BIOLOGICAL EFFECTS OF ULTRASOUND

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ABSTRACT

The use of diagnostic ultrasound in the clinical practice of medicine is generally recognized as not representing any hazard. Whereas agents such as drugs are generally not considered safe until proven so, radiation, such as ultrasound, is generally considered safe until proven otherwise. The studies necessary to assess the risk associated with human exposure to ultrasound have not been done. It is, however, important to recognize that ultrasound at sufficient energy levels is capable of totally destroying biological tissues. Thus the question of risk becomes (1) what biological systems are most sensitive to ultrasound and (2) what exposure levels impose a significant risk on those systems? The recent ultrasonic biological effect data has demonstrated a trend to lower exposure levels as the biological endpoint has become more sensitive. This review will examine the risk from ultrasound by discussing the extent to which it is used in clinical practice, presenting specific biological effects for which the cause may not be known, detailing those mechanism which are known to produce biological effects, introducing some thought relative to ultrasonic dosimetry and summarizing by briefly examining the situation of cumulative and synergistic effects.

INTRODUCTION

The use of ultrasound in the clinical practice of medicine is generally recognized as not representing any hazard. The studies, however, necessary to support a reliable assessment of the risks associated with human exposure to ultrasound have not been done (O'Brien et al, 1972, O'Brien, 1978a). In 1974, the Committee on Radiology of the American Academy of Pediatrics stated that ". . . There is no radiation hazard. . ." in the closing of the Academy's statement concerning the use of ultrasonography in children (Grossman et al, 1974). Since ultrasound is not ionizing radiation the statement is technically correct. Scheidt (1975), a pediatrician in the Food and Drug Administration, questioned the impression which could be obtained from such a statement and suggested that it is too early to judge the risk from diagnostic ultrasound and in particular with respect to infant and fetal exposure. Further, Scheidt and Lunden (1977) summarized nine studies which dealt with possible effects of ultrasonic energy exposure to the human fetus and concluded that little information exist about such effects. That is, their analysis of these studies produced insufficient information to document whether or not a positive finding was possible.

With our current understanding of ultrasonically induced biological effects, it is scientifically next to impossible to argue against the statements such as "diagnostic ultrasound is not harmful to the conceptus." Experimental studies will not prove diagnostic ultrasound, as it is employed in clinical medicine, safe. Rather, what these studies will provide , if properly planned and executed, are data which will aid in the overall assessment of risk associated with exposure to ultrasound. Safe implies absence of an effect, not involving risk, or the like. It simply is not possible to prove that ultrasound, or for that matter any agent, does not produce any effect at the levels employed diagnostically. Also, the actual use of the word safe is vaque since it almost never refers to the absence of an effect. A more useful and workable approach is to examine the " risk " associated with ultrasonic exposure.

It is important to recognize that ultrasound, at sufficient levels, is capable of destroying biological material. For, example, ultrasound has been used for many years as a surgical tool for the treatment of Meniere's disease (Kossoff, 1972). Therefore, it is useful to approach the question of assessing the risk from ultrasound in a two fold manner, (1) what biological systems are most sensitive to ultrasound and (2) what exposure levels impose a significant risk on these systems. But even this approach has its difficulties in that how does one determine significant risk. What is usually meant by significant risk is the risk which is greater than some upper limit of acceptability. One usually then involves the benefit vs risk concept which, in principal, is quite understandable but, in practice, poses severe implementation problems. As a simplistic start, let it be assumed that p is the probability of occurrence to an individual of a specific biological effect. Thus, the "risk to an individual" from a diagnostic ultrasound examination is p whereas the "risk to society" is p times the population which has received a diagnostic ultrasound examination. This review will not develop numerical values for p but rather discuss in some detail the known biological effects. The mechanisms by which ultrasound interacts with biological materials will also

be discussed but, at our present state of understanding, the responsible mechanisms for many of the identified ultrasonically-induced biological effects are unknown. First, however, the extent to which ultrasound is employed in clinical medicine will be reviewed.

EXTENT OF USE

While no statistically based survey has been conducted to document the extent to which ultrasound has been used over the past decade, a number of indicators do, however, support the view that it is increasing in use and that a large fraction of the human population will eventually be exposed, especially *in utero*. A market analysis in 1969 predicted that the dollar value of ultrasonics would increase 300 percent during the period between 1968 and 1973 (Harris, 1970). This represented an annual increase of 75 percent. The marketing survey company of Frost and Sullivan (1975) indicated that the annual rate of growth for all the medical ultrasound industry was 25% for the year 1974. Later, based upon discussions with clinical manufacturers, the Food and Drug Administration's Bureau of Radiological Health predicted that in 1976 the industry was growing at an annual rate of 50 percent and that annual dollar sales was around $40 million (Smith, 1976).

In 1971, the Bureau of Radiological Health surveyed 301 out of 6306 short-term general hospitals in the United States and found that 12 percent of the hospitals used diagnostic ultrasound (Landau, 1973). In an editorial in the *Journal of Clinical Ultrasound*, the editor (Holmes 1974) doubted the 12 percent figure since, he believed, that Doppler ultrasound was used by over 50 percent of the obstetricians in the United States. In 1976 the same government agency reported on the conduct of another hospital survey which showed that 35 percent of the surveyed hospitals used diagnostic ultrasound (Roney, 1976). While there are questions which could 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 percent.

Further, Roney and Albrecht (1977) reported on a survey of ultrasonic use for the diagnosis of obstetric conditions in 1974. The study, conducted by the Bureau of Radiological Health, Food and Drug Administration, showed an estimated 16 percent of the obstetric services in the U.S. used diagnostic ultrasound and that about one-third of all U.S. births for 1974 were delivered in these hospitals. Additionally, it was estimated that 470,000 pregnant women were exposed to diagnostic ultrasound in 1974 with about 35-40% of these women being examined more than once.

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 and, in part, showed that between 1963 and 1971, there was an average annual increase in use of clinical ultrasound of approximately 10 percent (Ziskin, 1972). And, in the United Kingdom, it has been estimated in the early 1970's that the number of ultrasonic diagnostic examinations was doubling every three years (Wells, 1973), thus representing an annual 26 percent increase.

The United States National Science Foundation, 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 percent (an increase from the 1969 market survey prediction), representing an annual increase of 73 percent. Another conclusion from the NSF survey team was an estimate that the sales of clinical ultrasonic devices will match those of x-ray devices by 1983 (Marx, 1974). The diagnostic x-ray market in 1976 was around 700 million annually (Electronics, January 8, 1976) and for the diagnostic ultrasonic sales to approach this level would require a 50 per cent annual market increase starting from 40 million, assuming, of course, the x-ray sales remained constant. In 1978, an increase in sales of at least 25% was predicted for ultrasonic diagnostic equipment from 1978 to 1981, thus suggesting a steady increase in ultra sound equipment sales (Electronics, p. 137, 1978).

Therefore, if one were to assume that at least one-half of all pregnant women are currently being examined with ultrasound and, additionally, assume that the use is increasing at an annual rate of 10 percent, then within the next decade, virtually every fetus could be exposed to ultrasound. In support of the extrapolation, Stratmeyer (1977) , of the Food and Drug Administration, has suggested that ". . . potentially all of the children born after the early 1980's will be exposed to ultrasound."

Use and trends of use information for therapeutic ultrasound are as difficult to obtain as with diagnostic ultrasound, even though it has been recognized as a potential hazard since the initial applications in the 1930's (DeForest, 1952). It is suggested that there is presently little change in the extent of use although such information is quite limited. Three surveys have

been conducted which may provide some guidance, but it is difficult to extract precise information since the surveys were limited in design (Remark, 1971; Stewart et al., 1973a; 1973b). The 1970 Pinellas County, Florida survey (Remark, 1971) indicated approximately 45,000 ultrasonic treatments per month to 6000 patients in a county population of 500,000. Assuming this is typical of the United States, and there is no supportive information for this assumption, in a population of 200 million, this would represent on an annual basis approximately 216 million treatments to 2.4 million patients.

The output power and intensity parameters from commerical pulse-echo and continuous wave ultrasonic diagnostic equipment have been measured and reported (Hill, 1969; Filipczynski, 1970; Carson et al., 1973; Rooney, 1973; Ziskin et al., 1974; Carson and Oughton, 1976; Carson et al., 1978). The measurements of pulse devices show that the spatial average ultrasonic power ranges from 60 microwatts to 21 mW, the spatial average, temporal average intensity ranges from 0.1 to 300 mW/cm**2, spatial average, temporal peak intensity ranges from 1 to 95 W/cm**2 and spatial peak, temporal peak intensity ranges from 2 to 1700 W/cm**2. For continuous wave devices, the spatial average ultrasonic power ranges from 1 to 37 mW and the spatial average intensity ranges from 0.2 to 375 mW/cm**2.

ULTRASONIC BIOEFFECTS

Diagnostic medicine has generated a large volume of clinical experience with ultrasound and, during this time, it is highly encouraging that no deleterious effects have been reported. However, one only needs to examine the history of ionizing radiation to realize that many effects, subtle as they may be, would not have been discovered without comprehensive experimental studies. Thus it must be quite apparent that comprehensive toxicity studies are an essential part of the determination of permissible dosage levels for the various medical applications of ultrasound. This type of information buttresses the clinical experience and aids the physician in decision making concerning a specific course of action.

Some thirty-five years after the Curies discovered piezoelectricity (Cady, 1946), the French scientist Paul Langevin developed the first use of ultrasonic energy wherein underwater acoustic echoes were bounced off of submerged objects (Urich, 1967; Van Went, 1954). During the course of this work, the first reported observation was made that ultrasonic energy had a lethal effect upon small aquatic animals (Graber, 1953). The first

extensive investigation of the phenomena observed by Langevin was conducted by Wood and Loomos (1927). Although the ultrasonic levels were not specified, they did confirm Langevin's observation that ultrasonic energy could kill small fishes and frogs by one to two minute 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 papers, Harvey (1930) examined the physical, chemical and biological effect of ultrasound wherein effects on cells, isolated cells, bacteria and tissues were summarized with a view towards 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 pioneering studies of W. J. Fry and colleagues (1950, 1951), 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 (1958) 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 (Hueter et al., 1956; Fry et al., 1970; Pond, 1970; Dunn and Fry, 1971; Robinson and Lele, 1972); adult rat and neonatal mouse spinal cord (Fry, 1958; Dunn, 1958; Taylor and Pond, 1972); adult mouse, rat and rabbit liver (Bell, 1957; Curtis, 1965; Frizzell, 1976; Taylor and Pond, 1969); adult frog muscle (Eggleton et al., 1965; Ravitz and Schnitzler, 1970); rabbit blood vessel (Fallon et al., 1972); rabbit kidney and testicle (Frizzell, 1976), and rabbit ocular tissue (Coleman et al., 1971; Sokollu, 1972). In most cases, these studies represented rather gross damage because the ultrasonic energy induced either immediate functional and/or structural alteration. While not to minimize the importance of such studies to the elucidation of fundamental interaction processes, they do represent 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.

An extensive literature documents the gross effects or actions of ultrasonic energy on biological materials. One of the earliest reviews (Grabar, 1953) examined the

action of ultrasound on macromolecules, microorganisms, cells, organs and tissues. Two University of Illinois conferences (Kelly, 1957; Kelly, 1965) dealt with many aspects of both biological effects and applications of ultrasound. El´piner (1964) has reviewed the literature on the physical, chemical and biological actions of ultrasound. Perhaps the most recent, yet dated, comprehensive review of ultrasonic bioeffect work was conducted during the Workshop on the Interaction of Ultrasound and Biological Tissues (Reid and Sikov, 1972). Later, the National Science Foundation funded a project to develop a priority list of research and development objectives in medical ultrasound (Busser, 1975). The number one priority focused on the need to detemine the risk associated with human exposure to ultrasound.

In 1970, Macintosh and Davey 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 biological effects which raised the distinct possiblity of risk to patients and fetuses. Following this report, a number of researchers attempted to determine the validity of the reported effect. Thacker (1973) published one of the most comprehensive reviews of the history of chromosome damage induced by ultrasonic irradiation and also discussed the negative reports which countered the findings of Macintosh and Davey (1970). During this period, a second report by Macintosh and Davey (1972) not only verified the original findings but also extended the study to show a "threshold intensity" of 8.2 mW/cm**2 for a one hour exposure below which no chromosome damage occurred. A follow-up study showed that the high incidence of aberrations previously found could not be reproduced (Macintosh et al., 1975). Additionally, it was suggested that the earlier positive findings may have been due to some, yet unidentified, artifact unrelated to the ultrasonic irradiation.

Although there have been no verified reports of "classical" chromosome aberrations at diagnostic or therapeutic intensity levels of ultrasound, chromosome anomalies of another type have been observed (Cataldo et al., 1973; Gregory et al., 1974) wherein chromosome damage was reported in the form of bridged prophases and metaphases and agglomerated mitotics. This work with Vicia faba roots (2 MHz, one minute exposure at 8 W/cm**2) suggested that these particular types of aberrations may not have been observed because the standard technique for scoring metaphases for chromosome

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, viz., one should not be confined to assessing the results of an experiment based solely upon knowledge and experience of how another agent interacts with the biological specimen.

It is relatively important to assess whether or not ultrasound has a mutagenetic effect. In Thacker's (1973) extensive review, it was concluded that a genetic hazard was unlikely from diagnostic ultrasound. While this statement is welcomed, it was based upon an extrapolation of the data which even the author judged as equivocal. Further, upon reviewing the mutagenesis data, Thacker suggested there is some evidence that ultrasound could induce mutagenesis although the increase in mutation frquencies was small when compared with ionizing radiation.

Lyons and Simpson (1974) did not detect any evidence of an ultrasonically-induced genetic effect in their experimental study with mice. Testes or ovaries were irradiated for 15 minutes, at 1.5 MHz, under three exposure conditions, one continuous wave (1.6 W/cm**2) and two pulsed (Iav = 1.6 W/cm**2, duty cycle = 25%, pulse width = 1 ms and 0.9 W/cm**2 2%, 30 microseconds). 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 which the authors cited was the small number of animals.

Yet questions continue to be raised in the literature as to whether or not low level ultrasound does indeed affect genetic material. Galperin-Lemaitre and colleagues (Galperin-Lemaitre et al., 1975; 1976) reported the degradation of purified DNA at intensities as low as 200 mW/cm**2. While Thacker (1975) and Coakley and Dunn (1975) 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.

One of the earliest studies to examine ultrasonically induced developmental abnormalities used the fruit fly Drosophila melanogaster. The specimens were irradiated with continuous wave (800 kHz) ultrasound under various exposure conditions (spatial average intensity reported to be 0.7 to 4.0 W/cm**2 with exposure times 30 sec to 25 min) at different stages of

development. The egg, larval and prepupal stages were more susceptable to damage than the later stages of development. The abnormalities reported were to be similar to those induced by ionizing radiation but unlike those caused by simply heat (Fritz-Niggli and Boni, 1950).

Additional studies with the fruit fly by another laboratory, showed that a large fraction of the embryos developed abnormally following exposure to continuous wave (1 MHz) ultrasound at the syncytial blastoderm stage, that is, two hours after laying the eggs (Selman and Counce, 1953). The spatial average intensity was reported to be between 0.3 and 0.5 W/cm**2 with an exposure time of 30 sec. Abnormalities ranged from abnormal segmentation, absence of gonads and other organs, to absence of organogenesis and delayed mortality. An extension of this study to various exposure conditions (spatial average intensities of 0.05, 0.1, 0.23, 0.5, 1.2 W/cm**2 for 30 sec exposures) and developmental stages of exposure (15, 60 and 120 minutes later laying) led the authors to suggest that ultrasonic exposures less than 100 mW/cm**2 at 30 sec had an insignificant effect upon the normal development of Drosophila (Counce and Selman, 1955). The number of abnormalities increased as the ultrasonic intensity was increased beyond 100 mW/cm**2, which is suggestive of a dose-effect response. In these Drosophila studies, the dosimetry must be suspect since few details were provided. However, these studies do represent a quest to obtain such data some twenty years ago and to that end suggests the possibility that prenatal exposure to ultrasonic energy may induce abnormal growth and development. Also, it should be noted that the current ionizing radiation protection guidelines are, to a large extent, based upon Drosophila studies (BEIR, 1972).

One study is widely quoted as evidence that the clinical use of ultrasound is safe. Fertilized eggs from frogs and perch were exposed with a commerical ultrasonic flow device. However, no ultrasonic intensity parameters were reported. The tadpoles and perch were reported to have developed normally (Andrew, 1964). The physiological significance of these studies cannot be ascertained. In another widely quoted study but which again only provided few experimental and dosimetric details (apparently never published) Smyth (1966) concluded that pulsed ultrasound from a specific prototype diagnostic pulse-echo device did not produce congenital anomalies in mice. Only the testes of the male and the right ovary of the female were irradiated daily, for 10 minutes, during the five day premating period and during the ten day mating period. Furthermore, the right ovary continued to be irradiated

during gestation through the seventeenth day. During the gestational irradiation, the intent was to irradiate only the right ovary although some of the fetuses may have been exposed. The ultrasonic intensity was reported to be 10 mW/cm**2, presumably temporal average, spatial average.

The research group at Guy's Hospital in London (Woodward et al., 1970; Warwick et al., 1970) reported on a study in which pregnant mice were exposed to pulsed ultrasound under a wide variety of exposure conditions. For example, the temporal peak, spatial peak intensity ranged from 20 to 490 W/cm**2; the temporal average spatial peak intensity ranged for 0.75 to 27 W/cm**2; the pulse width and duty cycle ranged from 10 microseconds to 10 milliseconds and 0.2 to 20 percent, respectively; and the exposure time was typically 300 seconds. A total of 297 mice were irradiated with an additional 179 controls. Of the 13 exposure conditions, 5 groups involved irradiating the mouse at the eighth day of gestation (80 mice), 3 groups at the fifth day of gestation (41 mice) and one group at both gestational ages (42 mice), which accounts for 73 percent of the 223 mice irradiated. The remaining three groups included exposures at the following ages of gestation: (1) 8 and 9 days, (2) 1, 7, 10 and 12 days and (3) 3, 5, 6, and 8 days. The major criticism of this work is the inability to directly associate group numbers with exposure parameters, preventing any dose-effect analysis other than that offered by the authors. They concluded that there was no significant effect on litter size, resorption rate or abnormality rate. However, examination of the litter size data suggests that it could be divided into two groups, one with an average litter size of 8 or less and the other with an average letter size of 10 or more. But the information provided does not permit the association of these two litter size groupings with exposure conditions. Also, a dose response is suggested by examining the abnormality rate in the two groups with the highest temporal average, spatial peak intensity levels (27.0 and 10.71 W/cm**2). These latter data show a statistically significant effect on fetal abnormalities. Unfortunately, this aspect was not pursued in the articles.

Perhaps one of the more controversial studies on the effects from prenatal ultrasonic exposure of pregnant mice has been reported by a group from Hokkaido University (Shoji et al., 1971; Shoji et al., 1972; Shimizu et al., 1973). Pregnant DHS mice were irradiated on the ninth day of gestation with continuous wave (2.25 MHz) ultrasound for a period of five hours at a reported intensity (presumably spatial average) of 40 mW/cm**2 . The signal source was a commercial fetal

Doppler device and no dosimetric details were provided. One of the invesitgators was not able to provide such information (Shimizu, 1973). Eight types of fetal abnormalities were observed in both the irradiated and control groups. These differences were not significant. However, the rate of fetal death was significant in the irradiated group (Shoji et al., 1971). Later, they reported a statistically significant increase in fetal abnormalities in a different mouse strain, A/He. In addition, they reported a significant effect of fetal mortality (Shoji et al., 1972; Shimizu et al., 1973). In both of these studies, one of the investigators indicated that the mice were given an initial dose of sodium nembutal which was effective for approximately one hour, after which the animals struggled (Shimizu, 1973). Also, Lele (1975) has suggested that these observations may be a result of prolonged induction of a moderate temperature rise. Thermocouples were implanted in mouse fetuses in utero and the time course of temperature was recorded. These measurements agreed with calculations and apparently explained the fetal abnormalities to be caused by hyperthermia. Nyborg (1979) observed that Lele's temperature measurements were similar to that predicted by theoretical plot of temperature at the center of an absorbing sphere.

Mannor et al. (1972) examined the effect of in utero continuous wave (2.28 MHz) ultrasonic irradiation of pregnant mice during gestational ages from 8 to 16 days. The data were pooled into eight exposure groups including spatial average intensities of 0.164, 0.272, 0.490 and 1.050 W/cm**2 and exposure times of 5 and 10 min. Each exposure group consisted of 10 pregnant mice with the possibility of each exposure group containing nine gestational ages at the time of irradiation. Some maternal death was attributed to heat at the higher exposure conditions. All fetuses examined in surviving females were reported to be normal.

McClain et al. (1972) studied the effects of multiple prenatal exposure (gestation ages of 8, 9 and 10 days or 11, 12 and 13 days) from a commercial prototype of a fetal Doppler device (continuous wave, 2.5 MHz, spatial average intensity of 9.1 mW/cm**2) in rats. Examination of the fetuses of the 20th day of gestation 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 percent of the irradiated and 36 percent of the controls. The authors suggest that there was no biological significance since the effect was predominantly in the 30 min exposure group, and not the 120 min group.

The more recent reviews (Dunn, 1979; Lele, 1979; Nyborg, 1979; Nyborg, 1978; O'Brien, 1978a, Stratmeyer, 1977 ; Frost and Stratmeyer, 1977) of the ultrasonic bioeffect literature appear to suggest that as more sensitive biological endpoints are studied, the ultrasonic exposure parameters required to produce measurable effects appear to decrease. This is further supported by the five articles which were published during 1979 which describe ultrasonically induced biological effects from ultrasonic diagnostic equipment, that is, ultrasonic energy from a commercial diagnostic device altered the biological system. One, of course, must be cautious in assessing the significance of single observations of such biological alterations--it could be beneficial or innocuous or it may represent some degree of risk--but one also must be sensitive to the fact that some or all of these alterations are occurring at diagnostic levels from commercial equipment. Such alterations include immunosuppressive effect from in vivo exposure of mouse spleenic region (Anderson and Barrett, 1979); altered DNA and growth patterns from in vitro exposure of animal cells (Liebeskind et al., 1979a); sister chromatid exchanges, a chromosome alteration, from in vitro exposure of freshly isolated human lymphocytes and cultured lymphoblasts (Liebeskind et al., 1979b); decreased ability of attachment to plastic Petri dish from in vitro exposure of cultured human cells (Siegel et al., 1979); and platelet aggragation from in vitro exposure of human platelets near microscopic air bubbles (Miller et al., 1979). These observations are not meant to imply that ultrasonic energy will eventually be shown to represent a substantial risk. Rather, they are observations, and whether or not ultrasound will be shown to represent a significant risk will depend upon the types of effects observed, the levels at which these effects occur, their dose-effect responses and the assessment of the mechanism from dose-effect data. 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 (BEIR, 1972).

Over the last few years, and especially within the past year or so, experimental observations have been made which suggest that subtle effects are occurring to rodent embryos and fetuses when exposed to ultrasound in utero. Stratmeyer (1977) reviewed ultrasonic toxicity studies from a public health point of view (including developmental, hematological and vascular, neurological and behavioral, immune system response and genetic effects) and observed that numerous studies have

reported effects at or near diagnostic ultrasonic intensity levels, that is less than 100 mW/cm**2.

Perhaps the first statistically based study which suggested that in utero ultrasonic irradiation affected prenatal growth and development was reported by O'Brien (1976). Time-mated CFI mice (an outbred non-Swiss albino) were irradiated to continuous wave (1 MHz) ultrasound on the eighth day of gestation. The fetuses were removed by laparotomy on the eighteenth day of gestation and individually weighed. The data showed a statistically significant weight reduction from about 6 to 18 percent, depending upon the exposure conditions. There were seven exposure groups, including a sham. Two hundred and seventy two litters (2866 fetuses) were exposed under conditions ranging from 0.5 to 5.5 W/cm**2 and 10 to 300 seconds. The non-parametric Kruskal-Wallis statistical test showed that the average fetal weight varied significantly with exposure condition. A dose-effect dependence of exposure condition versus average fetal weight was examined by defining the dose parameter as the product of the exposure intensity squared (I**2) and exposure time (t), viz., I**2t. This dose parameter is consistent with that which described the production by ultrasound of irreversible lesions in mammalian brain (Johnston and Dunn, 1976b).

The observation that in utero ultrasonic exposure of mice causes weight reduction compared to the sham has been reported by two other groups usng two different strains of mice, viz., LAF/J (Fry et al., 1976, 1977, 1978) and Swiss-Webster (Stolzenberg et al., 1978a, 1978b). In the former study, pulsed ultrasonic exposure conditions were employed where in the spatial peak, temporal peak intensity was as high as 2936 W/cm**2; the spatial average, temporal average intensity was approximately 10 W/cm**2; and the exposure time was 10 seconds with a nominal ultrasonic frequency of 1 MHz. In the latter study, continuous wave (2 MHz) ultrasound (1 W/cm**2 for 140 to 200 seconds) produced a 9 to 10 percent weight loss in exposed mice relative to the sham.

A preliminary study suggested that the fetal weight reduction is sustained post weaning (Stratmeyer, 1976). Time-mated CFl mice were irradiated at the thirteenth day of gestation with continuous wave (1 MHz) ultrasound. At the 55th day post conception (approximately two weeks post weaning) , a total of 162 pups from 21 litters were examined. There were three exposure groups (sham, 0.25 W/cm**2, and 0.80 W/cm**2 for 120 seconds) and the data yielded statistically significant weight reductions of 8.7 and 14.8 percent, respectively, relative to the sham. In a follow-up

study Stratmeyer et al. (1977) did not confirm this earlier finding of sustained post weaning weight reduction (although irradiation was performed at day ten of gestation). Rather, a weight gain compared to the sham was suggested. 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 which produced some mortality (Sikov, 1976; Sikov et al., 1976). There were, however, a few pups stunted but this observation is not statistically significant. Ziskin et al. (1978) irradiated pregnant guinea pigs to continuous wave, 2.25 MHz ultrasound (0-1.1 W/cm**2, 60 min) and observed a decrease in fetal brain weight which was attributed to the hyperthermic condition.

Curto (1976a) observed an increased post partum mortality of the offspring of time-mated mice irradiated in the thirteenth day of gestation to continuous wave ultrasound (1 MHz). Four spatially averaged intensities, sham, 0.13, 0.25 and 0.50 W/cm**2 (180 second exposure time), yielded respectively, 4.3, 14.4, 13.0 and 26.7 percent mortality and represent a statistically significant difference relative to the sham. The original observation has been confirmed by repeating the experiment (Curto, 1976b). Stratmeyer et al. (1977) observed an increase in mortality during the perinatal period (between 18 and 21 days postcoitus) of 2.3% for the sham versus 26.0% (0.25 W/cm**2, 2 min) and 31.3% (0.80 W/cm**2, 2 min). These mice were irradiated on the tenth day of gestation. The effect was not statistically significant owing, probably, to the small sample size. Edmonds et al. (1979) compared their study to that of Curto's and did not find an increased post partum mortality.

The studies of Murai and colleagues (1975a, 1975b) suggest that prenatal continuous wave (2.3 MHz) ultrasonic irradiation of rats at the ninth day of gestation produces subtle effects which were evident in the offspring. The pregnant rats were irradiated for 5 hours with a commercial Doppler instrument (reported intensity of 20 mW/cm**2). 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 and colleagues (Sikov et al., 1976) lends some support to these observations wherein rats were exposed in utero on the fifteenth day of gestation to continuous wave (0.93 MHz) ultrasound for five minutes at

intensities which range from 10 mW/cm**2 to 1 W/cm**2 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. (1976) irradiated hela cells in vitro with a pulse echo diagnostic device for 10 minutes (reported intensity of 4 mW/cm**2) and observed that DNA synthesis was inhibited. Harvey et al (1975a) on the other hand, irradiated human fibroblasts in vitro to therapeutic levels of ultrasound (temporal average, spatial peak intensity of 0.5 W/cm**2) under both continuous wave and pulsed (2 ms on: 8 ms off) for five minutes and caused a marked increase in the rate of protein synthesis as measured by tritium labeled 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 treating 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/cm**2 (Harvey et al., 1975b). This work was designed to elucidate the mechanism responsible for ultrasonically induced acceleration in the rate of wound healing (Dyson et al., 1968). The increased rate of tissue regeneration was accompanied by an increasing uptake of tritium labeled thymidine after irradiating experimentally produced wounds in rabbit ears. At 3.55 MHz, the optimum exposure conditions to stimulate growth was 100 mW/cm**2 (temporal averaged, 5 minutes exposure time) administered three times a week. The mechanism was not purely thermal since 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/cm**2, 2 ms on and 8 ms off) delivered to the skin surface surrounding varicose ulcers three times weekly stimulated healing (Dyson et al., 1976).

A decrease in the mitotic index of rat liver resulted from continuous wave (1.9 MHz) exposure for five minutes at 60 mW/cm**2 (spatial average). Two hours after the ultrasonic irradiation, a partial hepatectomy was performed and 28 to 30 hours later the mitotic index was determined for the regenerating liver tissue with mean mitotic indices ranging from 20 to 80% of shams (Kremkau and Witcofski, 1974). In an effort to repeat these results, ultrasonic intensities (spatial average) ranging from 60 mW/cm**2 to 16 W/cm**2 for one to five minute exposures (2.2 MHz) were employed (Miller et al., 1976). These authors were unable to confirm

experimental conditions. If the decrease in mitotic activity in the regenerating liver is, in fact, a real effect, then 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 (Williams et al., 1976). The samples were exposed for 5 minutes at 0.065, 0.33 or 1.6 W/cm**2. Immediately following 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/cm**2 exposed group. A follow-up study (Williams et al., 1977) suggested that a small population of cells had been disrupted, possibly by some form of cavitation-like activity.

INTERACTION MECHANISMS

It is appropriate to review briefly the three mechanisms by which ultrasonic biological effects are induced in biological 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 biological material, heat results. Biological tissues absorb ultrasound at a relatively high rate. In tissue, at the site where the ultrasonic intensity is I, the rate of heat generation per unit volume per unit time for a plane, progressive wave is

$$Q = 2\alpha I$$

where α is the frequency-dependent absorption coefficient. Table I lists the attenuation and, absorption coefficients for a number of biological materials. For example, at 1 MHz the attenuation in liver tissue is approximately 500 times greater than that for water whereas the absorption is about 160 times greater (Goss et al., 1979; Pinkerton, 1949). While adequate absorption coefficient data does not exist for many tissues, it is thought to be approximately two times greater in muscle tissue than in liver tissue, while fat tissue absorbs ultrasonic energy at about half the rate (Goldman and Hueter, 1956a, 1956b; Goss et al., 1978; Chivers and Parry, 1978).

Table I

Literature Values for the Amplitude Ultrasonic Absorption and Attenuation Coefficients in Biological Materials. The Ultrasonic Frequency f is in the Unit of MHz

Biological Material	Amplitude Absorption Coefficient (cm^{-1})	Amplitude Attenuation Coefficient (cm^{-1})	References
Water (37 C)	$0.00016\ f^{2}$	$0.00016\ f^{2}$	(Pinkerton, 1949)
Amniotic Fluid	$0.00081\ f^{1.4}$	$0.00081\ f^{1.4}$	(Zana and Lang, 1974)
Testis	$0.015\ f^{1.11}$	not determined	(Goss et al., 1979)
Brain	$0.024\ f^{1.18}$	$0.07\ f^{1.14}$	(Goss et al., 1979)
Liver	$0.026\ f^{1.17}$	$0.08\ f^{1.13}$	(Goss et al., 1979)
Heart	$0.028\ f^{1.04}$	$0.13\ f^{1.07}$	(Goss et al., 1979)
Kidney	$0.028\ f^{1.02}$	$0.10\ f^{1.09}$	(Goss et al., 1979)
Tendon	$0.14\ f^{1.17}$	$0.56\ f^{0.76}$	(Goss et al., 1979)

The heat distribution within tissue depends on the beam geometry as well as the absorption coefficient and intensity. The initial rate of rise of temperature is the same for both plane waves and focused beams (Nyborg, 1979). 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 a minute (Pond, 1968, 1970; Nyborg, 1979; AIUM/NEMA, 1979). The importance of thermal diffusion has also been shown to explain the apparent frequency independence of histological lesions in mammalian central nervous system tissue under focused ultrasonic conditions (Lerner et al., 1973).

It has been suggested that when the propagating longitudinal ultrasonic wave comes in contact with an interface, mode conversion results and selective heating at tissue interfaces results (O'Brien et al., 1972) because part of the longitudinal wave is converted to a shear wave. Shear waves have absorption coefficients orders of magnitude greater than longitudinal waves in tissues; specifically, such data are available for liver, kidney, and muscle (Frizzell et al., 1976). Frizzell (1976) showed that shear wave generation in soft tissue is negligible in its contribution to interfacial heating. The effect of mode conversion is much more pronounced at interfaces between bone and soft tissue than at interfaces between soft tissues. However, this is because substantial mode conversion occurs in the bone (Chan et al., 1974; Frizzell, 1976). The extent to which selective heating occurs during the applications of ultrasound is unknown, along with the role of reflections and scattering of the energy. As a result, during the application of ultrasound, temporal and spatial distribution of temperature in tissue is unknown.

Since ultrasound is the propagation of mechnical energy, particle mechanical properties such as displacement, velocity and acceleration, and peak acoustic pressure must be associated with the biological effects of ultrasonic energy. Consider numerical values as calculated from the idealized plane wave equations, at an ultrasonic frequency of 1 MHz. At a specific site and with the range of ultrasonic intensity from 10 mW/cm**2 to 100 W/cm**2 , the particle displacement ranges from 18 to 1800 angstroms, the particle velocity ranges from 12 to 120 cm/sec and the particle acceleration ranges from 7400 to 740,000 g's. 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 inducing biological damage (Rooney, 1972). The stresses cause stretching, twisting and, finally, rupture of membraneous structures. It has been reported that a steady shear stress in the range of 3000 to 4500 dynes/cm**2 is sufficient to hemolyze the erythrocyte (Rooney, 1970; Williams et al., 1970). Other consequences of these stresses have been eddying motions, rotations and other movements of intracellualar bodies within the cell (Nyborg, 1972). It is, however, difficult to determine the extent of these steady shearing stresses on dynamic biological processes in vivo, since 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. The bubble, once formed can either remain stable and radially oscillate or continue to grow, become unstable and collapse. The latter, known as transient cavitation, produces intense hydrodynamic shearing forces within the vicinity of the collapsing bubble which can disrupt the surrounding material. It is not known if extremely short pulses such as those encountered in diagnostic ultrasound, even at extremely high levels, will cause cavitation-induced

damage. However, to arrive at an estimate for the threshold intensity to cause tissue cavitation the basic assumption employed is that if the acoustic pressure in biological tissue exceeds the maximum static tensile strength of that tissue, then transient cavitation may result (AIUM/NEMA, 1979). The given assumption should be viewed as a worse case in which the process is not rate dependent. Of course, in diagnostic ultrasound, the pulses are sufficiently short such that a rate process should be considered which would increase the threshold intensity. For calculation purposes the maximum negative pressure (static tensile strength) for water which is reported to be 277 atmospheres (Nyborg et al., 1972) is employed. An additional assumption is that this static pressure is comparable to acoustic pressure from which an ultrasonic intensity may be determined. Thus, for an amplitude acoustic pressure of 277 atm, density of 100 kg/m**3 and velocity of 1500 m/s, the estimated threshold spatial peak, temporal peak intensity for collapse cavitation calculates to be 25,600 W/cm**2. In comparison Fry et al. (1970) and Dunn and Fry (1971) have showed that for an exposure time less than about 40 ms and for a spatial peak, temporal peak intensity above about 2000 W/cm**2. mammalian central nervous system tissue can be severly disrupted by what they describe as cavitation. At the shortest exposure time investigated, about 100 microseconds, the spatial peak, temporal peak intensity required for the cavitation-type in mammalian brain tissue lesion is about 20,000 W/cm**2, which is the same order of magnitude as the threshold value calculated above.

Stable cavitation has been investigated in biological materials (Rooney, 1970, 1972) 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 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 sent 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 utlilized in diagnosis and therapy. An acoustic streaming boundary layer is formed.

It has been shown (Rooney, 1970, 1972; Williams, 1972, 1977; et al., 1970; Crowell et al., 1977) that shearing stresses associated with acoustically induced fluid flow called acoustic microstreaming can cause biological effects. To date, most of these studies have

been conducted in the kilohertz frequency range. However, since the mechanism for causing bioeffects has been demonstrated, it is useful to calculate possible thresholds for similar effects that might occur in the megahertz frequency range.

In order to calculate the lowest threshold at which known types of bioeffects might occur (AIUM/NEMA, 1979), consider the situation of an air bubble resting near a rigid boundary serving as a very efficient source of acoustic streaming. This streaming near bubbles is well characterized and has been studied in detail (Nyborg, 1965). The shearing stresses occur in the region near the bubble called the boundary layer thickness where gradients in the streaming velocity exist. To calculate a value for the threshold amplitude, consider the bubble in body fluids driven by a 2.25 MHz sound field. For this example, the viscosity can be taken to be 0.04 P, the radius of a resonant bubble is 1.5 microns, and the fluid density is 1.0 gm/cc. Let the value of the stress be 1000 d/cm**2 which is typical for the bioeffects observed at lower frequencies. From these values, the relative amplitude A/R (where A is the displacement amplitude of the bubble surface and R is the equilibrium bubble radius) is determined to be around 0.03. This low value means that the bubble oscillation is occurring in a range where linear theory is valid. The pressure amplitude P in a sound field required to produce this displacement amplitude of the bubble is given by (Coakley and Nyborg, 1978)

$$P = 3GdhA/R$$

where G is the ratio of specific heats for the gas in the bubble, d is the damping constant of the bubble, and h is the hydrostatic pressure. Using the values where G is 1.4, d is 0.14, h is 1 atm and A/R is 0.03, one obtains a value for the driving pressure of 0.02 atm. In a plane travelling wave the intensity corresponding to this pressure amplitude is 0.1 mW/cm**2.

It is startling to postulate bioeffects from such low intensities. To date, the lowest levels at which bioeffects have been reported are much higher than this value. Martin et al., (1978) and Miller et al., (1979) have demonstrated this phenomena in plant and mammalian systems, respectively, with Doppler fetal heart monitors under experimental conditions intended to assure the existance of gas bubbles within the sound field. Here the continuous wave spatial peak intensities were at least a factor of 100 greater than 0.1 mW/cm**2. There are several possibilities for this discrepancy. One, the theory discussed may not be valid when extrapolated to the megahertz frequency range. Two, the postulated

bubbles may not be in suitable environments to be driven to serve as efficient sources of acoustic microstreaming. Three, the scale of distance over which the shearing stresses are effective is small at megahertz frequencies. Finally, perhaps the postulated effects do in fact occur but have not yet been detected.

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 or not cavitation occurs in biological tissue of diagnostic and therapeutic ultrasound is not yet resolved.

ULTRASONIC DOSIMETRY

Even though three mechanisms of ultrasonic action with biological material can be described, the current status of dosimetry does not permit adequate assessment of the dose-related biological consequences of these mechanisms (O'Brien, 1978b). 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 the assessment of risk associated with the exposure of ultrasound. It is, however, important to recognize that the lack of standardization and of dosimetry are common problems in all radiation bioeffect research.

A step or two removed from knowledge of the in situ ultrasonic field parameters, but yet an essential part of field characterization, is a thorough characterization of the ultrasonic field at the site where the specimen is placed but without the specimen in place. This provides a basis from which in situ ultrasonic field parameters can be calculated and a basis whereby duplication of the experimental arrangement is made possible. There are numerous techniques (O'Brien, 1978b) available to measure many of the ultrasonic field parameters when the medium of interest is macroscopic, homogeneous, isotropic and low-loss, such as water. But this does not hold for in vivo measurements and, as a result, both instrumentation and dosimetric concepts need to be developed.

Dosimetry has two important objectives. The first is to define physical quantities which properly reflect an interaction at some site in biological material which may be expressed in units such as joules/kgm, joules/m**3, etc. The second is to develop a concept or concepts of the quantity that is applicable for radiation protection purposes.

Typically, 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 dose-like concepts, is difficult, especially when the purpose is to include all possible physical and biological variables. Otherwise, and the more common, special quantities are developed for the specific case or biological 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 (ICRU, 1971). 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, threshold dose, etc. (BEIR, 1972). 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 (ICP, 1954; Rupert, 1974). Other quantities which have been used to characterize ultraviolet radiation were chosen to quantify a specific bioeffect. These included minimal erythema dose, minimal perceptible erythema, subvesicular dose, minimal color dose, etc. (Michaelson, 1972). There has been much discussion regarding microwave dosimetry. Terms such as specific absorption rate (Johnson, 1975; Susskind, 1975), absorbed power density and specific absorption density (Guy, 1975) and energy dose-rate (Susskind, 1975) have all been either used or suggested as a basis quantity to describe absorbed electromagnetic energy. Appropriateness of units has also not been agreed upon (Justensen, 1975).

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 W/cm**2. The principal reason for the use of intensity is, perhaps, convenience since it is an easily measured parameter. As a dosimetric quantity, intensity represents 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.

Through both calculations (Hall, 1973) and experimentation (Hall, 1973; Etienne et al., 1976; Hall and Robinson, 1974; Bang, 1972) 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 have yielded a total attenuation of 23 dB at a frequency of 2.5 MHz (Hall, 1973). One of the earliest *in vivo* experiments (Bang, 1972) showed an average loss between the skin and uterus to be around 2.5 dB at 2.25 MHz but later studies showed this to be higher, in the range from 9 to 20 dB (Hall, 1973; Hall and Robinson, 1974) or from 6 to 14 (Etienne *et al.*, 1976)

There have been ultrasonic dosimetric quantities which 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 (Purnell *et al.*, 1964). The dosimetric concept "damage ability index" with the unit of reciprocal seconds is a quantity intended to describe the effect of ultrasound on spinal cord hemorrhage (Taylor and Pond, 1972). Later, it was suggested (Johnston and Dunn, 1976b) 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" (J/mm**3) for histologically observable lesions at superthreshold levels (Fry *et al.*, 1970; Dunn and Fry, 1971) as a function of the "delivered intensity." It was shown that at two different ultrasonic frequencies, 3 and 4 MHz, identical constant volume curves result even though there were two different "threshold levels."

In the reduced average fetal weight study discussed under biological effects (O'Brien,1976), a linear dose-effect dependence of exposure condition versus average fetal weight was observed as a function of the dose parameter, defined as I**2t. In comparison to other forms of energy, a similar dose dependency has been observed for mammary neoplasms at low ionizing radiation doses wherein two x-ray secondary particles (produced by a single neutron) are required to elicit the effect (Rossi and Kellerer, 1972). Also, in photochemical and photobiological studies, at high energy concentrations, biphotonic excitation has been observed (Wang, 1976). Basically, a linear dependent effect upon the dose parameter I**2t suggests that two energy events are required to produce the observed effect, but there is insufficient information to speculate as to what are these ultrasonic energy events. However, it is essential to develop such mathematical dose-effect relationships as a first step in the understanding of the fundamental mechanism(s).

CUMULATIVE, SYNERGISTIC, ETC.?

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

There appears to be, at least, a reasonable doubt whether or not cumulative effects occur from exposure to ultrasound. Summation of subparalytic doses of ultrasound with sufficient time for temperature equilibrium to be re-established between pulses, produced paralysis in frog hind limbs (Fry *et al.*, 1950). 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 (Taylor and Pond, 1972).

There have been both positive (Woeber, 1965) and negative (Clark *et al.*, 1970) synergistic findings with ionizing radiation and positive findings with both hypoxia (Taylor and Pond, 1972) and one chemothrapy drug (Kremkau *et al.*, 1976).

Structural lesions in mammalian adult brain were initially thought to be frequency independent over the range from 1 to 9 MHz (Fry *et al.*, 1970; Dunn and Fry, 1971; Lerner *et al.*, 1973) but after further examination of the data, a weak oscillatory frequency dependence was shown (Dunn *et al.*, 1975). The explanation for this dependence has been identified as a capsular layer surrounding the brain (Johnston and Dunn, 1976a). It is interesting to observe that most organs possess some type of capsular layer and thus its dependence upon ultrasonic energy transmission. However, the intensity threshold responsible for lesion production appears to be frequency independent. Other types of frequency dependent examples, all based upon intensity as the reported ultrasonic parameter, include greater damage to liver at lower frequencies over the range 0.5 to 6 MHz (Taylor and Pond, 1972), greater change in the electrophoretic mobility of irradiated cells at lower frequencies over the range 0.5 to 3.2 MHz (Taylor and Newman, 1972) and greater susceptibility to the production of cataracts at higher frequencies over the range 5 to 15 MHz (Sokollu, 1972).

SUMMARY

The assessment of risk associated with exposure to ultrasound has primarily been viewed as a threshold concept. In terms of radiation protection this 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 (Wells, 1974; Ulrich, 1974). These curves were generated from selected biological effect data much of which was not threshold studies. Such compilations are important in that they provide the opportunity to assess the current information and possible trends. They imply that the concept of risk associated with exposure to ultrasound can be ascribed to a threshold phenomena, that is there exists a "Threshold" below which the use of ultrasound can be viewed as safe. There is no scientific basis to believe this is not the case. But in fairness there is no scientific basis to believe this is so.

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 the clinicians to be provided up to date information on potential risks so that they can continue to render an informed benefit-risk judgment. The AIUM/NEMA (1979) Safety Standard for Diagnostic Ultrasound Equipment is an important step in this diection.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the U. S. Food and Drug Administration, FD 927.

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