BIOLOGICAL EFFECTS OF ULTRASOUND

This article summarizes the interaction between sound waves and mammalian tissue and the resulting biological effects. We define these biological effects, or "bioeffects," as the distortion or destruction of tissue, or enhancement of artificial or natural biological phenomena within biological media. Our intention is to review the variety of phenomena that occur when sound interacts with living tissue without offering an exhaustive survey. There are excellent review articles and books that we reference on the subject of diagnostic ultrasound, that is, using sound for imaging, when bioeffects are to be minimized or avoided completely. Therefore, we lean heavily on these publications so that we can concentrate on relatively recent and largely unreviewed work on therapeutic ultrasound where sound is used to create bioeffects intentionally for useful purposes.

In the first section, we describe how sound interacts with tissue, emphasizing what can happen regardless of whether or not what happens is desirable. We start by describing the propagation, absorption, and scattering of sound within biological tissue. Scattered sound may be absorbed elsewhere in the tissue or be received eventually at a hydrophone, where it gives information about the tissue from which it ultimately scattered. The absorption of sound by biological tissue creates within it several physical and chemical processes, which we discuss in the second section. Ultrasound raises the temperature of the tissue. It adds momentum, which strains tissue and also repels it from the direction of the acoustic source. If the ultrasound is absorbed in fluids, it causes flow called "acoustic streaming." Ultrasound-induced heat and/or strain cause "cavitation," that is the generation and/or stimulation of bubbles. Cavitation can, in turn, produce local strains in the tissue and fluid that are close to the bubbles. It can increase the momentum absorbed from the incident sound field;

it can increase the scatter of sound; it can add heat; and it can generate free radicals. The biological effects of ultrasound have their ultimate source in these thermal, mechanical, and chemical processes.

With these basic acoustic facts in mind, we turn in the third section to diagnostic ultrasound. The field of diagnostic ultrasound rests primarily on creating interpretable images of insonified portions of the body using the fact that the quality of sound scattered back from biological tissues and fluids correlates with their intrinsic properties. We review the creation of different diagnostic ultrasound images along with some of the unanswered questions in this field and a sketch of its scientific frontiers.

Diagnostic ultrasound has attracted the attention of a large part of the biomedical acoustics community over the last few decades. Therefore, much research has been devoted to maximizing imaging quality while learning how to avoid acoustic bioeffects other than scattering. Finally, in the fourth section, we describe how many of these bioeffects have been reevaluated for their possible therapeutic benefits, in treating biological problems with ultrasound, rather than simply visualizing or diagnosing.

FUNDAMENTALS OF PROPAGATION AND ABSORPTION OF ULTRASOUND

Linear and Nonlinear Acoustic Waves

When sound propagates in fluids it creates local, periodic perturbations in density, pressure, and temperature and induces small-scale displacements. In a fluid those displacements and changes in pressure occur along the direction of wave propagation. When sound propagates in a simple solid, it generally does so via "longitudinal" pressure waves, just described, and via "shear" waves, where the displacements and changes in pressure occur transverse to the direction of wave propagation (1). Longitudinal waves dominate in the majority of biomedical applications, and we restrict our discussion to this mode.

Typical applications of ultrasound for diagnosis (2,3) use short pulses (generally one to a few acoustic cycles) of intense (up to 5 MPa of instantaneous pressure with instantaneous intensities of up to a few hundred W/cm^2) ultrasound spaced fairly far apart in time (typically once every 0.1 ms to 1.0 ms) at frequencies (1 MHz to 20 MHz) high enough to resolve fine-scale biological structure (with length scales from 0.1 mm to 1.5 mm) by generating and measuring acoustic backscatter. The specific choices of acoustic parameters balance the need to maximize the backscattered signal strength and imaging resolution, by increasing the intensity and frequency of the sound, with the need to avoid harmful biological effects. The latter is achieved by decreasing the length and instantaneous pressure amplitude of the pulses, and by increasing the spacing of the pulses, all to help minimize the production of heat and mechanical forces within the imaged tissue.

Therapeutic ultrasound generates beneficial bioeffects by using a wider range of frequencies (0.02 MHz to 10 MHz), focal pressures (0.01 MPa to 50 MPa) and intensities (0.1 W/cm^2 to 10,000 W/cm^2) applied either in pulsed mode as in diagnostic ultrasound, often with greater pulse lengths and more pulses per second than in diagnostic ultrasound, or with "continuous waves," where there is no break in applying ultrasound from the time it is first turned on until it is finally turned off.

Although sound propagation, in principle, is always a "nonlinear" process, that is, the properties of the sound as it propagates vary as a complex function of its amplitude, in many practical applications one may consider the properties of sound to vary linearly with amplitude. Under those circumstances simple and useful formulas exist (1,3) that relate the sound's frequency, amplitude, intensity, particle displacement, etc., assuming that the pressure wave varies sinusoidally in space and time. However, those formulas and the sinusoidal concept on which they rest break down when the amplitude of the sound increases sufficiently. For example, in an unbounded medium, the initial "sine wave" form of the acoustic wave evolves into a sharpened, symmetrical, sawtooth structure as the increase in amplitude of the wave generates harmonics of the initial single-frequency sinusoid. The addition of diffraction, absorption, and focusing breaks that symmetry (4). In lithotripsy, for example, where the application of high-intensity pulses of focused ultrasound breaks up calcified stones within the kidney and gallbladder, standard applications create a short acoustic pulse in the form of a shock wave whose shape is far from sinusoidal, with peak positive pressures of up to 50 MPa with rise times of less than a few nanoseconds, and peak negative pressures of up to 1 MPa that last a few microseconds (5). One can represent these nonlinear waves by a Fourier series. Within this description, one can say that the acoustic wave becomes nonlinear by generating harmonics of the fundamental wave, as the latter propagates and grows.

Acoustic Attenuation as Absorption Plus Scattering

As unfocused sound propagates through a medium, its amplitude decreases, in part because the medium absorbs the sound and in part because the acoustic energy is scattered in a direction away from the direction of propagation. The relative amount of absorption versus attenuation in biological tissue depends significantly on the type of tissue. To appreciate why tissue attenuates sound, we start by quantifying how much sound tissue attenuates. The attenuation coefficient describes how much the amplitude of a propagating wave decreases over a standard distance. For example, if a medium has an attenuation coefficient of 1 Np/cm (Np = Neper), this means that as the sound propagates 1 cm, its amplitude is reduced by a factor of $1/e$. Another standard unit is dB/cm. For this unit, an attenuation coefficient of 20 dB/cm means that in 1 cm the amplitude of a propagating wave is reduced by one-tenth.

At 1 MHz the attenuation coefficient in water at room temperature is 0.00025 Np/cm, a negligible amount in the laboratory and significant only over kilometers in the ocean. In pure water, attenuation occurs by thermally induced structural relaxation of the water molecule, with a few additional molecular relaxation mechanisms that correspond to each of the typical chemicals in salt water (6,7). However, attenuation in biological tissue at 1 MHz is significantly higher, at times owing to increased absorption and at other times to increased scattering (8–10). For example, the attenuation coefficient for whole blood is 0.024 Np/cm whereas for plasma (whole blood minus red and white cells and platelets) it is 0.008 Np/cm. The different attenuation values for plasma and whole blood

at the same frequency arise mostly because of scattering of the sound by the cells in whole blood, an attenuation mechanism missing from plasma. The different attenuation coefficients for plasma and water at the same frequency arise from the individual proteins in plasma that absorb sound more efficiently than water, because proteins have many more degrees of freedom available than water molecules (8–10). [Although the levels of attenuation at 1 MHz differ, as described, sound attenuates more quickly in water as a function of increasing frequency than in biological tissue. Attenuation increases as the square of the frequency in water but at only a little more than the first power of frequency in most tissue (9).] At 1 MHz, liver has an attenuation coefficient of 0.05 Np/cm, larger than that of plasma, because liver has a greater concentration of proteins. (Pureed liver has the same attenuation coefficient as whole liver (11), thus showing that absorption on the molecular scale rather than at the scale of tissue structure causes acoustic attenuation.) Collagen is the common protein in biological tissue, and its concentration in tissue correlates well with acoustic attenuation due to absorption. Fat also absorbs a significant amount of acoustic energy. Subcutaneous fat from a pig has an attenuation coefficient of 0.21 Np/cm at 1 MHz. The high fat content of liver contributes significantly to its absorption of sound, along with collagen. Finally, human lung tissue has an attenuation coefficient of 3.5 Np/cm at 1 MHz, almost entirely because of the scattering of sound rather than absorption, whereas bone at 1.0 MHz has an attenuation coefficient of 2.5 Np/cm, almost entirely from absorption rather than scattering.

These differences in absorption produce complications with important practical consequences. We now discuss one example.

The Derating Problem. Here we follow the discussion by Carstensen (5). Therapeutic applications require imposing a prescribed dose of ultrasound. Diagnostic ultrasound requires a dose less than that known to create damage for diagnostic ultrasound applications. Transducer characterization is typically done in water, even though transducer applications occur ultimately in tissue. "Derating" means using acoustic measurements in water to predict the acoustic fields in tissue for purposes of calibration. This works well when there is an appropriate linear model for propagation in tissue and when linear acoustics describes the propagation conditions that pertain to measurements in water. In particular, with acoustic measurements in water and mathematical models, one can translate those measurements into predictions of acoustic pressure in tissue, because the attenuation under these different conditions scales from one to the other.

However, many diagnostic and therapeutic devices produce nonlinear waves in both water and tissue. This creates several problems for derating. One has to worry about cavitation during measurements in water, although one can avoid it under many circumstances. Saturation of the amplitude of the propagating wave makes tenuous the one-to-one relationship between input voltage and measured pressure. This is so because as the acoustic waves become nonlinear, the higher harmonics in those waves attenuate significantly, stopping the continued rise in signal level. For a high enough initial signal, an equilibrium develops between the low-frequency waves that receive the initial energy and the highest harmonics that rapidly attenuate. The production of nonlinear acoustic waves also complicates the modeling step that connects the measurements in water to predictions in tissue. Because high-frequency waves attenuate more quickly in water than in tissue, acoustic saturation occurs at lower initial signal amplitudes in the former than in the latter. Therefore, under circumstances when the pressure amplitude increases in tissue, it levels out in water. If a given pressure occurs under conditions of acoustic saturation in water, using linear acoustic theory to translate that value to pressure in tissue, results in an underestimate. Fortunately, a solution to this problem exists. Calibration is carried out under conditions of no acoustic saturation, and the small-amplitude results are then extrapolated using a linear function. This works fine for calibrating diagnostic systems because it overestimates the signal level in tissue, a conservative estimate that avoids the production of bioeffects if one knows the actual acoustic pressure associated with those bioeffects. If one needs a better estimate of the incident pressure in tissue there is the option of more careful mathematical modeling (12).

Absorption of sound by tissue results in physical and chemical effects through the generation of heat, the addition of momentum, and the production and stimulation of bubbles, the latter known as "acoustic cavitation." We discuss these subjects in turn.

PHYSICAL AND CHEMICAL PROCESSES ENGENDERED BY MEDICAL ULTRASOUND

Heat Generation and Thermal Index

Sound absorbed by tissue generates heat at the site of absorption in a process described mathematically by the negative of the gradient of the energy flux vector of the sound field. When the acoustic waves are linear or weakly nonlinear, the heat-generation term reduces to a quantity proportional to the intensity of the signal and absorption (not attenuation) coefficient of the tissue, although for most applications the attenuation coefficient is used instead of the absorption coefficient. The "bio-heat" equation (13) describes how the heat generated by ultrasound produces a temperature rise within the tissue by codifying the combined effects of tissue diffusion, heat capacity, and density along with the spatially integrated action of capillary beds, which "perfuse" heat away from its acoustic source as long as the tissue remains undamaged. Arteries or veins also conduct heat away from a site, and their presence within real and modeled tissue severely alters the temperature effects induced by ultrasound (14). Under therapeutic conditions, the temperature can approach 100°C in a fraction of a second (15). This rapid temperature rise denatures tissue—useful for "cooking" cancer cells as a way to kill them—or even vaporizes tissue by the boiling of its constituent water or by cavitation—useful, for example, for ablation-based therapies for killing cancer or for reopening passages within the body. (We discuss these applications in the section on therapeutic ultrasound.) However, such effects are to be avoided when applying ultrasound diagnostically. The thermal index gives a measure of the temperature rise induced in tissue under diagnostic conditions. When using diagnostic ultrasound, values of the index less than a critical value are desired. The index is based on conservative estimates of the average heat generated within tissue and takes into account transducer characteristics that govern the intensity of sound

at the site of acoustic heat generation and the tendency for tissue to absorb sound (16).

Acoustic Radiative Pressure

Sound absorbed by tissue and fluids adds heat and also adds momentum to those media via a force known as the "acoustic radiative force," created by the negative of the gradient of "radiative pressure" induced by acoustic waves. For example, when a single-frequency acoustic wave deposits momentum in a substance away from boundaries, the sound effectively pushes the substance in a time-independent way in the acoustic-wave propagative direction. In water, this process shows up as a steady current moving away from the transducer, known as "acoustic streaming." Within tissue, this process strains the tissue by attempting to move it away from the acoustic source. The presence of a bubble or any large impedance mismatch increases the acoustic radiative force generated by the ultrasound. The presence of several discrete absorbers (such as several bubbles) also engenders forces between the absorbers, known as "Bjerknes" forces in the case of bubbles. Finally, the radiation force allows an isolated bubble to create a force on its surrounding liquid, known as "microstreaming" (discussed later).

Subtleties abound in the concept of radiative pressure, and Beyer's (17) oft-quoted comment remains valuable enough to quote again: "It might be said that radiation pressure is a phenomenon that the observer thinks he understands—for short intervals, and only every now and then." Moreover, most analysis rests on the study of acoustic-momentum absorption in fluids, not in tissue. We cannot address these subtleties here, many of which are based on the presence or absence of confining geometry and on whether or not one works in Eulerian or Lagrangian coordinate systems. Instead, we refer the reader to recent analyses (18–20) of acoustic radiative pressure in fluids that offer concise mathematical representations of radiative pressure and discuss radiative pressure in terms of its constituent energy densities: kinetic, potential, and "hydrostatic," the latter particular to the presence of boundaries.

Acoustic Cavitation

Excellent surveys of cavitation (21–23) review bubble formation and growth; bubble dynamics (the properties and behavior of isolated or communities of bubbles when stimulated by ultrasound, including bubble scattering and emission); mechanical effects of bubbles, including microstreaming and hydrodynamic jet and shock formation; and sonochemistry. All of these physical and chemical processes occur in vivo often with profound biological consequences. These processes and their effects therefore deserve an extensive presentation. Because we cannot do justice to this incredibly rich field, however, we content ourselves here with a cursory overview that highlights the essentials and most interesting aspects, and draws liberally from the references quoted in this paragraph, among others. Our general discussion of cavitation focuses on bubble behavior in solution, where current understanding has its firmest underpinnings. Observations in vivo support the utility of this approach, but the field could always use more in vivo measurements of cavitation.

Bubble Formation and Growth. In practice, water cavitates at tens of kilohertz at pressure amplitudes of a few tenths of a megapascal. However, in theory the threshold for cavitation of water is a hundred times this pressure, considering the tensile strength of pure water. The reason for this disparity is the impurities in water. Examples include dust particles, which trap minute quantities of gas within cracks on its surface, or microbubbles within the fluid that are stabilized by a skin of surfactant. These are nascent bubbles, or "cavitation nuclei." (Ionizing electromagnetic radiation in the form of gamma rays represents another source of cavitation nuclei, independent of the purity of the liquid.) The amplitude necessary to form a bubble from these sources—the "cavitation threshold"—increases, for example, with increasing frequency and surface tension, among a host of other parameters. Once created, the oscillating sound field causes the bubble's radius to oscillate within an acoustic cycle. Continued acoustic stimulation of a free bubble causes that bubble to grow via a process of "rectified diffusion" (24). This process describes the net effect on bubble size over a few to many acoustic cycles of changes in both the concentration gradient of diffusing gases near the bubble's surface (generally of prime importance) and the surface area of the bubble (generally of secondary importance) within an individual acoustic cycle. Briefly, as a bubble expands, the bubble's surface area grows as does the concentration gradient of the gas adjacent to the bubble's surface within the liquid. At the same time, the concentration gradient of the gas inside the bubble adjacent to the bubble surface decreases. All of these factors increase the flux of gas from the outside of the bubble to its inside. When the bubble's size decreases, the surface area decreases, and the changes in gas concentration gradient adjacent to the bubble's surface reverse. The net result is an increase in the flux of gas from the inside to the outside of the bubble. However, because of asymmetry in this process, the bubble grows minutely with each acoustic cycle and significantly over many acoustic cycles.

Bubble Dynamics. A bubble, like a spring, has a primary resonant frequency. For a bubble, this frequency varies inversely with its radius and also strongly depends on gas content and surface tension, among other factors. [A convenient formula for the resonant bubble's radius R_0 at a given frequency f_0 in water at room temperature is $f_0R_0 = 3.26$ MHz μm, as discussed in (21–23).] A newly formed bubble within a relatively weak acoustic field often has a resonant frequency that is off from the applied frequency, making its temporal variation in volume initially small, symmetric in shape, and simple within an acoustic cycle and over many acoustic cycles. In particular, when the bubble is larger than its resonant size, its volume will decrease when the applied acoustic field is large and will grow when the field is small. When the bubble is smaller than resonant size, its volumetric pulsations will be out of phase with the driving pressure. ("Stable" or noninertial cavitation refers to bubbles undergoing such relatively simple volumetric changes where factors in addition or instead of the inertia in the surrounding fluid govern the bubble behavior.) Under these circumstances the bubble scatters sound (because of its geometric properties and impedance mismatch with the surrounding fluid) and emits sound (via the compression and rarefaction of the liquid surrounding the bubble) at the frequency of the applied signal. Generally, the

emitted sound has a larger amplitude than the scattered sound. As the bubble grows toward its resonance radius (which can happen in only a few cycles) or, as the applied sound field increases, the volumetric changes in the bubble evolve to more complex functions of time within an acoustic cycle, as do the acoustic emissions, whether or not those changes remain radially symmetrical. As a function of growing bubble amplitude, those emissions first include the superharmonics of the applied signal. Eventually, the once stably oscillating bubble collapses violently and/or becomes asymmetrical. This generally occurs by a process known as "inertial cavitation"; it is called that because the inertia in the surrounding fluid governs the collapse of the bubble. Associated with inertial cavitation are broad-band acoustic emissions over a greater range of frequencies than evinced by stable cavitation, and, eventually, acoustic emissions at multiples of the subharmonic of the applied signal. Detection of these emissions via a hydrophone (25) offers a means of remotely assessing the level of cavitation activity within insonified material and often correlates with a variety of mechanical and chemical effects associated with cavitation. The initial "mother" bubble may break down at this point into a small cloud of microbubbles, known as "daughter" bubbles, which come from the original mother bubble. With continued acoustic stimulation, the process of bubble growth and eventual destruction resumes. Without continued acoustic stimulation, the daughter bubbles eventually dissolve or float away. Indeed, with an appropriately timed restart of the applied sound, as in pulsed applications of ultrasound, these daughters may be optimally configured for acoustically driven growth or violent collapse, as desired (26).

Microstreaming. Pulsating bubbles generate vorticity and hence a viscous boundary layer within the liquid adjacent to their surface. The shear in this layer stresses any material in the solute close to the bubble. The oscillations of the bubble also help bring material from afar into the vicinity of the bubble by inducing a generally steady flow in the fluid, known as acoustic microstreaming. The work of Nyborg (27) and that of his students, colleagues, and contemporaries is admirably reviewed by Miller (28). That review contains many examples of and references for microstreaming, from which we draw a few of the more interesting ones.

A number of studies exist whose central scientific principle is using an isolated bubble (mounted on the end of a minute tube, for example, or a collection of isolated bubbles (formed on hydrophobic membranes which contain gas-filled micropores) to allow controlled study of the stable-cavitation process. A fascinating study by Williams (29), using the longitudinally vibrating tip of an 85 kHz probe, shows the formation of symmetrical microstreaming-induced vortices within an intact blood vessel. Besides offering a clear visualization of microstreaming, the study shows a thrombus forming within one of the vortices. (Similar work (30) shows similar results achieved with a bubble mounted on a micropipette.) The forces associated with the controlled application of microstreaming bubbles or wires (whose circulation mimics that formed by stable cavitation) are amenable to analytic studies. Rooney (31) used this analysis and a 250 μm diameter bubble suspended at the end of a small tube within a vial of red blood cells stimulated by a 20 kHz sound source to measure the shear stresses necessary to create hemolysis (about 450 Pa). Several researchers have used the hydrophobic membrane apparatus described previously (28,32), to show that microstreaming brings red blood cells and platelets toward individual bubbles from a distance several pore diameters away from the center of the bubbles and both lyses cells and activates the platelets, which highlights the reach and effect of microstreaming. Coakley and Nyborg (33) make an instructive calculation of the strength and reach of microstreaming for a microbubble with a resting radius of 3.3 μm that is resonant at 1 MHz. For a weak driving pressure of 5000 Pa, the bubble's amplitude variation is one tenth of its resting radius. Platelets in saline drawn to the bubble's surface arrive there with a velocity of about 1.3 m/s, whereas platelets two resting radii away from the center of the bubble approach with a velocity of 0.0004 m/s, which gives a sense of the streaming field's reach. Nonetheless, these velocities are significant. They show that it would take about 0.003 s to clear the space around a vibrating bubble out to two resting radii from the center of the bubble, leaving a central bubble surrounded by a dense, close clump of cells.

Ignoring the gastrointestinal tract and lungs, bubbles are not ordinarily present within mammalian tissue (22,34,35), but can and have been introduced for a variety of purposes, as we discuss later. This means that without their introduction, for medical ultrasound to be dangerous in vivo it must initiate and stimulate acoustic bubbles. This generally requires producing "inertial cavitation," to which we turn now.

Formation of Hydrodynamics Jets. During inertial cavitation, bubbles generally collapse asymmetrically. This is particularly important, and spectacular, if the bubble is near an interface such as that formed by a container, tissue, or another bubble. The result is irregular and aperiodic microstreaming. More important, however, is the formation of hydrodynamic jets. There is an excellent image [(36), see also Young (21)] produced by Crum that shows an asymmetrically collapsing air bubble adjacent to a hard surface with a liquid jet piercing its heart. The bubble collapses asymmetrically near an interface because the liquid cannot approach the center of the bubble near the interface as effectively as away from the interface. Near a rigid interface, the vorticity in the fluid associated with collapsing bubble causes the in-falling liquid away from the interface to enter the bubble in the form of a jet that shoots through the bubble interior, striking the rigid interface on the opposite side of the bubble from which it started. These jets are violent physical processes capable of turning a few tenths of a megapascal of pressure, applied to the bulk of the fluid, into local (on submillimeter scales) generation of several to at least tens of megapascals of pressure, with extensive damage. The inertial collapse of a bubble can occur within a single acoustic cycle, and therefore the potential for inertial cavitation cannot be eliminated even for very short pulses of ultrasound (37). This fact forms the basis of the analysis behind the creation of the "mechanical index" (by Apfel and colleagues), used in diagnostic ultrasound machines to avoid the possibility of inertial cavitation in vivo. We discuss this later.

Heat Generation. As bubbles grow and shrink under the action of an applied acoustic field, they generate effects outside themselves directly via mechanical forces, and indirectly by altering their contents. To appreciate the results of the internal processes of bubbles, we first discuss heat generation

within the interior of bubbles and a possible example of biological effects associated with this process. This theoretical source of localized rather than bulk heating remains unobserved directly in vivo although in principle it is important in vivo. Because it is a process not within the purview of the thermal index, which concerns itself with bulk heating of tissue by ultrasound, and because internal heat generation represents the force driving other internal bubble processes under many circumstances, we discuss it here.

Compression of a bubble by an acoustic wave squeezes its contents, which warms by an increase in collisions between molecules within the gas residing in the bubble. If the characteristic timescale for bubble collapse is small compared to the timescale characteristic of thermal diffusion, the bubble's interior warms at least adiabatically (22) if not via more exotic mechanisms, such as those which cause single-bubble sonoluminescence (38). Single-bubble sonoluminescence is the production of light by squeezing a single, acoustically levitated bubble with sound. This squeezing ionizes the bubble's contents. The ionization can, in turn, produce free radicals which, when released, may be the direct cause of certain ultrasound-induced biological effects. We turn later to a discussion of this phenomenon and its implications. Instead, if the characteristic timescale for bubble collapse is large compared to the timescale describing thermal diffusion out of its interior, that bubble collapses isothermally and in principle acts as a local source of heat.

This mechanism may explain observations (39) of a significant 23°C increase in temperature generated within muscle in vivo by a 1 s burst of ultrasound at 0.56 MHz at a focal intensity of 250 W/cm^2. The ultrasound induces strong broadband acoustic emissions and hyperechogenicity within the tissue. This points to the presence and thermal significance of bubbles in the same area as the large temperature rise, which is too large to be explained by standard, bubble-free absorption of ultrasound by the tissue.

Free-Radical Generation. Rather than isothermally compressing their contents, cavitating bubbles may do so adiabatically, as mentioned earlier, resulting in a dramatic warming of the bubble's contents through a variety of mechanisms and the production of free radicals, such as singlet oxygen, hydrogen peroxide, and hydroxyl radicals, when the bubbles cavitate in water (40). Independent of their source, free radicals within biological tissue create significant biological damage (41) by inducing deleterious chemical reactions with carbohydrates, nucleic acids, lipids, and proteins. For example, in the presence of free radicals, enzyme activity reduces DNA, proteins cross-link, and DNA suffers single- and double-strand breaks.

Indeed, in vitro experiments have shown (42) that a cavitating ultrasound field induces single-strand breaks in Chinese hamster ovary (CHO) cells in suspension, presumably through a combination of free-radical generation and mechanically induced cell damage. To isolate the mechanical from the sonochemical effects, a more careful study (43) by the same researchers found that a cavitating ultrasonic field applied to a solution before the introduction of CHO cells generates single-strand breaks of DNA in those cells. Then these researchers showed that intertial cavitation induces these breaks via the production of hydrogen peroxide in the solute which persists long enough to affect the CHO cells. Thus, cavitation produces mechanical damage directly and also induces chemically-based damage. As to whether or not free-radical production by ultrasound occurs in vivo, in the section on therapeutic ultrasound we highlight in vivo research that points to this very possibility. Nonetheless, the body has natural antioxidant mechanisms (41) which, when not overwhelmed or circumvented, reduce or curtail completely the effects of ultrasonically induced free radicals.

Mechanical Index. For diagnostic purposes, cavitation poses an obvious danger when one considers the mechanical, thermal, and chemical effects associated with it. Apfel and Holland (44,45) developed a conservative measure, called the "mechanical index," for the onset of inertial cavitation of a preexisting bubble subjected to one cycle of applied acoustic pressure. They chose inertial cavitation because associated with it are the potentially deleterious processes one can expect from cavitation in the human body, where stable cavitation is quite unlikely (34). This measure is proportional to the peak negative pressure amplitude and inversely proportional to the square root of the frequency of the applied sound. Its governing assumptions include isothermal growth of an optimally sized bubble, the neglect of gas diffusion into the bubble, and incompressibility of the fluid surrounding the bubble. (All of these assumptions produce the most violent bubble collapse, making the mechanical index as conservative as possible.) Their theory predicts the value of the mechanical index when their theoretical bubble produces internal temperatures of 5000°C, which, they argue, diagnostic ultrasound machines should not exceed. Their basic approach has been accepted, and diagnostic ultrasound machines display a measure of the mechanical index that varies from application to application based on extensions of the original work of Apfel and Holland. Carstensen (16,35,46) offers excellent reviews of cavitation thresholds in tissue.

DIAGNOSTIC ULTRASOUND

Standard Diagnostic Imaging

There are useful and practical overviews of diagnostic ultrasound (2 and internal references). The basic concept of diagnostic ultrasound resides in the notion that sound backscattered from tissue does so as a function of the acoustic impedance and position of that tissue and that the acoustic impedance and position tell you something fundamental about the tissue. In standard applications, that "something fundamental" is the structure, position, and hence identity of the tissue. So-called "A" mode imaging produces a simple, one-dimensional trace of backscattered echoes. This imaging is useful for applications of diagnostic ultrasound to the eye, for example, where imaging a structure in more than one dimension is not the issue. In "B" mode imaging, a series of "A" mode scans from the diagnostic source are collected together to form two-dimensional maps of the backscatter values as functions of distance and angle relative to the acoustic source/receiver. In "M" mode imaging, an A mode scan is followed in time, producing a time-distance trace that finds particular use in imaging the heart and its periodic motion. Besides imaging stationary or moving structures, one can measure the speed and direction of moving tissue and fluids using "Doppler imaging." (Blood is by far the most analyzed in this fash-

ion.) Doppler imaging takes advantage of the fact that blood moves relative to the direction of acoustic wave propagation to create images based on the strength and direction of the Doppler shift in the backscattered signal.

The practical details of these existing imaging methods, the avoidance of bioeffects, and the search for new imaging modalities make diagnostic ultrasound a challenge. As an example of imaging complexity, diagnostic ultrasound based on acoustic backscatter requires the careful interleaving of sent and received acoustic energy with the assumptions that one can translate time of flight into distance using a standard speed of sound. Artifacts in acoustic images arise when this assumption breaks down. In addition, acoustic shadows can form within tissue because of the strong absorption of sound by tissue that lies between the acoustic source and other tissue.

Sonoelastic Imaging

A physician's palpation of tissue, essentially, a low-frequency interrogation of the elastic properties of tissue, gives information on tissue not contained within standard diagnostic images. Another imaging modality (47–50), called "sonoelastic imaging," works on the principle behind palpation by taking advantage of the fact that differences in low-frequency elasticity between tissue types range over several orders of magnitude, whereas differences in acoustic impedance (the sound velocity times the density of the material) vary by less than an order of magnitude at 1 MHz (47). To perform sonoelastic imaging, first one creates a standard B-mode image of the tissue in question. Then one changes the elastic strain on the tissue by compression, for example, or by applying a low-frequency vibration, to create another B-mode image. Direct comparisons of the two images highlight the regions with different elastic properties. A useful example (51) of such an "elastogram" showed a breast carcinoma within a tissue phantom that standard ultrasound could not adequately image.

Contrast Agents

The previous discussion of cavitation has laid the groundwork for this section, in which we discuss introduced and induced acoustic contrast agents. Here we define an "acoustic contrast agent" as a substance placed within the body to increase the usefulness of an ultrasonic diagnostic process by the difference in acoustic properties between the agent and the biological tissue or fluid. (We note that there are (52) therapeutic applications of acoustic contrast agents, whose details we do not discuss here.)

Manufactured acoustic contrast agents are typically micron-sized artificial bubbles placed into the blood stream to increase the echogenicity of desirable parts of images created with diagnostic ultrasound. Contrast agents used in this and other ways have become a burgeoning field of study and application (2,53). Its inspiration for imaging purposes lies in the serendipitous observation (54) of an improvement in the imaging of heart tissue after injecting a dye into the root of the aorta intended for measuring blood flow within the heart muscle itself. The solution carries within it acoustically bright bubbles generated hydrodynamically at the catheter tip which scatter more sound back to the diagnostic equipment than to the surrounding tissue.

Contrast agents give a larger acoustic signal than the tissue in which they reside, mostly because the contrast agents are acoustically resonant bubbles that are stimulated to emit sound at a variety of frequencies, including that of the incident sound wave. Because the acoustic emission of sound by the contrast agents at the incident acoustic frequency is more intense than the backscatter of the incident sound from surrounding tissues, biological structures in which the contrast agents gather appear bright or brighter than their surroundings in diagnostic ultrasound images.

The fact that acoustically stimulated acoustic contrast agents emit sound at frequencies in addition to the applied frequency has led to recent research to exploit those emissions for imaging. The procedure, known as "harmonic imaging" (55), consists of insonifying tissue perfused with contrast agents with pulses of ultrasound at a given frequency and listening for the emission by the contrast agents of sound at twice that frequency. Because those harmonic emissions have amplitudes much larger by a factor of 1000 than those emitted or scattered by tissue, the regions carrying the contrast agents stand out significantly in harmonic images. "Ultra harmonic imaging" is a variation of this approach based on detecting emissions other than at the second harmonic of the incident sound field (56).

There are currently more contrast agents under construction than are clinically available (56). Most are based on the paradigm of wrapping a gas bubble (that gas can be air, perfluorpropane, or fluorocarbon, among others) within a stabilizing shell of material (albumin, lipid bilayers, for example). Others are based on a means of introducing into the vasculature bubbles consisting entirely of gas that does not readily diffuse into blood.

Although many early applications of contrast agents lie in the field of cardiac studies, where contrast agents allow unprecedented detailed imaging of anatomic and physiological structure within the heart (57), contrast agents have made their way into other medical fields. An exciting example is the contrast enhancement of tumors, based on the observation that contrast agents preferably fill either the tumor or the immediately surrounding tissue (as a function of tumor type), thus allowing ultrasound to highlight the tumor itself (58,59). The field of gynecology (60) has also benefited greatly from the use of contrast agents. For example, pathological tissue within fallopian tubes and the intrauterine cavity resists standard ultrasonic imaging procedures but appears in contrast-agent-assisted ultrasonic images. (For these purposes an injection of sterile saline solution often acts as the source of the contrast agent because it is easily absorbed by the body after imaging is complete.) As one important example, ovarian tumors are difficult to image by standard diagnostic ultrasound because of their intrinsically low acoustic contrast relative to surrounding tissue. Their poor vascularity also precludes the use of standard Doppler imaging. These difficulties lead to the death of many women because a significant line of defense lies in diagnosing this rapidly proliferating cancer early. Recent successful work based on imaging small ovarian tumors with diagnostic ultrasound in its Doppler mode in conjunction with contrast agents offers hope to the many women stricken with this disease. Imaging fine structure and blood flow within a fetus in utero represents another exciting (and potentially dangerous) application of contrast agents with ultrasound for diagnostic purposes. A particu-

larly fascinating study (61) showed that contrast agents injected into one of a pair of fetal twins in utero led eventually to the appearance of those contrast agents in the other twin, thus confirming the diagnosis that the circulation system of each twin communicates with the other through their connection with the mother. The researchers reported no adverse side effects. Concern for fetal safety makes such applications the exception rather than the rule.

Finally, as noted earlier, the scientific roots of acoustic contrast agents lie in injecting saline or other liquids into the bloodstream, so as to introduce bubbles or cavitation nuclei. These bubble clouds rapidly spread out throughout the body, making it impossible to image an isolated portion of the circulation system within one cardiac cycle. To address this problem, Fowlkes and colleagues successfully explored the technique of using intense (4300 W/cm^2 to 19,000 W/cm^2), short (12 ms to 250 ms), individual pulses of ultrasound to induce transient and localized clouds of microbubbles (62,63).

THERAPEUTIC ULTRASOUND

The excellent book by Williams (64) offers an extensive survey of the desired and undesired bioeffects of ultrasound found in the literature prior to 1983. Readers interested in the roots of many aspects of therapeutic ultrasound should turn to this book.

Therapeutic ultrasound acts via the physical, chemical and thermal forces that it generates. Its efficacy is often affected in very specific ways by the biological disease and the biological tissue to which it is applied. In this section we summarize various modalities of therapeutic ultrasound and try to make clear how therapeutic ultrasound does what it is observed to do.

Rapid Heating with Ultrasound

For Tumor Destruction. Miller and Ziskin (65) offer an extensive review of the biological consequences of hyperthermia, because ultrasound has been used for generating elevated temperatures within tissue to kill unwanted tissue. Although they go into great detail about those biological consequences, an important point to keep in mind now is how much time is required to kill cells at a given elevated temperature, following the important work done by Dewey and Sapareto (66). Briefly, for every 1°C increase in temperature above 43°C, where it takes approximately an hour to kill a given percentage of cells, the time necessary to kill the same percentage of cells via denaturation of proteins decreases by a factor of 2 so that at 50°C it takes approximately two minutes to kill cells. Early work on the use of ultrasound to induce hyperthermia followed the strategy of raising the temperature of unwanted cells up to approximately 43°C to 45°C. This was not as successful as hoped for in practice because thermal diffusion and perfusion made it difficult to maintain the appropriate temperature for the desired length of time (67).

Starting a little more than a decade ago, researchers considered a new strategy, based on circumventing diffusion and perfusion by inducting rapid heat rises in tissue via ultrasound. One of several review articles from ter Haar (68) describes the early literature, its successes and failures, and what is being done now. For example, the treatment of liver and prostate (69) diseases has benefited from ultrasound-induced rapid hyperthermia. Because liver surgery often produces dangerous amounts of bleeding, the ability of this methodology to cauterize tissue around the edges where it kills tissue is particularly attractive. Indeed, "acoustic lesions" produced by focused ultrasound have sharp boundaries. The transition from destroyed cells to healthy ones measures only six to ten cells thick. Another attractive feature of acoustic surgery is its potential for extracorporeal application. Problems remain with this therapy, however. For example, when large volumes require treatment and one's acoustic source cannot cover the desired area in one application, one must take care that heat generated at one spot does not precondition adjacent spots by its diffusion. That preconditioning can cause the next application of ultrasound to generate cavitation, probably via vaporization of the water-saturated tissue rather than protein denaturation, because of the combined warming of the tissue by the first and second applications of ultrasound, and the fact that acoustic absorption generally increases with temperature (8). Ebbini (70) among others designed arrays of transducers and operating strategies to get around this problem. Cavitation, rather than cooking, also distorts the intended acoustic lesion, causing that lesion to grow toward the transducer by prefocal heating created by the backscatter properties of the bubbles formed at the initial site of cavitation (71,72).

The threshold for acoustic lesions created by *cavitating* high-intensity, focused ultrasound within the liver and brain occurs at higher applied intensities and shorter durations than that for creating thermally induced lesions (73,74). With the therapeutic success of heat-induced lesions, researchers (75,76) have intentionally created cavitation in liver and prostate with focused ultrasound to treat disease, making a virtue out of what is a problem for some techniques. This has been particularly useful in treating decreased or blocked urinary flow created by the prostate, where tissue removal rather than killing is the ultimate aim. However, at least one in vivo study (77) applying cavitating ultrasound fields to treat cancer in soft tissues showed that, in effect, an uneradicated cancer could be made worse in the long run, perhaps by the dispersion of the cancer cells by the explosive action of cavitation.

For Hemostasis. Rapid heating created by focused ultrasound has also successfully stopped blood flow in vivo. For example, Delon-Martin et al. (78) occluded exposed rat femoral arteries using several three-second bursts of 7.31 MHz ultrasound with a focal intensity of 167 W/cm^2. (Success was measured by Doppler ultrasound and histology.) Two days after exposure the blood vessels remained occluded by large blood clots. They offered this as a model for treating varicose veins, where current practice induces blood clots within a vein by various means, ultimately killing that section of blood vessel. The blood clot induced by the focused ultrasound arose from a thermally damaged portion of the endothelium. Unfortunately, the kind of thrombus they saw have been known to embolize upon repair of the endothelial layer.

Hynynen et al. (79) occluded blood flow in rabbit femoral arteries with ultrasound by creating of a transient mechanical constriction of the blood vessel (along with some unwanted hemmorhage). They did this by inducing cavitation in and around the arteries using an intense (4400 W/cm^2 to 8800 W/cm^2) one-second blast of ultrasound at 1.49 MHz. In a fol-

low-up study (80) they showed that the combination of an initial, cavitating pulse of ultrasound (as in their previous work) followed by rapid heating induced by ultrasound in the same area (using 10 second applications of 1.49 MHz ultrasound with an intensity of 2800 W/cm^2) occludes the renal artery (with diameters of 0.6 mm) of rabbits in vivo, all done noninvasively using MRI-guided focused ultrasound.

Vaezy et al. (15) demonstrated that high-intensity focused ultrasound at 3.3 MHz operated in continuous-wave mode and a peak focal intensity of about 3000 W/cm^2 for an average of 1.5 minutes successfully stops bleeding from deeply cut rabbit livers. In their work, they exposed the rabbit's liver in water, cut that liver with a scalpel, and then "painted" the surface of the incision with the focus of their transducer. Ultrasound coagulates the tissue from the surface down several millimeters and occludes blood vessels whose diameters are as large as 2.5 mm. They described this process as "volume cauterization," to contrast it with other cauterizing methodologies that act on just the surface of a bleeding area. This work points toward the possible application of focused ultrasound for bloodless liver surgery, a significant possibility given the extensive vasculature of livers. Finally, in a recent work, Vaezy at al. (81) showed that it is possible to stop blood flow from an exposed, punctured artery in vivo. They used a handheld, focused ultrasound device, equipped with a water-filled, conical cover for transmitting the ultrasound from the transducer to the area of interest. They applied ultrasound at 2.0 MHz and 3.5 MHz in continuous-wave mode using intensities ranging from 500 W/cm^2 to 3100 W/cm^2. They achieved cauterization in as little as a few seconds and more typically in about a minute. The diameters of the blood vessels ranged from 2 mm to 10 mm, marking a significant advance in acoustic hemostasis.

Acoustic Ablation Therapy

For Heart Disease. In the section on rapid heating by ultrasound we noted that besides heating tissue with focused ultrasound, where cavitation reduces the therapeutic effect, recent work shows that the intentional induction of cavitation rather than heating removes undesired tissue. These applications are examples of acoustic ablation therapy. This section presents two other examples: the treatment of certain forms of heart disease and the destruction of kidney stones.

There is evidence that ultrasound affects the heart. For example, shock waves in the form of individual high amplitude (5 MPa to 10 MPa), short pulses (5 ms) of sound generated by a lithotripter have caused premature ventricular contractions in vivo, as demonstrated in frogs (82). Longer pulses of sound with high amplitude also alter the contraction of heart muscle in frogs (83). As yet no mechanism to create these bioeffects has been identified, although cavitation is likely, given the acoustic regime brought to bear in their studies. In principle, these results could be of concern to those who wish to use ultrasound only for diagnostic purposes. This is particularly true given the trend to increase the pulse amplitude of diagnostic ultrasound machines, which would enhance the opportunity for cavitation. (However, Carstensen et al. (84) show that now this trend has not yet produced machines capable of damaging the heart.) Also of concern is the use of contrast agents to improve imaging, which increase the likelihood of cavitation. These are also words of warning for those who are treating diseases elsewhere in the body with therapeutic ultrasound, whose pressures are often higher than used in diagnostic ultrasound. However, these results also motivate therapeutic applications of ultrasound to treat heart disease.

In particular, recent work addresses the problem of cardiac arrhythmia, when the upper and lower chambers of the heart do not contract in time relative to each other as they should, thus reducing blood flow from the heart. Surgeons in Brazil in the 1980s discovered that removing a chunk of the heart muscle somehow resets the heart's contraction pattern. Motivated by this work, Kluiwstra et al. (85,86) demonstrated that ablative ultrasound applied to heart muscle achieves the same result, promising that this treatment for cardiac arrhythmia can be applied from outside of the body, thus avoiding open-heart surgery. Their technique merits some attention here because of the interesting biological and engineering problems they had to solve to create their desired bioeffect. There are significant difficulties in applying this technique to a living, beating heart from outside of the body. The ultrasound has to be aimed at a particular point on the heart while that point moves within the body behind the rib cage, which is, at times, a shield between the heart and the ultrasonic source and also a place for possible damaging heat generation. The solution by these scientists was to create a computer-controlled phased array of ultrasonic sources (that is, a series of individual acoustic sources which can be coordinated) whose malleable acoustic beam could be rapidly steered to follow the target on the heart while entirely avoiding the insonification of the ribs. They made the steering problem easier to solve by tying the time of acoustic output to the latter two-thirds of the cardiac cycle, when the heart is relatively quiescent, via feedback from an EKG.

For Destruction of Kidney Stones. Short, intense, focused pulses of sound in the form of shock waves destroy kidney stones in a process known as lithotripsy. Indeed, a hundred thousand cases of kidney stones are treated with lithotripsy each year in the United States. Amazingly, the mechanism or mechanisms by which it works remain unclear. Early theories include compressive failure of the stone (87) and a process known as "spalling" (88). The first mechanism would occur if the peak pressure associated with the acoustic shock wave exceeds the compressive strength of the stone. Simply, the shock wave directly crushes the stone. Spalling entails the entry into the stone of the peak positive pressure which, upon reflection from the back of the stone, inverts into a large negative pressure whose tensile stress fractures the stone. (This theory rests on the fact that most solids break more easily under tensile stress than under compressive stress.) A more recent theory (89) holds that the shock waves progressively develop microcracks in the material upon repeated insonation which eventually produce catastrophic failure. This theory is consistent with observations that more pulses of sound break up more stones. Its virtue is that it reduces the need for individual shock waves to exceed the compressive strength of the stone. The action of cavitation is also likely, because for example, the efficacy of lithotripsy is reduced by overpressure (90). (Overpressure dissolves cavitation nuclei (21–23), thus making cavitation more difficult to initiate.) Moreover, other recent results show that lithotripsy pulses created by inverting the temporal order of the peak positive and peak negative

pressure fail to break up kidney stones and also fail to create significant inertial cavitation relative to the standard lithotripsy pulse (91).

Even successful lithotripsy creates significant damage in the kidney and surrounding tissue. Delius and colleagues have done much to make lithotripsy successful, and to explore its wanted and unwanted bioeffects. In one important paper (92) they correlated hemorrhage in piglet livers in vivo with the production of gas-filled bubbles in the same area. In another (93) Delius showed that overpressure reduces both tissue damage and gallstone breakup, and that the reduction of tissue damage created by overpressure is larger than the reduction of stone destruction. This observation supports the contention that cavitation correlates with both tissue damage and stone destruction as mentioned before and also suggests a strategy for reducing damage while still achieving stone destruction. Our final point regarding the damage that lithotripsy can create is that it does so by a mechanism in addition to cavitation. This mechanism is the small-scale focusing of shock waves caused by subtle variations in the properties of the tissue through which the shock wave propagates (94).

Ultrasound-Affected Transport across Biological Barriers

Individual Cell Membranes. Ultrasound delivers chemicals across the ordinarily impermeable outer membranes of cells by transiently opening up holes in the cell membrane. This process has been called "sonoporation" because its effect on membranes is similar to the effects caused by electroporation. The work of Boa et al. (95) is a good introduction to this field because of its literature review and its coverage of the gamut of sonoportation's achievements. For their experiment they worked with CHO cells in suspension with an acoustic contrast agent. They added to this solution either fluorescent dextran (with a molecular weight of 580 kDa) or the plasmid for luciferase. Luciferase, when taken up by the cell's DNA and expressed, causes the cell to glow via the same mechanism as that used by fireflies. They applied sound in continuous-wave mode at 2.25 MHz over a range of incident pressures for one minute while rotating the container. [Both the rotation and addition of contrast agents maximize the production of cavitation (34).] In their system (without cells) they first measured the production of hydrogen peroxide, a free radical commonly produced by inertial cavitation. By this measure, inertial cavitation occurs for peak positive pressures greater than or equal to 0.4 MPa and increases steadily as the incident pressure increases. Then they measured the uptake of fluorescent dextran by the cells (by flow cytometry) and the viability of the cells as functions of increasing applied pressure via their successful exclusion of trypan blue. (This use of trypan blue quantifies cell viability by actually quantifying the integrity of the cell-membrane structure.) Cell viability decreased and fluorescent dextran uptake increased as functions of the applied pressure, each becoming statistically significant for pressures of about 0.2 MPa, less than at the onset of inertial cavitation. The rate of change of each parameter increased significantly as the applied pressure reached 0.4 MPa. This rate of change remained positive but became quite small for further increases in applied pressure. Then they quantified the uptake of the reporter plasmid for luciferase along with the ability of the cells to proliferate. Again they saw statistically significant decreases in cell proliferation and increases in the absolute value of luciferase production for applied pressures less than 0.4 MPa. They also saw a large increase in these bioeffects at 0.4 MPa, which then leveled off at larger values of applied pressure.

Among the conclusions one can draw from this and similar research is that ultrasound-mediated transfection works in vitro [and in vivo (96,97)] and that it correlates with inertial cavitation above a certain threshold of applied pressure. Support for cavitation as the mechanism of sonoporation also lies in the work of Gambihler et al. (98,99). They used lithotripter pulses to sonoporate leukemia cells in suspension with fluorescent dextran in vitro, over a range of molecular weights up to 2000 kDa. They also produced highly informative images of the results of sonoporation by using confocal microscopy. Without the lithotripter pulses, some low molecular weight fluorescent dextran shows up in the cells by endocytosis, as marked by the appearance of isolated, round, fluorescent patches. After applying ultrasound, the entire cell is fluorescent, suggesting that the intense pulses of ultrasound drive the dextran through the membrane bypassing endocytosis in a way that destroys almost half of the cells but leaves the other half able to reproduce.

However, sonoporation may be more complex than purely mechanical generation of transient holes in cell membranes. Lee et al. (100) measured the enhanced flux of dextran into human and chicken red blood cells created by a pressure wave induced in vitro by the rapid destruction of foil adjacent to the cells by an intense laser. They found a significant flux of dextran into human red blood cells relative to controls but not into chicken red blood cells. Chicken red blood cells lack a cell membrane structure known as "aquaporins." To see if this difference in cell-membrane structure correlates with the difference in sonoporation of the different cells, they applied a chemical to the human cells that blocks the action of their aquaporins. With these altered cells they found no significant uptake of fluorescent dextran caused by ultrasound.

Skin. Research over the last decade or so points to the possibility of using ultrasound to deliver therapeutic chemicals through the skin, a process known as "sonophoresis." This has been shown in vivo using 1 MHz sound sources operating at a few watts per square centimeter for a few minutes (101). Typical ratios of ultrasound-enhanced flux of permeants to the passive flux of permeants are less than or equal to a factor of 10. Even more successful (by a factor of 1000 or so) has been work (102,103) at lower frequencies (around 20 kHz) using pulsed ultrasound (with pulse lengths on the order of 100 ms applied every second for as long as one hour) with smaller average intensities, typically a few hundred milliwatts per square centimeter. [See also work by Tachibana and colleagues (104,105).] We take the time to discuss sonophoresis in some detail because its study sheds light on a number of bioacoustic phenomena and illustrates the complexity of identifying acoustic mechanisms behind bioeffects.

To understand the current mechanistic views of sonophoresis, first one must know the structure of the stratum corneum, the impermeable layer forming the top surface of the skin. Away from hair follicles, the stratum corneum has a thickness of 15 μm. It is made of a combination of keratinocytes (which are 1 μm thick and about 23 μm long) stacked like mortared bricks with lamellar lipid bilayers that have a net thickness of 50 nm acting as the mortar. Near the base of

the hair follicles (whose horizontal cross section measures about 50 nm), the stratum corneum thins considerably.

The best in vitro study of possible mechanisms behind sonophoresis at 1 MHz (106) involves a series of in vitro experiments using a special two-compartment tank, where a layer of stratum corneum taken from heat-stripped, then hydrated human cadaver skin separates the compartments. The drugs of interest go in the "donor compartment" along with the transducer. The arrival of these drugs in the "receiver compartment" marks their successful transport through the stratum corneum. Mitragotri et al. (106) tested the effects on drug flux of temperature change, alterations in the viscosity of the donor-compartment fluid, degassing the skin, and increases in frequency of the applied sound. They concluded that the source of sonophoresis in their in vitro system was cavitation within the keratinocytes that disorder the lipid bilayers within the stratum corneum. As a final test of this hypothesis, they measured the production of hydrogen peroxide in the skin by free radicals generated by cavitation. They did so by soaking the skin with a chemical marker that bleaches in the presence of hydrogen peroxide. Confocal microscopic analysis of the stratum corneum before and after the application of ultrasound (with the ultrasound applied directly to the skin, rather than through the fluid in the donor chamber) showed the production of hydrogen peroxide within the keratinocytes, thereby suggesting the action of cavitation within the keratinocytes. Then they developed an algebraic model of sonophoresis using these data, arguing that cavitation within the keratinocytes acts to partially disorder the lipid bilayers, which greatly enhances the net transport of chemicals across the stratum corneum and weakly increases the permeation of chemicals into the lipid bilayers. Without any free parameters, the model qualitatively captures the observed dependence of the enhancement ratio on molecular weight both in vitro (106) and in vivo (107).

Considering the significant differences between the stratum corneum in vitro and in vivo, the success of this model is remarkable. The controversial but well-tested (by in vitro means) hypothesis upon which it rests bears further analysis. Any alternative mechanism has to explain the crux of their results, namely, that ultrasound acts to disorder lipid bilayers within the stratum corneum consistent with the action of cavitation within the keratinocytes. Although we do not offer such an alternative analysis here, we note two of several persistent questions. For example, part of the controversy over the results is the likely difficulty for cavitation to occur easily within the confines of natural keratinocytes, whose water (50% by volume) is largely bound into the protein walls of the keratinocytes, compared with the hydrated keratinocytes used in their in vitro experiments. Also, even if there is sufficient proof that the source of in vitro sonophoresis is cavitation *within* the keratinocytes, there is no evidence to argue against the action of cavitation simply *near enough* to the stratum corneum in vivo, on either side of the skin, to induce lipid-bilayer disorder within the stratum corneum.

We close this section by briefly revisiting the in vitro work on sonophoresis simply referred to in the introduction of this section. That research shows that at 20 kHz the action of an unidentified acoustic mechanism creates a different bioeffect (103) than the disordering of lipid bilayers reported at 1 MHz. Specifically, the acoustic disruption of the stratum corneum appears more extensive at low frequencies than at higher frequencies, because the enhancement ratio for low-frequency sonophoresis is significantly larger than at higher frequencies, is independent of molecular weight, unlike at 1 MHz, and lasts for a time after the cessation of ultrasound, also unlike at 1 MHz. The proposed bioeffect behind sonophoresis at low frequencies that explains these observations calls for the formation of aqueous channels within the stratum that bypass the lipid bilayers and its effects on drug diffusion. Inertial cavitation is the hypothetical mechanism. This is because of its ability to dig deep into hard structures via hydrodynamic jets.

Blood Clots. Breaking up unwanted blood clots (thrombolysis) is a difficult and often invasive process. Studies during the last decade (108–110) indicate that 1 MHz ultrasound at intensities of 1 W/cm^2 to 8 W/cm^2 accelerates the enzymatic reactions in thrombolysis rather than causing irreversible mechanical fragmentation via a process known as ultrasound-enhanced thrombolysis. Ultrasound does so by enhancing the transport of reactants. Experiments in vitro demonstrate that ultrasound increases transport of plasminogen activators both into and within thrombi (111,112). This is important because transport of reactants into and within thrombi is a rate-limiting step in fibrinolysis in vitro and in vivo (113,114). The physical mechanism or mechanisms responsible for enhancing of fibrinolysis are unknown, but bulk heating alone is not a sufficient explanation (115). Cavitation could be important in systems exposed to air. Examples include most in vitro experimental systems and animal models that include surgical exposure of the vessel. However, ultrasound also accelerates the destruction of blood clots in deep vessels within animal models where cavitation is not likely to occur. One example is in animal models of small vessel injury (116). Another is electrically induced thrombosis (117), in which the method of vessel injury does not include introducing gas. Moreover, recent in vitro work (118) designed to assess the relative importance of cavitation versus other nonthermal acoustic mechanisms (acoustic streaming, for example) finds that only 50% of ultrasound-enhanced thrombolysis in vitro is explained by cavitation. This remains an active field of study that still lacks a basic understanding of its fundamentals.

Ultrasound Activation of Drugs

Studies motivated by the desire to assess biological damage from diagnostic ultrasound (to learn how to create damage and therefore avoid it) and therapeutic ultrasound (such as in lithotripsy, where the goal is to minimize collateral damage and maximize stone destruction) have quantified mechanical and chemical means of destruction with ultrasound. These mechanisms include sound-wave induced stress gradients, cavitation damage via a variety of specific mechanical and chemical processes, and heat generation. Chemotherapy represents another, time-honored way of killing undesired cells.

Umemura and colleagues (119) coined the phrase "sonodynamic therapy" to describe the therapeutic process that arises from the synergy between ultrasound and separately introduced chemicals and number among the first to explore this phenomenon (120,121). Briefly, these and other studies show that doses of ultrasound and chemicals that separately would do little or no harm to cancer cells, for example, act together

synergistically to create the desired therapeutic effect. Because ultrasound can be focused within the body and many drugs are too strong to be used in vivo, sonodynamic therapy represents a potentially powerful strategy for creating localized therapeutic effects.

The paper by Jeffers et al. (122) is a clear study of drug/ultrasound synergy with a good survey of the literature on the subject. Their in vitro study evaluates the enhancing effect of a polar solvent (dimethylformamide or DMF, a potent anticancer drug too potent to be used alone, generally) on the destruction of leukemia cells caused by applying ultrasound in conjunction with contrast agents. They applied continuous-wave ultrasound at 985 kHz with intensities ranging from 0.5 W/cm^2 to 2.5 W/cm^2 and total exposure times of 15 s. Cavitation was critical for the success of the ultrasound/drug synergy (enhanced cell death occurred when DMF, contrast agents, and ultrasound were applied simultaneously) and its role was confirmed by detecting subharmonics emitted by the insonified cell culture. They developed a useful test of the hypothesis that the synergy in their system arises from a "sonomechanical "effect, such as might occur if the solvent increases the susceptibility of the cell membrane to shear stresses produced by cavitation. They tested the sonomechanical effect by subjecting the cells and drug to shear between rotating concentric cylinders as in a viscometer. They found no significant difference in cell lysis in their viscometer with and without the drug, suggesting that the chemical does not increase the susceptibility of the cells to sound-induced shear. However, at the highest intensities explored they found that DMF enhances the amount of cavitation relative to that generated without the drug. Following the conclusions of Umemura et al. (119), they inferred but could not test the theory that short-lived sonochemical reactions lie at the heart of at least some sound/drug synergy. Riesz and colleagues (123) successfully searched for these sonochemical reactions in a similar experimental system. In particular, they identified the production of "carbon-centered radicals" (such as CH_3) as the source of the synergistic toxic effects between ultrasound and the chemicals considered by Jeffers et al. (122). In particular, the radicals formed by the interaction of ultrasound-generated H and OH radicals with the chemicals of interest or by the direct pyrolysis of the weak bonds in the solute molecules.

Other studies (124,125) show that ultrasound actuates some traditionally photoactivated chemicals (porphyrins, a class of chemicals that become therapeutic when stimulated by laser light) to kill tumors. The early hypothesis that cavitation-produced singlet oxygen (perhaps via sonoluminescence) lies behind this process (126) has given way in recent work to new arguments that cavitation activates porphyrins by producing free radicals other than singlet oxygen, probably directly through sonochemical reactions rather than through light production (127,128).

However, there are studies suggesting that the action of sonodynamic therapy is based in part on sonoporation. Essentially, ultrasound delivers chemicals into cells where the chemicals would not ordinarily go. There, they damage cells by means ordinarily unavailable to those chemicals. For example, Saad and Hahn (129) found that an increase in the accumulation of a chemotherapeutic agent (adriamycin) by CHO cells lies in part behind the drug/sound synergy they studied. They also found that exposure of the cells to ultrasound makes these cells more sensitive to adriamycin applied after insonation of the cells. Harrison and colleagues coined the phrase "sonopotentiation" to describe the activation of chemicals via ultrasound, especially under the relatively weak acoustic fields which they explored at length. Indeed, they developed a body of work (130–132) showing that sonodynamic therapy/sonopotentiation acts by intracellular drug accumulation enhanced by ultrasound in some drug/sound/cell systems, by free-radical production (such as hydroxal radicals) in others, and by a combination of both in yet others. They and others (133,134) argue that the common element among these examples is the action of cavitation as a source of both mechanical stress and free radicals. Therefore, one can say that the current view of sonodynamic therapy is that it is an example of both or either sonoporation and sonochemistry.

Ultrasound-Actuated Vehicles for Targeted Drug Delivery

Liposomes. We briefly mentioned the use of contrast agents in conjunction with targeted ultrasound, with and without additional chemicals, for targeted and enhanced ultrasound-induced biological effects (52). Liposomes, a lipid bilayer vesicle or collection of vesicles that contain within them aqueous solutions of pharmaceutical agents are another drug-carrying vehicle accessible to targeted ultrasound. The first study (135) on liposomes, motivated by the ability of ultrasound to produce localized hyperthermia, recognized that thermally activated liposomes in conjunction with targeted ultrasound could create a targeted drug-delivery system. This elegant study has an edifying and quotable delineation of the many ways in which local hyperthermia may make drug-containing liposomes efficacious, namely, "(1) by promoting selective drug release at temperatures near that of the lipid phase transition of the liposomes; (2) by increasing local blood flow; (3) by increasing endothelial permeability to particles, thereby enhancing accumulation of liposomes in the target tissues; (4) by increasing the permeability or susceptibility of target cells to the drug released from the liposomes; and (5) by increasing direct transfer of drug from vesicle to cells, for example, by fusion or endocytosis . . ." (135). The research was designed to test the first hypothesis in vitro by observing the inhibition of protein synthesis within bacteria through the hyperthermic release of inhibitory drugs carried by the liposomes.

Again, quoting from the text, their research strategy was as follows. "[N]ear their liquid-crystalline transition temperatures (T_c), liposomes become highly leaky to water-soluble contents . . . a phenomenon generally attributed to disorder at the boundaries between solid and fluid domains in the lipid. Our basic strategy was to design liposomes with T_c above physiological temperature but in a range attainable by mild local hyperthermia. On passing through the heated area in the circulation, the liposomes would be expected to release their contents at a greater rate than elsewhere and thus to develop higher local concentrations" (135). Indeed, their expectations were met, and the use of liposomes in conjunction with ultrasound for therapeutic applications is now an active field of research.

For example, this basic idea has met with success in vivo on implanted tumors in mice (136). Tacker and Anderson used ultrasound at 5 MHz to warm the implanted tumor before injecting liposomes carrying a chemotherapeutic agent

into the circulatory system of the mouse. After allowing the liposomes to circulate throughout the body of the mice, they removed the tumors and measured the amount of therapeutic agent accumulated within each tumor. Ultrasonically warmed tumors contained ten times the amount of chemicals contained within unwarmed tumors. As a further control for their experiment, the researchers injected free (rather than liposome-containing) therapeutic agents into the mice and found no significant difference in the accumulation rate of warmed versus unwarmed tumors.

As a final example, Ning et al. (137) explored the possibility that ultrasound applied simultaneously with the introduction of liposomes could release therapeutic chemicals from long-circulating liposomes in a targeted fashion, and also could enhance the therapeutic power of those chemicals at the same time. In this combined in vitro and in vivo experiment with doxorubicin (a porphyrin), the researchers found that the chemicals are delivered preferentially into tumors that are insonified and that the ultrasound enhances the action of doxorubicin. This approach offers a significant means, eventually, for targeted ultrasonic therapy.

Subdermal Implants. Another approach to targeted drug delivery with ultrasound involves a drug-soaked matrix implanted subdermally near the tissue or circulation system of interest, whose contents are released and/or activated via extracorporeal applications of ultrasound. In an example (138), Kost et al. placed a variety of polymer-matrix microspheres soaked with a marker chemical subdermally within rats. When released, the chemical appeared in the rats' urine. In one experiment, the background drug concentration in the urine without ultrasound was initially 35 μg/mL/h and declined to 20 μg/mL/h before the application of ultrasound. (This background rate was nonzero because the polymer they used was intrinsically leaky. That rate declined because the flux of drugs into the surrounding tissue from the surface of the polymer was faster than the flux of drugs from the interior of the polymer matrix to its surface.) Within 30 minutes after the transdermal application of ultrasound, drug concentrations within the urine increased by a factor of 4 to 6. Within 2 h after the application of ultrasound, the drug concentration returned to its background level. They explained the initial rise in chemical marker after the application of ultrasound by the action of cavitation on the implants, which they quantified by measuring pitting on the microspheres consistent with cavitation-induced damage. These pits are divots on the polymer surface that expose previously hidden portions of the drug-soaked polymer, which then leak the chemical marker. (Note that if this is the only mechanism at play this transient increase of chemical marker caused by cavitation would give a measure of the new surface area on the microspheres created by cavitation relative to the original surface area.) This and other (139) studies established that mechanical damage from acoustic cavitation is a mechanism for ultrasound-controlled release of chemicals from subdermal implants.

Another in vitro analysis (140) addressed how ultrasound causes the release of drugs from subdermal implants. Liu et al. studied the acoustic effects (from a 1 MHz continuous wave source with an intensity of 1.7 W/cm^2) on a drug-soaked polymer within both aqueous and nonaqueous solutions. Among other things they measured the drug-release rate, the amount of polymer hydrolysis and the factors that control it, and the amount of pitting on the polymer. They concluded that ultrasound accelerates both polymer hydrolysis and mechanically induced surface erosion. This, in turn, exposes new polymer surfaces for drug release. Liu et al. found the source of enhanced polymer erosion in the enhanced permeation of water into the polymer matrix that exposes more polymer cross-links to hydrolysis. This could have been induced by acoustic streaming within the liquid in which the polymer was suspended. If so, this may be an artifact of their in vitro study, because in many in vivo applications, the implant would be surrounded mostly by tissue, not liquid. Other mechanisms could be microstreaming or hydrodynamic jets associated with acoustically stimulated bubbles at the polymer surface, which are more likely mechanisms in vivo. Liu et al. also identified cavitating bubbles as the source of the mechanically induced erosion. However, they found no role for free radicals produced by cavitation, although presumably a different choice of polymer or polymer-saturating solute would have been receptive to sonochemical reactions.

Ultrasound-Accelerated Healing of Broken Bones, Flesh Wounds, and Cut Nerves

In this section we discuss three applications of therapeutic ultrasound for the healing "everyday" maladies—bone fractures, superficial skin wounds, and peripheral (versus central) nervous-system damage—and stimulating nerves for a variety of purposes.

Bone Fractures. First consider the use of ultrasound to accelerate the healing of bone fractures. Early work (141) on rabbits with holes introduced into their femurs found that they healed more quickly relative to those of controls when exposed to a daily regimen of 5 MHz and 10 MHz pulse ultrasound applied 10 min/day for as long as 15 days. At the 4 day mark, histological analysis suggested enhanced osteosynthesis. By 15 days, the insonified femur holes healed completely whereas the controls were still in the early healing stages.

Consistent with those results are others (142) showing that fractured rat fibulae healed more quickly than those of controls when pulsed 1.5 MHz or 3.0 MHz ultrasound with an average intensity of 0.5 W/cm^2 was applied for five minutes per day for several days. Moreover, Dysen et al. found a difference in biological response depending on when the treatment was applied relative to the time of fracture. For example, both repair quality and rate were significantly better if the ultrasound was applied during the first two weeks after fracture. If the treatment started at the third week, only more collagen formed, with an ambiguous impact on fracture healing.

These experiments suggest that the effects of ultrasound on the bone-healing process are subtle. For example, most acoustic protocols reduce the amount of heating (by pulsing the sound) relative to what could be produced by ultrasound applied continually. However, even under these conditions, one could expect at least some increase in temperature, because bone is such a good absorber of ultrasound. However, heat production has been insufficiently quantified in these studies, although at least one early study reported complications associated with excessive temperature elevations. Fi-

nally, cavitation seems unlikely under the typical acoustic protocol applied in these studies.

A recent study (143) by Greenleaf and colleagues explains these early results. They worked with rat models of fracture using an intensity of 50 mW/cm^2 (spatial and temporal average) at 0.5 MHz, with pulse sound 200 μs long, separating at 1 kHz. This work starts with an excellent and concise introduction to the spatial and temporal patterns of the processes involved in bone healing independent of ultrasound. One important point is that the healing mechanisms involve spatial and temporal variability in the gene expression of matrix proteins within the forming and solidifying union of the bone fracture. The other point is that mechanical stimulation of bone translates into metabolic and structural changes in the bone cells. Because ultrasound induces mechanical forces on bone via acoustic radiative force and/or cavitation (and can heat bone and surrounding tissue), this study measured changes in gene expression as a function of acoustic protocol and asked whether or not that expression is related to beneficial changes in bone structure and function. Quoting from their abstract, they note that "[t]hese data suggest that ultrasound stimulation increased the mechanical properties of the healing fracture callus by stimulating earlier synthesis of extracellular matrix proteins in cartilage, possibly altering chondrocyte maturation and endochondral bone formation" (143). Because many of these biological effects arise early in the bone-healing process, this result explains the observation noted before that therapeutic ultrasound is better at healing bone when applied soon after fracture formation, rather than later, and the rapid and early acceleration of bone healing induced by ultrasound. On the basis of these results it appears that mechanical stimulation of the bone by ultrasound accelerates bone healing, possibly by acoustic radiative pressure or by small-scale oscillations of the tissue with each acoustic cycle.

Wound Healing. Dyson's group in London pioneered the application of ultrasound to accelerate wound healing. An example of her early work (144) showed that ultrasound increases the repair rate of holes cut out of rabbit ears. They used 3.6 MHz ultrasound applied either continually or pulsed, with peak intensities ranging from 0.1 W/cm^2 to 8.0 W/cm^2 applied for 5 min three times per week. The insonified holes closed more quickly than the controls (by a factor of 1.3 in the best case). Varicose ulcers on the skin surface have also been successfully treated with ultrasound, under an acoustic regimen comparable to that just discussed. Following Williams' discussion (64), the repair of soft tissue occurs in three consecutive phases. The first is associated with inflammation of the wound, when the clotted tissue and debris are cleared out by leucocytes. The second phase consists of the invasion of fibroblasts, cells necessary for the production of new tissue, which also occurs at this time. During this phase collagen fibers connect healthy and newly forming tissue, bridging and contracting the wound. During the third and extended phase, the scar tissue previously formed undergoes continual modification via collagen creation and destruction as that tissue heals. Dyson and her colleagues found that therapeutic ultrasound acts on each of these three different stages of wound healing and that it is beneficial to apply therapeutic ultrasound soon after wound formation. Paraphrasing a review paper by Dyson (145), among other things, ultrasound stimulates protein synthesis, creates a variety of cellular-level changes, and decreases electrophoretic mobility. The stimulation of protein synthesis by ultrasound has been observed both in vivo and in vitro. Many of the cellular changes induced in vitro by ultrasound (quoted by Dyson) have been suppressed in vitro when ultrasound was applied to the cells in an overpressure system. Thus, cavitation is a likely mechanism for these ultrasound-induced cellular changes, probably (according to Dyson) in the form of stable cavitation with its microstreaming (and, we add, possibly acoustic radiative pressure with or without the presence of bubbles). Dyson argues against inertial cavitation as a mechanism because it is a violent process inconsistent with the accelerated growth of cells and structure observed during ultrasound-enhanced wound healing.

Effect of Ultrasound on Nerves

Diagnostic Stimulation of Nerves. A review article (146) on the interaction of ultrasound and the central and peripheral nervous system cites the application of ultrasound to stimulate nerves noninvasively for both diagnostic and therapeutic purposes. This would be useful, especially for problems associated with deep nerves, because there are a variety of diseases, which they summarize, associated with changes in perceiving different sensations, such as pain, cold, and pressure for which stimulation by ultrasound either induces or removes the sensation, as desired.

They also note that ultrasound may help with physiological research in many ways. For example, ultrasound can delineate the function of various parts of the brain via the stimulation of those parts and the correlation of the physical response to that stimulation. It has been known since at least the late 1950s that ultrasound applied to the central nervous system can do this. Early seminal work in vivo included the transient dilation of the pupil of cat's eyes and the transient depression or enhancement of the spinal-cord reflex, also in cats (147). The Fry brothers were involved in much of the ground-breaking research in applying therapeutic ultrasound, including applications to the brain. In one extensive review article (148), William Fry notes, among other things, that ultrasound applied to the visual cortex of cat's brains repeatedly suppresses in a transient manner various phases of cortical potentials normally evoked by flashing light into the cat's eye. Other quite remarkable work summarized in that article deals with the treatment of tremors in patients with Parkinson's disease. The researchers report that they repeatedly created the reversible alleviation of tremors, within a given human patient which they eventually removed permanently by a larger dose of ultrasound than that required to create the transient effects. Apparently the side effects of this procedure were minimal. (They successfully treated 18 different patients in this fashion.) Given the importance of these findings, it is unclear to us why this procedure has not become commonplace or at least gotten more press.

As to how ultrasound creates these transient effects, the researchers removed both temperature effects (via both theoretical arguments and in vivo measurements) and cavitation (by insonifying the animals under hydrostatic pressure while creating the same biological effects) from consideration. Through this and a similar process of elimination, one can argue that at least acoustic radiative force is at play. This force may act by exerting strain on the membranes of the neu-

rons and supporting cells, thereby transiently changing the permeability of those membranes, and allowing the greater flux of ions (and the changes in the cellular potential thereby induced) which would alter the electrical potentials of the neurons.

Therapeutic Ultrasound for Accelerated Nerve Regeneration. With regard to the peripheral nervous system, researchers (149) have found that ultrasound (with a frequency of 1 MHz at an intensity of 0.5 W/cm^2 applied continually over a period of one minute three times per week) accelerates the healing of crushed bilateral tibial nerves in rats relative to that of controls. They found an increase in subcutaneous temperature of only 1°C near the point of application of ultrasound. Also, doubling the intensity decreased the healing rate of the nerves relative to that of controls. Finally, they found that both the nerve's conduction velocity and the amplitude of the invoked compound muscle action potential (associated with the action of the tibial nerves) transiently increase after applying therapeutic ultrasound. Although they did not try to measure cavitation, these conditions appear unlikely to create significant bubble activity. Because of the low temperature rise and because of the change in electrical potential of the nerve, one can speculate that acoustic radiative force might create this effect, simply because, in principle, it is a way of inducing stress on the membranes of the nerves without the action of bubbles, which could (again in principal) transiently increase their permeability, thereby changing the ion flux across the cell membranes and therefore its action potential. However, much work needs to be done before this hypothesis will have any merit.

Is Ultrasound Safe for Obstetrics?

We have saved this section for last because we felt that its conclusions would be best appreciated after our extended discussion of the myriad positive and negative biological effects that ultrasound creates.

Many people experience diagnostic ultrasound in the context of fetal monitoring and imaging in utero. Is it safe to use ultrasound for this purpose? In brief, epidemiological studies say "yes" (at least within their ability to assess the answer to this question) based on current diagnostic ultrasound machines and how they are used. For example, a study [one of several quoted in (2)] included over 800 children, half of whom had been exposed to diagnostic ultrasound in utero. An exhaustive survey of the health of these children over a period of 12 years (including their birth weight and length, congenital abnormalities, and cognitive function among many other measures) found no statistically significant differences between insonified and uninsonified children.

One must remain vigilant, however, because the desire for increased imaging resolution leads naturally to considering more powerful pulses of ultrasound and the use of contrast agents, both of which may lead to cavitation, acoustic radiative force, or significant heat generation. The latter is especially problematic for tissues near bone. Also, the plethora of subtle biological effects induced by ultrasound recounted here (transient increases in the permeability of cell membranes; alteration of gene expression in bone; nerve stimulation; damage due to cavitation induced by the interaction of ultrasound and contrast agents, etc.) continue to hold the attention of researchers in case diagnostic ultrasound creates these effects in ways that elude epidemiological studies. A recent, excellent, and comprehensive review of this subject [see (150) and its extensive references] addresses the results and concerns discussed in this last section and highlights specific observations worthy of additional study but not as yet of clinical concern.

Here we mention just two that they discuss at greater length in their article. For example, studies with rat embryos have shown without explanation the production of heat-shocked proteins and retarded embryonic development when the embryos are subjected to mild hyperthermia (their temperatures were raised by 1.5°C) in conjunction with pulsed, 1 MHz ultrasound with a spatial peak, temporal average intensity of 1.2 W/cm^2 applied for 15 minutes. Animal studies (on sheep and primates) also show that diagnostic ultrasound evokes transient fetal neural responses without any detected biologically significant or deleterious consequences, however.

CONCLUSIONS

We find that as a discipline medical ultrasound offers a continuing source of challenge and excitement, significant intellectual rewards, and the opportunity to make a welcome impact on people's lives. Success in this field requires experimental acumen, penetrating insight into complex biophysical systems and interactions, a willingness to span many disciplines, and unbridled curiosity coupled with a willingness to act on that curiosity. It can be considered a place for fruitful synthesis of many of the tools, concepts, and techniques discussed in this encyclopedia.

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