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Safety of Ultrasound

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Experimental studies will not prove diagnostic ultrasound, as it is employed in clinical medicine, safe. Rather, these studies will provide, if properly planned and executed, data that will aid in the overall assessment of risk associated with exposure to ultrasound. Safe implies absence of an effect, not involving risk, or the life. It simply is not possible to prove that ultrasound, or any agent, does not produce any effect at the levels employed diagnostically.

A more realistic approach is to examine the "risk" associated with ultrasonic exposure. This risk, coupled with the benefits derived from the diagnostic examination, must then be weighed by the clinician, who must make the benefit versus risk judgment. Given that there are definite benefits derived from diagnostic ultrasound, as is evidenced by many of the contributions in this book, the following text will concentrate on the information available to assess the risk.

It is important to recognize that ultrasound, at sufficient levels, is capable of deleterious effects on biologic materials. For example, ultrasound has been used for many years as a surgical tool for the treatment of Menière's disease (1). Therefore, it is necessary to aproach the question of ultrasonic risk assessment in a twofold manner. First, what biologic systems are most sensitive to ultrasound? Second, what exposure levels impose a significant risk on these systems?

In attempting to assess the risk, not only must the experimental studies be evaluated but also the extent to which ultrasound is used along with information on the output parameters from diagnostic equipment must be included in the evaluation.

While no statistically based survey has been conducted to document the extent to which ultrasound is being used, several indicators do, however, support the fact that its use is increasing.

A market analysis in 1969 predicted that the dollar value of the ultrasonic market would increase 300% during the period between 1968 and 1973 (2). This figure represents an annual increase of 75%. More recently, based on discussions with clinical manufacturers, the United States Food and Drug Administration's Bureau of Radiological Health predicted that in 1976, the ultrasonic industry would grow at an annual rate of 50% and that annual dollar sales will be around 40 million (3).

In 1971, the Bureau of Radiological Health surveyed 301 of 6306 short-term general hospitals in the United States and found that 12% of the hospitals used diagnostic ultrasound (4). In an editorial in the *Journal of Clinical Ultrasound*, the editor (5) doubted the 12% figure, because he believed that Doppler ultrasound was being used by more than 50% of the obstetricians in the United States. In 1976, the same government agency reported that a hospital survey showed that 35% were using ultrasound (6). While questions may be raised as to the validity of the 1971 and 1976 survey estimates, they do show, however, an approximate annual increase in use of 24%.

An international mail survey was conducted under the sponsorship of the IEEE Group on Engineering in Medicine and Biology's Subcommittee on Ultrasound Safety and Standards, with the help of the American Institute of Ultrasound in Medicine, the Biomedical Engineering Society, the Bureau of Radiological Health, the United States Public Health Service, and the United Kingdom Medical Research Council (7). In part, the survey showed that, on the average, between 1963 and 1971, there was an annual increase in use of clinical ultrasound of approximately 10%.

It has been estimated that, in the United Kingdom, the number of ultrasonic diagnostic examinations is doubling every three years (8). This figure represents an annual 26% increase.

The United States National Science Foundation (NSF), through its Office of Experimental Research and Development Incentives, conducted an international state-of-the-art survey of diagnostic ultrasound in March 1973. One of the conclusions of the survey team was that between 1971 and 1973, the number of ultrasonic diagnostic instruments sold in the United States increased by 300%, which represents an annual increase of 73%. Another conclusion of the NSF survey team was an estimate that the sales of clinical ultrasonic devices will match those of x-ray devices by 1983 (9). And, Kossoff (10) estimated that by 1980, the sales volume of ultrasonic diagnostic equipment will exceed that of nuclear imaging equipment.

If one were to assume that presently 50% of all pregnant women were subjected to an ultrasonic examination for the determination of the well-being of the fetus [recall that Holmes (5) indicated that 50% of United States obstetricians have Doppler devices; in addition, Ziskin's (7) survey showed that 17% of all pulse-echo examinations were to the gravid uterus], and additionally assume that the use is increasing at an annual rate of 10%, in eight years, virtually the entire population from that point on will be exposed to ultrasonic energy in utero.

The output power and intensity parameters from commercial pulse-echo and continuous-wave ultrasonic diagnostic equipment have been measured and reported as shown in Table 1 (11-16). The measurements of pulse-echo devices show that the spatial average ultrasonic power ranges from about 60 μW to 21 mW and that the spatial average temporal peak intensity ranges from 1.4 to 95 W/cm^2, while the spatial peak temporal peak intensity ranges from 2 to 177 W/cm^2. For Doppler continuous-wave devices, the spatial average ultrasonic power ranges from 1 to 37

mW, and the spatial average intensity ranges from 1 to 305 mW/cm².

BIOLOGIC EFFECTS

About 35 years after the Curies discovered piezoelectricity (17), the French scientist Paul Langevin developed the first use of ultrasonic energy, wherein underwater acoustic echoes were bounced off of submerged objects (18,19). During the course of this work, the first reported observation was made that ultrasonic energy had a lethal effect on small aquatic animals (20).

The first extensive investigation of the phenomena observed by Langevin was conducted by Wood and Loomis (21). Although the ultrasonic levels were not specified, they did confirm Langevin's observation that ultrasonic energy could kill small fishes and frogs by 1–2-min exposures. Qualitatively, they reported death of small animals and unicellular organisms in terms of ". . . tearing to pieces . . . ," ". . . cells ruptured . . . ," and ". . . torn apart. . . ." In perhaps the first review paper, Harvey (22) examined the physical, chemical, and biologic effects of ultrasound wherein effects on cells, isolated cells, bacteria, and tissues were summarized with a view toward identifying the responsible mechanism. The ultrasonic exposure conditions of this early work were not well characterized, but the intensity levels were undoubtedly quite high.

The early studies of W. J. Fry (23,24), in which the ultrasonic exposure conditions were carefully controlled and specified, examined the production and mechanism of sciatic nerve paralysis in the frog. Again, the ultrasonic intensities were quite high. Later, Fry (25) reviewed the production of ultrasonically induced lesions in central nervous system tissue using focused ultrasonic energy. In addition to these studies, intense ultrasound had been employed to produce lesions in adult cat and rat brain (26–30), adult rat and neonatal mouse spinal cord (25,31,32), adult mouse, rat, and rabbit liver (33–36), adult frog muscle (37,38), rabbit blood vessel (39), rabbit kidney and testicle (35), and rabbit ocular tissue (40,41). In most cases, these studies represented rather gross damage, because the ultrasonic energy induced either immediate functional and/or structural alteration. While not minimizing the importance of such studies to the elucidation of fundamental interaction processes, they do possess some limitations for extrapolation to potential effects, or lack of, at ultrasonic levels employed in clinical medicine. These studies do, however, support the view that the ultrasonic exposure conditions employed diagnostically more than likely will not produce acute, gross, irreversible damage to the irradiated tissue.

Table 1. Summary of Measured and Reported Output Parameters from Commercially Available Ultrasonic Diagnostic Equipment

						Intensity			
Reference	*Nominal Frequency (MHz)*	*Pulse Repetition Frequency (MHz)*	*Beam Area (cm²)*	*Average Power (mW)*	*Pulse Power (W)*	*Temporal Average Spatial Average (mW/cm²)*	*Temporal Average Spatial Peak (mW/cm²)*	*Temporal Peak Spatial Average (W/cm²)*	*Temporal Peak Spatial Peak (W/cm²)*
					Pulsed				
	1	1538	1.3	4.6	1.5*	3.5	6.8		26
	2.25	1538	1.3	4.4	3.2*	2.7	9.6		8.6
	3.5	1538	1.3	1.12	1.3*	1.08	11.0		25.0
	5	1538	0.8	1.82	3.0*	2.32			
	1	1538	1.3	2.7	0.9*	2.0			
	2.25	1538	1.3	5.3	3.9*	4.0			
	3.5	1538	1.3	5.3	3.9*	4.0			
	5	1538	0.8	2.6	4.2*	1.95			
	5	1538	2.8	0.5	0.8*	0.17	35		
	2.25	520	1.3						50
11	2.0	520	1.3	4.2	8.1	3.2			
	2.0	520	1.3	2.8	5.4*	2.1			
	2.0	676	1.3						49
	2.25	964	1.3						44
	2.25	1924	1.3						27
	7.5	48,000	0.07	21	1.6*	296.			4.9
	2.25		2.8	0.83		0.29			
	2.25		2.8	6.3		2.22			
	2.25		2.8	11.4		4.0			
	2.25		1.3	1.01		0.78			
	2.25		1.3	5.1		3.8			
	2.25		1.3	6.4		4.8			
	8		≃0.1	0.61		4.9			
	10		≃0.3	0.060		0.21			
		770		2.5		1.9	13.9		57
		770		1.3		1.0	7.2		30
12		1520		3.3		2.5	18.3		38
		400		4.0		3.0	22.2		177
		1270		7.1		5.4	39.4		98
		770		1.6		1.2	14.5		59

Table 1. (*Continued*)

Reference	Nominal Frequency (MHz)	Pulse Repetition Frequency (MHz)	Beam Area (cm^2)	Average Power (mW)	Pulse Power (W)	Intensity: Temporal Average Spatial Average (mW/cm^2)	Intensity: Temporal Average Spatial Peak (mW/cm^2)	Intensity: Temporal Peak Spatial Average (W/cm^2)	Intensity: Temporal Peak Spatial Peak (W/cm^2)
13	1.5	484	1.31	3.2	10	2.4†		7.6	
	2	940	0.49	5.4	11.5	11.0†		23.5	
	2	1000	0.49	0.8	1.6	1.6†		3.3	
	1.5	1200	1.0	1.1	1.4	1.1†		14	
	2	455	0.96	21	91	21.9†		95	
	1	500	0.7	7	14	10.0†		20	
	1	735	0.7	15.4	21	22.0†		30	
	2	735	0.43	14.4	40	33.5†		93	
	1.5	700	0.28	1.3	4	4.6†		14	
	5.0	700	0.54	<0.3	<3	<0.6†		<6	
	1.5	700	1.31	1.0	3	0.8†		2.3	
14	3		1	0.7‡		0.7		6.0	
	10		1	0.1‡		0.1		1.4	
					Continuous				
11	5		0.8	9.8		25			
	9.3		0.8	9.8		305			
	2.25		4.9	37.0		15.9			
12			4.4		7.2				
13	2		0.84	19		22.6			
	2		1.16	24		20.7			
	5					3			
15	9.5			3.37		33.7			
16	5		0.8	1.03		1.29	4.35		
	8.8		0.32	30		93.8	300		

* Calculated: pulse power $= \frac{\text{average power}}{(\text{PRF})\,(\text{PW})}$, where PW is assumed to be two cycles.

† Calculated: average power per beam area.

‡ Calculated: (temporal average spatial average intensity) (beam area).

The more recent bioeffect literature appears to suggest that as more sensitive biologic end points are studied, the ultrasonic exposure conditions required to produce measurable effects appear to decrease. This is not to imply that ultrasonic energy will eventually be shown to represent a substantial risk. Rather, it is simply an observation, and whether ultrasound will be shown to represent a significant risk will depend on the types of effects observed and at what levels of exposure they have occurred. It is interesting to observe that even with ionizing radiation, deleterious effects are attributed to individuals and populations of living organisms from natural background levels only by inference. Such effects have not been directly observed (42). For now, this review will focus on the ultrasonically induced biologic effects that have been observed to occur at relatively low ultrasonic exposure conditions or that have been somewhat controversial.

In 1970, Macintosh and Davey (43) reported a significant increase in the number of chromosomal aberrations after in vitro irradiation of cultured human lymphocytes to continuous-wave ultrasound. This report caused concern and controversy, in that the ultrasonic sources were commercial fetal heart detectors and represented one of the first ultrasonically induced biologic effects that raised the distinct possibility of risk to patients and fetuses. Following this report, many researchers attempted to determine the validity of the reported effect. Thacker (44) reviewed the history of chromosomal damage induced by ultrasonic irradiation and also discussed the negative reports that countered the findings of Macintosh and Davey (43). During this period, a second report by Macintosh and Davey (45) not only verified the original findings but also extended the study to show a threshold intensity of 8.2 mW/cm^2 for a 1-hour exposure, below which no chromosomal damage occurred. A follow-up study showed that the high incidence of aberrations previously found could not be reproduced (46). Additionally, it was suggested that the earlier positive findings were due to some, yet unidentified, artifact unrelated to the ultrasonic irradiation.

Although there have been no verified reports of "classic" chromosomal aberrations at diagnostic or therapeutic intensity levels of ultrasound, chromosomal anomalies of another type have been observed (47,48), wherein chromosomal damage was reported in the form of bridged prophases and metaphases and agglomerated mitotics. This work with *Vicia faba* roots (2 MHz, 1-min exposure at 8 W/cm^2) suggested that these particular types of aber-

rations may not have been observed because the standard technique for scoring metaphases for chromosomal aberrations would select against mitotic figures that were not well spread; thus, a welded chromosome would not be scored as abnormal, for example. The importance of this observation is not necessarily in terms of assessing the genetic effects of ultrasound but rather philosophical; namely, one should not be confined to assessing the results of an experiment based solely on knowledge and experience of how another agent interacts with the biologic specimen.

It is relatively important to assess whether ultrasound has a mutagenic effect. In Thacker's (44) extensive review, it was concluded that a genetic hazard was unlikely from diagnostic ultrasound. While this statement is welcomed, it was based on an extrapolation of the data, which even the author judged as equivocal. Further, on reviewing the mutagenesis data, Thacker suggested that there is some evidence that ultrasound could induce mutagenesis, although the increase in mutation frequencies was small when compared with ionizing radiation.

Lyon and Simpson (49) did not detect any evidence of an ultrasonically induced genetic effect in their experimental study with mice. Testes or ovaries were irradiated for 15 min, at 1.5 MHz, under three exposure conditions, one continuous wave (1.6 W/cm²) and two pulsed (I_{av} = 1.6 W/cm², duty cycle = 25%, pulse width = 1 msec and 0.9 W/cm², 2%, 30 μsec). Principal tests included dominant lethal mutations and translocations with the results compared to both positive (x-ray) and negative controls. The principal weakness of the study that the authors cited was the small number of animals.

Yet, questions continue to be raised in the literature as to whether low-level ultrasound does, indeed, affect genetic material. Galperin-Lemaitre et al. (50,51) reported the degradation of purified DNA at intensities as low as 200 mW/cm². While Thacker (52) and Coakley and Dunn (53) agreed that ultrasound could degrade purified DNA, they questioned, and rightly so, the appropriateness of whether this is "mutagenetic or purified DNA." Also, they argued that other physical factors could be responsible for affecting the purified DNA, such as reflections, which would increase the intensity at the discontinuity.

The early studies with the fruit fly *Drosophila melanogaster* eggs showed that developmental abnormalities could be induced by ultrasound (54–56). The most recent of these studies (56) suggested that ultrasonic exposures of less than 100 mW/cm² for 30 sec had an insignificant effect on the normal development, whereas the number of anomalies increased as the ultrasonic intensity exceeded 100 mW/cm², suggestive of a dose-effect response.

Even though the dosimetry of these early fruit fly studies is suspect, they do represent a quest to obtain such data more than 20 years ago. Also, it should be noted that the current ionizing radiation protection guidelines are, to a large extent, based on *Drosophila* studies (42).

Perhaps some of the more controversial findings of adverse effect from prenatal ultrasonic exposure of pregnant mice (at the ninth day of gestation) were those of Shoji et al. (57, 58). The signal source was a commercial fetal Doppler device operating under continuous-wave conditions (2.25 MHz) with a reported intensity (presumably spatially averaged) of 40 mW/cm², and the mice were exposed for a period of 5 hours. In this earlier study with DHS mice, eight types of fetal abnormalities were observed, but the difference between exposed and control mice was not significant. However, the rate of fetal death was significant in the irradiated group (57). Their later study with a different strain of mouse, A/He, yielded a statistically significant increase in both fetal abnormalities and fetal mortality (58). In both of these studies, the mice were given an initial dose of nembutal which lasted about 1 hour, after which time the animals struggled. Lele (59) has suggested that these observations may be a result of prolonged induction of a moderate temperature use.

There were earlier reports of negative findings. Pregnant mice were exposed to pulsed ultrasound under a wide variety of exposure conditions, namely, temporal peak spatial peak intensity ranged from 20 to 490 W/cm², temporal average spatial peak intensity ranged from 0.75 to 27 W/cm², pulse width and duty cycle ranged from 10 μsec to 10 msec and from 0.2 to 20%, respectively, and the exposure times typically were 300 sec (60,61). Of the 13 exposure conditions, five groups were irradiated at the eighth day of gestation, three groups at the fifth day of gestation, one group at both of these gestational ages, and the remaining three groups at mixed gestational ages of 8 and 9; 1, 7, 10, and 12; and 3, 5, 6, and 8. The study concluded that there were no significant effects on litter size, resorption rate, or abnormality rate. However, a dose response could be suggested by examining the abnormality rate of the two groups with the highest temporal average spatial peak intensity that show a statistically significant effect on fetal abnormalities. Unfortunately, this aspect was not pursued in the article.

The effects of multiple prenatal exposure in rats from a prototype fetal Doppler device (continuous wave, 2.5 MHz, spatial average intensity of 9.1 mW/cm²) showed no significant differences in fetal and maternal weight, viability, death, litter size, implants, and external and soft-tissue abnormalities. Incomplete or absent ossification of strenebrae was present in 50% of the irradiated and in 36% of the controls, but the authors suggested that there was no biologic significance, since the effect was predominantly in the 30-min exposure group and not in the 120-min group (62).

Within the past year or so, experimental observations appear to suggest that subtle effects are occurring to rodent embryos and fetuses when exposed to ultrasound in utero.

Time-mated mice exposed to continuous-wave (1 MHz) ultrasound at the eighth day of gestation showed a statistically significant weight reduction from about 6 to 18%, depending on the exposure conditions. There were seven exposure groups, including a sham, and 272 litters (2866 fetuses), with exposure conditions ranging from 0.5 to 5.5 W/cm² and from 10 to 300 sec (63). The observation that in utero ultrasonic exposure of mice causes a weight reduction has also been reported in a different strain of mouse (64). Here, the mice were also irradiated on the eighth day of gestation but this time to pulsed ultrasonic energy (spatial peak temporal peak intensity of 1500 W/cm², spatial average temporal average intensity of 10 W/cm² for 10 sec at a nominal ultrasonic frequency of 1 MHz). Fetal weight reduction was not observed in rats exposed on the ninth day of gestation to continuous-wave (3.2 MHz) ultrasound, even at exposure conditions that produced some mortality (65). However, a few pups were stunted, but this observation was not statistically significant. A preliminary study suggested that the fetal weight reduction is sustained beyond weaning when time-mated mice were irradiated at the 13th day of gestation with continuous-wave (1 MHz) ultrasound and examined at the 55th day after conception (66). There were a total of 162 pups from 21 litters in three

exposure groups (sham, 0.25 W/cm², and 0.80 W/cm² for 120 sec), and they yielded statistically significant weight reductions of 8.7 and 14.8%, respectively, relative to the sham.

Curto (67) observed an increased postpartum mortality of the offspring of time-mated mice irradiated at the 13th day of gestation to continuous-wave (1 MHz) ultrasound. Four spatially averaged intensities, sham, 0.13, 0.25, and 0.50 W/cm² (180-sec exposure time), yielded, respectively, 4.3, 14.4, 13.0, and 26.7% mortalities, and these mortalities represent a statistically significant difference relative to the sham. The original observation has been confirmed by repeating the experiment (68).

The studies of Murai et al. (69,70) suggest that prenatal continuous-wave (2.3 MHz) ultrasonic irradiation of rats at the ninth day of gestation produced subtle effects that were evident in the offspring. The pregnant rats were irradiated for 5 hours with a commercial Doppler instrument (reported intensity of 20 mW/cm²). The responses to the grasp reflex, the vibrissa placing response, visual placing response, and acceleration righting reflex were statistically significant relative to the shams. No differences were detected for the righting reflex, negative geotaxis, and cliff drop aversion. The authors suggested that prenatal exposure to low-intensity ultrasound may affect brain development. The work of Sikov et al. (71) lends some support to these observations, wherein rats were exposed in utero on the 15th day of gestation to continuous-wave (0.93 MHz) ultrasound for 5 min at intensities that ranged from 10 mW/cm² to 1 W/cm², with neuromuscular development effects, even at the lowest levels.

Some of the lowest ultrasonic intensity effects reported have been somatic effects; that is, the effect is manifested in the exposed specimens themselves. Prasad et al. (72) irradiated HeLa cells in vitro with a pulse-echo diagnostic device for 10 min (reported intensity of 4 mW/cm²) and observed that DNA synthesis was inhibited. Harvey et al. (73), on the other hand, irradiated human fibroblasts in vitro to therapeutic levels of ultrasound (temporal average spatial peak intensity of 0.5 W/cm²) under both continuous-wave and pulsed (2 msec on, 8 msec off) ultrasound for 5 min and caused a marked increase in the rate of protein synthesis, as measured by [³H] proline incorporation. They also suggested that the effect may be inversely related to ultrasonic frequency, at least over the 1–5 MHz range and that treatment of the fibroblast cells with cortisol prior to irradiation eliminates the stimulation. They earlier had reported the stimulation of protein synthesis in human fibroblasts at levels as low as 0.25 W/cm² (74). This work was designed to elucidate the mechanism responsible for ultrasonically induced acceleration in the rate of wound healing (75). The increased rate of tissue regeneration was accompanied by an increasing uptake of [³H]thymidine after irradiating experimentally produced wounds in rabbit ears. At 3.55 MHz, the optimum exposure conditions to stimulate growth was 100 mW/cm² (temporal averaged, 5-min exposure time) administered three times a week. The mechanism was not purely thermal, because varying pulse regimes did not produce the same degree of wound healing with the same temperature changes. A beneficial application of the phenomena has been reported in which pulsed ultrasound (3 MHz, spatial averaged intensity of 1.0 W/cm², 2 msec on, 8 msec off) delivered to the skin surface surrounding varicose ulcers three times weekly stimulated healing (76).

A decrease in the mitotic index of rat liver resulted from continuous-wave (1.9 MHz) exposure for 5 min at 60 mW/cm² (spatial average). Two hours after the ultrasonic irradiation, a partial hepatectomy was performed, and 28–30 hours later, the mitotic index was determined for the regenerating liver tissue, with mean mitotic indices ranging from 20 to 80% of those of shams (77). In an effort to repeat these results, ultrasonic intensities (spatial average) ranging from 60 mW/cm² to 16 W/cm² for 1–5-min exposures (2.2 MHz) were employed (78). These authors were unable to confirm Kremkau and Witcofski's (77) observation under essentially identical experimental conditions.

If the decrease in mitotic activity in the regenerating liver is, in fact, a real effect, there appears to be a contradiction in that ultrasound also stimulates tissue regeneration.

It has been shown that when fresh human platelet-rich plasma is exposed to 1 MHz ultrasound, a time-dependent traumatic insult to the platelet population results (79). The samples were exposed for 5 min at 0.065, 0.33, or 1.6 W/cm². Immediately after irradiation, no changes were detected in the recalcification process, as assayed by the recalcification time. Incubation of the samples at room temperature, however, resulted in a time-dependent decrease in the recalcification time to an asymptotic value of, for example, 9% less than the control for the 0.065 W/cm² exposed group. A follow-up study (80) suggested that a small population of cells had been disrupted, possibly by some form of cavitation-like activity.

MECHANISMS OF INTERACTION

It is appropriate to review briefly the three mechanisms by which effects are induced in biologic material. The mechanisms are termed thermal, mechanical, and cavitation. Operating definitions of the mechanisms, as used in ultrasound bioeffects research, are developed in the discussions below.

Whenever ultrasonic energy is absorbed by any biologic material, heat results. Biologic tissues absorb ultrasound at a relatively high rate. For example, at 1 MHz and 37°C, the absorption in liver tissue is approximately 600 times greater than that for water. Absorption is approximately two times greater in muscle than in liver tissue, while fat absorbs ultrasonic energy at about half of the rate (81).

The heat distribution within tissue depends on the beam geometry and on the absorption coefficient and intensity. The initial rate of rise of temperature is the same for both plane waves and focused beams. However, the heat-diffusing effects make the temporal development of temperature markedly different. The time dependence of temperature in focused and plane-wave beams at 1 MHz is such that the time constant for a focal beam is of the order of 0.1 sec. For plane waves, the time constant can be up to 1 min (28). For the therapeutic use of ultrasound, around 1 MHz, plane continuous-wave techniques are typically utilized. However, reflections and scattering could cause focusing.

The selective heating that occurs at tissue interfaces can be ascribed to a thermal mechanism. The propagating longitudinal ultrasonic wave comes in contact with an interface, and mode conversion results. Thus, part of the longitudinal wave is converted to a shear wave. Shear waves have absorption coefficients orders of magnitude greater than those of longitudinal waves in tissues. Consequently, the wave energy quickly dissipates as heat within the immediate neighborhood of the interface. The effect of mode conversion is much more pronounced

at interfaces between bone and soft tissue than at interfaces between soft tissues. The extent to which selective heating occurs during the application of ultrasound is unknown, as is the role of reflections and scattering of the energy. As a result, during the application of ultrasound, the temporal and spatial distributions of temperature in tissue are unknown.

Since ultrasound is the propagation of mechanical energy, mechanical properties, such as displacement, velocity, acceleration, and peak acoustic pressure, must be associated with the biologic effects of ultrasonic energy. Consider numerical values as calculated from the idealized plane-wave equations, at an ultrasonic frequency of 1 MHz. Within the intensity range from 10 mW/cm² to 100 W/cm², the displacement in tissue ranges from 18 to 1800 Å, the velocity ranges from 12 to 120 cm/sec, and the acceleration ranges from 7400 to 740,000*g*. That such extremely high acceleration forces could possibly shake something loose would not be at all surprising. At the higher intensity levels, finite amplitude effects occur, causing distortion of the wave shape, and acoustic streaming results. Ultrasonically induced shearing stresses associated with acoustic streaming have been implicated as a mechanism that induces biologic damage (82). The stresses cause stretching, twisting, and, finally, rupture of membranous structures. It has been reported that a steady shear stress in the range of 3000–4500 dyn/cm² is sufficient to hemolyze the erythrocyte (83, 84). Other consequences of these stresses have been eddying motions, rotations, and other movements of intracellular bodies within the cell (85). It is, however, difficult to determine the extent of these steady shearing stresses on dynamic biologic processes in vivo, because much of this experimental work has been performed in the low kilohertz frequency range in vitro.

Cavitation is the general term used to describe the growth and subsequent dynamic behavior of gas bubbles in an ultrasonically irradiated medium (86,87). The bubble, once formed, can either remain stable and radially oscillate or continue to grow, become unstable, and collapse. The latter phenomenon, known as "transient cavitation," produces intense hydrodynamic shearing forces within the vicinity of the collapsing bubble that can disrupt the surrounding material. Transient cavitation has been reported to occur in central nervous system tissue at very high intensity levels at 1500 W/cm² or greater (27,29).

Stable cavitation has been investigated in biologic materials (82,83), at lower frequencies than those utilized in the healing arts. In the event that such oscillating bubbles occur adjacent to a cell, both rotational and irrotational forces could be induced, with consequent localized vibration of the cell surface, resulting in a steady stress field toward the oscillating bubble, and particles within the cell would tend to accumulate near the vibrating area. Additionally, the particles would be set into steady rotation and move in circular paths. Motion pictures have demonstrated the existence of these phenomena in isolated cells at frequencies well below those utilized in diagnosis and therapy. An acoustic streaming boundary layer is formed. A minimum exposure time ($\simeq$1 msec) has been suggested as necessary to elicit a biologic effect from stable-type cavitation (88,89).

Because transient cavitation has been reported at much higher ultrasonic intensities and stable cavitation has been studied at ultrasonic frequencies much lower than those used in the healing arts, the question of whether cavitation occurs in biologic tissue under the influence of diagnostic and therapeutic ultrasound is not yet resolved.

Even though three mechanisms of ultrasonic action with biologic material can be described, the current status of dosimetry does not permit adequate assessment of the dose-related biologic consequences of these mechanisms. Ideally, the spatial distribution of the instantaneous values of particle velocity and particle pressure, along with their relative phase, is required to completely characterize the ultrasonic field. The lack of adequate ultrasonic dosimetry is a most serious obstacle to assessment of the risk associated with the exposure of ultrasound. The importance of dose-effect relationships in experimental studies and in humans becomes critical when one attempts to extrapolate, as imprecise as this procedure may be, from experimental data to presumed effects in humans. Such extrapolation will hopefully be possible when there exists a complete understanding of the underlying mechanisms responsible for ultrasonically induced biologic effects, and also when there exists a similar level of understanding regarding differences in *interspecies* response; however, this level of understanding has not yet been reached and probably will not be reached within the foreseeable future.

Other biophysical phenomena that need to be at least considered, especially when aimed at radiation protection, include the possibility of a cumulative effect of ultrasonic biologic actions, the role of synergism, frequency dependence of an effect, the critical organ or tissue concept, and, perhaps, others. Although ultrasonic energy does not have an analogy to ionizing radiation's "quality of radioation," the relative biologic effectiveness represents an important radiology concept and thus should be kept in mind.

There appears to be, at least, a reasonable doubt as to whether cumulative effects occur from exposure to ultrasound. Summation of subparalytic doses of ultrasound, with sufficient time for temperature equilibrium to be reestablished between pulses, produced paralysis in frog hind limbs (23). It has also been demonstrated that under pulsed ultrasonic exposure conditions, by varying only the duty cycle with a constant pulse width, spinal cord hemorrhage occurred only when the total sum of on-time of pulses reached the same value (32).

There have been both positive (90) and negative (91) synergistic findings with ionizing radiation and positive findings with both hypoxia (32) and one chemotherapy drug (92).

Structural lesions in mammalian adult brain were initially thought to be frequency independent over the range from 1 to 9 MHz (27,29,93), but after further examination of the data, a weak oscillatory frequency dependence was shown (94). The explanation for this dependence has been identified as a capsular layer surrounding the brain (95). It is interesting to observe that most organs possess some type of capsular layer, and thus its role in ultrasonic energy transmission should be considered. By compensating for the capsular layer effect, the intensity thereshold responsible for lesion production appears to be frequency independent.

Other types of frequency-dependent examples, all based on intensity as the reported ultrasonic parameter, include greater damage to the liver at lower frequencies over the range 0.5–6 MHz (32), greater change in the electrophoretic mobility of irradiated cells at lower frequencies over the range 0.5–3.2 MHz (96), and greater susceptibility to the production of cataracts at higher frequencies over the range 5–15 MHz (41).

DOSIMETRY

Dosimetry is concerned with the quantitative determination of energy interaction with matter, or, in other words, defining the

quantitative relationship between some physical agent and the biologic effect it produces. In one sense, dosimetry is the determination of a dose, or similar type of physical parameter, that characterizes the physical agent as to its potential or actual interaction with the biologic material of interest.

In the case of ultrasonic dosimetry, the object is to relate magnitudes of specific parameters, such as intensity, acoustic pressure, and particle displacement, or perhaps some parameter yet to be developed, to the likelihood of occurrence of a biologic alteration. To accomplish this objective, it is necessary to quantify the output parameter(s) of the source, to determine the effect of the material on the propagating energy, namely, reflections, refraction, scattering, or absorption, and to relate the first two items to quantitative parameter determination at the site of interest (97).

Thus, dosimetry has two important objectives. The first is to define physical quantities that properly reflect an interaction at some site in biologic material, which may be expressed in units, such as joules per kilogram or per cubic meter. The second is to develop a concept(s) of the quantity that is applicable for radiation protection purposes.

Typically, the term "dose" connotes something that is given or imparted in a quantitative manner. The history of other forms of radiation has documented that defining dose, or doselike concepts, is difficult, especially when the purpose is to include all possible physical and biologic variables. Otherwise, and more commonly, special quantities are developed for the specific case or biologic action under consideration. In ionizing radiation, for example, dose generally refers to the quantity "absorbed dose," which has been specifically defined as the "energy imparted to matter by ionizing radiation per unit mass of irradiated material at the site of interest" (98). But, other dose quantities have been defined for specific purposes, such as "genetically significant dose," which is the gonad dose from medical exposure, or "cumulative dose," "dose equivalent," and "threshold dose" (42). In photobiology, dose sometimes refers to the quantity "dose of ultraviolet radiation," which has been defined as the "energy per unit surface area applied to an object" (99,100). Other quantities that have been used to characterize ultraviolet radiation were chosen to quantify a specific bioeffect. These quantities included minimal erythema dose, minimal perceptible erythema, subvesicular dose, and minimal color dose (101). There is currently much discussion regarding microwave dosimetry. Such terms as "specific absorption rate" (102,103), "absorbed power density" and "specific absorption density" (104), and "energy dose-rate" (103) have all been either used or suggested as a basic quantity to describe absorbed electromagnetic energy. Appropriateness of units has also not been agreed upon (105).

By comparison, the field of ultrasonic dosimetry has not developed to the extent of ionizing radiation dosimetry. The most widely used dosimetric parameter in ultrasonic bioeffect and biophysical studies is intensity in the mixed unit of watts per square centimeter. The principal reason for the use of intensity is, perhaps, convenience, since it is an easily measured parameter. As a dosimetric quantity, "intensity" presents many of the same problems as did the ionizing radiation quantity "exposure," in that it is not a measure of dose, or the like. Yet, the majority of bioeffect and biophysical reports use intensity as the measured physical parameter of the ultrasonic field. An extensive literature documents the actions of ultrasound but, in most cases, phase between these two field parameters at the site(s) of interest (106).

Through both calculations (107) and experimentation (107–110), attempts have been made to determine in utero ultrasonic intensity in both the gravid and nongravid human uterus. A model of the tissue layers between the skin surface and fetal sac has yielded a total attenuation of 23 dB at a frequency of 2.5 MHz (107). One of the earliest in vivo experiments (110) showed that the average loss between the skin and uterus is about 2.6 dB at 2.25 MHz, but more recent studies have shown that this loss is higher, in the range from 9 to 20 dB (107,109) or from 6 to 14 db (108).

There are ultrasonic dosimetric quantities that are noteworthy of comment in that they represent, in concept, the basic approach to dosimetry. The "cataract-producing unit" (CPU) was a quantity defined as the "length of exposure necessary to produce a grossly observable cataract and expressed in units of seconds" (111). The dosimetric concept "damage ability index", with the unit second^{-1}, is a quantity intended to describe the effect of ultrasound on spinal cord hemorrhage (32).

More recently, it has been suggested (112) that a universal dosimetric response to ultrasonic exposure may exist for different tissues, but the response has only been demonstrated, in a limited manner, in mammalian brain tissue. The response is in terms of the "energy absorbed per unit volume" (joules per cubic millimeter) for histologically observable lesions at suprathreshold levels (27,29) as a function of the "delivered intensity." It is shown that at two different ultrasonic frequencies, 3 and 4 MHz, identical constant volume curves result, even though there are two different "threshold levels."

SUMMARY

The assessment of risk associated with exposure to ultrasound has primarily been viewed as a threshold concept. In terms of radiation protection, this risk is of tremendous practical importance. Two threshold-type curves have suggested that there are hazardous exposure conditions and not so hazardous, or safe, exposure conditions (113,114). These curves were generated from selected biologic effect data, much of which was not derived from threshold studies. Such compilations are important in that they provide the opportunity to assess the current information and possible trends. Additionally, they also imply that the concept of risk associated with exposure to ultrasound can be ascribed to a threshold phenomenon and that there exists a "threshold" below which the use of ultrasound can be viewed as safe. There is no scientific basis to believe that this is not the case. But, in fairness, there is no scientific basis to believe this is so.

The American Institute of Ultrasound in Medicine's (AIUM) Bioeffect Committee adopted the following statement, which was also approved by the AIUM Governing Board (see *J Clin Ultrasound* 5:2, 1977):

Statement of Mammalian in vivo Ultrasonic Biological Effects, August 1976

In the low megahertz frequency range there have been (as of this date) no demonstrated significant biological effects in mammalian [illegible] intensities below 100 mW/cm^2. Furthermore,

higher intensities, when the product of intensity* and exposure time** is less than 50 joules/cm².

* Spatial peak, temporal average as measured in a free field in water.

** Total time; this includes off-time as well as on-time for a repeated-pulse regime.

This quotation is a factual account of the known information as of August 1976, relative only to mammalian in vivo ultrasonically induced biologic effects. This statement is not meant to imply specific advice on safe levels that might be universally applied.

In conclusion, it appears that the available information suggests that the risk associated with the clinical use of ultrasound is quite low. However, our knowledge regarding ultrasonic bioeffects and biophysical interaction is rather incomplete at this time. Because of this apparent paradox, it is essential for clinicians to be provided up-to-date information on potential risks, so that they can continue to render an informed benefit-risk judgment.

REFERENCES

1. Kossoff G: Current status of ultrasonic treatment of Meniere's disease, in Reid JM, Sikov MR (eds.), Interaction of Ultrasound and Biological Tissues. Washington, D.C., U.S. Government Printing Office, DHEW Publ. (FDA) 73-8008, 1972, p. 293.
2. Harris JY: Electronic Product Inventory Survey. Bureau of Radiological Health, Report 70-29, Rockville, Md., 1970.
3. Smith SW: Diagnostic equipment and its use. Paper presented at the 8th Annual National Conference on Radiation Control, May 2-7, Springfield, Ill., 1976.
4. Landau E: Are there ultrasonic dangers for the unborn? *Pract Radiol* 1:27, 1973.
5. Holmes JH: Editorial. *J Clin Ultrasound* 2:87, 1974.
6. Roney P: Hospital survey of obstetrical ultrasound. Ad hoc review panel on ultrasound bioeffects and measurements. Bureau of Radiological Health, Apil 9-10, Rockville, Md., 1976.
7. Ziskin MC: Survey of patient exposure to diagnostic ultrasound, in Reid JM, Sikov MR (eds.), Interaction of Ultrasound and Biological Tissues. Washington, D.C., U.S. Government Printing Office, DHEW Publ. (FDA) 73-8008, 1972, p. 205.
8. Wells PNT: What future for ultrasonics? Biomedical ultrasonics. *Ultrasonics* 11:16, 1973.
9. Marx JL: Diagnostic medicine: the coming ultrasonic boom. *Science* 186:247, 1974.
10. Kossoff G: Technical procedures and imaging, in Donald I, Levi S (eds.), Present and Future of Diagnostic Ultrasound. New York, John Wiley & Sons, 1976, p. 1.
11. Carson PL, Oughton TV: Survey of acoustic power and intensities produced by diagnostic ultrasound. Paper presented at the International Conference on Medical Physics, Ottawa, 1976.
12. Carson PL, Hendee WR, Leung S: Performance evaluation and dosimetry in diagnostic ultrasound. *Quart Bull AAPM* December:200, 1973.
13. Hill CR: Acoustic intensity measurements on ultrasonic diagnostic devices, in Bock J, Ossoinig K (eds.), Proceedings of the 1st World Congress on Ultrasonic Diagnostics in Medicine and SIDUO III. Verlag der Wiener Medizinisches Akademy, 1969, p. 21.
14. Filipczyński L: Ultrasonic measurement methods and instrumentation developed in Poland for medical diagnostics, in Filipczyński L (ed.), *Proceedings of the Conference on Biology and Medicine.*
15. Ziskin MC, Romayananda N, Harris K: Ophthamologic effect on ultrasound at diagnostic intensities. *J Clin Ultrasound* 2:119, 1974.
16. Rooney JA: Determination of acoustic power outputs in the microwatt-milliwatt range. *Ultrasound Med Biol* 1.13, 1973.
17. Cady WG: Piezoelectricity. New York, Dover Publications, 1964, vol. 1.
18. Urick RJ: Principles of Underwater Sound for Engineers. New York, McGraw-Hill Book Company, 1967.
19. Van Went JM: Ultrasonic and Ultrashort Waves in Medicine. New York, Elsevier, 1954.
20. Graber P: Biological aspects of ultrasonic waves. *Advan Biol Med Phys* 3:191, 1953.
21. Wood RW, Loomis AL: The physical and biological effects of high-frequency sound-waves of great intensity. *Phil Mag* 4:417, 1927.
22. Harvey EN: Biological aspects of ultrasonic waves, a general survey. *Biol Bull* 59:306, 1930.
23. Fry WJ, Wulff VJ, Tucker D, et al: Physical factors involved in ultrasonically induced changes in living systems: I. Identification of non-temperature effects. *J Acoust Soc Amer* 22:867, 1950.
24. Fry WJ, Tucker D, Fry FJ, et al: Physical factors involved in ultrasonically induced changes in living systems: II. Amplitude duration relations and the effect of hydrostatic pressure for nerve tissue. *J Acoust Soc Amer* 23:364, 1951.
25. Fry WJ: Intense ultrasound in investigations of the central nervous system. *Advan Biol Med Phys* 6:281, 1958.
26. Hueter TF, Ballantine HT Jr, Cotter WC: Production of lesions in the central nervous system with focused ultrasound: a study of dosage factors. *J Acoust Soc Amer* 28:192, 1956.
27. Fry FJ, Kossoff G, Eggleton RG, et al: Threshold ultrasonic dosages for structural changes in the mammalian brain. *J Acoust Soc Amer* 48:1413, 1970.
28. Pond JB: The role of heat in the production of ultrasonic focal lesions. *J Acoust Soc Amer* 47:1607, 1970.
29. Dunn F, Fry FJ: Ultrasonic threshold dosage for the mammalian central nervous system. *IEEE Trans Biomed Eng* BME-18:253, 1971.
30. Robinson TC, Lele PP: An analysis of lesion development in the brain and in plastics by high-intensity focused ultrasound at low-megahertz frequencies. *J Acoust Soc Amer* 51:1333, 1972.
31. Dunn F: Physical mechanisms of the action of intense ultrasound on tissue. *Amer J Phys Med* 37:148, 1958.
32. Taylor KJW, Pond JB: A study of the production of haemorrhagic injury and paraplegia in rat spinal cord by pulsed ultrasound of low megahertz frequencies in the content of the safety for clinical usage. *Brit J Radiol* 45:343, 1972.
33. Bell E: Some changes in liver tissue which survives irradiation with ultrasound, in Kelly E (ed.), Ultrasound in Biology and Medicine. Washington, D.C., American Institute of Biological Sciences, 1957, p. 203.
34. Curtis JC: Action of intense ultrasound on the intact mouse liver, in Kelly E (ed.), Ultrasonic Energy. Urbana, Ill., University of Illinois Press, 1965, p. 85.
35. Frizzell LA: Ultrasonic heating of tissues. Ph.D. Thesis, The University of Rochester, Rochester, N.Y., 1976.
36. Taylor KJW, Pond J: The effects of ultrasound on varying frequencies on rat liver. *J Pathol* 100:287, 1969.
37. Eggleton RC, Kelly E, Fry FJ, et al: Morphology of ultrasonically irradiated skeletal muscle, in Kelly E (ed.), Ultrasonic Energy. Urbana, Ill., University of Illinois Press, 1965, p. 117.
38. Ravitz MJ, Schnitzler RM: Morphological changes induced in the frog semitendinosus muscle fiber by localized ultrasound. *Exp Cell Res* 60:78, 1970.
39. Fallon JT, Stehbens WE, Eggleton RC: Effect of ultrasound on

40. Coleman DJ, Lizzi F, Burt W, et al: Some properties of ultrasonically induced cataracts. *Amer J Ophthalmol* 71:1284, 1971.
41. Sokollu A: Destructive effect of ultrasound on ocular tissue, in Reid JM, Sikov MR (eds.), Interaction of Ultrasound and Biological Tissue. Washington, D.C., U.S. Government Printing Office, DHEW Publ. (FDA) 73-8008, 1972, p. 129.
42. BEIR: The Effects on Populations of Exposure to Low Levels of Ionizing Radiation, Report of the Advisory Committee on the Biological Effects of Ionizing Radiation, National Academy of Science/National Research Council, Washington, D.C., 1972.
43. Macintosh IJC, Davey DA: Chromosome aberrations induced by an ultrasonic fetal pulse detector. *Brit Med J* 4:92, 1970.
44. Thacker J: The possibility of genetic hazard from ultrasonic radiation. *Curr Topics Radiat Res Quart* 8:235, 1973.
45. Macintosh IJC, Davey DA: Relationship between intensity of ultrasound and induction of chromosome aberrations. *Brit J Radiol* 45:320, 1972.
46. Macintosh IJC, Brown RC, Coakley WT: Ultrasound and 'in vitro' chromosome aberrations. *Brit J Radiol* 48:230, 1975.
47. Cataldo FL, Miller MW, Gregory WD: A description of ultrasonically-induced chromosomal anomalies in *Vicia faba*. *Radiat Bot* 13:211, 1973.
48. Gregory WD, Miller MW, Carstensen EL, et al: Non-thermal effects of 2 MHz ultrasound on the growth and cytology of *Vicia faba* roots. *Brit J Radiol* 47:122, 1974.
49. Lyon MF, Simpson GM: An investigation into the possible genetic hazards of ultrasound. *Brit J Radiol* 47:712, 1974.
50. Galperin-Lemaitre H, Kirsch-Volders M, Levi S: Ultrasound and mammalian DNA. *Lancet* Oct 4:662, 1975.
51. Galperin-Lemaitre H, Kirsch-Volders M, Levi S: Fragmentation of purified mammalian DNA molecules by ultrasound below human therapeutic doses. *Humangenetik* 29:61, 1975.
52. Thacker J: Ultrasound and mammalian DNA. *Lancet* Oct 18:770, 1975.
53. Coakley WT, Dunn F: Ultrasound and DNA. *The Lancet* Nov 22:1037, 1975.
54. Fritz-Niggli H, Boni A: Biological experiments on *Drosophila melanogaster* with supersonic vibrations. *Science* 112:120, 1950.
55. Selman GG, Counce SJ: Abnormal embryonic development in *Drosophila* induced by ultrasound treatment. *Nature (London)* 172:503, 1953.
56. Counce SJ, Selman GG: The effects of ultrasonic treatment on embryonic development of *Drosophila melanogaster*. *J Embryol Exp Morphol* 3:121, 1955.
57. Shoji R, Momma E, Shimizu T, et al: An experimental study on the effect of low-intensity ultrasound on developing mouse embryos. *J Fac Sci Hokkaido Univ Ser VI* 18:51, 1971.
58. Shoji R, Momma E, Shimizu T, et al: Experimental studies on the effect of ultrasound on mouse embryos. *Teratology* 6:119, 1972.
59. Lele PP: Ultrasonic teratology in mouse and man, in Kazner E, et al (eds.), Ultrasound in Medicine. Amsterdam, Excerpta Medica, 1975, p. 22.
60. Warwick R, Pond JB, Woodward B, et al: Hazards of diagnostic ultrasonography—a study with mice. *IEEE Trans Sonics Ultrasonics* SU-17:158, 1970.
61. Woodward B, Pond JB, Warwick R: How safe is diagnostic sonar? *Brit J Radiol* 43:719, 1970.
62. McClain RM, Hoar RM, Saltzman MB: Teratologic study of rats exposed to ultrasound. *Amer J Obstet Gynecol* 114:39, 1972.
63. O'Brien WD Jr: Ultrasonically induced fetal weight reduction in mice, in White D, Barnes R (eds.), Ultrasound in Medicine. New York, Plenum Press, 1976, p. 531.
64. Fry FJ, Dunn F, Brady J, et al: Ultrasonic toxicity study of the mouse reproductive system and the pregnant uterus, in White D, Barnes R (eds.), Ultrasound in Medicine. New York, Plenum Press, 1976, p. 533.
65. Sikov MR, Hildebrand BP: Effects of ultrasound on the prenatal development of the rat. Part I. 3.2 MHz Continuous wave at nine days of gestation. *J Clin Ultrasound* 4:357, 1976.
66. Stratmeyer ME: What do we know about the bioeffects? Paper presented at the 8th Annual National Conference on Radiation Control, May 2–7, Springfield, Ill., 1976.
67. Curto KA: Early postpartum mortality following ultrasonic radiation, in White D, Barnes R (eds.), Ultrasound in Medicine. New York, Plenum Press, 1976, p. 535.
68. Curto KA: Personal communication, 1976.
69. Murai N, Hoski K, Nakamura J: Effect of diagnostic ultrasound irradiated during fetal stage on development of orienting behavior and reflex ontogeny in rats. *Tohoku J Exp Med* 116:17, 1975.
70. Murai N, Hoshi K, Kang C, et al: Effects of diagnostic ultrasound irradiated during foetal stage on emotional and cognitive behavior in rats. *Tohoku J Exp Med* 117:225, 1975.
71. Sikov MR, Hildenbrand BP, Stearns JD: Postnatal sequelae of ultrasound exposed at fifteen days of gestation in the rat. Paper presented at the 1st World Federation for Ultrasound in Medicine and Biology, August 3–7, San Francisco, 1976.
72. Prasad N, Prasad R, Bushong SC, et al: Ultrasound and mammalian DNA. *Lancet* May 29:1181, 1976.
73. Harvey W, Dyson M, Pond JB, et al: The '*in vitro*' stimulation of protein synthesis in human fibroblasts by therapeutic levels of ultrasound, in *Proceedings of the 2nd European Congress on Ultrasonics in Medicine*. Amsterdam, Excerpta Medica, 1975, p. 10.
74. Harvey W, Dyson M, Pond JB, et al: Metabolic changes induced by ultrasound in fibroblasts in vitro. *Ultrasound Med Biol* 1:473, 1975.
75. Dyson M, Pond JB, Joseph J, et al: The stimulation of tissue regeneration by means of ultrasound. *Clin Sci* 35:273, 1968.
76. Dyson M, Franks C, Suckling J: Stimulation of healing of various ulcers by ultrasound. *Ultrasonics* 14:232, 1976.
77. Kremkau FW, Witcofski RL: Mitotic reduction in rat liver exposed to ultrasound. *J Clin Ultrasound* 2:123, 1974.
78. Miller MW, Kaufman GE, Cataldo FL, et al: Absence of mitotic reduction in regenerating rat liver exposed to ultrasound. *J Clin Ultrasound* 4:169, 1976.
79. Williams AR, O'Brien WD Jr, Coller BS: Exposure to ultrasound decreases the recalcification time of platelet rich plasma. *Ultrasound Med Biol* 2:113, 1976.
80. Williams AR, Sykes SM, O'Brien WD Jr: Ultrasonic exposure modifies platelet morphology and functions *in vitro*. *Ultrasound Med Biol* in press, 1977.
81. Goldman DE, Hueter TF: Tabular data of the velocity and absorption of high-frequency sound in mammalian tissue. *J Acoust Soc Amer* 28:35, 1956.
82. Rooney JA: Biological effects from sonic shearing—a review, in Reid JM, Sikov MR (eds.), Interaction of Ultrasound and Biological Tissues. Washington, D.C., U.S. Government Printing Office, DHEW Publ. (FDA) 73-8008, 1972, p. 93.
83. Rooney JA: Hemolysis near an ultrasonically pulsating gas bubble. *Science* 169:868, 1970.
84. Williams AR, Hughes DE, Nyborg WL: Hemolysis near a transversely oscillating wire. *Science* 169:871, 1970.
85. Nyborg WL: Microsonation and physical mechanisms for biological effects of ultrasound, in Reid JM, Sikov MR (eds.), Interaction of Ultrasound and Biological Tissues. Washington, D.C., U.S. Government Printing Office, DHEW Publ. (FDA) 73-8008, 1972, p. 99.
86. Flynn HG: Cavitation dynamics. I. A mathematical formulation. *J Acoust Soc Amer* 57:1379, 1975.

87. Flynn GH: Cavitation dynamics. II. Free pulsations and models for cavitation bubbles. *J Acoust Soc Amer* 58:1160, 1975.

88. Clarke PR, Hill CR: Physical and chemical aspects of ultrasonic disruption of cells. *J Acoust Soc Amer* 47:649, 1969.

89. Child SZ, Carstensen EL, Miller MW: Growth of pea roots exposed to pulsed ultrasound. *J Acoust Soc Amer* 58:1109, 1975.

90. Woeber K: The effect of ultrasound on the treatment of cancer, in Kelly E (ed.), Ultrasonic Energy. Urbana, Ill., University of Illinois Press, 1965, p. 137.

91. Clarke PR, Hill CR, Adams K: Synergism between ultrasound and x-rays in tumor therapy. *Brit J Radiol* 43:97, 1970.

92. Kremkau FW, Kaufman JS, Walker MM, et al: Ultrasonic enhancement of nitrogen mustard cytotoxicity in mouse leukemia. *Cancer* 37:1643, 1976.

93. Lerner RM, Carstensen EL, Dunn F: Frequency dependence of thresholds for ultrasonic production of thermal lesions in tissues. *J Acoust Soc Amer* 54:504, 1973.

94. Dunn F, Lohnes JE, Fry FJ: Frequency dependence of threshold ultrasonic dosages for irreversible structural changes in mammalian brain. *J Acoust Soc Amer* 58:512, 1975.

95. Johnston RL, Dunn F: Influence of subarachnoid structures on transmeninges ultrasonic propagation. *J Acoust Soc Amer* 60:1225, 1976.

96. Taylor KJW, Newman DL: Effects of electrophoretic mobility of Ehrlich cells exposed to ultrasound of variable parameters. *Phys Med Biol* 17:270, 1972.

97. O'Brien WD Jr: Ultrasonic dosimetry, in Fry FJ (ed.), Ultrasound: Its Application in Medicine and Biology. Amsterdam, Elsevier, 1977, in press.

98. ICRU: Radiation quantities and units. International Commission on Radiation Units and Measurements. Report 19. Washington, D.C., 1971.

99. ICP: Proposals concerning definitions and units for the biological and medical use of UV radiation, in *Proceedings of the 1st International Photobiological Congress*, Amsterdam, 1954, p. 440.

100. Rupert CS: Dosimetric concepts in photobiology. *Photochem Photobiol* 23:203, 1974.

101. Michaelson SM: Human exposure to nonionizing radiant energy—potential hazards and safety standards. *Proc IEEE* 60:389, 1972.

102. Johnson CC: Recommendations for specifying EM wave irradiation conditions in bioeffects research. *J Microwave Power* 10:249, 1975.

103. Susskind C: Correspondence on D. R. Justesen's "Prescriptive grammar for the radiobiology on nonionizing radiation." *J Microwave Power* 10:357, 1975.

104. Guy AW: Correspondence on D. R. Justesen's "Prescriptive grammar for the radiobiology of nonionizing radiation." *J Microwave Power* 10:358, 1975.

105. Justesen DR: Toward a prescriptive grammar for the radiobiology of nonionizing radiation: quantities, definitions, and units of absorbed electromagnetic energy—an essay. *J Microwave Power* 10:343, 1975.

106. O'Brien WD Jr, Shore ML, Fred RK, et al: On the assessment of risk to ultrasound, in de Klerk J (ed.), 1972 Ultrasonics Symposium Proceedings. New York, IEEE Cat. 72 CHO 708-8SU, 1972, p. 486.

107. Hall AJ: Ultrasonic dosimetry in obstetrics. Paper presented at the 2nd World Congress on Ultrasonics in Medicine, Rotterdam, June 4–8, 1973.

108. Etienne J, Filipczyński L, Firek A, et al: Intensity determination of ultrasonic focused beams used in ultrasonography in the case of gravid uterus. *Ultrasound Med Biol* 2:119, 1976.

109. Hall AJ, Robinson HP: Transmitted ultrasonic energy levels in the nongravid uterus. Paper presented at the Colloquium on Ultrasound Bioeffects and Dosimetry, London, July 22–24, 1974.

110. Bang J: The intensity of ultrasound in the uterus during examination for diagnostic purposes. *Acta Pathol Microbiol Scand* 80A:341, 1972.

111. Purnell EW, Sokollu A, Torchia R, et al: Focal chorioretinitis produced by ultrasound. *Invest Ophthalmol* 3:657, 1964.

112. Johnson RL, Dunn F: Ultrasonic absorbed dose, dose rate, and produced lesion volume. *Ultrasonics* 14:153, 1976.

113. Wells PNT: The possibility of harmful biological effects in ultrasonic diagnosis, in Reneman RS (ed.), Cardiovascular Applications of Ultrasound. New York, Elsevier, 1974, p. 1.

114. Ulrich WD: Ultrasound dosage for nontherapeutic use on human beings—extrapolations from a literature survey. *IEEE Trans Biomed Eng* BME-21:48, 1974.