n commercial equip-
to control it for pa-
oto-Optical Instrumen-
, 1974.
thermal parameters
nts by a calorimetric

of ultrasonic expo-
nethods, *Ultrasonics*,

ucher und technik in
ceedings of the three
in medicine, Davos,

4

Selected Biological Effects of Ultrasound

F. Dunn

University of Illinois, Urbana, Illinois

1. Introduction

That ultrasound could produce effects in biological systems became apparent at its inception near the end of World War I when techniques for locating submarines were being developed. Among such pursuits were those of Langevin (Wood and Loomis, 1927) who was investigating an acoustic method in which a piezoelectric transducer was excited by a Poulsen arc converter to vibrate at the resonance frequency of the structure and emit ultrasound into the bay at Toulon. Since the electric potentials applied to the quartz plate at times were as high as 40,000 V, the amplitude of the acoustic wave was appreciable and small fish and other marine animals were found dead in the vicinity of this radiation. Because of its inherent instability, the Poulsen arc was unsuitable for detailed investigation of these phenomena, and serious study awaited the development of the vacuum-tube oscillator for use as the piezoelectric transducer driver.

The first extensive investigation of the phenomena observed by Langevin was conducted by Wood and Loomis (1927). They described the destruction of *Spirogyra* and the killing of small fish and frogs exposed to 300 kHz ultrasound for several minutes at an intensity believed to be in the neighborhood of 10 W/cm^2. Subsequently, events were viewed with a light microscope and, at 406 kHz, streaming within cells and cellular destruction were observed, with an attendant increase in temperature (Harvey and Loomis, 1928).

Following the introduction of piezoelectric materials into acoustics and the subsequent rapid developments in electronics during the next three decades, it became feasible to construct instruments for precise measurement of the velocity of propagation and the absorption coefficient of ultrasonic waves in liquids and liquid-like media. An additional advance occurred after 1945 when the adaptation of radar techniques yielded pulsed ultrasonic instrumentation operating in the multi-megahertz range. Numerous techniques have been developed since that time permitting utilization of ultrasound in the frequency range from about 2×10^4 Hz (the arbitrary upper bound for the "audio" range) to 10^9 Hz; extension to beyond 10^{11} Hz is emerging with the continued development of Brillouin scattering techniques. Simultaneous development of piezoelectric materials, lens-focusing systems, and field-measuring schemes, have allowed high-intensity ultrasonics to be employed for the precision production of reversible and irreversible effects in biological media.

As a result, two distinct modes of operation have emerged, viz., passive uses, in which the acoustic field does not significantly alter structure and/or function of the interrogated systems, and active uses, in which reversible or irreversible alteration of the system is the objective. For the latter, an initial motivation for serious study was the early observation that ultrasound provided an opportunity for true deep heating in tissues and not simply the superficial heating that attended irradiation with infrared and the like (Dunn and O'Brien, 1976). In attempts to understand details of the mechanisms of the interaction of ultrasound with biological materials, experimental studies have been conducted at various levels of biological complexity. Some attention has been devoted to interaction studies in solutions of macromolecules and suspensions of microorganisms and cells, with the hope that these would provide simpler models. Herein, a principal question deals with the necessity for the presence of cavitation to affect the biological endpoint. By experimental design, thermal mechanisms are gen-

henomena observed
mis (1927). They de-
ling of small fish and
eral minutes at an in-
of 10 W/cm^2. Subse-
croscope and, at 406
estruction were ob-
erature (Harvey and

ectric materials into
ments in electronics
sible to construct in-
locity of propagation
waves in liquids and
occurred after 1945
ded pulsed ultrasonic
hertz range. Numer-
that time permitting
range from about
the "audio" range) to
ging with the contin-
niques. Simultaneous
focusing systems, and
-intensity ultrasonics
of reversible and irre-

ration have emerged,
does not significantly
gated systems, and ac-
teration of the system
notivation for serious
und provided an op-
nd not simply the su-
with infrared and the
to understand details
sound with biological
conducted at various
n has been devoted to
olecules and suspen-
nope that these would
al question deals with
o affect the biological
mechanisms are gen-

erally minimized in these systems. Cavitation is the general term used to describe the growth and subsequent behavior of cavities in an acoustically perturbed medium. It is useful to think in terms of two types of cavitation. The violent type, called *transient* or *collapse cavitation,* produces intense hydrodynamic forces within the vicinity of the collapsing bubble, which are capable of severely disrupting biological structures. Highly reactive free radicals can also be byproducts of transient cavitation. For the less violent type, called *stable cavitation,* the bubble (or cavity) does not collapse, but rather grows to a resonant size and oscillates or pulsates under the influence of the ultrasonic field. The hydrodynamic forces in the vicinity of the oscillating bubble are considered responsible for affecting biological materials (Flynn, 1964; Nyborg 1965).

Interest in the interaction of ultrasound with biological tissues, organs, and whole animals has been dominated by investigations of the role of thermal events in the production of irreversible structural changes and by determinations of levels of threshold for such effects. The motivation for these pursuits has, of course, been the probable application to medical problems and the choice of central nervous tissue as an often-employed tissue specimen has also been promoted by its relatively static acoustic and biological properties.

The purpose of this chapter is to identify findings that have emerged from these kinds of studies, with progression extending, for convenience, from whole organism studies, through tissues and organs, to the cellular and molecular levels. No attempt has been made to be exhaustive, rather to illustrate the nature of the studies undertaken and the character of the results obtained at the low megahertz frequencies. Thus, two otherwise important topics are omitted completely, viz., studies dealing with the interaction of ultrasound and plant systems and kilohertz ultrasound investigations.

Regarding the latter, individuals exposed to substantial levels of high audible frequency noise complain of an unpleasant pressure in the ears, headaches that do not remain after exposure, tinnitus, nausea, and mild vertigo (Skillern, 1965; Acton and Carson, 1967). These effects do not appear to be intimately related to the duration of exposure. Permanent physiological and psychological changes in users of industrial ultrasonic equipment, compared with a matched control group, were not found in a retrospective survey (Knight, 1968). Industrial workers exposed to levels of up to 110–120 dB low frequency ultrasound exhibited neither temporary (Acton and Carson, 1967) nor permanent (Knight, 1968)

hearing loss. Temporary threshold shifts can occur at subharmonic frequencies associated with exposure to 148–154 dB levels at discrete frequencies in the range 17–37 kHz (Parrack, 1966). Acton (1974) has reviewed the effects of industrial airborne ultrasound and readers are referred to his study for more detail.

Since sufficient detail cannot be included for all readers, important literature references are provided. Studies were carried out for a variety of purposes, satisfying specialized interests in specific topics, and leading to a very scattered literature. Thus, crucial questions directed toward a specified purpose may not be answerable simply because the pertinent experiments and measurements have not been carried out.

2. Whole-Body Radiation

In the low megahertz frequency range, the wavelength of sound in soft tissues is of the order of a millimeter. Since the half-power beam width of transducers designed for clinical purposes rarely extends beyond about a centimeter, it is apparent that whole body exposure of animals to ultrasound will be limited to a few cases. Foremost among these is the mammalian fetus, though model studies have included insects and microorganisms.

2.1. Vertebates

A number of studies have been conducted following the report of Shoji et al. (1972) that pregnant mice irradiated for 5 h with 40 mW/cm^2, 2.25 MHz ultrasound on the ninth day of gestation exhibited a significant increase in fetal mortality. They also observed some increase in fetal abnormalities, but no significant alteration in fetal weight. Lele (1976) has suggested that the unusual irradiation conditions could result in a uterine temperature increase sufficient to produce the observed abnormalities. Mannor et al. (1972) were unable to detect differences in abnormality rates in fetuses between days 8 and 20 of gestation, as well as in subsequent brother–sister cross-matings, of mice irradiated for as much as 60 min/day for up to 5 days, to intensities as high as 1 W/cm^2 with 2.25 MHz ultrasound. Taylor and Dyson (1974) found early chick embryos (corresponding to approximately 3-week human development) to be affected by 5 min exposure to pulsed 1 MHz ultrasound of 2.5 W/cm^2 average intensity, but not to 1 W/cm^2 average intensity. More advanced embryos (corresponding to approximately 6-week human development) were unaffected at 10 W/cm^2 average intensity. They further found no effects upon developing

ifts can occur at
posure to 148–154 dB
17–37 kHz (Parrack,
of industrial airborne
tudy for more detail.
ed for all readers, im-
Studies were carried
alized interests in spe-
erature. Thus, crucial
e may not be answera-
ts and measurements

on

avelength of sound in
Since the half-power
inical purposes rarely
arent that whole body
imited to a few cases.
fetus, though model
anisms.

ollowing the report of
diated for 5 h with 40
h day of gestation ex-
ty. They also observed
o significant alteration
at the unusual irradia-
perature increase suf-
alities. Mannor et al.
bnormality rates in fe-
s well as in subsequent
ated for as much as 60
h as 1 W/cm^2 with 2.25
found early chick em-
week human develop-
o pulsed 1 MHz ultra-
ot to 1 W/cm^2 average
sponding to approxi-
naffected at 10 W/cm^2
ffects upon developing

embryos exposed for 24 h to irradiation from a 2.25-MHz Doppler diagnostic instrument having an electrical input power of 100 mW/cm^2. Others have reported finding no effects upon mice (Kirsten et al., 1963; Smyth, 1966; Warwick et al., 1970), rats (McClain et al., 1972; Takeuchi, 1970; Woodward et al., 1970), or rabbits (Holmes, 1962).

More recently O'Brien (1976) has shown that the mean weight per fetus is reduced significantly when pregnant mice are exposed to 1 MHz ultrasound for 5 min at an average intensity as low as 1 W/cm^2. Further, while Curto (1976) found irradiation of pregnant mice with 1 MHz, 0.35 W/cm^2 average intensity for 3 min to result in a significant increase in mortaility in litters observed up to 21 days post partum, Edmonds et al. (1979) find no such increase. This discrepancy may be accounted for by the day of gestation on which exposure to the ultrasound was perpetrated and other differences in specimen preparation and procedure.

Sikov et al. (1976), in a reporting of work-in-progress, believe they observed delayed neuromuscular development in the rat on exposure to 5 min of cw ultrasound of 10 mW/cm^2.

2.2. Insects

An early study with insects involved exposure of Drosophila eggs to 1 MHz, 0.5 W/cm^2 ultrasound with demonstration of a variety of developmental abnormalities appearing (Selman and Counce, 1953), most likely associated with undetected cavitation. In a more recent study involving large-scale breeding experiments, Drosophila survived the irradiation procedure and exhibited no significant increase in the frequency of recessive lethal mutations and chromosomal nondisjunction, even under exposure conditions sufficient to kill a substantial portion of the flies (Thacker and Baker, 1976).

Table 4.1 lists some of the significant findings regarding whole body ultrasound exposure.

3. Tissues and Organs

Much activity has occurred in the area of identification of specific effects to selected tissues and organs irradiated by particular ultrasonic exposure regimes, and of quantitative determination of threshold levels at which unique events manifest themselves. The following discussion illustrates the very appreciable range of interests exhibited by investigators and the breadth of their findings.

TABLE 4.1
Effects of Whole-Body Exposure to Ultrasound

F, MHz	*I*(SPTA), W/cm^2	T, min	Species	Effect	Reference
1	1 cw	5	Pregnant mouse	Fetal weight reduction	O'Brien, 1976
1	0.4 cw	3	Pregnant mouse	Post partum fetal mortality increase	Curto, 1976
2	0.5 cw	3	Pregnant mouse	No post partum fetal mortality increase	Edmonds et al., 1979
1	2.5 p	5	Chicken embryo= 3 week human development	Effected	Taylor and Dyson, 1974
1	10 p	5	Chicken embryo= 6 week human development	No effect	Taylor and Dyson, 1974

3.1. Central Nervous System

The mammalian central nervous system provides an acoustically static organ for study, viz., that the ultrasonic propagation properties remain largely unchanged in response to physiological and behavioral stimuli. Thus three laboratories have exhibited remarkable agreement in the determination of the relationship between the acoustic intensity in the tissue and the single-pulse duration necessary to produce threshold lesions in the brain (Fry et al., 1970; Pond, 1970; Robinson and Lele, 1972). The relationship $It^{1/2} = 200$, where I is the acoustic intensity (W/cm^2) at the site of interest in the tissue and t is the duration of the single-pulse exposure (in seconds), defines a threshold in that exposures greater than 200 W/cm^{2}s$^{1/2}$ always produce optical-microscopically identifiable lesions, while those less than this value do not (Fig. 1). This relation has been determined experimentally to describe the threshold events over the range of exposure from 100 μs to 10 min, beyond which it alters to approach an infinite-time exposure condition. Thermal processes have been shown to dominate in the low intensity–long pulse exposure region (Lerner et al., 1973), while evidence abounds for transient cavitation events to be most important in the highest intensity–shortest pulse exposure region. In the mid-intensity region, viz., about 700–1500 W/cm^2, other mechanical mechanisms are believed to occur. Histologically, white matter exhibits a lower threshold than does gray matter, with the vascular structures being most resistant (Fry, 1958). The

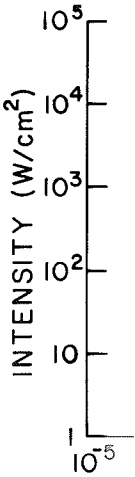

Fig. 1.
pulse durat
lian brain.
observed
1976) of th
region, fro
upon freq
quency de
other, mai
pendent o
posure of
mice (mai
variation
structural
eighth of
Fry, 1971)
Taylo
as a functi
25 W/cm^2
tor. They
quency an

A recent st
irreversibl
Kremkau
the freque

Ultrasound

	Reference
t	O'Brien, 1976
n etal rease	Curto, 1976
n fetal rease	Edmonds et al., 1979
	Taylor and Dyson, 1974
	Taylor and Dyson, 1974

tem

vides an acoustically propagation proper- physiological and be- e exhibited remarka- relationship between ingle-pulse duration he brain (Fry et al., 2). The relationship W/cm^2) at the site of he single-pulse expo- at exposures greater microscopically iden- e do not (Fig. 1). This ally to describe the e from 100 μs to 10 nfinite-time exposure vn to dominate in the Lerner et al., 1973), ion events to be most ulse exposure region. –1500 W/cm^2, other ccur. Histologically, an does gray matter, tant (Fry, 1958). The

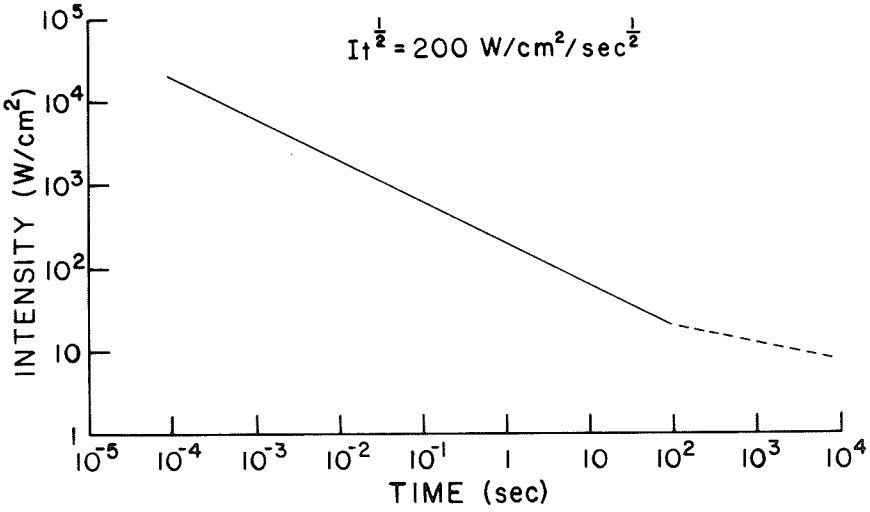

Fig. 1. Delivered acoustic intensity at the irradiation site vs single pulse duration of exposure to produce threshold lesions in the mammalian brain.

observed lack of frequency dependence (Johnston and Dunn, 1976) of the threshold boundary may result, at least in the thermal region, from the combined effects of the nearly linear dependence upon frequency of the absorption coefficient and the inverse frequency dependence of focal volume, which tend to balance each other, maintaining a relatively constant lesion volume that is independent of frequency (Lerner et al., 1973). A study involving exposure of the lumbar enlargement of the spinal chord of neonatal mice (maintained at 37°C), a preparation permitting temperature variation of the specimen and involving a functional rather than structural endpoint, yields threshold levels approximately one-eighth of those (above) for mature brain (Dunn, 1958; Dunn and Fry, 1971).

Taylor and Pond (1970) irradiated exposed rat spinal cords, as a function of ultrasonic frequency in the range 0.5–6 MHz, with 25 W/cm^2 in a pulsing regime of 10 μs pulses and a 10% duty factor. They observed a decrease in damage with increase in frequency and increased damage under hypoxic conditions.

3.2. Liver

A recent study by Chan and Frizzell (1977) shows the threshold for irreversible structural changes in cat liver to be twice that for brain. Kremkau and Witkofski (1974) reported a significant reduction in the frequency of mitotic cells in surgically stimulated rat liver in re-

sponse to exposure to 60 mW/cm^2, 1.9 MHz ultrasound. However, Miller et al. (1976) were unable to confirm these findings with surgically stimulated rat liver irradiated 1 and 5 min with 2.2 MHz ultrasound in the range 0.06–16 W/cm^2. One major difference in the procedures employed by these two groups is that the latter involved a circular motion of the transducer over the animal's ventral surface, while the transducer was held stationary in the former case. Barnett and Kossoff (1977) have also obtained negative results for exposure of regenerating rat liver to 2.5 MHz ultrasound pulsed at 10–50 kHz pulse repetition frequency (prf) and 33 W/cm^2 peak intensity. Taylor and Pond (1970), however, noted an increase in the frequency of hemorrhage at the lower frequencies (in the range 0.5–6 MHz) in surgically exposed liver, to 56 W/cm^2 peak intensity ultrasound for 5 min under a pulsing regime, wherein a 10% duty factor and 10 ms pulses were employed. Though the temperature rise did not exceed 5°C, damage was particularly severe in the neighborhood of the central vein.

3.3. Testes

Conflicting reports have resulted from animal studies of the ultrasonic effects upon testes, viz., Kamocsay et al. (1955) and Fahim et al. (1975) have reported observing effects upon spermatogenesis and fertility while Lyon and Simpson (1974) and Urry et al. (1979) failed to make such observations. In a more recent study, mouse testes were exposed sequentially for 30 s at a spatial peak ultrasonic intensity (SPTA) of 25 W/cm^2 at 1 MHz, removed at varying times post irradiation from immediately to 10 days, and examined histologically. The results suggest that two types of ultrasonically induced damage occur for different specimens under identical exposure conditions, viz., either seminiferous tubule disruption occurs with a suggestion of minor intertubule space involvement or a more severe form of tubule damage occurs with significant *interstitial* tissue involvement (O'Brien et al., 1979). Spermatocytes appear to be affected earlier than spermatogonia, contrary to the situation following exposure to ionizing radiation.

3.4. Blood Stasis

Dyson et al. (1974) have demonstrated that blood flow in the vessels of chick embryos can be arrested by exposure to ultrasound in the range of 1 to 5 MHz. Both cw and pulsed ultrasound are effective and the SATA intensity necessary to produce the stasis may be as low as 0.5 W/cm^2 (at 3 MHz), depending upon blood vessel size,

type, and orientation. The stasis is reversible upon cessation of the sound exposure, though electron microscopy has revealed damage to some endothelial cells lining the embryonic vessels in which the stasis is produced. Since the production of the stasis is associated with standing waves, it can be avoided by either continual movement of the sound source or employment of sufficiently short pulses of irradiation in the exposure procedure (ter Haar and Wyard, 1978).

3.5. Tissue Regeneration

Tissue regeneration in response to ultrasonic irradiation has been studied by Dyson et al. (1968). The rate of repair of 10 mm square holes in rabbit ears exposed to 3.6 MHz ultrasound for 5 min three times per week, under either 0.1 W/cm^2 cw application or various pulsing regimes with the intensity in the range 0.25–8 W/cm^2, was significantly more rapid than the untreated control ear. The attending temperature rise was considered too small to be responsible for the effects. Subsequently, patients with chronic varicose ulceration were treated with 3 MHz ultrasound at 1 W/cm^2 for 10 min three times per week for 4 weeks with encouraging results (Dyson and Suckling, 1978). Other reports of enhanced tissue regeneration in response to ultrasonic irradiation have also appeared, e.g., Pizzarello et al. (1975) wherein slightly faster tissue renewal of the amputated prelimb of a newt occurred, over that of the opposite also amputated prelimb, though details are sparse and conditions complicated for identifying crucial dosage conditions.

3.6. Neoplastic Tissues and Synergism

The effects of ultrasound on neoplastic tissues have involved at least two lines of inquiry, viz., a direct effect upon the tissues possibly involving hyperthermic effects and a synergistic involvement with other modalities. A recent example of the former deals with the irradiation of subcutaneously implanted rat Wilm's tumors with 1 MHz ultrasound at 1.5 W/cm^2 to achieve reduction in tumor volume, reduction in tumor weight, and increase in mean rat survival time (Longo et al., 1975). Histological observation revealed nuclei with condensed chromatin patterns. Substantial temperature increases also occurred. With regard to synergism, Lehmann and Krusen (1955) noted that the X-ray dosage required to produce regression in an experimental tumor was substantially reduced when simultaneously irradiated with 1 MHz ultrasound at 8.4 W/cm^2, and they believed the heating effect owing to attendant

sound absorption was responsible. Woeber (1965) has reported marked improvement in the treatment of human superficial cancer from simultaneous employment of ultrasound and X-rays. However, Clarke et al. (1970) failed to observe such synergistic effects using either cultured mouse lymphoma cells or implanted tumors in rats. Gavrilov et al. (1975), however, have found that a preliminary irradiation of transplanted sarcoma 37 tumors in mice with 1 MHz ultrasound in the range 0.5–2.5 W/cm^2 for periods of 1–5 min enhances the sensitivity of the tumor cells to subsequent gamma radiation. A synergism with chemotherapy has been suggested by Heimburger et al. (1975), who irradiated malignant brain tumors simultanously through a bone flap with 1 MHz ultrasound at 3 W/cm^2. Though the patient population was small, they believed the effectiveness of the chemotherapy was improved using ultrasound.

Enhanced DNA synthesis has been reported by Elmer and Fleischer (1974) in neonatal mouse tibiae exposed for 5 min, three times in 24 h, to 1.8 W/cm^2, 1 MHz ultrasound. Observations revealed that growth, protein accumulation, and ^{3}H-proline incorporation remained unaffected. The DNA synthesis may have been affected by the temperature rise measured, attending absorption of the ultrasonic energy in the high absorbing bone, though the authors were not so convinced.

Table 4.2 summarizes effects observed upon tissues and organs.

4. Cells and Microorganisms

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

Coakley et al. (1971) have been able to associate the destruction of amebae with the specific number of discrete cavitation events occurring during the irradiation procedure. This apparent necessity for the presence of cavitation is most important in attempts to determine risk in clinical employment of ultrasound

TABLE 4.2
Ultrasound-Induced Effects on Various Tissues and Organs

F, MHz	I(SPTA), W/cm^2	t, min	Organ	Effect	Reference
1–9	2×10^4–1 cw	10 μs–10 min	Brain	Lesion threshold; $It^{1/2} = 200$ W/cm^2/s$^{1/2}$	Lerner et al., 1973 Fry et al., 1970; Pond, 1970;

(1965) has reported
man superficial can-
asound and X-rays.
e such synergistic ef-
ells or implanted tu-
ave found that a pre-
37 tumors in mice
W/cm^2 for periods of
r cells to subsequent
erapy has been sug-
rradiated malignant
ıp with 1 MHz ultra-
ation was small, they
rapy was improved

orted by Elmer and
sed for 5 min, three
nd. Observations re-
d ^{3}H-proline incor-
hesis may have been
ttending absorption
g bone, though the

upon tissues and

ns

de model systems of
being comprised of
hrony, but with the
ıe architectural fea-
rtance of this. Such
ng with the physical
alterations in more
ultrasonic exposure
n lead to cell death
s.

sociate the destruc-
discrete cavitation
ture. This apparent
st important in at-
ment of ultrasound

TABLE 4.2
Ultrasound-Induced Effects on Various Tissues and Organs

F, MHz	I(SPTA), W/cm^2	t, min	Organ	Effect	Reference
1–9	2×10^{4}–1 cw	10 μs–10 min	Brain	Lesion threshold; $It^{1/2} = 200$ W/cm^2/s$^{1/2}$	Lerner et al., 1973 Fry et al., 1970; Pond, 1970; Robinson and Lele, 1972
3	2×10^{4}–3×10^{2} cw	10 μs–1 s	Liver	$It^{1/2} = 400$ W/cm^2/s$^{1/2}$	Chan and Frizzell, 1977
1	200–1 cw	10^{-2}–10	Neonatal cord	Hind limb paralysis, ⅛ that of brain	Dunn, 1958; Dunn and Fry, 1971
0.5–6	25 p	0.5–2	Laminectomized cord	Greater damage at lower frequencies	Taylor and Pond, 1972
1	25 p	0.5	Testis	Spermatocytes affected earlier than spermatogonia (contra. ioniz. rad.)	O'Brien et al. 1979
1–5	1 cw	Instantaneous	Chick embryo	Blood flow stasis	Dyson et al., 1974
3.6	0.1 cw	5(3/week)	Rabbit ear	Hole repair	Dyson et al., 1968
3	1 cw	10 (3/week, 4 weeks)	Human	Treatment for varicose ulcerations	Dyson and Suckling, 1978
1	1.5 cw		Wilm's tumor	Hypothermia/reduced tumor weight	Longo et al., 1975
1	8 cw		Experimental tumors	Reduced X-ray dosage for tumor regression (synergism)	Lehman and Krusen, 1955
1	1.8 cw	5(3/day)	Neontal mouse tibia	Enhanced DNA synthesis (hyperthermia)	Elmer and Fleischer, 1974

since virtually nothing is known of cavitation phenomena in tissues. It appears that cell disintegration occurs preferentially, at least when cavitation is allowed to occur, during the mitotic phase of the cell cycle, e.g., mouse leukemia cells in aqueous suspension were more susceptible to damage in M phase when exposed to 1 MHz ultrasound having spatial peak intensity of 15 W/cm^2 for 10 s (Clarke and Hill, 1969). It has been suggested that there may occur variations in the mechanical strength of the cell membrane during the cell cycle. An interesting report is that of Brown and Coakley (1975) who exposed gel-caged suspensions of an ameba to 1 MHz ultrasound sufficient to produce irreversible alterations in mammalian tissues. Though they employed cw and pulsed regimes, samples from logarithmically growing and from synchronous cultures treated in free field and standing wave field conditions failed to exhibit differences in their growth patterns compared to controls.

Nonlethal effects upon cells have also been investigated. Repacholi et al. (1971) and Taylor and Newman (1972) observed a reduction in the electrophoretic mobility of Ehrlich ascites cells following exposure to low megahertz ultrasound, implying alteration of the electric charge density of the cellular surface. Ultrasonic irradiation, at 1.8 MHz with intensities greater than 1 W/cm^2, of rat thymocytes was followed by an immediate decrease in potassium content, suggesting a sublethal alteration in the structures intimate to permeability (Chapman, 1974). Additionally, investigation of the details of ultrastructure has revealed mitochondrial modifications in cells exposed to ultrasound (Hrazdira, 1970).

Microorganisms have been employed in genetic studies without positive results. Thus Combes (1975) did not find an increase in the back-mutation of an auxotrophic strain of *Bacillus subtilis* in response to 2-MHz ultrasonic irradiation for 5 min at intensities up to 60 W/cm^2, in a pulsing regime. Also, Thacker (1974) found that abnormal genetic effects did not occur in ultrasonicaly irradiated yeast cells, even when treated in such a manner than the cells were killed to 0.1% of the survival rate of the controls.

The positive findings above are listed in Table 4.3.

5. Biomacromolecules and Their Assemblages

The response of large molecules of biological importance, to ultrasonic exposure, has been studied in aqueous solution for details of tissue interaction mechanisms. The findings that ultrasonic absorption is largely attributable to tissue protein content (Car-

on phenomena in tis-
curs preferentially, at
ring the mitotic phase
n aqueous suspension
se when exposed to 1
y of 15 W/cm^2 for 10 s
l that there may occur
cell membrane during
f Brown and Coakley
of an ameba to 1 MHz
e alterations in mam-
and pulsed regimes,
rom synchronous cul-
field conditions failed
atterns compared to

o been investigated.
an (1972) observed a
hrlich ascites cells fol-
d, implying alteration
urface. Ultrasonic ir-
than 1 W/cm^2, of rat
ecrease in potassium
e structures intimate
ally, investigation of
ochondrial modifica-
ra, 1970).
genetic studies with-
not find an increase
of *Bacillus subtilis* in
5 min at intensities
hacker (1974) found
ultrasonicaly irradi-
anner than the cells
e controls.
Table 4.3.

ssemblages

ical importance, to
ous solution for de-
dings that ultrasonic
otein content (Car-

TABLE 4.3
Reported Effects of Ultrasound on Various Cell Lines

F, MHz	I, W/cm^2	T, min	Cells	Effect	Reference
1	15 (SPTA)	0.1–0.2	Mouse leukemia	Disintegration preferentially during mitosis	Clark and Hill, 1969
1	1 (SATA)	5	Ehrlich ascites	Reduction in electrophoretic mobility	Repacholi et al., 1971 Taylor and Newman, 1972
2	10 (SPTA)		Rat thymocytes	Immediate decrease in potassium content	Chapman, 1974

stensen et al., 1953) and that tissue interactions resulting in irreversible structural changes must occur at levels of structure below that identifiable with the light microscope (Dunn, 1958), encouraged some of these studies.

5.1. Biomacromolecules

For molecules having molecular weights below about 10^4, i.e., proteins, in aqueous solution, it appears that degradation occurs only in the presence of cavitation in the ultrasonic frequency range 1–27 MHz (Macleod and Dunn, 1968). For larger molecules in aqueous solution, e.g., DNA with molecular weights greater than about 10^6, it has been possible to demonstrate degradation in the absence of any phenomena suggesting the presence of cavitation (Hawley et al., 1968). Herein intensities as high as 30 W/cm^2 were employed and essentially monodisperse fragments were produced with the limiting value depending upon intensity, i.e., greater intensities of exposure produced smaller fragments. The molecular weight dependencies on cavitation are illustrated in Table 3.4. This sequential halving of the molecules, with continued irradiation time, is also a characteristic feature of the much more prevalent studies of degradation of DNA in the presence of cavitation (Peacocke and Pritchard, 1968). The breaking of the DNA molecules preferentially at the midpoints of their extended conformation in solution suggests a mechanical mechanism being responsible, though chemical effects, largely resulting from free radical production in the presence of cavitation, have been described extensively (El'piner, 1964), in particular in the low megahertz frequency range (Hill, 1972). Nonetheless, although it is an easy task to degrade nucleic acid molecules in solution, it has not been possible to produce mutagenic lesions following in vitro irradiation of transforming DNA (Combes, 1975). The apparent necessity for the extended conformation of DNA molecules in solution to affect degradation implies the much lesser opportunity for denaturation of cellular DNA to occur.

5.2. Macromolecular Assemblages

An interesting finding from two laboratories is that the order of ultrasonic reactivity obtained by observing spectral changes in nucleic acid bases in solution at 1 MHz and less than 5 W/cm^2, viz., Thy > Ura > Cyt > Gua > Ade (McKee et al., 1977) seems to be the same as that obtained by chromatography in dilute solutions of nucleic acids at 800 kHz and approximately 10 W/cm^2 (Bragin-

ions resulting in irre-
els of structure below
Dunn, 1958), encour-

es

ow about 10^4, i.e., pro-
gradation occurs only
onic frequency range
r larger molecules in
weights greater than
te degradation in the
presence of cavitation
igh as 30 W/cm^2 were
ments were produced
ensity, i.e., greater in-
nents. The molecular
strated in Table 3.4.
th continued irradia-
ne much more preva-
presence of cavitation
ng of the DNA mole-
extended conforma-
nism being responsi-
ng from free radical
ve been described ex-
e low megahertz fre-
ough it is an easy task
it has not been possi-
n vitro irradiation of
pparent necessity for
es in solution to affect
nity for denaturation

blages

s is that the order of
spectral changes in
ss than 5 W/cm^2, viz.,
et al., 1977) seems to
hy in dilute solutions
y 10 W/cm^2 (Bragin-

TABLE 4.4
Effects of Ultrasound on Macromolecules Exposed "In Vitro"

F, MHz	I(SPTA), W/cm^2	T, min	Structure	Effect	Reference
1–27			Proteins, mw $< 10^4$	Cavitation necessary for destruction	Macleod and Dunn, 1968
1	30		DNA, mw $> 10^4$	Degradation in absence of cavitation	Hawley et al., 1968

skaya et al., 1964). No information exists with regard to the occurrence of such events intracellularly.

A very considerable attention has been devoted to the concern over possible ultrasonic effects on chromosomes. Much of this interest has been associated with studies involving human lymphocyte chromosomes from cultured preparations, with overwhelming evidence from the results that ultrasound does not produce an increase in aberrations even at much greater exposure intensities and longer irradiation times than are likely to occur during medical diagnostic procedures (Coakley et al., 1972; Hill et al., 1972; Watts et al., 1972; Rott and Soldner, 1973; Bucton and Baker, 1972; Macintosh et al., 1975), though a synergistic effect with X-ray may occur (Kunze-Mühl, 1975). Liebeskind et al. (1979a) report an increased immunoreactivity to antinucleoside antibodies in G_1 cells and repair replication in the DNA of mammalian HeLa cells exposed for 20–30 min to 2.5 MHz ultrasound with a prf of 200 Hz, pulse duration 200 μs, SPTA intensity of 6.6 mW/cm^2 and SPTP intensity of 35.4 W/cm^2. However, problems arise with the interpretation of the results in the antiguanosine antibody method since it is subject to high background and high variability. Further, although increased repair synthesis of DNA is reported, the authors were unable to demonstrate DNA strand damage by the sensitive technique of alkaline–sucrose gradients.

Although these investigators were unable to demonstrate an increase in sister chromatid exchanges (SCEs) from exposure of HeLa cells to diagnostic ultrasound, they later reported (Liebeskind et al., 1979b) SCEs in human lymphocytes exposed for 30 min to 2 MHz ultrasound at a SATP intensity of 5 mW/cm^2. This is in contrast to the previously reported work of Morris et al. (1978), who were unable to demonstrate any effect on SCE in human leucocytes exposed for 10 min to 1.05 MHz cw ultrasound at intensities from 15.3–36 W/cm^2. More recently, Wegner et al. (1980) found no increase in the frequencis of structural chromosomal aberrations or in SCEs in Chinese hamster ovary cells treated with 2.2 MHz ultrasound from a fetal pulse detector operated at 10mW/cm^2 for substantial periods of time. Also, there was no indication of single-strand breaks induced in the G_2 phase.

A few studies have treated membranes and membrane models. Thus Rohr and Rooney (1978) were able to increase the permeability of membranes formed from oxidized cholesterol exposed to 1 MHz ultrasound at intensities greater than 1.5 W/cm^2. Liver plasma membranes exhibited decreased 5′-nucleotidase ac-

tivity and altered morphology in response to 0.87 MHz ultrasound in the range 0.75–3 W/cm^2 for exposures ranging from 2 to 10 min (Montmory and Pourhadi, 1976b). Coble and Dunn (1976) found an unlinking of the membrane potential and short circuit current to occur in that their time courses in response to ultrasound differed, i.e., the short circuit current increased continuously for exposures of 0.5 s and longer, while the membrane potential reached its maximum within 0.5 s and did not alter with increased duration of exposure. This occurred at 1 MHz in the intensity range 1–100 W/cm^2 for isolated frog skin preparations. Williams et al. (1976) were able to alter the recalcification time of platelet-rich plasma with 1 MHz ultrasound at approximately 0.2 W/cm^2 (spatial peak) in 5 min.

6. Concluding Remarks

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

Since ultrasound appears to induce embryological effects, treatment of pregnant women in the abdominal area, e.g., possibly for lower back pain, should be avoided (see Chapter 6).

A systematic analysis of existing reliable data for mammalian tissues has led to the following two summary statements (Nyborg, 1977):

> No substantial bioeffects have been demonstrated for spatial peak–temporal average intensities less than 100 mW/cm^2.
>
> No substantial bioeffects have been demonstrated for which the product of It is less than 50 J/cm^2 where, for pulsed operation, t is the total ("on" + "off") time.

The spatial peak intensities referred to in the statements are typically very much greater than the spatial average values of intensity used in the specification of ultrasonic instrumentation (see Chapter 3). The statements, while providing terse and compendious rendering of a huge literature, may need to be modified as new data appear since most of the data are from mammals other than man and the extrapolation to man is not always clear, the influence

of exposure factors such as pulsing conditions and acoustic frequency are not included, and the most sensitive biological tests may not have been employed.

No fully satisfactory epidemiological study has as yet been performed. However, a retrospective survey, but not case-controlled, of more than 1000 apparently normal women examined with ultrasound during various stages of pregnancy exhibited a 2.7% incidence of congenital abnormalities on newborn physical examination, as compared with a figure of 4.8% exhibited in a separate and unmatched survey of women not having received ultrasonic diagnosis (Hellman et al., 1970). Neither the gestation period at which the first ultrasonic examination occurred, nor the number of examinations, appeared to increase the risk of fetal abnormality occurring. A smaller study has also yielded no indication of either congenital malformations or chromosomal aberrations in the fetus (Koranyi et al., 1972).

Finally, though not scientifically objective, it must be noted that a very substantial number of persons receiving ultrasonic diagnosis also undergo subsequent clinical examinations, and undesirable effects from such procedures, or suspicions thereof, have not been reported (Dunn and Fry, 1971).

Acknowledgments

The author acknowledges, gratefully, support from the National Science Foundation and the National Institutes of Health for the portion of the work described herein performed in his laboratory.

References

Acton, W. I., The effects of industrial airborne ultrasound on humans, *Ultrasonics* **12,** 124–128, 1974.

Acton, W. I., and Carson, M. B., Auditory and subjective effects of airborne noise from industrial ultrasonic sources, *Brit. J. Indust. Med.* **24,** 297, 1967.

Barnett, S., and Kossoff, G., Negative effect of long duration pulsed ultrasonic irradiation on the mitotic activity in regenerating rat liver, in *Ultrasound in Medicine,* White, D., and Brown, R., eds., Plenum, New York, 1977, pp. 2033–2044.

Braginskaya, F. I., and El'piner, I. Y., Metachromatic reaction of nucleic acids (DNA and RNA) native and irradiated with ultrasonic waves, *Biofizika* **9,** 31–40, 1964.

Brown, R. C., and Coakley, W. T., Unchanged growth patterns of *Acanthamoeba* exposed to intermediate intensity ultrasound, *Ultrasound Med. Biol.* **2,** 37–41, 1975.

Bucton, K. E., and Baker, N. V., An investigation into possible chromosome damaging effects of ultrasound on human blood cells. *Brit. J. Radiol.* **45,** 340–342, 1972.

Carstensen, E. L., Li, K., and Schwan, H. P., Determination of the acoustic properties of blood and its components, *J. Acoust. Soc. Am.* **25,** 286–289, 1953.

Chan, S., and Frizzell, L. F., Ultrasonic thresholds for structural changes in the mammalian liver, *Proc. IEEE Sonics Ultrasonics Symp.,* Cat. # 77CH1264-1SU, 1977, pp. 153–156.

Chapman, I. V., The effect of ultrasound on the potassium content of rat thymocytes in vitro, *Brit. J. Radiol.* **47,** 411–413, 1974.

Clarke, P. R., and Hill, C. R., Biological action of ultrasound in relation to the cell cycle, *Exptl. Cell Res.* **58,** 443–444, 1969.

Clarke, P. R., Hill, C. R., and Adams, K., Synergism between ultrasound and X-rays in tumor therapy, *Brit. J. Radiol.* **43,** 97–99, 1970.

Coakley, W. T., Hampton, D., and Dunn, F., Quantitative relationships between ultrasonic cavitation and effects upon amoebae at 1 MHz, *J. Acoust. Soc. Am.* **50,** 1546–1553, 1971.

Coakley, W. T., Slade, J. S., Braeman, J. M., and Moore, J. L., Examination of lymphocytes after exposure to ultrasonic irradiation, *Brit. J. Radiol.* **45,** 328–332, 1972.

Coble, A. J., and Dunn, F., Ultrasonic production of reversible changes in the electrical parameters of isolated frog skin, *J. Acoust. Soc. Am.* **60,** 225–229, 1976.

Combes, R. D., Absence of mutation following ultrasonic treatment of *Bacillus subtilis* cells and transforming deoxyribunucleic acid, *Brit. J. Radiol.* **48,** 306–311, 1975.

Curto, K., Early postpartum mortality following ultrasound radiation, in *Ultrasound in Medicine,* White, D., and Barnes, R., eds., Plenum, New York, 1976, Vol. 2, pp. 535–536.

Dunn, F., Physical mechanisms of the action of intense ultrasound on tissue, *Am. J. Phys. Med.* **37,** 148–151, 1958.

Dunn, F., and Fry, F. J., Ultrasonic threshold dosages for the mammalian central nervous system, *IEEE Trans. Biomed. Engr.* **BME-18,** 253-256, 1971.

Dunn, F., and O'Brien, W. D., Jr., eds., *Ultrasonic Biophysics,* Dowden, Hutchinson, and Ross, Stroudsburg, 1976.

Dyson, M., and Suckling, J., Stimulation of tissue repair by ultrasound: a survey of the mechanisms involved, *Physiotherapy* **64,** 105–108, 1978.

Dyson, M., Pond, J. B., Joseph, J., and Warwick, R., The stimulation of tissue regeneration by means of ultrasound, *Clin. Sci.* **35,** 273–285, 1968.

Dyson, M., Pond, J. B., Woodward, B., and Broadbent, J., The production of blood cell stasis and endothelial damage in blood vessels of chick embryos treated with ultrasound in a stationary wave, *Ultrasound Med. Biol.* **1,** 133–148, 1974.

Edmonds, P. D., Stolzenberg, S. J., Torbit, C. A., Madan, S. M., and Pratt, D. C., Post partum survival of mice exposed *in utero* to ultrasound, *J. Acoust. Soc. Am.* **66,** 590–593, 1979.

Elmer, W., and Fleischer, A., Enhancement of DNA synthesis in neonatal mouse tibial epiphyses after exposure to therapeutic ultrasound, *J. Clin. Ultrasound* **2,** pp. 191–195, 1974.

El'piner, I. P., *Ultrasound: physical, chemical, and biological effects,* Consultants Bureau, New York, 1964.

Fahim, M. S., Fahim, Z., Der, R., Hall, D. G., and Harman, J., Heat in male contraception (hot water, 60°C, infrared, microwave, and ultrasound), *Contraception* **11,** 549–562, 1975.

Flynn, H. G., Physics of acoustic cavitation in liquids, in *Physical Acoustics,* Vol. IB, Mason, W. P., ed., Academic Press, New York, 1964, pp. 57–172.

Fry, F. J., Kossoff, G., Eggleton, R. C., and Dunn, F., Threshold ultrasonic dosages for structural changes in mammalian brain, *J. Acoust. Soc. Am.* **48,** 1413–1417, 1970.

Fry, W. J., Intense ultrasound in investigations of the central nervous system, In *Advances in Medical and Biological Physics,* Lawrence, J. H., and Tobias, C. A., eds., Academic Press, New York, 1958, Vol. 6, pp. 281–348.

Gavrilov, L. R., Kalendo, G. S., Ryabukhin, V. V., Shaginyan, K. A., and Yarmonenko, S. P., Ultrasonic enhancement of the gamma radiation of malignant tumors, *Sov. Phys. Acoust.* **21,** 119–121, 1975.

Harvey, E. N., and Loomis, A. L., High frequency sound waves of small intensity and their biological effects, *Nature* **12,** 622–624, 1928.

Hawley, S. A., Macleod, R. M., and Dunn, F., Degradation of DNA by intense, noncavitating ultrasound, *J. Acoust. Soc. Am.* **35,** 1285–1287, 1968.

Heimburger, R. F., Fry, F. J., Franklin, T. D., and Eggleton, R. C., Ultrasound potentiation of chemotherapy for brain malignancy, in *Ultrasound in Medicine,* White, D. N., Plenum, New York, 1975, Vol. 1, pp. 2743–281.

Hellman, L. M., Duffus, G. M., Donald, I., and Sunden, B., Safety of diagnostic ultrasound in obstetrics, *Lancet* **1,** 1133–1135, 1970.

Hill, C. R., Ultrasonic exposure thresholds for changes in cells and tissues, *J. Acoust. Soc. Am.* **52,** 667–672, 1972.

Hill, C. R., Joshi, G. P., and Revell, S. H., A search for chromosome damage following exposure of Chinese hamster cells to high intensity, pulsed ultrasound, *Brit. J. Radiol.* **45,** 333–334, 1972.

Holmes, J., Ultrasonic visualization of living tissues, *Abstr. Fed. Proc.* **21,** 304, 1962.

Hrazdira, I., Changes in cell ultrastructure under direct and indirect action of ultrasound, in *Ultrasonographia Medica,* Bock, J., et al., Academy of Medicine, Vienna, 1970, pp. 457–463.

Johnston, R. L., and Dunn, F., Influence of subarachnoid structures on transmeningeal ultrasonic propagation, *J. Acoust. Soc. Am.* **60,** 1225–1227, 1976.

Kamocsay, D., Rona, G., and Tarnoczy, T., Effects of ultrasonics on testicles: experimental studies on white rats, *Arztliche Forschung* **9,** 389–395, 1955, in German.

Kirsten, E. G., Zinssler, H. H., and Reid, J. M., Effect of 1 Mc ultrasound on the genetics of mouse, *IEEE Trans. Ultrason. Engr.* **UE-10,** 112–116, 1963.

Knight, J. J., Effects of airborne ultrasound on man, *Ultrasonics* **6,** 39–42, 1968.

Koranyi, G., Falus, M., Sobel, M., Pesti, E., and van Bao, T., Follow-up examination of children exposed to ultrasound *in utero, Acta Paed. Acad. Scient. Hungar.* **13,** 231–238, 1972.

Kremkau, F. W., and Witkofski, R. L., Mitotic reduction in rat liver exposed to ultrasound, *J. Clin. Ultrasound* **2,** 123–126, 1974.

Kunze-Mühl, E., Chromosome damage in human lymphocytes after different combinations of X-ray and ultrasonic treatment, in *Ultrasonics in Medicine,* Kazner, E., et al., eds., Excerpta Medica, Amsterdam, 1975, pp. 3–9.

Lehmann, J. F., and Krusen, F. H., Biophysical effects of ultrasonic energy on carcinoma and their possible significance, *Arch. Phys. Med. Rehabil.* **36,** 452–459, 1955.

Lele, P. P., Ultrasonic teratology in mouse and man, in *Proceedings of the 2nd European Congress on Ultrasonics in Medicine,* Excerpta Medica, Amsterdam, 1976, pp. 22–27.

Lerner, R. M., Carstensen, E. L., and Dunn F., Frequency dependence of thresholds for ultrasonic production of thermal lesions in tissue, *J. Acoust. Soc. Am.* **54,** 504–506, 1973.

Liebeskind, D., Bases, R., Elequin, F., Neubort, S., Leifer, R., Goldberg, R. and Koenigsberg, M., Diagnostic ultrasound: effects on the DNA and growth patterns of animal cells, *Radiology* **131,** (1), 177–184, 1979a.

Liebeskind, D., Bases, R., Mendez, F., Elequin, F., and Koenigsberg, M., Sister chromatid exchanges in human lymphocytes after expossure to diagnostic ultrasound, *Science* **205,** 1273–1275, 1979b.

Longo, F., Tomashefsky, P., Rivin, B. D., Longo, W. E., Lattimer, J. K., and Tannenbaum, M., Interaction of ultrasound with neoplastic tissue, *Urology* **6,** 631–634, 1975.

Lyon, M. F., and Simpson, G. H., An investigation into the possible genetic hazards of ultrasound, *Brit. J. Radiol.* **47,** 712–722, 1974.

Macintosh, I. J. C., Brown, R. C., and Coakley, W. T., Ultrasound and *in vitro* chromosome aberrations, *Brit. J. Radiol.* **48,** 230–232, 1975.

Macleod, R. M., and Dunn, F., Effects of intense noncavitating ultrasound on selected enzymes, *J. Acoust. Soc. Am.* **44,** 932–940, 1968.

Mannor, S. M., Serr, D. M., Tamari, I., Meshorev, A., and Frei, E. H., The safety of ultrasound in fetal monitoring, *Am. J. Obstet. Gynec.* **113,** 653–661, 1972.

McClain, R. M., Hoar, R. M., and Saltzman, M. B., Teratologic study of rats exposed to ultrasound, *Am. J. Obstet Gynec.* **114,** 39–42, 1972.

McKee, J. R., Christman, C. L., O'Brien, W. D., Jr., and Wang, S. Y., Effects of ultrasound on nucleic acid bases, *Biochem.* **16,** 4651–4654, 1977.

Miller, M. W., Kaufman, G. E., Cataldo, F. L., and Carstensen, E. L., Absence of mitotic reduction in regenerating rat livers exposed to ultrasound, *J. Clin. Ultrasound* **4,** 169–172, 1976.

Montmory, E., and Pourhadi, M., Action d'ultra-sons sur des plasmalemmes isolés á partir de foies de souris adultes: étude biochimique et cytochimique, *C. R. Acad. Sci. Paris* **283,** 1743–1745, 1976.

Morris, S. M., Palmer, C. G., Fry, F. J., and Johnson, L. K., Effect of ultrasound on human leucocytes: sister chromatid exchange analysis, *Ultrasound Med. Biol.* **4,** 253–258, 1978.

Nyborg, W. L., Acoustic streaming, in *Physical Acoustics,* Vol. IIB, Mason, W. P., Academic Press, New York, 1965, pp. 265–331.

Nyborg, W. L., Physical mechanism for biological effects of ultrasound, HEW Publ. FDA 78-8062, US Gov't. Print. Office, Washington, DC, 1977.

O'Brien, W. D., Jr., Ultrasonicaly induced fetal weight reduction in mice, in *Ultrasound in Medicine,* White, D., and Barnes, R., Plenum, New York, 1976, Vol. 2, pp. 531–532.

O'Brien, W. D., Jr., Brady, J. K., and Dunn, F., Morphological changes to mouse testicular tissue from *in vivo* ultrasonic irradiation (preliminary report), *Ultrasound Med. Biol.* **5,** 35–43, 1979.

Parrack, M. O., Effects of airborne ultrasound on humans, *Int'l. Audiology* **5,** 294 (1966).

Peacocke, A. R., and Pritchard, N. J., The ultrasonic degradation of biological macromolecules under conditions of stable cavitation. II. Degradation of deoxyribonucleic acid, *Biopolymers* **6,** 605–623, 1968.

Pizzarello, D. J., Wolsky, A., Becker, M. H., and Keegan, A. F., A new approach to testing the effect of ultrasound on tissue growth and differentiation, *Oncology* **31,** 226–232, 1975.

Pond, J. B., The role of heat in the production of ultrasonic focal lesions, *J. Acoust. Soc. Am.* **47,** 1607–1611, 1970.

Repacholi, M. H., Woodcock, J. P., Newman, D. L., and Taylor, K. J. W., Interaction of low intensity ultrasound and ionizing radiation with the tumor cell surface, *Phys. Med. Biol.* **16,** 221–227, 1971.

Robinson, T. C., and Lele, P. P., An analysis of lesion development in the brain and in plastics by high-intensity focused ultrasound at low-megahertz frequencies, *J. Acoust. Soc. Am.* **51,** 1333–1351, 1972.

Rohr, K., and Rooney, J., Effect of ultrasound on a bilayer lipid membrane, *Biophys. J.* **23,** 33–40, 1978.

Rott, H. D., and Soldner, R., The effect of ultrasound on human chromosomes in vitro, *Humangenetik* **20,** 103–112, 1973.

Selman, G. G., and Counce, S. J., Abnormal embryonic development in Drosophila induced by ultrasonic treatment, *Nature* **172,** 503–504, 1953.

Shoji, R., Momma, E., Shimizu, T., and Matsuda, S., Experimental studies on the effect of ultrasound on mouse embryos, *Teratology* **6,** 119, 1972.

Sikov, M. R., Hildebrand, B. P., and Stearns, J. D., Postnatal sequelae of ultrasound exposure at fifteen days of gestation in the rat (work in progress). Presented before the First Meeting of the World Federation for Ultrasound in Medicine and Biology, San Francisco, August 1976.

Skillern, C. P., Human response to measured sound pressure levels from ultrasonic devices, *J. Amer. Indust. Hyg. Assoc.* **26,** 132, 1965.

Smyth, M. G., Animal toxicity studies with ultrasound at diagnostic power levels, in *Diagnostic Ultrasound,* Grossman, C. C., Holmes, J. H., Joyner, C., and Purnell, E. W., eds., Plenum, New York, 1966, pp. 296–299.

Takeuchi, H., Experimental studies on ultrasonic Doppler method in obstetrics, *Acta Obstet. Gynec. Jap.* **17,** 11–16, 1970.

Taylor, K. J. W., and Dyson, M., Toxicity studies on the interaction of ultrasound on embryonic and adult tissues, in *Ultrasonics in Medicine,* de Vlieger, M., White, D. N., and McCready, V. R., eds., Excerpta Medica, Amsterdam, 1974, pp. 353–359.

Taylor, K. J. W., and Newman, D. L., Electrophoretic mobility of Ehrlich suspensions exposed to ultrasound of varying parameters, *Phys. Med. Biol.* **17,** 270–276, 1972.

Taylor, K. J. W., and Pond, J. B., The effects of ultrasound of varying frequencies on rat liver, *J. Path.* **110,** 287–293, 1970.

Taylor, K. J. W., and Pond, J. B., A study of the production of haemorrhagic injury and paraplegia in rat spinal cord by pulsed ultrasound of low megahertz frequencies in the context of safety for clinical usage, *Brit. J. Radiol.* **45,** 343–353, 1972.

ter Haar, G., and Wyard, S. J., Blood cell banding in ultrasonic standing wave fields: A physical analysis, *Ultrasound Med. Biol.* **4,** 111–123, 1978.

Thacker, J., An assessment of ultrasonic radiation hazard using yeast genetic systems, *Brit. J. Radiol.* **47,** 130–138, 1974.

Thacker, J., and Baker, N. V., The use of Drosophila to estimate the possibility of genetic hazard from ultrasound irradiations, *Brit. J. Radiol.* **49,** 367–371, 1976.

Urry, R. L., Dougherty, K. A., Child, S., Fernandez, F., Cockett, A. T. K., Linke, C., and Carstensen, E. L., Vasocystostomy: II Effects of Ultrasound on spermatogenesis in the rat, *Fertil. Steril.,* in press (1982).

Warwick, R., Pond, J. B., Woodward, B., and Connolly, C. C., Hazards of diagnostic ultrasonography—a study with mice, *IEEE Trans. Sonics Ultrason.* **SU-17,** 158–164, 1970.

Watts, D. L., Hall, A. J., and Fleming, J.E.E., Ultrasound and chromosome damage, *Brit. J. Radiol.* **45,** 335–339, 1972.

Wegner, R. D., Obe, G., and Meyenberg, M., Has diagnostic ultrasound mutagenic effects? *Hum. Genet.* **56,** 95–98, 1980.

Williams, A. R., O'Brien, W. D., Jr., and Coller, B. S., Exposure to ultrasound decreases the recalification time of platelet-rich plasma, *Ultrasound Med. Biol.* **2,** 113–118, 1976.

Woeber, K., The effect of ultrasound in the treatment of cancer, in *Ultrasonic Energy,* Kelly, E., ed., University of Illinois Press, Urbana, 1965, pp. 137–147.

Wood, R. W., and Loomis, A. L., The physical and biological effects high-frequency sound waves of great intensity, *Phil. Mag.* **4,** 417–436, 1927.

Woodward, R., Warwick, R., and Pond, J. B., How safe is diagnostic sonar? *Brit. J. Radiol.* **43,** 719–725, 1970.