete retinal necrosis with a dense underlying choroiditis; bradyzoites are seen in the *inset* (×100; *inset,* ×400). (**A,** Courtesy Hans Grossniklaus, MD; **C,** Courtesy Narsing Rao, MD.)

Subperiosteal Abscess

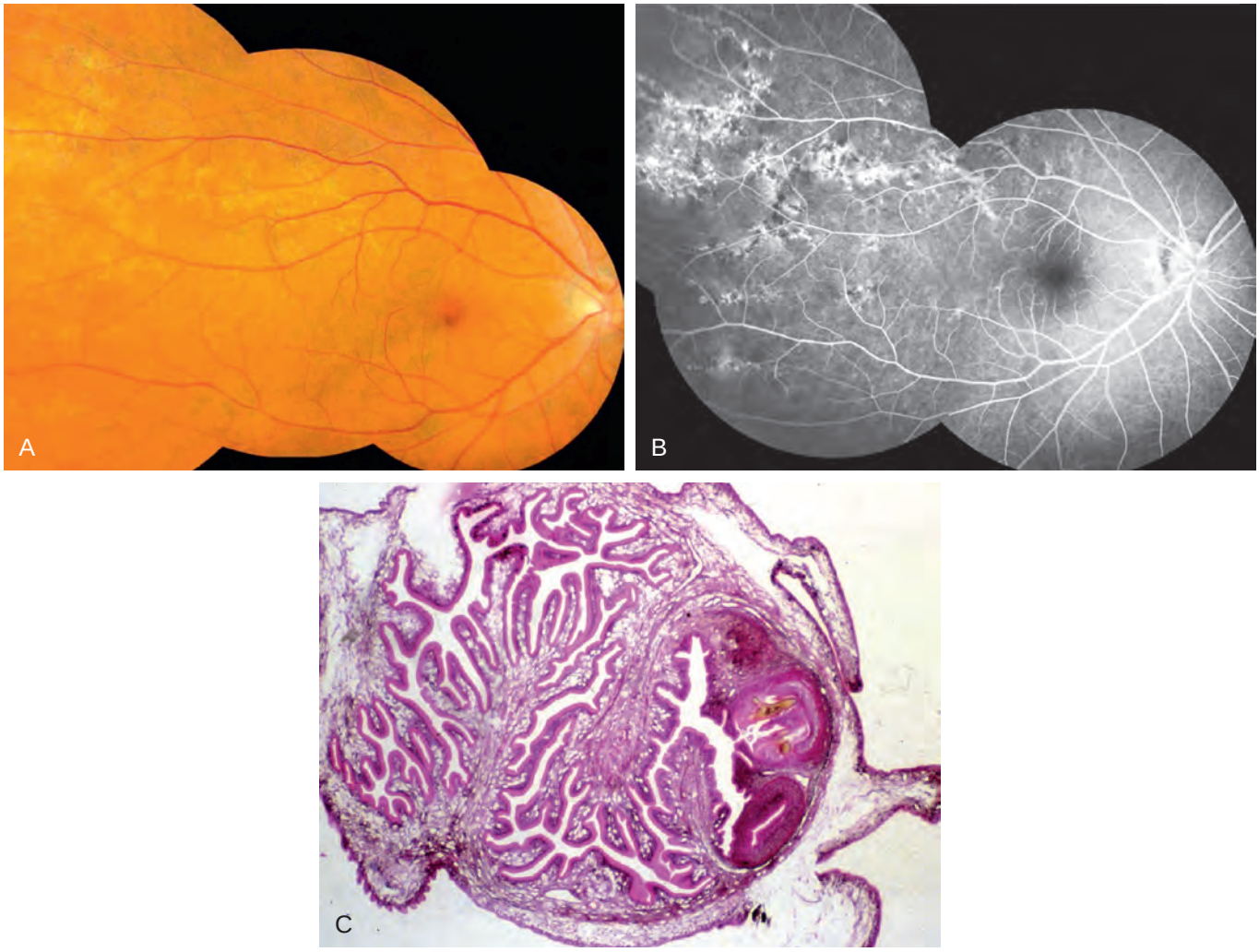
Subperiosteal abscess (see Fig. 21.38D) is a common orbital complication of bacterial sinusitis and orbital cellulitis. Prior to the mid-1990s, all abscesses were surgically drained. However, data have shown that patients younger than 9 years often have purely aerobic infections that can resolve with medical therapy alone. Patients older than 9 years or any patient at risk for anaerobic infection, those with frontal sinus involvement (risk for intracranial spread), and/or evidence of retinal or optic nerve compromise should undergo emergent drainage.[90] Histopathologic study reveals liquefactive necrosis surrounded by fibrous periosteal tissue and orbital fat and associated fibrous septa (Fig. 21.39A). Gram-positive organisms are most commonly identified (see Fig. 21.39B).

Necrotizing Fasciitis

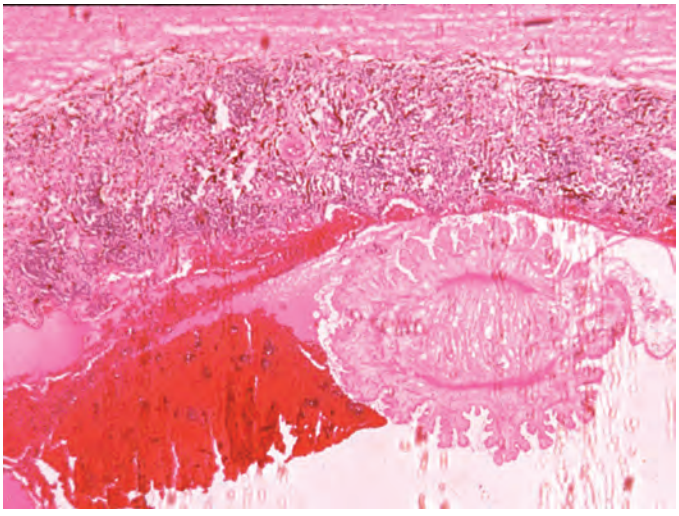
This condition rarely affects the eyelids, periocular tissues, and orbital contents due to group A β-hemolytic streptococci infection. Necrosis is likely mitigated by the rich vascular supply of the eye and orbit. Only 10% of cases of necrotizing fasciitis arise in the head and neck and fewer than 100 cases have been well documented in the ophthalmic literature. Patients may be immunocompromised (chronic alcoholism, diabetes) or have a normal immune status.[91] Bilateral eyelid and/or orbital involvement is often encountered, and in many cases débridement may be needed, including orbital exenteration. Histopathologic examination discloses suppurative inflammation with necrosis that extends from the eyelids into the orbit between the orbicularis oculi muscle and conjunctiva along the orbital fascial planes. Dense collections of gram-positive cocci can be displayed with Gram staining. Perivascular orbital inflammation with thrombosis and infarction of the optic nerves or ischemic necrosis of the retina, choroid, and iris may be present. Fundoscopic examination may reveal a central retinal artery occlusion, making the decision to exenterate the orbit easier.

Bacterial Dacryocystitis, Canaliculitis, and Dacryoadenitis

Dacryocystitis is produced by obstruction of the nasolacrimal duct with resultant tear stasis. Clinically, patients display erythema and



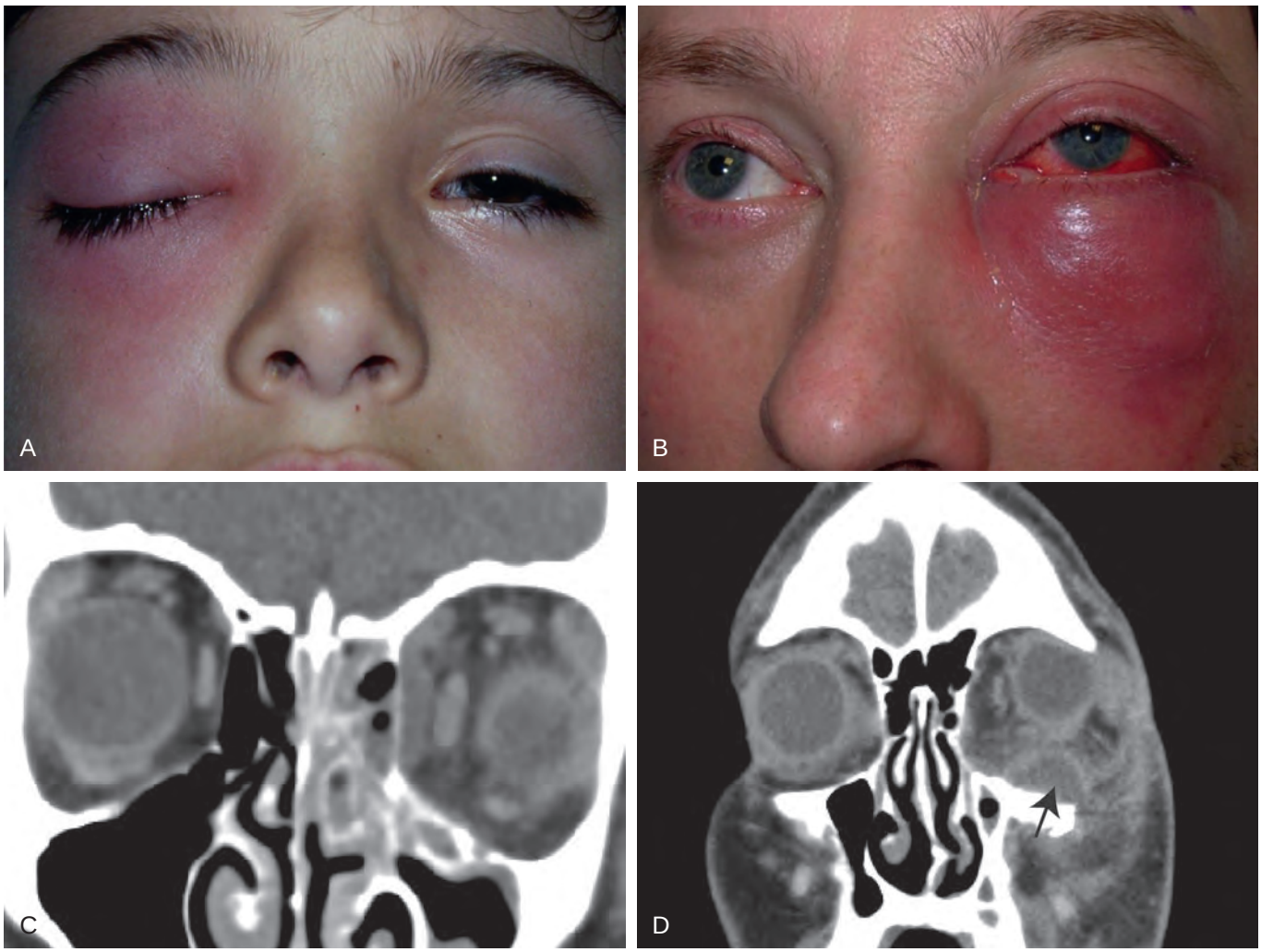
• **Figure 21.36** Diffuse unilateral subacute neuroretinitis (DUSN). **A,** The retina appears pale in areas of retinal pigment epithelium atrophy where the nematode has tracked through the subretinal space (×50). **B,** Late-phase fluorescein angiography highlights the pigment epithelial disturbance. **C,** Cross section of *Cysticercus cellulosae*, another cause of DUSN (*Taenia solium*, pork tapeworm) (×40). (**A** and **B,** Courtesy Emmett Cunningham, MD.)



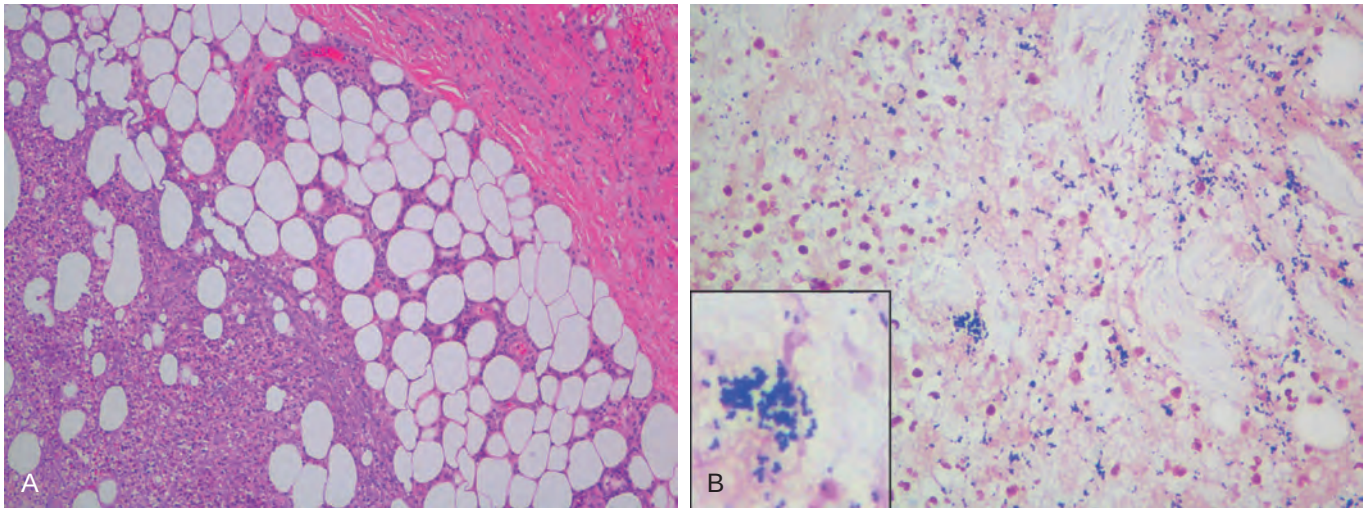
• **Figure 21.37** Intraocular cysticercosis. The branching body cavity of likely *T. solium* is seen in the anterior chamber of the eye, posterior to the iris. Hyphema (blood in the anterior chamber) is also present (×40). (Courtesy Narsing Rao, MD.)

swelling of the lacrimal sac, creating a mass in the medial canthal area, centered below the medial canthal tendon (Fig. 21.40A). Infected dacryocystoceles can become massive.[92] In contrast to orbital cellulitis, gram-negative organisms make up a large proportion of acute, purulent dacryocystitis. The most commonly isolated pathogens are *P. aeruginosa* and Staphylococcus species (especially *S. aureus*).[93] Cultures from chronic dacryocystitis more commonly yield coagulase-negative staphylococci and *K. pneumoniae*.[94] Along with antibiotic therapy or external drainage of an abscess through the skin, definitive treatment consists of dacryocystorhinostomy (DCR) surgery to create a new passageway for tears into the nasal cavity, eliminating the predisposing stagnant tear fluid in the lacrimal sac that is the basis for infection. Microscopic study of DCR specimens reveals chronic, and occasionally acute, inflammation of the fibrous wall of the lacrimal sac with varying degrees of fibrosis and granulation tissue. Organisms are occasionally identified (see Fig. 21.40B).

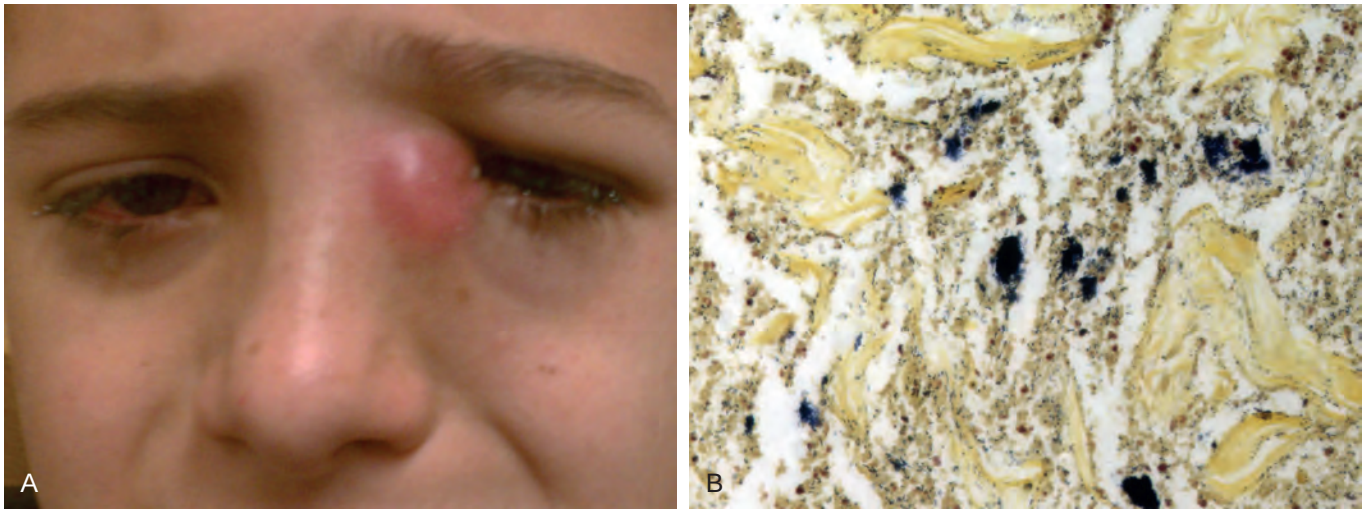
Infection of the canaliculus (a thin canal extending from the puncta of the eyelid margin to the lacrimal sac) is relatively uncommon and presents with epiphora (tearing) and edema and reddening of the puncta (usually lower punctum, seen as a



• **Figure 21.38** Bacterial orbital cellulitis. **A,** Preseptal cellulitis with erythema and edema of the upper and lower eyelids and accompanying ptosis. **B,** Orbital cellulitis resulting in massive orbital erythema and edema with proptosis, chemosis, and restricted upgaze. **C,** A concurrent sinusitis is almost always present (seen here as left ethmoid sinusitis on coronal computed tomography [CT] scanning). **D,** Coronal CT scan discloses a large subperiosteal abscess *(arrow)* of the maxillary bone in the inferior orbit (**A** and **C,** Courtesy Gerald Harris, MD; **B** and **D,** Courtesy Aaron Fay, MD.)



• **Figure 21.39** Histopathology of orbital cellulitis and subperiosteal abscess. **A,** A subperiosteal abscess displays central liquefactive necrosis (×100). **B,** Gram-positive cocci in clusters are demonstrated on Gram stain shown at higher magnification in the *inset* (×400) (**A** and **B,** Courtesy Valerie White, MD.)



• **Figure 21.40** Acute dacryocystitis. **A,** Massive swelling and overlying erythema of the medial lacrimal sac in a child. **B,** Histopathologic evaluation reveals gram-positive cocci within the sac (×400). (**A,** Courtesy Michael Yoon, MD.)

"pouting punctum"; Fig. 21.41A and B). Purulent discharge may be expressed (see Fig. 21.33A). Historically, the most common causative organisms of canaliculitis are *Actinomyces* species, although streptococcal and staphyloccal species have increasingly been identified.[95] Curettage of the canaliculi frequently produces stones or concretions that are histopathologically positive for *Actinomyces* (see Fig. 21.41C). These concretions are also called sulfur granules, are gritty in nature, and histopathologically display GMS- (see Fig. 21.41D) and gram-positive bacilli as branching filaments.[96] Canaliculotomy (opening of the canal through the skin) with removal of infected concretions is the most common treatment.

Dacryoadenitis (inflammation of the lacrimal gland located in the anterior superotemporal orbit just beneath the conjunctiva and behind the orbital rim) is typically idiopathic and noninfectious. Many infectious cases are viral mediated (Epstein-Barr virus, mumps), although *S. aureus* may cause an ascending bacterial dacryoadenitis via the excretory ducts of the gland that empty into the conjunctival sac.[97] Rare cases of granulomatous dacryoadenitis have been reported due to such organisms as *Schistosoma haematobium*[98] or Sporothrix or tuberculosis.

Mycobacterial Infections and Other Unusual Bacterial Infections of the Eyelids and Orbit

Tuberculous infections of the orbit are rare but may occur as a periostitis or orbital soft tissue tuberculoma; disease may also spread from the paranasal sinuses.[99] Atypical mycobacteria,[100] commonly found in the soil and water of the Southern coastal United States, have emerged as pathogens responsible of postoperative infections. They can involve the eyelid skin, creating unique clinical and histopathologic appearances. Patients present weeks after surgery (usually blepharoplasty) with erythematous, nodular eyelid lesions along the healing surgical incision (Fig. 21.42A), which may be mistaken for a suture granuloma or chalazion.[101] In nearly all cases, the acid-fast bacteria are found within the empty lipid vacuoles of a lipogranuloma, which may simulate chalazion histopathologically (see Fig. 21.42B and C).[102]

M. leprae has a high rate of ocular involvement.[103] Nodular skin lesions (Fig. 21.43A) and thickening of the eyelids and eyebrows caused by direct infiltration of *M. leprae* can be seen and can lead to loss of eyelashes (madarosis). A leonine facies eventually emerges. Ptosis caused by cranial nerve III palsy and ectropion and lagopthalmos by CN VII involvement are also noted in leprosy patients. Biopsy of eyelid lesions reveals acid-fast bacilli within the dermis (see Fig. 21.43B).

Malakoplakia is a histiocytic disease most commonly isolated from the bladder, usually in patients with some underlying immunosuppressive state or a chronic disease. Cutaneous cases are rare but have been reported in the ocular adnexa as hyperkeratotic, dome-shaped lesions resembling keratoacanthoma (Fig. 21.44A).[104] Defects in the function of histiocytes in response to bacterial colonies (often *Escherischia coli*) result in intracellular accumulation of iron and calcium (known as Michaelis-Gutmann bodies; see Fig. 21.44B). Diffuse sheets of histiocytes containing phagosomes with nondigested bacteria (known as von Hansemann cells) occupy the dermis.

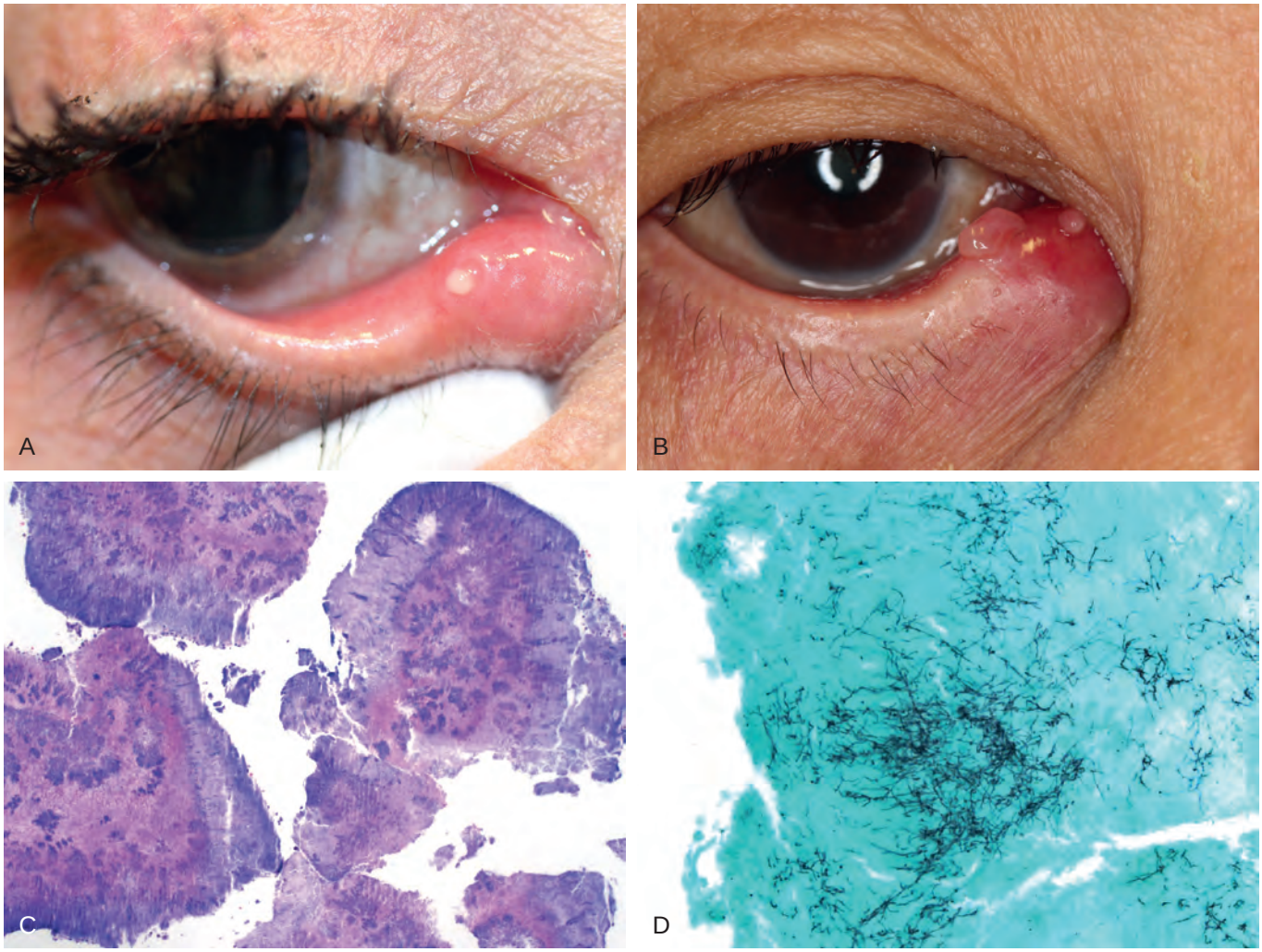
Botryomycosis or pseudomycosis, a rare morphologic expression of infection, which can occur in immunosuppressed patients, is due to chronic bacterial infection (usually with *S. aureus*) and displays histopathologic features similar to that of actinomycosis; conglutinated colonies of organisms appear to be encapsulated in granules. In these cases, a prominent eosinophilic ring (Splendore-Hoeppli phenomenon) surrounds the organisms. Rare cases involve the orbit.[105-107]

Infection of the adnexa by *Blastomyces dermatitidis* may follow systemic dissemination of a primary pulmonary infection. The infection causes pseudoepitheliomatous hyperplasia, evident as large hyperkeratotic verrucous plaques. The dermis contains granulomatous inflammation with microabscesses that harbor large budding yeast forms. Some infected patients have had contact with beaver dams.[108,109]

Viral Infections

Eyelid Molluscum Contagiosum

This affection, caused by a DNA poxvirus, commonly infects the face, including the eyelid margin. Pale, round smooth papules with a central umbilication at the eyelid margin may cause a



• **Figure 21.41** Canaliculitis. **A,** There is edema of the lower eyelid punctum (a "pouting punctum"). With minimal pressure over the area a purulent discharge can be expressed. **B,** A small pyogenic granuloma has formed on the lower eyelid margin due to chronic canaliculitis. **C,** An actinomyces concretion was removed during canaliculotomy with curettage (×100). **D,** The filamentous bacteria are well-seen with Gomori methenamine silver staining (×200). (**A** and **B,** Courtesy Daniel Lefebvre, MD.)

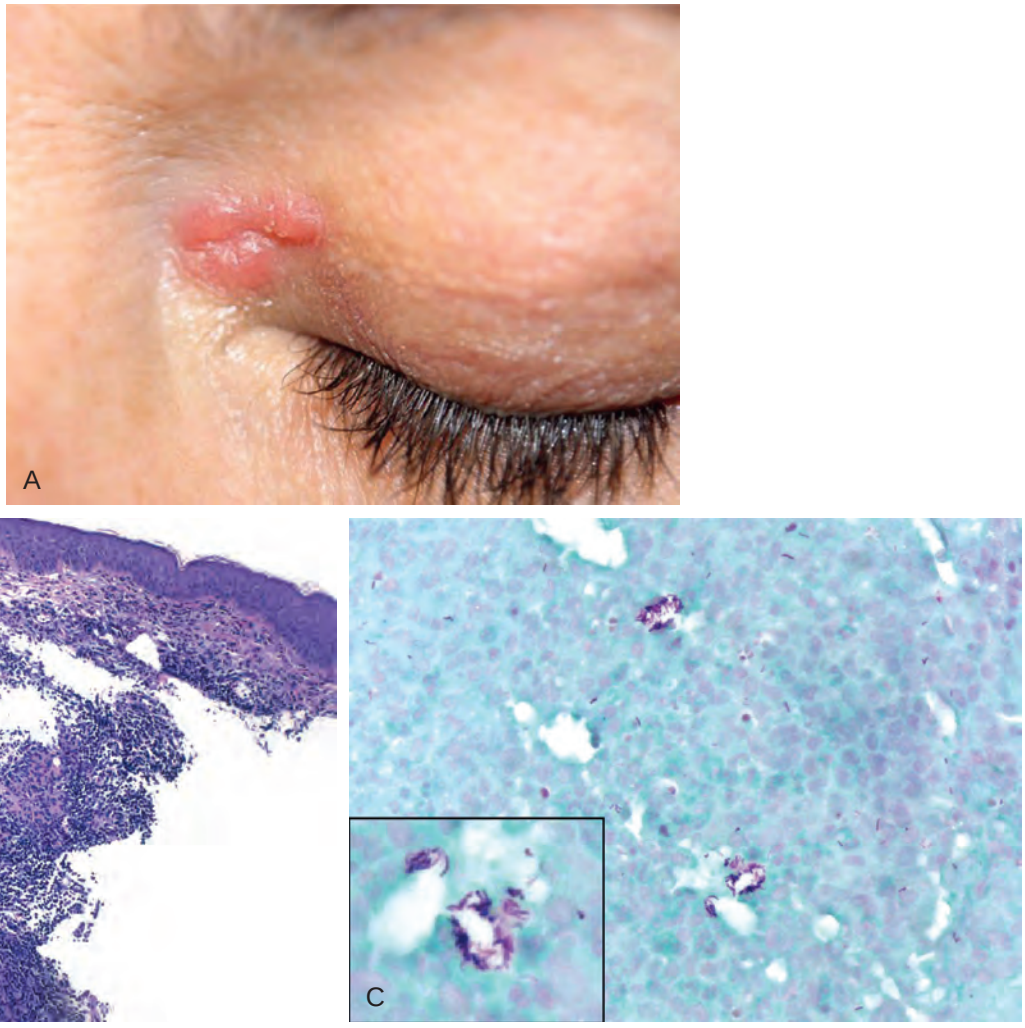
chronic, unilateral follicular conjunctivitis due to shedding of the viral particles into the inferior conjunctival sac (Fig. 21.45A). Numerous lesions may be seen in immunocompromised (especially HIV/AIDS) patients. These patients, as well as immunocompetent patients, may develop exceptionally rare epibulbar conjunctival molluscum lesions.[110] Histopathologically, the excised lesions show numerous molluscum bodies (Henderson-Patterson corpuscles) within an acanthotic, hyperkeratotic infected epithelium with surrounding chronic, nongranulomatous dermal inflammation (see Fig. 21.45B). Immunopathologic studies have revealed that the inflammatory infiltrate is composed almost entirely of T lymphocytes.[111] Eyelid lesions are not uncommonly excised and sent for histopathologic examination; their removal cures the chronic conjunctivitis.

Herpesvirus Family

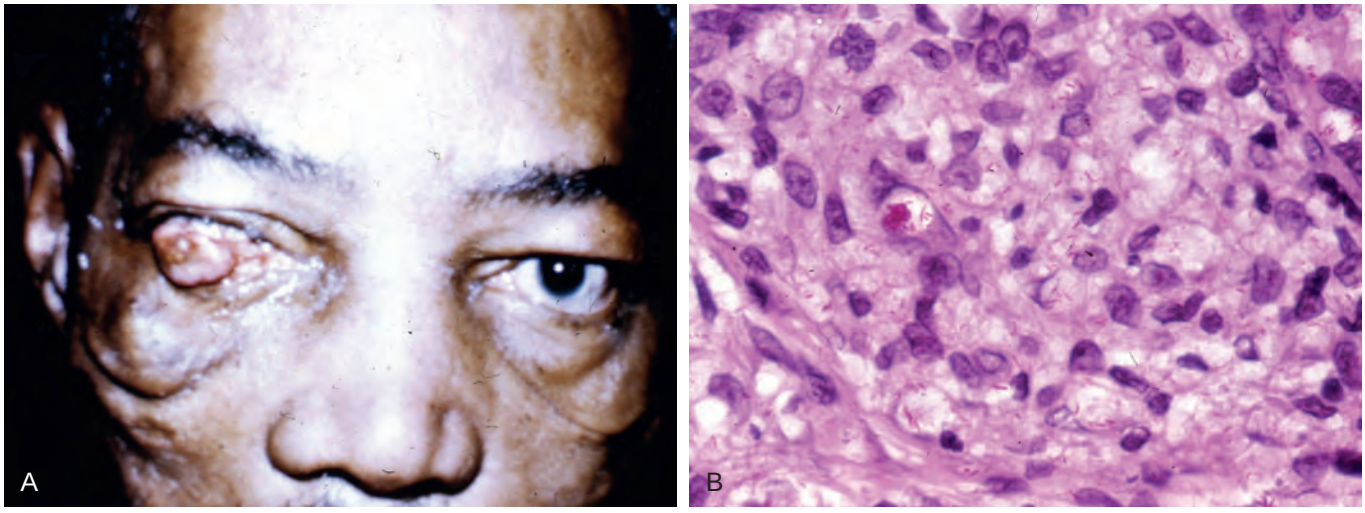
As discussed in the conjunctival and cornea sections, primary HSV often manifests as a unilateral vesicular rash with a blepharoconjunctivitis (Fig. 21.46A). Angular blepharitis (inflammation of the angles of the eyelids temporally), usually caused by staphylococci or Morax-Axenfeld *diplobacilli*, may be present.[112] Herpes zoster ophthalmicus from reactivation of the latent VZV in the trigeminal (gasserian) ganglion can present in a similar fashion, usually in middle-aged, elderly, or immunocompromised individuals. The painful vesicular rash always respects the midline. Occasionally, only a single vesicular lesion is observed.[113] Histopathologic evaluation demonstrates multinucleated, acantholytic keratinocytes with distinct intranuclear inclusions; full-thickness epidermal necrosis follows (see Fig. 21.46B). PCR or immunohistochemistry distinguishes HSV1, HSV2, and VZV (see Fig. 21.46C).

Fungal Infections

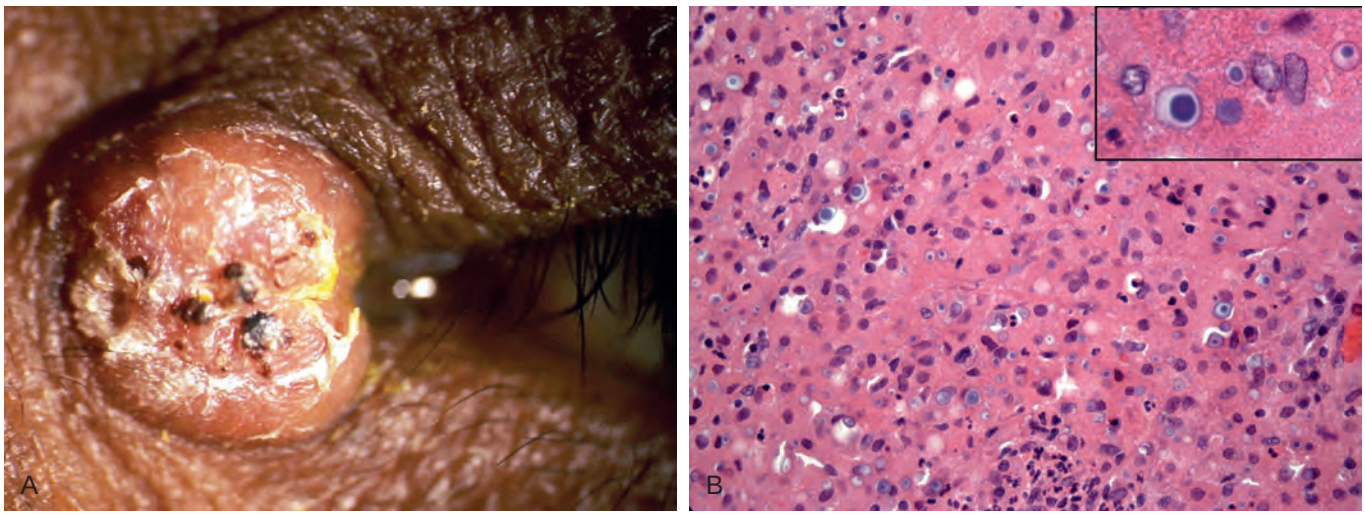
Invasive orbital fungal infection with *Mucor* or *Rhizopus* fungi (class Zygomycetes), like most cases of bacterial orbital cellulitis, begins in the nasal cavity or sinuses and extends into the orbit.[114] Patients with invasive fungal rhinosinusitis are nearly always immunosuppressed due to hematologic malignancy, diabetes, immunosuppressive therapy after solid organ transplantation,



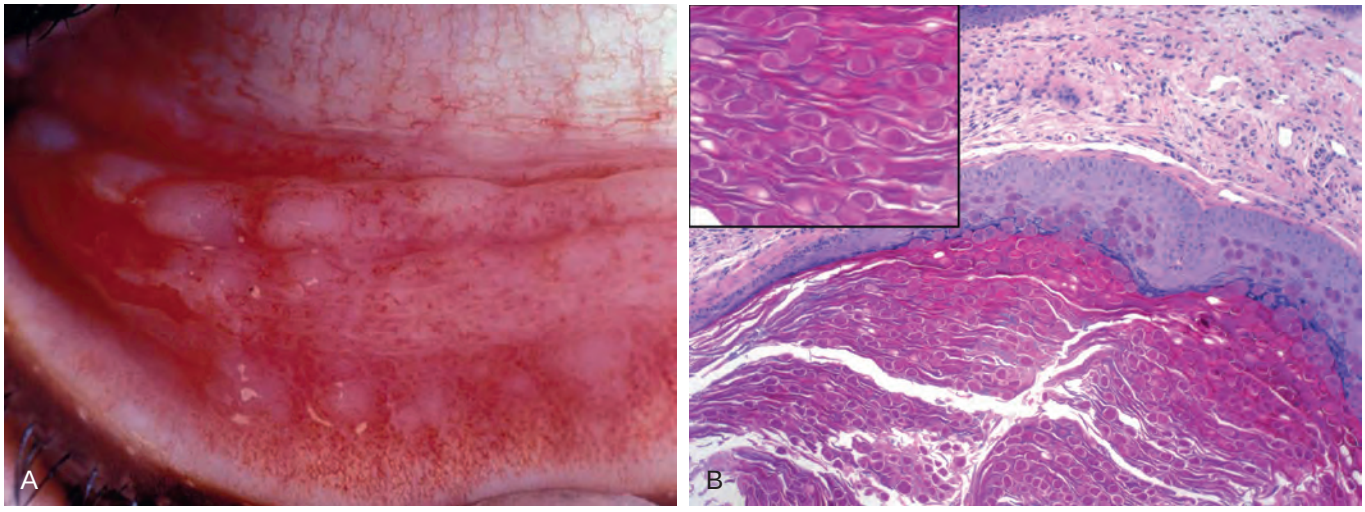
• **Figure 21.42** Atypical mycobacterial infection of the eyelid. **A,** Nodular erythema has developed at the site of a prior blepharoplasty incision. **B,** There is dermal lipogranulomatous inflammation, mimicking a chalazion (×100). **C,** Acid-fast bacilli (seen at higher power in the *inset*) are clustered within dissolved out lipid locules, highly characteristic of this infection (×400). (**A,** Courtesy Daniel Lefebvre, MD.)



• **Figure 21.43** Ocular leprosy. **A,** A nodular thickening of the lower eyelid with madarosis due to *Mycobacterium leprae*. Patients are sometimes characterized as having a leonine facies. **B,** Acid-fast bacteria are seen in foamy macrophages (Virchow cells) in the dermis of a skin biopsy (×400).



• **Figure 21.44** Malakoplakia. **A,** A medial canthal lesion somewhat similar in appearance to a keratoacanthoma. **B,** Sheets of von Hansemann histiocytes occupy the dermis (×200). Michaelis-Gutmann bodies are seen at high power in the *inset* (×600).



• **Figure 21.45** Molluscum contagiosum. **A,** A follicular conjunctivitis has resulted from chronic shedding of viral particles into the inferior fornix. **B,** Molluscum bodies fill a central pore of this eyelid lesion (×100). The so-called Henderson-Patterson bodies highlighted at higher power in the *inset* (×200). (**A,** Courtesy Dan Jones, MD.)

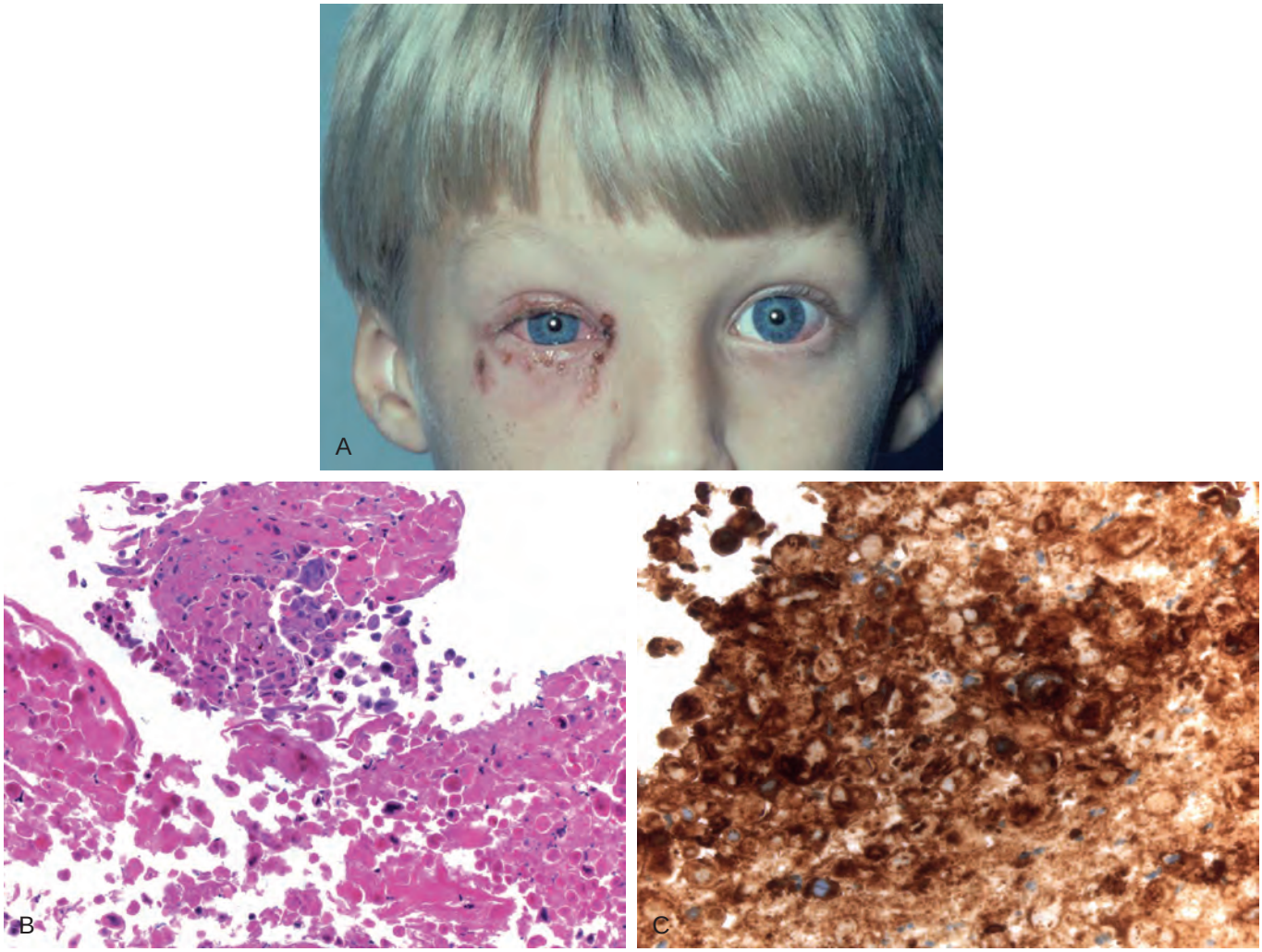
long-term corticosteroid use, HIV/AIDS, etc. These fungi can invade blood vessel walls, causing thrombosis and tissue ischemia (eschar formation, Fig. 21.47A), which leads to further invasion by reducing tissue oxygen levels. Diagnosis is often suspected from necrotic tissue seen within the nasopharynx, using nasal endoscopy. Orbital disease presents with proptosis, ophthalmoplegia, and decreased vision. Optic neuropathy and other cranial neuropathies may be detected and central retinal artery occlusion is relatively common. The infection can be life-threatening. The role of exenteration in the management of orbital fungal infection is debated and is not guaranteed to preserve life.[115]

The hyphae of Mucor species may be seen within the walls of orbital blood vessels with H&E staining (unlike other fungi; see Fig. 21.47B). They are nonseptate, wide (7 to 30 μm) and often branch at 90-degree angles. Invasive *Aspergillus*, on the other hand, displays hyphae of uniform width that are narrow (2 to 4 μm) and septate (see Fig. 21.47C). They stain with PAS or GMS and branch at acute, often 45-degree angles. Cases of rhino-orbital-cerebral disease have been found to be due to a related fungus of the genus *Apophysomyces*. They have been reported in immunocompetent patients following minor trauma with implantation of soil or decaying vegetable matter; these infections may be more indolent.[116]

Parasitic Infections

Trichinosis

Infection with *Trichinella spiralis* and other *Trichinella* species[117] occurs after ingestion of contaminated meats containing larvae. The usual offender is contaminated pork, but a variety of sources, including horse meat, has been indicted. Adult worms leave the intestinal mucosa to invade striated muscle.[118] During this larval migration, patients often have systemic symptoms, such as fever, periorbital edema, and conjunctivitis (thought to reflect allergy



• **Figure 21.46** Herpes simplex and herpes zoster of the eyelid. **A,** A child with primary herpes simplex virus infection displays multiple skin vesicles, especially along the eyelid margin. **B,** Biopsy from a patient with herpes zoster ophthalmicus reveals acantholysis of the entire epidermis (×200). **C,** Immunohistochemistry is positive for varicella zoster virus (×400). (**A,** Courtesy Dan Jones, MD.)

rather than direct migration through tissue). Larvae may rarely migrate into retinal vessels but rather encyst in the extraocular muscles (Fig. 21.48A), where they cause pain with eye movement or paresis with diplopia. Histopathologic examination reveals a basophilic change to the infected muscle cells (see Fig. 21.48B).[119] Calcification of *Trichinella* cysts may be seen on imaging studies of involved extraocular muscles.[120]

Dirofilaria

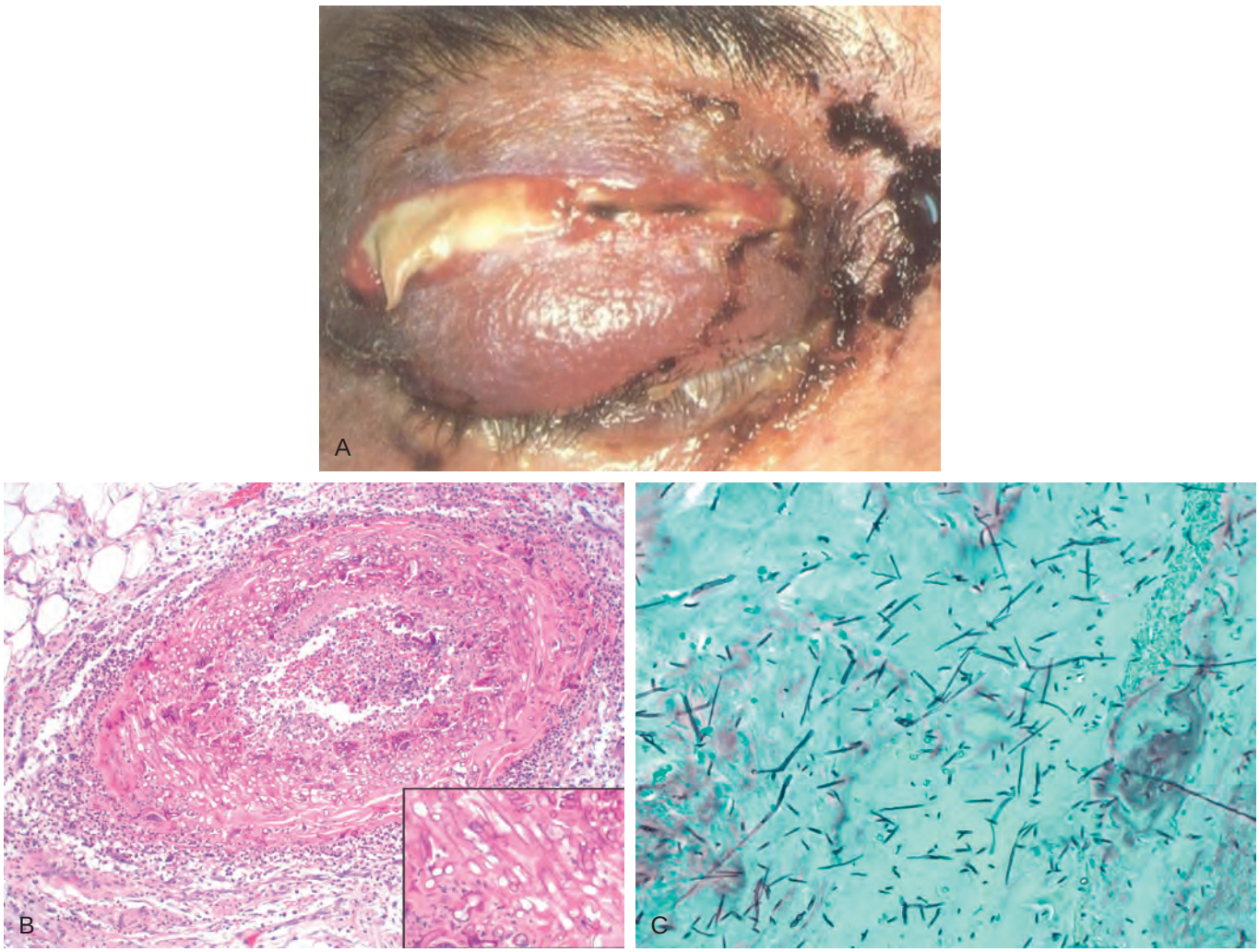
Dirofilaria species are zoonotic filariae that are transmitted to humans through an infected vector (blood-sucking insects) that have fed on an animal with a filarial infection. The most common presentation is a periocular subcutaneous nodule filaria that may be found subconjunctivally or within the eyelid or orbital tissues, clinically and radiographically mimicking preseptal or orbital cellulitis or orbital "pseudotumor" (Fig. 21.49A).[121] Eyelid/brow or orbital biopsies[122] may reveal necrotizing granulomas.[123] A moderate number of eosinophils may be seen along with foreign body giant cells. Surrounding microabscesses with degranulated eosinophilic leukocytes (Splendore-Hoeppli phenomenon) may also be seen. The organisms can be found in the center of a necrotizing granuloma (see Fig. 21.49B) and display a characteristic thick cuticle (5 μm thick) (see Fig. 21.49C) with prominent external ridges and numerous tall muscle cells that extend far into the body cavity. Removal of the subcutaneous nodule or subconjunctival worm is curative.

Echinococcosis

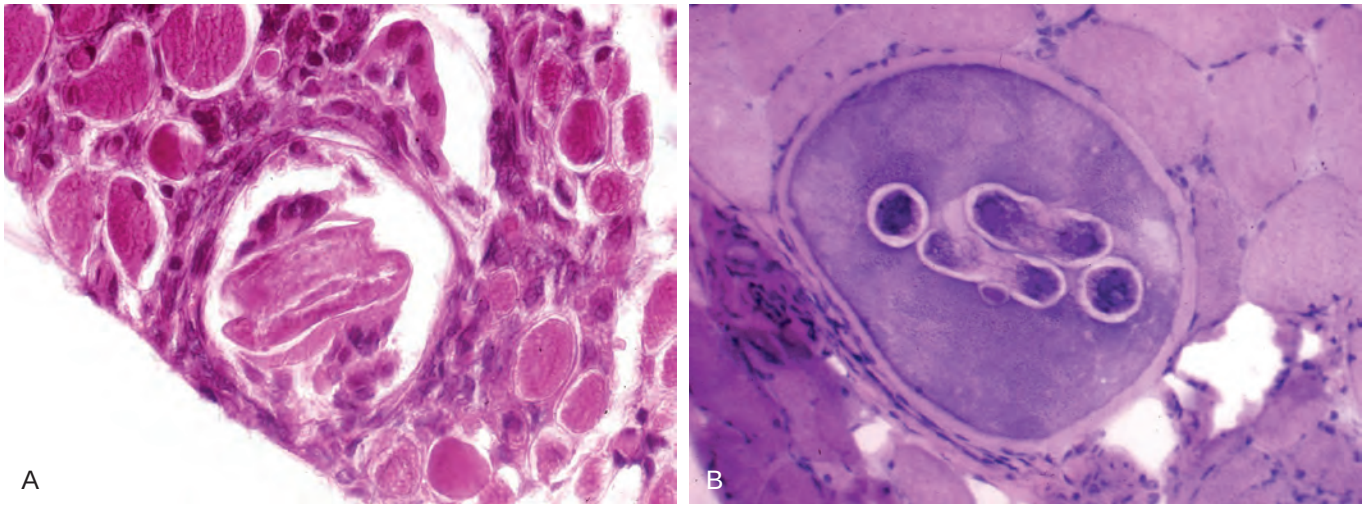
The adult tapeworm of *Echinococcus granulosa* causes hydatid cyst of the orbit. Patients (usually children) present with unilateral slowly progressive proptosis and a quiet eye in areas where the disease is endemic such as the Middle East.[124,125] The hydatid cyst forms a thick fibrous capsule in the orbit as it enlarges. Treatment of orbital disease is surgical. Histopathologic examination reveals multiple scolices with hooklets within a PAS-positive cyst wall.[125]

Myiasis

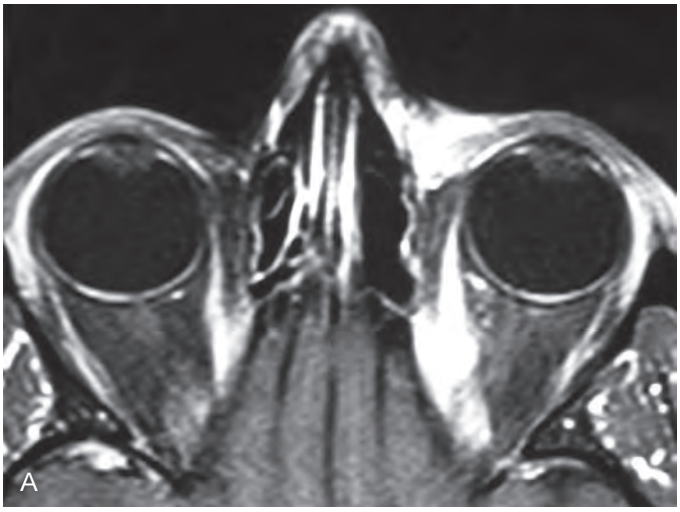
Ophthalmomyiasis is due to invasion of the ocular and adnexal tissue by fly larvae and can be classified as external (conjunctiva or eyelid), internal (subretinal space or vitreous cavity), or orbital.[126] The larval tracks may be seen in the subretinal space. In the eyelid a gelatinous head often develops (Fig. 21.50A). The

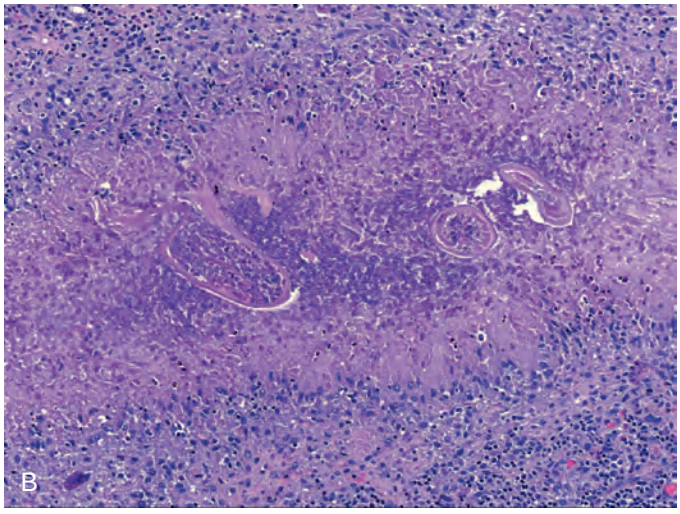


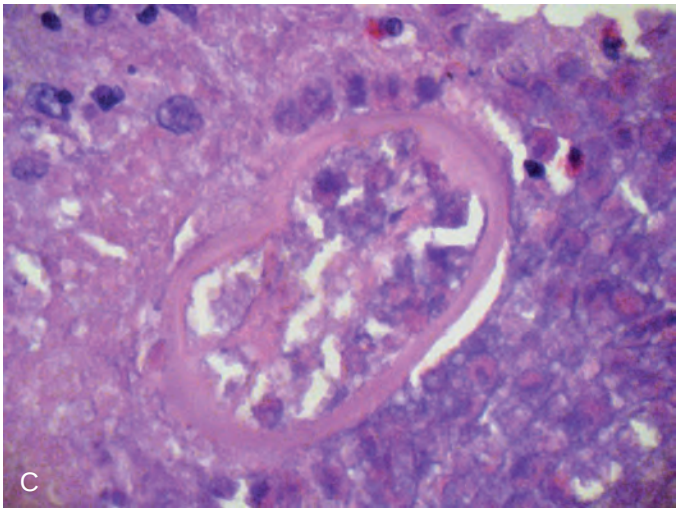
• **Figure 21.47** Fungal orbital cellulitis. **A,** Necrosis of the eyelid skin and purulent discharge are observed in this case of invasive fungal cellulitis. **B,** In mucormycosis, the broad hyphae have invaded the wall of an orbital vessel (×100), seen at higher power in the *inset* (×400). **C,** Invasive aspergillosis displays acutely branching hyphae that are narrower than those of mucor (×100). (**A,** Courtesy Aaron Fay, MD.)



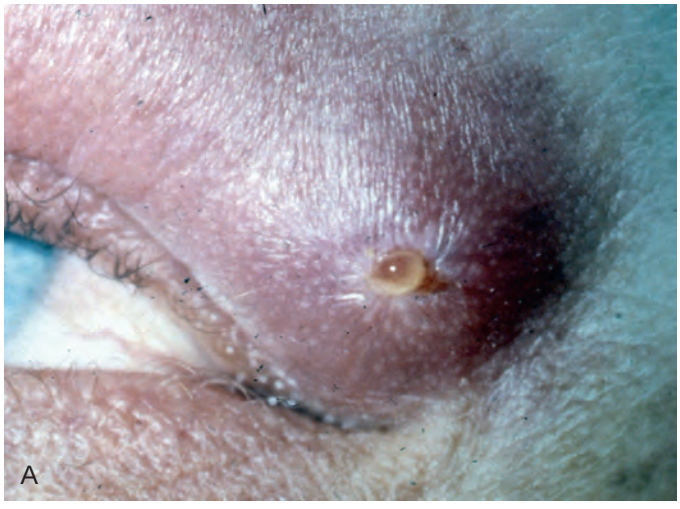
• **Figure 21.48** Orbital trichinosis. **A,** Biopsy of the extraocular muscle shows the encysted organism, *Trichinella spiralis* (×200). **B,** The larvae are seen in cross section inside a muscle cell that has undergone basophilic transformation, a characteristic finding of infestation (×400).







• **Figure 21.49** Dirofilaria. **A,** Post-contrast T1-weighed magnetic resonance imaging reveals enhancement in the medial anterior orbit. **B,** A necrotizing granuloma is disclosed on biopsy (×200). **C,** The organism displays a characteristically thick cuticle (×400). (**A** to **C,** Courtesy Thaddeus Dryja, MD.)



• **Figure 21.50** Ophthalmomyiasis. **A,** Ophthalmomyiasis externa due to fly larval infestation of the medial eyelid with a characteristic gelatinous cap. **B,** Gross photograph of the larva after removal.

larvae may spontaneously appear or after chemical immobilization may be removed from the eyelid, orbit, or aqueous/vitreous cavity (see Fig. 21.50B).[127]

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