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Bioeffects of Ultrasound

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Introduction

In the beginning of Chapter 8, the inherent non-linear equations of acoustics were linearized to obtain a tractable approach to sound wave propagation in fluid media. This led to a benign interaction between the wave process and the propagating medium, where neither was affected by the other. The attenuation (absorption) factor was then introduced to the wave equation to describe the decrease in amplitude of the acoustic parameters as the wave process propagates through the medium. At the extreme in non-linear phenomena are the strong shock waves characterized by discontinuities at the wave front. The interest here, however, is in the intermediate range of non-linear acoustic fields, where a number of distinct phenomena which are not observed in low-amplitude acoustic fields become apparent. These may account for both the reversible and irreversible biological effects produced by ultrasound.

Mechanisms of Interaction

In this chapter, phenomena associated with thermal effects, radiation force, and cavitation events are described briefly, and quantitative relations are presented which are useful for obtaining estimates of the magnitudes of the effects.

THERMAL PHENOMENA

As shown in Chapter 8, the decrease in intensity of a plane acoustic wave in a free field may be approximated by

$$I = I_0 e^{-2\alpha x} \tag{1}$$

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where I is the acoustic intensity at the position x, I_0 is the intensity at $x = 0$, and α is the ultrasonic absorption coefficient of the propagating medium, provided that $\alpha \ll k$, where the wave number $k = \frac{\omega}{c} = 2\pi/\lambda$. The coefficient α is often expressed in nepers (Np) per unit length. For example, if $\alpha = 1$ Np/cm, the intensity is reduced in each centimeter of propagation to $e^{-2} = 0.135$ of its previous value (note that the wave amplitude is reduced to $e^{-1} = 0.37$ of its value, see Equation *4*, Chapter 8. Depending upon the experimental measuring schema, it is often convenient to express α in decibels (dB) per unit length; the conversion is that 1 Np/cm = 8.686 dB/cm.

Equation *1* suggests that if α represents the portion of attenuation ascribed to absorption processes (energy irreversibly converted to heat in the body of the propagating medium), the rate of energy transfer per unit volume from the sound field is

$$-\frac{dI}{dx} = 2\alpha I \tag{2}$$

For the situation where heat conduction or radiation processes are unimportant, the rate of heat production per unit volume results in an initial time rate of increase of temperature in the medium

$$\left(\frac{dT}{dt}\right)_0 = \frac{2\alpha I}{\rho C K} \tag{3}$$

where (ρC) is the heat capacity per unit volume of the medium (ρ is the density and C is the heat capacity per unit mass per °C), and K is the mechanical equivalent of heat, equal to 4.2 J/cal. As an example of the use of this equation, consider that muscle tissue, for which α (at 1 MHz) is 0.15 Np/cm and $\rho C \simeq 1$ cal/cc°C, is exposed to a 1 MHz ultrasonic field of intensity 1 W/cm^2. The initial time rate of temperature increase is approximately 0.07°C/s, and will remain so throughout the exposure, until thermal conduction processes work to establish thermal equilibrium with the surrounding tissue.

Where heat conduction plays a significant role, the following equation is used instead of Equation *3*:

$$2\alpha I = \rho C K \frac{dT}{dt} + \kappa \frac{d^2T}{dx^2} \tag{4}$$

where κ is the thermal conductivity of the medium. It is typical of therapeutic ultrasound that the dimensions of the acoustic field are always small in comparison with the specimen being irradiated, so that heat is conducted away from the center of the heated region. This results in a temperature increase lower than that calculated from Equation *3*, and which approaches an equilibrium value. Additionally, blood perfusion affects the flow of heat and may be considered, to a first approximation, to have the effect of increasing the value of κ in Equation *4*.

Boundary Layer Heat Generation

In addition to heat being generated in the body of a medium due to absorption of ultrasonic energy, it is also possible for heat to develop in boundary layers, and thus preferentially heat small regions dispersed throughout an exposed volume. Very small "hot spots" could then develop which may not be revealed by macroscopic temperature measurements. These result from particle velocity gradients and associated viscous shear effects. Suppose the particle velocity along x to be u_x and to vary primarily with z. The particle velocity gradient is $\frac{\partial u_x}{\partial z}$; let its magnitude be G. Heat is generated by this motion at the time-averaged rate per unit volume of $\eta(G_{av})^2$, where η is the coefficient of viscosity of the fluid medium and G_{av} is the time average value of the magnitude of the particle velocity gradient (1). For the situation where the boundary layer is established near a rigid surface, *e.g.*, by a plane wave at grazing incidence in the x-direction, it can be shown that on the surface $G = u_a/B_L \cos \omega t$ and $G_{av}^2 = \frac{1}{2}\left(\frac{u_a}{B_L}\right)^2$. Here u_a is the amplitude of the particle velocity and $B_L = \left(\frac{2\eta}{\rho\omega}\right)^{1/2}$, where ω is the angular frequency (1). The boundary layer is characterized by the thickness B_L and has the value of approximately 0.6 μm at 1 MHz for water. Using the previous relations (recalling also that for a plane wave $I = \frac{1}{2}\rho c u_a^2$) the rate of heat deposition is $\eta G_{av}^2 = \frac{k}{2} I$, where k is the wave number, which would substitute for $2\alpha I$ in Equations *3* and *4*. Since $k \gg \alpha$ for most biological media at megahertz frequencies, the heat generation rate at a boundary layer with a rigid surface is much greater than the heating rate due to absorption within the medium. Boundary heating, however, occurs in a very small volume so that the resultant temperature elevation is drastically reduced by heat conduction, governed by Equation *4*. In the human body these conditions (a boundary layer near a rigid surface) will likely only exist at bone-tissue interfaces, and it may be only there that such significant heat generation rates occur. For interfaces between soft tissues, the rigid surface condition does not obtain such that G, and consequently the heat produced by this particle velocity gradient are appreciably reduced.

Heating Due to Mode Conversion

The contribution to heating in bone by energy converted to the shear propagation mode is discussed in Chapter 8. A quantitative example of this phenomenon is developed here.

In Figure 9.1, the ratio of energy in the wave of interest to the energy of the incident wave is plotted for each of the waves leaving a muscle-bone

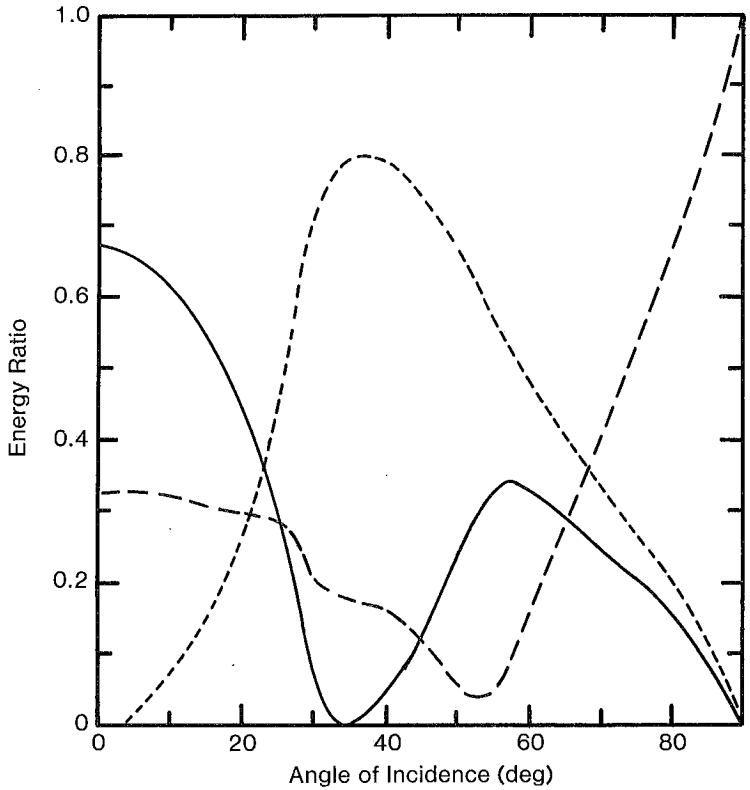

Fig. 9.1. Energy ratios (energy in wave of interest/energy in incident wave) versus angle of incidence for a longitudinal wave incident upon a muscle-bone interface (transmitted longitudinal——, reflected longitudinal— —, transmitted shear - - - - - -).

interface for a 1 MHz longitudinal wave incident from the muscle (2) (see Table 9.1 for the values used in the calculations). The energy ratio for the reflected shear wave is always less than 0.01 so that it does not appear in the figure, and the contribution to heating from the wave is neglected. It is apparent from Figure 9.1 that over a significant range of angles of incidence, the energy in the transmitted shear wave exceeds the energy in the transmitted longitudinal wave. Additionally, Table 9.1 shows that the absorption coefficient for shear waves in bone is almost twice that for longitudinal waves. As an example of the relative contribution to heating by these two waves, the ratio of heating rates at the interface, $2\alpha_s E_s/2\alpha_L E_L$, is 4.3 for an angle of incidence of 50°. Here, E_s and E_L are the energy fluxes normal to the interface for the shear and longitudinal waves, respectively, and α_s and α_L are the absorption coefficients for the shear and longitudinal waves, respectively. Details of the heating rates in a three layer, fat-muscle-bone system can be found in the literature (3).

TABLE 9.1. *Properties of Tissues at 1 MHz Used for the Mode Conversion Computations (3)*

Tissue	Longitudinal Velocity (m/s)	Longitudinal Absorption Coefficient (Np/cm)	Shear Velocity (m/s)	Shear Absorption Coefficient (Np/cm)	Density $\left(\frac{g}{cm^3}\right)$
Muscle	1530	0.12	22[a]	5000[a]	1.07
Bone	3380	1.52	1940	2.6	1.79

[a] Data from Frizzell *et al.* (26).

RADIATION FORCE

Radiation force results when the momentum transported by the acoustic wave process changes with position in the medium. It can occur at an interface between two media of different acoustic properties and also within an absorbing homogeneous medium. The rate at which momentum per unit volume is transported along the direction of propagation of the wave is the product of the momentum per unit volume (in the medium traversed by the plane traveling wave) and the particle velocity, viz. $\rho U \times U = \rho U^2$. For a traveling wave of intensity I and cross-sectional area A, the time-average rate of momentum transport is IA/c, where c is the velocity of propagation of the wave. The force on a material object is the rate at which momentum is transported to it. Thus, for a perfectly absorbing object, all the momentum of the sound wave is transferred to the object, which experiences a force

$$F_r = \frac{IA}{c} \tag{5}$$

For a perfect reflecting object, the sound wave is reversed in direction at the interface, its momentum is changed by $2IA/c$ per unit time, and

$$F_r = \frac{2IA}{c} \tag{6}$$

Radiation force also produces flow of a homogenous viscous medium. This occurs because absorption of sound in the medium results in a spatial gradient of the acoustic energy and conservation of momentum requires that the momentum disappearing from the field manifest itself as a steady force in the medium. The gradient of the radiation force is

$$\frac{dF_r}{dx} = -\frac{2\alpha IA}{c} \tag{7}$$

which produces streaming in the fluid medium. For the case where $2L \leq 3$, where L is the "length" of the acoustic beam (L can be taken as several times the axial length of the focal region for a focused field and at least ten times the beam diameter for an unfocused beam), the streaming speed v_{ST} can be computed (1) from

$$v_{ST} \simeq \frac{\alpha\rho a^2 U_a^2}{2\eta} \tag{8}$$

Here U_a is the acoustic particle velocity amplitude (axial value for a focused beam and average value for an unfocused beam), a is the beam radius and α, ρ, and η are the pressure absorption coefficient, the density and the shear viscosity coefficient of the medium, respectively. For example, consider a 1 MHz focused beam in water at room temperature for $a = 0.2$ cm, $\rho = 1$ gm/cm^3, $\alpha = 2 \times 10^{-4}$ cm^{-1}, $\eta = 0.01$ poise, and $U_a = 11.5$ cm/s (approximately 1 W/cm^2). This yields a streaming speed $v_{ST} = 0.05$ cm/s.

CAVITATION

Cavitation phenomena are produced in liquid media subjected to acoustic disturbances when the acoustic pressure during the rarefaction phase of the cycle reduces the hydrostatic pressure to a specific "threshold" value. This threshold value of the acoustic pressure amplitude is a function of a number of physical parameters which describe the state of the medium. These include temperature, pressure, frequency, kind and amount of dissolved gas, and previous history of the medium. Two types of cavitation phenomena can be distinguished, stable and transient cavitation, each of which exhibits different kinds of behavior of a gas or vapor bubble in response to an acoustic field. Stable cavitation of a medium containing dissolved or entrained gas prevails when a bubble(s) oscillates in a radial mode about a resonance size for a number of cycles without leaving the field. Transient cavitation occurs during the compression phase, in media experiencing a tension stress during a portion of the rarefaction phase of the acoustic disturbance, following growth of the bubble over several cycles to an unstable size. Here, the bubble collapse is very rapid and the bubble disintegrates, possibly by surface instability, and is thus of transient existence.

Transient cavitation is known to occur in tissues, but only at the highest intensities attainable in the frequency range of 1 to 10 MHz. Cavitation-mediated lesions have been produced in cat brain at 1 MHz at a peak intensity of 5,000 W/cm^2 and exposure times of at least 2 ms (4). Subsequent studies have implied that transient cavitation may occur at intensities beyond about (1,000 W/cm^2, related to the time of exposure by $It^{1/2} = 200$. Here, I is the acoustic intensity in W/cm^2 delivered to the site in the tissue, and t (s) is the exposure time.

Evidence for the existence of stable cavitation in tissues is lacking, except for blood (5) *in vitro*. This suggests a relatively high threshold level, 130 to 260 W/cm^2, although details of the extraction and handling of the specimen material are insufficient to determine the gas content relative to that expected *in vivo*. Stable cavitation has been associated with the production of irreversible effects on cells in suspension, macromolecules in solution, etc.

Discussion of Biological Effects

That ultrasound could produce effects in biological systems became apparent at its inception near the end of World War I, when techniques for locating submarines were being developed. An acoustic method was inves-

ed in which a piezoelectric transducer was shock excited to vibrate at esonant frequency of the structure and emit ultrasound into the bay at on. As the electric potentials applied to the quartz plate at times were gh as 40,000 V, the amplitude of the acoustic wave was appreciable and l fish and other marine animals were found dead in the vicinity of the ducer (6).

e first extensive investigation of these phenomena had to await the lopment of the vacuum-tube oscillator. The destruction of *Spirogyra* the killing of small fish and frogs exposed to 300 kHz ultrasound for al minutes at an intensity believed to be in the neighborhood of 10 W/ was observed (6). Subsequently, streaming within cells and cellular uction were observed at 406 kHz, when viewed with an optical micro-e. These events were also accompanied by an increase in temperature

e early observation that ultrasound provided an opportunity for true heating in tissues, and not simply the superficial heating that attended iation with infrared and the like (8), spawned attempts to understand nteraction of ultrasound with biological materials. Consequently, ex-nental studies were conducted at various levels of biological complexity. e attention has been devoted to interaction studies in solutions of omolecules and suspensions of microorganisms and cells, with the hope these would provide simpler models. Herein, a principal question deals the necessity for the presence of cavitation to affect the biological end-. By experimental design, thermal mechanisms are generally minimized ese systems. Interest in the interaction of ultrasound with biological es, organs, and whole animals was dominated by investigations of the of thermal events in the production of irreversible structural changes by determinations of threshold levels for such effects. The motivation hese pursuits was, of course, the probable application to medical lems. The choice of central nervous tissue as an often-employed tissue men was promoted by its relatively static acoustic and biological erties.

the following, findings are identified that have emerged from these s of studies, progressing from whole organism studies, through tissues rgans, to cellular and molecular levels. The attempt has not been made exhaustive, but rather to illustrate the nature of the studies undertaken he types of results obtained at the low megahertz frequencies. Studies carried out for a variety of purposes, satisfying specialized interests in fic topics, and leading to a very scattered literature. Thus, crucial tions directed toward a specified purpose may not be answerable simply use the pertinent experiments and measurements have never been ed out.

WHOLE BODY RADIATION

the low megahertz frequency range the wavelength of sound in soft es is on the order of a millimeter; and the half-power beam width of

transducers designed for clinical purposes is approximately a centimeter. Thus, it is apparent that whole body exposure of animals to ultrasound will be limited to a few cases. Foremost among these is the mammalian fetus, though model studies have included insects and microorganisms.

Pregnant mice irradiated for 5 hours at 40 mW/cm^2, 2.25 MHz ultrasound on the 9th day of gestation, and sacrificed on Day 18, exhibited a significant increase in fetal mortality (9). Some increase in fetal abnormalities were also observed, but there was no significant alteration in fetal weight. It has been suggested that the unusual irradiation conditions could have resulted in a uterine temperature increase sufficient to produce the observed abnormalities (10). Others were unable to detect differences in abnormality rates in fetuses between Days 8 and 20 of gestation, as well as in subsequent brother-sister cross-matings, of mice irradiated for as much as 60 minutes per day, for as many as 5 days, for up to 1 W/cm^2 with 2.25 MHz ultrasound (11). Early chick embryos (corresponding to approximately three weeks human development) were affected by 5 minutes of exposure to pulsed 1 MHz ultrasound of 2.5 W/cm^2 average intensity, but not to 1 W/cm^2 average intensity (12). More advanced embryos (corresponding to approximately 6 weeks human development) were unaffected at 10 W/cm^2 average intensity. Further, no effects were found on developing embryos exposed for 24 hours to irradiation from a 2.25 MHz Doppler diagnostic instrument having an electrical input power of 100 mW/cm^2. Others have reported finding no effects on mice (13–15), nor on rats (16–18), nor on rabbits (19).

More recently (20), it has been shown that the mean weight per fetus is reduced significantly when pregnant mice are exposed to 1 MHz ultrasound for 5 minutes at an average intensity as low as 1 W/cm^2. Another study (21) found irradiation of pregnant mice with 1 MHz, 0.35 W/cm^2 average intensity for 3 minutes resulted in a significant increase in mortality in litters observed at 21 days post-partum; however, others (22) found no such increase. This discrepancy may be related to the day of gestation on which exposure to the ultrasound was perpetrated and to other differences in specimen strain, specimen preparation and procedure. A report of work-in-progress suggests that neuromuscular development in the rat is delayed on exposure to 5 minutes of CW ultrasound at 10 mW/cm^2 (23).

An early study with insects involved exposure of Drosophila eggs to 1 MHz, 0.5 W/cm^2 ultrasound. This demonstrated a variety of developmental abnormalities, most likely associated with undetected cavitation (24). A more recent study involved large scale breeding experiments with Drosophila. Those surviving the irradiation procedure exhibited no significant increase in the frequency of recessive lethal mutations and chromosomal nondisjunction, even under exposure conditions sufficient to kill a substantial portion of the flies (25).

TISSUES AND ORGANS

Much activity has occurred in identifying specific effects to selected tissues and organs irradiated by particular ultrasonic exposure regimes and

in the quantitative determination of threshold levels at which unique events occur. The following illustrates the considerable range of interests of the investigators and the breadth of their findings.

The mammalian CNS provides an acoustically static organ for study in that the ultrasonic propagation properties remain largely unchanged in response to physiological and behavioral stimuli. Remarkable agreement has been reached in determining the relationship between the acoustic intensity in the tissue and the single-pulse duration necessary to produce threshold lesions in the brain (4, 27, 28). The relationship $It^{1/2} = 200$, where I is the acoustic intensity at the site of interest in the tissue in (W/cm^2), and t is the time duration of the single-pulse exposure in (s), defines the threshold. That is, exposures greater than 200 W/cm^2 s$^{1/2}$ always produce lesions identifiable under the optical microscope, while those less than this value do not. This relation has been determined experimentally to describe threshold events over the range of exposure from 100 μs to 10 minutes, beyond which it alters to approach an infinite time exposure condition. Thermal processes have been shown to dominate in the low intensity-long pulse exposure region (29), while transient cavitation events are believed to be of greatest importance at the highest intensity-shortest pulse exposure region. In the mid-intensity region, about 700 to 1500 W/cm^2, other mechanical mechanisms are believed to occur. Histologically, white matter exhibits a lesser threshold than does gray matter, with the vascular structures being most resistant (30). The observed lack of frequency dependence (31) of the threshold boundary may be due, at least in the thermal region, to the combined effects of the nearly linear dependence of the absorption coefficient on frequency and the inverse dependence of focal volume on frequency. These tend to balance each other, maintaining a relatively constant lesion volume independent of frequency (29). A study involving exposure of the lumbar enlargement of the spinal cord of neonatal mice (maintained at 37°C), a preparation permitting temperature variation of the specimen, and involving a functional rather than structural endpoint, yielded threshold levels approximately one-eighth of the above for the mature brain (32, 33).

Rat spinal cords were exposed to ultrasonic frequencies in the range 0.5 to 6 MHz at 25 W/cm^2 using 10-μsecond pulses with a 10 percent duty factor (34). A decrease in damage with increase in frequency and increased damage under hypoxic conditions were observed. Recent studies show the threshold for irreversible structural changes in cat liver to be about twice that for brain (35) and thresholds for kidney and testis to be higher still (36). A significant reduction in the frequency of mitotic cells has been reported in surgically stimulated rat liver in response to exposure to 60 mW/cm^2, 1.9 MHz ultrasound (37). However, others (38) were not able to confirm these findings with surgically stimulated rat liver irradiated 1 and 5 minutes with 2.2 MHz ultrasound in the range 0.06 to 16 W/cm^2. One major difference in the procedures employed by these two groups was that the latter used a circular motion of the transducer over the animal's ventral surface, while the former maintained the transducer stationary. Negative results were also obtained for exposure of regnerating rat liver to 2.5 MHz ultrasound pulsed at 10 to 50 kHz prf and 33 W/cm^2 peak intensity (39). However, an increase was noted in the frequency of hemorrhage at the lower frequencies, in the range 0.5 to 6 MHz, in surgically exposed liver to 56 W/cm^2 peak intensity ultrasound for 5 minutes where 10-ms pulses were used with a 10 percent duty factor (40). Although the temperature rise did not exceed 5°C, damage was particularly severe in the neighborhood of the central vein.

Conflicting reports have resulted from animal studies of the ultrasonic effects on testes, viz., effects on spermatogenesis and fertility (41, 42), while others failed to make such observations (43, 44). In a more recent study, mouse testes were exposed sequentially for 30 s at a spatial peak ultrasonic intensity of 25 W/cm^2 at 1 MHz. The testes were removed at varying times post-irradiation from immediately to 19 days, and were then examined histologically. The results suggest that two types of ultrasonically induced damage occur for different specimens under identical exposure conditions: either seminiferous tubule disruption occurs with a suggestion of minor intertubule space involvement or a more severe form of tubule damage occurs with significant interstitial tissue involvement (45). Spermatocytes appear to be affected earlier than spermatogonia, contrary to the situation following ionizing radiation.

Blood cell stasis has been demonstrated in the vessels of chick embryos exposed to ultrasound in the range of 1 to 5 MHz (46). Both CW and pulsed ultrasound were found to be effective; the intensity necessary to produce stasis may be as low as 0.5 W/cm^2 (at 3 MHz), depending upon blood vessel size, type, and orientation. The stasis is reversible upon cessation of the sound exposure, although electron microscopy has revealed damage to some endothelial cells lining the embryonic vessels in which stasis is produced. As the production of stasis is associated with standing waves, it can be avoided by either continually moving the sound source or by using sufficiently short irradiation pulses (47).

Tissue regeneration in response to ultrasonic irradiation has been studied (48). The rate of repair of 10 mm square holes in rabbit ears exposed to 3.6 MHz ultrasound for 5 minutes three times per week, under either 0.1 W/cm^2 CW application or various pulse regimes with the intensity in the range 0.25 to 8 W/cm^2, was significantly more rapid than the untreated control ear. The attending temperature rise was considered to be too small to be responsible for these effects. Subsequently, patients with chronic varicose ulceration were treated with 3 MHz ultrasound at 1 W/cm^2 for 10 minutes, three times per week for 4 weeks with encouraging results (49). Other reports of inhibited tissue regeneration in response to ultrasonic irradiation have also appeared. In one, slightly slower tissue renewal of the amputated

prelimb of a newt occurred, following ultrasound exposure, compared to that of the opposite amputated prelimb, although details were sparse and conditions were complicated for identifying the crucial dosages (50).

The effects of ultrasound on neoplastic tissues has involved at least two lines of inquiry: a direct effect on tissue possibly involving hyperthermic effects, and a synergistic involvement with other modalities. A recent example of the former deals with the irradiation of subcutaneously implanted Rat Wilm's tumors. The use of 1 MHz ultrasound at 1.5 W/cm^2 resulted in a reduction in tumor volume and weight, and an increase in mean rat survival time (51). Histological observation revealed nuclei with condensed chromatin patterns. Substantial temperature increases also occurred. In regard to synergism, it was noted that the X-ray dosage required to produce regression in an experimental tumor was substantially reduced when simultaneously irradiated with 1 MHz ultrasound at 8.4 W/cm^2; the effect was believed to be due to heating resulting from sound absorption (52). Marked improvement in the treatment of human superficial cancer was reported from the simultaneous use of ultrasound and X-rays (53). However, synergistic effects were not observed with either cultured mouse lymphoma cells or implanted tumors in rats (54). It has been reported that a preliminary irradiation of transplanted sarcoma 37 tumors in mice with 1 MHz ultrasound in the range 0.5 to 2.5 W/cm^2 for periods of 1 to 5 minutes enhances the sensitivity of the tumor cells to subsequent gamma radiation (55). A synergism with chemotherapy has been suggested (56), where malignant brain tumors were irradiated simultaneously, through a bone flap, with 1 MHz ultrasound at 3 W/cm^2. Although the patient population was small, they believed the effectiveness of chemotherapy improved.

Enhanced DNA synthesis has been reported in neonatal mouse tibiae exposed for 5 minutes, three times in 24 hours, to 1.8 W/cm^2, 1 MHz ultrasound (57). Observations revealed that growth, protein accumulation, and ^{3}H-proline incorporation remained unaffected. The DNA synthesis may have been affected by the substantial temperature rise which accompanied absorption of the ultrasonic energy in the highly absorbing bone tissue, though the authors are not so convinced.

CELLS AND MICROORGANISMS

Cells and microorganisms in suspension provide model systems of tissues and organs. They have the advantage of being composed of single cell lines, possibly even in mitotic synchrony, but with the disadvantage of not being constrained by tissue architectural features, though gel-caging can reduce the importance of this. Such systems have been attractive for studies dealing with the physical mechanisms by which ultrasound can produce alterations in more complex structures. Thus, it has emerged that ultrasonic exposure of cells and microorganisms in suspension can lead to cell death, and that cavitation is important to the process. Indeed, some investigators (58) have been able to associate the destruction of an amoeba with the specific number

of discrete cavitation events occurring during the irradiation procedure. This apparent relationship of cavitation to cell destruction is important in attempts to determine risk in the clinical use of ultrasound, especially since virtually nothing is known of cavitation phenomena in tissues. It appears that cell disintegration occurs preferentially, at least when cavitation is allowed to occur, during the mitotic phase of the cell cycle. Mouse leukemia cells in aqueous suspension were most susceptible to damage in M-phase when exposed to 1 MHz ultrasound having a spatial peak intensity of 15 W/cm^2 for 10 seconds (59). It has been suggested that the mechanical strength of the cell membrane may vary during the cell cycle. In one interesting case, gel-caged suspensions of an amoeba were exposed to 1 MHz ultrasound sufficient to produce irreversible alterations in mammalian tissues (60). They employed samples from logarithmically growing and synchronous cultures treated in free field and standing wave field conditions in both CW and pulsed regimes. The treated samples, however, failed to show differences in growth patterns compared to controls.

Non-lethal effects on cells have also been investigated. A reduction in the electrophoretic mobility of Ehrlich ascites cells was observed following exposure to low megahertz ultrasound, implying alteration of the electric charge density of the cellular surface (61, 62). Ultrasonic irradiation at 1.8 MHz with intensities greater than 1 W/cm^2 of rat thymocytes was followed by an immediate decrease in potassium content, suggesting a sublethal alteration in the structures intimate to permeability (63). Additionally, investigations of ultra-structural details has revealed mitochondrial modifications in cells exposed to ultrasound (64).

Microorganisms have been employed in genetic studies without positive results. An increase was not found in the back-mutation of an auxotrophic strain of *Bacillus subtilis* in response to 2 MHz ultrasonic irradiation for 5 minutes at intensities up to 60 W/cm^2, in a pulsed regime (65). Also, abnormal genetic effects did not occur in ultrasonically irradiated yeast cells, even when treated in such a manner that the cells were killed to 0.1 percent of the survival rate of the controls (66).

BIOMACROMOLECULES AND THEIR ASSEMBLAGES

The response of large molecules of biological importance to ultrasonic exposure has been studied in aqueous solution to determine details of tissue interaction mechanisms. The findings showing that ultrasonic absorption is largely attributable to tissue protein content (67) and that tissue interactions resulting in irreversible structural changes must occur at levels of structure below that identifiable with the light microscope (32) encouraged some of these inquiries.

For molecules having molecular weights below about 10^4, *i.e.*, proteins, in aqueous solution, degradation appears to occur only in the presence of cavitation in the ultrasonic frequency range of 1 to 27 MHz (68). For larger

molecules in aqueous solution, *e.g.*, DNA with molecular weights greater than about 10^6, it has been possible to demonstrate degradation in the absence of any phenomena suggesting the presence of cavitation (69). Using intensities as high as 30 W/cm^2, essentially monodisperse fragments were produced with the limiting value depending upon intensity, *i.e.*, greater intensities of exposure produced smaller fragments. This sequential halving of the molecules with continued irradiation time is also a characteristic feature of the much more prevalent studies of degradation of DNA in the presence of cavitation (70). The breaking of DNA molecules preferentially at the midpoints of their extended conformation in solution suggests a mechanical mechanism being responsible. However, chemical effects, largely due to free radical production in the presence of cavitation, have been described extensively (71), in particular in the low megahertz frequency range (72). Nonetheless, while it is an easy task to degrade nucleic acid molecules in solution, it has not been possible to produce mutagenic lesions following *in vitro* irradiation of transforming DNA (65). The apparent necessity for the extended conformation of DNA molecules in solution for degradation to occur implies a much lesser opportunity for denaturation of cellular DNA to occur.

An interesting finding from two laboratories is that the order of ultrasonic reactivity obtained by observing spectral changes (73) in nucleic acid bases in solution at 1 MHz and less than 5 W/cm^2 (viz., Thy > Ura > Cyt > Gua > Ade) seems to be the same as that obtained by chromatography (74) in dilute solutions of nucleic acids at 800 kHz and approximately 10 W/cm^2. No information exists about the occurrence of such events intracellularly. A recent study of the effect of 1 MHz ultrasound in the range 15 to 36 W/cm^2 for 10 minutes on human leucocytes failed to increase sister chromatid exchange frequency, implying no effects to chromosomes (75).

Considerable attention has been devoted to possible ultrasonic effects on chromosomes. Much of this interest has been associated with studies involving human lymphocyte chromosomes from cultured preparations, with the overwhelming result that ultrasound does not produce an increase in aberrations even at much greater exposure intensities and longer irradiation times than are likely to occur during medical diagnositic procedures (76–81), though such a synergistic effect with X-rays may occur (82).

A few studies have treated membranes and membrane models. The permeability of membranes formed from oxidized cholesterol was increased by exposure to 1 MHz ultrasound (83) at intensities greater than 1.5 W/cm^2. Liver plasma membranes exhibited decreased 5′ nucleotidase activity and altered morphology in response to 0.87 MHz ultrasound in the range 0.75 to 3 W/cm^2 for exposures ranging from 2 to 10 minutes (84). An unlinking of the membrane potential and short circuit current was found to occur in that their time courses in response to ultrasound differed, *i.e.*, the short circuit current increased continuously for exposures of 0.5 seconds and longer while the membrane potential reached its maximum within 0.5 seconds and did not alter with increased duration of exposure (85). This occurred at 1 MHz in the intensity range 1 to 100 W/cm^2 for isolated frog skin preparations. Alteration of the recalcification time of platelet-rich plasma occurs with 1 MHz ultrasound at approximately 0.2 W/cm^2 (spatial peak) in 5 minutes (86).

Summary of Biological Effects

From the investigations that have been conducted, it appears that ultrasound can be considered a very inefficient mutagenic agent. Chromosome damage in response to ultrasonic irradiation is most likely to be lethal. Because of the particular molecular conformation necessary to bring about effects *in vitro*, it does not appear likely that selective effects can be produced in cellular nucleic acids.

As ultrasound appears to induce embryological effects, the treatment of pregnant women in the abdominal area., *e.g.*, possibly for lower back pain, should be avoided.

A systematic analysis of existing reliable data for mammalian tissues has led to the following two summary statements (87)

a) No substantial bioeffects have been demonstrated for spatial peak-temporal average intensities less than 100 mW/cm^2.
b) No substantial bioeffects have been demonstrated for which the product $I \cdot t$ is less than 50 J/cm^2 where, for pulsed operation, t is the total ("on" + "off") time.

(It should be noted that the spatial peak intensities referred to in the statement are typically very much greater than the spatial average values of intensity used in the specification of ultrasonic instrumentation.) The statements may need to be modified as new data appear, since a) most of the data are from mammals other than man, and the extrapolation to man is not always a clear procedure, b) the influence of exposure factors such as pulsing conditions and acoustic frequency are not included, and c) the most sensitive biological tests may not have been used yet.

No fully satisfactory epidemiological study has as yet been performed. However, a retrospective survey which was not case-controlled of more than 1000 apparently normal women examined diagnostically with ultrasound during various stages of pregnancy exhibited only a 2.7 percent incidence of congenital abnormalities on newborn physical examination. This is to be compared with the figure of 4.8 percent from a separate and unmatched survey of women who did not receive ultrasonic diagnosis (88). Neither the gestation period at which the first ultrasonic examination occurred nor the number of examinations appeared to increase the risk of fetal abnormality. A smaller study has also yielded no indication of either congenital malformations or chromosomal aberrations in the fetus (89).

Finally, although not scientifically objective, it must be noted that a

stantial number of persons receiving ultrasonic diagnosis also undergo sequent clinical examinations; undesirable effects from such procedures, uspicions thereof, have not been reported (33).

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