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WFUMB Scientific Workshop on Safety of Ultrasound in Medicine
Utsunomiya, Japan, 11–15 July, 1994

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PII S0301-5629(98)00081-7

Organization

WFUMB Symposium on Safety of Ultrasound in Medicine
Kloster-Banz, Germany, 14–19 April, 1996

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PII S0301-5629(98)00080-5

Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. xi–xii, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00072-6

Preface

This document provides a consensus of current scientific opinion pertaining to the safety of diagnostic ultrasound. It is the result of two workshop-style interactive meetings of internationally renowned scientists, chosen for their expertise in acoustic physics and the interactions of ultrasound with biological tissue. The initial workshop was held in 1994 in Utsunomiya, Japan. The proceedings of that meeting were compiled, edited and distributed to other scientists, ultrasound societies and regulatory authorities. This allowed an independent critical peer-review process. The subject was reevaluated and conclusions and recommendations were drawn up during a symposium on thermal and nonthermal mechanisms for biological effects of ultrasound, held April 14–19, 1996, in Kloster-Banz, Germany. Participants in this meeting included scientists and clinicians. A number of observers from industry participated in the discussions.

The Kloster-Banz symposium was the culmination of a series of WFUMB Symposia on Ultrasound Safety, beginning in June 1985. Early WFUMB symposia focussed on the selection and standardisation of pertinent physical parameters in specifying the acoustic outputs of diagnostic ultrasound machines. Appropriate tissue models also were discussed. In 1991, a symposium held in Hornbaek, Denmark, considered biological effects of ultrasound resulting from the capability of ultrasound to heat tissues. This resulted in the publication of the WFUMB-approved document entitled ''WFUMB Symposium on Safety of Ultrasound in Medicine: Issues and Recommendations Regarding Thermal Mechanisms for Biological Effects of Ultrasound, which was published in *Ultrasound in Medicine and Biology* in 1992. (WFUMB 1992)

The most recent symposium held in Kloster-Banz expanded the focus to include all mechanisms. Because of the previous concentration on thermal mechanisms, this symposium placed a special emphasis on nonthermal mechanisms. The work was divided into seven tasks:

(1) Update on thermal bioeffects issues
(2) Nonthermal issues: Cavitation—Its nature, detection and measurement
(3) Other nonthermal mechanisms: Acoustic radiation force and streaming
(4) Free-radical production: Its biological consequences
(5) Other nonthermal bioeffects: Organs, Tissues and Cells
(6) Thresholds for nonthermal bioeffects: Theoretical and experimental basis for a threshold index
(7) Clinical implications

Each task group, consisting of a task group leader and four to six members, was responsible for preparing a review of the salient facts known about each assigned topic and generating a list of conclusions and recommendations. Although the initial generation of each report was the product of the assigned task group, sufficient interaction amongst all the participants warrants that the final document be viewed not as seven separate parts with individual authorship but as one integrated multi-authored document.

The process for obtaining consensus was a purposely deliberate and lengthy one to best ensure consideration of all points of view. The overall plan was initially approved by the WFUMB Council in 1993. A preliminary workshop was held in Utsunomiya, Japan, in July 1994, in which each task group reviewed and summarised its work to date. Voting, indicating level of enthusiasm, was carried out to indicate to each group the degree of approval and support for each conclusion and recommendation. The conclusions provided a summary of the general content of the chapter; thus, acceptance indicated support for the subject matter and its relevance to ultrasound safety.

The proceedings of the Utsunomiya Symposium Workshop were circulated to national society members of the WFUMB and individual scientists for their review and comment. Members of the task groups continued to work on the content of their chapters during the interim period between the initial workshop and the 1996 symposium. A revised list of conclusions and recommendations was intensively reviewed and discussed during the Kloster-Banz symposium, culminating in voting to ascertain acceptance. Each participant voted on each conclusion and recommendation in a closed manner with ''approve,'' ''not-approve'' or ''abstain'' choices. For any statement to be accepted, it had to receive more ''approve'' votes than the sum of ''not-approve'' and ''abstain'' votes. All of the statements in the final document were accepted in this manner. In voting, the participants were instructed to base their decision on their individual best scientific opinion and not necessarily according to any national organisational interests.

With one exception, task groups were not limited to

what they might propose in their list of recommendations. The one exception was they should avoid listing areas requiring additional research. Initially many such areas were identified, but to include them in their document would have the appearance of being self-serving. Nevertheless, it was strongly felt by the participants of this symposium that there are many important gaps in our knowledge and that it is vital that sufficient funding for ultrasound bioeffect research continue to be made available.

On behalf of the WFUMB Safety Symposium Organising Committee, I wish to thank the WFUMB Administrative Council for their support, without which none of this would be possible. I am grateful to my wonderful colleagues on the Organising Committee and to the participants who so tirelessly and expertly created this document. Our Japanese colleagues, including the JSUM, provided gracious hospitality during the initial scientific workshop meeting in Utsunomiya. Special thanks are due to Hans-Dieter Rott, who single-handedly and most competently took care of the local arrangements in preparation for, and during, the symposium in Kloster-Banz.

Finally, I wish to thank Stanley B. Barnett for his expert handling of the editing of the Symposium Proceedings.

Marvin C. Ziskin
Chairman
WFUMB Safety Symposium

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PII S0301-5629(98)00084-2

● *WFUMB Consensus Statements on Thermal Issues*

WORLD FEDERATION FOR ULTRASOUND IN MEDICINE AND BIOLOGY STATEMENTS ON THERMAL EFFECTS IN CLINICAL APPLICATIONS

The following safety statements were endorsed as policy of the World Federation for Ultrasound in Medicine and Biology following recommendations from the 1991 WFUMB Symposium on Safety of Ultrasound in Medicine: Thermal Issues. The conclusions from the 1996 WFUMB Symposium on Safety of Ultrasound in Medicine are that there is no scientific evidence to alter the existing safety statements on thermal issues. Hence, the WFUMB Safety Statements for Thermal Bioeffects are reiterated to complete the current safety guidelines.

B-mode imaging
Known diagnostic ultrasound equipment as used today for simple B-mode imaging operates at acoustic outputs that are not capable of producing harmful temperature rises. Its use in medicine is therefore not contraindicated on thermal grounds. This includes endoscopic, transvaginal and transcutaneous applications.

Doppler
It has been demonstrated in experiments with unperfused tissue that some Doppler diagnostic equipment has the potential to produce biologically significant temperature rises, specifically at bone/soft tissue interfaces. The effects of elevated temperatures may be minimised by keeping the time for which the beam passes through any one point in tissue as short as possible. Where output power can be controlled, the lowest available power level consistent with obtaining the desired diagnostic information should be used. Although the data on humans are sparse, it is clear from animal studies that exposures resulting in temperatures $<38.5°C$ can be used without reservation on thermal grounds. This includes obstetric applications.

Transducer heating
A substantial source of heating may be the transducer itself. Tissue heating from this source is localised to the volume in contact with the transducer.

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PII S0301-5629(98)00085-4

● *WFUMB Symposium Recommendations*

WFUMB SYMPOSIUM ON SAFETY OF ULTRASOUND IN MEDICINE RECOMMENDATIONS ON THE SAFE USE OF ULTRASOUND

At the completion of the symposium in Kloster-Banz, 1996, the following recommendations were agreed as representing international consensus on thermal and nonthermal issues. These were approved by the WFUMB Executive Council in March 1997.

Recommendations: Thermal effects

- A diagnostic exposure that produces a maximum *in situ* temperature rise of no more than 1.5°C above normal physiological levels (37°C) may be used clinically without reservation on thermal grounds.
- A diagnostic exposure that elevates embryonic and fetal *in situ* temperature above 41°C (4°C above normal temperature) for 5 min or more should be considered potentially hazardous.
- For diagnostic ultrasound systems that are capable of producing a tissue temperature increase >1.5°C above normal, users should be provided with worst-case estimates of the temperature increase for all pertinent operating modes.
- The risk of adverse effects of heating is increased with the duration of exposure. Thus, safety guidelines should include an appropriate duration factor.
- Care should be taken to avoid unnecessary additional embryonic and fetal risk from ultrasound examinations of febrile patients.
- If temperatures above 41°C may occur at the surface of an intracavitary transducer when in use, such equipment should provide temperature information to the user during equipment operation.

Recommendations: Nonthermal effects

- The possible occurrence of cavitation, either inertial or noninertial, should be considered in assessing the safety of diagnostic ultrasound and of other forms of medical ultrasound.
- Caution is required in applying results of *in vitro* ultrasound biological effect studies to medical ultrasound exposures *in vivo.*
- It has been shown experimentally that acoustic cavitation can alter mammalian tissues. It is therefore important to consider its significance in medical applications of ultrasound.
- Currently available animal data indicate that it is prudent to reduce ultrasound exposure of human postnatal lung to the minimum necessary to obtain the required diagnostic information.
- Estimates of tissue field parameters at the point of interest should be based on derated values calculated according to an appropriate specified model and be extrapolated linearly from small signal characterisation of source–field relationships.
- The acoustic *pressure amplitude* at the point of interest for a given frequency should be used as a *primary* index of the potential for adverse, nonthermal biological effects of an ultrasound exposure.
- A risk–benefit analysis should be performed if anticipated acoustic pressure amplitude at the surface of postnatal lung tissue exceeds 1 MPa.
- Safety evaluations should consider the characteristics of the site of ultrasound exposure. Thresholds for nonthermal biological effects are lowest in:
  (a) tissues that naturally contain gas bodies, *e.g.*, postnatal lung and intestine, and
  (b) all tissues in the presence of introduced gas bodies, *e.g.*, ultrasonic contrast agents.
- *In vitro* studies: Because the probability of cavitation is much greater for *in vitro* conditions, one must be cautious in applying *in vitro* experimental results to the clinical situation.
- Contrast agents: Gas bodies introduced by a contrast agent increase the probability of cavitation. A physician should take this into account when considering the benefit–risk ratio of an examination.
- Pulmonary capillary bleeding: In considering the significance of ultrasound-induced pulmonary red blood cell extravasation and capillary bleeding, one must realize that these effects also can occur as a result of coughing and can occur spontaneously in neonates. For the most part, unless there is extensive hemorrhage, the clinical significance is negligible, and its occurrence would be difficult to detect. Nevertheless, it is prudent to reduce ultrasound exposure to the postnatal lungs to the minimum necessary to obtain the required diagnostic information.
- B-mode imaging: When tissue–gas interfaces or contrast agents are not present, the use of B-mode imaging

need not be withheld because of concern for ultrasound safety. This statement also applies to endoscopic, transvaginal and transcutaneous applications. When tissue–gas interfaces or contrast agents are present, ultrasound exposure levels and durations should be reduced to the minimum necessary to obtain the required diagnostic information.

- Doppler: When tissue–gas interfaces or contrast agents are not present, and where there is no risk of significant temperature elevation, the use of diagnostic Doppler equipment need not be withheld because of concern for ultrasound safety. When any of the above conditions might be present, ultrasound exposure levels and durations should be reduced to the minimum necessary to obtain the required diagnostic information.
- Information about clinically relevant quantities describing, for example, the anticipated temperature elevation or the potential for cavitation should be made available to health care professionals. Methods for making this information available include, for example, the continuously updated display of a thermal index and mechanical index, and the classification of ultrasound fields.
- Education: Diagnostic ultrasound has potential for both false-positive and false-negative results. Misdiagnosis is far more dangerous than any effect that might result from the ultrasound exposure. Therefore, diagnostic ultrasound should be performed only by persons with sufficient training and education.

Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. S1–S10, 1998
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PII S0301-5629(98)00078-7

Chapter 1

UPDATE ON THERMAL BIOEFFECTS ISSUES

INTRODUCTION

The previous WFUMB Symposium on Safety and Standardisation of Ultrasound in Medicine addressed various issues relating to thermal mechanisms for biological effects of ultrasound. The topic stimulated extensive debate during the initial workshop in Geneva (1990) and the subsequent symposium at Hornbaek, Denmark (1992). International consensus was achieved, resulting in the formulation of key statements and recommendations on thermal effects in clinical applications of ultrasound. Three statements on 1) B-mode imaging, 2) Doppler ultrasound and 3) transducer heating have since been formally adopted as WFUMB policy on safety guidelines. The established WFUMB guidelines on safety with regard to thermal issues are as follows:

B-mode imaging

Known diagnostic ultrasound equipment as used today for simple B-mode imaging operates at acoustic outputs that are not capable of producing harmful temperature rises. Its use in medicine is therefore not contraindicated on thermal grounds. This includes endoscopic, transvaginal and transcutaneous applications.

Doppler

It has been demonstrated in experiments with unperfused tissue that some Doppler diagnostic equipment has the potential to produce biologically significant temperature rises, specifically at bone/soft tissue interfaces. The effects of elevated temperatures may be minimised by keeping the time for which the beam passes through any one point in tissue as short as possible. Where output power can be controlled, the lowest available power level consistent with obtaining the desired diagnostic information should be used.

Although the data on humans are sparse, it is clear from animal studies that exposures resulting in temperatures < 38.5°C can be used without reservation on thermal grounds. This includes obstetric applications.

Transducer heating

A substantial source of heating may be the transducer itself. Tissue heating from this source is localised to the volume in contact with the transducer.

Since the publication of the proceedings and conclusions of the WFUMB 1991 Symposium (WFUMB 1992), there have been two substantial reports from within the USA (AIUM 1994; NCRP 1992) and some publications (detailed within this chapter) on ultrasound-induced heating and bioeffects of hyperthermia. Nevertheless, significant limitations remain in the scientific data base on bioeffects of ultrasound-induced hyperthermia, and this chapter draws attention to these important issues. It presents an updated review of the topic that can be understood without referring to the previous WFUMB publication (WFUMB 1992). The conclusions reflect the current status of research on biological effects of ultrasound-induced heating.

It should be noted that, since 1991, the acoustic outputs from ultrasound scanners in clinical use have increased substantially. During the same time, the regulation of exposure from diagnostic equipment has changed, placing greater responsibility on the user for exposure control and risk–benefit assessment. The trend for increasing acoustic output has continued. Henderson et al. (1995) reported that the measured I_{spta} values from diagnostic equipment in current clinical use in the UK have increased by approximately a factor of five in B-mode applications since 1991. The total acoustic power output has doubled in pulsed Doppler mode during that period. This increase in available acoustic power will provide greater opportunity for ultrasound-induced heating and biological consequences. It is important to evaluate the extent of heating that may be produced by diagnostic equipment (Barnett 1998; Barnett et al. 1994).

MEASURED TEMPERATURE INCREASE INDUCED BY ULTRASOUND

In vitro soft tissue

Exposures from commercial equipment or laboratory instruments simulating diagnostic scanners have produced substantial temperature increases in biological

tissues when used in pulsed Doppler mode. There is a consistency in the results obtained from studies that use similar fine wire thermocouples in unperfused tissues. Exposure conditions of a 5-MHz mechanical sector scanner (beam width = 1.9 mm, I_{spta} = 2.0 W·cm^{-2}) gave a maximum temperature increase (ΔT_{max}) of 1.9°C after 2 min in fresh pig liver (ter Haar et al. 1989). The maximum temperature increase in freshly excised sheep brain was found to be 2.5°C after 5-min exposure using similar equipment (WFUMB 1992). Exposure in a water tank to 3.2-MHz (simulated pulsed Doppler, −6 dB beam width = 2.5 mm, I_{spta} = 2.95 W·cm^{-2}) increased the temperature in freshly excised guinea pig brain by 2.5°C after 2 min (Bosward et al. 1993). These experiments in excised tissue do not allow for cooling by vascular perfusion. However, this may be compensated by substantial cooling effects from the bulk fluid streaming in the water tank exposures, as demonstrated by Wu et al. (1994).

In vitro bone

Substantial temperature increases occur when bone is situated within the ultrasound beam. Temperature increases of approximately 5°C have been reported in the brain close to the parietal bone in guinea pig fetuses (Bosward et al. 1993; Horder et al. 1993). Although the peak temperature has been shown to increase as a function of gestational age in fetal guinea pig brains (Bosward et al. 1993) and in fetal human femurs (Drewniak et al. 1989), it is not certain how much is due to the increasing mineral composition compared to the increasing thickness and lateral dimensions of the bone targets.

In vivo cranial

The susceptibility of the central nervous system (CNS) to damage by increased temperature creates particular interest in measurements of heating at the bone interface and in adjacent brain tissue. Using the mouse skull as a model for human fetal insonations, Carstensen et al. (1990) recorded temperature elevations > 5°C after 90 s in anaesthetised animals exposed to either continuous-wave (cw) or pulsed-wave ultrasound at an intensity (I_{spta}) of 1.5 W·cm^{-2}. The largest temperature increase was observed in older mice and approached 4°C within 15 s. The −6 dB focal beam width was 2.75 mm. Temperature measurements after death were approximately 10% higher, indicating that perfusion had a minimal cooling effect beyond that of conduction. Similarly, Horder et al. (1997) found little difference in temperature increase (ΔT) measured at the skull–brain interface before and after death in guinea pig fetuses insonated *in utero* at 57–61 days of gestational age (dga). A mean peak temperature increase of 4.9°C was measured at the inner aspect of the skull parietal bone of 60 dga fetuses during exposure to 2.5 W·cm^{-2} I_{spta} for 120 s. The −6 dB beamwidth was 2.7 mm. However, in fetuses near to term (62–67 days of gestation), the mean peak temperature increase was reduced by approximately 12% to 4.3°C as a result of cooling from more substantially developed cerebral vasculature. In experiments using a relatively large beam (−6 dB beam width of 16 mm), cooling effects of vascular perfusion were found to be dominant (Duggan et al. 1995) in limiting the magnitude of temperature increase in the cerebral cortex in sheep fetuses insonated *in utero*. Exposure to 0.3 W·cm^{-2} spatial average, temporal average intensity (I_{sata}) for 120 s produced a mean temperature increase of 1.7°C, which was approximately 40% lower than the postmortem value, giving support to the theory proposed by Thomenius (1990) to predict the steady-state temperature rise. In this study, thermocouples and a transducer were surgically attached to the fetal skull and insonation was applied after a recovery period of approximately 7 days. Hence, ultrasound was applied during the normal behavioural state without the need for anaesthesia. Temperature was measured in soft tissue at a depth of 1–2 mm from the parietal skull bone.

Another study using large animals measured the intracranial temperature in ketamine-sedated monkeys (Tarantal et al. 1993) during insonation from a diagnostic scanner. Both the thermocouple and the transducer were manually held in place by the operator during the procedure. A thermocouple was inserted to some depth in the brain to the medial aspect of the left cerebral hemisphere. The depth of the structure of interest was 3.6–4.6 cm. The greatest temperature elevation recorded was 0.6°C, and this did not differ between pulsed Doppler and B-mode imaging. It should be noted that the quoted I_{spta} (in water) values were 54 and 27 mW·cm^{-2}, respectively. These output values are very low compared to the maximum free-field values reported by Henderson et al. (1995) for current equipment; pulsed Doppler ≈ 9,000 mW/cm^2 and B-mode ≈ 990 mW·cm^{-2}. The median I_{spta} intensity value for pulsed Doppler clinical equipment used in the UK is given as 1180 mW·cm^{-2} (Duck and Henderson 1997).

Tissue-mimicking phantoms

Water-based gels have been used to provide a homogeneous material with acoustic properties similar to soft tissue. For heating due to ultrasound absorption at or near to the geometric focus, Wu et al. (1992) reported reasonable agreement between measured and predicted values using the NCRP (1992) model, within the range of 1–3.5 MHz. A similar result was reported by this group (O'Neill et al. 1994) when measurements at a bone sample embedded in soft tissue-mimicking medium were

compared to the worst-case estimated value for unscanned beams (NCRP 1992).

In a study on temperature rises generated by diagnostic equipment in a phantom model of transcranial exams (using human temporal bone), Wu et al. (1995) found that the calculation of cranial thermal index (TIC) consistently underestimated the temperature rise measured at the external bone interface. They demonstrated a difference in heating that may be due to conduction from surface heating of the transducer based on the results of their measurements in the phantom. The study by Wu et al. (1995) used a modified HP Sonos 1000 diagnostic imaging system operating at 2 MHz in combined (color, sector, pulsed Doppler) modes where the measured total power ranged from 95–295 mW. With the transducer positioned 0.2 cm from the phantom and operating at the highest power, a temperature increase of 11°C above ambient (20°C) was measured on the external surface of the temporal bone after 20 min of exposure. An increase of 8°C was recorded on the internal aspect. The combined mode that produced only 95 mW acoustic power generated a temperature increase of 5.1°C on the external surface of the bone, which is a factor of 2.7 higher than the temperature increase predicted by TIC. A temperature increase of 3.9°C was measured on the internal surface of the bone. The heat generated in bone was reduced by 33% when the water path distance between the transducer and phantom was increased by 1 cm.

Most studies on tissue path models use a single piece of bone as an absorbing biological target. Thus, they do not show the wide variability in temperature increase that is commonly associated with ultrasound-induced heating in bone. Furthermore, it should be noted that Wu et al. (1995) used human temporal bone that had been embalmed for 2 years. This process causes leaching of mineral content from the bone and also may alter the geometric patterns of the trabecular bone so that the results obtained may considerably underestimate temperature increase occurring with fresh vital human skull bone.

ESTIMATED ULTRASOUND-INDUCED TEMPERATURE INCREASE

A large amount of data has been published on the estimated temperature elevation in tissue for unfocused (Nyborg and Steele 1983) and focused (NCRP 1992; Thomenius 1990) beams. The NCRP model estimates the *worst-case* steady-state heating that would *never be exceeded* in diagnostic ultrasound examinations. The AIUM/NEMA values were derived using a tissue path attenuation coefficient of 0.3 dB/cm.MHz and are *not expected to be exceeded in the majority* of ultrasonographic examinations. In a report of an analysis by the AIUM Bioeffects Committee (AIUM 1994), comparison of the NCRP (1992) model for the worst-case maximum estimated temperature increase (ΔT_{max}) and the AIUM/NEMA (1992) estimate of temperature increases in homogeneous soft tissue showed differences by as much as a factor of 3. Measurements with test objects using tissue-mimicking materials and thin film thermometry have found the AIUM/NEMA predictions of the thermal index for soft tissue (TIS) to underestimate the temperature increase by up to a factor of 2 (Bacon and Shaw 1993).

The comparison between estimated and measured ΔT were based on a soft tissue absorption coefficient of 0.05 Np $cm^{-1}MHz^{-1}$ as specified in the NCRP report (NCRP 1992). The experimental procedures used nine focussed transducers covering a range of 2–10 MHz. About half of the transducers gave values of a factor of two higher than that predicted by the AIUM/NEMA soft tissue TI model. There was closer agreement with the NCRP model. The difference may be due to variations in the choice of attenuation properties for the target tissue. All thermal measurements were performed at the focus of the beam, which is not necessarily the position of greatest heating. Also, the comparisons were only made for soft tissue so that the results cannot be applied directly to estimates of bone heating.

First trimester heating

Bly et al. (1992) applied the NCRP (1992) model to estimate temperature increases in obstetric examinations, based on the maximum outputs of pulsed Doppler (non-fetal) equipment used in Canada. He found a maximum value in the first trimester of pregnancy of 1.6°C, whereas the majority of exposures would give ΔT_{lim} (*i.e.*, the maximum value) below 1°C. For equipment operating at the FDA regulated intensity limit of $I_{spta} = 720$ mW·cm^{-2} (derated), the best available estimate of maximum temperature rise in the conceptus is reported as approximately 2°C (AIUM 1994).

Fetal heating

When bone is present in the second and third trimester fetus, the ultrasound-induced temperature rise is substantially increased. Bly et al. (1992) calculated ΔT_{lim} of 8.7°C in the third trimester using the worst-case fixed path attenuation model. Patton et al. (1994) calculated the worst-case temperature increase in bone (ΔT_{Blim}) for fetal exposures to be 5.9°C. This was based on output data obtained from diagnostic equipment approved by the FDA under section 510(k) requirements during the period 1990–1991. This value was found to be a factor of four greater than that obtained using the FDA homogeneous tissue model.

BIOLOGICAL EFFECTS OF HEATING

This overview is intended to complement the report of the 1991 WFUMB Symposium on Safety of Ultrasound in Medicine and to support the conclusions of this chapter. *In vivo* and *in vitro* data resulting from studies focusing on the effects of heating on biological systems have been reported in the literature. It is generally accepted that tissues containing a large component of actively dividing cells are sensitive to the effects of heat (Barnett et al. 1997; Edwards et al. 1995; WFUMB 1992). Abnormalities in cell physiology or the rate of DNA synthesis can occur following exposure to increased temperatures above normal basal levels. Commonly reported effects of heating on embryonic development are the apparent retardation of growth of systems such as the heart, brain and skeleton (Kimmel et al. 1993a). Generalised fetal weight reduction is often associated with intrauterine heating or maternal stress.

Brain development

Developing embryos may mount a protective response to adverse sublethal environmental conditions, such as hyperthermia, which temporarily arrests mitosis. This phenomenon has been observed in the brains of rodents in which division lapsed for as much as 8 h following a single heat treatment (Edwards et al. 1974; Upfold et al. 1989) and heat shock proteins were synthesised in place of normal neural proteins. Normal cell division resumed after 8 h, with the fetus appearing morphologically normal albeit smaller and with a substantial neural deficit. Nondeforming retardation of brain growth and reduced learning performance are common abnormalities in the offspring of moderately heat exposed pregnant guinea pigs. These defects can be caused during both early and later fetal growth (Edwards 1993). In general, embryos are more susceptible to damage than fetuses due to the high rate of cellular activity during organogenesis. However, continually developing organ systems, such as the brain, remain susceptible to heat throughout pregnancy.

The biological consequences of a hyperthermic episode depend on the magnitude of temperature elevation and the duration of exposure. Data are available for whole-body exposures (generally resulting in severe abnormalities) where it has been shown that rat embryos exposed to a temperature increase of 4°C for 5 min developed encephaloceles (Edwards 1993; Germain et al. 1985). Other gross malformations commonly reported include anencephaly, microphthalmia, micrencephaly, maxillary hypoplasia and facial clefting. These results are statistically robust and repeatable, and the bioeffects are irreparable.

THRESHOLDS FOR BIOLOGICAL EFFECTS

The effects of increased temperature on biological systems have been reviewed extensively (Barnett et al. 1994; Edwards 1993; Miller and Ziskin 1989; NCRP 1992; WFUMB 1992). Recently, the Bioeffects Committee of the AIUM prepared a report on thermal and nonthermal effects of ultrasound (AIUM 1994), which includes recommendations with a time factor, stating that:

(a) ''for exposure durations up to 50 hours, there have been no significant adverse biological effects observed due to temperature increases less than or equal to 2°C above normal'', and

(b) ''for temperature increases of 4°C and 6°C (above normal), the corresponding limits for the exposure duration (t) are 16 min and 1 min, respectively.''

However, contrary to the conclusions of the report, table 4.3 in the accompanying text provides data for the lowest reported thermal exposures associated with teratogenic effects and lists 26 abnormalities in mammals where the temperature was increased by $\leq$ 2°C above the normal physiological temperature. The abnormalities were mostly reported in guinea pigs heated in incubators where the absolute air temperature was 41°C (*i.e.*, 1.5°C above normal for guinea pig species). This apparent discrepancy may be due to the Bioeffects Committee's reliance on an approximate data table from a veterinary clinical handbook (Kirk and Bistner 1995) rather than taking data from peer-reviewed publications in the scientific literature. Edwards (1969) measured the core temperature of 86 guinea pigs and obtained a mean resting temperature of 39.4° $\pm$ 0.28°C. Table 6–19 of the handbook (Kirk and Bistner 1995) contains a number of other inaccuracies. For example, it shows a gestation range of 62–72 days for guinea pigs when experience with many hundreds of pregnancies shows it to be 66–68 days. There also are problems in conversion of Fahrenheit to Celsius temperature scale, where the body temperature for gerbils is shown as 100.8°F and 32.8°C (an error of 5.4°C).

It should be noted that the studies reporting effects after temperature elevations of $<$ 2°C were not actually designed *a priori* to identify threshold levels. They typically involved heat exposures of 60 min because it takes approximately 30 min to overcome the normal maternal homeostatic response and elevate the maternal core temperature. In most cases, neither the temperature elevation nor the duration of hyperthermic exposure within the fetus was measured. The heat dose was estimated from the maternal core temperature measured *per rectum.* Recent studies with rats, heated in a hot air incubator, have reported time constants on the order of 13 min per 1°C elevation in core temperature (Kimmel et al. 1993a).

This is an extremely slow rate of heating compared to that caused by ultrasound absorption in which substantial elevations occur within seconds.

A study that was designed to identify a threshold of heat exposure used water immersion body heating and the development of encephalocoeles in rats as a gross endpoint (Germain et al. 1985). The results found the shortest exposure to be 1 min at 43.5°C (5° ΔT for rats). The same effect occurred after 5 min of exposure to a temperature elevation of 4°C. This threshold core temperature of 42.5°C for 5 min has been confirmed recently in rats (Sasaki et al. 1995) in which hyperthermia was achieved using a water bath. The majority of malformations involved microphthalmia and encephalocoeles. The resting core temperature measured in all rats prior to heat treatment was $> 38° < 39$°C.

Webster and Edwards (1984) also have reported development of a major brain abnormality, exencephaly, in mice following intrauterine exposure for 5 min at 42.3°C (*i.e.,* the normal body temperature was increased by 4.3°C ΔT). The resting temperature for rats was established from measurement of the core temperature of 50 rats during daylight (*i.e.,* when less active, resting) and gave a mean value of $38.5° \pm 0.5$°C (Germain et al. 1985). This temperature of 38.5°C has since been used to successfully culture rat embryos in test tubes with consistent growth and development (Angles et al. 1990; Walsh et al. 1987).

The shortest duration of exposure resulting in a significant biological effect was reported recently in a study in which pregnant rats were heated, on day 10 of gestational age (dga), in air incubators until their core temperature reached 41°C (an increase of 2.5°C above normal). Upon evaluation of the embryos 24 h later, the total number of somites was significantly reduced (Cuff et al. 1993). When the ''dose'' to the conceptus was increased to 42°C ($\Delta T = 3.5$°C) for 5 min, decreased head length and protein content also were observed. Abnormal skeletal development was reported when the core temperature of pregnant rats was raised above normal by 2.5°–3.4°C (*i.e.,* 41°–41.9°C). In this study 90% of pups exhibited malformed axial skeleton, including fused ribs and vertebrae (Kimmel et al. 1993a). The rats were heated in a warm air chamber until the core temperature reached the required elevated level and then were immediately withdrawn. Unfortunately, no information was provided on the time required for heat dissipation so that the total duration of exposure to elevated temperature is uncertain. It is probable that this would have taken $\approx$ 40 min, and that for 20 min of the time the temperature was above 40.5°C. On termination of heating, the maternal core temperature may have remained above 40°C for an additional 20 min.

In 1988 Shiota reported a threshold for exencephaly in mice as being 5 min at 4.5°C increase above their normal body temperature. There is evidence from repeated studies of major maldevelopment of brain structures in a range of rodent species when the body temperature was increased by $\approx$ 4°C for 5 min (Edwards et al. 1995).

Impaired embryo development also has been reported in a study using an embryo culture system (Kimmel et al. 1993b) where reduced crown–rump length and morphological abnormalities in the brain occurred in rats after 10 min of exposure to 42°C. Delayed development of the branchial bars and forelimbs also was observed. It is important to note the absolute temperature because this study chose to use 37°C as the culture temperature, whereas the resting temperature for rats has been shown to be 38.5°C (Germain et al. 1985). The temperature at which the effects were reported is 3.5°C above the normal body temperature for rats. The same exposure conditions (whole body hyperthermia at 3.5°C above normal for 10 min) produced exencephaly in mice (Shiota 1988) and microphthalmia in rats (Edwards et al. 1995).

Studies with rat embryo culture systems have demonstrated a stress-evoked response to elevated temperature and ultrasound interaction. The production of heat-shock proteins (HSP) and retarded embryonic development has resulted from exposure to hyperthermia alone (Walsh et al. 1987), or from exposure to pulsed ultrasound (spatial peak, temporal average intensity I_{spta} of 1.2 $W \cdot cm^{-2}$, for 15 min) together with a 1.5°C elevation in culture temperature during the insonation (Angles et al. 1990; Barnett et al. 1990). The mechanism for ultrasound-mediated effects has not been identified but bulk heating of the culture medium is not responsible.

Mirkes et al. (1994) used a specialised electrophoresis gel technique to produce cleavage of DNA into oligonucleotide fragments that are associated with apoptosis in the neuroepithelium of rat embryos. This occurred following exposure in culture to a temperature increase (ΔT) of 3.5°C above normal for 15 min. The result may be impaired development of the fetal brain. It is possible that a similar biological mechanism may occur at lower temperature thresholds when ultrasound is applied together with an elevated temperature. This subject of potential synergism between ultrasound and elevated maternal core temperature requires further research.

Within the fetal hematopoietic system, bone marrow is the main site of blood formation in the third trimester of pregnancy. Abnormalities have been reported in the nuclei of neutrophils in proliferating bone marrow cells in adult guinea pigs following exposure to systemic heating or localised ultrasound-induced hyperthermia. The former study exposed animals to 42.5°–43.5°C in a hot air incubator for 60 min and reported abnormal nuclear segmentation in neutrophils (Edwards

and Penny 1985). The duration of exposure needed to cause the effect was reduced considerably in the ultrasound study. When ultrasound was used to elevate the temperature in the marrow, the same cellular abnormalities were observed following exposures to 43°C (ΔT 3.5°C) for 4 min (Barnett et al. 1991).

Studies at the cellular level have been undertaken in cell culture protocols in which cells were isolated from the effect of surrounding tissue so that bulk heating effects are unlikely. The endpoints studied usually include gross effects on cell survival and/or mutagenicity. There are insufficient data currently available to assess risk at the subcellular level where the most sensitive site may be the cell membrane and the signal transduction pathway. The kinetics of biochemical processes are clearly temperature sensitive, but little research has been directed toward such endpoints. It is difficult to perceive of a biological interaction that does not involve some amount of heating, at least at the molecular level. The existing data base draws mainly on gross effects of bulk heating.

There is evidence that mild heat shock can elicit cellular responses that do not alter the apparent structural integrity of the cell but alter normal biological processes. Roberts and Sandberg (1979) reported an enhanced response in human leukocytes to specific migration factors by incubating these cells at a temperature 1.5°C above normal, thus demonstrating that modest elevations in temperature are capable of altering normal cellular functions. In addition, it has been shown that increasing the temperature 1°C is sufficient to induce thermotolerance in some mammalian cells growing in culture (Joshi and Jung 1979).

The evidence from studies on laboratory animals of the effects of ultrasound-induced temperature increase in embryonic and proliferating tissue indicates that biologically significant abnormal events occur after exposures of 4°C above normal for 5 min.

ASSESSMENT OF RISK TO HUMAN HEALTH

Hyperthermia is accepted as a teratogen in mammalian biological systems (Bell 1987; Edwards 1986) and is considered to be a human teratogen (Edwards et al. 1995; Jones 1988; Shepard 1982, 1989). Many of the defects caused by hyperthermia in animal studies also have been found in children following *in utero* febrile episodes (Erickson 1991; Jones 1988). Neural tube defects following exposure to hyperthermia in early pregnancy was reported in a small study (Fisher and Smith 1981). More recently, in a study population exceeding 23,000 women, Milunsky et al. (1992) reported that exposure to heat above 38°C (from sauna, hot tub, electric blanket or fever) during the first 8 weeks of pregnancy was associated with a significant increase in the risk of neural tube defects. A difficulty with human studies is the absence of quantitative measure of ''thermal dose'', *i.e.,* internal body temperature elevation and the duration for which it was maintained. Nevertheless, data from the study by Milunsky et al. (1992) are in agreement with findings of other studies in both nonhuman primates (Hendrickx et al. 1979; Poswillo et al. 1974) and humans. Smith et al. (1978) reported that maternal febrile illness that caused the human body temperature to rise above 38.9°C in early stages of pregnancy was associated with fetal anomalies. Thus, long-term hyperthermia above 39°C may be teratogenic to the human fetus. Data from retrospective studies indicate that mothers of babies with various CNS malformations experienced increased prevalence of febrile illness during early pregnancy (Layde et al. 1980; Pleet et al. 1981; Shiota 1982; Spraggett and Fraser 1982). Whilst these studies demonstrate that hyperthermia is a common teratogen, the important question to be answered relates to the duration and degree of exposure required to produce the effect.

There are uncertainties in the extrapolation of biological effects of whole body hyperthermia in small laboratory animals to human risk from ultrasound induced heating. One of the greatest difficulties lies in determining the equivalent exposure conditions in humans. Normal resting body temperature varies by up to 3°C among homeothermal mammalian species. A fundamental issue is whether or not to adopt the notion that an absolute temperature is critical for normal biochemical processes and development of all mammalian biological systems. It may be more acceptable that the catalysts for biochemical reactions, enzyme systems and biomolecular messengers have operating temperatures that are optimised for each species, with similar limited ranges of tolerances. The conservative approach would be to adopt the latter proposition and translate the lowest threshold values, in terms of elevated temperature above normal basal levels for any mammalian species, into the threshold for human safety. In fact, some eminent researchers have stated that there is a common misconception that ''there is a single absolute threshold for all species of animals, regardless of their normal temperature'' (Edwards et al. 1995).

Recent evidence from studies on molecular biology show that the heat-shock response is related directly to a temperature increase and not to an absolute temperature in mammalian species. Heat-shock factor (HSF), which mediates the expression of HSP genes, is activated at a temperature set-point that varies among species. Rather than responding to an absolute temperature within the mammalian cell, HSF is activated by a temperature change (Abravaya et al. 1991). This individual response to temperature elevation was taken a step further when

Sarge et al. (1995) showed that HSF1 was activated at different absolute temperatures, but similar temperature increases, for different organs in the same animal. They reported that, in mice, HSF binds to HSP at 42°C in the liver (37°C organ temperature) and at 36°C in the testis (30°C organ temperature). One explanation may be that the HSF activation temperature is directly related to the protein denaturation profile characteristics of each cell type. The incubation period for these experiments was 60 min.

There is some evidence that heat-shock gene expression is not entirely genetically determined but also correlates with the normal body temperature or temperature at which the organism lives. Dietz and Somero (1992) reported a 4°C difference in the temperature at which HSP 90 gene is induced in eurythermal goby fish, depending on whether they were acclimatized to 10° or 20°C. Differences have been reported within the same animal for organs with varying physiological temperature.

The lowest temperature at which teratogenic effects have been repeatedly reported in mammals is 41°C. However, the conditions of long-term exposure to whole body heating make it difficult to extrapolate to risk from heating in human examinations. A further complication is the extent of heating. If a 41°C temperature is translated directly to humans as an absolute temperature threshold, it represents a temperature increase of $\approx$ 4°C or an increase in fetal temperature of $\approx$ 3.5°C. However, it is equivalent to a temperature increase of only 2.5°C in the rat. The bulk of the literature shows adverse developmental effects require exposure to a temperature increase greater than 1.5°C above normal body temperature.

Most experiments have involved placing pregnant animals in a hot air incubator, thereby subjecting them to the stress of whole body heating for protracted durations (typically 60 min) to overcome the normal maternal homeostasis. However, the potential for consequent effects of compromised maternal physiology are not reported. Similarly, the teratogenic effects of fever in humans result from periods of many hours at elevated body temperature. Similar biological effects are reported in embryo culture systems, and these may offer the best available methods to determine thresholds of short-duration exposures.

Current estimates of risk from ultrasound-induced heating are mostly based on existing data on whole body heat exposure in animal studies, in the absence of ultrasound. The effect on cell development probably differs from that due to the rapid onset of ultrasound-induced heating. Acoustic absorption is immediate, resulting in deep tissue temperature elevation within seconds of exposure, compared to tens of minutes required to elevate fetal temperature under whole-body heating conditions. It takes some time to synthesise HSPs; therefore, potential protective mechanisms that may be mounted by cell reactions to whole body hyperthermia would be overridden in rapid ultrasound-induced heating. The degree of temperature increase is directly dependent on the acoustic absorption coefficient of the target tissue. The heated tissue volume is small and usually restricted to a volume of < 0.5 cm^3. The effects of damaging a small volume depend on the sensitivity of the target tissue. From a safety perspective, it is essential that the worst-case situation be considered.

International committees and working groups are currently developing test objects to measure the maximum temperature elevations in fields emitted by diagnostic scanners. The biophysical basis for safety classification will be determined by information available on the effects of whole body hyperthermia, which may not necessarily be accurate. Estimates of risk based on whole body heating do not give any information on the probable potentiating effects of ultrasound interactions when accompanied by moderate temperature elevations.

Exposure to some diagnostic equipment operating in pulsed Doppler mode has been shown to produce biologically significant temperature increases in tissue, particularly when bone is present. Under the latest Track 3 option for FDA compliance in the USA, manufacturers may obtain market approval for equipment having an output display in which the only limit is a maximum I_{spta} intensity of 720 mW·cm^{-2} estimated in tissue. This means that there would be no application-specific limit on output, and that the fetal exposures could be increased by almost a factor of 8 over the previously allowed value. This could occur with multimode imaging systems. Note that the fetal body temperature is reported to be normally about 0.5°C higher than the maternal temperature in humans (Walker et al. 1969; Wood and Beard 1964).

DISCUSSION

There are many reports of gross effects of hyperthermia on prenatal development in animals. Hyperthermia is recognised as a potential human teratogen. It is probable that the same effects would occur following ultrasound exposure, producing similar elevations of temperature above normal physiological levels. The scale of effect may be expected to be less in humans compared to small animal exposures due to the ratio of embryonic/fetal body size to beam width. However, subcellular effects of heating within the intense focal zone of ultrasound beams have not been studied adequately. There is a substantial data base of evidence that fetal brain development is significantly affected in laboratory animals by whole body exposure of the pregnant mother

to a temperature elevation > 2°C. The effect of hyperthermia is dependent on a combination of temperature rise and the duration for which it is maintained. The lowest exposure conditions producing repeatedly observed major malformations in embryonic development is a 4°C increase above normal body temperature maintained for 5 min.

Embryonic development has been shown to be delayed significantly in rats exposed to whole body heating until the maternal core temperature was elevated by 4°C. Although the pregnant rats were removed immediately from the heat when core temperature increase was achieved, the actual duration of embryonic exposure to the 4°C elevated temperature was uncertain. However, rat embryo culture studies that controlled exposure duration found a threshold value of 42°C for 10 min of exposure. The endpoint was a change in the crown–rump length (Kimmel et al. 1993b). Thus, the effect was produced by an increase above the normal body temperature of 3.5°C in rats.

There is evidence from studies using embryo culture systems that the effects of ultrasound may be enhanced by a moderate temperature increase (Angles et al. 1990; Barnett et al. 1990). The minimum exposure conditions were ΔT 1.5°C (absolute temperature 40°C) together with an ultrasound intensity I_{spta} of 1.2 $W \cdot cm^{-2}$ applied for 15-min duration.

Theoretical models or tissue-mimicking test objects for calculating temperature increases in tissues at the focal zone of the ultrasound beam are most reliable when the cooling function of tissue perfusion is less important than thermal diffusion. Perfusion is not likely to be a dominant factor in clinical situations in which narrow beams and small heated volumes occur for short dwell times. When bone is in the ultrasound path, perfusion is even less significant.

The transducer is a substantial source of heating, by conduction, in soft tissue examinations (WFUMB 1992; Wu et al. 1995). This is particularly important for pulsed transducers, which are inefficient in converting electrical to acoustic energy. Heating is localised close to the transducer. This has implications for safety for intracavitary applications, particularly where there is a trend toward increased power outputs in gynaecological examinations using the endovaginal route. The risk of inadvertently exposing an unknown pregnancy to heat cannot be excluded.

Some of the conclusions of the AIUM report (1994) are inconsistent with the findings of this report. This is due, in part, to recent data that were not available at the time of writing the AIUM report. There also are apparent inconsistencies and uncertainties in the present data base relating to thresholds for modest increases in temperature and the importance of duration of exposure.

SUMMARY

Most of the bioeffect studies describe the effects of whole body heating in the absence of ultrasound exposure. Few studies have been designed to specifically determine threshold values for abnormal embryonic or fetal development. Nevertheless, there are sufficient data on which to draw conclusions. Results of a number of studies on rats and mice have demonstrated that exposure for 5 min to a temperature increase of 4°C above their normal body temperature (*i.e.*, 42.5°C for rats) is hazardous to embryonic and fetal development.

There is some difficulty in directly extrapolating from whole body hyperthermia experiments in animals to humans. However, there are no new data to alter the conclusion arrived at in the 1991 WFUMB Safety Symposium that, regardless of duration, a diagnostic exposure that produces a maximum temperature rise of 1.5°C above normal physiological levels (37°C) does not present a risk from thermal effects in humans.

There are limited data available on the biological effects of interaction of ultrasound with tissues that have a preexisting temperature elevation. Specialised studies using rat embryo culture techniques have demonstrated that a modest temperature increase (1.5°C above normal body temperature) combined with pulsed ultrasound resulted in significant changes in embryo development and neural protein synthesis. The results imply that ultrasound-induced biological effects can be potentiated by an existing elevated core temperature. This subject of potential synergism between ultrasound and elevated maternal core temperature requires further research.

In scanned beams used in imaging procedures, any single tissue target is interrogated for only fractions of a second each time the beam sweeps past. Thus, there is little opportunity to heat a specific tissue target. To achieve significant amounts of heating requires that the ultrasound beam must be fixed in relation to a tissue target so that all the energy in the beam is directed onto that target. This occurs in pulsed spectral Doppler applications.

The effect of damaging a small volume depends on the sensitivity of the target tissue. It is questionable whether or not the effects of damage to small discrete volumes of neural tissue would ever be detected by studies of gross morphological structure. Techniques in molecular biology are becoming very sensitive and need to be adapted to provide assays to detect such small biological effects.

Thresholds for teratogenic effects of hyperthermia are determined by a combination of the induced temperature elevation and its duration. Most ultrasound diagnostic procedures involve brief dwell times that help to widen the safety margin. There are uncertainties in esti-

mates of *in situ* tissue temperature increase caused by ultrasound; thus, it is prudent to minimise exposure, particularly in pulsed Doppler applications.

WFUMB SYMPOSIUM CONCLUSIONS ON THERMAL ISSUES

These conclusions were accepted by vote of the participants at the WFUMB Safety Symposium, Kloster-Banz 1996.

- Assessment of risk from tissue heating is based largely on data from whole body hyperthermia studies that are qualitatively different from ultrasound-induced heating of small volumes of tissue.
- Although few studies have been designed to specifically determine thresholds, data from whole body hyperthermia (*in utero* and in embryo culture) studies with rats demonstrate that exposure for 5 min to a temperature increase of 4°C above their normal body temperature (*i.e.*, 42.5°C) is hazardous to embryonic and fetal development. Similar thresholds have been reported for ultrasound-induced heating of actively dividing cells in bone marrow.
- Developmental abnormalities have been observed in animals when the embryonic or fetal temperature is increased by 2°C or more above their normal body temperature for extended duration.
- Under specialised laboratory conditions using rat embryo culture, exposure to pulsed ultrasound combined with a temperature increase of 1.5°C above normal (*i.e.*, at 40°C) affects embryonic development.
- Biologically significant temperature increases have been measured, at or near bone/soft tissue interfaces, during exposure to conditions similar to those used in Doppler diagnostic equipment. The effects of elevated temperatures may be minimised by keeping the time for which the beam passes through any one point in tissue as short as possible.
- It has been demonstrated in studies with unperfused tissue that, for transcranial applications, the transducer itself can be a substantial source of heating.
- The acoustic output from simple B-mode imaging is controlled to levels that are not capable of producing harmful temperature rises in tissue. Ultrasound scanning in B-mode is not contraindicated on thermal grounds.

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Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. S11–S21, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00074-X

● *Chapter 2*

NONTHERMAL ISSUES: CAVITATION—ITS NATURE, DETECTION AND MEASUREMENT

INTRODUCTION

Acoustic cavitation is defined as sonically induced activity of gas-filled cavities; it is either *inertial* or *noninertial.*

Inertial cavitation occurs when a gas-filled cavity in a liquid, *i.e.,* a bubble, expands during part of an acoustic cycle, then contracts very rapidly (''collapses'') to a small fraction of its original volume. During contraction, liquid is drawn inward and, as pointed out by Flynn (1964), its inertia largely controls the bubble motion.

Toward the end of the collapse, the predicted wall speeds and accelerations are high, and shock waves can propagate through the gas and be emitted into the liquid. Polytropic models of the collapse of a bubble containing homogeneous gas predict pressures in the range of hundreds of megapascals and temperatures in the range of thousands of degrees Kelvin when the volume is minimum. These transient temperatures and the gas shocks are capable of generating free radicals by hydrolysis, which subsequently yield reactive chemicals. Electronically excited species, in turn, can cause the emission of a light flash, a phenomenon known as *sonoluminescence.*

After reaching minimum volume, the bubble rebounds, emitting a pressure pulse into the liquid. It then may fragment, immediately or after undergoing a small number of rebounds. However, it recently has been found (Gaitan and Crum 1990) that, under special conditions, a spherical bubble maintains its identity during collapse and repeats an expansion–contraction cycle during each period of a continuous sound field for an indefinite number of cycles. The entire repeated cycle may include several reduced rebounds, as shown in Fig. 2.1.

Under other conditions the bubble collapses asymmetrically, then disintegrates into smaller fragments, which may or may not dissolve. A liquid jet may form which projects through the bubble with high speed. This is seen in Fig. 2.2, which shows computed surface profiles of a bubble located near a solid surface, at various times after the deformation begins. The jet may deform or erode the solid surface.

Noninertial cavitation includes various types of sonically induced activities involving bodies of gas. The latter may be either relatively free to move, as for bubbles in a liquid (*e.g.,* echo-contrast agents in large blood vessels), or more constrained, as for gas-filled channels in plant tissues, insects and (nonfetal) mammalian lung. Examples of noninertial cavitation activity are: translational motion of free bubbles, surface distortions (including formation of liquid jets and gaseous microbubbles), growth by rectified diffusion or by coalescence, heat generation, radiation forces exerted on neighbouring particles (including other bubbles) and small-scale acoustic streaming, *i.e., microstreaming,* of the gas on the interior and of the liquid in the exterior.

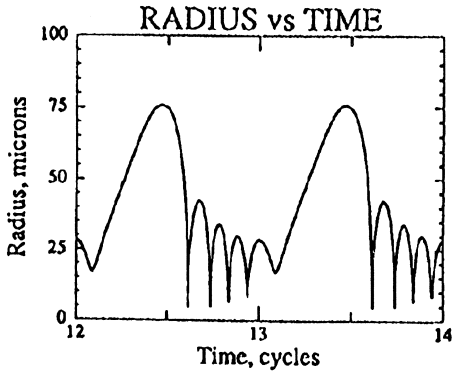

Fig. 2.1. Repeated inertial cavitation at 22.3 kHz. [Reproduced with permission from Gaitan and Crum (1990).]

Figure 2.3 shows the results of radiation force exerted by a vibrating gas body (H) of microscopic size, in a pore of hydrophobic Nuclepore® membrane, on human red cells (C) in a saline suspension, exposed to a 2.1-MHz continuous-wave ultrasound field of low pressure amplitude. The cells have gathered around the gas-filled pore, and each cell is somewhat distorted as a part of its membrane nearest the pore is pulled toward it.

Figure 2.4 shows stream-lines followed by small indicator particles in the vicinity of a gas bubble in liquid; the bubble rests on a boundary while exposed to a 10-kHz sound field. The four patterns apply for different liquids and different vibration amplitudes. Analogous

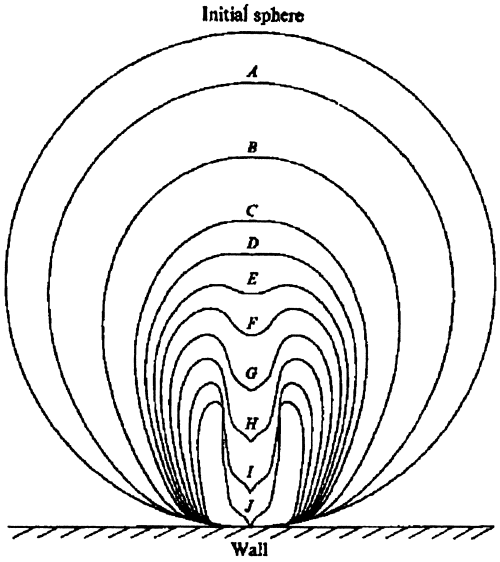

Fig. 2.2. Formation of liquid jet at various times after the deformation begins. A = 0.63 ms; C = 0.97 ms; E = 1.03 ms; J = 1.12 ms. [Reproduced with permission from Plesset and Chapman (1971).]

microstreaming occurs near bubbles set into vibration by ultrasound in the megaHertz frequency range and, together with the bubble–cell attraction illustrated in Fig. 2.3, leads to effects on cells in the vicinity of the bubbles at moderate vibration amplitudes. Special forms of cavitation, such as those depicted in Figs. 2.3 and 2.4, can occur when stabilized gas bodies are present. Natural biological structures present such gas bodies in the form of plant tissue gas spaces, insect larvae gas channels, mammalian lung tissue and gas pockets in the mammalian gut. In clinical practice, gas bubbles are to be expected during infusions and intravenous injections and are present when gaseous echo-contrast agents are used (since the latter consist of suspensions of small encapsulated bubbles). These forms of cavitation are of particular interest here because of their potential for producing effects under conditions where acoustic pressures are below the threshold for inertial cavitation.

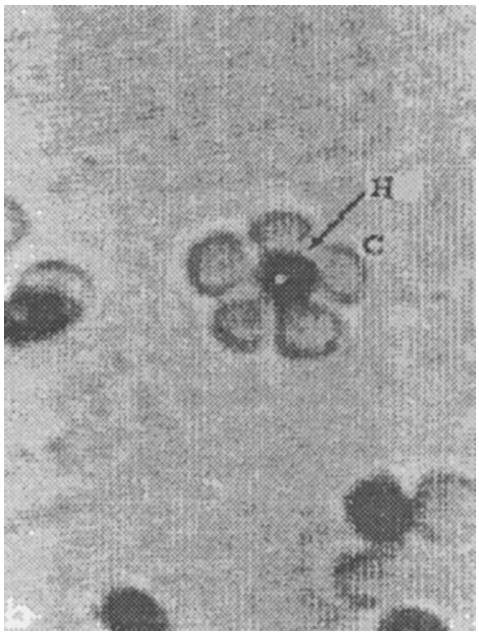

Fig. 2.3. Cells are drawn to a vibrating gas body. C = human red cell; H = vibrating gas body. [Reproduced with permission from Nyborg and Miller (1982).]

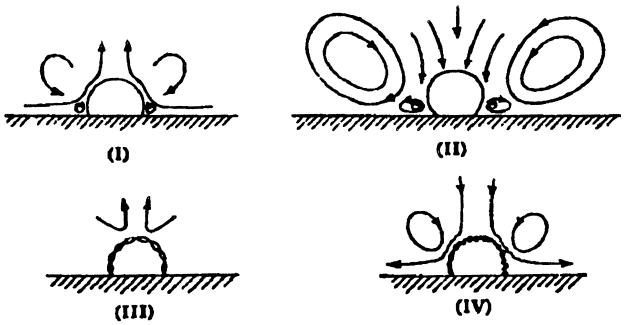

Fig. 2.4. Patterns of flow in the microstreaming observed near a vibrating bubble. [Reproduced with permission from Elder (1959).]

Whether a given bubble responds to a sound field with inertial or noninertial cavitation depends on the pressure amplitude in the local sound field, the initial bubble size, the acoustic frequency and other factors, including physical properties of the liquid. Below the inertial cavitation threshold of a sample only noninertial cavitation occurs, whereas above the threshold a complex variety of phenomena can occur. Based on theory, proposed criteria for distinguishing inertial from noninertial cavitation include: 1) the profile of the major collapse portion of the radius vs. time curve; 2) the maximum relative radius achieved; and 3) the maximum temperature reached by the gas. Proposed criteria based on experiment include detection of chemical reaction products and of sonoluminescence. Typical cavitation fields are complex. Commonly, there are many bubbles present, of varying size, and the sound field is nonuniform. In many examples where inertial cavitation is produced in a liquid sample, noninertial cavitation occurs simultaneously.

BIOPHYSICAL CONSIDERATIONS

A wide variety of physical, chemical and biological effects can be produced by ultrasound through the action of inertial or noninertial cavitation. In general, the cavitation activity is very complex, but considerable insight

has been obtained from physical studies of simplified models. In particular, much attention has been given to acoustic theory for the volume oscillations of a spherical gas-filled bubble, surrounded by liquid and subjected to a continuous-wave sound field. In the following discussion of cavitation activity, the aspects dealt with first are those of noninertial cavitation, which occur when the oscillation amplitude is in the small-to-moderate range.

Linear resonance

Although the general differential equation for the vibratory motion of a bubble is nonlinear, it reduces to a linear equation when the oscillation amplitude is not too large. For a spherical gas bubble, if the acoustic pressure in the liquid varies sinusoidally in time with frequency f, the bubble radius also varies sinusoidally in time with the same frequency.

Under linear conditions, a bubble responds as a harmonic oscillator with a resonance frequency that is inversely related to its equilibrium radius. For example, the resonance frequency f_o of a free spherical gas bubble in water at 20°C and atmospheric pressure is given, for bubbles of radius R_o about 10 μm or larger, by:

$$f_o = 3.3/R_o, \quad R_o > \approx 10\ \mu\text{m} \qquad (1)$$

where f_o is in megaHertz when R_o is in micrometers. According to this equation, if the radius is 10 μm, the resonance frequency is 0.33 MHz. This simple formula overestimates f_o increasingly as R decreases; for gas bubbles in water whose radius is only a few micrometers or smaller, resonance properties depend on surface tension at, and heat transport across, the gas–liquid interface (Coakley and Nyborg 1978; Flynn 1964; Leighton 1994).

Other expressions have been derived for resonance frequencies of particles or structures containing undissolved gas. Some of these expressions apply to gas bodies encapsulated with viscoelastic shells and hence to particles used for contrast agents (Church 1995; de Jong and Hoff 1993); others apply to small bodies of gas trapped in pores (Miller and Nyborg 1983) or contained in intercellular channels of plant tissue (Miller 1979).

Scattering

When an ultrasound wave impinges on a small gas body, such as a free bubble or a gas-filled cavity contained in a structure, much of the energy is reradiated, *i.e., scattered,* into the surrounding medium, especially under resonance conditions. It is for this reason that gas-containing particles have proven highly effective as contrast agents for diagnostic ultrasound (Bleeker et al. 1990).

Heat production

Of the energy extracted from an ultrasound wave by a gas body, the part that is not scattered is converted into heat. Calculations, based on linear theory for pulsations of a spherical bubble, have been made for the heat generated by free resonant bubbles (about 3 μm in radius) at a frequency of 1 MHz. It is estimated that if such bubbles were present in soft tissue at a concentration of 100 mL^{-1} (corresponding to a volume fraction of about 10^{-8}), the absorption coefficient would double and, hence, the rate of heat production by an ultrasound beam of given intensity also would double (Nyborg 1991). Hynynen (1991) has shown experimentally that the temperature rise produced by ultrasound in mammalian tissue is increased when cavitation occurs. (The temperature rise referred to here is the average over a sonic cycle or over a repetition period and, of course, differs greatly from the submicrosecond duration temperature ''spikes'' that occur during the instant of minimum radius, as in Fig. 2.1, during inertial cavitation.)

Diffusion and rectified diffusion

As a bubble expands and contracts in response to the time-varying pressure in a sound field, gas diffuses inwardly and outwardly. Even in a sound field in which the acoustic pressure varies sinusoidally with time the net inward flow during a cycle often is positive, causing the bubble to grow in a process called *rectified diffusion* (Crum and Hansen 1982). In experiments with supersaturated gels, rectified diffusion is believed responsible for the appearance of gas bubbles during exposure to ultrasound fields from equipment designed for physical therapy (Crum et al. 1987). The bubbles evidently are enlarged to detectable sizes (10 μm or larger in diameter) from smaller gas bodies normally present in the gel. Evidence also has been given for bubble growth produced by therapeutic ultrasound in mammalian tissues (ter Haar 1986; ter Haar and Daniels 1981). The time-averaged intensities used were comparable to those used in some applications of Doppler ultrasound. However, it has not been demonstrated that Doppler ultrasound can produce such bubble growth; the typical frequency, beam width and pulsing characteristics for diagnostic Doppler are different than those for therapy.

Theory shows that diffusion also plays a major role in the bubble dynamics induced by lithotripsy fields (Church 1989). Here the diffusion is primarily ''one way,'' that is, into the bubble during its initial prolonged expansion phase.

Radiation forces on bubbles

Cavitation activity is influenced strongly by the net forces, called *acoustic radiation forces,* which act on bubbles. According to theory (based on the linear ap-

proximation for the vibrational motion), the radiation force on a bubble in a sound field is proportional to the square of the local pressure amplitude; its direction and magnitude depend on the nature of the field and on the size of the bubble relative to that required for resonance. These forces cause free bubbles to move about and combine with each other. For example, in a standing wave the radiation force is toward pressure-amplitude maxima for bubbles smaller than resonance size and in the opposite direction for larger bubbles. Near a solid boundary, the radiation force on a gas bubble may draw it toward its ''image.''

In a plane travelling ultrasound wave at large distances from the source, the radiation force on a bubble is in the direction of propagation and is maximum for resonance conditions. A free bubble may be caused to move at high speed, which is an important consideration for *in vitro* research. In a progressive-wave field, air bubbles rapidly cross the exposure chamber and remain there unless the exposure chamber is rotated to continually recycle the bubbles (Miller and Williams 1989). This type of behaviour exemplifies the many differences between *in vitro* and *in vivo* conditions that complicate the interpretation of bioeffect reports.

Bubbles smaller than resonance size will attract each other and may coalesce. Such coalescence is an important process for bubble growth caused by ultrasound in a liquid medium.

Attraction of cells to a vibrating bubble

Radiation forces in the liquid near a small gas body executing volume oscillations attract any small particle, such as a biological cell, to the vibrating body, especially if the density of the particle is greater than that of the liquid (Nyborg and Miller 1982) (see Fig. 2.3). In an erythrocyte suspension of high hematocrit, cell collection at vibrating bubbles can influence cavitation damage (Brayman and Miller 1993).

Bubble-associated acoustic microstreaming

Investigators have used special arrangements for studying biological implications of the microstreaming that occurs near vibrating bubbles (Fig. 2.4). According to reviews by Williams (1983) and Miller (1987), the following effects have been attributed to microstreaming generated by bubbles set into vibration by ultrasound: hemolysis; release of protein from bacteria; degradation of DNA in solution; release of ATP from erythrocytes; and mechanical disruption of plant cells. The pressure amplitudes required were usually low, sometimes as low as 10^4 Pa. In experiments with plant cells, the pressure-amplitude threshold for damage was found to be approximately proportional to the square root of the frequency and given by 0.95 MPa/MHz$^{1/2}$. The damage was attributed to shear stress associated with intracellular microstreaming resulting from vibration of intercellular gas bodies (Miller and Thomas 1993b).

Surface waves and jet formation

Sound fields typically cause patterns of transverse waves to be set up at gas–liquid interfaces, including bubbles. Even at moderate amplitudes the waves may become distorted and chaotic, with microbubbles forming on the liquid side of the interface, while jets of liquid are formed on the other side. Large bubbles can completely break up into microbubbles. The microbubbles serve as new sites for cavitation activity. For a bubble pulsating on a solid boundary, the liquid-jet impact discussed earlier (Fig. 2.2) has been proposed as a contributing process in the destruction of kidney stones by ultrasonic lithotripsy (Coleman et al. 1987).

Harmonics, subharmonics and other frequency components in cavitation spectra

At even moderate levels of the pressure amplitude, features of bubble response appear that are absent under linear conditions. For example, if the driving acoustic field is a continuous wave of frequency f, the acoustic pressure field scattered by the bubble contains spectral components of harmonic frequency ($2f$, $3f/2$, etc.), subharmonic frequency ($f/2$, $f/3$, etc.) and ultraharmonic frequency ($3f/2$, $2f/3$, etc). When the acoustic pressure is measured in a medium (such as an aqueous suspension) exposed to ultrasound, the appearance of harmonic or subharmonic components in the pressure spectrum can be used as an indicator of nonlinear bubble motion.

Inertial cavitation

The phenomena associated with inertial cavitation (see Introduction) can occur in water or other aqueous media, even at frequencies and pressure amplitudes commonly used in diagnostic ultrasound, if small gas bodies (cavitation nuclei) of appropriate size are present. These phenomena include the sonochemical reactions resulting from the generation of free radicals, and the production of sonoluminescence; the emission of rebound pressure pulses and their contribution to erosion and acoustic emissions; and a range of phenomena associated with motion of the bubble wall (*e.g.*, extensive expansion and fragmentation). Measurements of the light generated from sonoluminescence, and of the products resulting from chemical reactions, can be used as measures of inertial cavitation activity. Sonoluminescence has been observed in aqueous solutions exposed to ultrasound under acoustic conditions comparable to those characteristic of diagnostic fields (Fowlkes and Crum 1988).

Theoretical values of the threshold for inertial cavitation depend on the specific definition for the threshold,

on the initial bubble sizes and, for pulsed ultrasound, on the center frequency and pulse shape (Apfel and Holland 1991; Flynn 1982; Sponer 1991).

Cavitation nuclei

Small bodies of gas are ubiquitous in water and other liquids used for *in vitro* experiments with biological systems. The gas bodies provide sites for cavitation, are commonly called *cavitation nuclei,* and would dissolve or float out of the region of interest if they were not stabilized by some means. Nuclei may exist in the liquid as small bubbles of gas coated with organic material that slows or prevents diffusion, or as gas stabilised in cracks or pores at solid surfaces (which may exist in container walls, small impurity particles, etc.) that reduce or eliminate excess internal pressure caused by curvature of the gas–liquid interface. Typically, the nature and distribution of nuclei vary from one experiment to another, unless steps are taken to control them (Apfel 1981; Crum 1982). Media sterilised by filtering or autoclaving *in vitro,* or the biologically clean conditions in a living animal, are examples of situations with a paucity of cavitation nuclei for which relatively high cavitation thresholds are typically found.

In one method for making nuclei available in a controlled manner, use is made of hydrophobic membranes with uniform gas-containing pores a few micrometers in diameter. Vigorous acoustic microstreaming occurs near the pores during the exposure to ultrasound, even at relatively low pressure amplitudes. At higher amplitudes the gas leaves the pores and probably forms nuclei for inertial cavitation. Other methods for providing nuclei make use of ultrasound contrast agents (Williams et al. 1991) or polystyrene particles (Atchley et al. 1988). Prediction of the likelihood of inertial cavitation *in vivo* in the absence of such artificial nuclei would require knowledge of the availability of naturally occurring nuclei. Characterisation of this natural nuclei population *in vivo* has not been achieved with current techniques.

MECHANISMS FOR CAVITATION-MEDIATED BIOEFFECTS

If appropriate nuclei are present, exposure to ultrasound may cause alterations of cells through mechanisms of noninertial cavitation, at pressure amplitude levels below the inertial cavitation threshold. Direct information on how noninertial cavitation affects biological systems has come from use of arrangements in which nuclei of effective size are provided and observations are made through an optical microscope during ultrasonic exposures, as in obtaining Fig. 2.3. In particular, effects of microstreaming and radiation forces associated with vibrating gas bodies have been studied in this way, often at relatively low values of the pressure amplitude, so that inertial cavitation is absent. In experiments in which stabilized gaseous nuclei are provided, cellular effects have been observed from exposures to ultrasound under acoustic conditions comparable to those characteristic of diagnostic fields (Miller 1987).

In most experiments in which larger quantities of cell suspension are exposed to ultrasound (as when the cells are contained in a test tube or other vessel of comparable size), it is difficult, if not impossible, to make direct optical observation of the activity occurring at individual sites. It then is necessary to use other methods to characterize the activity (see later section on Cavitation Detection). In such experiments it often is determined that little cell damage occurs, unless inertial cavitation is present. This is probably because effective nuclei for noninertial cavitation are not present initially. At acoustic pressure amplitudes exceeding the threshold for inertial cavitation, bubbles of microscopic and submicroscopic size move about at high speed and interact in complex patterns. Hydrodynamic stresses resulting from collapse or from rapid translational movement of bubbles then may be the dominant mechanisms for cell lysis (Carstensen et al. 1993). Chemical activity involving free-radicals produced during collapse does not appear to be an important factor in cell lysis (see Chapter 4) although active sonochemicals that are produced can cause DNA damage in cells. For example, H_2O_2 produces strand breaks in DNA (Miller et al. 1991a, b, e).

In organized tissues, *gas body activation,* a form of noninertial cavitation, has been demonstrated for medically relevant conditions and has known bioeffects in plants (Miller and Thomas 1993a), insects (Child et al. 1981) and mammalian lung (Child et al. 1990; Frizzell 1994). In plants, membrane damage occurs and is attributed to viscous shear stresses associated with acoustic microstreaming observed in adjoining liquid-filled vacuoles, as noted earlier. The microstreaming results from vibrations of the cell wall that, in turn, result from volume oscillations of the intercellular gas-filled channels. In insects and mammals, structures analogous to the plant vacuoles are not seen in the immediate vicinity of the gas-filled respiratory spaces, and the mechanisms for ultrasonically produced damage are not known. The possible consequences of gas body activation associated with gas-filled lung tissue, intestinal gas pockets and gas-containing echo-contrast agents represent specific instances of cavitation considerations relevant to clinical practice.

CAVITATION DETECTION

Investigations of biological effects attributed to acoustic cavitation, discussed in Chapters 4 through 6,

Table 2.1a. Cavitation detection methods and applications.

Acoustical:	**Reference:**
Imaging (B-scan);	
Diagnostic, *in vivo*	ter Haar et al. 1982
Diagnostic, *in vitro*	Watmough et al. 1991
Hyperthermia, *in vivo*	Hynynen 1991
Lithotripsy, *in vitro*	Zeman et al. 1990a
Lithotripsy, *in vivo,* human	Zeman et al. 1990b; Coleman et al. 1995
Decompression, *in vivo*	Mackay and Rubissow 1978; Daniels et al. 1979, 1980
Ultrasound scatter;	
In vitro	Atchley et al. 1988; Roy et al. 1990
In vivo	Holland et al. 1996
Subharmonic emission;	
In vitro	Neppiras 1968a, 1968b; Coakley 1971; Coakley et al.1971; Edmonds and Ross 1986, 1988; Morton et al. 1982
In vivo	Lele 1977; Sommer and Pounds 1982; Hynynen 1991
Second harmonic emission;	
In vitro	Miller 1981
In vivo	Gross et al. 1985; Christman et al. 1986
Two-frequency, *in vitro*	Newhouse and Shankar 1984; Chapelon et al. 1988; Leighton et al. 1991
Broadband noise emission;	
In vitro	Neppiras 1968a, 1968b; Holland and Apfel 1990; Coleman et al. 1992
In vivo	Hynynen 1991
Doppler;	
in vitro	Nishi 1972
In vivo	Gillis et al. 1968; Evans and Walder 1970; Belcher 1980
Biological:	
Damaged mammalian tissues in vivo;	
Focal lesions	Fry et al. 1970; Frizzell 1994
Endothelial cell damage	Dyson et al. 1974
Hemorrhaging (by lithotripsy)	Delius et al. 1990a, b; Hartman et al. 1990a, 1990b; Miller and Thomas 1995
Hemorrhaging (by ultrasound)	Child et al. 1990; Frizzell 1994
Hind limb paralysis, influenced by hyperpressure	Frizzell 1994
Sonoluminescence:	
Ultrasound *in vivo*	Leighton et al. 1990
Light scattering:	
Fibre optics, *in vivo*	Huber et al. 1994

in vivo = Exposures of living mammals; in vivo, human = human exposures.

have depended on a variety of methods for detecting and characterizing cavitation. Under some conditions, the occurrence of cavitation during an exposure to ultrasound is marked by the occurrence of intermittent or continuous ''hissing'' or ''clicking'' sounds that are audible to the unaided ear. When cell suspensions contained in a transparent vessel are exposed to ultrasound while illuminated in a suitable manner, arrays or cloudy regions composed of bubbles, as well as streaks or streamers formed by rapidly translating bubbles, sometimes can be seen. Under other conditions, such direct methods either cannot be used or are inadequate.

Lauterborn (1980), ter Haar (1986), Young (1989) and, more recently, Leighton (1994) have published comprehensive reviews of physical, chemical and biological techniques that have been used to detect the presence of bubbles and monitor cavitation activity. A list of the detection methods that are particularly suitable for *in vivo* work is given in Table 2.1a. Methods generally confined to use with *in vitro* work are listed in Table 2.1b. The one or two references associated with each method are given as examples only and are not intended to represent a comprehensive review of the literature.

Detection of bubbles and cavitation in living mammals

Diagnostic ultrasound. Ultrasound imaging methods have been used in laboratory animals to study bubble growth produced by decompression (Daniels et al. 1979; Mackay and Rubissow 1978), therapeutic ultrasound (Hynynen 1991; ter Haar et al. 1982) and lithotripsy (Coleman et al. 1995; Zeman et al. 1990, 1990b). They also have been used to track boluses formed of gas-containing contrast agents in diagnostic procedures (Wang and Shung 1994). (As noted above the latter agents are effective because of the exceptional backscatter characteristics of small gas bodies.) Single bubbles of 10 μm diameter can be detected.

Ultrasound backscatter. A system capable of sending and receiving pulses of focused 30-MHz ultrasound (Roy et al. 1990) has been used to detect short-lived cavitation events that result from *in vivo* exposure of rat

Table 2.1b. Cavitation detection methods and applications (*in vitro*).

Sonochemical:	**Reference:**
Oxidation reactions from OH radical release;	
Teraphthalic acid	McLean and Mortimer 1988; Price and Lenz 1993; Wang et al. 1993; Miller and Thomas 1993a
Fricke solution Weissler reaction	Ciaravino et al. 1981; Weissler 1950
Hydroethidine	Suhr et al. 1991
Dichlorofluorescin diacetate	Suhr et al. 1991
Spin trapping	Makino et al. 1983
Other sonochemical methods	Suslick 1988
Optical:	
Sonoluminescence;	Daniels and Price 1991; Roy et al. 1985; Saksena and Nyborg 1970; Coleman et al. 1993; Negishi 1962
Chemiluminescence;	Miller and Thomas 1993a; Crum and Fowlkes 1986
Imaging;	
Holography, high-speed photography	Neppiras and Coakley 1976; Lauterborn 1980
Electrical impedance:	
Changes in impedance	Lubbers and van den Berg 1976; Neppiras and Coakley 1980; Manley 1969; Kryachko et al. 1989; Kovovin and Semenova 1989
Biological damage:	
Plants and insects	Carstensen et al. 1983; Miller 1987
Cells (hemolysis) by ultrasound	Esche 1952; Carstensen et al 1993; Miller and Thomas 1993a, 1993b

lung to four-cycle bursts of focused 4-MHz ultrasound (Holland et al. 1996).

Acoustic emissions: subharmonic and noise. Evidence of cavitation produced *in vivo* by ultrasound in tissues such as cat brain, dog thigh muscle and pork muscle have been obtained by monitoring emissions of subharmonic or wide-band noise emitted from the exposed regions (Hynynen 1991; Lele 1977; Sommer and Pounds 1982). It is believed that the emissions observed at the higher intensity levels are produced mainly by inertial cavitation, whereas at the lower levels the emissions probably come from noninertial cavitation. By using focused detectors the spatial distribution of the cavitation can be studied, with the resolution being dependent on the applicable acoustic wavelengths.

Acoustic emission: second harmonic. Techniques based on monitoring the second harmonic emissions from bubbles (Miller 1981) have been used to detect bubbles produced in dogs by decompression (Christman et al. 1986), ultrasound (Gross et al. 1985) and lithotripsy (Williams et al. 1989). The device used in these studies is a cuff that is placed around a blood vessel (or other fluid-containing tube) of interest. It is capable of counting bubbles passing along the tube and is particularly sensitive to bubbles of resonance size within a few millimeters of the detector. Blood perfusion has been measured with methods based on detection of second harmonic emission from gas-containing contrast agents (Schrope and Newhouse 1993).

Nonacoustic methods. Methods other than those discussed above have provided basis for identifying cavitation as a cause of biological effects (see Chapter 5). One method involves histologic examination of damaged tissue. The appearance of ''holes,'' or of irregularities in focal lesions, has been taken as evidence of cavitation. In another method, test animals are exposed to ultrasound in a closed vessel, and the time required to produce an effect (such as hind-leg paralysis) is determined for different values of the ambient pressure. Cavitation is indicated as a mechanism if increase of the ambient pressure leads to an increase of the time required to produce the effect. A third method is association of observed tissue damage with bodies of gas known to exist in the tissue. It has been found, for example, that ultrasound produces hemorrhage preferentially in adult lung and in the intestine.

Detection of bubbles and cavitation in other systems

Biological cell suspensions, plants and insects provide convenient systems for studying effects of acoustic cavitation as they occur in different situations. Most of the techniques listed in Table 2.1a and 2.1b have been used to detect cavitation in experiments involving exposures of these systems to ultrasound.

Optical methods. Direct observations have been made through an optical microscope during ultrasound exposures to study microstreaming and other effects of noninertial cavitation in plant tissue containing gas-filled channels and in cell suspensions under the influence of gas-filled pores in Nuclepore® membranes; see review by Miller (1987). Other optical methods involve use of photodetectors to receive light from sonoluminescence or chemiluminescence associated with inertial cavitation in aqueous solutions and suspensions (Daniels and Price 1991; Leighton et al. 1988, 1990; Pickworth et al. 1988,

1989a, 1989b; Roy et al. 1985; Vona et al. 1995; Walton and Reynolds 1984) and light-scattering methods for studying the dynamics of single bubbles in water and other liquids (Bradley and Putterman 1992; Holt and Crum 1992; Keller 1972).

Sonochemical methods, including chemiluminescence. Many tests for cavitation are possible, based on measurements of free radicals and chemical reaction products (Ciaravino et al. 1981; Christman et al. 1987; Crum and Fowlkes 1986; Makino et al. 1983; McLean and Mortimer 1988; Miller and Thomas 1993a; Price and Lenz 1993; Riesz and Kondo 1992; Suslick 1988; Suhr et al. 1991; Wang et al. 1993; Weissler 1950). Since free radical production is required for such chemical effects, these tests are means for detecting inertial cavitation.

Biological methods. Biological effects of cavitation can be viewed alternatively as means of cavitation detection. Observation of hemolysis (breakage of red blood cells) in a saline suspension is an adaptable method that has been used *in vitro* (Esche 1952; Miller and Thomas 1993a, 1993b). Since hemolysis can result from cavitation at relatively low levels, it is a means for detecting either noninertial or inertial cavitation.

Electrical and mechanical methods. An electrical test is based on the change cavitation causes in the electrical conductivity of a liquid (Kovovin and Semenova 1989). A test for the ability of cavitation to produce mechanical point impacts on a solid surface makes use of a film whose emulsion is sensitive to individual implosions or jets associated with asymmetrical inertial cavitation (Coleman et al. 1987; Geise et al. 1990).

WFUMB SYMPOSIUM CONCLUSIONS ON CAVITATION; NATURE, DETECTION AND MEASUREMENT

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz 1996.

- For the purposes of this report, acoustic cavitation is defined as ultrasonically induced activity of gas bodies. It is conveniently classified as *inertial* (formerly termed ''transient'') or *noninertial.*
- Phenomena associated with inertial cavitation include direct collapse stress, the production of free radicals and other chemically active species and sonoluminescence. It is predicted theoretically and has been verified with *in vitro* experiments that ultrasound fields, including fields with characteristics similar to those used in diagnostic ultrasound, can produce these phenomena under some circumstances. Inertial cavitation therefore warrants consideration in assessing the safety of diagnostic ultrasound and other applications of medical ultrasound.
- Phenomena associated with noninertial cavitation include oscillation of gas-body-related structures (*e.g.*, plant cell walls), the translational motion of bubbles, bubble growth by rectified diffusion or coalescence, heat generation, radiation forces on neighbouring particles and small-scale streaming. These phenomena are predicted theoretically and their biological effects, as in some cases of cell lysis, have been observed after *in vitro* exposures to ultrasound fields, for some of which the characteristics were similar to those of fields used in diagnostic ultrasound. Noninertial cavitation therefore warrants consideration in assessing the safety of diagnostic ultrasound and other applications of medical ultrasound.
- Present models for cavitation, whether inertial or noninertial, have not proven adequate for explaining the rupture of capillaries, leading to bleeding, by ultrasound in animal lung. However, the occurrence of this effect is consistent with findings that plant and insect tissues, which are known to contain structurally stabilized gas bodies, also are susceptible to damage by diagnostic ultrasound.
- The extent to which cavitation effects occur in water and other aqueous media is determined strongly by stabilized gas bodies, typically of micrometer size, that may be present and serve as nuclei. Little is known about the concentration and characteristics of naturally occurring nuclei in living mammals. Echo-contrast agents that contain undissolved gas can serve as cavitation nuclei under some conditions and may do so in mammals. Present techniques are not sufficiently sensitive and accurate for detection and characterization of small nuclei *in vivo.*

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Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. S23–S28, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00075-1

● *Chapter 3*

OTHER NONTHERMAL MECHANISMS: ACOUSTIC RADIATION FORCE AND STREAMING

INTRODUCTION

The propagation of ultrasound stresses the medium supporting the wave; the medium experiences not only forces in the direction of wave propagation, but also shear and torque. When averaged over time, these forces are small at medical diagnostic intensities, although, owing to the pulsed nature of the exposure, instantaneous forces are higher than time-averaged forces. Whilst at higher intensities biological effects may be dominated by cavitation and heating, at intensities that are below a cavitation threshold or give rise only to small temperature rises (< 1°C), it may be suggested that these forces could affect cells in a way that could modify their behaviour and future development. For this reason the study of acoustic radiation forces is necessary for a complete evaluation of the risks associated with clinical ultrasound.

General reviews providing useful background include those by Dunn and Pond (1978), Dunn and O'Brien (1976), Fry and Dunn (1962), NCRP (1983) and Rooney (1988).

The acoustic forces can be grouped conveniently into two classes. The first is associated with the mechanical oscillations of the wave itself in which, under linear conditions, the local time-averaged force is zero. The second class includes stresses whose time averages are not zero; the tissues and fluids experience both a unidirectional force, usually but not always away from the transducer, and rotational forces. In addition, it is useful to consider several broad ''ideal'' categories of target or tissue structure that may be operated upon by the acoustic forces. These may be grouped conveniently as follows.

1. **Objects Larger in Scale than a Wavelength**

(a) An absorbing continuum, without local acoustic inhomogeneities, and whose dimensions exceed those of the ultrasonic beam. Liquid volumes such as urine or amniotic fluid are examples. Also, to a first approximation, many large organs such as liver, spleen or brain often are considered to be homogeneous in this context.

(b) Surfaces separating two different acoustic materials where the extent of the surface is significantly larger than a wavelength. Skin–air and soft tissue–bone interfaces are examples.

2. **Objects Smaller in Scale than a Wavelength**

(c) Small structural inclusions, of dimensions less than a wavelength, with acoustic properties that are different from those of the surrounding liquid medium. Blood cells or cells in suspension *in vitro* are examples.

(d) Liquid inclusions in a surrounding matrix. The intra- and intercellular liquid spaces are examples.

A macroscopic volume of tissue may include targets in any or all of these categories, experiencing both oscillatory and steady forces. Mammalian tissue consists of a complex acoustic structure, and at present there is only a poor understanding of the acoustic forces that may act within its structure and their biological significance. Any proper evaluation must take into account other forces at the molecular and cellular levels, for example, osmotic pressure, surface tension and cell adhesion, whose effects might be modified by the imposition of external forces of acoustic origin.

FORCES ON LARGE-SCALE OBJECTS

Oscillatory forces

Consider first the oscillatory forces due to the passage of an ultrasonic wave acting on the particles of the medium, causing microscopic displacements from their null positions. Peak particle velocities in diagnostic pulses are of the order of 1 m·s^{-1}, nearly three orders of magnitude smaller than the mean thermal velocity of water molecules at 37°C, 660 m·s^{-1}. Thus, it should not be expected that extra forces due to mechanical wave movements would directly produce molecular damage.

When a sound wave is incident at grazing incidence on a liquid–solid boundary, a gradient in the particle velocity occurs in a thin layer in the liquid adjacent to the boundary. In water at 1 MHz at room temperature, the boundary layer thickness is about 0.6 μm. Associated

Table 3.1. Calculated time-averaged (ta) and pulse-averaged (pa) forces and pressures for maximum diagnostic intensities exerted on several target arrangements, where force = D (IS)/c.

Arrangement	Coefficient *D*	Time-averaged force F_{ta} (μN)	Time-averaged pressure P_{ta} (Pa)	Pulse-averaged force F_{pa} (mN)	Pulse-averaged pressure F_{pa} (kPa)
Perfect absorber, normal	1.0	65	6.5	32.5	3.25
Perfect reflector normal	2.0	130	13	65	6.5
Acoustic velocity boundary $c_1 \neq c_2$	1 - c_1/c_2 0.093	6.1	0.6	3.0	0.3
1 mm thick absorbing medium 1	2α.dx 0.03	0.2	0.02	0.1	0.01
1 mm thick absorbing medium 2	2α.dx 0.10	0.66	0.66	0.33	0.33

The direction of the force is towards the medium of higher acoustic velocity. The numeric calculations assume $c_1 = 1450$ m·s^{-1} and $c_2 = 1600$ m·s^{-1} modelling a fat–muscle interface. The absorber is assumed to be thin enough for intensity through the slice to be approximately constant. Absorbing medium 1, $\alpha = 0.015$ mm^{-1}, models a 3-MHz beam. Absorbing medium 2, with $\alpha = 0.05$ mm^{-1} and S = 1 mm^2, models a 10-MHz diagnostic beam at its focus.

Time-average intensity = 1 W·cm^{-2}; pulse average intensity = 500 W·cm^{-2}; beam area = 10 mm^2 except for absorbing medium 2, in which beam area = 1 mm^2.

oscillatory viscous stresses at typical medical ultrasound intensities are of the order of 100 Pa.

Steady forces

Greater attention has been given to those forces whose time average is not zero. The origin of these forces lies in a second-order property of the acoustic wave, the acoustic radiation pressure. The radiation pressure field varies throughout the region of influence of the ultrasonic source and, in a pulsed beam, varies with time. Gradients in this pressure field are associated with a transfer of momentum to the tissue and its components.

For a plane progressive wave of intensity *I*, the force F exerted on an area *S* depends on the energy density *(I/c)*, and a coefficient *D* (the force per unit area per unit energy density [Hueter and Bolt 1955]):

$$F = D\frac{IS}{c}. \quad (1)$$

Values for *D*, and estimates of time-averaged and pulse-averaged forces for a number of conditions, are given in Table 3.1. For a perfect absorber and normal incidence $D = 1$, whilst for a perfect reflector $D = 2$. No perfectly absorbing interface exists *in vivo*. The closest approximation is a soft tissue–bone interface. A lung or bowel gas boundary most closely approximates a perfectly reflecting boundary. The acoustic radiation force exerted on a target is the basis for a standard method of measuring total acoustic power. Forces at interfaces between two liquids, or at a liquid–gas interface, can result in displacement of the interface. These forces are most likely to manifest themselves *in vivo* at highly reflecting targets such as those involving lung and bowel gas.

Irrespective of the presence of large interfaces, absorption of sound energy of the beam by the medium results in a force, the associated coefficient

$$D = 2\alpha$$

For the pulse intensities used in extracorporeal lithotripsy, forces and radiation pressure gradients are greater. Starritt et al. (1991) gave estimates of radiation pressure gradients exerted on a soft tissue target during a pulse of intensity 10^5 W·cm^{-2}, mean frequency 0.5 MHz, of about 10 kPa·mm^{-1} depending on the level of nonlinear distortion. The acoustic radiation force during such a pulse on a perfectly absorbing target is 67 N within a beam of 1 cm^2 cross-sectional area, equivalent to the gravitational force of a mass of approximately 6.5 kg.

There has been little attempt to explore the significance of these forces from large-scale pressure gradients in tissues, or tissue-like materials, although Dyer and Nyborg (1960) demonstrated that a weak agar gel was displaced slightly in an acoustic beam (25 kHz), but the gel recovered its original position when the beam was turned off.

The forces exerted at a given intensity increase with frequency because absorption does so. Starritt et al. (1991) have carried out calculations giving estimates of radiation pressure gradients in tissues in ''typical'' medical pulsed beams. They calculate ''worst-case'' pressure gradients caused by the absorption of the full spectrum of frequencies in fully shocked pulses at liquid–tissue interfaces as 1 kPa·mm^{-1} for a diagnostic pulse and 14.5 kPa·mm^{-1} for a lithotripsy pulse.

If the medium is a liquid, and so free to move under the influence of the force, it will do so, resulting in acoustic streaming, the so-called ''quartz wind.'' This is one of the most easily observable outcomes of the acoustic forces under discussion. This topic is reviewed by Nyborg (1965) and Rooney (1988).

The equations giving estimates of maximum streaming velocity *u* in a beam of radius *r* and intensity *I* are of the form:

$$u = (\alpha I/\eta c) r^2 A \quad (2)$$

where η is the bulk viscosity and α is the attenuation coefficient (Eckart 1948; Nyborg 1965; Tjotta 1959). The constant A is a geometric factor that depends on the dimensions of the beam and container. Both viscous and boundary forces limit the velocities that may be achieved. It only recently has been appreciated that diagnostic intensities of ultrasound are capable of causing significant streaming; velocities up to about 10 $cm \cdot s^{-1}$ in water have been reported (Duck et al. 1993; Starritt et al. 1989). Streaming velocities of the order of 1 $cm \cdot s^{-1}$ were observed in imaging beams.

An exploration of the dependency of the streaming velocity on absorption coefficient and viscosity [eqn (2)] was undertaken by Mitome et al. (1993). Under the highly nonlinear conditions characteristic of most diagnostic pulses in water (WFUMB 1992), absorption also is locally increased, which also results in increased streaming velocities. Streaming in biological fluids will differ from streaming in water because of differing absorption, viscosity and nonlinear parameters. Attenuation in blood, for example, is about 100 times that in water, whilst its relative viscosity is only about 5, leading to the conclusion that streaming velocities in blood could exceed those in water. However, in nonlinear pulsed beams, other factors may dominate, and in these conditions preliminary observations suggest that streaming velocities in blood may be of a similar magnitude to those in water.

The time required to establish a stable stream depends, amongst other factors, on the width of the beam. For pulsed Doppler beams this time is of the order of 100 ms in water and thus is well within the dwell times of clinical practice (Starritt et al. 1989).

Remembering that the radiation pressure also varies radially in an acoustic beam, it is clear that shear also is experienced by the medium. Little exploration of acoustic shear forces has yet been carried out.

FORCES ON STRUCTURAL INCLUSIONS

The second general category of target is that of a small structural inclusion that differs in density and/or compressibility from the liquid space surrounding it. The forces experienced by the target structure will depend on its attachment to the surrounding medium. Streaming will exert a small unidirectional force. The particle velocities due to the wave propagation will exert oscillatory forces of greater magnitude, the ''Stokes'' force. There will be an additional steady component in the direction of the wave because the particle velocity waveform is not sinusoidal (the Oseen force). In addition to these viscous forces, the target itself will experience a direct force due to the radiation pressure gradient at its surface. Estimates of the magnitudes of some of these forces on a 2- μm spherical inclusion of appropriate properties suggest values of the order of 10^{-2} μN in a beam of intensity 4×10^7 $W \cdot m^{-2}$ (Dunn and Pond 1978).

A special case in this category is that of a small bubble. The behaviour of bubbles is dealt with separately (see Chapter 2).

Radiation torque can be experienced by small targets (NCRP 1983). Spinning of intracellular particles in ultrasonic fields has been observed in a number of special arrangements involving small-scale field inhomogeneities. For example, Martin et al. (1978) examined the rotation of organelles in plant cells produced in the field of a Doppler fetal heart detector.

Streaming within small liquid-filled spaces has been studied using local acoustic sources, such as oscillating gas bubbles (see Chapter 2). A number of articles have reported flow induced near a small gas-filled channel in contact with a cell membrane. The gas body is induced to vibrate and, in turn, to cause local vibration of the membrane, thus generating acoustic streaming in the cell protoplasm (Nyborg 1968). At frequencies below 100 kHz, using either bubbles or vibrating needles as the acoustic sources, it was possible to demonstrate that flow could be induced in plant cell cytoplasm, and this flow was attributed to acoustic streaming arising from nonuniform vibration of the cell membranes. Williams (1977) and Frizzell et al. (1986) have demonstrated thrombus formation induced by a vibrating wire or micropipette, apparently caused by shear associated with microstreaming.

Dunn and Pond (1978) have evaluated theoretically ''quartz wind'' streaming through narrow ducts. A number of authors have suggested that it might be possible to cause liquid diffusion through tissue down the acoustic pressure gradient, so-called ''sono- or phonophoresis.'' However, experimental support for this direct action of radiation force is very weak. Some reports of ultrasound-enhanced transport of drugs through the skin may be explained most plausibly by thermal vasodilatation, especially at megaHertz frequencies and when the transducer is in contact with the skin. Many vasodilating agents have been shown to enhance drug diffusion in a similar manner. Conversely when the skin is bathed in an aqueous medium, cavitation-mediated processes seem most probable (Mitragotri et al. 1995; Tachibana and Tachibana 1993).

OTHER ISSUES

In the pulsed fields characteristic of diagnostic applications, all the forces discussed will be modulated by the pulsing regime, operating on the constituent tissues

only during the duration of the pulse, of the order of 1 μs. The medium will experience very brief periods of stress, at repetition rates of the order of 1 kHz, separated by long unstressed periods.

The variation of radiation pressure in a stationary wave can give rise to greater forces than would be expected in travelling waves. For example, in the laboratory, artificial arrangements have been used to demonstrate these forces causing banding of erythrocytes in capillaries of a chick embryo (Dyson et al. 1974) and in small mammals. Cell banding was demonstrated at therapeutic intensities, but it is unclear if banding could ever arise *in vivo* at the lower intensities used in continuous-wave Doppler fields. Banding also has not been demonstrated with diagnostic pulses, probably because the pulse length is insufficient for equilibrium to be achieved.

Streaming may affect the outcome of other bioeffects mechanisms. Wu et al. (1994) report a reduction in ultrasound-induced bone heating when bone is in direct contact with water compared with the heating under a layer of tissue-equivalent material. The difference in heating was ascribed to the convective cooling caused by the water stream flowing across on the surface. In experiments studying cavitation in the presence of travelling waves, streaming will tend to transport bubbles through the field, although the convection velocities are considerably slower than those that may be achieved due to direct radiation forces on bubbles of resonant size (Carstensen et al. 1993). An example has been given by Coleman et al. (1993). Shear has been suggested as a potentiating factor in cell damage caused by heating (Dunn 1985).

POSSIBLE BIOPHYSICAL SIGNIFICANCE OF ACOUSTIC RADIATION FORCES

The question remains whether the forces predicted are of biological significance at clinical diagnostic levels and, if so, how they are manifested. Some approximate values for radiation forces and associated pressures for conditions appropriate to the upper end of diagnostic range are presented in Table 3.1. One example where radiation force could, with some confidence, be identified as the biophysical mechanism responsible for an observed effect is the transient blanching of the choroid layer of the eye with associated depletion of blood flow reported by Lizzi et al. (1981). Whilst this effect occurred at time-averaged intensities that were capable of causing tissue destruction, the pulse average intensities were of the order of 100 $W \cdot cm^{-2}$, *i.e.*, comparable with diagnostic pulses.

''Quartz wind'' streaming must occur within large liquid spaces *in vivo* (urine, amniotic fluid, vitreous humor, ventricular cerebrospinal fluid) and may be observed during clinical scanning at high output levels. This phenomenon is of diagnostic value in distinguishing solid from liquid masses during diagnostic ultrasound examinations. Examples of fluids within which streaming has been reported *in vivo* include intracranial haemorrhage (Betheras 1990), cystic breast fluid (Nightingale et al. 1995) and abscess (Meire, personal communication). However, the process of stirring appears unlikely to be harmful. Shear at the boundary of a 2-mm-diameter stream with maximum velocity 10 $cm \cdot s^{-1}$ in water is about 10 Pa, below the threshold for haemolysis for erythrocytes given by Leverett et al. (1972) as 150 Pa. Nevertheless fluid motion induced close to a membrane can reduce the depth of the unstirred layer close to it and so alter the concentration gradients of diffusing substances there. When substances rapidly permeating across a membrane are rate-limited by diffusion across the unstirred layer, membrane permeability can increase due to ultrasound streaming (Pohl et al. 1993a). The same mechanism could be responsible for the altered membrane transport reported by Mortimer and Dyson (1988). Pronounced alterations of concentration profiles within the membrane unstirred layers (Pohl et al. 1993b) suggest that local alterations of messengers and surface potential can be caused by the same mechanism. This is supported by the observation of transient enhancement of erythrocyte agglutination and aggregation by ultrasound (Pohl et al. 1995). The possibility of liquid transport within the tissue structure remains speculative and deserves further study because of the importance of local fluid balance on a variety of cell functions.

Disturbance of soft tissue depends on its local mechanical properties, which are complex (Fung 1987). The ultimate tensile strength of the weakest tissue noted by Yamada (1970), kidney, is 50 kPa. It also was noted that fetal tissue strengthened with gestational age, although the measurements reported were only on fetal connective tissue, with strengthening occurring later in pregnancy. Further data on other strengths, including shear strength, of a wider range of tissues are needed, together with a deeper understanding of nonlinear mechanical properties including thixotropy and hysteresis. Recently, evidence has been reported of hemorrhage within mouse fetal tissue in the apparent absence of gas bodies (Dalecki et al. 1997b), caused by an experimental piezoelectric lithotripter (0.3 MHz, $P < 1$ MPa). Soft tissue damage was observed near developing bone and cartilaginous structures whilst adjacent soft tissues remained free of damage. In the absence of other known mechanisms for damage, the hemorrhage could have resulted from relative motion between partially ossified bone and surrounding soft tissue caused by differential radiation force. However, the consensus remains that mechanical

damage to tissue from radiation force and streaming at current diagnostic intensities appears to be unlikely. If it were to occur, it would probably occur within weak tissue close to a tissue–air or tissue–bone boundary. On the other hand, relative displacement of cells, altering their expected channels of communication, could be postulated as a realistic outcome of the weak forces described. If this were to occur, and the cells failed to return to their undisturbed position so that cellular links could be reestablished when exposure ceased, the subsequent development of the cellular structure might be disturbed. The relationship between the radiation stresses and stress gradients associated with pulse propagation and the intercellular cohesive forces is poorly quantified. There is a need to explore thresholds for permanent cell displacement, particularly for weakly bound tissue structures.

Another reference point might be the forces associated with surface tension. The interfacial tensions for water against olive oil and water against air are 20 and 71.2 $\mu N \cdot mm^{-1}$, respectively. Cell processes that depend on surface tension could be modified by the additional stresses implied in Table 3.1.

The direct action of ultrasound on skin and on deep-seated nerves causing sensations of warmth and pain has been related to a threshold in displacement amplitude (Gavrilov 1984), whilst thresholds for tactile sensations have been related directly to radiation force (Dalecki et al. 1995). Foster and Wiederhold (1978) and Magee and Davis (1993), amongst others, have reported the direct stimulus of auditory responses by pulsed ultrasound. Magee and Davis demonstrated that the pulse repetition frequency of a pulsed Doppler beam could be sensed, when the beam was directed from the base of the skull, upwards towards the ear. The mechanism seems to be direct mechanical action via acoustic radiation pressure. Ultrasound pulses of sufficient magnitude and duration can alter cardiac function. Dalecki et al. (1997a) have shown that a reduction in aortic blood pressure can be related to radiation force exerted on the myocardium. Estimated radiation force thresholds were about 3 mN for 5-ms pulses and 1 mN for 10-ms pulses.

WFUMB SYMPOSIUM CONCLUSIONS ON RADIATION FORCE AND STREAMING

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz 1996.

- Ultrasonic beams exert radiation forces on tissues and biological fluids. In currently used clinical diagnostic procedures, these forces are of the order of milliNewtons during the pulse.
- Liquid flow can be caused by radiation forces in diagnostic ultrasound fields. Maximum streaming velocities measured *in vitro* in water and blood are of the order of 10 $cm \cdot s^{-1}$ at pulsed Doppler intensities and frequencies. Acoustic streaming has been observed *in vivo* within abscesses, cysts and intracranial haemorrhage during ultrasound scanning.
- Acoustic radiation forces increase with intensity. Streaming velocities increase with time-averaged intensity and with acoustic attenuation and decrease with increasing shear viscosity. Forces and streaming also are enhanced at larger ultrasound amplitudes because of nonlinear propagation.
- Radiation forces are the most probable cause of some observed physiological responses to ultrasound; for example, retinochoroidal blanching, tactile sensation and auditory stimulation in the adult. Of these, only auditory stimulation has been reported at diagnostic exposure levels.
- Neither acoustic radiation forces nor streaming are likely to cause mechanical damage under diagnostic conditions.

Note that these conclusions concern the acoustic radiation forces exerted throughout an ultrasonic beam, excluding considerations of any soft tissue–gas boundaries such as those found in the lung, intestines and echo-contrast agents.

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Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. S29–S34, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00076-3

● *Chapter 4*

FREE-RADICAL PRODUCTION: ITS BIOLOGICAL CONSEQUENCES

INTRODUCTION

In biology and medicine, free-radicals are now of wide interest because they appear to be involved in many different aspects of metabolism and disease (Halliwell and Gutteridge 1989). A free-radical is any species capable of independent existence that contains one or more unpaired electrons. It is well known that some free-radicals, especially hydroxyl radicals (OH radicals) react rapidly with almost all biological molecules at nearly diffusion-limited rates (rate constants are more than 10^9 $dm^3 \cdot mol^{-1} \cdot s^{-1}$) and can alter biomolecules, such as DNA, proteins, and lipids (von Sonntag 1987). Consequently, ultrasonically induced free-radical formation is of considerable interest in terms of: 1) potential risks associated with patient exposure to diagnostic and/or therapeutic ultrasound, 2) exploring possible uses in cancer therapy in combination with ionizing radiation and/or anticancer drugs, and 3) understanding the mechanisms whereby ultrasound can affect cells. Such research also can provide important information on the alteration of sonicated samples and the acceleration of reactions via free-radical processes for laboratory application.

FREE-RADICAL PRODUCTION BY ULTRASONIC CAVITATION

Biophysical modes of ultrasonic action can be classified into two categories: 1) thermal mechanisms, and 2) nonthermal mechanisms. The nonthermal mechanisms involve two processes: cavitational and noncavitational. When an acoustic wave propagates through a liquid, pressure fluctuations occur, whereby the liquid is successively subjected to compression and tension. Such tension, or negative pressure, can cause the rapid growth of previously existing seed-gas nuclei (which may be microscopic gas pockets stabilized through attachment to solid motes in the liquid or proteinaceous shells stabilized through the presence of a hydrophobic skin, or unstabilized gas pockets recently generated by ionizing radiation). This bubble-growth phase may be followed by a rapid adiabatic collapse, which serves to concentrate energy from the sound wave into the bubble system. The compressed gas within the bubble may attain temperatures of several thousands Kelvin and pressures of several hundreds atmospheres. A gas shock may propagate through the bubble. On rebound, a shock wave may be generated in the liquid. The high temperatures and the passage of shock waves can generate free-radicals within a gas (Leighton 1994). It is not certain whether either of these mechanisms or some other mechanism dominates for the production of free-radicals during a given cavitation event. However, what is known is that the cavitation that accompanies the passage of high-amplitude ultrasonic waves in a liquid can readily generate free-radicals (Suslick et al. 1986).

It has been shown that sonochemical reactions take place in three different regions (Riesz and Kondo 1992). The first is the high-temperature region within the gas contained by the collapsing bubbles. In this region, hydrogen atoms and OH radicals are formed by thermal dissociation of water. Any volatile solutes in this region will give rise to radicals typical of combustion processes. The second region is the interface between the hot gas bubbles and the bulk of the liquid, where large temperature and pressure gradients as well as high radical concentrations exist. Nonvolatile solutes with charged groups that anchor them in the aqueous phase also may give rise to radicals by pyrolysis (*i.e.*, degradation due to high temperature), if they are present at high concentrations in the interfacial region. The third region is the bulk of the solution at ambient temperature where the radicals that have escaped from the hot zones react with solutes to give rise to products that are similar to those observed in aqueous radiation chemistry (Riesz and Kondo 1992).

Sonochemistry of water

Hydroxyl radical formation induced by ultrasound in vitro. When cavitation occurs in response to the passage of high-intensity ultrasonic waves through water, the dissociation of water vapour into H atoms and OH radicals is readily observed. The H atoms and OH radicals either combine to form H_2, H_2O_2 and water or attack solute molecules that are reduced or oxidized. Ultrasound exposure in the presence of oxygen leads to the formation of oxygen atoms. Hydrogen atoms

react with oxygen to form HO_2 radicals, the acid form of superoxide anion radicals. Recently, it has been shown that HO_2 radicals are formed directly by the sonolysis of water in the absence of O_2 (Kondo and Riesz 1996). There are several reviews on different aspects of free-radical formation induced by ultrasound (Henglein 1987; Riesz and Kondo 1992; Riesz et al. 1985; Suslick 1988, 1989, 1990).

The H and OH radicals are extremely reactive and consequently survive in aqueous media for only about 10^{-6} s. Some molecules (called spin-trapping agents) have chemical structures that can stabilize an unpaired electron by delocalization, so that the free-radical form of those molecules may be stable for prolonged periods. Thus, one of these molecules could interact with an OH radical to form a new macroradical that is stable; the accumulated stable macroradicals are then identified and quantified by electron spin resonance (ESR) spectroscopy. ESR spin-trapping with 5,5-dimethyl 1-pyrroline N-oxide (DMPO) has been applied to aqueous sonochemistry, and DMPO-OH and DMPO-H adducts induced by ultrasound have been observed (Makino et al. 1983). When the yield of OH radicals was measured by spin trapping with DMPO after sonication of water saturated with different rare gases, the DMPO-OH yields were in the order Xe > Kr > Ar > Ne > He (Kondo et al. 1988), *i.e.*, in the sequence of their vapour pressures. When the relationship between the ESR signal intensity of the DMPO-OH adduct and estimates of the final temperature of the collapsing cavitation bubbles was examined, the ESR signal intensity increased with increasing values calculated for the final temperatures of the inertially cavitating bubbles containing these noble gases (Fig. 4.1).

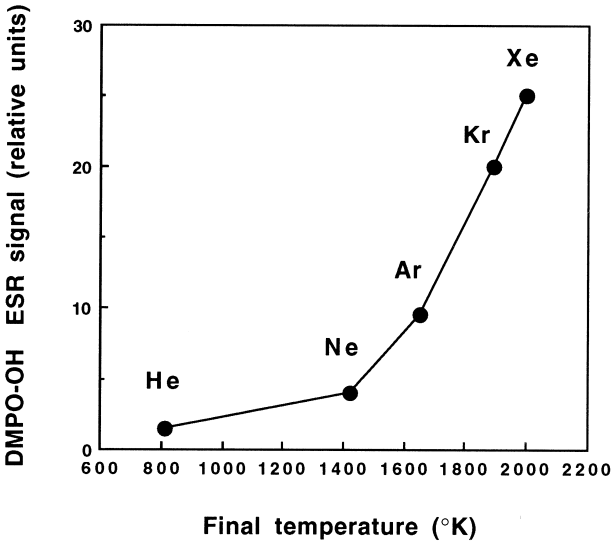

Fig. 4.1. The relationships between the electron spin resonance (ESR) signal intensity of the DMPO-OH adduct and the final temperature of the gas contained within the collapsing cavity, as estimated by Young (1976). For the assumptions inherent in these estimations, see Leighton (1994: 472). Redrawn from data of Kondo et al. (1988).

Free-radical formation in amino acids and DNA constituents in vitro

In spin-trapping studies of the sonochemistry of dilute aqueous solutions of amino acids, only radicals formed by abstraction of specific atoms and/or additions to double bonds by OH radicals and H atoms were detected. The radicals were similar to those observed in aqueous radiation chemistry (Krishna et al. 1988). In the sonochemistry of dilute aqueous solutions of pyrimidine, both nucleoside and nucleotide radicals formed by the addition of OH radicals to the 5,6 double bond of the pyrimidine ring were identified by ESR spin-trapping. However, at higher solute concentrations, additional new radicals (typically methyl radicals) produced by thermal decomposition of solutes in the interfacial high-temperature regions were observed for amino acids and nucleotides (Kondo et al. 1989b, 1990). The occurrence of thermal decomposition due to high temperature in the interfacial regions between the gas and the aqueous phase of cavitation bubbles was observed in the aqueous sonochemistry of various polymers including DNA. Production of carbon monoxide, formed by thermal decomposition of the polymer, was greatest for polyethylene glycol and least for DNA (Henglein and Gutierrez 1988).

BIOLOGICAL EFFECTS OF ULTRASOUND-INDUCED FREE-RADICALS

DNA, Enzymes and lipids, in vitro

The review by Elsner and Linblad (1989) is useful for understanding the history of ultrasonic degradation of DNA. It has been shown that the decrease in transforming activity of *Haemophilus influenzae* DNA induced by ultrasound was inhibited by aminoethyl isothiouronium bromide (Bach 1971). The contribution of ultrasonically induced free-radicals to DNA degradation and to the loss of biological activity of DNA was suggested. Ultrasound exposure induced single- and double-strand breaks in aqueous solutions of calf thymus DNA, but when sonication occurred in the presence of cysteamine, a free-radical scavenger, the number of single-strand breaks decreased, whereas the number of double-strand breaks remained virtually unaffected (Kondo et al. 1985). Therefore, in aqueous DNA solutions, the double-strand breaks were exclusively induced by the mechanical shearing stress and the majority of single-strand breaks were produced by

Table 4.1. Protection on cell killing induced by ultrasound in the presence of cysteamine.

Cells	Frequency (MHz)	Experimental Set-up	Intensity $W \cdot cm^{-2}$	Temperature (°C)	Cysteamine concentration (mM)	Biological endpoint	Results (+/−)	Change of % survival with cysteamine	Reference
V-79	1.1	Rotating tube	20	37	8	c.f.u.	+	(1–2.5)	Fu et al. (1979)
			10				+	(7–10.5)	
						lysis	−		
CHO	1.1	Chamber with thin film	1	3–37	50	c.f.u.	+	(15–90)	Armour and Corry (1982)
						lysis	+		
EMT6	1	Rotating tube	5	37	8	c.f.u.	+	(3.4–13)	Dooley et al. (1984)
						lysis	−		
L	1	Rotating tube	5.8	20	2	c.f.u.	+	(80–100)	Kondo and Kano (1988)
						lysis	−		
CHO	0.05	Fixed tube		25	50	lysis	−		Kondo et al. (1988)
CHO	1	Chamber with thin film	1.8	3–37	14.5	c.f.u.	+	(10–70)	Inoue et al. (1989)
			7.2			lysis	+	(75–100)	

c.f.u. = colony-forming unit.

OH radicals and H atoms resulting from sonolysis of water. In addition to causing strand breakage, free-radicals can attach to the bases of DNA and change them, so that repair enzymes then may substitute an incorrect base at that location.

The protection by cysteamine against thymine base damage induced by ultrasound inside EMT6 mouse mammary sarcoma cells *in vitro* is an example of intracellular DNA damage due to ultrasonically induced free-radicals (Dooley et al. 1984). Although single-strand breaks in the intracellular DNA were readily observed to be induced by ultrasound by inertial cavitation (see Chapter 2), the DNA damage appeared to reside primarily in Chinese hamster ovary (CHO) cells, which did not survive sonication (Miller et al. 1991a). However, some formation of single-strand breaks in DNA of fresh cells added to sonicated medium resulted from residual hydrogen peroxide (Miller et al. 1991b). Induction of single-strand breaks in DNA of mouse mammary carcinoma FM3A cells was confirmed in cells not surviving ultrasonic cavitation but double-strand breaks were not observed (Kondo et al. 1993). Recently, DNA damage was detected by ''comet'' assay (single-cell gel electrophoresis) in cells surviving inertial cavitation (Miller et al. 1995).

The inhibition by 2-mercaptoethanol, acting as a free-radical scavenger, of the inactivation of alcohol dehydrogenase, induced by 20-kHz ultrasound, has been shown (Coakley et al. 1973). Additional effects of free-radicals induced by 0.88-MHz ultrasound on the inactivation of the enzymes hexokinase and erythrocyte acetylcholine esterase also have been reported (Braginskaya and Zorina 1987; Braginskaya et al. 1990).

The formation of malondialdehyde (MDA) can be used as an indicator of lipid peroxidation. Its formation in the liposomal membrane under the influence of 20-kHz and 3.5-MHz ultrasound has been shown to be inhibited by several OH radical scavengers (Jana et al. 1990) but not by superoxide dismutase (SOD), histidine, dimethylfuran or β-carotene. Acting through a mechanism of OH radical scavenging, HEPES buffer can protect against lipid peroxidation, as demonstrated for model membranes, when polyunsaturated phospholipids are subjected to ultrasound (Fiorentini et al. 1989).

The results of the collective studies in this section indicate that biomolecules *in vitro* can be inactivated or altered not only by mechanical breakdown but also by ultrasonically induced free-radicals. There is a possibility of DNA damage due to free-radicals in cells that survive inertial cavitation.

Effects of free-radicals on cell killing

The contribution of mechanical and sonochemical effects to cell killing of mouse L5178Y cells *in vitro* induced by 1-MHz ultrasound was investigated initially by (Clarke and Hill 1970). Although prolonged sonication of the medium can reduce the capacity to support cell growth, such indirect sonochemical effects are insignificant in the short duration of sonication required to kill 99% of cells, and the mechanical action of ultrasound is suggested as the primary mechanism of cell damage.

There are several reports on the use of sulfhydryls, such as cysteamine, as a radical scavenger for the estimation of free-radicals induced by ultrasound on cell killing (Armour and Corry 1982; Dooley et al. 1984; Fu et al. 1979; Inoue et al. 1989; Kondo et al. 1988; Kondo and Kano 1988). The contribution of cysteamine to the protective effect in cell killing induced by ultrasound varied from about 1.5% to more than 70% depending on acoustic fields, intensity and cell type (Table 4.1).

Reduction of cavitation-induced cellular damage by vitamin E has been reported recently (Suhr et al. 1994). After an *in vitro* exposure to shock waves, increased amounts of free-radicals were found in intact MGH-U1 cells, in which mitochondria and numerous intracellular

vacuoles were damaged. Excess free-radicals may have originated from within the cells, or they may have come from damage produced intracellularly by extracellular bubble activity, without damage to the cell membrane.

The effects of shear stress and free-radicals induced by 50-kHz ultrasound on damage to erythrocytes have been examined. The membrane fluidity, permeability and the deformability of remaining unlysed cells after sonication with or without free-radical formation were unchanged and identical to those of the control cells (Kondo et al. 1989a).

Cell lysis induced by ultrasound appears not to be due to a free-radical mechanism because several reports showed no protection by cysteamine, except for those studies that used a chamber with a thin anechoic film and range of temperatures (Table 4.1).

It appears unlikely that free-radicals form directly inside cells from ultrasound exposure, since the higher viscosity of cytoplasm inside the cell tends to mitigate against cavitation and the potential for nucleation appears to be less. The short lifetime of the free-radicals produced extracellularly means that they have a limited diffusion distance and so are unlikely to be able to migrate from outside the cell to sensitive sites within the cell, such as nuclear DNA. Additionally, the serum in culture medium contains large amounts of proteins that are likely to scavenge extracellular free-radicals. Free-radicals could, however, form long-lived sonochemicals (*e.g.,* H_2O_2) that might penetrate cells and affect intracellular target molecules in the absence of catalases, which are ubiquitous in mammalian tissues.

The migration of free-radicals from outside to the inside of cells may be facilitated by a transient increase in the permeability of the cell membrane, for example, as a result of cavitation-related shearing forces. Introduction of macromolecules (*e.g.,* plasmid DNA) into living mammalian cells by sonication has been reported (Fechheimer et al. 1987). To explain the protective effect of cysteamine, a cell-permeable scavenger, it has been proposed that extracellularly produced free-radicals may be injected into the cell by cavitation microjets (Inoue et al. 1989); however, this mechanism is only known to operate near rigid surfaces. There are some difficulties in estimating the free-radical contribution to cell killing by using only the radical scavenger cysteamine. Further studies of cell survival in the presence of different types of free-radical scavengers with various diffusion rate constants would be useful.

With regard to a combined effect of ultrasound with drugs, the involvement of single oxygen was suggested as a causative factor, since cell damage as evidenced by decreased viability was enhanced by the combination of ultrasound and the photosensitizer hematoporphyrin; the damage was suppressed by the addition of histidine but not by mannitol. Since SOD had some protective effect against cell damage, it was postulated that superoxide anion radicals also are involved in the enhancement mechanism (Umemura et al. 1990). Kessel et al. (1994) observed enhancement of ultrasound cytotoxicity by porphyrins in the incubation medium but not by intracellular porphyrins, and there was no correlation between the efficacy of a given porphyrin for light- versus ultrasound-induced cytotoxicity. Although the mechanism of the enhancement *in vivo* is not well understood, future developments are expected.

It seems reasonable to conclude that the cell lysis and a large fraction of cell killing (so-called reproductive death) are not due to free-radicals induced by ultrasound. However, a very modest role for free-radicals is projected for induction of deleterious effects by sonochemicals for those cells that survive the inertial cavitation events.

Free-radical formation induced by ultrasound simulated for medical use

There is evidence for free-radical formation in aqueous solutions, or in biological fluids, induced *in vitro* by exposure to ultrasound at acoustic frequencies and pressures similar to those used for therapeutic and diagnostic purposes. This includes continuous-wave and tone-burst exposures (Armour and Corry 1982; Clarke and Hill 1970; Crum et al. 1987; Dooley et al. 1984; Eastwood and Watmough 1976; Edmonds and Sancier 1983; Fu et al. 1979; Inoue et al. 1989; Jana et al. 1990; Kondo and Kano 1988; Kondo et al. 1985; Leighton et al. 1988; McLean and Mortimer 1988; Miller et al. 1991a, 1991b; Pickworth et al. 1988).

Theoretical models of Flynn (1982) and Apfel (1986) have predicted that inertial cavitation can occur in water with microsecond pulses above a certain intensity threshold for an assumed optimal nucleation situation. For water exposed to a pulse duration of 6.5 μs of 1-MHz ultrasound and temporal maximum intensities from 8.4–140 W/cm^2, significant ESR signals of DMPO-OH spin adducts were found (Carmichael et al. 1986). The cavitation threshold for chemoluminescence resulting from microsecond pulses in a buffered aqueous solution at pH 10.9 was shown to be within the range of some imaging and Doppler units (Crum and Fowlkes 1986). As an example of a biological effect of diagnostic pulsed ultrasound, the suppression of cultured cell growth after sonication of 3-μs pulsed ultrasound at an intensity (I_{spta}) of 240 mW/cm^2 with 1000-Hz repetition frequency for 30 min has been demonstrated (Maeda et al. 1986).

FUTURE SUBJECTS TO BE INVESTIGATED

Although comparisons among different experimental protocols and results are difficult and more research is required, the expected overall concentrations of free-radicals resulting from cavitation are small compared with those produced biochemically in the body (*e.g.*, from smoking, inflammatory response) (Halliwell and Gutteridge 1989); also lifetimes are very short. However, preliminary experiments on ultrasound-induced H_2O_2 production suggest that the local concentration of ultrasound-induced free-radicals may be comparable to the concentrations that can be biologically significant, if found at a biochemical sensitive site (Miller and Thomas 1994).

The quantification of free-radicals formed by ultrasound and the possibility of intracellular production of free-radicals by bubble activity, which is highly likely to be extracellular, remain subjects for further investigation.

Now becoming popular are certain diagnostic procedures in which the tissues to be interrogated with ultrasound are deliberately infused with a large number of stabilized microbubbles, which are just about the ''right size'' for explosive growth at the frequencies and amplitudes used in diagnostic ultrasound. Thus, what was once considered a ''not-gas-nucleated'' medium has become richly endowed with such nuclei, thus raising the potential for inertial cavitation to occur *in vivo.*

WFUMB SYMPOSIUM CONCLUSIONS ON FREE-RADICAL PRODUCTION AND BIOLOGICAL EFFECTS

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz, 1996.

- Free-radical formation by ultrasound is due to inertial cavitation and therefore is strongly dependent on its threshold acoustic pressure at specific frequencies.
- Free-radical formation by ultrasound cavitation in aqueous systems is initially due to the dissociation of water and any solutes. Radicals can chemically modify biomolecules (*e.g.*, enzymes, DNA and lipids) with potentially serious consequences.
- Free-radical formation has been observed *in vitro* in aqueous solutions and biological fluids following exposure to continuous-wave, tone-burst and microsecond pulses of ultrasound at acoustic pressures similar to those that may be encountered during medical applications.
- Free-radicals produced by ultrasound are associated with cell killing *in vitro,* although the extent of their role is not established. Free-radicals or their chemical products have been shown to cause intracellular DNA damage *in vitro.*
- Since inertial cavitation has not been shown to occur *in vivo* under diagnostic exposure conditions, free-radical formation is not expected *in vivo* under the same conditions.

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Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. S35–S39, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00077-5

● *Chapter 5*

OTHER NONTHERMAL BIOEFFECTS: ORGANS, CELLS AND TISSUES

INTRODUCTION

The nonthermal mechanism that has been studied most widely in the search for biological effects resulting from ultrasonic exposures is cavitation. Interpretation of the published literature must be carried out with caution, since effects produced by cavitation may be introduced from the experimental technique itself. Differences in exposure conditions and cellular environment usually mean that samples exposed to ultrasound *in vitro* may be more likely to exhibit cavitation activity than those exposed *in vivo*. For example, cells exposed to ultrasound in suspension are in an aqueous medium in which any bubbles may freely expand and contract, unlike those that may be trapped within intercellular spaces in tissues *in vivo*. It also is clear from published work that design, position in the field and rotation of the sample holder are all significant factors in the amount of cavitation produced. However, results from *in vitro* exposures give valuable insight into the mechanisms by which ultrasound can produce biological effects.

Therefore, this chapter will review the evidence for biological change induced by ultrasound both in cell cultures *in vitro* and in organised tissues *in vivo*.

NONTHERMAL BIOLOGICAL EFFECTS

Suspensions of single cells

One advantage of using cell culture for the study of biological effects is that it is possible to examine the direct effect of ultrasound on a specific cell type without the reaction being affected indirectly by a systemic response to the exposure. In addition, it is possible to design experimental conditions such that the bulk temperature does not rise during the ultrasonic treatment.

Where violent cavitation takes place, cells may come into contact with either the oscillating bubbles or with the high shear stresses created by them, and lysis is a common consequence. Wherever genetic damage has been reported (Doida et al. 1990, 1992; Kaufman et al. 1985; Miller et al. 1991), inertial cavitation has been implicated. It seems probable that genetic damage is due to the free-radicals produced during violent bubble collapse.

Altered transport of ions across cell membranes has been attributed to a form of ''mixing'' caused by the streaming patterns set up by stable cavitation bubbles. This may be the basis for the therapeutic action of ultrasound in enhancing wound healing (Dyson 1990; Mortimer and Dyson 1988). It also has been suggested that intracellular cavitation may occur (Inoue et al. 1989).

Organised tissues in vivo

There are two main problems in the study of cavitation *in vivo*. The first is that absorption of the incident sound beam leads inevitably to a local rise in temperature. It is possible, however, to arrange the incident energy levels and/or the pulsing regime in such a way that this is kept below a biologically significant level. The second problem is that many of the methods used to detect cavitation *in vitro* are not suitable for use in opaque media such as tissue (see Chapter 2). The majority of evidence for the occurrence of cavitation events in mammalian tissues comes from histology.

Hug and Pape (1954) found groups of ''bubbles'' in rat and mouse liver and in brain following exposure to ultrasound. Bell (1957) reported so-called ''pseudocavitation'' holes in murine liver, which also occurred even when the tissue was cooled sufficiently during exposure to ultrasound to prevent the temperature from rising to damaging levels. Mechanical disruption (including ''holes'') in histologic sections has been attributed to cavitation damage (Curtis 1962).

The hind limb paralysis reported by Lee and Frizzell (1988) following exposure to ultrasound could be suppressed by the application of excess hydrostatic pressure. This is commonly believed to be an indication that the observed effect is due to a cavitational mechanism.

There is a considerable body of evidence pointing to the occurrence of cavitation *in vivo* as a result of the high-intensity tone burst or continuous-wave exposures such as those used in focused ultrasound surgery. For example, Fry et al. (1970) described lesions in cat brain

that were attributed to cavitation damage, as did Frizzell et al. (1988) in cat liver.

Hynynen (1991) reported cavitation during hyperthermia treatment of dogs. Lee and Frizzell (1988) showed that the intensity at which ultrasound-induced cavitation events were seen was reduced as the ambient temperature increased. These results are not surprising. As the tissue temperature increases, it is to be expected that gas solubility in tissue decreases, and so the availability of gas nuclei improves, as would their ability to grow in an ultrasonic field. The probability of seeing a cavitation event would therefore rise.

Effects due to existing gas bodies

It has been reported that biological effects may be induced when ultrasound is incident on organised tissues containing large stabilised gas bodies. Effects have been demonstrated both in plant (*Elodea*) leaves and in fruit flies (*Drosophila melanogaster*) [see, for example, Carstensen (1987) and Miller (1987)] and also in the murine intestine (Dalecki et al. 1995). This work will not be dealt with in detail here, but it has demonstrated that considerable cellular damage may be induced in organised structures at diagnostically relevant exposure levels if stabilised gas bodies are present.

Recently, evidence has been presented that pulsed ultrasound can lead to hemorrhage at the surface of mammalian lungs (Child et al. 1990). This occurred at levels that were low compared to those required to produce cavitation in other tissues that do not contain gas bodies, a finding substantiated by Frizzell et al. (1994) in neonatal mice. Alveolar flooding and protein leakage into alveolar spaces were seen in the alveoli that contained erythrocytes. Vascular lesions were seen, with endothelium, epithelium and basal laminae disrupted (Penney et al. 1993). It seemed clear that the damage occurred during exposure and did not progress afterwards. Cell death appeared to be due to lysis. Raeman et al. (1993) found that a brief exposure of the lung at a high repetition frequency was less likely to cause damage than an exposure with the same total on-time applied over a longer duration. Also, lung hemorrhage has been produced in the lung of *Macaque* monkeys following exposure to diagnostic ultrasound (Tarantal and Canfield 1994). The mechanism for this (clearly nonthermal) effect is not clear, although it does appear to be mediated by an existing stabilised gas body in tissue (Table 5.1).

Cavitation due to lithotripsy pulses

Lithotripters produce pulses of microsecond length and of several hundred atmospheres peak pressure. The acoustic pulse energy of 10–150 mJ is repeated at a low pulse repetition rate of 1–3 Hz. This output is unique in that there is no significant tissue heating because of the low total power used. Thus, results from lithotripsy studies are of interest. However, there are significant differences between lithotripsy pulses and the short pulses used in diagnostic ultrasound. Lithotripsy pulses have a longer negative pressure portion and a greater peak positive pressure. Therefore, biological effects may occur with lithotripter pulses in tissue that do not occur in the same way with diagnostic ultrasound.

Tissue effects of lithotripters are best characterised in the liver, kidney and lung (Delius et al. 1987, 1988b, 1990b) and recently in the gut (Miller and Thomas 1995a) (Table 5.1). The effects generally consist of vessel wall damage, especially of capillaries and medium-sized veins; focal lesions in arterial walls do occur, but less frequently. The consequences of this type of damage are hemorrhage of a variable degree, which may range from being either only detectable microscopically, up to being severe enough to necessitate surgical intervention, or to the formation of venous thrombi.

Cavitation from lithotripsy pulses can be visualized by diagnostic ultrasound since the pulses generate relatively stable gas pockets in tissues (Delius 1990a). It occurs along the axis of the shock wave field and persists long enough to enable the interaction of a shock wave with the remnant gas from the preceding pulse. The importance of this shock wave–gas bubble interaction for the generation of tissue damage has been derived from an increase in tissue damage at higher shock wave administration frequencies of 15 or 100 Hz (Delius 1988a, 1990b). This is further supported by the fact that effects of lithotripters and the cellular level (hemolysis and membrane permeabilisation) are considerably reduced by minimal static excess pressures as low as 100 kPa. Moreover, the administration of preformed, stabilised gas bubbles during the shock wave application led to a dramatic increase in tissue damage (Prat et al. 1991).

The lung is the organ most sensitive to shock waves. Hartman et al. (1990) showed that fetal murine lung had a higher threshold for lithotripsy damage than did adult lung. This again was presumed to be due to the absence of preformed gas bubbles in the fetal lung, as compared with the air-filled alveoli in adult lung.

CONTRAST AGENTS

A rapid development of gas body-based ultrasound contrast agents has occurred over the last few years (Goldberg et al. 1994; Fowlkes 1995). Albunex® (Molecular Biosystems Inc., San Diego, CA, USA) and Levovist® (Schering AG, Berlin, Germany) have been approved for

Table 5.1. Intensities at which cavitation has been observed in organised mammalian tissues.

	Effect studied (Reference)		Frequency (MHz)	Pulse length	Exposure time (s)	Intensity (Pressure)
Mammalian tissues						
in vivo	Liver damage	(1)	1	cw	480	1.6–2.0 W/cm^2
	Brain damage	(1)	1		480	1.3–1.6 W/cm^2
	Lens damage	(1)	1		480	1.8–2.2 W/cm^2 (SA)
	Mouse liver ''pseudocavitation''	(2)	1	cw	15	$<$ 20 W/cm^2 (SA)
	Cat brain	(3)	1–3	cw	$<$ 0.04	2000 W/cm^2
	Cat liver	(4)	3	cw	0.03	2500 W/cm^2
	Cavitation in dog thigh	(5)	0.2–1.68	cw	1	700 @ 1 MHz (5 MPa/MHz)
Lithotripsy	Shock wave damage; in pig liver	(6)				80 MPa
	in dog liver and gall bladder wall	(7)				80 MPa
	Murine lung haemorrhage; Adult	(8)				1.7 MPa
	Fetal	(8)				20 MPa
System containing pre-existing gas	Murine lung haemorrhage	(9)	1.0–3.7	10 μs (1:1,000)	180	0.007–0.1 W/cm^2 (TA) (P^- 0.4–1.4 MPa; P^+ 0.7–3.0 MPa). (1.1–3.7 MHz)
	Murine subscapular lung haemorrhage	(10)	1.1	10 μs (1:1,000)	180	0.04 W/cm^2 (TA) (1.6 MPa)
	Neonatal lung haemorrhage		1.0	10 μs (1:1,000)	2.4	300 W/cm^2 (SPPA)
				(1:10,000)	180	30 W/cm^2 (SPPA)
	Neonatal hind limb paralysis	(11)	1.0	10 μs (1:200)	2.4	125 W/cm^2 (SPPA)
	Murine lung damage	(12)	1.1		0.03	27 W/cm^2 (SPPA) (1 MPa)

SA = spatial average; TA = temporal average.

(1) Hug and Pape 1954; (2) Bell 1957; (3) Fry et al. 1970; (4) Frizzel 1988; (5) Hynynen 1991; (6) Delius et al. 1990a; (7) Delius et al. 1990b; (8) Hartman et al. 1990; (9) Child et al. 1990; (10) Penney et al. 1993; (11) Frizzell et al. 1994; (12) Raeman et al. 1993.

clinical use. These companies and several others are pursuing development of second-generation agents that use special gases to improve the persistence of the contrast effect. Imaging benefits of gas body contrast are thought to apply to a quarter of all ultrasound examinations, which implies a large potential market for these agents by the year 2000 (Wight 1995). The newer agents show promise for diagnostic ultrasound, particularly for power Doppler modes to display and quantify actual perfusion of organs. In addition, a second harmonic technique is being evaluated on newer diagnostic instruments, which takes advantage of the nonlinear scattering of the gas bodies at twice the imaging frequency to enhance greatly the contrast effects (Burns 1995).

The advent of gas body contrast agents has remarkable implications for the consideration of nonthermal bioeffects of ultrasound. Circulating blood normally contains very few gas bodies or cavitation nuclei (Williams et al. 1989). Gas body-based contrast agents are certainly resonant gas bodies as shown by theory (de Jong et al. 1992), measurement (Bleeker et al. 1990) and the second-harmonic contrast technique *in vivo* (Schrope et al. 1992). In addition, these agents can serve as inertial cavitation nuclei in otherwise cavitation-free systems (Miller and Thomas 1995b). Therefore, injection and ultrasound exposure of gas body contrast agents introduces the potential for cavitation into the body under otherwise cavitation-free conditions. Recent research indicates that a gas body contrast agent can cause hemolysis upon exposure to 1-MHz ultrasound under some conditions, even in whole blood (Brayman et al. 1995; Miller et al. 1995; Williams et al. 1991). The ability of gas body contrast agents to initiate ultrasonic cavitation, and the known potential for biological consequences *in vitro* and *in vivo,* enhances the relevance of nonthermal bioeffects considerations for risk evaluation and sonographer guidance during diagnostic ultrasound examinations with contrast agents.

Table 5.1 summarises the work on mammalian tissue referred to in this chapter. The acoustic parameters given are quoted from the articles as those at which some form of cavitation has been seen. Intensities quoted are a mixture of pulse average, temporal average and spatial average intensities. Exposure times are given. These are total times, not on-times. It is not clear what role exposure time plays in determining cavitation threshold.

WFUMB SYMPOSIUM CONCLUSIONS ON OTHER NONTHERMAL BIOEFFECTS

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz, 1996.

- *In vitro* studies of ultrasound biological effects have demonstrated cellular effects that include lysis and changes in ion transport across membranes. These studies give useful insight into the nonthermal mechanisms by which ultrasound can produce biological effects. However, caution is required in applying these results to medical ultrasound exposures *in vivo.*
- Cavitation effects have been shown to occur *in vivo* in mammalian tissues. The exposure threshold for cavitation effects decreases with increasing temperature. Effects seen include mechanical disruption of tissue structure (obvious under histologic examination) and hind limb paralysis. Such effects have not been observed with diagnostic exposures.
- In the air-filled mammalian lung, bleeding from alveolar capillaries has been induced experimentally by ultrasound at diagnostic exposure levels. This effect has not been observed in the fluid-filled mammalian fetal lung. To date there is no direct evidence as to whether or not this effect can occur in humans.
- Current evidence indicates that the lowest thresholds for nonthermally induced mammalian tissue damage are found where gas bodies are present, such as in lung alveoli and intestine.

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Ultrasound in Med. & Biol., Volume 24, Supplement 1, 1998, S41–S49
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00078-7

● *Chapter 6*

THRESHOLDS FOR NONTHERMAL BIOEFFECTS: THEORETICAL AND EXPERIMENTAL BASIS FOR A THRESHOLD INDEX

INTRODUCTION

In addition to heating, there are at least two physical phenomena associated with ultrasonic radiation that have been implicated causally in biological effects. The most important of these involves the interaction of ultrasound with gas bodies or bubbles called acoustic cavitation (Chapters 2 and 5) and the second involves the unidirectional radiation force that is exerted by the acoustic field on tissues and tissue components (Chapter 3). No adverse biological effects have been related directly to radiation force up to the present time and, therefore, there is no motivation to develop an index to describe diagnostic ultrasound exposures from the point of view of radiation force. This topic examines physical processes as well as relevant bioeffects data leading to criteria that may be useful in predicting possible adverse, cavitation-related, biological effects from exposure to diagnostic ultrasound. For the purposes of this discussion, diagnostically relevant exposure conditions include carrier frequencies above 1 MHz, pulse lengths up to 10 μs and repetition rates small enough that tissue heating is not an important factor.

CAVITATION THRESHOLD

As discussed in Chapter 2, the transition from comparatively stable bubble oscillations (noninertial cavitation) to the condition of violent collapse (inertial cavitation) may occur with a very small increase in acoustic pressure amplitude. This is in sharp contrast to macroscopic heating by absorption, in which temperature increments for a given source are related linearly to local intensities over a very wide range of acoustic intensities. This highly nonlinear response of bubbles to acoustic excitation leads to a physical threshold for inertial cavitation, the point in the relationship between the bubble response and the driving acoustic pressure at which a very small increase in acoustic pressure causes an abrupt increase in amplitude of the bubble oscillation. If there are sharp thresholds for biological effects of heat, they arise from the strong temperature dependence of critical chemical processes in the biological material rather than from an abrupt change in the temperature itself with increase in acoustic intensity.

There are other interesting differences between the biological effects of heat and cavitation. Heating is a relatively slow process. Typically, even the most extreme exposures encountered in conventional medical applications of ultrasound must last for seconds to produce biologically significant tissue temperature changes. In contrast, the collapse associated with inertial cavitation may occur within a single pressure cycle, and a single collapse may cause tissue damage. Whereas the heating rate is directly related to *temporal average* intensity or power, the occurrence of cavitation depends in a general way upon the *temporal peak* pressure in the sound field. Although there are exposure conditions in which both heating and cavitation should be considered, it is possible to have significant heating from the absorption of sound without cavitation. Furthermore, cavitation may occur at temporal average intensities of pulsed ultrasound that are much too small to produce significant heating.

Frequency dependence of the cavitation threshold

Flynn (1982) computed thresholds for inertial cavitation for microsecond length pulses of ultrasound with center frequencies in the range from 1–10 MHz. The threshold values depend upon the equilibrium size of the nucleus. For optimally sized bubbles and for the low diagnostic frequency range, *e.g.,* 1–2 MHz, Flynn's threshold levels are of the order of 0.3 MPa. In a general sense, his threshold values increase with center frequency but, in addition, for frequencies above 5 MHz, the change in collapse pressure with increase in acoustic pressure becomes less abrupt and the ''threshold'' itself is more difficult to define.

Apfel and Holland (1991) developed a simple relationship between acoustic pressure and the onset of inertial cavitation under the assumption that optimally sized bubbles are present. The theory assumed isothermal growth, adiabatic collapse, an incompressible host fluid and neglected gas diffusion into the bubble. In addition, they assumed that the pulse of ultrasound con-

sisted of a single cycle. Their thresholds were of the order of 0.5 MPa for optimally sized bubbles at 1 MHz. They showed that the threshold acoustic pressure level increases approximately inproportion to the square root of the center frequency. Šponer (1991) addressed the same problem but used a ''Gaussian'' envelope for the acoustic pulse. He found that the threshold for inertial cavitation for an optimally sized bubble increases approximately as the first power of the center frequency.

Whereas other investigators (including those mentioned above) have based their analyses on single spherical bubbles in an infinite fluid, Miller and Thomas (1993) used a linear model to compute the shear stresses generated by ultrasonically driven oscillations of cylindrical gas bodies trapped in the intercellular spaces of *Elodea* leaves (a form of noninertial cavitation). They found that the acoustic pressure required to maintain a given shear stress increases as the square root of the frequency in the range from 1–10 MHz. Thus, although the details of the models of bubble interaction with ultrasound vary, there is general agreement that the acoustic pressures required to produce effects increase with frequency.

NONTHERMAL BIOEFFECTS

Cavitation-related effects in tissue

There is evidence from both lithotripsy and continuous-wave (cw) ultrasound that cavitation can occur in tissues in which stabilized gas bodies are not known to be present. With current knowledge and understanding of the mechanisms and processes involved, it is not possible to extrapolate from studies either with cw or with lithotripter shocks to pulsed ultrasound. However, the research community should be alert to the possibility that cavitation may occur in tissues even when there are no obvious gas bodies present.

Assuming that the appearance of bubbles following exposure is a result of growth from preexisting smaller ones, evidence that gaseous nuclei do exist *in vivo* comes from such disparate sources as exposures of guinea pigs to megaHertz frequency ultrasound (ter Haar and Daniels 1981) and from exposures of pigs to lithotripter shock waves (Delius et al. 1990a). Typically, the increased scattering develops only after many shock waves. This suggests that the bubble population is built up slowly by the treatment from a relatively nucleus-poor medium and/or the medium itself is modified by shock wave damage in such a way that cavitation is enhanced.

There are many examples of cavitation damage to soft tissues as side effects of lithotripsy. Delius and coworkers showed damage to kidney (Delius et al. 1988a, 1988b), liver (Delius et al. 1990a, 1990b) and lung (Delius et al. 1987). Extravasation of blood cells has been observed in kidneys exposed to 10 spark-generated, spherically diverging shocks at peak positive pressures as low as 3 MPa (Mayer et al. 1990). However, when the same organ was exposed to pulsed ultrasound, no evidence of hemorrhage could be found with peak positive pressures in excess of 10 MPa even though the total numbers of individual shock waves in the pulsed ultrasound exposure exceeded one million (Carstensen et al. 1990c). Clearly, it is impossible to extrapolate from studies with lithotripter shocks to pulsed ultrasound given the present knowledge of these processes. Chick embryos exposed to three spark-generated shocks at pressure amplitudes of 10 MPa had significantly elevated rates of early deaths, delayed deaths and malformations (Hartman et al. 1990a). Rat and mouse fetuses are damaged by exposure to commercial lithotripters (Dalecki et al. 1997b; Ohmori et al. 1994; Smith et al. 1992).

The pressure threshold for adult murine lung hemorrhage with spark-generated shock waves is comparable to that with pulsed ultrasound (Hartman et al. 1990b). In contrast, fetal murine lung did not show signs of damage at 20 MPa (Hartman et al. 1990b). Similar threshold levels have been found lethal for the larvae of the fruit fly *Drosophila melanogaster* (Carstensen et al. 1990a). Peak positive pressure thresholds for hemorrhage in murine intestine exposed to a piezoelectric lithotripter are of the range of 1–3 MPa (Dalecki et al. 1995a). Many soft tissues become susceptible to hemorrhage in lithotripter fields of the order of 1 MPa when microbubbles are injected into the blood (Dalecki et al. 1997d).

Hynynen (1991) used 1-s exposures to cw ultrasound during which the temperature of the tissue increased significantly. His thresholds for cavitation in dog muscle in the frequency range from 0.25–1.7 MHz were 5 MPa·MHz^{-1}. Fry et al. (1970) observed what appear to be cavitation-generated lesions in cat brain with a single 40-ms exposure of 1-MHz ultrasound with focal intensities of approximately 2000 W·cm^{-2} (8 MPa). Related studies by Lele (1977, 1978) provided similar results and additional evidence to support the cavitation mechanism. With similar exposure conditions, Frizzell and coworkers (Chan and Frizzell 1977; Frizzell 1988) produced lesions in cat liver that were histologically similar to the cavitation-generated lesions in cat brain. Other studies using much longer exposure times suggest that cavitation can occur in other tissues. Suppression of hind limb paralysis in neonatal mice by application of hydrostatic pressure supports the conclusion that cavitation is in part responsible for this effect at cw ultrasound exposures as low as 60 W·cm^{-2} (1.3 MPa) (Lee and Frizzell 1988). O'Brien and Zachary (1994a, 1994b) produced hemorrhage in lungs of mice, rabbits and swine with 30-kHz ultrasound. Taylor and Pond (1972) reported hemorrhages in rat spinal cord with long pulses having peak intensities $>$ 25

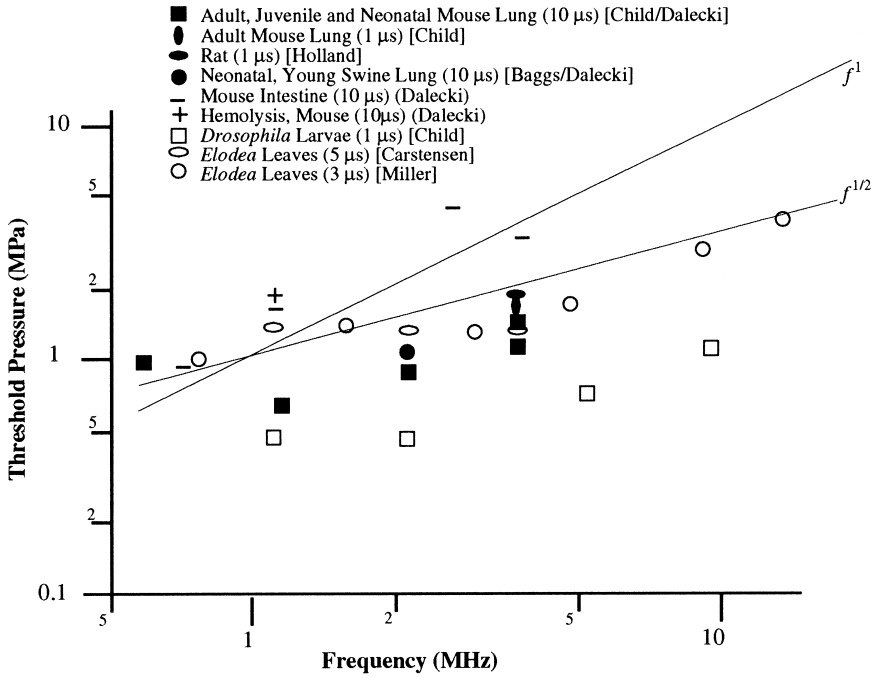

Fig. 6.1. Threshold, negative acoustic pressures *in situ* for known nonthermal biological effects *in vivo* of diagnostically relevant ultrasound exposures. Pulse durations are shown in parentheses in the legend. All exposures consisted of repetitive short pulses (≤ 10 μs). Total exposure times were <5 min. In all cases, the tissues contain identifiable, small, stabilized gas bodies. Sources: adult, juvenile and neonatal murine lung hemorrhage (Bailey et al. 1996; Child et al. 1990; Dalecki et al. 1997a; Raeman et al. 1993, 1996); rats (Holland et al. 1996); neonatal and young swine lung (Baggs et al. 1996; Dalecki et al. 1997e); mouse intestine (Dalecki et al. 1995b); hemolysis in mice (Dalecki et al. 1997c); killing of larvae (Bailey et al. 1996; Child et al. 1981); lysis of cells in leaves of aquatic plants (Carstensen et al. 1990b; Miller and Thomas 1993). To help in the assessment of frequency dependence of the thresholds, lines have been drawn through the point (1 MPa, 1 MHz) with slopes proportional to frequency to the one half and to the first power of frequency.

$W \cdot cm^{-2}$ (0.8 MPa). Lehmann and Herrick (1953) reported petechiae in the intestines of mice with therapeutic exposures of cw ultrasound ($\approx$ 3 $W \cdot cm^{-2}$; 0.3 MPa). Hemorrhage is suggestive of acoustic cavitation, but it must be granted that heat development may have been a factor in most of the studies cited here. In fact, after replicating and extending the study of Lehmann and Herrick, Miller and Thomas (1994) concluded that heating is the dominant mechanism in intestinal hemorrhage with cw exposures. Heating and cavitation may interact synergistically under diagnostic conditions. But, since the detailed nature of cavitation activity is unknown, none of the observations cited here can be extrapolated to applications of diagnostic ultrasound.

Bubble-related biological effects at diagnostically relevant exposures

Biological systems that contain bubbles are far more complex than the models that have been used in most theoretical and experimental studies of acoustic cavitation. In studies that are most directly relevant to the question of hazards associated with cavitation produced by diagnostic ultrasound, the exposures were applied in repeated short pulses with temporal average intensities low enough to make it possible to eliminate heating as the primary mechanism of the effect. Fig. 6.1 provides a summary of the thresholds for reported effects that satisfy these criteria. In each case, even though inertial cavitation in the classic sense may not be involved, it is clear that gas bodies stabilized in the tissue play an essential role in the events.

Miller and Thomas (1993) found a threshold for lysis of the cells in leaves of the water lily *Elodea* to be approximately 1 $MPa \cdot MHz^{1/2}$ in the frequency range 0.7–15 MHz. This was with a 3-μs pulse and total on-time of 60 ms. The threshold was not critically dependent on pulse duration. Carstensen et al. (1990b) found the same threshold levels for similar exposure conditions in the 1- to 3-MHz frequency range. This occurs only with gas channels in the leaves. However, it is unlikely that inertial cavitation is involved (Miller and Thomas 1993).

Studies have shown that there is a sharp threshold for killing of larvae of the fruit fly *Drosophila*, with pulsed ultrasound at peak pressures of 0.3–0.5 MPa using diagnostically relevant pulse durations and repetition frequencies (Child et al. 1981). A statistically significant lethal effect occurred at temporal average intensities as low as 3 $mW \cdot cm^{-2}$. However, as long as the peak pressure was held constant, the temporal average intensity could be increased 100 times (by increasing the pulse repetition rate) with little change in the killing effect. That there is a threshold for killing and that it depends upon temporal peak as opposed to temporal average exposure has been demonstrated by experiments showing that, with a temporal maximum intensity of 4 $W \cdot cm^{-2}$ and pulse repetition rates great enough to bring the temporal average intensities up to 80 $mW \cdot cm^{-2}$, there was no evidence of an effect even with total exposure times as long as 20 min. In a study of the effects of pulsed ultrasound at low temporal average intensity on *Drosophila* eggs, it was found that the sensitivity of the organism increased dramatically at the stage of development when the respiratory apparatus of the fully developed larva within the egg shell fills with air (Child and Carstensen 1982). This suggests that the sites of action for ultrasound are gas bodies within the organism. However, killing of larvae does not have characteristics expected for inertial cavitation of unconstrained bubbles. The biological response is no greater for negative pressures than it is for purely positive pressures (Bailey et al. 1996).

Although general knowledge and confidence comes from theory and from experiments with plants, insects and experiments with cells *in vitro*, specific decisions about thresholds in patients must be based primarily on studies with mammals. Thresholds for lung hemorrhage in mice (Child et al. 1990) fall between those for cell lysis in *Elodea* leaves and killing of *Drosophila* larvae. When the mouse lung is exposed to pulsed ultrasound at threshold levels, the damage is limited to subcapsular petechiae near the surface of the lung corresponding to the site of incidence of the sound beam. The extent of damage increases markedly with exposure level. At twice threshold pressure levels, the lesion penetrates the entire murine lung and its lateral extent increases (Raeman et al. 1993). Lesion boundaries are sharply defined. No histologic abnormalities have been seen outside the regions that are characterized by extravasation of blood cells (Penney et al. 1993). Studies of the repair of suprathreshold focal lesions in murine lung indicate that damage is limited to the original hemorrhagic region and that there are no clinically significant long-term alterations to the lung tissue (Penney et al. 1996).

Thresholds for extravasation of blood cells in lung with center frequencies in the range 0.5–5 MHz are of the order of 1 MPa. The threshold levels are approximately the same for neonatal, 2-week-old and adult mice (Dalecki et al. 1997a), rats (Holland et al. 1996) and neonatal and 10-day-old swine (Baggs et al. 1995; Dalecki et al. 1997e). As with fruit fly larvae, the threshold is only very weakly dependent upon pulse repetition rate (temporal average intensity), *i.e.*, temporal peak parameters rather than temporal average parameters of the sound field are predictors of this biological effect. Thresholds for lung hemorrhage (expressed in terms of spatial peak pressures) are the same for focused or unfocused fields. Positive and negative pressures are equally damaging to murine lungs (Bailey et al. 1997).

Tarantal and Canfield (1994) have presented evidence that lung hemorrhage may be produced in monkeys using a commercial diagnostic ultrasound system. In their studies, the transducer was held in direct contact with the chest wall in a reasonable simulation of standard clinical procedures. Holland et al. (1996) demonstrated hemorrhage in rats with a commercial scanner and detected cavitation activity in association with the lung damage. Zachary and O'Brien (1995) produced lung hemorrhage in mice and rabbits using a commercial diagnostic ultrasound system. Although their studies were not designed specifically to determine thresholds, their results are consistent with the threshold data for mice described above. They did not find the effect in $\approx$ 30-kg swine at the highest levels ($>$ 4 MPa at 3 MHz) available with their system. Frizzell (1994) reported thresholds at 1 MHz ranging from 0.3 to $>$ 1 MPa for lung hemorrhage in cold neonatal mice. Dalecki et al. (1997a) found no significant differences in the thresholds for lung hemorrhage in warm neonatal mice, 2-week-old mice or adult mice.

Superficially, cavitation is perhaps the most obvious mechanism to be examined when considering biological effects in the lung. Holland et al. (1996) recently reported detecting the signs of cavitation in or near rat lung with an active bubble detector at roughly the level that corresponds to hemorrhage. Leighton et al. (1995) addressed the behavior of cylindrical bubbles in pulmonary capillaries.

As bioeffects information continues to accumulate, it becomes less obvious that cavitation is responsible for lung hemorrhage. Thresholds are somewhat lower for lung hemorrhage than for hemorrhage in tissues with obvious cavitation nuclei available to support the phenomenon. Negative pressures cause no greater effect than positive pressures (Bailey et al. 1996) as would be expected for classic cavitation. Adding nuclei to the blood has no apparent effect on the severity of hemorrhage (Dalecki et al. 1997d). The frequency dependence (above 1 MHz) of bioeffects that are clearly related to

acoustic cavitation is stronger than the frequency dependence of lung hemorrhage (Fig. 6.1).

The intestine also contains gas bodies. It can be speculated that the range in sizes for these bubbles includes those that would be resonant at the frequencies used in diagnostic ultrasound. However, almost nothing is known about the distribution of these bubbles throughout the organ. The thresholds for hemorrhage in the intestine of adult mice range from approximately 1 MPa at 1 MHz to 3 MPa at 3 MHz (10-μs pulse, 100-Hz repetition frequency) (Dalecki et al. 1995a). Fetal intestine is essentially free of gas bodies (Caspi et al. 1993). The immunity of fetal murine intestine to hemorrhage in a lithotripter field at levels that cause massive damage to the maternal intestine supports the hypothesis that cavitation is involved in this biological effect (Dalecki et al. 1996a).

Hemolysis has been observed in mice exposed to 1-MHz pulsed ultrasound when microbubbles were injected into the blood (Dalecki et al. 1997c). No hemolysis was seen without the addition of microbubbles. At a frequency of 1 MHz, the threshold was approximately 2 MPa for the negative pressure. No significant hemolysis was observed at more than twice that pressure amplitude when the exposure was performed at a frequency of 2.4 MHz. The high pressure threshold and the strong dependence of the threshold on frequency suggest that hemolysis is unlikely to be a concern with existing diagnostic ultrasound systems. Brayman et al. (1996) reported a ''pseudothreshold'' for hemolysis *in vitro* at approximately the value shown in Fig. 6.1 for mice. In view of this similarity of threshold values *in vivo* and *in vitro,* an important finding of Brayman et al. (1996) should be mentioned. They used the term ''pseudothreshold'' because they observed small, but statistically significant, levels of hemolysis below the ''pseudothreshold'' pressure level.

Thus, we must acknowledge the possibility that a small, undetectable amount of hemolysis may occur even *in vivo* below the threshold levels reported in Fig. 6.1. The question is largely academic.

The experimental data summarised in Fig. 6.1, like the models for the interaction of bubbles with ultrasound, show an upward trend with frequency. Two lines have been added to Fig. 6.1 to assist in evaluating that frequency dependence. Hemolysis and intestinal hemorrhage, in which the physical mechanism may approach the classic phenomenon of inertial cavitation, are strongly dependent upon the frequency of the ultrasound. Thresholds for these effects are high in comparison with the maximum output levels of commercially available ultrasound systems and are strongly dependent on frequency. Thresholds for lung hemorrhage—as well as lysis of cells in *Elodea* leaves or the killing of *Drosophila* larvae—in which classic inertial cavitation is less likely to be responsible for the effects, are less strongly dependent upon frequency.

It is interesting that, in each case in which nonthermal effects have been observed under diagnostically relevant conditions, the organisms contain rich collections of stabilized gas bodies. This suggests that other regions known to contain gas bodies, such as the follicles of the ovary following flushing maneuvers used in collecting oocytes, may be susceptible to biological effects of ultrasound.

NONLINEAR PROPAGATION

Thus far, the discussion has ignored the ambiguity that arises in specifying acoustic pressure when nonlinear propagation distorts the waveform. Particularly at high pressure levels, the positive portion of the wave tends to travel more quickly than the negative portion. So, instead of the wave propagating as the pure sinusoid that was generated by the source, the wave becomes progressively distorted as it moves through the medium. Under conditions typical for diagnostic ultrasound, diffraction and dispersion phenomena interact with this nonlinear propagation process in such a way that the wave becomes asymmetrical, with the positive pressures being somewhat greater than the negative pressures. It appears that neither positive nor negative pressures can be ignored when predicting the occurrence of biological effects in tissues containing bubbles (Bailey et al. 1996). Furthermore, nonlinear propagation produces complex changes in the attenuation of the wave that make it very difficult to relate measurements made in water at high ultrasound levels with the levels that would be found if the wave had propagated through tissue. In the extreme, the attenuation arising from nonlinear propagation becomes so great that the acoustic levels in the field *saturate, i.e.,* the field pressures reach levels at which they no longer increase with increase in the source levels.

These ambiguities can be avoided if we rely on the small-signal, experimental characterization of the sound field as the basis for tissue field predictions that was recommended by World Federation of Ultrasound in Medicine (WFUMB 1992). Specifically, the fields of each source should be characterized by hydrophone measurements at levels low enough that it is possible to relate linearly each point in the sound field, including the focus, to the source power. Basing estimates of tissue fields on small-signal field determinations avoids experimental errors associated with hydrophone measurements of sound fields at high output

levels and simplifies the theoretical and computational aspects of the derating problem as well. Once the field has been characterized through measurements in water for a given low source power, it can be derated linearly by an appropriate attenuation factor for the tissue path. These derated fields are linearly related to source power. Since, for almost all diagnostic systems, the source acoustic power is linearly related to the electrical input to the transducer, it is relatively simple to provide a real-time indication of the linearly derated field values for any operating condition. These linearly derated estimates of tissue fields are good approximations of the true fields in tissues for a large range of input values and, at very high field strengths at which nonlinear propagation does modify the output, the linearly derated values are conservatively large, *i.e.*, the estimates tend to err on the high rather than the low side of the true values.

The common practice of linear derating from high-level measurements in water is undesirable because it can lead to substantial underestimates *of the tissue fields.*

Because it is now clear that the acoustic output of some diagnostic ultrasound equipment is capable of producing biological effects, it is essential that manufacturers provide a convenient way to transmit output information to the user. Various strategies are possible. For a relatively low-power uncomplicated device, it might be satisfactory simply to report upper limits for various operating conditions in the user's manual. For these devices, the user might determine after reading the manual that no foreseeable application would require concern for adverse biological effects. At the other extreme, manufacturers of more advanced systems that have high outputs may choose to provide a real-time, on-screen, output indicator. This type of linear output indicator is relatively simple to implement and may be adequate for all but the highest acoustic levels. When greater accuracy is desired, corrections for the effects of nonlinear propagation can be made either theoretically (Christopher and Carstensen 1996) or experimentally (Preston et al. 1991a, 1991b).

INDICES OF EXPOSURE

Whether or not cavitation-related damage occurs when tissues are exposed to ultrasound depends first upon the preexistence of suitable gaseous nuclei. All of the biological effects shown in Fig. 6.1 occur in tissues that are known to contain stabilized, microscopic gas bodies and, in each case, there is independent evidence that the effects either disappear or are markedly reduced in the absence of these bubbles. Given the present state of knowledge, it appears that the thresholds for nonthermal damage to most of the tissues of the body are higher than those used in diagnostic procedures. Yet, almost all of the soft tissues are subject to hemorrhage if microbubbles are injected into the blood (Dalecki et al. 1997d). This says first of all that cavitation can occur *in vivo* if appropriate nuclei are present. However, it also tells us that cavitation nuclei in the normal mammal are extremely rare. Much of the safety of ultrasound in normal practice depends upon this fortuitous property of tissues.

In communicating the field conditions so that users can estimate the probability of the occurrence of specific nonthermal biological effects, the most direct approach would be to describe completely the pressure, center frequency, pulse duration, repetition rate and total exposure time. Of these parameters, however, pressure is by far the most critical. Changes in pressure by as little as a factor of 2 can make the difference between no effect and extensive damage to lung tissue. It is important to specify pressure as described in the preceding section. Measurements of pressure in water at saturation levels have limited predictive value for fields in tissues. Acoustic pressure alone can serve as a first-order index of the exposure conditions.

The next most sensitive parameter is the center frequency. There are theoretical arguments suggesting that pressure thresholds for bubble activity should increase with frequency to a power between 0.5 and 1. Thresholds for hemolysis and intestinal hemorrhage have a relatively strong dependence upon frequency. That, plus the absolute magnitudes of the thresholds, make it unlikely that either of these effects will be a significant concern in normal diagnostic procedures. For three of the effects summarized in Fig. 6.1, an increase in center frequency by a factor of 4 doubles the threshold for its occurrence. Lung hemorrhage could be a practical concern in some diagnostic procedures.

Consensus groups in the United States have combined the two exposure parameters, pressure p (in MPa) and frequency f (in MHz), in a single mechanical index (MI) defined as:

$$MI = \frac{p}{\sqrt{f}} \tag{1}$$

where p is the derated peak negative pressure (in MPa) at the location of the maximum pulse intensity integral (AIUM/NEMA 1992). By taking account of the observed frequency dependence of diagnostically relevant, nonthermal biological effects, the mechanical index tends to simplify the description of thresholds

for bioeffects in tissues known to contain stabilized gas bodies (Fig. 6.1) and, therefore, is useful for certain purposes such as on-screen labelling. Thresholds for known effects in mammals in the diagnostic frequency range are at $MI > 0.6$.

As defined by the FDA derating process, the mechanical index fails to account for the effects of nonlinear propagation and, as a result, may underestimate conditions *in situ.* This problem could be remedied by using linearly derated pressures based on source power as defined above. In this way, the value of the pressure in the index will be conservatively high. For more precise predictions of the true pressure, appropriate correction factors may be applied (Christopher and Carstensen 1996; Preston et al. 1991a, 1991b). A second limitation of the mechanical index lies in the fact that it describes conditions only at the focus, not necessarily the point of interest. The most common way for lung to be exposed in diagnostic procedures is in echocardiography, in which the transducer is applied directly to the chest of the patient. Typically, the focus of the sound field is well below the surface of the lung during this procedure. Because the attenuation of lung tissue is much greater than the present values used for derating the field, a knowledge of the mechanical index would have little meaning in this application.

At present there is no single number (index) that can be used to predict the occurrence of known nonthermal biological effects. The acoustic source and focal pressures and the mechanical index may be helpful in making risk–benefit decisions that are appropriate for specific clinical applications. However, there is no substitute for an informed user who is aware of the kind of data shown in Fig. 6.1 and the biophysics of the application.

WFUMB SYMPOSIUM CONCLUSIONS ON THRESHOLDS FOR NONTHERMAL BIOEFFECTS

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz, 1996.

- When considering ultrasonic fields in tissues, estimates of tissue field parameters should be based on small-signal characterization of source–field relationships. Once the field has been characterized through measurements in water for a given low source power, it can be derated linearly using a specified model and an appropriate attenuation factor for the tissue path. If these derated fields are extrapolated linearly from the source power, estimates of tissue fields are good approximations of the true fields in tissues for a large range of input values and, at very high field strengths at which nonlinear propagation does modify the field, the linearly derated values are conservatively large. When greater accuracy is desired, corrections for the effects of nonlinear propagation can be made either theoretically or experimentally. The common practice of linear derating from high-level measurements in water is undesirable because it can lead to substantial *underestimates* of tissue fields.
- Thresholds for nonthermally induced biological effects of diagnostically relevant ultrasound are related more to the temporal peak than to the temporal average properties of the ultrasound field. For a given tissue, the threshold of known potentially adverse biological effects depends strongly on the *in situ* acoustic pressure amplitude at a given frequency, but only weakly on other acoustic parameters such as the pulse duration and repetition rate, the exposed volume of tissue and the total exposure time.
- Thresholds for confirmed nonthermally induced biological effects in mammalian tissues in the diagnostic frequency range of 2–10 MHz are above approximately 1 MPa. All of the biological effects that have been confirmed under diagnostically relevant exposure conditions involve tissues that are known to contain gas bodies, such as lung and intestine. For tissues *not* known to contain such gas bodies, thresholds for nonthermal effects can be assumed to be greater.
- Capillary bleeding has been observed in the lung after exposure of neonatal, young and adult mice, swine and adult rats, rabbits and monkeys to diagnostically relevant, pulsed ultrasound. Thresholds for capillary bleeding in adult mice and neonatal and young swine are of the order of 1 MPa at 2 MHz, which is within the range of output values of commercially available diagnostic ultrasound systems.
- Bleeding in murine intestine and hemolysis in mice injected with contrast agents have been reported. Thresholds for these effects (*e.g.,* > 3 MPa at 2.5 MHz) are close to the highest estimated *in situ* exposure levels from commercially available diagnostic ultrasound systems.

Note that these conclusions refer specifically to diagnostically relevant ultrasound exposures.

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Ultrasound in Med. & Biol., Vol. 24, Supplement 1, pp. 51–55, 1998
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0301-5629/98 $19.00 + .00

PII S0301-5629(98)00079-9

● *Chapter 7*

CLINICAL IMPLICATIONS

INTRODUCTION

Ultrasonic imaging has been used clinically as an effective diagnostic tool over the past 25 years. In spite of literally millions of examinations, there is no verified documented evidence of adverse effects caused by exposure to ultrasound in patients (Ziskin and Petitti 1988). There have been a number of epidemiological studies of intrauterine ultrasound exposure, including several case-control and prospective randomized-control studies. In some studies, an association was identified of one or another bioeffect, such as low birth weight (Moore et al. 1982; Newnham et al. 1993), delayed speech (Campbell et al. 1993), or increased incidence of left handedness (Salvesen et al. 1993). However, with the exception of low birth weight, these findings have not been duplicated, and the majority of such studies have been negative. There is no experimental basis for the positive findings, and they cannot be explained by known effects of diagnostic ultrasound. Based on the evidence to date, there is insufficient justification to warrant a conclusion of a causal relationship between diagnostic ultrasound and adverse effect.

Although the apparent safety of clinical diagnostic ultrasound is comforting, acoustic outputs of diagnostic instruments continue to increase (Duck and Henderson 1998; Duck and Martin 1991), and laboratory studies clearly show that ultrasound is capable of producing serious biological damage if the intensity is sufficiently high (Barnett et al. 1994) and the tissues sufficiently sensitive (Barnett et al. 1997). Biological damage can result from both thermal and nonthermal mechanisms. Both mechanisms are important, and either may predominate depending on the exposure conditions. Knowledge of the mechanisms is important to any attempt to extrapolate research findings in the laboratory to predictions of effects in human exposures.

CONCERNS INVOLVING THERMAL EFFECTS

Thermal mechanisms underlying ultrasonic bioeffects have been reviewed in several publications (Barnett 1996; Barnett et al. 1994; Miller and Ziskin 1989; NCRP 1992; WFUMB 1992). Especially notable is a previous WFUMB symposium on ''Issues and Recommendations Regarding Thermal Mechanisms for Biological Effects of Ultrasound'' (WFUMB 1992). There were several major recommendations that resulted from debate by the international expert group. A recommendation based solely on a thermal criterion was that a diagnostic exposure that produces a maximum temperature rise of 1.5°C above normal physiological levels (37°C) may be used without reservation in clinical examinations. The longer the temperature elevation is maintained, the greater is the likelihood for damage to occur. Exposure of adult proliferative tissues to heat can cause damage comparable to that occurring in the embryo and fetus, but with exposures to 42°C for up to 2 h, complete recovery is expected.

Following extensive review of the subject of thermal bioeffects (Chapter 1) the current WFUMB Symposium agreed on a recommendation: A diagnostic exposure that elevates embryonic and fetal *in situ* temperature above 41°C (4°C above normal body temperature) for 5 min or more should be considered potentially hazardous.

CONCERNS INVOLVING NONTHERMAL EFFECTS

Nonthermal mechanisms are not as well understood as thermal effects. They include such phenomena as acoustic cavitation, acoustic streaming, radiation force, radiation pressure and acoustic torque. These phenomena set up stresses on cells and, if sufficiently intense, are capable of lysing cell membranes and destroying tissues. In general, these effects are studied in aqueous media in the laboratory under conditions that may be different from *in vivo* conditions. Consequently, predominant physical and biological interactions and mechanisms involved in an *in vitro* effect may not pertain to the *in vivo* situation, and extrapolation to the clinical situation is difficult.

Cell lysis

Cells in aqueous suspension are lysed readily when exposed to ultrasound at sufficiently high intensities at which inertial cavitation occurs. A number of studies have shown that even less violent cavitation can cause

changes in cell membrane permeability, temporary intracellular ion concentrations and increased cellular uptake of anticancer drugs. These effects have been reviewed recently (Barnett et al. 1994). The clinical consequences of such events depend on the type of cells involved. For example, the destruction of several red blood cells (RBCs) would be insignificant compared to the estimated 2.5×10^{11} RBCs normally destroyed each day. However, the destruction of even a very few cells in the embryonic central nervous system may result in devastating biological effects (WFUMB 1992). In addition to that in the embryo and fetus, destruction of cells in the adult brain and eye would have serious consequences. To date, there is no direct evidence that this has occurred in diagnostic clinical practice.

Cellular and tissue lesions

RBC stasis has been observed *in vivo* in chick embryos during insonation with 1 W/cm^2 (I_{sata}) continuous-wave (cw) ultrasound in which a standing wavefield was created (Dyson et al. 1974). Capillary endothelial damage was reported and thought to be due to direct effect of cavitation, or to the anoxic effect of prolonged RBC stasis. This effect would not be expected in diagnostic examinations utilizing pulsed ultrasound in the absence of standing wave fields. However, intimal lesions resulting from cavitation could lead to thrombosis formation, which would constitute a significant clinical hazard. There is no evidence that this has occurred in clinical practice.

SPECIAL CONCERNS RELATING TO CAVITATION

The presence of a gas bubble is a requisite for cavitation. Gas collections are normally found in pulmonary alveoli and in the lumen of the intestinal tract. Therefore, these structures are sites for which nonthermal cavitation-related bioeffects are most likely to occur. However, bubbles also are to be expected in certain clinical conditions such as infusions, injections of contrast agents, surgical procedures, decompression illness, gas gangrene, and traumatic lesions with penetration of the skin, lungs or bowels. The first finding of RBC extravasation in lung tissue was reported at an acoustic pressure threshold of 1 MPa in mice (Child et al. 1990). Similar results were subsequently found in mice, pigs, rabbits, rats and monkeys (Holland et al. 1996; Tarantal and Canfield 1994; Zachary and O'Brien 1995). The threshold for extravasation in neonatal mouse lung is approximately 0.5 MPa at a frequency of 1 MHz. The mechanism is not fully understood, but is considered to be nonthermal. Acoustic output measurements reported by Duck (1989) and by Duck and Martin (1991) indicate that the peak rarefactional pressure amplitudes of B-mode pulse echo equipment range from 0.58–4.3 MPa. Henderson et al. (1995) reported a range of peak negative values of 0.45–5.54 MPa, with similar values for pulsed Doppler systems. These high pressures would appear to be sufficient to lead to adverse effects. So far, there has been no evidence of pulmonary extravasation following human diagnostic ultrasound procedures, but small amounts of extravasation would be clinically insignificant and would, no doubt, go undetected. The same would be true for small amounts of extravasation in the intestinal tract. However, greater amounts of extravasation, should it occur in neonatal examinations, could be of concern.

A number of other cavitation-related bioeffects in mammals have been reported (ter Haar et al. 1986). They include focal lesions in the brain (Dunn and Fry 1971), irreversible hind limb paralysis in neonatal mice (Borrelli et al. 1968; Frizzell et al. 1985), as well as a variety of adverse effects caused by exposure to lithotripsy devices (Delius et al. 1987 and 1988). For the most part, these effects occur only when the acoustic intensities exceed those used for diagnostic purposes (Carstensen 1987). However, they do provide insight into the relationship and relative importance of physical parameters to the production of biological damage. In addition to physical variables, exposure thresholds also depend on tissue properties. Because of the limited amount of available data, it is not possible to specify precise thresholds at which acoustic cavitation will occur in mammals.

During the collapse of a bubble, the internal temperature can exceed 1500°C and free-radicals can be created (Suslick 1988; Christman et al. 1987). The free-radicals are highly active chemical species that have the potential to disrupt normal cellular function. The most serious effect is damage to DNA (Miller et al. 1991a, 1991b). For this to occur the free-radicals must reach the DNA within the cell nucleus. Laboratory evidence indicates that free-radical production by ultrasound occurs outside the cell, and if sufficiently close the free-radicals can penetrate through the membrane. However, since the lifetime of a free-radical is short, there is little chance for one to penetrate through the cell membrane and survive the naturally occurring intracellular radical scavengers long enough to do any damage. It is uncertain if free-radicals can be produced by ultrasound within cells.

Beneficial effects

Any discussion of risks of ultrasound also should mention its beneficial effects. Besides the invaluable use of ultrasound in diagnostic medicine, there are a number of therapeutic uses that result from nonthermal mechanisms. Microspheres of encapsulated gas bubbles can be injected in blood vessels to enhance the effect of therapeutic applications as well as to increase the contrast in ultrasonic imaging. Renal calculus lithotripsy and gall-

stone dissolution are used commonly in clinical practice. Other applications include clot lysis, sonophoresis, sonodynamic chemotherapy, wound healing acceleration and bone fracture healing acceleration (Dyson 1985; Dyson et al. 1974).

SAFE CLINICAL DIAGNOSTIC PRACTICE

To aid health care practitioners to maximize patient safety, incorporation of continuously updated on-screen indices in clinical ultrasound instruments has been recommended (AIUM/NEMA 1992). These indices, the thermal index (TI) and the mechanical index (MI), provide dimensionless numbers giving information on the likelihood of an adverse biological effect resulting from the current ultrasound examination. The indices were designed so that if either index exceeded a predefined value, there was the potential for harm. When this happens, the clinician should assess if the examination could be performed with a lower acoustic intensity, or consider mitigating factors in reevaluating the benefit–risk ratio. Mitigating factors include the absence of gas-containing structures, anatomical sites that would be particularly invulnerable to damage and the perfusion rate in the region being examined. Also, the duration of the examination should be kept to a minimum to avoid any unnecessary exposure. However, it is important to recognise that the potential harm from misdiagnosis can have greater consequences than that of ultrasound-induced bioeffects, and the examination, if performed, should not be foreshortened to the point where the needed diagnostic information is compromised (Ziskin 1990).

SUMMARY

Diagnostic ultrasound has been in use worldwide since the late 1950s. During this time, various diagnostic techniques have been developed for numerous clinical applications. These techniques use a wide range of ultrasound exposure levels. There is a continuing trend towards increased acoustic outputs from diagnostic equipment that has become evident in recent years (Duck and Martin 1993; Duck and Henderson 1998; Henderson et al. 1995).

It is important to note that no established adverse effects on patients or instrument operators caused by exposure to diagnostic ultrasound instruments have been reported. This is consistent with the body of knowledge of how ultrasound interacts with biological tissues (NCRP 1983 and 1992; WFUMB 1992), including thermal and nonthermal mechanisms, and the knowledge of experimental biological effects and epidemiological studies. Although the possibility exists that such effects may be identified in the future, current data indicate that the benefits to patients of the prudent use of diagnostic ultrasound outweigh the risks, if any, that may be present.

WFUMB SYMPOSIUM CONCLUSIONS ON CLINICAL IMPLICATIONS

The following conclusions were adopted by voting by participants at the WFUMB Safety Symposium, Kloster-Banz, 1996.

- *In vitro biological effects:*
  Studies of *in vitro* biological effects provide valuable information about the underlying physical mechanisms by which ultrasound can alter tissue.
- *Noncavitational, nonthermal mechanisms:*
  Noncavitational, nonthermal phenomena, such as acoustic streaming and acoustic radiation force, may be involved in producing bioeffects. Red blood cell stasis, retino-choroidal blanching and accelerated wound healing are examples of bioeffects that most likely are due to these mechanical mechanisms at intensities above the diagnostic range. Ultrasound at diagnostic intensities can induce body fluid movements in cysts, abscesses and other fluid collections. These movements can be visualized during ultrasound examinations and can be helpful in diagnosis.
- *Tissue–gas interfaces:*
  Tissue–gas interfaces are known to exist in the postnatal lung and intestinal lumina. These interfaces greatly increase the potential for nonthermal biological effects. Evidence from mammalian studies shows that pulmonary capillary bleeding can occur at diagnostic ultrasonic pressure levels. Within the frequency range of present diagnostic instruments (2–10 MHz), the threshold for pulmonary capillary bleeding is approximately 1 MPa, and the threshold for intestinal bleeding is approximately 3 MPa. Ultrasonically induced lung damage in the fluid-filled lungs of fetuses is not to be expected.
- *Cavitation:*
  Inertial cavitation is a threshold phenomenon. The acoustic outputs of current diagnostic instruments have not been demonstrated to exceed this threshold in mammalian tissue.
- *Free-radical production:*
  The acoustic generation of free-radicals will occur only as a result of inertial cavitation.
- *Contrast agents:*
  The use of a contrast agent to enhance the diagnostic value of an ultrasound examination is increasing. Although no ultrasonically induced adverse effects from

these agents have been reported in humans, their use is relatively new and their safety is currently under scientific evaluation.

- *B-mode imaging:*
  Known diagnostic ultrasound equipment as used today for simple B-mode imaging operates at acoustic outputs that have not been shown to cause harmful temperature rises. In addition, these instruments have not been shown to cause adverse nonthermal effects, provided tissue–gas interfaces or contrast agents are not present in the regions being exposed.

- *Doppler:*
  In the absence of tissue–gas interfaces or contrast agents, currently available diagnostic Doppler instruments have not been shown to cause adverse nonthermal effects. However, some Doppler diagnostic equipment has the potential to produce biologically significant temperature rises, especially at bone–soft tissue interfaces.

- *Transducer self-heating:*
  Some transducers, because of self-heating, can produce surface temperatures as high as 41°C or higher.

- *Continuously updated display of biophysical quantities:* such as anticipated temperature rise or potential for cavitation, can help health care professionals monitor patient exposure during each examination. Should these quantities exceed predefined values, the user may choose, for example, to:

(a) reduce the acoustic output level,
(b) consider mitigating factors, such as local perfusion and obesity,
(c) minimize the duration of the examination, or
(d) reevaluate the benefit–risk ratio.

- *Epidemiology:*
  A number of epidemiological studies of intrauterine ultrasound exposure has been reported in the past three decades, including several case-control and prospective randomized-control studies. Epidemiological evidence provides no justification for claiming a causal relationship between diagnostic ultrasound and any adverse effect.

- *Clinical safety:*
  No adverse effects on patients or instrument operators caused by exposure at acoustic output levels typical of currently-available, properly-operating diagnostic ultrasound instruments have ever been established. Current data indicate that the benefits to patients of the prudent use of diagnostic ultrasound outweigh the risks, if any, that may be present.

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