STUDIES OF THE CAVITATIONAL EFFECTS OF
CLINICAL ULTRASOUND BY SONOLUMINESCENCE.

by


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Abstract.

Ultrasound is now a widely used technique in medicine. The precise mechanisms by which ultrasound interacts with tissues are still not fully understood, however, and it is possible that ultrasound presents a small hazard to those who receive it. An understanding of the possible mechanisms by which ultrasound may be hazardous is necessary if reliable safety levels are to be set.

One possible damage mechanism is transient cavitation, which is the creation, expansion and collapse of small bubbles in tissues in response to the variations in pressure produced by the ultrasound wave. During the collapse stage, very high temperatures can be produced within the bubble and it is likely that free radicals are formed. Sonoluminescence is the name given to the light emissions that accompany transient cavitation, and is an indication that cavitation has occurred. In this thesis the phenomenon of sonoluminescence, and various factors that influence it, are investigated under similar conditions to those obtaining in clinical practice.

When the effect of physiotherapeutic ultrasound on a tank of water was investigated and light output was detected using either a photomultiplier or an image intensifier, sonoluminescence was found to increase with increasing ultrasonic intensity above a well defined threshold. Sonoluminescence also increased with increasing temperature and was found to depend on duty cycle and standing wave ratio. Subsequently sonoluminescence was also recorded from water
after insonation with quite short pulses of ultrasound, but thresholds were much higher than with long pulses. The effect of ultrasound on monolayers of cells growing in culture was found to depend on the position of the monolayer in the standing wave field. Finally a direct attempt to measure sonoluminescence from the human cheek was made, but none was observed.
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Chapter 1. Introduction.

Ultrasound is sound at a frequency that is too high to be heard by humans. In the most common medical applications, the frequencies are between 500 kHz and 10 MHz. Ultrasound has been used as an aid to physical therapy for over thirty years, and the advantages of using ultrasound for diagnosis have become widely recognised.

For techniques in physiotherapy, continuous wave or pulsed ultrasound of frequency either 1 MHz or 3 MHz, and intensities of up to 3 W cm⁻² are typical. It needs to be made clear, however, that a simple statement of the intensities encountered with clinical ultrasound is not possible. In general, these vary both spatially and temporally. The above figure is a spatial average, so there will be parts of the beam where the intensity is higher than this. If the ultrasound is pulsed, the figure of 3 W cm⁻² is a temporal peak, whereas with continuous wave ultrasound, of course, the temporal peak and the temporal average intensities are the same. One piece of physiotherapeutic equipment which features in this thesis is the Therasonic 1030. This can generate continuous wave ultrasound (figure 1.1a), and pulsed ultrasound of duty cycle (or mark space ratio) 1:2, 1:4 or 1:7. In each case the on-time of the ultrasound is 2 ms (figure 1.1b-d) so there will be several cycles (2000 at 1 MHz) in each pulse. These regimens are fairly typical of commercially available physiotherapy ultrasound machines.

For the majority of diagnostic techniques, pulse-echo systems with average (temporal and spatial) intensities of the order of a few
milliwatts per square centimetre are used. The pulse will typically last for about a microsecond, while echoes resulting from reflection at interfaces and from scattering at tissue discontinuities are observed for the remainder of one millisecond (figure 1.1e). Spatial and temporal peak intensities can therefore be much higher than physiotherapeutic intensities.

Another application is Doppler ultrasound where the shift in frequency on reflection of ultrasound from a moving target is detected. Both continuous wave and pulsed systems are used with peak intensities of the order of 100 mW cm$^{-2}$. Doppler ultrasound is used to measure, amongst other things, blood flow rates in the arteries and can detect abnormal flow patterns due to occlusions.

Ultrasound can also be used in surgery where intensities are of the order of 100 W cm$^{-2}$. One of the main applications is to break up kidney stones by means of a device known as a lithotripter.

1.1 "Is Ultrasound Safe?"

Ultrasound is classified as a non-ionising radiation along with radiations such as radio waves and microwaves. As such it is commonly described as being non-invasive. However any investigative technique must to some degree interact with the object under investigation. If there was no interaction, no information could be obtained; if ultrasound did not interact with the tissues during a diagnostic scanning procedure, there would be no image. It must therefore be asked, in what way does ultrasound affect the patients examined? Are
Figure 1.1. Diagram showing typical on- and off-times for therapeutic ultrasound (a-d) and diagnostic ultrasound (e).
there any significant side effects, and, in particular, are they adverse? Although it is frequently stated that ultrasonic techniques are without risk to the patient, absolute safety cannot be proved, and certainly should not be assumed. There may be only a small possibility of any harm being caused to an individual, but many people may be involved, and there may be quite a high probability that a small percentage of patients who have received ultrasound have experienced some undesirable effect. Diagnostic ultrasound needs to be subjected to particularly close scrutiny because it is now widely used to examine the unborn child.

In order to appreciate the physical characteristics of an ultrasonic field which may cause harm to biological systems, it is necessary to understand the physical and biological mechanisms involved. One way in which ultrasound can affect a biological system is by raising its temperature; a thermal mechanism is involved and effects that originate with elevated temperatures are termed thermal effects. The second mechanism is cavitation; since ultrasound is a pressure wave, mechanical in nature, it can produce changes via its fields of mechanical force. In the case of small bubbles in suspension, the changes are the creation, expansion, contraction and collapse of the bubbles. The effects produced by rapidly oscillating bubbles are termed cavitational effects and are discussed in detail in chapter 3. A third class of effects is termed non-thermal non-cavitational effects and takes account of all processes not included in the first two categories. An example of such an effect is streaming where the ultrasound radiation force causes particles in the insonated medium to move away from the source of the ultrasound.
The effects of ultrasound have been investigated in a wide range of biological systems. The work is reviewed in chapter 2. Some of this work is indicative of an adverse effect, while some is relatively comforting, but it has not been possible to answer the most difficult question: "is ultrasound safe?".

1.2 Aims of this Research.

A National Council on Radiation Protection and Measurements Report (no. 74, 1983) recommends that "A sustained research effort should be maintained to widen the data base and to increase the understanding of biological effects of ultrasound. The activities should include a balance between (a) those whose aim is to gain general insight and (b) those directed to immediate clinical needs. Among the former are investigations of basic principles of the interaction between ultrasound and living matter. Clearly indicated here are thermal and cavitation mechanisms but investigators should be alert to others, such as the effects of radiation forces...". The work in this thesis is in the first of the two categories mentioned.

It can be shown that the spatial peak temperature rise within a fetus or organ of interest during a typical diagnostic investigation will be less than one degree Kelvin (Wells 1987). Temperature fluctuations of this magnitude can occur within the course of a diurnal cycle, so it seems unlikely that thermal effects pose a significant biological threat under normal circumstances. A more likely mechanism by which ultrasound may cause damage is the second class of effects mentioned.
in section 1.1, cavitation. Cavitation can be a gentle process involving only the oscillation of bubbles about a mean radius. This is known as stable cavitation. However it can also be a very violent process involving separating the molecules of the insonated medium to create a bubble, followed by the rapid collapse of the bubble. This is transient cavitation.

The problems addressed in this thesis, therefore, are those that have to be answered in order to gain a better understanding of the circumstances under which the effects of transient cavitation can be eliminated, or at least minimised. In order to have a chance of predicting the likelihood of cavitation occurring in a system as complicated as the human body, it is necessary to have a good understanding of the conditions that favour transient cavitation in simpler systems.

1.3 Assessing the Possibilities of Cavitation.

The method adopted for identifying cavitation events is to try and detect sonoluminescence, the light emissions which accompany transient cavitation. The evidence for believing that transient cavitation can be assessed by studying sonoluminescence is discussed in section 3.8.

The main question that needs to be answered is whether cavitation can occur in-vivo. Four approaches are possible. Firstly, an attempt can be made to discover whether enough bubbles of a suitable size exist, or can be created, in biological materials to generate a significant
amount of sonoluminescence. Secondly, an attempt can be made to look
for evidence of biological damage in in-vitro systems that have been
insonated with therapeutic or diagnostic ultrasound with simultaneous
monitoring of cavitational activity via sonoluminescence. Thirdly, if
cavitation is a threshold phenomenon, an assessment of the conditions
under which sonoluminescence is unlikely to occur in-vivo can be
attempted. This may lead to safe working limits being imposed on the
variables in an ultrasound field. Finally a direct observation of
sonoluminescence in-vivo can be attempted. All four approaches are
valid and have been pursued in this project, but it will become
apparent that the third approach is probably the easiest, and has
provided the predominant methodology in this work.

The first experiments reported in this thesis investigate the
conditions under which cavitation occurs in in-vitro systems. The
system initially investigated is a tank of water, largely because it
is the simplest system to work with; it is transparent and therefore
any sonoluminescence produced in the water has a good chance of
reaching the detector. As the work progresses, agar gel is
investigated briefly. The next step is to investigate a biological
system, and monolayers of mouse mammary tumour cells grown in culture
are used. Finally the effects of insonating an in-vivo system; human
tissue in the form of the cheek; are investigated. At each stage the
significance of the results of earlier experiments becomes clearer,
and gradually a picture can be built up of the probability of
cavitation events occurring in certain systems.

Physiotherapeutic ultrasound is employed for the majority of the
experiments performed. The longer on-times and larger temporal average intensities mean that cavitation effects can be observed more easily, and the result of changing parameters such as intensity and duty cycle more easily investigated. However a project such as this should not restrict itself to consideration of the effects of therapeutic ultrasound, and sonoluminescence from water insonated with short pulses of ultrasound is also investigated. It would be a very large step to try and detect cavitation from a system with a duty cycle of 1:1000 and an on-time of the order of only 1 µs (figure 1.1.e), so the problem of whether short pulses of ultrasound can produce cavitation is tackled by gradually reducing the pulse duration and measuring the sonoluminescence produced at each pulse length (chapter 6).

Other specific problems addressed in this thesis include the effect of ultrasonic intensity on sonoluminescence, and the question of whether there is a threshold for cavitation. The effect of temperature on sonoluminescence is investigated, as is the effect of changing the duty cycle (chapter 4). The duty cycle is a subject of investigation because many authors believe that, with pulsed ultrasound, the behaviour of oscillating bubbles depends on the off-time of the ultrasound as well as the on-time. The effect of the gassiness of the water sample is investigated (chapter 4) because a gassy sample will contain more bubbles than a degassed one, and viscosity receives some attention because a viscous sample will withstand the tensions produced by an ultrasound field more easily than a less viscous one. The importance of standing wave ratio becomes clear during the course of the project so this is also
investigated in detail, both in the water tank and on the work with monolayers of cells in culture (chapters 5 and 7).

At the conclusion of this thesis it will be possible to summarise the factors that have the greatest effect on the generation of sonoluminescence and to give some guidelines on threshold levels for various combinations of ultrasound field parameters below which transient cavitation seems unlikely to occur to any substantial degree.
Chapter 2. The Biological Effects of Ultrasound.

The safety of clinical ultrasound has been the subject of controversy ever since Macintosh and Davey (1970) reported an increased incidence of chromosome aberrations in insonated human lymphocytes. Possible damage mechanisms were a cause for concern for many years before this, and had already been reviewed by Hill (1968). Since the Macintosh and Davey report there have been hundreds of investigations attempting to identify the mechanisms of potential adverse effects (see for example the review by Stewart et al. 1985), and many definite effects are reported in the literature. In the U.K., a Minister's warning about routine use of ultrasound in pregnancy appeared in the Lancet in 1984. Mr. John Patten, junior health minister, was quoted as saying, "given the publicity there has recently been about the possible risk of ultrasound scanning we would not expect any health authority to be advocating screening for all mothers as a routine procedure". Although it is rare that adverse effects have turned out to be reproducible, the abundance of data has led to a number of statements being made by national and international bodies on the safety of diagnostic ultrasound (Wells 1987), most of these being published between 1982 and 1985. Perhaps one of the most often quoted of these is the National Council on Radiation Protection and Measurements (1983) report, which recommends inter alia that "research should be carried out to investigate the possibility that biologically significant cavitation or bubble activity occurs in human tissue under conditions of diagnostic and therapeutic ultrasound".

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It is now thirty years since Doppler techniques were used to measure blood flow, and it is twenty years since ultrasonic methods began to be widely used. However the N.C.R.P. report acknowledges that "there is no firm evidence that any physiological change...is produced". Should we not, then, be reassured by the fact that no clinically obvious adverse effect has been reported? Davies (1984) is one author who takes this view: "The accumulated clinical experience of the past quarter century should be reassuring enough" he says.

However, this "experience" has not reassured all those who are voicing their concerns about ultrasound. Past evidence has shown that it tends to be non-clinicians who conduct research and identify adverse effects of drugs or radiation, for example. As Chalmers (1984) points out, X-rays were introduced into obstetric practice in 1899; they became widely used during the 1920's and by 1935 it had been recommended that they be used routinely. It was not until 1956 that it was suggested that practice might increase the chance of the development of leukaemia in childhood. The fact that there are several groups currently working towards a better understanding of the possible risks associated with ultrasound is comforting evidence that researchers have learnt from past experience with X-rays.

In view of the fact that clear clinical evidence of harmful effects could lag some way behind experimental evidence, it is important to examine the latter critically for any effects that could be harmful under the various regimens currently used with diagnostic and therapeutic ultrasound. The following sections deal with biological effects of ultrasound that have been reported to date.
2.1 Chromosome Aberrations.

Macintosh and Davey (1970) were the first workers to make a startling report on the possible biological consequences of diagnostic ultrasound. They insonated human blood cultures with 2.25 MHz ultrasound of not more than 30 mW (based on manufacturer's figures). The aberrations they reported were chromatid breaks and achromatic lesions or gaps, and chromosome fragments and chromosome (or isochromatid) gaps. This work was followed up by a second report (Macintosh and Davey 1972) which confirmed the results of the first and established a link between ultrasound intensity and the number of observed aberrations. In this work they used 2 MHz ultrasound of 8.2–40 mW cm⁻². Several workers tried to repeat the findings, and most presented inconclusive data. Coakley et al (1971) repeated the experiments and obtained the same results, but proposed that the aberrations were due to a chemical agent released from the sample container rather than a direct result of ultrasound interaction. They also reported that damage showed no detectable dependence on ultrasound intensity. When the work was repeated using a different container they obtained a negative result (Coakley et al 1972). Thacker (1973) reviewed the possibility of genetic hazard and concluded that "it seems that current diagnostic procedures are unlikely to result in a genetic hazard". Thacker himself (1974) obtained negative results from his experiments. The debate virtually ended when Macintosh et al (1975), working in Coakley's laboratory, performed a double-blind experiment (this had not been the case with the earlier work), found no increase in chromosomal abnormalities,
and Macintosh admitted that he no longer believed his original results.

2.2 Other Effects on DNA and Cells.

Liebeskind et al (1979b) described an increase in the sister chromatid exchange rate of human lymphocytes after exposure to 2 MHz ultrasound of an intensity $2.7 \text{ mW cm}^{-2}$ (spatial average, temporal average) which is comparable to that of a diagnostic system (Duck et al 1985). This intensity was calculated on the basis of certain assumptions: Liebeskind et al never actually measured it. A sister chromatid exchange is the exchange of homologous portions of each of the two chromatids in a chromosome, and increased rate of these exchanges is often presumed to be indicative of chromosomal damage. The report by Liebeskind et al again stimulated several workers to try and reproduce the results.

A few articles have supported this claim (Haupt et al 1981, Ehlinger et al 1981, Barnett and Kossoff 1984), but far more articles report a negative effect (e.g. Wegner et al 1980, Au et al 1982), while Barrass et al (1982) report a positive effect at $43^\circ\text{C}$ but negative results at $37^\circ\text{C}$. Brulfert et al (1984) went to some lengths to reproduce the conditions under which Liebeskind et al (1979b) performed their experiments, but still found no increase in sister chromatid exchange effects compared to the controls. Recently Barnett et al (1988) have reported increased sister chromatid exchanges in Chinese hamster cells with $10\ \mu\text{s}$ pulses of $2.5 \text{ MHz}$ ultrasound of nominal peak intensity $2500 \text{ W cm}^{-2}$. This is the only report of sister
chromatid exchange effects at such short pulse lengths.

In the papers reporting an increase in exchanges, it is difficult to criticise the methodology. In general, the studies used widely differing experimental methods, and it is hard to draw any firm conclusions on the possible effects of ultrasound on sister chromatid exchange.

There have been several other investigations relating to the effects on DNA, and showing relevant cellular and subcellular interactions. They are too numerous to discuss all the findings, but some merit mention. Liebeskind et al (1979a) found that treatment of G1 phase HeLa cells with 11 mW cm\(^{-2}\) for 20 minutes at 2.25 MHz resulted in alterations in DNA and its synthesis, namely: increased immunoreactivity to antinucleoside antibodies in G1 cells; strongly suggestive of unwinding of the helix or single-stranded break induction, and low levels of non-semiconservative synthesis in logarithmically growing cells treated with hydroxyurea, indicating repair synthesis. C\(_3\)H mouse embryonic cells treated similarly lost the capacity for contact inhibition. Kremkau and Witcofski (1974) exposed rat livers to 1.90 MHz ultrasound at 60 mW cm\(^{-2}\) continuous wave (spatial average) for five minutes and found that the mitotic index was reduced. Miller et al (1976) failed to reproduce this result however.

If effects similar to the above are associated with ultrasound exposure, it ought to be possible to detect them as a change in mutation rate. However, Combes (1975), with 2 MHz ultrasound "in the
diagnostic regime" was unable to detect mutations in a strain of Bacillus subtilis, and Thacker (1974), with 20 kHz and 1-2 MHz ultrasound, also found no evidence for increased mutations in yeast.

The work of Liebeskind et al (1982) is of interest because a commercial diagnostic instrument was used to insonate 3T3 fibroblast cells. Differences in cell motility between exposed and unexposed cells were reported, and again exposed cells showed less contact inhibition. Siegel et al (1979) exposed three human cell lines (kidney, embryonic kidney and amniotic fluid cells) growing in tissue culture on plastic Petri dishes, to 1.76 mW of 2.25 MHz ultrasound for between 0.25 and 60 minutes. They concluded that there was a significant reduction in the attachment of the sonicated cells to the dish surface even after exposure times of only 0.5 minutes, and postulated that alterations in attachment may affect implantation in situ.

2.3 Effects on the Reproductive System and the Development of the Fetus.

Since one of the main uses of ultrasound is observation of the fetus, the effect of ultrasound on the reproductive system and fetal development is a principal area of interest for possible biohazards. A large number of developing embryonic systems have been investigated in attempts to ascertain whether ultrasound could have adverse effects on their growth and differentiation. Again they are too numerous to mention in their entirety, and a more complete review of these and other effects of diagnostic ultrasound is given in Wells.
McClain et al (1972) reported a slight increase in skeletal variations in the offspring of Charles Rivers rats exposed to 10 mW cm\(^{-2}\) (spatial average) of 2.5 MHz continuous wave ultrasound for thirty minutes on days 8, 9, 10, 11 and 13 of gestation. No increase was observed after two hours exposure, suggesting the increase in skeletal variations after thirty minute exposures was not really significant. Sarvazyan et al (1982) explored the effect of both continuous wave and pulsed ultrasound of low intensity (0.025-0.1 W cm\(^{-2}\), spatial average, temporal average) and discovered that the damage suffered by chick embryos depended on the pulse repetition frequency. Dyson et al (1974) also investigated chick embryos and reported damage to the luminal aspect of the plasma membrane, using continuous wave ultrasound of 500 mW cm\(^{-2}\). Garrison et al (1973) and Barnett (1983) both used pulsed ultrasound of higher intensities (1000 mW cm\(^{-2}\) and 4500 mW cm\(^{-2}\) respectively) on chick embryos and reported no observable effect. The work by Stolzenberg et al (1980a) may throw some light on these apparent discrepancies. They reported that the threshold for undesirable and lethal effects is between 100 and 200 seconds of exposure at 1 W cm\(^{-2}\). However, many reports of positive effects indicate much shorter exposure times.

There are many reports of morphological changes to the reproductive organs of the female mouse (e.g. ter Haar et al 1979, Bailey et al 1983) and even one of damage to the male reproductive organs in the mouse (O'Brien et al 1979). Other reports (e.g. Sikov and Hildebrand 1976) mention malformations to the rat fetus, and one (Sikov 1973)
even discusses the possible use of ultrasound in the termination of pregnancy. There are just as many negative reports in the literature. Of these, Glick et al (1979) and Adamovics and Edmonds (1981) not only reported an absence of an effect on the mouse reproductive system, but also on human amniotic fluid.

One theme that does recur in the literature is the question of whether ultrasound may lead to reduced fetal weight (Stolzenberg et al 1980b, O'Brien 1983, Kim et al 1983), and again there are many contradictory reports. Another area of contention is the relationship between ultrasound exposure and fetal movement (David et al 1975, Gettinger et al 1978, Hertz et al 1979). Murai et al (1975a,b) report that exposure to ultrasound during the fetal stage affects emotional behaviour in young rats, and may affect the brain of the offspring. These reports give some cause for concern although they do not necessarily prove that there is a biohazard. As Brodie and Hanson (1960) have pointed out, the physiological and psychological effects of restraint on conscious animals may mask or exaggerate any effect due to ultrasound.

A few workers have carried out controlled trials of obstetric ultrasound in humans. Bernstine (1969), Cartwright et al (1984) and Bakketeig et al (1984) all report that there is no difference in risk of childhood cancer between offspring whose mothers receive ultrasound and control groups. Kinnier Wilson and Waterhouse (1984), who carried out their own controlled trial, argued that while results tend to show ultrasound to be safe with respect to cancer and leukaemia for offspring of up to six years of age, this question is
unresolved for older children. This is because ultrasound used for children who were older than this was selectively related to abnormal pregnancies.

2.4 Effects on Blood Cells and Blood Vessels.

One of the effects of ultrasound on blood is the production of blood cell stasis or banding (Dyson et al 1974, ter Haar and Wyard 1978), where bands of red blood cells appear. The bands are separated by half a wavelength. Such an effect requires continuous wave ultrasound and depends on the presence of a standing wave field (see section 3.6). Dyson et al also report endothelial damage to the blood vessels of chick embryos. Both groups reported their effects at 0.5 W cm$^{-2}$ (spatial peak) and above. Miller and Williams (1983) investigated ATP release from human erythrocytes on exposure to continuous wave, and 1μs pulses of, 2 MHz ultrasound at 5-100 mW cm$^{-2}$. The response was approximately proportional to the square of the spatial peak temporal average intensity and to the duration of exposure. Glick et al (1981) looked at chemical changes in the blood of exposed mice using continuous wave 2 MHz ultrasound of 1000 mW cm$^{-2}$ (spatial average). They reported changes in blood constituents suggesting cell damage. These changes are too numerous to mention, but included the white blood cell count and triglyceride concentration. Eastwood and Watmough (1976) recorded sonoluminescence (implying the presence of free radicals, see section 3.8) from human blood plasma using therapeutic ultrasound of frequencies 0.75 and 1.5 MHz and intensity 2 W cm$^{-2}$ (spatial average). Bause et al (1983) reported that erythrocytes of women who were exposed to Doppler
were more osmotically fragile, although an alternative explanation of these results is that women whose labour lasted more than seven hours received medication or other treatments which could have caused a change in cell volume.

Several investigators have subjected blood in-vivo to therapeutic intensities of continuous wave ultrasound and have been unable to detect significant amounts of damage to any of the cellular elements of blood (Howkins and Weinstock 1970, Williams et al 1981, Chater and Williams 1982).

2.5 Immunological Effects.

A few workers have reported effects on the immunological system due to ultrasound. Anderson and Barrett (1979) insonated mouse spleens in-vivo with 2 MHz ultrasound for 1.6, 3.3 and 5 minutes (spatial average temporal average intensity $8.9 \text{ mW cm}^{-2}$, spatial peak temporal peak $28 \text{ W cm}^{-2}$, pulse repetition frequency 691 Hz). The authors reported a slight decrease in the IgM compartment of immunoglobulin response, and a decrease in the number of direct antibody plaque-forming cells in the spleen, compared to the controls. Child et al (1981) and Berthold et al (1982) attempted to reproduce these findings but reported no immunosuppressive effect.

Anderson and Barrett (1981) also investigated the effect of ultrasound on phagocytosis of inert particles and bacteria by the reticuloendothelial system. They used the same system as in their
earlier work, and in one series of experiments looked at the rate at which particles of colloidal carbon were being cleared from the bloodstream. They reported that the decay time of the exponential clearance curve was reduced when assayed after exposure to ultrasound. Surprisingly, they found that longer exposures had a lesser effect. Wells (1987) severely criticises their experimental protocol, however, on the grounds that the carbon particles were injected as a suspension in 0.25 ml of water containing 0.5% gelatine, whereas the entire blood volume of a mouse is only about 3 ml. The injection of 0.25 ml of water therefore results in immediate osmotic lysis of a significant number of erythrocytes, and in a significant reduction in the osmolarity of the blood plasma. Thus the measured clearance rates reflect the rate at which carbon particles coated with gelatine were removed in a hypotonic environment in competition with free haemoglobin and cellular debris. Saad and Williams (1982) injected 0.3 ml of blood-borne colloidal particles in isotonic saline into rats, and reported that the rate of clearance was affected by continuous wave ultrasound (1.65 MHz, spatial average) provided that the intensity was greater than 0.8 W cm\(^{-2}\).

2.6 Effects on the CNS, Organs, Muscle, and other Soft Tissue.

There have been countless further reports of damaging effects to tissues, organs etc. Dunn and Fry (1971) and Frizzell et al (1983), using 1-6 MHz and 1 MHz ultrasound respectively, reported hindlimb paralysis after exposure to ultrasound, although the intensities used would be described as high (compared to physiotherapeutic and
diagnostic levels) for Dunn and Fry's work (5.4 W cm\(^{-2}\) spatial average) and phenomenal for Frizzell et al. (289 W cm\(^{-2}\) spatial average). Hu and Ulrich (1976) used 2.25 MHz and 5 MHz pulsed ultrasound at 3 mW cm\(^{-2}\) (spatial and temporal average), applied to the brains of anaesthetised squirrel monkeys, and were able to detect additional evoked potentials. Mortimer et al (1980) have reported changes in the resting cardiac muscle tension in rats (2.3 MHz, 1.1, 2.2 and 3.3 W cm\(^{-2}\), spatial and temporal average), and Lizzi et al (1978a,b) observed cataract and lesion production in the eyes of rabbits after exposure to 9.8 MHz ultrasound of between 200 and 1000 W cm\(^{-2}\) (spatial average).

Stark et al (1984) investigated short- and long-term risks after exposure to diagnostic ultrasound in utero. They used ultrasound with a spatial average temporal average intensity in the range 2.1-7.1 W cm\(^{-2}\), and a spatial peak temporal peak intensity in the range 200-1000 W cm\(^{-2}\), and reported an increase in the incidence of dyslexia in young children, although they investigated so many possible manifestations, that statistically, it would have been surprising if they had not been able to report at least one adverse effect. Indeed, the authors themselves warn against overinterpretation of their data.

2.7 Summary.

There is clearly an abundance of data indicating a potential for biological damage due to ultrasound. In some cases poor experimental procedures mean the data can be dismissed, but most of it has to be
taken seriously. In order to attain a greater understanding of the problem, it is necessary to look at the mechanisms by which ultrasound may cause the effects that have been discussed in the preceding sections. The next chapter looks at cavitation, which has been a focus of attention for some time as one of the possible mechanisms. Later on in the chapter, sonoluminescence, which is one of the methods by which cavitation may be detected, is introduced and discussed.
3.1 Mechanisms by which Ultrasound may cause Damage.

Carstensen (1987), in a review of the safety of diagnostic ultrasound, states that despite the abundance of reported effects, there is currently no reason to reduce or cease clinical ultrasound investigations. As we have seen, all the reported effects have failed to find enough support among other research workers to class them as proven effects. However, the temporal and spatial shapes of ultrasound beams vary widely, and it is almost impossible to reproduce faithfully the work of other researchers. For this reason, it should not be assumed that two or three negative results mean that a positive result is wrong, unless a methodological error can be pinpointed.

It was stated in chapter 1 that the dominant interaction mechanisms which have been identified in the case of ultrasound are the heating of tissues and the production of cavitational activity. Any other effects are usually grouped under the general heading "non-thermal, non-cavitational effects". It was also stated that the temperature rise within a region of interest during an ultrasound investigation is small.

In the following section, therefore, we will turn our attention to cavitation and the behaviour of bubbles. The creation, expansion and contraction of bubbles containing gas or vapour in a liquid, under the influence of an ultrasonic field, is termed acoustic cavitation.
A time-varying pressure, added to the steady ambient pressure, causes the oscillation. This may be gentle or violent, and cavitation is often described as being stable or transient, although the distinction between the two is often far from clear-cut.

The question of whether cavitation can occur in-vivo is a subject of controversy. Ter Haar and Daniels (1981) have observed cavitation in guinea pigs and ter Haar et al. (1982) report the production of gas bubbles in mammalian tissue, although they may only have caused already present bubbles to grow to a detectable size. The conditions under which bubbles may be produced in-vivo has recently been assessed by Daniels et al. (1987) and Crum et al. (1987a) using agar gels. Ziskin et al. (1972) and Meltzer et al. (1980) have mentioned ultrasound contrast imaging, whereby a fluid is injected rapidly into the blood vessels, producing bubbles, which act as a contrast agent in ultrasound imaging. Gas bubbles are thus deliberately injected into an acoustic beam, so this is one situation where bubbles may occur in-vivo. In order to appreciate the other conditions (if any) that may lend themselves to cavitation in-vivo, bubble dynamics and cavitation will now be discussed in some detail.

3.2 Transient and Stable Cavitation.

Transient cavitation occurs when regions of high negative pressure generated in the liquid by the sound field, force the liquid molecules apart. A bubble is created, and it collapses when the compressional part of the wave arrives. Theoretically, transient cavitation requires a sound field with a peak pressure amplitude of
100 MPa, this being the tensile strength of water at room temperature (Walton and Reynolds 1984). However, pressures much lower than this have been seen to produce the effect, because water contains several weak spots, such as pre-existing bubbles. Such bubbles often expand to many times their original size and then collapse violently and disintegrate into a mass of smaller bubbles. The duration of the whole process is less than a wave period.

Stable cavitation, on the other hand, is a relatively long-lasting process. It can occur at lower pressure amplitudes than those required for transient cavitation, and requires the pre-existence of bubbles. Stable cavities oscillate in phase with the sound wave in a non-linear fashion around some equilibrium size, and may continue oscillating for several acoustic pressure cycles.

3.2.1 Cavitation Thresholds.

Any discussion of threshold phenomena must take account of the limitations of the method by which a threshold is detected. Cavitation can be detected by (see Hill et al 1969) the accompanying degradation of DNA, the release of free iodine from KI solution in the presence of CCl₄, observations of the first subharmonic of the driving frequency, and, as will be discussed in section 3.8, observations of sonoluminescence. If any of these effects cannot be detected, there are two explanations: that there is no cavitation, or that the detection system is not sensitive enough to pick up cavitation that is present.
The intensity above which transient cavitation can occur and below which only stable cavitation can take place, is called the transient cavitation threshold (Neppiras, 1980). This threshold is usually taken as being the threshold of detection, and of course, it can never be proved that a cavitation threshold does exist. At intensities near this threshold, it is hard to distinguish between a stable cavity and a transient cavity, and transient cavitation is usually assumed to set in when, during the collapse phase, the bubble wall velocity attains the speed of sound in the liquid. There is another threshold, the stable cavitation threshold, above which stable cavities can grow and become transients.

Cavitation thresholds have to be carefully defined as they depend on several factors, and the question of whether a certain set of conditions will produce cavitation is a subject of great complexity. Experimentally, a cavitation threshold is the dividing line between detection and non-detection of cavitation. If we are to define conditions under which cavitation may occur, it is not enough merely to define the intensity of the beam. In the case of diagnostic ultrasound, the beam is on for typically the order of a microsecond, followed by an off-period of about a millisecond, so while the temporal average intensity of diagnostic ultrasound is low, the peak intensity is often very high (Carson et al. 1978, Duck et al. 1985). In contrast, in physiotherapy, continuous wave ultrasound is often used. In this case, the temporal average is equal to the temporal peak, and is of the order of $1 \text{ W cm}^{-2}$ (spatial average). Cavitation can easily be detected when physiotherapeutic ultrasound is used. Whether cavitation can occur under diagnostic conditions is more difficult to
ascertain and the question remains unanswered, although Flynn (1982) has predicted that microsecond pulses of ultrasound can generate cavitation in water if the peak intensity is $10-30 \text{ W cm}^{-2}$, a situation that does arise with some diagnostic equipment (Carstensen and Flynn 1982, NCRP report 1983).

3.2.2 Factors affecting Cavitation.

Apart from the spatial and temporal profiles of the ultrasound beam, there are several other factors which affect the onset of cavitation. The standing wave pattern in the medium is one factor, and this is discussed in section 3.6. Clearly the state of the medium is important; its compressibility and viscosity determine the ease with which it is pulled apart (see equation 3.10). Cavitation can be suppressed both by distillation and by degassing (Strasberg, 1959), or by raising the ambient pressure (Hill, 1972). It has also been shown that cavitation thresholds increase with frequency (Esche, 1952), and when a tank of water is insonated, even the volume of water used may be important (Iernetti, 1971). This is a consequence of the distribution of the cavitation nuclei (see section 3.3). The degree of growth and collapse of a bubble also depends on the acoustic duty cycle (Hill et al 1969, Ciaravino et al 1981, and Chapter 4, this thesis).

3.3 Cavitation Nuclei and Bubble Dynamics.

In this section the behaviour of bubbles in a liquid is discussed. This is necessary in order to be able to understand many aspects of
sonoluminescence (section 3.8) and the ways in which bubbles behave in an ultrasonic field (sections 3.4, 3.5 and 3.7).

One factor mentioned in section 3.2 is the gas content of the insonated medium. This determines the number and size distribution of cavitation nuclei; centres for cavitation. A small bubble in a liquid is just one example of a cavitation nucleus, although such a bubble is never really in equilibrium as it would float to the surface. Larger bubbles are expelled from the liquid in this way faster than smaller bubbles, and tap water which has been left to stand for a few hours will contain no bubbles larger than $10^{-5}$ m in diameter (Flynn, 1964). Larger bubbles may be present if they are trapped in an angled crevice on a solid particle or on the container walls (Harvey et al, 1944), and indeed, tap water contains between 50000 and 100000 solid particles per cm$^3$ (Apfel 1981a), and these can act as cavitation nuclei.

Consider a single bubble in equilibrium in a liquid. For the present, we are not concerned with its stabilisation mechanism, and indeed, discussion of nucleation sites in living organisms is not inhibited to the same extent by stabilisation problems (i.e. any bubbles present do not float upwards). The pressure in a bubble in equilibrium is given by $P_0 + (2\sigma/R_0)$, where $P_0$ is the hydrostatic pressure, $R_0$ is the equilibrium bubble radius, and $\sigma$ is the surface tension in the liquid. If $P_0$ changes to a new value $P_1$, causing the bubble radius to change to a new value $R_1$, the new gas pressure in the bubble is

$$ (P_0 + (2\sigma/R_0))(R_0/R_1)^3 $$

(3.1)
and

\[ P_1 = (P_0 + (2\sigma/R_0))(R_0/R_1)^3 - (2\sigma/R_1) \]  

(3.2)

This assumes an isothermal process and ignores the effect of vapour pressure. Differentiating and taking \( \partial P_1/\partial R_1 = 0 \), it is easily seen that there is a minimum value of the acoustic pressure required for bubble growth when the initial radius is \( R_0 \). The minimum value of the acoustic pressure required for growth is called the Blake threshold pressure and is given by

\[ P_B = P_0 - P_1 = P_0 + (8\sigma/9)(3\sigma/(2P_0 + (2\sigma/R_B))^3)]^{1/2} \]  

(3.3)

For small bubbles, surface tension dominates, so that \( 2\sigma/R_B > P_0 \), and

\[ P_B = P_0 + (0.77\sigma/R_B) \]  

(3.4)

whereas for large bubbles

\[ P_B = P_0 + (8\sigma/9)(3\sigma/2P_0)^3)^{1/2} \]  

(3.5)

In this derivation, inertial and viscous forces have been neglected and it has been assumed that gas diffusion occurs slowly compared to the rate of mechanical growth of the bubble. (The effect of inertial forces, for example, may mean that significant growth does not occur over an acoustic period, and that the equations are not satisfactory for describing the behaviour of bubbles in a diagnostic ultrasound field.)

Now consider a bubble of radius \( R_0 \) in an incompressible viscous liquid. The liquid pressure is of the form \( P_{\infty} = P_0 + \dot{P}(t) \), and the radius of the bubble changes to a new value, \( R \), in response to the changing hydrostatic pressure. If the work done by \( P_{\infty} \), the hydrostatic pressure at infinity, minus the work done by \( P_L \), the hydrostatic pressure in the liquid layer adjacent to the bubble, is equal to the kinetic energy surrounding the bubble then
\[ \int_{R_0}^{R} (P_L - P_\infty) 4\pi R^2 \, dR = \rho/2 \int_{R}^{\infty} R^2 4\pi R^2 \, dR \]  \tag{3.6}

where \( \rho \) is the liquid density.

For an incompressible liquid \( \dot{r}^2 = \dot{R}^2 \), so the right-hand side of the equation becomes \( 2\pi \rho R^3 \dot{R}^2 \).

The gas pressure in the bubble is \( P_0 + (2\sigma/R_0) \) when the radius is \( R_0 \), and \( (P_0 + (2\sigma/R_0)) (R_0/R)^{3K} \) when the radius is \( R \). \( K \) is the polytropic index of the gas, which varies between 1 and \( \gamma \) for the isothermal and adiabatic limits respectively.

Therefore we have

\[ P_L = [P_0 + (2\sigma/R_0)] (R_0/R)^{3K} - 2\sigma/R \]  \tag{3.7}

Equation 3.6 gives, on differentiation,

\[ (P_L - P_\infty) 4\pi R^2 = 6\pi R^2 \dot{R}^2 + 4\pi R^3 \rho \dot{R} \]  \tag{3.8}

Substituting equation 3.7 into equation 3.8, and dividing by \( 4\pi R^2 \rho \) gives

\[ \ddot{R} + 3\dot{R}^2/2 = 1/\rho [([P_0 + (2\sigma/R_0)] (R_0/R)^{3K} - 2\sigma/R - P_0 - P(t)) \tag{3.9} \]

(Noltingk and Neppiras 1950, Neppiras and Noltingk 1951), and if the vapour pressure and viscosity terms are included, we have

\[ \ddot{R} + 3\dot{R}^2/2 = 1/\rho [([P_0 + (2\sigma/R_0) - P_v] (R_0/R)^{3K} - 2\sigma/R - 4\mu \dot{R}^2/R - P_0 - P(t)) \tag{3.10} \]

where \( P_v \) is the vapour pressure and \( \mu \) is the coefficient of viscosity.

Equation 3.10 facilitates the study of single bubbles, and accurately describes the motion of a bubble wall for all types of stable cavity, and also transients except where the wall velocity approaches the speed of sound, when the assumption of liquid incompressibility ceases to hold (Neppiras 1980). The derivation of this equation makes several assumptions about the behaviour of the bubble, but the equation is considered by Walton and Reynolds (1984) to be entirely
satisfactory for discussing most aspects of sonoluminescence. Also, apart from being suitable for computing the form of the radius-time plot for a bubble for a single cycle pulse (Lauterborn 1976), or for several periods (Lewin and Bjorno 1982), the equation can be used in conjunction with well known thermodynamic relations for finding the maximum temperature and pressure reached by a gas in a cavity. Lewin and Bjorno (1981) calculated the thresholds of the incident ultrasonic wave at which stable cavitation may be induced and rectified diffusion (see section 3.5) may begin.

3.4 Resonant Frequency of a Bubble.

Assuming that a bubble of equilibrium radius \( R_0 \) contains gas in a liquid at an ambient pressure \( P_0 \), it is possible to derive an equation of motion for linear oscillations under the influence of a superimposed sinusoidal pressure. Neglecting damping terms, and substituting \( R=R_0+r \) into equation 3.9, where \( r< R_0 \), expanding in powers of \( 1/R_0 \) and retaining only first-order terms leads to:

\[
\ddot{r} + \left(\frac{r}{P_0 R_0^2}\right)[3K[P_0+(2\sigma/R_0)]-(2\sigma/R_0)] = \left(\frac{P_A}{P_0 R_0}\right) \sin \omega_A t \tag{3.11}
\]

where \(-P_A \sin \omega_A t\) replaces \( P(t) \) in equations 3.9 and 3.10. Equation 3.11 may be written in the form

\[
\ddot{r} + \omega_r^2 r = \left(\frac{P_A}{P_0 R_0}\right) \sin \omega_A t \tag{3.12}
\]

where the resonant frequency of the bubble, \( \omega_r \), is given by

\[
\omega_r^2 = \left(\frac{1}{P_0 R_0^2}\right)[3K[P_0+(2\sigma/R_0)]-(2\sigma/R_0)] \tag{3.13}
\]

If the vapour pressure and viscosity are also considered

\[
\omega_r^2 = \left(\frac{1}{P_0 R_0^2}\right)[3K[P_0+(2\sigma/R_0)-P_v]-(2\sigma/R_0)-(4\mu/P_0 R_0^2)] \tag{3.14}
\]

For small bubbles, where the surface tension effects dominate,
equation 3.13 becomes
\[ \omega_r^2 = \left( \frac{2a}{\rho R_0^3} \right)(3K-1) \] (3.15)
and for large bubbles, surface tension effects are negligible and
\[ \omega_r^2 = 3K \rho_0 \rho R_0^2 \] (3.16)
(Minnaert 1933).

For large air bubbles in water, this reduces to the form
\[ f_r R_0 \approx 3 \] (3.17)
where \( f_r \) is the linear frequency in Hertz and \( R_0 \) is in metres.

In the experimental sections which follow, the frequency of the ultrasound used is 1.1 MHz, so in the systems described, the resonant size of an air bubble in water is 2.7 \( \mu \)m.

3.5 Rectified Diffusion.

Acoustic thresholds for bubble growth that are less than those described by equation 3.3 can arise when a process known as rectified diffusion takes place. Small oscillations of a bubble occur for acoustic pressure amplitudes ranging from a fraction of an atmosphere to a few atmospheres. The gas inside the bubble, usually air, acts as a spring to limit large changes from the equilibrium radius of the bubble. Since bubbles are mechanically unstable in a liquid, they will slowly change equilibrium size at a rate determined by the diffusion of gas across the interface. This diffusion is, of course, accelerated by the presence of the sound field. The mechanism for rectified diffusion arises because during the compression phase of the sound cycle, the gas concentration in the bubble (i.e. the number of moles of gas per unit volume) increases, and so gas tends to leave
the bubble for the liquid. During the expansion phase the gas concentration in the bubble decreases, and the gas tends to diffuse into the bubble. These two rates of diffusion are not the same, however, since the bubble has a greater surface area during the expansion phase than during the contraction phase. Diffusion rates are proportional to surface area, and thus over a complete cycle the bubble will gain gas. This gain more than offsets the loss due to diffusion from the bubble as a consequence of the excess pressure due to surface tension. Safar (1968) has given a general result for the acoustic pressure threshold for rectified diffusion, $P_D$, for a bubble of radius $R_D$:

$$P_D/P_0 = \left[ 3K\left[ 1+(2\sigma/P_0 R_D)\right]-\left(2\sigma/P_0 R_D\right)\right] [1-(\omega_A/\omega_D)^2][1-\left(\omega_D/\omega_0\right)^2]^{\frac{1}{2}}$$

where $C$ is the ratio of the gas concentration in the liquid at a point remote from the bubble to the saturation concentration.

Crum and Hansen (1982) have shown that the production of ultrasonically induced stable cavitation bubbles in tissues, at the frequency and intensities used by ter Haar and Daniels (1981) (see section 3.1), can be predicted on the basis of bubble growth by rectified diffusion.

3.6 Standing Wave and Travelling Wave Fields.

Whenever an ultrasound beam strikes an interface between two lossless media, the beam will be partially reflected and partially transmitted. The particle pressure reflection and transmission coefficients are given by
\[ R = \frac{P_r}{P_i} = \frac{(Z_2 \cos \theta_i - Z_1 \cos \theta_t)}{(Z_2 \cos \theta_i + Z_1 \cos \theta_t)} \]  
(3.19)

and

\[ T = \frac{P_t}{P_i} = \frac{2Z_2 \cos \theta_i}{(Z_2 \cos \theta_i + Z_1 \cos \theta_t)} \]  
(3.20)

where \( P_i, P_r, \) and \( P_t \) are the pressure amplitudes of the incident, reflected and transmitted waves, \( \theta_i \) and \( \theta_t \) are the angles of incidence and of transmission, and \( Z_1 \) and \( Z_2 \) are the acoustic impedances in the medium containing the incident beam, and in the medium containing the transmitted beam, respectively.

It can be seen from equation 3.19 that \( R \) can be either positive or negative, the negative case corresponding to a \( \pi \)-phase change in the reflected component. It can also be seen that if the two media have very similar acoustic impedances, \( R \) will be insignificant compared to \( T \) (equation 3.20), and virtually all the energy will be transmitted into the second medium. In this case a travelling wave will exist in the first medium. The other limiting case occurs when \( Z_1 \gg Z_2 \), and \( T \ll R \). All the energy is reflected, and in a lossless medium, a complete standing wave field will be set up. In a standing wave ultrasonic field, pressure and displacement are \( 180^\circ \) out of phase, and care must be taken when discussing nodes and antinodes. The pressure antinodes, where there are large changes in pressure, are displacement nodes; while displacement antinodes, where there are large deviations from the equilibrium position, are pressure nodes. For the remainder of this thesis, unless otherwise stated, the terms node and antinode will mean pressure node and pressure antinode respectively.

In cavitation studies, it is often an advantage, and sometimes even
necessary, to use a standing wave field. Bubbles below resonant size can move to pressure antinodes where they can grow by rectified diffusion to a size at which transient cavitation occurs. Another advantage is that the peak intensity in a standing wave field is four times that in the component travelling waves. In practice, it is very hard to be sure what type of field is present in any experimental situation, and the wave generally has a standing wave and a travelling wave component.

The proportion of a sound field which is standing wave, expressed as a percentage, is calculated from the peak-to-peak acoustic pressure maximum at an antinode, \( P_{\text{max}} \), and the pressure minimum at the adjacent node, \( P_{\text{min}} \), from the expression

\[
\frac{(P_{\text{max}} - P_{\text{min}})}{(P_{\text{max}} + P_{\text{min}})} \times 100\% \quad (3.21)
\]

Close to the reflecting surface, this is numerically equal to the acoustic reflectivity of the surface.

Another factor to consider in the experimental situation, is that all media are attenuative. Often, the attenuation is small, as is the case for water, but in the human body, for example, attenuation is about \( 1 \text{ dB cm}^{-1} \text{ MHz}^{-1} \), so the standing wave percentage will be considerably higher near an interface than away from it.

Numerically \( Z \) is the product of the liquid density, \( \rho \), and the speed of ultrasound in the liquid, \( c \). Under conditions that cause cavitation, the average values of both \( \rho \) and \( c \) will change as the liquid becomes, in effect, a different medium, acoustically lossier and more compressible. Thus \( Z \) will change. The properties of the new
medium control the course of subsequent events, and the concept of acoustic impedance as a unique property of the medium is invalid. However it is still useful, under such conditions, to refer to an effective acoustic impedance.

3.7 Bjerknes Forces.

When a bubble that is growing by rectified diffusion reaches a size that will make it resonate at close to the resonant frequency, it undergoes large oscillations. In most cases the bubble grows near a region of an acoustic pressure antinode, because acoustic radiation forces move it to one of these regions.

A body of volume V in a field where a pressure gradient \( \nabla P \) exists will experience a force \(-\nabla P\). If \( P \) and \( V \) are time-varying, we have

\[
F = -\langle V(t) \nabla P(r,t) \rangle \quad (Walton \ and \ Reynolds \ 1984) \quad (3.22)
\]

Walton and Reynolds use this as a basis for their derivation of the forces on bubbles in sound fields and arrive at the expressions

\[
F_x = \pm(3PAk \varepsilon V_0/R_0) \cos kx \quad (3.23)
\]

the sign depending on whether bubbles are smaller or larger than resonant size. However, the author has examined this expression and its derivation critically; the final expression of Walton and Reynolds does not lead to their subsequent conclusions on the movement of bubbles, and there are a number of inaccuracies in their derivation, the most serious of which is the assumption that the amplitude of oscillation of the bubble is independent of its position in the ultrasound field. Reconsideration of the problem indicates that the final expression for \( F_x \) should depend on \( \sin 2kx \) instead of
on cos kx. The full derivation is as follows;

For a standing wave pressure field of the form

\[ P(x,t) = P_0 + 2P_A \sin k_A x \cos \omega_A t \]  \hspace{1cm} (3.24)

where \( k_A = \frac{2\pi}{\lambda_A} \),

the volume of a bubble at a position x may be written as

\[ V(t) = V_0 \left[ 1 - \left( \frac{3\xi}{R_0} \right) \cos(\omega_A t + \alpha) \right] \]  \hspace{1cm} (3.25)

where \( V_0 = 4\pi R_0^3/3 \),

\[ V(t) = 4\pi R(t)^3/3, \]

\[ R(t) = R_0 - \xi \cos(\omega_A t + \alpha), \]

\( \xi \) is the amplitude of oscillation and itself varies as

\[ \xi = \xi_0 \sin k_A x \]

where \( \xi_0 \ll R_0 \) (the point missed by Walton and Reynolds), and \( \alpha \) is a phase term which allows for the fact that oscillation of the bubble radius may not be in phase with the oscillation of the pressure.

For bubbles driven with \( \omega_A < \omega_r \), the sound pressure and volume are in phase and \( \alpha = 0 \) in equation 3.25, whereas for bubbles driven with \( \omega_A > \omega_r \) the sound pressure and volume are out of phase and \( \alpha = \pi \). (This follows from the solution of equation 3.12.) Substituting equations 3.24 and 3.25 into equation 3.22 gives

\[ F_x = -2P_A k_A V_0 \cos k_A x \left[ 1 - \left( \frac{3\xi}{R_0} \sin k_A x/R_0 \right) \cos(\omega_A t + \alpha) \right] \cos(\omega_A t) \]  \hspace{1cm} (3.26)

or

\[ F_x = 3P_A k_A \xi_0 V_0 \sin 2k_A x /2R_0 \]  \hspace{1cm} (3.27)

for \( \omega_A < \omega_r \), and

\[ F_x = -3P_A k_A \xi_0 V_0 \sin 2k_A x /2R_0 \]  \hspace{1cm} (3.28)

for \( \omega_A > \omega_r \).

Now \( \omega_A > \omega_r \) implies that the bubbles are greater than resonant size, so comparing equations 3.27 and 3.28 with the \( \sin k_A x \) variation of the amplitude of the pressure field (equation 3.24), leads to the
conclusion that bubbles larger than resonant size bubbles will experience a force directed from a pressure antinode towards a pressure node. Such bubbles have continued to grow by rectified diffusion and have become slightly larger than the resonant size, at which point the driving frequency has become nearly \( \pi \) out of phase with the motion of the bubble. The bubble is forced toward a local pressure minimum, and at such a position in a true standing wave field, rectified diffusion can no longer occur and bubble growth ceases.

The case \( \omega < \omega_r \) implies that the bubbles are smaller than the resonant size, and these bubbles experience a force towards a pressure antinode. These forces on bubbles in standing wave fields are called primary Bjerknes forces.

The situation becomes much more complex when we consider a number of bubbles oscillating in the sound field. Such bubbles also experience secondary Bjerknes forces, which arise because one bubble experiences a force due to the field radiated by the others around it. If two bubbles are either both larger or both smaller than the resonant size, they oscillate in phase and attract each other. If one bubble is larger than the resonant size and one is smaller, they oscillate out of phase and repel one another.

3.8 Sonoluminescence.

When a bubble in a sound field collapses in such a way that the speed of the bubble wall is comparable with the speed of sound, the
collapse is said to be transient. The gas inside the bubble cannot escape into the surrounding liquid because the collapse is too rapid and the diffusion process is too slow. This rapid compression is therefore adiabatic, and the temperature of the gas and vapour mixture in the bubble may increase to a few thousand degrees Kelvin as the bubble radius reaches a minimum value in the sound cycle. The high temperatures cause the production of electronically excited molecules, and of .H and .OH free radicals (Edmonds and Sancier 1983), which can either radiate back to the ground state, or recombine radiatively. The radiation emitted on recombination is in the visible region of the spectrum and is termed sonoluminescence (Harvey 1939). Spectral measurements, and experiments that employ radical scavengers, confirm that sonoluminescence does indeed originate from this free radical recombination (Sehgal et al 1980c).

Since the discovery of sonoluminescence in 1934, many other theories as to its origin have been expounded (see for example Finch 1963), but there is now almost universal agreement that sonoluminescence is generated by free radicals produced during bubble collapse.

3.8.1 Recording Sonoluminescence.

Under some circumstances, sonoluminescence can be observed with dark-adapted eyes (Chambers 1937), and early sonoluminescence experiments were carried out using this method of assessment. Marinesco and Trillat (1933) discovered that latent images were produced in silver halide emulsions on photographic films and plates immersed in an ultrasonic bath. This actually pre dated the discovery of
Photomultipliers were first used to record sonoluminescence by Griffing and Sette (1955). The use of photomultipliers led to the discovery that sonoluminescence occurs as discrete flashes, the frequency of the flashes being twice the frequency of the ultrasound (Gunther et al. 1957a). This frequency doubling occurs because in a standing wave field alternate antinodes are 180° out of phase. Also there was a spread in emission times of approximately 10% of the sound period, just before the sound pressure goes into its negative half-cycle (Jarman and Taylor 1970), due to the fact that there is a range of bubble sizes present in the liquid.

Flynn (1964) first demonstrated the potential of an image intensifier to record sonoluminescence. An image intensification system is described by Walton and Debenham (1980) and this is the system which is used in some of the work in chapters 5-7. The tremendous advantage to be gained from image intensification is that it gives information on the spatial distribution of sonoluminescence.

3.8.2 The Effect of the Liquid Medium on Sonoluminescence.

The intensity of sonoluminescence depends on the nature of the liquid medium (see for example Golubnichii et al. 1971; Singal and Pancholy 1967). Jarman (1959), after investigating fourteen different liquids, concluded that sonoluminescent intensity correlated closely with \( \sigma^2 / P_v \), where \( \sigma \) is the surface tension and \( P_v \) the vapour pressure in the liquid. Golubnichii et al. (1971) found that there was also a
correlation with the viscosity \( \mu \), and the inverse of the adiabatic compressibility \( 1/\beta \). These dependencies are not surprising since \( \sigma \), \( P_v \) and \( \mu \) all appear in equation 3.10, and this equation results from a derivation which assumes liquid incompressibility.

Dissolved substances may also affect the intensity of sonoluminescence. In the case of suspensions, this intensity is independent of the suspended substance (Harvey 1939), although suspended substances do increase the number of nucleation sites. Electrolytic solutions can increase or decrease sonoluminescent intensity, depending on the concentration of the salt, and on the salt itself (Jarman 1959). Negishi (1961) showed that sonoluminescent emission could be enhanced many times using a solution of 0.2g of luminol and 5g of sodium carbonate per litre of water, and many workers have deliberately employed luminol to facilitate observations of sonoluminescence e.g. Crum and Fowlkes (1986). In cases where luminol is used, the light observed is really the result of chemiluminescence – light produced by the oxidation of luminol through a reaction that is initiated by free radicals – rather than of sonoluminescence.

Sonoluminescence can be produced from a very wide range of liquids, and these include blood plasma (Eastwood and Watmough 1976) and amniotic fluid (Crum et al 1987b).
3.8.3 Dependence of Sonoluminescence on Various Factors.

3.8.3.1 Acoustic Pressure.

The way in which the sound pressure amplitude, \( P_A \), affects the maximum temperature attained inside a bubble during collapse, \( T_{\text{max}} \), can be deduced from the equation

\[
T_{\text{max}} = \left( \frac{T_0}{R_0^3} \right) \left( \frac{4}{3} \right)^{3/2} \left[ \left( \frac{P_A - P_0}{P_0} \right)^3 \right] \left[ \frac{I + 2(P_A - P_0)}{3P_0} \right] (P_0 + P_A)(K-1)
\]

(3.29)

where \( T_0 \) is the equilibrium temperature, and the other symbols have the same meaning as in sections 3.3 and 3.4 (Walton and Reynolds 1984). Transient cavitation demands, of course, that \( P_A > P_0 \) and the effect of \( P_A \) on sonoluminescence arises because the range of initial bubble radii which can give rise to transient cavitation is a function of \( P_A \) (and also of \( P_0 \)) (Apfel 1981b.)

Griffing and Sette (1955) and Parke and Taylor (1956) found an approximately linear relationship between sonoluminescence and acoustic pressure. Jarman (1959) also reported an increase in sonoluminescence with power up to 35 W. Negishi (1961) however, observed a sudden cut-off at around 2 W cm\(^{-2}\) for a number of liquids. This effect arises because intense cavitation in the region of the transducer can prevent transmission of ultrasound to the region of the liquid under observation.

More recent work by Margulis and Akopyan (1978), at a frequency of 0.88 MHz, reported that at low power levels the sonoluminescent intensity is proportional to the square of the acoustic intensity,
but this changes to a linear dependence at about 0.7 W cm\(^{-2}\). This relationship held for a number of liquids including water saturated with oxygen and for a luminol solution.

3.8.3.2 Frequency.

Surprisingly there is very little data available on the dependence of sonoluminescence on frequency. Griffing and Sette (1955) made a quantitative study at frequencies of 0.66 MHz, 1 MHz and 2 MHz, and found that sonoluminescent output was greatest at 0.66 MHz and least at 2 MHz. Gunther et al (1957b) also reported a decrease of sonoluminescence with frequency, using frequencies of 30, 60, 80 and 100 kHz. Equation 3.29 shows a dependence on the angular frequency, \(\omega_A\), of the sound field, and indeed predicts that sonoluminescent intensity should decrease as the frequency increases. The decrease is not as rapid as would be expected from equation 3.29, but, as Walton and Reynolds (1984) point out, this equation is derived on the basis of the dynamics of a single bubble, and increasing the frequency will increase the number of antinodal points in a standing wave, thereby lessening the predicted intensity decrease (see section 3.8.3.5).

Another feature of sonoluminescent output is that since sonoluminescence occurs over one tenth of the sound period, higher frequency ultrasound will result in shorter bursts of sonoluminescence, but a greater number of them (Jarman and Taylor 1970).
3.8.3.3 Hydrostatic Pressure.

It can be seen from equation 3.29 that sonoluminescent intensity is strongly dependent on the term \((p_A - P_0)\) and to a lesser extent on \((P_A + P_0)\). The dependence of sonoluminescent intensity on \(P_0\) should thus, in general terms, be similar to its dependence on \(P_A\).

Harvey (1939) noted that sonoluminescent intensity increased up to \(P_0 = 2.3\) bar and then fell to zero. Finch (1965) obtained a similar result, with the maximum occurring between \(P_0 = 1.5\) bar and \(1.7\) bar. He investigated the problem by changing \(P_0\) by hydraulic and gas-applied pressure, while Harvey only applied the pressure hydraulically. Chendke and Fogler (1983) measured the sonoluminescent intensity from nitrogen-saturated water as \(P_0\) was increased hydraulically from \(1\) bar to \(15\) bar. They also took measurements of the sonoluminescence spectrum throughout this pressure range, and fitted the spectra to black-body curves and deduced the temperatures. These were found to be independent of \(P_0\), and Chendke and Fogler inferred that the sonoluminescent intensity of a cavitation event was independent of \(P_0\), and that any rise in intensity was due to a growth in the number of suitable cavitation nuclei.

3.8.3.4 Temperature.

Several workers have looked at the effect of temperature on sonoluminescence. Work by Sehgal et al (1980b), Chendke and Fogler (1985), Jarman (1959) and Iernetti (1972), among others, has led to the general statement being made that sonoluminescence decreases with
temperature. However, there is poor agreement amongst these workers as to the shape of the intensity-temperature function. Sehgal et al reported a very sharp decrease at temperatures near 10-20°C, with a gradual flattening off of the curve towards 70°C. Chendke and Fogler measured the initial and final temperatures of air-saturated water and plotted sonoluminescence against time. They found that for colder water samples, the insonation heated up the water and the sonoluminescent output was less at the end of the experiment than at the beginning. For hotter samples, the liquid cooled during the course of the experiment and sonoluminescence increased.

Jarman and Iernetti, on the other hand, reported the appearance of a maximum in their plots of sonoluminescence flux against temperature. In other words, they only found an increase in sonoluminescence with temperature over part of the temperature range studied. Jarman studied fifteen different liquids, including water, and selected secondary butyl alcohol as exhibiting "typical" behaviour. For this liquid the peak was at 25°C. Iernetti reported the presence of a peak at 12°C for an air-water mixture.

The earlier work of Blake (1949) is inconsistent with the above findings. He showed that cavitation thresholds decrease with temperature, although he did not look for sonoluminescence.

All the above workers used different frequencies, ranging from 16.5 kHz to 0.7 MHz, and other factors likely to affect the amount of sonoluminescence were not always well controlled. The way in which sonoluminescence varies with temperature for the systems used in this
thesis, is one of the subjects investigated in Chapter 4.

3.8.3.5 Standing Wave Pattern.

One of the problems in interpreting the results of cavitation experiments is that one needs to know how the beam is made up in terms of its standing and travelling wave properties. It is at present uncertain whether sonoluminescence requires the presence of standing waves, and this situation is complicated by the fact that, as noted in section 3.6, the peak intensity in a standing wave is four times that in the component travelling waves. This in itself will make cavitation more likely to occur in a standing wave field. Also Bjerknes forces play a part in increasing the likelihood of cavitation. The question of the role played by standing wave patterns in cavitation studies is one of the problems addressed in this thesis.

Wagner (1958) demonstrated that sonoluminescence increased from a minimum at a pressure node to a maximum at an adjacent antinode. In a purely standing wave field the minimum will be a zero, whereas in a real field, it may again be zero or it may have a finite value.

Some workers have claimed to have recorded sonoluminescence from pure travelling waves. Graham et al (1980), for example, terminated their tank with sound-absorbing rubber, but they did not quantitate the nature of the sound field with a hydrophone, so they may have had some standing wave component present.
Standing waves may be avoided by using short length pulses of ultrasound. The length in the liquid must, of course, be less than the dimensions of the cell. While there may still be standing waves momentarily set up near a reflecting surface, the number of cycles in the pulse train may be insufficient to allow processes such as bubble motion due to Bjerknes forces.

3.8.3.6 Dissolved Gas.

Harvey (1939) was the first to investigate the effect of dissolved gases on sonoluminescent intensity. He found that sonoluminescence appeared in water containing air, oxygen, neon and nitrogen, but not if it contained hydrogen or carbon dioxide. Srinivasan and Holroyd (1961), Parke and Taylor (1956), Gunther et al (1957b), Prudhomme and Guilmart (1957) and Young (1976) have all produced lists of the relative intensity of sonoluminescence from water containing various dissolved gases. Comparison of the five sets of results again indicates that sonoluminescence data needs to be treated with caution as there are some marked discrepancies. However there are some aspects of the data which are generally agreed; in particular, sonoluminescence is enhanced when the dissolved gas is xenon or krypton, and to a lesser extent when it is argon. For the rare gases, the higher the thermal conductivity of the gas, the lower the sonoluminescent intensity. This is not surprising, since a higher thermal conductivity will tend to decrease the value of $K$ in equation 3.28 from $\gamma$ towards 1. A more detailed discussion of this aspect of sonoluminescence is given by Walton and Reynolds (1984). Here, it is noted that sonoluminescent intensity may be increased by bubbling
xenon, krypton or argon through water, and that by using xenon, the intensity may be increased by between 20 and 200 times (Prudhomme and Guilmart 1957, Gunther et al 1957b).

3.8.3.7 History of the Liquid.

It has been noted that cavitation, and therefore sonoluminescence, may be influenced by the previous history of the liquid being insonated. This is because the cavitation history affects the liquid tensile strength (Overton et al 1984), and in turn the liquid tensile strength influences the cavitation threshold of the liquid (Trevena 1984). For this reason it is important that liquid samples are frequently changed in sonoluminescence studies, and this procedure has been adhered to in the following chapters.

3.8.4 Sonoluminescence from Stable Cavitation?

As stated in section 3.1 the distinction between transient and stable cavitation is imprecise, so it would not be unexpected if under some conditions, stable cavitation could produce sonoluminescence. Certainly during stable cavitation the bubble wall velocity is great enough to produce high temperatures and pressures in the bubble, as the gas in a stable bubble will behave adiabatically (Walton and Reynolds 1984).

Saksena and Nyborg (1970) first reported the appearance of sonoluminescence from stable cavitation. The evidence they give is firm, but not conclusive. They introduced air bubbles of about
resonant size into a glycerine-water mixture. The bubbles would slowly rise under gravity, and would halt when a 30 kHz magnetostrictive oscillator was turned on. Light emitted from the line of bubbles was detected by a vertical photomultiplier mounted at the top of the test cell. The authors described the effect as being "sonoluminescence without the visible and audible manifestations often ascribed to cavitation". Saksena and Nyborg failed to observe sonoluminescence from stable cavitation in water.

Later work by Crum and Reynolds (1985) also reported the appearance of sonoluminescence from stable cavitation. They observed sonoluminescence using an image intensifier, and insonated a tank of water with a 20 kHz ultrasonic horn. Visual observations of the tank via the image intensifier indicated to the authors the presence of activity resembling stable rather than transient cavitation. However, oscillation of stable bubbles is by no means the only explanation for the appearance of the light patterns described. Despite the authors refutation, they may be due to transient cavities, or indeed to "ion spots", an artefact of the image intensification system.

There are no other studies pointing to the existence of sonoluminescence from stable cavitation, and this is a question which must, for the present, be regarded as unresolved.

3.8.5 Sonoluminescence Spectra.

Paounoff (1939) first considered the sonoluminescence emission spectra from liquids containing various dissolved gases. He recorded
spectra from water saturated with air, oxygen or nitrogen, and using a plate spectrograph, found a continuum stretching from 445 to 558 nm. Prudhomme and Guilmart (1957) studied sonoluminescence in the ultra-violet region, and found that emission was greatest with water saturated with xenon, and that krypton, argon, neon and helium gave rise to progressively decreasing intensity. Taylor and Jarman (1970) and Sehgal et al (1977, 1980a), have shown that the sonoluminescence spectra of water containing dissolved noble gases show a peak at 310 nm on a broad continuum extending from 240 nm to the far infra-red. The spectral distribution changes with frequency of insonation (Verrall and Sehgal 1987); at a frequency of 333 kHz the emission peak at 310 nm is strong, and smaller peaks are seen at 270, 290 and 340 nm, while at 459 kHz the peak at 310 nm is the only one visible, and is itself weaker. The prominence of the peaks decreased with increasing molecular weight of the dissolved gas, but their positions in the spectrum were unchanged, showing that the gases do not take part in the emission process.

Sehgal et al (1980a) explained the observations as follows: the emission band at 310 nm is due to light emission from excited hydroxyl radicals due to the transition $A^2\Sigma^+ \rightarrow X^2\Pi$. The emission in the near ultra-violet is due to transitions from the triplet $^3B_1$ state of water. The cut-off at 240 nm gives the heat of the reaction $H_2O \rightarrow H + OH$. Sehgal et al (1980c) were able to quench the OH band with a NO$_3^-$ scavenger and thereby uncover the predicted H$_2$O emission band. The broad continuum is due to radiative recombination of free radicals of the type

$$H + OH + M \rightarrow H_2O + M + h\nu$$
(Saksena and Nyborg 1970, Sehgal et al 1977 and 1980a) where M is a gas or water molecule.

Finally, Sehgal et al assert that the dependence of the spectra on ultrasound frequency may be due to the dependence of the relative number of transient and stable cavities on frequency, a dependence implied by Apfel (1981b).

3.9 Summary.

Some of the ideas introduced in this chapter, in particular the concepts of resonant sized bubbles, rectified diffusion and Bjerknes forces, will be referred to regularly in this thesis. Other aspects will be focussed on and will form the subject of experimentation and discussion in subsequent chapters. In chapter 4 the question of cavitation thresholds will be addressed, as will the effect of temperature on sonoluminescence. In later chapters beam profiles and standing wave patterns will be discussed (chapter 5), and their effect on sonoluminescence examined.
Chapter 4. Light Output from a Therapeutic Ultrasound Beam and the Effect of Temperature and Duty Cycle.

It was seen in the last chapter that sonoluminescence depends on many factors. A direct search for sonoluminescence in vivo is attempted later in this thesis (see chapter 8), but it is not normally fruitful and even an assessment of the number and size of bubbles in body fluids and tissues that might be sites for sonoluminescence is not easy (ter Haar and Daniels, 1981).

The approach adopted in this chapter is to try to establish threshold levels below which sonoluminescence is unlikely to occur. Some conditions under which sonoluminescence will occur in aqueous media are established and pulse height analysis is used to ascertain the range of light outputs. An assessment is made of the amount of light produced in a given volume of liquid in a given time to provide an indication of the maximum amount of cavitation occurring under the conditions specified.

The effects of temperature and duty cycle have also been investigated. For the temperature investigations, experiments have been done in the range 22-45°C. For the duty cycle experiments, continuous wave ultrasound and three pulsed regimens commonly employed by physiotherapists have been compared. The mechanisms proposed to explain the observed effects of duty cycle depend on the evidence from pulse height analysis that there are many different sized bubbles in the insonated medium.
4.1 Generation and Measurement of Ultrasound.

An ultrasound generator of the type widely employed in physiotherapy departments, the Therasonic 1030 (Electro-Medical Supplies), was used for the experiments described here. This generator has a nominal intensity range of 0-3 W cm\(^{-2}\) and can operate in a continuous wave mode or in a pulsed mode at either 1 MHz or 3 MHz. Only the 1 MHz setting was used. The available duty cycles in pulsed mode are 1:2, 1:4 and 1:7, where the on-time is 2 ms in each case (figure 1.1).

The importance of precise measurement of ultrasonic power output is now well recognised (see for example Duck et al 1987). The output of the generator was checked using a Bio-tek UWII Force Balance, and the manufacturer’s figure of 440 mm\(^2\) for the effective radiating area of the transducer was used to calculate spatial average temporal average intensities. The figures agreed with the meter readings on the Therasonic to within 10% and the meter readings are therefore quoted in this thesis. The manufacturer's figures for the duty cycles, given above, were found to be accurate to within 5% using a needle hydrophone (Dapco NP10-3). The spatial average temporal average intensities and the duty factors were used to calculate the spatial average temporal peak intensities for the pulsed regimens.

4.2 Pulse Height Analysis.

To study sonoluminescence, the liquid to be investigated was placed in a glass tank on top of a photomultiplier tube (RCA 8575, photocathode diameter 50 mm) (figure 4.1) with the transducer facing...
Figure 4.1. Schematic diagram of apparatus.
downwards in the liquid. The transducer, tank and tube were enclosed in a wooden box which was covered with layers of blackout cloth. The reflection coefficient of the base of the glass tank was around 54% (see section 5.1).

Some, if not all, of the collapsing bubbles produced a short burst of light, which appeared as a current pulse in the photomultiplier. A pulse height analyser with differential discriminator was used to count the pulses, and to analyse the distribution of pulse heights in the light burst. For some of the studies of the effect of temperature, described in section 4.6, this counting system was replaced by a Levell multitester type TM11 nanoammeter, and an EMI 9781B side window photomultiplier with light guide was used. In some experiments the light intensity reaching the photomultiplier was reduced by using neutral density filters inserted between the glass tank and the photomultiplier tube.

For the main set of experiments reported, freshly drawn tap water was used. This was allowed to settle, and experiments were performed between one and five hours later. In some cases, distilled or degassed water was used. The gas content of the water samples was measured using a Corning 178 pH/blood gas analyser. Tap water that had been left to stand for a few days was also measured. In one experiment, a solution of 1.875% (w/v) agar was used. This was prepared by adding agar to boiling water and allowing the agar to cool, when it set to a fairly sloppy consistency (measured as 170 cp).
The brightness of the sonoluminescence produced was compared with that of a calibrated beta light, which gave a photon flux of \(5 \times 10^8\) photons per second. The beta light had a faint yellow appearance when viewed in the dark, so the wavelength of the light it emitted was in the region of 580 nm. Sonoluminescence is essentially a black body radiation over the range 400-700 nm (Gunther et al 1959). Thus the 580 nm of the beta light is representative of the middle of the wavelength range of sonoluminescence, and the overall detection efficiency of the system will be similar for the two sources of light.

The effect of temperature was investigated using a thermoregulator and thermocouple (FH 15-V, Grant Instruments (Cambridge) Ltd.). The water was heated to 37.9°C and circulated around the water bath. To allow for clear viewing the thermoregulator was then switched off while readings were taken. When the temperature dropped to 36.4°C, the thermoregulator was switched on again. This range of temperatures centres on the normal body temperature of around 37°C.

4.3 Background Spectrum.

The first step was to obtain a background spectrum, where the count rate is due to photomultiplier noise and amplifier noise. Since the ultrasonic vibrations could be transmitted to the photomultiplier tube, in the experimental arrangement of figure 4.1, the background was measured in two ways:

1) with the ultrasound switched off,
2) with a continuous wave, high power beam switched on, but with a
piece of black card placed between the water tank and the photomultiplier tube to intercept any visible light generated. Both methods produced the same spectrum of counts/second vs. pulse height. This spectrum is shown in figure 4.2a. The pulse height is measured in arbitrary linear units.

4.4 Threshold Measurements.

With freshly drawn tap water in the tank, no difference was found between the pulse height spectrum generated at low intensities of ultrasound \( <0.1 \text{ W cm}^{-2} \) and that produced under background conditions. At higher intensities (figure 4.2a) there was a marked increase in count rate for a given pulse height, and a shift in the mean pulse height to a higher value. The increases continued with intensity, up to \( 3 \text{ W cm}^{-2} \), the highest intensity measured.

At \( 0.25 \text{ W cm}^{-2} \) (figure 4.2b), it can be seen that there is a slight, but not insignificant, increase in the recorded count rate over a range of pulse heights. Thus at \( 0.25 \text{ W cm}^{-2} \), sonoluminescence is present in tap water at room temperature, and the threshold for cavitation, under the conditions described lies between 0.1 and \( 0.25 \text{ W cm}^{-2} \). \( 0.25 \text{ W cm}^{-2} \) corresponds to pressure amplitudes of 126-140 kPa at an antinode, and 33-48 kPa at a node, with this standing wave system (the range of figures for the pressure amplitude arises because of the uncertainty in the reflection coefficient due to spurious reflections; see chapter 5). This figure of \( 0.25 \text{ W cm}^{-2} \) is primarily a threshold and not simply a limit on the sensitivity of the detection system. This is demonstrated by figure 4.3 which shows
Figure 4.2. Sonoluminescence spectra from fresh tap water insonated with continuous wave, 1 MHz ultrasound, at various intensities.

a) (++) background; (▲ ▲ ▲) 0.5 W cm\(^{-2}\); (○ ○ ○) 1 W cm\(^{-2}\).

b) (++) background; (■ ■ ■) 0.25 W cm\(^{-2}\).

Every third point is shown on these graphs and on figures 4.5, 4.6 and 4.9. This is sufficient to indicate the forms of the spectra, even on this logarithmic scale. Error bars are not shown as they are less than the size of the symbols; this also applies to figures 4.5-4.7 and 4.9.
Figure 4.3 (left). Percentage increase in count rate for different pulse heights at an intensity of 0.25 W cm$^{-2}$.

Figure 4.4 (right). Percentage increase in count rate for different intensities at a pulse height of 1.0.
the percentage increase in light output over background for an ultrasonic output of 0.25 W cm\(^{-2}\). It indicates that the most sensitive pulse height for observations of sonoluminescence is 1.0. Figure 4.4 shows the percentage increase in sonoluminescence at this pulse height for the full range of powers investigated. Above the detection limit, light output rises extremely rapidly with increasing intensity.

When distilled or degassed water was used in place of freshly drawn tap water, the threshold for sonoluminescence was found to be higher, and for a given set of conditions the spectra were more similar in form to the background spectrum than with freshly drawn tap water (figure 4.5). The results of measurements of partial pressures of O\(_2\) and CO\(_2\), for the various types of water are shown in table 4.1 and are discussed in section 4.9.2.

4.5 Assay of Light Output.

This assay was carried out by introducing calibrated neutral density filters into the system, between the tank of fresh tap water and the photomultiplier tube. A neutral density filter of number 1.0 will, on average, allow one in ten photons to pass through it. A filter with number 2.0 will allow one photon in a hundred through. Because of the random nature of the interaction of photons with neutral density filters, the exact effect on pulses of different heights is difficult to predict, but on successive insertion of neutral density filters, increasing in value by 0.2, the spectrum gradually returns to its background form. At an ultrasound intensity of 1 W cm\(^{-2}\), a neutral
counts per second

Figure 4.5. Spectra obtained by insonating

(Δ Δ Δ) freshly drawn tap water,

(□ □ □) old tap water,

(○ ○ ○) degassed water,

(⊕ ⊕ ⊕) distilled water

with 1 MHz, continuous wave ultrasound of intensity 1 W cm⁻².

(× × ×) is a background spectrum.
Table 4.1

Partial pressures of oxygen and carbon dioxide for various samples of water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$P_{O_2}$ (kPa)</th>
<th>$P_{CO_2}$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly drawn tap water</td>
<td>17.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Old tap water (left to stand for a few days)</td>
<td>19.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Distilled water</td>
<td>24.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Degassed water</td>
<td>11.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>
density filter of value 2.0 was the minimum required to produce a spectrum that was indistinguishable from background. Thus by attenuating 99 in every hundred photons, the limit of detection of the system has been reached. When neutral density filters were inserted between the beta light and the photomultiplier, it was found that a neutral density filter of value 5.0 was required indicating that the sonoluminescence was 1000 times smaller than the photon flux from this source. Hence the photon flux from sonoluminescence was of the order of $5 \times 10^5$ photons per second at $1 \text{ W cm}^{-2}$, in a volume of $20-25 \text{ cm}^3$ of water.

4.6 Effect of Temperature.

Figure 4.6 shows that increasing the temperature to $37^\circ\text{C}$ always produced more sonoluminescence for 1 MHz continuous wave ultrasound at $1 \text{ W cm}^{-2}$. The same effect was observed at $0.5 \text{ W cm}^{-2}$, and also with pulsed ultrasound. It will be noticed that the background count is greater at the higher temperature, an effect that is primarily due to warming of the photomultiplier, but inspection of the curves at a pulse height where the background is negligible at both temperatures shows that the increase in sonoluminescence is real, and not an artefact caused by increased noise in the tube.

Figure 4.7 is an expanded version of the results at $37^\circ\text{C}$ when 1:4 pulsed ultrasound was used. This spectral curve is not smooth but contains several peaks, which correspond exactly to readings taken at $37.9^\circ\text{C}$, the subsequent readings being at successively lower temperatures until the next heating (see section 4.2). This is a further confirmation that sonoluminescence is greater at higher
Figure 4.6. The effect of temperature on the sonoluminescence spectra. Samples were insonated with 1 MHz continuous wave ultrasound at 1 W cm$^{-2}$.

(++) background at 22°C,

(XXX) background at 37°C,

(⊙⊙⊙) with ultrasound at 22°C,

(△△△) with ultrasound at 37°C.
Figure 4.7. Part of the background-corrected sonoluminescence spectrum from pulsed ultrasound (duty cycle 1:4) at 1 W cm$^{-2}$ and 37°C. All the points are shown. The vertical arrows indicate readings taken at 37.9°C, with subsequent readings (to the right) being taken at lower temperatures until the next arrowed point.
Figure 4.8. Total photomultiplier current as a function of temperature.

lower curve: 1 W cm$^{-2}$,
upper curve: 3 W cm$^{-2}$.  

66
temperatures, and shows that the effect can be observed even when the change in temperature is small.

The variation of sonoluminescence with temperature for pulse heights below 0.5 is not amenable to testing with the above methodology because of saturation effects in the photomultiplier. The temperature variation was therefore also tested simultaneously over the entire range of pulse heights, by replacing the photomultiplier and counting system with the EMI 9781B photomultiplier and nanoammeter. Sonoluminescence produced in the water bath was carried to the tube by a light guide, so that temperature variations in the bath did not affect the tube.

The results show an increase of sonoluminescence with temperature in the range 22-45°C (figure 4.8), for intensities of 1 W cm$^{-2}$ and 3 W cm$^{-2}$. In all cases a constant dark current of 0.11 nA has been subtracted.

4.7 Effect of Duty Cycle.

The effect of changing the duty cycle was investigated, keeping the frequency fixed at 1 W cm$^{-2}$. The available duty cycles were continuous wave, 1:2 (2 ms on and 4 ms off), 1:4 (2 ms on and 8 ms off) and 1:7 (2 ms on and 14 ms off). The continuous wave beam would be expected to give the highest count rate for all pulse heights, with the 1:2 duty cycle next, followed by the 1:4, then the 1:7, and the count rate should be in the ratio 1:1/3:1/5:1/8. This is indeed observed for the smaller pulse heights, but as the discriminator
Figure 4.9. The effect of duty cycle on the sonoluminescence spectra. Samples were insonated with 1 MHz ultrasound at 1 W cm$^{-2}$.

- (XXX) continuous wave,
- (+ + +) pulsed ultrasound of duty cycle 1:2,
- (O O O) duty cycle 1:4,
- (△ △ △) duty cycle 1:7.

a) results from fresh tap water,

b) results from degassed water,

c) results from 1.875% agar gel.
level is set higher, the continuous wave spectrum falls off faster than the others (figure 4.9a). At very large pulse heights, the 1:7 duty cycle produces most light, followed by 1:4, and then 1:2, the continuous wave ultrasound giving the least light.

When degassed water was insonated, the curves still crossed over but at lower light levels (figure 4.9b). If an agar gel was used instead of freshly drawn tap water, no crossing over of the four curves was seen, and the continuous wave ultrasound always produced more light (figure 4.9c). When using agar the insonated medium contained less gas, because preparation of the gel involved boiling, but it was also more viscous.

Note that because of the log scale in figure 4.9, the differences in light output are actually quite large. They were also consistently reproducible.

4.8 Effects at Low Pulse Heights.

It will be noticed that in figures 4.2, 4.5, 4.6 and 4.9 that there is an anomalous effect at pulse heights of less than about 0.5. To find out whether this was an effect inherent in the photomultiplier, a variable LED was shone towards the photomultiplier which was protected by a neutral density filter of number 5.0. The discriminator was set at various levels, and the brightness of the LED increased. It was found that the counts always increased to a maximum and then decreased, except at low discriminator levels, when the count rate decreased with increasing brightness, i.e. in this
case the "peak" has already been passed. This is a strong indication that the tube is paralysing at high count rates, and the shape of the pulse height spectra at low pulse heights (say below 0.5) can be ignored.

4.9 Discussion.

4.9.1 Threshold Measurements.

When tap water is insonated with 1 MHz continuous wave ultrasound, light output is a function of ultrasound power output (figure 4.2a). If the ultrasound power is gradually reduced, the minimum intensity at which sonoluminescence is just discernible is about $0.25 \text{ W cm}^{-2}$ (figure 4.2b). No sonoluminescence was ever detected at output intensities below $0.1 \text{ W cm}^{-2}$. The steep rise in sonoluminescence output with intensity in the most sensitive part of the detector range suggests that this value of $0.1 \text{ W cm}^{-2}$ may be a threshold for practical purposes.

4.9.2 Gas Content.

Freshly drawn tap water always gives more sonoluminescence than either degassed tap water or distilled water (figure 4.5). There are few measurements reported in the literature on the gaseous condition of liquids during sonoluminescence experiments. The data in table 1 and figure 4.5 indicate that in our work the partial pressures of both oxygen and carbon dioxide may affect light output. Comparison of the first three sets of figures suggests that dissolved CO$_2$ is an
important source of gas required for bubble growth prior to unstable collapse. Although distilled water showed a higher \( \text{PO}_2 \) than degassed water, it should be noted that the process of distillation is likely to remove a number of potential nucleation sites as well as dissolved gas.

4.9.3 Light Output Measurements.

At 1 W cm\(^{-2}\) and 1 MHz, the photon flux from sonoluminescence will be of the order of \( 5 \times 10^5 \) photons per second. The region of water under investigation has a volume of about 20-25 cm\(^3\), so the photon flux is \( 2 \times 10^4 \) photons per cm\(^3\) per second. If it is assumed that each free radical pair produces one photon when it recombines, the photon count then gives an estimate of the number of free radicals produced per cm\(^3\) per second. Using different insonation conditions, Carmichael et al. (1986) have reported free radical concentrations from sonoluminescence comparable to those produced by 10 \( \mu \)Gy of cobalt-60 gamma radiation. It is shown in the next chapter that sonoluminescence, and hence presumably the free radicals, can be highly localised at the pressure antinodes in a standing wave field.

It is of interest to note that background radiation produces free radicals as a result of ionisation at a rate of approximately \( 10^4 \) ion pairs per cm\(^3\) per second. If the number of free radicals produced by ultrasound is only twice this, then why is the photon flux detected from non-insonated water not above the background levels when the photomultiplier is blacked off? The answer would appear to lie in the mechanisms of free radical production and recombination. Indeed, no
luminescence was detected even when a source of $^{137}$Cs was placed in the water tank.

4.9.4 Temperature.

Results in figures 4.6, 4.7 and 4.8 show conclusively, using two independent systems, that sonoluminescence increases with temperature in the range 22-45°C for the experimental system of figure 4.1. Such a conclusion is consistent with an early observation of Blake (1949), who showed that cavitation thresholds decreased with temperature. There are a number of reports in the literature suggesting that over most, if not all, of the temperature range studied here, sonoluminescence falls with temperature (Sehgal et al, 1980b; Chendke and Fogler, 1985; Jarman, 1959; Iernetti, 1972). However, all these workers used frequencies lower than 1 MHz, most used liquids other than water, and some found an increase in sonoluminescence over part of the temperature range they studied. Furthermore, other factors (such as the previous history of the liquid, concentration of dissolved gases and of foreign substances) likely to affect the amount of sonoluminescence were different in each case. The results shown in figures 4.7 and 4.8, which were repeated on several occasions, appear to show conclusively that sonoluminescence increases with temperature.

4.9.5 Duty Cycle.

The effects of changing the duty cycle are the most difficult to interpret. The duty cycles used were continuous wave, 1:2 (2 ms on
and 4 ms off), 1:4 and 1:7, and for small pulse heights the continuous wave ultrasound produces the most light. For large pulse heights the 1:7 duty cycle produces the greatest number of counts per second from water, even though the ultrasound is on for the shortest time. In seeking an explanation for this anomalous behaviour it should be noted that when the ultrasound intensity is just above the threshold for transient cavitation, the bubbles most likely to collapse will be those of resonant size. As the intensity is increased, bubbles above and below the resonant size will also collapse, but the bubbles of resonant size will always collapse most violently giving the brightest sonoluminescent flashes. In addition, it will be shown in chapter 5 that sonoluminescence often appears as bands of light, and that the position of these bands corresponds to the pressure antinodes. Thus the explanation for the appearance of the spectra produced by different duty cycles in freshly drawn water must be one that a) is associated with collapse cavitation, b) requires bubbles of non-resonant size to behave as might be expected from simple theory of "on" to "off" ratios, and c) results in more light output from resonant sized bubbles when the ultrasound is pulsed.

This work differs in significant detail from previous work on the effect of pulsed ultrasound. For example, Hill et al (1969) looked at the effect of changing pulse length on DNA degradation, sonochemical changes (the release of free iodine from KI solution) and observations of the first harmonic of the driving frequency. Working at 1 MHz they found that pulsing had a large influence on the observed cavitation. Decreasing the pulse length increased the
effect, down to pulse lengths of about 30 ms, but by 1 ms the effects of pulsing were again negligible. The explanation offered was based on bubble growth by rectified diffusion. It was postulated that in the continuous wave field bubbles grew to above resonant size. With very short pulses they grew little, but a maximum effect was observed when the pulse interval was just sufficient for the bubbles to grow through resonant size.

The mechanism of Hill et al is not thought to apply to the work described in this chapter because:

1) The results of Hill et al were obtained with travelling waves while the spectra of figure 4.9 were obtained with standing wave fields.

2) At the pulse lengths used in this study, Hill et al reported a very small effect.

Later work by Ciaravino et al (1981) and Flynn and Church (1984), also at about 1 MHz, involved varying the pulse length, but fixing the intensity and duty cycle. Iodine release was observed and a maximum occurred when the pulse length was of the order of 10 ms. The appearance of a maximum in this work only occurred at intensities greater than 10 W cm$^{-2}$, so it was assumed by the authors to arise from transient cavitation. These authors suggested that the maximum in iodine release was due to the persistence of unstabilised nuclei generated in the previous pulse. In continuous wave operation a steady state distribution of nuclei that are unstabilised against diffusion (they are products of a collapsed cavity) is established during the first ten cycles after the sound field is switched on. In
pulsed operation the distribution of nuclei generated by a pulse is the same as that for continuous wave operation when the pulse length is either very long or very short: with a long pulse length, i.e. greater than $10^{-5}s$, an equilibrium state will be reached during the "on" period but the unstabilised nuclei disappear during the "off" period; with a short pulse length, the nuclei which have persisted from the previous pulse are almost unchanged in size. The maximum occurs for pulses of intermediate length when the nuclei grow during the "on" period and contract during the "off" period, thereby being repeatedly cycled through resonance. This mechanism may well be occurring in the present study, but it is difficult to understand why survival of unstabilised nuclei should favour resonant sized bubbles in preference to off-resonant bubbles during the next "ultrasound on" period.

Two possible mechanisms acting alone or together may explain the results (Leighton 1988). Firstly, consider again the mechanism of rectified diffusion (section 3.5). During the expansional part of the sound cycle, gas is drawn into the bubble from the surrounding liquid. However, in a system where there is a large standing wave component, Bjerknes forces draw smaller than resonant size bubbles to the pressure antinodes where they feel the full effect of the maximum pressure variations, whilst large bubbles are drawn to the nodes and are maintained there (see section 3.7).

The first mechanism by which pulsing the ultrasound can enhance sonoluminescence involves ultrasonic degassing (Blake 1949) and bubble migration. Here, liquid in the locality of a bubble undergoing
growth by rectified diffusion becomes partially degassed. For atmospheric gases in water, the diffusion length after a time \( t \) is \((4 \times 10^{-5} t)^{1/2}\) cm (Jost 1960). For example, if \( t = 2 \) ms, the diffusion length is \(2.8 \times 10^{-3}\) mm. If partial degassing occurs, therefore, bubble growth becomes slower, and a bubble requires more acoustic cycles to reach resonant size. Since in each bubble oscillation there is a chance of transient collapse, the slower growth would result in more bubbles collapsing before they reach resonant size. If the sound is pulsed, large bubbles from the pressure nodes would be ejected, these being the only bubbles that can migrate during the off-times. These large bubbles pass through the antinodal regions, mixing up the fluid there. Aerated water would be flushed into the region of the antinodal bubbles enabling them to grow more quickly to resonance. This would produce an increase in the number of the brightest sonoluminescent flashes (which are from resonant size bubbles) and a decrease in the number of flashes of lower intensity, as seen in figure 4.9a. This idea is supported by an observation of Blake, who obtained strong cavitation in a tank in which the water was continually stirred, but the intensity of cavitation showed a marked decrease a few seconds after the stirrer was switched off.

The second mechanism concerns the effect of bubbles on the nature of the medium. If a liquid contains large numbers of bubbles, it becomes a two-phase medium with an effective acoustic impedance that is different from that of the liquid when bubbles are not present (Neppiras 1980). The acoustic impedance depends on the concentration and distribution of bubbles. Thus if a liquid contains two regions of differing bubble concentrations, there will be a discontinuity in the
effective acoustic impedance, and there will be partial reflection at the "interface" due to this mismatch. Thus ultrasound will be attenuated to a lesser extent when it propagates through a liquid containing a uniform bubble distribution than if the liquid contains inhomogeneous bubble concentrations. At 1 MHz, pulsing enables the large bubbles to migrate significantly away from the pressure nodes. Thus at the start of the next pulse of sound the medium no longer contains concentrated large bubble-aggregates at each node which would change the effective acoustic impedance there and so hinder the propagation of ultrasound. The sound can reach the near-resonant sized bubbles at the antinodes without significant attenuation, and so causes the most intense cavitation there. Thus, as seen in figure 4.9a, pulsing increases the number of the brightest sonoluminescent flashes, whilst continuous wave enhances the lower energy light emissions.

Figure 4.9a is consistent with these two mechanisms. It can be seen that for off-times up to 14 ms, the longer the off-time the greater the enhancement effect. Calculations by Leighton (1988) show that large bubbles continue to migrate for 25 ms, while resonant-sized bubbles migrate for a few microseconds only, and so do not contribute to this effect.

Two aspects of these postulated mechanisms which are amenable to testing are the gassy nature of the water, and the viscosity of the insonated medium. When the experiments were repeated in degassed water (figure 4.9b), the results were qualitatively similar to those in tap water, although as in other experiments in degassed water, the
light output level was lower. The crossing over effect may be less marked, but gassiness would not appear to provide a complete explanation for figure 4.9a. When the experiments were repeated in agar, no crossing over of the four curves was seen (figure 4.9c). The continuous wave ultrasound always produced more light, and this result is consistent with the mechanisms proposed above, since the mobility of bubbles when the ultrasound field was switched off would be greatly reduced, and facilitated rectified diffusion could no longer occur in the pulsed modes.

The work of Hill et al (1969), and Ciaravino et al (1981) has already indicated that the common physiotherapeutic practice of using pulsed ultrasound, may be potentially more harmful than using continuous wave ultrasound. This work supports this view to some extent, but at the same time questions some of the conclusions that might be drawn from it. The anomalous behaviour of pulsed ultrasound might not occur in body tissues, which would be analogous to quite stiff agar, although the behaviour of blood, for example, which is of intermediate viscosity, cannot be easily predicted.
Chapter 5. Standing Wave Patterns Produced by a Therapeutic Unit and Their Correlation with Sonoluminescence.

Physiotherapeutic ultrasound directed into the human body may undergo relatively strong partial reflection by discontinuities such as bone and air sacs, causing standing waves to be set up. The work in this chapter investigates the standing wave patterns that are set up when continuous wave ultrasound is reflected off various surfaces. Then by using three reflectors, chosen from the sample as being a good reflector, a good absorber and an intermediate reflector, the sonoluminescence produced from some standing wave patterns is observed using high-gain image intensification.

Many workers have presented data on sonoluminescence which purports to be from a travelling wave system or a standing wave system, without checking to see whether they really do have such a system. For example Graham et al (1980) reported the presence of considerable sonoluminescence from travelling waves, but they did not quantitate the nature of the sound field, and it is therefore possible that there was a standing wave component present. In sections 5.1 and 5.2 it will be shown that the sound field set up by a Therasonic transducer, in laboratory simulations, is complex and can contain a range of standing wave components, presumably as a result of reflections from the liquid surface, container walls and the transducer's front plate. Therefore the sound fields in the region where sonoluminescence is being recorded in section 5.4 are measured using a needle hydrophone. The aim of this part of the chapter is to correlate the spatial distribution of sonoluminescence with the
standing wave component of the sound field.

5.1 Standing Wave Patterns in a Large Water Tank.

The transducer of the Therasonic 1030, the needle hydrophone that was used in chapter 4, and a reflecting plate were arranged in a water bath as in figure 5.1. It can be seen that the hydrophone is orientated at 90° to the axis of the transducer, an orientation at which the hydrophone sensitivity is high (Filmore and Chivers 1986). The attenuation coefficient for water is 0.0022 dB cm\(^{-1}\) at 1 MHz (Wells 1969), so the beam is only attenuated by about 2% over 30 cm, and a good reflector should give rise to a high standing wave component as given by equation 3.21.

The hydrophone can also be used to give an accurate reading of the ultrasound frequency, with the aid of an oscilloscope, and this was found to be 1.09±0.03 MHz. This means that the wavelength of the ultrasound in water is 1.36 mm, and there will be a pressure antinode every 0.68 mm in a plane wave field. In these experiments, the peak-to-peak hydrophone voltage was measured at a pressure maximum every 2 or 3 mm, and the adjacent peak-to-peak voltage minima were also measured. The tank measured 440 x 210 mm and was 160 mm in depth, and the transducer reflector distance was 17 cm for most of the experiments and in some cases 5 cm. The spatial average temporal peak intensity of the ultrasound was 0.5 W cm\(^{-2}\) as measured by the force balance described earlier.

The reflectors investigated were an aluminium plate of 1 mm
Figure 5.1. Schematic diagram of the apparatus used to measure standing wave patterns in the large tank.
thickness, an aluminium plate of 3 mm thickness, a 5 cm thick polystyrene block, a corrugated foam sponge bilayer, a corrugated rubber mat, a 2 mm thick glass plate, a 6 mm thick glass plate, a 10 mm thick glass plate, a 6 mm brass plate, and a wire wool scouring pad. The variation of maximum and minimum hydrophone voltages measured allows several values of standing wave component to be calculated for each material, and for all the reflectors a wide range of values was obtained. In saying that one material produces a higher standing wave component, therefore, it is important to be clear that it is only meaningful to talk of the average standing wave component for these experiments. The materials with the highest average standing wave component were the brass plate, the thicker of the two aluminium blocks and the polystyrene, whilst the rubber and the sponge had the lowest. Even so, at one position in the field produced by the sponge, the standing wave component is 50%, whereas at one position in the field produced by the thick aluminium block, the standing wave component is 36%. For some reflectors the variation in standing wave component was as much as 40%, although for the good absorbers it was considerably less than this. These standing wave components are calculated from equation 3.21.

For the case of the 2 mm glass plate, with a transducer reflector separation of 5 mm, the average standing wave proportion was 45-62%. This is the situation described in section 4.1. By placing the 6 mm brass in front of the glass plate and touching it, the average standing wave proportion is increased to nearly 70%. This value relates to the insonation of cells described in chapter 7.
5.2 Standing Wave Patterns in a Small Cell.

Higher standing wave components can be obtained if a much smaller container is used, and for two of the three experiments performed in section 5.4, a sample cell measuring 10.1 cm by 2.6 cm, and 4.2 cm deep, was employed. When using this system, the transducer insonates from outside the sample cell, and the end of the cell nearest the transducer is an acetate sheet window, which is, to a good approximation, acoustically transparent. Various materials were again placed at the far end of the cell, and the hydrophone was used to measure the standing wave components on the axis of the transducer. The position of the hydrophone relative to the transducer was determined, to within 0.1 mm, using an adapted micropositioner. The reflectors investigated were a 27.5 mm thick brass block, a car mat, a foam sponge bilayer, a wire wool scouring pad, a 1 cm thick steel plate, a 2 mm thick glass plate and a 0.75 mm brass plate.

In the experiments described in this section and section 5.1, the highest average standing wave component was obtained from the 27.5 mm brass block (79%) in the small cell (although again the range of standing wave components obtained ranged from 68% to 92%). The lowest was obtained from the wire wool in the large tank, the actual value being very hard to measure, but it was estimated that the average standing wave component was not more than 2%. (The next best absorber, the corrugated rubber mat, gave a value of 6%). Since the 0.75 mm brass plate gave a value of 40% in the small cell, this was selected as the intermediate reflector for the experiments described in section 5.4. For these three reflectors, the above experiments
were repeated at 37°C. This had no noticeable effect on the standing wave component produced by the wire wool absorber, but the average standing wave component from the brass block fell to 58%, while that for the brass plate rose to 61%.

5.3 The Image Intensifier.

In order to investigate the spatial distribution of sonoluminescence within the small cell, an image intensifier was used. The principal item is a high-gain image tube (EMI type 9912), which is a magnetically focussed four-stage tube, of phosphor-photocathode sandwich construction, with a bialkali input and a P11 phosphor output. Its maximum gain is such that approximately 65% of the photoelectrons leaving the input photocathode are recorded as discrete points on the film in the camera viewing the output phosphor. This camera may be a Nikon F2 still camera with f1.4 lens, or a television camera (Hamamatsu C1000 type 12). A rotating mirror allows the specimen to be viewed through a ground glass screen or brought to focus on the input photocathode.

5.4 Correlation of Sonoluminescence with Standing Wave Patterns.

To investigate the spatial distribution of sonoluminescence, the transducer was immersed in a large water bath as shown in figure 5.2. The sample cell was included for the measurements made with the good reflector (the brass block), and the intermediate reflector (the thin brass plate described in section 5.2. Note that this is not the brass plate mentioned in section 5.1). For experiments on the absorber (the
Figure 5.2. Schematic diagram of the apparatus used to observe spatial patterns of sonoluminescence.
layers of wire wool), the sample cell was removed. The whole cell was painted black to prevent sonoluminescence originating inside the large water bath being scattered up into the optical system. The water temperature in the large water bath was thermostatically controlled to within 1°C of either 22°C or 37°C. After the hydrophone had been used to make the measurements described in section 5.2, it was removed from the cell while sonoluminescence was observed.

Sonoluminescence was recorded by photographing the output phosphor of the image intensifier with the Nikon camera. The film (Kodak Tri-X) was developed in a standardised manner, and the densities across each negative measured, along a line corresponding to the axis of the transducer, using a microdensitometer (Joyce, Loebl and Co. Ltd. Mk IIIB), using the known H-D curve for the film-developer combination. Thus the relative light intensities could be measured and compared. The light intensity noise level was found to be 0.08 arbitrary units, this figure being relevant to the figures for light intensity given subsequently.

The majority of the experiments described in this section were done at an intensity of 3.3 W cm⁻², although in the final experiment a spontaneous malfunction in the Therasonic 1030 produced an output of 10 W cm⁻². Again these measurements were made using the force balance.

The transducer was placed at approximately 5 mm from the sample cell window, so the measurements were done in the near field, as in clinical practice. The transducer insonated the region of interest
from the right as seen in figures 5.3 to 5.6. Average values of the standing wave component across the region of the sound field where bands are seen in figures 5.3 and 5.6 are quoted.

5.4.1 Thick Brass Block.

When the 27.5 mm brass block was placed at the end of the cell (figure 5.2), and the water bath was maintained at 22°C, clear bands of light were seen (figure 5.3b) with maxima of intensity of up to about 1000 units (figure 5.3c). The bands of light appeared approximately 10 seconds after switching on the ultrasound. The hydrophone trace (giving an average standing wave component of 79%) is shown in figure 5.3a.

The acoustic field became 58% standing wave at 37°C (figure 5.4a), and sonoluminescence was uniform in the field of view with an intensity of around 200 units (figures 5.4b and 5.4c).

5.4.2 Thin Brass Plate.

When the 0.75 mm brass plate was placed at the end of the cell (40% standing wave, figure 5.5a), sonoluminescence was again uniform (figure 5.5b) and the average intensity of sonoluminescence was around 5 units.

At 37°C (61% standing wave, figure 5.6a), clear bands of light were again seen (figure 5.6b) peaking at intensities of 100 units (figure 5.6c). As in the case of the thick block at 22°C, bands of light were
Figure 5.3. Results obtained with the 27.5 mm brass block reflector at 22°C, showing: (a) peak-to-peak acoustic pressure as measured with the needle hydrophone; (b) appearance of the corresponding sonoluminescence as seen through the intensifier; (c) light intensity (in relative units). (a), (b) and (c) have a common abscissa.
Figure 5.4. Results obtained for the 27.5 mm brass block at 37°C. a), b) and c) are as in figure 5.3.
Figure 5.5. Results obtained for the 0.75 mm brass plate at 22°C. a), b) and c) are as in figure 5.3.
Figure 5.6. Results obtained for the 0.75 mm brass plate at 37°C. a), b) and c) are as in figure 5.3. In (a) the mean pressure amplitude is about 1 MPa. This is consistent with the output of 10 W cm$^{-2}$ from the generator noted in section 5.4. The occurrence of bands is not solely due to these augmented pressures, because bands were also seen from this geometry with mean acoustic pressures of about 0.4 MPa.
positioned at regions of the sound field where the standing wave component was greatest.

5.4.3 Wire Wool Absorber.

In this experiment the sample cell was removed and the experimental arrangement was that described in section 5.1. No bands of light were seen either at 22°C or 37°C. Little or no sonoluminescence was present apart from an initial burst, which lasted for less than one second. It was not possible to measure the intensity of this flash, since photographs of the output phosphor require exposures of typically twenty seconds.

5.4.4 Rocking the Transducer.

Whichever of the three reflectors was used, an oscillatory rotation, or rocking of the transducer, so that it deviated by up to 20° from its normal axis, always helped to initiate sonoluminescence. For the two brass reflectors, sonoluminescence sometimes did not appear until a minute or so after first switching on the ultrasound, and rocking would accelerate its appearance. The effects of rocking the transducer were not due to the removal of bubbles from the face of the transducer, as the transducer was regularly cleaned of bubbles during the course of the experiments. The transducer was rocked in order to try and simulate the motion of a transducer over a patient's body in physiotherapy. Where light appeared in bands, cessation of rocking did not affect the banding pattern, although in the absence of bands, sonoluminescence would fade when rocking was stopped. As
long as the transducer was rocked if necessary, the presence or absence of bands of light in any given sound field showed 100\% reproducibility.

5.5 Discussion.

In figure 5.6 the light intensity maxima correspond exactly with the hydrophone maxima. In figure 5.3 this is again the case for most of the maxima, although in some instances (between 17 and 20 mm in particular) the peaks of figures 5.3a and 5.3c do not line up. However there will occasionally be errors in measurement of the hydrophone position, and a further check can be made on the lining up of these peaks. Figures 5.3a, 5.4a, 5.5a and 5.6a are all sections of traces which cover a greater distance on the axis of the transducer. (The sections were selected because they corresponded to the field of view of the image intensifier.) The full hydrophone trace for the brass block at 22°C is shown in figure 5.7. All five hydrophone traces show regions where the spacing between antinodes is not uniform. Clearly this spacing must be uniform in reality, so figure 5.7 can be used to provide a "best fit" for the true antinode positions, with the constraint that the wavelength is unknown but constant. (Note that this exercise could be based on a calculated value of the wavelength, but the speed of sound is not really known with sufficient precision, due to the presence of bubbles in the water (see section 3.6), and any error in the wavelength would be cumulative across the field of view of the image intensifier.) In figure 5.8 the best fit points are shown as crosses superimposed on figure 5.3c. It can clearly be seen that there is excellent agreement
Figure 5.7. Complete hydrophone trace for the 27.5 mm brass block reflector at 22°C. Figure 5.3a is a section of this figure corresponding to the field of view of the image intensifier.
Figure 5.8. Copy of figure 5.3c with crosses indicating the antinodal positions as found from the "best fit" treatment described in section 5.5.
between the calculated antinode positions and the light intensity maxima. Figure 5.7 is useful for demonstrating the large variation in standing wave ratio in an ultrasound field, and can also be used to calculate a value for the speed of sound in the water bath. Taking an average value of the wavelength from this figure, 1.36 mm, and the manufacturer's figure for the frequency, 1.09 MHz, gives a value of 1480 m s$^{-1}$.

Figures 5.3, 5.6 and 5.8 show conclusively that bands of sonoluminescence appear at the pressure antinodes in a standing wave field. The standing wave field is complex but the intensity of light in the bands correlated well with the magnitude of the standing wave ratio at different points in the field. At 22°C the acoustic reflectivity of the brass block is higher than that of the brass plate and since the appearance of sonoluminescence is associated with the standing wave pattern, it would be expected that bands of light would appear when the average standing wave proportion was highest. Bands are indeed seen when the brass block is used at 22°C (79% standing wave) or when the brass plate is used at 37°C (61%), but not with the block at 37°C, nor with the plate at 22°C.

In section 4.6 it was shown that the sonoluminescence produced by a Therasonic unit increased with temperature in the range 22-45°C. The most probable explanation for the absence of bands when the brass block was used at 37°C is that cavitation had been confined to a region very close to the transducer, effectively creating a barrier against the transmission of the ultrasound into the bulk of the medium. That such an effect occurs is well established and is

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Strong sonoluminescence has been shown to be associated with standing waves and to occur in bands. Furthermore, experiments in which there was little or no standing wave component failed to produce any persistent sonoluminescence, and if there is any sonoluminescence associated with travelling waves it is far smaller than that associated with standing waves.

Other observations from the experiments of section 5.4 were that there was an immediate, brief flash of light on switching on the ultrasound for all reflecting surfaces, and that rocking the transducer caused similar flashes of light. When the sound is switched on, or when the beam scans across the bubble field, bubbles start to oscillate. The initial motion of the bubble wall contains transients which decay to leave the steady state oscillation. Leighton (1988) has produced solutions to equation 3.9, which take account of these transients. The transients enhance some of the compressions, with the result that the temperature of some bubbles reaches more than 2000 K.

Sacks et al (1982) noted that if a test-tube filled with biological material was insonated, bioeffects were greatly increased by slowly rotating the tube. Church et al (1982) postulated that, in stasis, acoustically active gas bubbles and the biological material would be well separated. Acoustically active gas bubbles are situated at the pressure antinodes (see section 3.7) whilst single cells are larger than resonant size and therefore collect at the pressure nodes.
Church et al then argued that motion of the transducer disturbs the acoustic field, resulting in cells coming into close proximity with violently oscillating bubbles, leading to increased damage. The observations of section 5.4 suggest that this mixing process may not be the whole explanation, and that rocking the transducer increases the bubble activity itself.

The acoustic reflectivities encountered biologically range from a few per cent (e.g. 3% between muscle and kidney) to values comparable with those in the experiments described in this chapter. Higher acoustic reflectivities are found for interfaces involving the lung (of order 70%) or bone (60%) (Wells 1977). Reflectivities of nearly 100% will be present at the tissue-air interface diametrically opposite the transducer, although a 1 MHz beam will be attenuated by about 60% (pressure amplitude) after passing through 10 cm of tissue.

In conclusion, sonoluminescent intensity is related to the standing wave ratio, and when the standing wave component is high, strong banding effects at the pressure antinodes are frequently observed. Sonoluminescence can be produced in aqueous media at the power levels, and with the transducer movements, employed in physiotherapy, and with the acoustic reflectivities occasionally encountered in practice.
Chapter 6. Cavitation from Pulses of a few Microseconds Duration.

The two preceding chapters have shown that sonoluminescence is readily seen when water is insonated with therapeutic ultrasound, and that this sonoluminescence is primarily associated with standing waves. With the duty cycles encountered in physiotherapeutic ultrasound, the shortest pulses are typically 2 ms, and a standing wave pattern will be set up whenever a reflection occurs. The question remains, however, as to whether cavitation can occur under diagnostic ultrasound conditions. A standing wave system may not be set up, but other cavitation mechanisms may operate e.g. non-linear distortion causing shock excitation (Bacon 1984, Duck and Starritt 1984). Crum and Fowlkes (1986) have observed luminescence flashes characteristic of violent cavitation from ultrasonic pulses as short as one cycle at a frequency of 1 MHz, implying that there is a mechanism for cavitation when standing waves are not present. However they used duty cycles of between 1:3 and 1:20, i.e. pulse repetition frequencies of between 250 and 50 kHz, whereas in the diagnostic situation, duty cycles are more typically 1:1000 (Duck et al 1985). It is shown by the present author in chapter 4 that pulsed enhancement of cavitation can occur if the length of the off-time is carefully chosen.

The work in this chapter describes an alternative approach to that of Crum and Fowlkes. The frequency of the ultrasound from a generator built at the University of Surrey was set at 1 MHz to provide comparisons with chapters 4 and 5, and the pulse repetition frequency was fixed at 1 kHz, which is a typical value for a diagnostic
machine. The ultrasound intensity (I), and the number of cycles in each pulse (N) were then varied and for each value of N, the threshold value of I for sonoluminescence was found. Sets of threshold values for two transducers have been obtained and possible implications for the diagnostic use of ultrasound are discussed.

6.1 The Signal Generator.

The ultrasound generator system comprised three separate modules; a signal generator and control module, a power supply providing stabilised H.T. and E.H.T. voltages, and an ultrasonic power output unit with tuning and amplitude controls.

The high frequency pulse generator provides a continuous train of pulses which feeds the power output stage via a 50 Ω line driver. An additional feed controls a count down timer stage which in turn provides a gating signal to the mode control switch. Pulsed or continuous wave modes are provided. The output of the mode control switch feeds the control input of the power output stage.

In pulsed mode, the counter stage is initiated by a trigger pulse from a rate oscillator. When the counters reach zero another pulse is generated, thus the initiating trigger pulse and the "count complete" pulse define the exact number of pulses within the pulse envelope. The two pulses toggle a bistable which controls the output power amplifier.

The output stage was complicated by the techniques used to generate
the variable frequency drive and pulse counting and gating circuitry for the pulsed mode. The grid of the power output valve is driven by rectangular pulses which, due to the band pass characteristics of the tuned circuit loading the anode, produce a sine wave output signal. The quality of the sine waves produced will depend on the "Q" of the tuned circuit loading the output. A high "Q" tuned circuit gives good waveforms but produces a slow build-up and decay envelope in the pulsed mode.

To enhance the build-up of the pulse, the output valve is overdriven by modulating the cathode with a decaying pulse at the start of each pulse. The decay of the pulse is damped by applying a short circuit to the tuned circuit by way of a hydrogen thyratron which, when fired, absorbs the energy in tuned circuit.

The mode control stage gates the ultrasonic oscillator to produce the required high frequency pulse burst. This is amplified and buffered by a unity gain push-pull stage before feeding the grid of the output valve. Amplitude control is achieved by varying the H.T. voltage of the amplifying stage. The signal across the transducer is capacitatively attenuated and rectified to provide a peak RF voltage detector, the voltage being shown on a meter.

Electrical tuning was achieved using plug-in ferrite cored fixed inductors and an additional variable capacitor in parallel with the transducer for fine tuning control. The design enables the generator to be robust enough to survive being driven at maximum output into a mismatched load.
Thus the properties of the signal generator are such that it can produce:

i) a variable intensity output, from zero to the maximum limit for the transducer (typically 0.1-0.5 W cm\(^{-2}\), spatial and temporal average),

ii) a sound wave of frequency approximately 1 MHz tunable over a narrow range,

iii) continuous wave or pulsed ultrasound,

iv) in pulsed mode, any number of complete cycles in each excitation pulse between 1 and 99,

v) a pulse repetition rate of between 100 Hz and 1 kHz.

6.2 The Transducers.

The signal generator was used to drive two different transducers. One was the Therasonic 1030 transducer of section 4.1. To drive this transducer, the frequency of the generator was set to 1.09 MHz, the exact resonant frequency of this transducer. The second transducer was a diagnostic transducer NE4161 (Nuclear Enterprises), with an effective radiating area of 299 mm\(^2\) (quoted by the manufacturers) and designed to operate at 1.5 MHz. Since this frequency could not be obtained with the generator, the needle hydrophone and oscilloscope were used to find the maximum value of the spatial peak temporal peak intensity within the frequency range of the generator. This was obtained with a driving frequency of 0.97 MHz.

The needle hydrophone was also used to determine the exact temporal shapes of the pulses produced by the two transducers. The appearance
of the pulse as measured by the hydrophone was recorded on the oscilloscope so that build-up and decay times could be recorded.

6.3 Calibration of Output.

Two methods were used to calibrate the ultrasound output. Firstly, for each transducer, the position of the spatial peak was found, using the needle hydrophone, to be 10 cm from the transducer. The value of the peak negative pressure was then found at this point as the peak RF power output was varied. Hydrophone calibration measurements were carried out with the hydrophone orientated at 90° to the axis of the transducer, as is the arrangement in figure 5.1. The spatial average temporal average intensity was also found from radiation pressure measurements using a calibrated force balance described by Anson et al (1988). For these measurements a transducer-target distance of 10 cm was selected as this corresponded to the position of the focus of the diagnostic transducer. The measurements were carried out at pulse lengths of 10, 20, 40, 60, 80 and 99 cycles per pulse.

The hydrophone was cross-calibrated against a membrane hydrophone that had been calibrated at the National Physical Laboratory. The calibration factor of the needle hydrophone was found to be $2.25 \pm 0.05$ kPa/mV at 1 MHz (the frequency response is flat for frequencies up to 2 MHz; see Smith 1986). Results of the calibration of the RF voltage scale on the generator, in terms of peak negative pressures, for the two transducers are shown in figures 6.1 and 6.2. A word of caution is needed in the interpretation of these results;
Figure 6.1. Peak negative hydrophone pressure against scale reading of RF voltage output for the therapeutic transducer.
Figure 6.2. Peak negative hydrophone pressure against scale reading of RF voltage output for the diagnostic transducer.
Figure 6.3. Spatial average temporal average intensity against scale reading of RF output for the therapeutic transducer for several pulse lengths:

- (× × ×) 99 cycles per pulse,
- (+++ ) 80 cycles per pulse,
- (○ ○ ○) 60 cycles per pulse,
- (△ △ △) 40 cycles per pulse,
- (□ □ □) 20 cycles per pulse,
- (···) 10 cycles per pulse.
Figure 6.4. Spatial average temporal average intensity against scale reading of RF output for the diagnostic transducer for several pulse lengths. Symbols refer to the same pulse lengths as in figure 6.3.
the above mentioned paper by Smith (1986) came to light only when this work was well advanced, and it severely criticises Dapco hydrophones because their sensitivity falls off rapidly with frequency above 2 MHz. For physiotherapeutic ultrasound measurements this does not present a problem, but for diagnostic ultrasound there are harmonic frequencies present. The errors caused in measurement of the intensities of diagnostic pulses would be considerable, but are not believed to be very great for the work in this chapter for three reasons. Firstly the slow build-up of the pulse (see section 6.4) will mean that the proportion of harmonic frequencies produced at the transducer will be low. Secondly, as the measurements of sonoluminescence are made well within the near field the distortion of the waveform, which is caused by the transfer of the wave energy to the harmonic frequencies, will not be significant (see Bacon 1984). Thirdly, Fourier analysis of a wave of ten cycles duration (see results section) indicates that the spread in frequency of such a pulse is only 0.1 MHz. The spatial average temporal average intensities measured using the force balance are shown in figures 6.3 and 6.4.

Since the pulse repetition frequency of the generator is known, a value for the spatial average pulse average intensity can be found from the relation

$$I_{sapa} = I_{sata} \times 1000 / N$$  \hspace{1cm} (6.1)

$I_{sapa}$ can be equated to $I_{satp}$, although this and equation 6.1 are only approximations due to build-up and decay times. The approximations are more satisfactory at 99 cycles per pulse than at 10 cycles per pulse. $I_{satp}$ is the intensity figure given on the
Table 6.1.

Average values of $I_{sapa}$ for the diagnostic transducer over the range of pulse lengths. The pulse lengths used to obtain these averages were $N=10, 20, 40, 60, 80$ and $99$.

<table>
<thead>
<tr>
<th>Pk RF o/p (V)</th>
<th>$I_{sapa}$ ± S.D. (W cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>70</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>80</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>90</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>100</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>110</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>120</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>130</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>140</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td>150</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
<td>160</td>
<td>1.06 ± 0.05</td>
</tr>
<tr>
<td>170</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td>180</td>
<td>1.32 ± 0.08</td>
</tr>
<tr>
<td>190</td>
<td>1.54 ± 0.09</td>
</tr>
<tr>
<td>200</td>
<td>1.69 ± 0.11</td>
</tr>
<tr>
<td>210</td>
<td>1.84 ± 0.14</td>
</tr>
<tr>
<td>220</td>
<td>2.04 ± 0.12</td>
</tr>
</tbody>
</table>
Figure 6.5. Temporal pulse shapes of (a) the therapeutic transducer, (b) the diagnostic transducer, (c) the diagnostic generator and transducer used by Crum and Fowlkes (1986), at their respective spatial peaks.
Therasonic 1030 and most other therapeutic units. Values of $I_{sape}$ may be calculated from figures 6.3 and 6.4 and equation 6.1. Table 6.1 shows results for the diagnostic transducer and similar results were obtained for the therapeutic transducer. The errors quoted include the systematic error introduced by pulse build-up and decay and this can be seen to be small. The error due to pulse shape was somewhat greater for the therapeutic transducer.

6.4 Pulse Shapes.

The temporal shapes of the pulses produced by the two transducers are shown in figure 6.5. Both pulses reached 95% of their maximum amplitude within a few microseconds (8-9 $\mu$s for the therapeutic transducer, 6-7 $\mu$s for the diagnostic transducer). From this point until the pulse from the generator is terminated and the hydrogen thyatron is fired, there is only around 5% variation in the maximum and minimum pressure values. So far the two pulse types are very similar. However with the therapeutic transducer the decay time significantly lengthens the effective pulse duration. The pressure has only decreased to about 80% of its maximum value 10 $\mu$s after the end of the generator's pulse, and only after about 17 $\mu$s has there been a significant reduction. Thus any cavitation effect observed at short pulses will be exaggerated with this transducer. In contrast, with the diagnostic transducer the pulse stops almost instantaneously (figure 6.5). The slow build-up now causes greater inaccuracies than the decay period, and any cavitation effect will be underestimated.
6.5 Experimental Set-up.

The experimental arrangement is that shown in figure 4.1 and described in section 4.2 (the generator is, of course, the one described in section 6.1 rather than the one used in chapter 4). For the present experiments, a limited range of pulse heights from the middle of the pulse height spectrum was chosen (between 1.2 and 1.8); large enough to be above the region of electronic noise and pulse pile-up but not so high that the background count has become insignificant. Light pulses were counted for thirty seconds. The background count for the middle of the selected range of pulse heights was found to be 950±200. Sonoluminescence was assumed to be present either when the count obtained was greater than 1550 (i.e. average background count plus three standard deviations), or when the count was consistently between one and three standard deviations above background at three or more pulse heights.

The presence or absence of sonoluminescence was investigated for both transducers using a fixed frequency (1.09 MHz for the therapeutic transducer, 0.97 MHz for the diagnostic transducer), and fixed pulse repetition frequency (1 kHz). The number of cycles in each pulse varied between 1 and 99, and the peak RF output varied between 0 and 300 V. Observations were made as the pulse length was both increased and decreased to its final value as the intensity was kept constant, and as the intensity was increased and decreased to its final value as the pulse length was kept constant. For each pulse duration peak output combination, several tests of whether or not sonoluminescence was present were made.
Figure 6.6. Peak RF output against the number of cycles per pulse for the therapeutic transducer, showing three distinct regions. The shaded area represents settings where sonoluminescence sometimes occurs.
Figure 6.7. Peak RF output against the number of cycles per pulse for the diagnostic transducer, the shaded area again representing the region of uncertainty.
6.6 Observations of Sonoluminescence.

The results obtained using the therapeutic transducer are summarised in figure 6.6. On a graph of RF power output from the generator against the number of cycles in each pulse, three regions can be seen. In the upper region, sonoluminescence could always be detected. In the lower region, sonoluminescence did not occur, or the photon flux was below the level necessary for detection (section 4.5). There is also a broad middle region where sonoluminescence occurred sometimes, while at other times it did not. The principal feature of the graph is that as the number of cycles per pulse, $N$, is decreased, the ultrasound intensity, $I$, required to generate cavitation is greater. With the powers that could be generated with the present system, the smallest value of $N$ for which sonoluminescence could be observed was $N=7$. In particular, no sonoluminescence could be observed for $N=1$. Since it was not possible to obtain a 1 MHz diagnostic transducer, a 1.5 MHz transducer was used. The fact that this transducer was not being driven at its resonant frequency meant that it was not possible to get as much power out of it. The results for the diagnostic transducer are shown in figure 6.7. This figure has the same form as figure 6.6, but there are some differences. The minimum value of $N$ at which sonoluminescence can be observed is now 20, and the band of uncertainty is narrower.

In both figures 6.6 and 6.7 the appearance or non-appearance of sonoluminescence in the middle region seems to be in part random, although it can be influenced by whether the intensity is being turned up or down to reach the values specified. As shown in
Figure 6.8. Expanded section of figure 6.7 showing how the appearance of sonoluminescence depends on whether $I$ or $N$ is being turned up or down;

- $(\triangle)$ sonoluminescence present, $I$ or $N$ being increased,
- $(\triangledown)$ sonoluminescence absent, $I$ or $N$ being increased,
- $(\triangledown)$ sonoluminescence present, $I$ or $N$ being decreased,
- $(\downarrow)$ sonoluminescence absent, $I$ or $N$ being decreased.
figure 6.8, if the intensity is being increased to its final value, or if the number of cycles per pulse is being increased to its final value, sonoluminescence is more likely to be absent, whereas if the power is first turned up to its maximum value before being turned down to its final value, or if the number of cycles per pulse is decreased from 99 to its final value, the sonoluminescence, once initiated, is sustained at the lower values. It should be noted that figures 6.6 and 6.7 show the boundaries between the three regions and not experimental points.

6.7 Short Pulses and Standing Waves.

If the image intensifier is set up as shown in 5.2, the generator being that described in section 6.1 and the transducer being the therapeutic one, an attempt can be made to look for standing waves with this generator. The longest pulses that can be generated would be \((1.36 \times 99)\) mm or 135 mm, so if the separation of the transducer and the reflecting brass block is 50 mm (the same as the distance of the transducer above the bottom of the water tank in the arrangement of figure 4.1) a standing wave could, in principle, be set up for a short time and bands of light may be visible. At 99 cycles per pulse clear bands of light can be seen, although they fade rapidly as \(N\) decreases and disappear at around \(N=90\). Some standing wave bubble activity (localised, but not banded, sonoluminescence) is apparent down to about \(N=60\). If standing waves are a requirement for sonoluminescence, then as \(N\) decreases, it might be expected that the banding pattern is visible for longer near the reflecting surface, since, in this region, there may still be overlapping of the incident
and reflected waves. However this is not what is observed, and the banding pattern disappears at around N=90 at all points on the axis of the main beam. The implication is that there is some sonoluminescence associated with travelling waves.

6.8 Discussion.

The work in this chapter shows that it is possible to record sonoluminescence from water on insonation with pulses of a few microseconds duration when relatively long time intervals between pulses are used. In general, the results with the pulses from the diagnostic and therapeutic transducers are similar. The threshold for sonoluminescence detection remains virtually unchanged between 60 and 99 cycles per pulse. For the therapeutic transducer the threshold power output is 70-130 V (figure 6.6), corresponding to an intensity (spatial average temporal average) at 99 cycles per pulse of between 0.03 and 0.07 W cm$^{-2}$ (figure 6.3), while for the diagnostic transducer the threshold power output is 135-155 V (figure 6.7), corresponding to an intensity of 0.06-0.08 W cm$^{-2}$ at 99 cycles per pulse (figure 6.4). The corresponding peak negative pressures are 330-660 kPa for the therapeutic transducer (figure 6.1) and 270-330 kPa for the diagnostic transducer (figure 6.2). If the threshold is expressed as a pulse average (0.76 W cm$^{-2}$, table 6.1) it is of the same order of magnitude as that found for the Therasonic 1030 (which utilises long pulses of 2000 cycles or continuous wave ultrasound, see section 4.4).

In the regions of uncertainty in figures 6.6 and 6.7, the appearance
of sonoluminescence depends partly on whether N and/or I is being decreased or increased in order to reach their final values. If N or I is decreased towards its final value, sonoluminescence is more likely to be present than if N or I is increased to a final value. This indicates that sonoluminescence is more easily sustained than initiated.

When the number of cycles in the pulse becomes very small the peak RF output required for sonoluminescence rises sharply. For the diagnostic transducer this rise is at N=20. For the therapy transducer the sharp rise appears to be for much shorter pulses (N=10). However because of the long decay time for the therapy transducer (figure 6.5) the effective pulse duration is about 10-15 cycles greater than the recorded value of N. Thus the steep rise in peak RF output occurs when the pulse contains less than about 20 cycles for each transducer. Using the data in figures 6.3 and 6.4 it may be shown that the sharp rise in RF power required to induce sonoluminescence as the pulse length is reduced occurs at an ultrasound intensity (sata) of about 0.02-0.03 W cm$^{-2}$ for each transducer.

Crum and Fowlkes (1986) mention a ringing down effect in their pulses. Their acoustic pressure waveform was not a single cycle. However, they point out that their pulse closely resembles the scanning mode pulse of a diagnostic ultrasound system, and that it is similar to that used by Flynn in his theoretical studies of the cavitation effects of pulses of nominally one microsecond duration. A diagnostic scanner will produce a very short pulse but it is still
not a single cycle. All work in which pulses of 1 or 2 cycles are used is subject to practical limitations on pulse shape. It may then also be of importance whether the pulse is initially positive- or negative-going.

It was shown in chapter 5 that strong sonoluminescence depends on the presence of a high standing wave content. Observation of sonoluminescence at low values of N implies that any light must have come from the bottom of the water tank if standing waves are a prerequisite for sonoluminescence. For example, with a pulse of 20 cycles there can only be standing waves within 13.6 mm of the bottom of the tank (for a wavelength of 1.36 mm). The insonated tank was therefore examined with the image intensifier: as either N or I is decreased, bands of sonoluminescence gradually fade away, but they are never stronger near the reflecting surface. The implication is that there is some sonoluminescence which is, after all, associated with travelling waves.

Flynn (1982) has calculated that microsecond pulses of ultrasound can generate transient cavitation in water, but the peak intensities required are 10–30 W cm$^{-2}$. In this chapter, a practical limit to the lowest value of N at which sonoluminescence could be recorded was set by the power that could be achieved with the generator. Extrapolation of figures 6.6 and 6.7 to one or two cycles per pulse is difficult but the results of this chapter do not contradict this calculation. Some diagnostic systems now in clinical use generate microsecond length pulses of ultrasound with temporal peak intensities of greater than 100 W cm$^{-2}$ (Carstensen and Flynn 1982) and with peak negative
pressures of 2 MPa (Duck et al 1985) whereas figures 6.1 and 6.2 indicate maximum values of peak negative pressure of 1.4 MPa for the therapeutic transducer and 480 kPa for the diagnostic transducer. For Doppler equipment, pulse durations of 20 or 30 cycles per pulse are not uncommon (Duck et al 1987), pulse repetition frequencies are between 1 and 10 kHz, peak negative pressures up to 2 MPa are found and peak intensities may be as much as 300 W cm$^{-2}$. These figures indicate that cavitation could occur under conditions encountered with diagnostic and Doppler ultrasound in water.

It has been demonstrated that sonoluminescence can occur when the time interval between pulses is relatively long. At short pulse lengths, the ultrasonic intensity required to initiate sonoluminescence is high and the light output is low. The output characteristics of diagnostic imaging and Doppler equipment should be such that the peak intensity and the pulse duration are at their lowest practicable value, so that the chance of sonoluminescence occurring is minimised.
Chapter 7. The Effect of Therapeutic Ultrasound on EMT/Ca/VJAC Cells in a Standing Wave Field.

The last three chapters have investigated the effect of ultrasound on water, and in section 4.7, on agar gel. In this chapter the effect of therapeutic ultrasound on cultures of mouse mammary tumour EMT/Ca/VJAC cells (EMT6) will be investigated. Following the results of chapter 5, where strong sonoluminescence was seen to occur at the pressure antinodes in a standing wave field, an attempt is made here to culture a layer of EMT6 cells, place them at either the nodes or the antinodes in a standing wave field, and identify any damage or inhibition of cell growth.

The first part of this chapter describes how, using the image intensifier, a system was devised by which the cells could be irradiated under sterile conditions in a geometry where good standing waves were known to exist. This is followed by a discussion of how the cell layer was to be placed exactly in a nodal or an antinodal plane, and of the cell culture techniques used. Finally results of the insonation will be presented and discussed.

7.1 Setting up a Standing Wave Field for the Insonation of Cells.

In order to insonate cell layers, it was necessary to place them in an ultrasound field in a water tank. Clearly this would present problems with sterility if the cell layer was not isolated from the water in some way. The other problem that had to be overcome was that it was necessary to be able to identify banded sonoluminescence in
the region where the cells were to be situated.

The set-up shown in figure 7.1 was deemed to be the best way of simultaneously overcoming both difficulties. The cells were to be deposited on the base of a culture bottle (Falcon 3013E Becton-Dickinson (UK) Ltd., surface area 25 cm$^{-2}$, capacity 50 ml, sterilised by gamma irradiation), which was supported at its edges by a stand whose height could be precisely adjusted by means of three screws. The stand rested on a 6 mm thick brass plate in order to provide a good reflector to establish standing waves, and the Therasonic 1030 transducer insonated this arrangement from the top of a water tank. To test the arrangement a culture bottle was filled with water, and the image intensifier was used to view the arrangement from the side. Even at quite low intensities (1 W cm$^{-2}$), bands of sonoluminescence, similar to those in figures 5.3 and 5.6, could be seen at positions corresponding to the region between the brass reflector and the culture bottle, and to the region inside the bottle. Further confirmation of a standing wave pattern could be obtained by viewing the arrangement with the naked eye, and noting that as the water was insonated lines of bubbles began to form. This occurred above, below and inside the culture bottle. The visible bubbles were presumably large bubbles that had migrated to the pressure nodes as a result of Bjerknes forces (section 3.7).

By using this arrangement therefore, good standing waves are obtained, the cells can be cultured on the base of the bottle, and the bottle can be filled with culture medium, to minimise unwanted reflections, and tightly closed so that the cells do not become
Figure 7.1. Schematic diagram of apparatus.
infected as a result of contact with any non-sterile materials. Once this system was adopted, tests were carried out with culture medium in the bottle instead of water. A standing wave pattern could still be observed above and below the culture bottle with the image intensifier.

7.2 Positioning of the Cell Layer.

In order for the cells to be at a pressure antinode the culture bottle had to be positioned so that the top side of its base was at an exact number of half wavelengths from the brass reflector (the top side of the reflector is at a displacement node i.e. a pressure antinode). For the cells to be at a pressure node this spacing had to be an odd number of quarter-wavelengths. The cells were placed as close as possible to the reflecting surface (any error in calculating the wavelength of ultrasound in this system is magnified the further away the cells are from the reflector), this being limited by the width of the lip of the stand (1.65±0.01 mm), the thickness of the base of the culture bottle (1.00±0.01 mm) and the fact that the rim of a culture bottle has a lip of thickness 0.58±0.01 mm. Thus the cells could not be placed nearer to the reflector than 3.23 mm.

Before it was possible to calculate the actual positions at which the cells should be placed, it was necessary to know the speed of ultrasound in the base of the culture bottle in order to calculate the wavelength in this region. This was difficult to ascertain as the manufacturers will only specify that the bottles are made from "a special type of polystyrene". The inside of the bottle is coated with
a substance which aids cell attachment but this and the actual material comprising the bottle are trade secrets. However Miller (1986) lists values for the speed of ultrasound in various materials, including polystyrene and water. Using his values it can be shown that 1 mm of polystyrene is equivalent to 0.48 of a wavelength, while 1 mm of water is equivalent to 0.73 of a wavelength. Thus by introducing the polystyrene, a quarter of a wavelength was effectively removed from the gap between the cells and the reflector.

The change in the positions of the nodes and the antinodes was also investigated by means of the experiment shown in figure 5.1. Using this arrangement the 6 mm brass reflector was 5 cm from the transducer and the positions of the nodes and the antinodes were recorded by means of the hydrophone. This series of measurements was repeated with a culture bottle, with its top sawn off, introduced between the hydrophone and the reflector. The node-antinode positions were then shifted by 0.35 mm, i.e. the positions of the antinodes when the base of the culture bottle was not present became the positions of the nodes when it was introduced. These measurements were, of course, only accurate to 0.05 mm whereas the error on the measurements of the thickness of the base of the culture bottle, width of the lip of the stand and the thickness of the rim of the bottle was 0.01 mm. However the measurements do seem to support the calculations based on the figures of Miller.

Since the wavelength of the ultrasound in water at room temperature is 1.36 mm at 1.09 MHz (section 5.5), a cell-reflector spacing of 4.08 mm (1.00 mm being the base of the culture bottle and 3.08 mm
being in water) is equivalent to a spacing of 2.75 wavelengths (0.48 wavelengths in the base of the flask and 2.27 wavelengths in water) and the cells would be at a node, while a cell-reflector spacing of 3.74 mm (1.00 mm in the flask and 2.74 mm in the water) corresponds to 2.50 wavelengths (0.48 wavelengths in the flask and 2.02 wavelengths in water) and the cells would be at an antinode. The height of the stand could be precisely adjusted using screw driven feet, to within 0.01 mm. Thus in all the experiments where cells were insonated, they were either at 3.74 mm or 4.08 mm from the brass reflector.

7.3 Cell Culture Techniques.

One of the objects of cell culture is to keep a cell line growing continuously in a healthy environment, feeding the cells as they use up the components of their culture medium. Cultures need to be regularly subcultured or restarted with a low concentration of cells when the original culture becomes overcrowded, thereby producing a steady supply of identical cells for a long period of time. When they are not undergoing subculture, nor being used for experimentation, cells are kept in a humidified incubator gassed with CO$_2$ at 37°C. EMT6 cells were chosen for this experiment because they are tolerant of changes in their environment, because they are a well established, extensively characterised cell line, they grow reproducibly with a twelve hour cell doubling time, they reliably have a high plating efficiency and they are anaplastic. EMT6 cells originated in a mouse alveolar tumour nodule and were successively transplanted between animal and in-vitro culture by Rockwell et al (1972), and have been
grown in continuous culture for several years.

For cells grown in a monolayer culture, subculturing involves detaching the cells from the surface on which they are growing. In the present work this was done by aspirating the culture medium from the subculture bottle and washing with 5 ml of a solution of 0.1% trypsin (Gibco (UK) Ltd.) in a phosphate buffer solution (Dulbecco's "A", Oxoid Ltd.). The trypsin was then aspirated and a fresh sample of 5 ml trypsin added. This was again aspirated and the bottle was returned to the incubator for fifteen minutes. After this period the cells had become sufficiently loosened that addition of culture medium washed them off the surface of the bottle.

When the cells were removed from the base of the bottle, 5 ml of culture medium was added. The medium used was 400 ml Eagles MEM (Gibco (UK) Ltd.) supplemented with 100 ml new-born calf serum (Sera Lab Ltd.), 5 ml antibiotics (penicillin and streptomycin) and 5 ml glutamine. The medium is pipetted about eight times in order to break up any cell clusters and give a suspension of single cells. A drop of this cell suspension is placed onto a haemocytometer, thereby enabling a small volume of cells to be counted and an estimate of the cell concentration obtained. The cell suspension can be added to a known volume of fresh culture medium to obtain the required concentration of cells.

Each week about twenty to twenty-five culture bottles were seeded, this being the maximum that could be handled at a time. Two methods of seeding the cells in preparation for insonation were adopted, both
being carried out in a laminar air flow cabinet. The first method involved pipetting 5 ml of a cell suspension containing $2 \times 10^4$ cells per ml (i.e. $10^5$ cells) into a bottle. The bottle was then returned to the incubator for two hours to allow the cells to become attached. The second method involved pipetting 0.1 ml of a cell suspension containing $10^5$ cells per ml (i.e. $10^4$ cells), and returning to the incubator for two hours. The major difference between the two methods is that with the first, the cell suspension spreads out over the base of the bottle, while with the second the suspension remains as a small blob in the centre of the bottle. After two hours the cells had become attached to the base of the culture bottle, (a fact that could be ascertained by looking at the bottles under a microscope,) and the bottle was completely filled with culture medium. This was done whichever of the two seeding methods was adopted. The bottles were tightly closed and sealed with Parafilm (American Can Co.).

The reason for using two different methods of seeding was that by seeding only a small "blob", it was certain that the ultrasound must have affected all the cells that were seeded, whereas it was not certain what the intensity of the ultrasound was at the bottle's edges, which did not lie directly under the transducer. As stated in chapter 1, the intensity pattern is spatially varying although with the arrangement used in these experiments, it is difficult to ascertain the degree of spatial variation. By pipetting a small blob onto the base of the flask, this unknown variation is eliminated. On the other hand the error in pipetting 0.1 ml of culture medium onto the base of the culture bottle may have been significant, whilst when 5 ml was the volume pipetted, differences in the actual number of
cells in each bottle were likely to be insignificant.

7.4 Insonation of the Cells.

The cell cultures were left in the incubator for 20-24 hours. This gives the cells time to re-establish their normal metabolism (for example, the process of trypsinisation will affect the protein structure of the cell membrane). Insonation of the cell cultures was then carried out. Before each insonation, the height of the stand above the brass plate was adjusted or checked to enable the cell layer to be placed at a node or an antinode. The culture bottles were divided into groups for insonation. Some bottles were insonated at 3 W cm\(^{-2}\), some at 2 W cm\(^{-2}\) and some at 1 W cm\(^{-2}\). The treatments were all with 1 MHz continuous wave ultrasound for ten minutes, the bottles being held firmly down on the stand in a tank of degassed water by means of two brass wedges (thus preventing any tendency for the bottles to float). Control bottles were put through exactly the same procedures but received no ultrasound. All the bottles were dried immediately after their treatment and returned to the incubator.

7.5 Assessment of Response.

The total number of cells in each bottle was counted 72 hours after insonation. The medium from each bottle was aspirated, the base of each bottle washed twice with trypsin-phosphate buffer solution, and the bottles were replaced in the incubator for fifteen minutes, after which 5 ml fresh culture medium was added. So far this is the exact
procedure described in section 7.3. The next step depended on which method had been used to seed the cells. If the cells had been uniformly seeded over the base of the culture bottle, they were now broken up into a single cell suspension and counted on a haemocytometer, as before. However, if the cells had been seeded as a small blob, there were too few of them to be counted on a haemocytometer immediately. In this case, the 5 ml of medium was transferred to a culture tube. This was centrifuged for five minutes, at which time the cells had all settled at the bottom of the culture tube. The medium was decanted off, leaving just the cells, to which 0.5 ml of fresh medium was added. This sample was then broken up into a single cell suspension and counted on a haemocytometer as previously.

Each set of experiments therefore yielded a number (usually four or five) of cell concentrations for each ultrasound treatment, which could be compared with the counts from the control bottles. Results are shown in tables 7.1 and 7.2. For cells placed at the antinodes, cell concentrations were significantly lower after 2 W cm$^{-2}$ and 1 W cm$^{-2}$ of ultrasound than for the controls. For every 100 cells present in the control bottles at the time of counting, there were 78 cells ($\pm 4$) in the bottles that had received 1 W cm$^{-2}$, and 68 cells ($\pm 4$) in those that had received 2 W cm$^{-2}$. However, for cells placed at the nodes there were 97 cells ($\pm 5$) in the bottles that had received 1 W cm$^{-2}$, and 101 cells ($\pm 2$) in the bottles that had received 2 W cm$^{-2}$ at the nodes. The results did not appear to be dependent on the seeding method used.
Table 7.1.

Numbers of cells per ml counted in each bottle 72 hours after treatment at antinodes.

<table>
<thead>
<tr>
<th></th>
<th>No. of cells after 2 W cm$^{-2}$</th>
<th>No. of cells after 1 W cm$^{-2}$</th>
<th>No. of cells in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical set of 3.5 x 10$^5$</td>
<td>4.5 x 10$^5$</td>
<td>4.9 x 10$^5$</td>
<td>6.0 x 10$^5$</td>
</tr>
<tr>
<td>results taken 5.4 x 10$^5$</td>
<td>4.9 x 10$^5$</td>
<td>5.6 x 10$^5$</td>
<td>5.5 x 10$^5$</td>
</tr>
<tr>
<td>in a week 4.5 x 10$^5$</td>
<td>5.1 x 10$^5$</td>
<td>5.1 x 10$^5$</td>
<td></td>
</tr>
<tr>
<td>4.3 x 10$^5$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average number of cells remaining in each bottle, per 100 cells in the control bottles for (a) experiments where 5 ml of cells in culture medium were originally seeded, (b) experiments where a small blob was originally seeded, (c) average over all samples encompassing both methods.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>63±8</td>
<td>75±6</td>
<td>100</td>
</tr>
<tr>
<td>b</td>
<td>70±5</td>
<td>82±7</td>
<td>100</td>
</tr>
<tr>
<td>c</td>
<td>68±4</td>
<td>78±4</td>
<td>100</td>
</tr>
</tbody>
</table>
Numbers of cells per ml counted in each bottle 72 hours after treatment at nodes.

<table>
<thead>
<tr>
<th>No. of cells after $2 \text{ W cm}^{-2}$</th>
<th>No. of cells after $1 \text{ W cm}^{-2}$</th>
<th>No. of cells in controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical</td>
<td>9.1 x 10$^5$</td>
<td>9.6 x 10$^5$</td>
</tr>
<tr>
<td>set of results</td>
<td>9.4 x 10$^5$</td>
<td>10.0 x 10$^5$</td>
</tr>
<tr>
<td></td>
<td>9.9 x 10$^5$</td>
<td>9.1 x 10$^5$</td>
</tr>
<tr>
<td></td>
<td>9.7 x 10$^5$</td>
<td>9.5 x 10$^5$</td>
</tr>
</tbody>
</table>

Average number of cells remaining in each bottle, per 100 cells in the control bottles for (a) experiments where 5 ml of cells in culture medium were originally seeded, (b) experiments where a small blob was originally seeded, and (c) average over all samples encompassing both methods.

<table>
<thead>
<tr>
<th></th>
<th>(a) 104±5</th>
<th>100±8</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) 99±6</td>
<td>95±7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>(c) 101±2</td>
<td>97±5</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
To be certain that any observed difference in numbers of cells present was significant, it was important to verify the variability in the observed cell concentration from a single control bottle. This was done on a number of occasions, six haemocytometer counts being performed each time. The variation in cell concentration was never more than 2% either side of the mean value.

7.6 Observations of Cells Before and After Treatment.

There are several possible reasons why fewer cells may be present in the treated flasks than in the untreated flasks. One possibility is that there is inhibition of growth analagous to that which occurs after treatment with ionising radiation. Another is that the cells are mechanically removed from the base of the flask during treatment. To exclude the second possibility, and to examine whether the first possibility is feasible the culture bottles were observed under the microscope immediately before and immediately after insonation. The base of each bottle was covered with black masking tape in such a way as to leave uncovered a square of area 1 mm$^2$. The exact positions of any cells in this area were noted on a diagram (typically there would be 20-30 cells) on each occasion. Whether the treatment given was 1 W cm$^{-2}$ or 2 W cm$^{-2}$, the diagram of the cells for each bottle before insonation was always identical to the diagram drawn after insonation, with respect to both the number of cells present and their relative positions. If the treatment was 3 W cm$^{-2}$, the two diagrams were again often identical, but in some cases there were fewer cells visible after treatment, and in some cases there were no cells visible. The implication is that some of the cells were being
shaken off the surface on which they had been growing. This occurred whether the 3 W cm\(^{-2}\) had been received at the nodes or the antinodes. If the cells had been at the antinodes they had been subjected to extremely large pressure variations during treatment, whereas if they had been at the nodes, which are displacement antinodes, they had been subjected to large displacements. There was no clear difference between the numbers of cells that had been affected in this way after treatment at the nodes, and the numbers affected after treatment at the antinodes.

If a radiation-like effect is taking place, it would be expected that cells would continue to divide, but on counting the cells there would be fewer in the insonated bottles than in the controls. To assess whether the cells are dividing after insonation, cells in the 1 mm\(^2\) area were counted and mapped every 24 hours until it was time for the cells to be counted. It was apparent that the cells were dividing, so long as they had remained attached to the base of the bottle, although it was often difficult to keep track of individual cells and their progeny. In no case, other than in the flasks that had been given 3 W cm\(^{-2}\), could it be definitely stated that any particular cell had disappeared from a position at which it had previously been mapped. The results of the follow-ups of the numbers of cells in each 1 mm\(^2\) are given in tables 7.3 and 7.4.

7.7 Response of EMT6 Cells to Ionising Radiation.

In order to compare the results obtained with surviving numbers of cells that have been exposed to ionising radiation, a further
Table 7.3.

Numbers of cells present in a 1 mm² area at various stages just prior to, and after, treatment at antinodes.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Just before</th>
<th>Just after</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>insonation</td>
<td>insonation</td>
<td>later</td>
<td>later</td>
<td>later</td>
</tr>
<tr>
<td>3 W cm⁻²</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>20</td>
<td>31</td>
<td>59</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>13</td>
<td>31</td>
<td>43</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 W cm⁻²</td>
<td>31</td>
<td>31</td>
<td>44</td>
<td>72</td>
<td>~100</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>27</td>
<td>65</td>
<td>132</td>
<td>~300</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>17</td>
<td>60</td>
<td>~180</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>41</td>
<td>100</td>
<td>~300</td>
<td>-</td>
</tr>
<tr>
<td>1 W cm⁻²</td>
<td>38</td>
<td>38</td>
<td>60</td>
<td>70</td>
<td>~110</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>37</td>
<td>72</td>
<td>~200</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9</td>
<td>32</td>
<td>90</td>
<td>~300</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>16</td>
<td>33</td>
<td>75</td>
<td>~120</td>
</tr>
<tr>
<td>control</td>
<td>22</td>
<td>-</td>
<td>40</td>
<td>58</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>-</td>
<td>65</td>
<td>~150</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-</td>
<td>36</td>
<td>~120</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>-</td>
<td>23</td>
<td>47</td>
<td>~110</td>
</tr>
</tbody>
</table>
Table 7.4.

Numbers of cells present in a 1 mm² area at various stages just before, and after, treatment at nodes.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Just before insonation</th>
<th>Just after insonation</th>
<th>24 hours later</th>
<th>48 hours later</th>
<th>72 hours later</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 W cm⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>8</td>
<td>28</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>9</td>
<td>22</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>40</td>
<td>~100</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2 W cm⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>47</td>
<td>66</td>
<td>~200</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>37</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>29</td>
<td>60</td>
<td>~200</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>35</td>
<td>~100</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1 W cm⁻²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>29</td>
<td>45</td>
<td>~200</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>31</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>30</td>
<td>~110</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>27</td>
<td>80</td>
<td>~250</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>26</td>
<td>45</td>
<td>~200</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>50</td>
<td>~150</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>-</td>
<td>50</td>
<td>~150</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>19</td>
<td>65</td>
<td>~200</td>
<td></td>
</tr>
</tbody>
</table>
experiment was performed. Fourteen culture bottles were seeded with 5 ml of culture medium containing $10^5$ cells as described in section 7.3. Twenty-four hours after seeding they were exposed to X-rays from a 250 kV 15 mA Pantak X-ray unit. Four of the bottles were retained as controls, and the remaining bottles received a dose of 0.25, 0.5, 1, 2, 5 or 10 Gy. As with the ultrasound-treated bottles, these bottles were left in the incubator for 72 hours, when the number of cells per ml in each bottle was ascertained as described in section 7.5. The number of cells, as a percentage of the number in the control bottles was plotted as a function of dose (figure 7.2), and from this graph it can be seen that a dose of 2.0 Gy has approximately the same effect as an ultrasound intensity of $2 \text{ W cm}^{-2}$ for cells at the antinodes, and a dose of 1.4 Gy has about the same effect as an ultrasound intensity of $1 \text{ W cm}^{-2}$.

7.8 Discussion.

There is clearly a difference in the response of EMT6 cells to ultrasound treatment depending on whether those cells were placed at the antinodes (where the most sonoluminescence occurs) or the nodes (where there is least sonoluminescence) during their treatment. The effects observed at 1 and $2 \text{ W cm}^{-2}$ have occurred with a minimal change in the conditions to which control bottles were subjected, thereby eliminating some of the criticisms made about the experiments of past workers. It must be remembered that everything possible has been done in setting up this experiment to maximise any effect (e.g. by using a known good reflector to increase the intensity of the ultrasound, by ensuring strong bands of sonoluminescence were
Figure 7.2. Graph of number of cells in irradiated bottles as a percentage of the number of cells in control bottles 72 hours after irradiation, as a function of the dose of 250 kV X-rays received by the irradiated bottles.
It has been shown that it is not a mechanical displacement of the cells that is responsible for this effect except at \( 3 \text{ W cm}^{-2} \), and the fact that mechanical displacement at this intensity is approximately the same at the nodes as at the antinodes would indicate that the difference in the observed effects between cells at the nodes and cells at the antinodes is not due to any mechanical displacement effect. By observation of the cells over the next 72 hours, it has been shown that the cells are capable of division after treatment. The effect may therefore be a delayed non-mechanical effect with similarities to ionising radiation, although the possibility that the effect is the result of the release of chemicals from the base of the culture bottle cannot be ruled out.

It is possible that some form of mechanical damage that did not result in the removal of cells from their surface may have taken place. It must be recognised that mechanical damage is more likely in cells that are trapped against a surface than in cells in suspension and this possibility has not been entirely eliminated in the present studies, and requires further investigation. Other reasons for a reduction in cell numbers would be that some of the cells have died; that the cells have survived but have become no longer viable; that there has been some slowing of growth rate of the cells; or some combination of any of these effects. Any of these explanations is possible from results of the actual numbers of cells present four days after seeding. Typically \( 10^5 \) cells would be seeded on day one, and \( 5 \times 10^6 \) would be counted on day five. This multiplication rate is in agreement with the observations of the proliferation kinetics of EMT6 cells by Twentyman et al (1975). The numbers do not exactly correspond to the twelve hour cell doubling time mentioned in section 7.3 because there is a period of about 24 hours after seeding.
when the cells are not multiplying.

Tables 7.3 and 7.4 indicate that treated cells have divided a number of times, whilst table 7.1 indicates that there has been a reduction in cell numbers after 72 hours with respect to the controls. At low doses of ionising radiation most types of cell do not show evidence of radiation damage until they attempt to divide and, following doses of less than about 10 Gy, lethally damaged cells may divide a few times before their progeny undergo lysis and disappear from the population. Radiation damage also delays the progression of cells through the cell cycle. There is a rapid drop in the mitotic index in an irradiated cell population because both lethally damaged and surviving cells cease to enter mitosis. After a period of time known as the mitotic delay cells recommence mitosis. This affects cells in different ways depending on the phase which they were in when radiation occurred with the result that the cell population is partially synchronised as a consequence of the radiation induced cell cycle delay. Even after doses as high as 10 Gy most cells divide at the end of the cycle in which they were irradiated, although surviving cells may continue to experience delays in their progression through subsequent cycles.

Twentyman and Bleehan (1975) have produced survival curves for EMT6 cells after irradiation with up to 15 Gy of 250 kV X-rays. Cultures which had received a dose of 1-2 Gy showed a 70-80% survival rate four days after irradiation. This in itself does not mean that the ultrasound treatment received by the cells at the antinodes was equivalent to a radiation dose of 1-2 Gy because Twentyman and
Bleehan were investigating the number of cells that had actually survived. The ultrasound treatments described in this chapter may not have killed any cells. The equivalent radiation dose of 2 Gy noted in section 7.7 is surprisingly large, and means that there must be a very large microdosimetric effect associated with the sonoluminescence banding phenomenon (although the effect of the ultrasound on cells at the nodes may have been equivalent to an ionising radiation dose of 0.5 Gy or more, no effect being detectable at this dose). It is important to make a distinction between a reduction in cell numbers (which the work in this chapter has investigated) and a reduction in cloning efficiency. The radiation dose required for a reduction in cell numbers is less than for a reduction in cloning efficiency. Very little work has been done on cell numbers although Nias et al (1965) investigated the effect of ionising radiation on HeLa cells. After a single dose of 6 Gy, or after doses of 0.3 Gy each day for six or eight days, the total number of cells present was less than in the controls. The reason for this was that although the cells had survived, their growth rates had become more variable. Colony sizes were analysed and it was found that there was a greater spread in the size of the colonies after irradiation. A slowing of the rate of proliferation of some cells may be the type of effect that was observed in the experiments described above. The experiments in section 7.7 were carried out because of the paucity of data concerning the reduction in cell numbers as a result of exposure to ionising radiation, but the method described is not a standard procedure and the data presented here cannot easily be compared to the work of others.
There are two problems which remain with this work. Firstly, as mentioned above, there are not enough follow-up results to rule out the possibility that the effect is due to some sort of mechanical damage. Secondly there is the uncertainty of how the ultrasound intensity varies across the base of the culture bottle.

In order to gain further insight into the processes that actually affect the cells in the ultrasound field, it would be necessary to count the cells at different stages after insonation. A time period of 72 hours from insonation to counting has been selected in these experiments. It may be that the cells have gone through a few divisions unaffected by this stage, and that at 72 hours an observable effect is only just beginning. Also the cells may undergo something akin to sublethal damage.

There will presumably be more chance of damage to cells that are irradiated with ultrasound if they are placed in a gaseous environment where cavitation can occur near the cells and there may be free radicals present. Many workers (e.g. Vivino et al 1985) have gone to some lengths to ensure that stabilised gas bodies are present in a system where cells are to be insonated. This usually involves the introduction of hydrophobic Nuclepore membranes into the sound field. In the experiments described in this chapter, no attempt was made to introduce stabilised gas bubbles. However, as mentioned in section 7.1, when using the system shown in figure 7.1, rows of bubbles, which are presumably bubbles of larger than resonant size congregating at the nodes, will gradually appear. It is interesting to observe how "straight" these rows of bubbles are. The antinodal
and nodal planes appear to be parallel to the transducer face and to the base of the culture bottle. It would be instructive to try and repeat the above experiments deliberately introducing stabilised gas bubbles. This would alter the amount of sonoluminescence expected, but would not affect the amount of cell damage sustained due to artefacts such as mechanical effects.

The results presented in this chapter may give some insight into the irreproducibility of some of the work discussed in chapter 2. To be sure of exactly reproducing the work of others, not only must the standing and travelling wave components be quantified, but the positioning within the standing wave field must also be ascertained. To achieve this measurements of the positions of insonated samples in the ultrasound field must be made with an accuracy of a few hundredths of a millimetre.

It has been shown that ultrasonic standing waves affect monolayers of cells grown in culture. At an intensity of $3 \text{ W/cm}^2$ cells are mechanically displaced from their surface by the ultrasound, but at lower intensities, mechanical displacement does not occur. If the cell monolayer is situated at a node there is no observable damage, whereas if it is at an antinode there is clearly an effect.
Chapter 8. Absence of Sonoluminescence in the Human Cheek.

Generally speaking it is not possible to observe sonoluminescence in-vivo because there is no way of detecting it short of inserting an optical fibre into the tissue. However if a bright pen torch is held against the hand, or to the inside of the cheek, red light is transmitted to the other side. Cutler (1929) described how the application of a light torch to the female breast could provide a simple non-invasive method of visualising lesions (a method known as diaphanography). More recently this technique has been investigated by Watmough (1982a,b). Cartwright (1930) observed a spectrum of the light transmitted through the cheek by means of a very fast spectrograph. He found that the flesh of the cheek is most penetrable to radiation of wavelength 1150 nm. The transmitted light was found to be mostly in the infra-red but did extend into the visible region. At 700 nm there was approximately 8% transmission and at 600 nm about 4%. The cheek would therefore seem a reasonable part of the human body in which to look for sonoluminescence; it has another advantage in that the inside of the cheek is living tissue. Looking for sonoluminescence in any other region would involve investigation of layers of dead skin, which would seem unlikely cavitation sites.

8.1 Detection of Sonoluminescence.

The method used for the detection of sonoluminescence was the same, in principle, as that used in chapters 4 and 6, and is shown in figure 8.1. The photomultiplier used was now an EMI 9658R, chosen because of its relatively high photocathode radiant sensitivity at
Figure 8.1. Schematic diagram of the apparatus.
the high wavelengths (it will detect light up to a wavelength of around 850 nm). The photomultiplier was placed inside a cooled housing (EMI-Gencom Inc.) and operated at -20°C, to reduce the large amounts of noise often present at the red end of the visible light spectrum. The photon counting system used in the previous chapters was not compatible with this photomultiplier so a 5C1 photon counting system and 5C14 dual channel pulse height analyser (EG & G Brookdeal Electronics Ltd.) were used. With this system individual counts are taken not as part of a series of discrete pulse heights, but as a single count corresponding to the photon peak of the photomultiplier. The 5C14 can be adjusted so that a suitable counting window, whose limits are on a slowly changing part of the photopeak, can be set. Counts can be recorded over any period of time between $10^{-6}$ seconds and $10^5$ seconds, and if a counting time of one second is selected a number of readings can be taken fairly rapidly.

8.2 Experimental Procedures.

In order to try and quantify any light from the inside of the cheek, some experiments were also carried out on small polythene bags filled with ordinary tap water. The Therasonic 1030 transducer was pressed against the outside of the cheek or a water bag, and a light guide leading to the photomultiplier was held to the inside of the cheek or to another part of the water bag. When investigating the cheek some care had to be taken as to the relative orientation of the transducer and the light guide. The light guide was sensitive to the direction of the incoming light, and by using a weak beta light and protractor, as shown in figure 8.2, and varying the angle $\theta$, it was found that
Figure 8.2. Arrangement used to find angles of acceptance of the light guide.
75% of the light accepted by the light guide comes from within a cone described by θ=10°.

As in the experimental procedures described in chapter 4, the first step was to obtain a background count with the end of the light guide remote from the photomultiplier in darkness. This was 60±20 counts in one second. As in section 6.5, sonoluminescence was assumed to have occurred whenever the count was greater than three standard deviations above the average (120 counts per second).

The various experimental arrangements employed are shown in figure 8.3. In some arrangements a water bag was insonated, and in some cases sonoluminescence was observed through a glass plate of thickness 6 mm in order to mimic the relative orientation of the transducer and light guide in the experiments on the cheek. The thickness of the cheek was found using a micrometer to be 6±1 mm. In all the experiments in this chapter, the intensity of the ultrasound was not higher than 2 W cm⁻². Above this intensity, some discomfort could be felt by the subject, and pain was perceived at the highest intensities or after long exposures.

8.3 Observations of Sonoluminescence.

With the arrangement of figure 8.3a, the basic transducer-cheek-light guide set-up, no sonoluminescence was observed after several minutes at intensities of 2 W cm⁻², the photon count being 60±20 per second, the same as the background count.
With the transducer, water bag and light guide arranged as in figure 8.3b, sonoluminescence could be detected at 1.5 $\text{W cm}^{-2}$ if the water bag was large enough to allow the light guide to view a region more than 2-3 mm from the transducer. If the glass plate was employed to allow on-axis viewing (figure 8.3c), sonoluminescence could be observed without difficulty even if a small water bag was used, at intensities greater than 1 $\text{W cm}^{-2}$. Although this set-up is similar in its orientation to that of figure 8.3a, the glass plate has introduced two acoustic reflection boundaries which will increase the standing wave component and make sonoluminescence more likely.

If a weak torch is held against the outside of the cheek, and the light guide against the inside (figure 8.3d), the photon flux was between 4 and $5 \times 10^6$ counts per second. A neutral density filter of 6.0 was placed between the same torch and the light guide. Very little light was detected so the filtration was removed in steps of 0.5. When the filtration was 3.0 the photon flux was $4.5 \times 10^6$ counts per second, so the cheek behaves approximately as a neutral density filter of number 3.0. A neutral density filter of number 3.0 placed between the light guide and the glass plate in the arrangement of figure 8.3c will again cut out any observable sonoluminescence (where a filter of number 2.5 will not). These experiments rule out the possibility of being able to detect any sonoluminescence with the arrangement shown in figure 8.3e, unless there is a minimum distance from the transducer before sonoluminescence will be observed. This experiment was tried and, as expected, no increase in the photon flux above background was seen.
Figure 8.3. Diagram showing the various orientations of the transducer, cheek, water bag, glass plate and light guide.
(Figure 8.3. continued.)
Finally, an attempt to find sonoluminescence with the orientation shown in figure 8.3f was made. Very low levels of light, barely above the required 120 counts per second, were observed at an ultrasound intensity of $2 \text{ W cm}^{-2}$. On this occasion the water bag used was necessarily small, in order to fit comfortably in the mouth for the duration of an investigation.

8.4 Discussion.

Of the experimental arrangements involving the cheek (figures 8.3 a, e and f), there was a small amount of sonoluminescence observed with the figure 8.3f arrangements; and this almost certainly came from the water bag; and none from the other two arrangements. No sonoluminescence was observed at any time from within the cheek. There are two possible explanations for this; either there is no cavitation in the cheek, or there is too small a quantity of sonoluminescence to detect. The experiments of figure 8.3b indicate that there may be some small stand-off distance within which sonoluminescence does not occur, although there was no sonoluminescence observed from the cheek even when a stand-off distance was created as in figure 8.3e.

A calculation of the amount of light that could be emanating from the cheek without the detection system being able to see it can be attempted. To do this, it is assumed that all the light originates at antinodes. Since the speed of ultrasound in tissue is the same (to within 5%) as the speed of ultrasound in water (Wells 1969), the wavelength of ultrasound in the cheek is taken as being 1.36 mm (the
value found for water in section 5.5). The first antinode thus occurs 0.68 mm from the air boundary, the second at 1.36 mm and so on. In the preceding section, it was seen that the cheek behaves as a neutral density filter of number 3.0. Any sonoluminescence emanating from the cheek would have to traverse a portion of the cheek which corresponded to a neutral density filter of some value between zero and 3.0 depending on the actual distance travelled in the cheek. For each antinodal position the "equivalent neutral density filter" through which the light must travel can be calculated. For example the third antinode occurs at 2.04 mm, which is almost exactly one third of the thickness of the cheek, so light from this region will have to travel through the equivalent of a neutral density filter of number 1.0. From these assumptions and from geometrical considerations (see appendix) a simple computer programme was written to calculate the probability of a photon, originating at any point in the cheek that can be seen by the light guide, being detected. It was found that, on average, the chances of detecting a photon emanating from the cheek were 20% of the chances of detecting a photon from the same volume of water. As mentioned in the preceding section, approximately the same volume of water will, when insonated, produce light that is a thousand times above background (a neutral density filter of number 3.0 is needed to bring the light down to the level of the background). Thus if a similar amount of cavitation were produced in the cheek, it should generate light which is two hundred times brighter than background. Therefore there must be correspondingly less sonoluminescence in the cheek than in water. In section 4.9.3, it was estimated that $2 \times 10^4$ photons per second per cm$^3$ are produced in water at an acoustic intensity of 1 W cm$^{-2}$. 
Admittedly this result was obtained with a different arrangement, but the reflector was a glass plate in both cases. Also in the experiments described in section 5.1, glass plates of thicknesses 2 mm and 6 mm were investigated and were found to produce very similar standing wave patterns. In the cheek, therefore, a maximum of \( 3 \times 10^3 \) photons per second per \( \text{cm}^3 \) are produced. Even this maximum is a conservative estimate because, of course, if there is any light produced, not all of it will come from the antinodes: on the above model, most of the light that is detected (in either the cheek or the water bag) will have originated at the antinode that is 0.68 mm from the light guide. If the model had assumed an even distribution of light production, the filtration effects of those parts of the cheek that are less than 0.68 mm from the light guide, which are clearly less than the filtration effects at 0.68 mm, would have been taken into account.

Of the parts of the body, the cheek would seem a likely site for cavitation on standing wave considerations. Some data has been produced on the attenuation coefficients of human tissues and organs (see for example, Wells 1969), and at 1 MHz, most parts of the body will attenuate an ultrasound beam by about 1 dB cm\(^{-1}\). It is a conservative estimate to say that about 88% of the ultrasound will be transmitted to the side of the cheek remote from the transducer, at which point an air gap will enable standing waves to be set up. Even if cavitation does not occur, the standing waves will enhance the ultrasound intensities present, and this may cause pain at the higher intensities.
If there is no cavitation occurring in the cheek at 2 W cm\(^{-2}\), it is a matter of speculation as to whether there is any at higher intensities. Cavitation may have been the reason for the perception of pain at intensities between 2 and 3 W cm\(^{-2}\), but because of this pain, no experiments were carried out at these levels. Alternatively, the pain may have been the direct result of heating of the transducer. Whatever the explanation for the perception of pain, the high percentage transmission through the cheek and the air boundary on the inside of the mouth will have produced standing waves which may have exacerbated it.

In this chapter an attempt has been made to look directly for sonoluminescence in-vivo. No sonoluminescence was observed but it has been shown that if there is any cavitation occurring in the cheek, the incidence of it must be low.
Chapter 9. Conclusions.

Ultrasound has been a major investigative and therapeutic tool for some years. In diagnostic use, very high temporal peak intensities occur, and in therapy, high temporal average intensities are found. The mechanisms of the interactions of ultrasound with biological materials at these intensities are still not fully understood, and it is important to try and establish whether there are any adverse effects of clinical ultrasound. In this thesis one possible mechanism for biological hazard, unstable cavitation (as evidenced by the production of sonoluminescence), has been investigated in depth in order to gain an understanding of the conditions under which this mechanism is likely to occur.

The conditions which favour cavitation in water have been investigated, and knowledge gained has been used to assess the likelihood of cavitation in more complex systems. For most of the experiments physiotherapeutic ultrasound has been used, but some work with shorter pulses has been done to assess the implications for diagnostic ultrasound. When the factors that affect cavitation in water were investigated, an important consideration was the standing wave pattern set up in the insonated medium. This finding was used subsequently when the effects of therapeutic ultrasound on cell monolayers were examined. The work has provided a greater understanding of the conditions under which cavitation is unlikely to occur in-vivo. At the end of the thesis, an attempt to detect sonoluminescence from an insonated human cheek has been made.
9.1 The Main Findings.

Ultrasonically induced transient cavitation in ordinary water would appear to be a threshold phenomenon. The intensity threshold has been investigated only for continuous wave physiotherapeutic ultrasound of 1 MHz, and obviously the threshold will not be the same for all regimens. However, sonoluminescence can readily be seen at the fairly modest intensity of 1 W cm\(^{-2}\) even if the water is distilled or degassed.

Sonoluminescence in water increased with increasing temperature over the range 22-45°C. This contradicts the work of some other authors, but the evidence presented in section 4.6 is fairly conclusive. The increase in sonoluminescence with temperature can be observed even over a small temperature range.

The duty cycle of a physiotherapy ultrasound beam also affected sonoluminescence, the pulsed regimens producing more light than the continuous wave at some pulse heights. This result is one of many that have indicated pulsed enhancement of cavitation effects. However pulsed enhancement did not occur when the insonated medium was a slightly more viscous agar gel. These findings are consistent with theories on bubble migration and localised gas depletion in continuous wave and pulsed fields. Thus it may be preferable to use pulsed ultrasound for physiotherapy, where the insonated "medium" is the "viscous" tissues. However the effect of continuous wave and pulsed ultrasound on blood, which is of intermediate viscosity, needs to be examined.
The standing wave patterns set up when therapeutic ultrasound insonates a tank of water are extremely complicated. A single measurement of acoustic reflectivity is not sufficient and standing wave patterns need to be meticulously investigated. A field with a high proportion of standing wave had significant effects on the spatial distribution of bubbles, and if the conditions favoured standing waves sufficiently, banded patterns of strong sonoluminescence were observed with an image intensifier. When sonoluminescence occurred in bands, the bands appeared at the pressure antinodes in the standing wave field. Sonoluminescence could not be detected with the image intensifier in a travelling wave field, or in a field with a low standing wave component.

For pulse lengths near to 100 μs, sonoluminescence was observed at about the same levels as with the therapeutic pulse lengths of 2 ms, but as the pulse length was decreased, keeping the pulse repetition frequency constant at 1 kHz, the threshold for sonoluminescence increased. The system used for investigating the effect of short pulses of ultrasound produced sonoluminescence at pulse durations of about 20 μs. No sonoluminescence could be detected at shorter pulse durations than this, but the peak pressure level from the generator was lower at shorter pulses. Other workers have demonstrated sonoluminescence with the pulse durations often encountered with commercially available pulse-echo equipment, but with unrealistic pulse repetition frequencies, while the work in this thesis has demonstrated sonoluminescence with the pulse repetition frequencies often encountered with this equipment, and with pulse durations that are close to diagnostic pulse durations.
When the effect of physiotherapeutic ultrasound with a large standing wave component on animal cells was investigated, the number of cells surviving for four days after culturing and three days after insonation was only affected by the ultrasound if the cells had been situated at the pressure antinodes, the sites for strong sonoluminescence, in the standing wave field. Cells placed at the pressure nodes survived just as well as those that had received no ultrasound.

From the work of others in the fields of diaphanography and transmission of light through human flesh, it seemed probable that if insonation of the human produced cavitation, the accompanying sonoluminescence should be detectable unless it was occurring at extremely low levels. Again therapeutic ultrasound of up to $2 \text{ W cm}^{-2}$ was used. No sonoluminescence was observed from the cheek at any time.

9.2 Implications for the use of Ultrasound in Physiotherapy and Diagnosis.

All the experiments described in this thesis were carried out at a frequency of 1 MHz. In physiotherapy, the most common frequencies are 1 and 3 MHz, and 1 MHz would be used for deep seated lesions. In using a frequency of 1 MHz it must be remembered that cavitation will decrease with increasing frequency. For the experiments in this thesis with therapeutic ultrasound, a range of intensities is used: a threshold for cavitation in water was found at $0.25 \text{ W cm}^{-2}$, duty
cycle and temperature effects were investigated at 1 W cm$^{-2}$, standing wave effects at 3.3 W cm$^{-2}$, and effects on cell monolayers and on the cheek at 1 and 2 W cm$^{-2}$. The implications of the work in this thesis for physiotherapy is that intensities above 1 W cm$^{-2}$ may produce cavitation.

Diagnostic examinations employ frequencies varying, in general, between 2.25 and 10 MHz. The output characteristics of diagnostic ultrasound equipment were discussed in section 6.8. The implications of the work presented in chapter 6 for the use of diagnostic and pulsed Doppler ultrasound, is that the peak intensity, and the duration of the pulse, should be kept as low as possible.

9.3 Suggestions for Future Work.

Answering the specific problems addressed in chapters 4-8 has generated a number of new questions. In chapter 4 it was stated that sonoluminescence from water was found to increase with increasing temperature. This finding disagreed with other workers, but these other workers did not agree among themselves as to how sonoluminescence varied with temperature. The questions that require further investigation are whether the variation of sonoluminescence with temperature is frequency dependent, or is dependent on the gassiness of the water insonated, for example?

The way in which sonoluminescence varies with viscosity, and in particular, the way in which pulsing of the ultrasound affects this, require further attention. One point that is still in doubt is the
regimens that may result in harm being done to the blood. The conditions that least favour cavitational and streaming effects in blood need to be identified.

Another question raised in chapter 4 was the absence of any kind of luminescence from water that is being insonated with a source of ionising radiation. Are the mechanisms of free radical production and recombination different to those that operate when ultrasound is involved? In this context it may be tempting to look for an alternative explanation of the origin of sonoluminescence to free radical recombination, but if this is to be attempted, some very powerful evidence which supports the explanation currently favoured must be overcome.

The work on cell monolayers could be extended in several ways. Cell numbers could be counted at different stages after insonation, or the cells could be plated out singly and the number of colonies formed could be counted, or a histogram of the number of cells per colony could be generated. Different reflectors which produce different standing wave patterns could be used, and the effects on cells in systems which produced no sonoluminescence bands could be assessed. Experiments where stabilised gas bodies are introduced could be carried out to ascertain whether any damage sustained is really due to sonoluminescence. Also cells could be cultured in suspension instead of being attached to a surface. This might cause the cells to move to the nodes or antinodes, thereby sustaining less or more damage than might have been thought from the results of experiments carried out on monolayers. The mechanism of the cell damage described
in chapter 7 could also be investigated. Much has been written on the biological effects of ultrasound and this has been reviewed in chapter 2, although rarely is a damage mechanism for a particular effect suggested. An exception to this is found in the recent work of Barnett et al (1988) on sister chromatid exchange effects. They speculate that the exchanges are caused by sonoluminescence itself: part of the sonoluminescence spectrum lies in the ultraviolet (Sehgal et al 1980a) and it has been shown by Kato (1974) that ultraviolet light is a strong sister chromatid exchange inducer.

The experiments described in chapters 4, 5, 7 and 8 could all be carried out using diagnostic ultrasound equipment, or equipment such as the generator used in chapter 6, which produces pulse regimens with a wide range of on-times. For experiments on short pulses of ultrasound it would be useful to build a generator like the one in chapter 6, but with a facility for producing a pulse of 1 or 2 cycles of an intensity comparable with the peak intensities produced by diagnostic equipment.

9.4 Summary.

The question of whether clinical ultrasound can present a potential hazard to a patient is extremely complex and remains unresolved. Many groups around the world are working on some aspect of the problem, and any project such as this can only aim to add some pieces to the jigsaw. A better understanding of the circumstances that favour cavitation in water has been obtained; it has been shown that, if the conditions are right, physiotherapy ultrasound can affect the growth
of monolayers of animal cells; but no evidence of sonoluminescence, which might be indicative of harmful effects, have been observed in the human cheek.


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Appendix 1. Probability of a Photon being Detected by a Light Guide.

To calculate the reduction in the amount of light emanating from the cheek of thickness 6 mm, compared to the amount of light emanating from a water bag of the same thickness, it is assumed that all the light originates at pressure antinodes. We will consider a point 0, and calculate the chances of a photon originating at 0 being detected by the light guide. The chance of detection depends on the direction in which the photon travels.

If the radius of the light guide is a, the angle subtended by the internal diameter of the light guide at 0 (see figure A.1) is $\theta$, and b and c are the other two sides of the triangle described in figure A.1, then

$$4a^2 = b^2 + c^2 - 2bc \cos \theta$$  \hspace{1cm} (A.1)

If x is the perpendicular distance of 0 from the light guide, and y is the distance of 0 from the axis of the light guide, then

$$b^2 = (a+y)^2 + x^2$$  \hspace{1cm} (A.2)

and

$$c^2 = (a-y)^2 + x^2$$  \hspace{1cm} (A.3)

Now the solid angle subtended by the light guide at 0 will be $2\pi r^2 (1-\cos(\theta/2))$ so the chance of a photon from 0 reaching the light guide is

$$2\pi r^2 (1-\cos(\theta/2))/4\pi r^2 = \frac{1}{2}(1-\cos(\theta/2))$$  \hspace{1cm} (A.4)

but

$$\frac{1}{2}(1-\cos(\theta/2)) = \frac{1}{2}(1-\left[\frac{1}{2}(1+\cos\theta)\right]^\frac{1}{3})$$  \hspace{1cm} (A.5)

and from equation A.1

$$\cos \theta = \frac{b^2 + c^2 - 4a^2}{2bc}$$  \hspace{1cm} (A.6)
Figure A.1. Geometrical representation of a point O at a perpendicular distance x from a light guide.
so from equations A.2 and A.3
\[
\cos \theta = \frac{(x^2 + y^2 - a^2)}{[x^2+(a+y)^2]^{\frac{3}{2}}[x^2+(a-y)^2]^{\frac{3}{2}}} \quad (A.7)
\]
therefore the chance of a photon from 0 reaching the light guide are
\[
\frac{1}{2} \left[ 1 - \frac{1}{2} \left( \frac{1+((x^2+y^2-a^2)/[x^2+(a+y)^2]^{\frac{3}{2}}[x^2+(a-y)^2]^{\frac{3}{2}}) \right)^{\frac{1}{2}} \right] \quad (A.8)
\]
In this equation, a, the internal radius of the light guide is known to be 3 mm, y can take any value between 0 and 3 mm, and x can be any multiple of 0.68 mm, but is always less than 6 mm.

It is a simple matter to write a computer programme such that y takes a large number of distinct values. Sonoluminescence is assumed to occur with equal likelihood at all points on an antinode described by -a<y<a and x<6 mm. The above treatment is, of course, the same for the cheek as for a bag of water. For the cheek, the series of values for the expression given in equation A.8, have a correction factor applied to them to account for attenuation in the cheek according to the method described in section 8.4. The correction factor is \(10^{-n/3}\) for the \(n^{th}\) antinode from the light guide.

The result of these calculations is that the chances of a photon emanating from the cheek being detected by the light guide are 20% of the chances of a photon from the water bag reaching the light guide.
Appendix 2. Papers Prepared for Publication.

1. Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 1. Correlation of sonoluminescence with the standing wave pattern in an acoustic field produced by a therapeutic unit.


3. Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 3. Cavitation from pulses of a few microseconds in length.
   M.J.W. Pickworth, P.P. Dendy, T.G. Leighton, E. Worpe and R.C. Chivers.
Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 1. Correlation of sonoluminescence with the standing wave pattern in an acoustic field produced by a therapeutic unit

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Abstract. A Therasonic 1030 (Electro-Medical Supplies) therapeutic ultrasound generator operating at 1 MHz continuous wave was used to insonate aerated water at two temperatures, 22 °C and 37 °C. Using various acoustically reflecting materials, sound fields were set up with different standing wave components. Measurements of the acoustic pressure variations on the axis of the sound fields were made using a needle hydrophone and the results were compared with photographs of the spatial distributions of the image intensified sonoluminescent light output. The near field region was used, thereby simulating the clinical situation.

Sustained sonoluminescence was observed for nominal intensities of 3 W cm⁻², and acoustic reflections of greater than 40%. Under these conditions, if sonoluminescence did not appear spontaneously it could always be induced by rotating the transducer. Whenever bands of maximum light output formed they correlated closely with the pressure antinodes in the standing wave pattern. Very little light was produced by travelling wave fields.

1. Introduction

When gas bubbles of a suitable size in a liquid are subjected to high intensity acoustic pressure fields, they grow isothermally during the expansion phase of the sound cycle and collapse adiabatically. As the bubble radius reaches a minimum value, the gas contained within the bubble may reach temperatures of several thousand degrees; high enough to produce electronically excited molecules and free radicals, which can either radiate back to the ground state, or recombine radiatively to produce sonoluminescence (Walton and Reynolds 1984). Spectral measurements, and experiments that employ radical scavengers, show that sonoluminescence originates mainly from the recombination of free radicals (Sehgal et al 1980). Since the production of light indicates the presence of free radicals, sonoluminescence would indicate at least the potential for biological damage.

Therapeutic ultrasound is currently used in continuous wave or pulsed modes, with frequencies of 1 or 3 MHz, and nominal output intensities of a few W cm⁻² (Allen and Battye 1978). Sound directed into the human body may undergo partial reflection by discontinuities (e.g. bone, tissue variations, fluid sacs), thereby setting up standing waves. This study simulates such conditions using a physiotherapeutic unit with 1 MHz continuous wave ultrasound at an intensity of about 3 W cm⁻² (spatial average), and high gain image intensification to observe any sonoluminescence.
Wagner (1958) suggested that sonoluminescence originates at the pressure antinodes of a standing wave field but did not check the positions of these pressure antinodes. Conversely Graham et al. (1980) reported the presence of considerable sonoluminescence from travelling waves, but they too did not quantitate the nature of the sound field, so it is possible that there was a standing wave component. The sound field set up by a Therasonic transducer, in laboratory simulations, can contain a range of standing wave components as a result of reflections off the liquid surface, container walls and absorbers (Tyszka, personal communication). To obtain meaningful results, it is necessary to measure the sound field with a hydrophone in the region where sonoluminescence is being recorded. Therefore this study aims to correlate the spatial distribution of sonoluminescence with the standing wave component of the sound field.

2. Methods

The apparatus was arranged as in figure 1 and the ultrasound generator was a Therasonic 1030 (Electro-Medical Supplies). The water temperature in the large bath was thermostatically controlled to within 1 °C of 22 °C or 37 °C. A sample cell, measuring 10.1 cm by 2.6 cm and 4.2 cm deep, containing aerated water was used for two of the three experiments. The end of the cell nearest the transducer was an acoustically transparent acetate window and the whole cell was painted black to prevent sonoluminescence originating inside the large water bath being scattered up into the optical system. An acoustic reflector was positioned at the back of the cell—either a thick brass block (27.5 mm) of high acoustic reflectivity, or a thin brass plate (0.75 mm) of intermediate reflectivity. To obtain the lowest acoustic reflectivities, i.e. most similar to a travelling wave system, the sample cell was removed and the experiment was performed in the large water bath, which was lined with several layers of wire wool to act as an acoustic absorber.

The acoustic pressure on the axis of the transducer in the region of interest was determined using a needle hydrophone (Dapco NP10-3). This had to be calibrated against a membrane hydrophone (Duck, personal communication) which had been calibrated at the National Physical Laboratory. The position of the hydrophone relative to the transducer was determined, to within 0.1 mm, using an adapted micropositioner.

Figure 1. Schematic diagram of apparatus.
Cavitational effects of clinical ultrasound

The output characteristics of the Therasonic were measured using a UWII Biotec force balance. Further details are given in the paper by Peckworth et al (1988). For the majority of these experiments the acoustic intensity was 3.3 W cm$^{-2}$, although in the final experiment a spontaneous malfunction in the Therasonic 1030 produced an output of 10 W cm$^{-2}$. For a travelling wave, a Therasonic intensity of about 3.3 W cm$^{-2}$ corresponded to a reading of between 0.3 and 0.4 MPa on the hydrophone.

The hydrophone was removed while sonoluminescence was recorded. This was done by photographing the output phosphor of the image intensifier (EMI type 9912, with a bialkali photocathode) with a Nikon F2 camera. Each point of light recorded

![Graph](image.png)

**Figure 2.** Results obtained with the brass block reflector at 22 °C, showing (a) Peak-to-peak acoustic pressure as measured with the needle hydrophone; (b) Photograph of the appearance of the corresponding sonoluminescence as seen through the intensifier; (c) Light intensity (as measured in relative units). (a), (b) and (c) have a common abscissa.
on the negative represents a single photon which entered the microscope objective, and released a single photoelectron from the input photocathode of the image intensifier. Walton and Debenham (1980) describe the image intensifier system and its associated optics in more detail. The film (Kodak Tri-X) was developed in a standardised manner, and the densities across each negative measured by a microdensitometer (Joyce, Loebl and Co. Ltd MkIII B), using the known H-D curve for the film-developer combination. Thus the relative light intensities could be measured in arbitrary units and compared. With the ultrasound off, the light intensity noise was 0.08 units.

All measurements were taken in the near field (i.e. within 10 cm of the transducer) as in clinical practice, and the transducer ionated the region of interest from the right as seen in figures 2-5.

The proportion of a given sound field which is standing wave, expressed as a percentage, is calculated from the peak-to-peak acoustic pressure maximum at an
Figure 4. Results obtained for the thin brass plate at 22 °C. (a), (b) and (c) are as in figure 2.

antinode $P_{\text{max}}$ and the pressure minimum at the adjacent node $P_{\text{min}}$, from the relation

$$
\frac{(P_{\text{max}} - P_{\text{min}})}{(P_{\text{max}} + P_{\text{min}})} \times 100\%.
$$

Where bands of light are seen, average values are quoted across this region of the sound field.

Rocking the transducer often encouraged sonoluminescence. This was not due to the removal of bubbles from the face of the transducer, as the transducer was regularly cleaned of bubbles.

3. Results

3.1. Thick brass block

When the 27.5 mm thick brass block was placed at the end of the cell (figure 1) and the water bath was maintained at 22 °C, the sound field was found to be 79% standing
wave on average (figure 2(a)). Clear bands of light were seen (figure 2(b)), with maxima of intensity of up to about 1000 units (figure 2(c)). The bands appeared approximately 10 s after switching on the ultrasound although unbanded sonoluminescence was observed sooner. The light bands coincide closely with the positions of the pressure antinodes as calculated from the complete hydrophone trace. Occasional apparent mismatches are due to errors in precise measurement of the position of individual pressure antinodes with the hydrophone.

Figure 5. Results obtained for the thin brass plate at 37 °C. (a), (b) and (c) are as in figure 2. In figure 5(a) the mean pressure amplitude is about 1 MPa. This is consistent with the spontaneously high output of 10 W cm⁻² from the generator noted in the methods section. The occurrence of bands is not solely due to these augmented pressures, because bands were also seen from this geometry with mean acoustic pressures of about 0.4 MPa.
Cavitational effects of clinical ultrasound

At 37 °C, the acoustic field was on average 50% standing wave (figure 3(a)), and the sonoluminescence was roughly uniform over the field of view with an intensity of around 200 units (figures 3(b) and 3(c)).

3.2. Thin brass plate

When the 0.75 mm brass plate was placed at the end of the cell, the acoustic field was 40% standing wave at 22 °C (figure 4(a)). No bands of light were seen (figure 4(b)): the average intensity of sonoluminescence was around 5 units (figure 4(c)).

At 37 °C, the sound field was 61% standing wave (figure 5(a)), and clear bands of light were seen (figure 5(b)) peaking at intensities of 100 units (figure 5(c)). As with the thick brass block, light bands were positioned at regions of the sound field where the standing wave component was greatest. Good correlation between the position of the light bands and the pressure antinodes is again observed.

3.3. Absorber

From the hydrophone measurements, the standing wave component was estimated to be less than 2%, at either 22 °C or 37 °C. No bands of light were seen in either case. At both temperatures, when the ultrasound was first switched on, sonoluminescence was seen to flash evenly over the screen (but at very low intensities) for 1–2 s. Because our comparative measurements of intensity require a long time exposure (typically 20 s), no difference could be measured between exposures with the ultrasound on and those with it off. However, the subjective estimate was that light intensities during the flash were below one unit of intensity.

Regardless of the reflector used, sonoluminescence sometimes did not appear until a minute or so after first switching on the ultrasound. An oscillatory rotation (rocking) of the transducer about a vertical axis passing through its centre, of order ±20°, and with a period of 1–2 s, always helped to initiate sonoluminescence. Rocking is not an arbitrary motion introduced into this experiment, but an attempt to simulate the motion an ultrasonic transducer makes over a patient's body in physiotherapy. If clear light bands formed, they did not fade when rocking stopped. In experiments where bands did not occur, the sonoluminescence always faded once rocking ceased. Provided that the transducer was rocked if necessary, the presence or absence of bands of light in any given sound field showed 100% reproducibility.

4. Discussion

These results show conclusively that bands of sonoluminescence appear at the pressure antinodes in a standing wave field. The standing wave field is complex (for example, with the brass block at 22 °C the proportion of standing wave in the region of the bands varied between 68% and 92%), but the intensity of the light in the bands correlated well with the magnitude of the standing wave ratio at different points in the field. The acoustic reflectivity of the brass block was higher than that of the brass plate and since the appearance of sonoluminescence is associated with the standing wave pattern, it would be expected that bands of light would appear when the average standing wave proportion was highest. Bands were indeed seen when the brass block
was used at 22 °C (79% standing wave) and when the brass plate was used at 37 °C (61%), but not with the block at 37 °C, nor with the plate at 22 °C.

Pickworth et al (1988) found that the sonoluminescence produced by a Therasonic unit increased with temperature between 22 °C and 45 °C. The most likely explanation for the absence of light bands when the brass block was used at 37 °C is that cavitation had been confined to the region close to the transducer (Walton and Reynolds 1984). The presence of bubbles close to the transducer could also contribute to the apparently anomalous behaviour of the standing wave ratio.

Experimental arrangements in which there was little or no standing wave component failed to produce any persistent sonoluminescence and any sonoluminescence associated with travelling waves was very small. Furthermore, the banding pattern suggests that strong sonoluminescence is associated with standing waves.

Two factors contribute to the time delay before bands appear. First, bubbles may need to grow to resonant size by rectified diffusion before unstable cavitation can occur. However, theoretical calculations suggest that this process should take no longer than a few milliseconds. Second, bubbles will have to migrate to pressure antinodes.

Two other observations/related discussion:

(1) On switching on the ultrasound there was an immediate light flash that was quickly extinguished. This applies to all reflecting surfaces.

(2) Rocking the transducer caused similar flashes of light.

The most likely explanation is that when sound is switched on, or when the beam scans across the bubble field, previously non-oscillating bubbles start to oscillate. Initially, the radial motion of the bubble wall contains transients which decay to leave the steady-state oscillation. One of the authors (TGL) has produced numerical solutions of the equations describing bubble wall motion (Neppiras 1980), which take account of these transients. Results show that the transients enhance some compressions, with the temperature of some bubbles reaching more than 2000 K. High-speed photography of bubbles in a 10 kHz acoustic field has recently confirmed the action of these transients.

Sacks et al (1982) noted that if a test-tube filled with biological material was insonated, bioeffects were greatly increased by slowly rotating the test-tube. Church et al (1982) postulated that, in stasis, the acoustically active gas bubbles and the biological material would be well separated. The former, being below resonant size, gather at pressure antinodes, whilst the single cells collect at the pressure nodes as they are greater than resonant size. Church et al then suggested that motion of the test-tube disturbs the acoustic field, resulting in the possibility of cells coming into close proximity with violently oscillating bubbles, leading to increasing damage. Our observations suggest that this mixing process may not be the whole explanation and that any disturbance of the sound field resulting from relative motion of the transducer and specimen may increase the bubble activity itself.

The acoustic reflectivities encountered biologically range from a few percent (e.g. 3% between muscle and kidney) to values comparable with those in the above experiments. The higher acoustic reflectivities are found for interfaces between lung and any adjacent biological material (of order 70%) and for interfaces involving bone (of order 60% reflectivity) (Wells 1977). Reflectivities of nearly 100% will be present at the tissue-air interface diametrically opposite the transducer in a body, although a 1 MHz beam will have been attenuated by about 60% (pressure amplitude) after passing through 10 cm of tissue. In conclusion, the intensity of sonoluminescence is related to the standing wave ratio and when the standing wave component is high, strong banding effects at the pressure antinodes are frequently observed.
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cence can be produced in water at the power levels, and with the transducer movements, employed in physiotherapy, and with the acoustic reflectivities occasionally encountered in practice.

Acknowledgements

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Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 2. Thresholds for sonoluminescence from a therapeutic ultrasound beam and the effect of temperature and duty cycle

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Abstract. Sonoluminescence, produced when a therapeutic ultrasound generator operating at 1 MHz was used to insonate a tank of water, was detected using a photomultiplier tube and analysed using pulse height analysis. Spectra of the number of counts per second were obtained for the complete range of observed pulse heights, under exposure conditions similar to those used in clinical practice. Water containing different concentrations of dissolved gases and an agar solution were investigated during the course of the experiments. Measurements were made to establish a threshold for sonoluminescence and the total sonoluminescent light output from tap water insonated with continuous wave ultrasound at 1 W cm⁻² was estimated. The density of free radicals produced under these conditions was also estimated. The effects of temperature and duty cycle were investigated. Sonoluminescence increased with temperature over the range 22-45 °C and pulsed regimens produced more sonoluminescence than continuous wave ultrasound over a significant part of the pulse height spectrum.

1. Introduction

Sonoluminescence is the light emitted when a liquid is cavitated in a particular, violent manner (Harvey 1939) and the conditions that obtain during collapse cavitation may cause biological damage. The amount of sonoluminescence will depend on the nature of the liquid (Singal and Pancholy 1967), and its dissolved gases (Finch 1963), its temperature (Chendke and Fogler 1985), and hydrostatic pressure (Finch 1965), and the frequency (Griffing and Sette 1955), power and duty cycle of the ultrasound. A direct search for sonoluminescence in vivo is not normally possible and an assessment of the number and size of bubbles in body fluids and tissues is not easy (ter Haar and Daniels 1981). An alternative approach is to try to establish levels below which sonoluminescence is unlikely to occur. It should then be possible to state with greater confidence safe levels for work with ultrasound in patients. In this paper, some conditions under which sonoluminescence will occur in aqueous media are established and pulse height analysis is used to find the range of light outputs. An assessment is made of the amount of light produced in a given volume of liquid in a given time to provide an indication of the maximum amount of cavitation occurring under the conditions specified.
Temperature effects have been investigated in the range 22-45 °C, and duty cycle experiments have compared continuous wave ultrasound and three pulsed regimens commonly employed by physiotherapists.

2. Materials and methods

A 1 MHz ultrasound generator (a Thersonics 1030, Electro-Medical Supplies) was used to generate intensities up to 3 W cm\(^{-2}\). The available duty cycles were continuous wave, 1:2 (2 ms on and 4 ms off), 1:4 (2 ms on and 8 ms off) and 1:7 (2 ms on and 14 ms off). The power output of the generator was checked using a Biotec UWII Force Balance, and the manufacturer's figure of 440 mm\(^2\) for the effective radiating area of the transducer was used to calculate spatial average temporal average (sata) intensities. These figures agreed with the meter readings to within 10%. Using a needle hydrophone (Dapco NP10-3), the manufacturer's figures for the duty cycles were found to be accurate to within 5%, and are therefore adopted in this paper. The sata intensities and the duty factors were used to calculate the spatial average temporal peak intensities for the pulsed regimens.

To study sonoluminescence the liquid to be investigated was placed in a glass tank on top of a photomultiplier (RCA 8575, photocathode diameter 50 mm) (figure 1) with the transducer facing downwards in the liquid. From hydrophone measurements of
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The acoustic reflection coefficients of a glass plate, the average reflection coefficient from the base of the glass tank was found to be in the range 45–62%. The transducer, tank and tube were enclosed in a wooden box which was covered with layers of blackout cloth.

Each collapsing bubble produced a short burst of light which appeared as a current pulse in the photomultiplier. A pulse height analyser with differential discriminator was used to count the pulses and to analyse the distribution of pulse heights in the light burst. For some of the temperature studies described in §3.4, this counting system was replaced by a Levell multimeter type TM11 nanoammeter and an EMI 9781B side window photomultiplier with light guide was used. In some experiments the light intensity reaching the photomultiplier was reduced by inserting neutral density filters between the glass tank and the photomultiplier tube.

For the main set of experiments reported, freshly drawn tap water was used. This was allowed to settle and experiments were performed between 1 and 5 h later. In some cases tap water that had been left to stand for a few days, distilled or degassed water was used. The gas content of the water samples was measured using a Corning 178 pH/blood gas analyser. In one experiment, a solution of 1.875% agar, prepared by adding agar to boiling water, was used. After cooling, its viscosity was 170 cp.

The sonoluminescence produced was compared with a calibrated beta light, which gave a photon flux of $5 \times 10^8$ photons per second. The beta light has a faint yellow appearance so the wavelength of the light emitted is in the region of 580 nm. Sonoluminescence is essentially a black body radiation over the range 400–700 nm (Gunther et al. 1959). Thus the 580 nm of the beta light is representative of the middle of the wavelength range of sonoluminescence and the overall detection efficiency of the system will be similar for the two sources of light. The effect of temperature was investigated using a thermostimulator and thermocouple (FH 15-V, Grant Instruments (Cambridge) Ltd). The water was heated to 37.9°C and circulated around the water bath. To allow for clear viewing the thermostimulator was then switched off while readings were taken. When the temperature dropped to 36.5°C, the thermostimulator was switched on. This range of temperatures centres on the normal body temperature of around 37°C.

3. Results

3.1. Background spectrum

Since the ultrasonic vibrations could be transmitted to the photomultiplier tube in the experimental arrangement of figure 1, the background was tested in two ways: (1) with the ultrasound switched off; (2) with a continuous high power beam switched on, but with a piece of black card placed between the water tank and the photomultiplier tube to intercept any visible light generated. Both methods produced the same spectrum (figure 2(a)).

3.2. Threshold measurements

With freshly drawn tap water at room temperature in the tank, no difference was found between the pulse height spectrum generated at low intensities of ultrasound (<0.1 W cm$^{-2}$) and that produced under background conditions. At higher intensities (figure 2(a)) there was a marked increase in count rate for a given pulse height and
Figure 2. Sonoluminescence spectra from fresh tap water insonated with continuous wave, 1 MHz ultrasound, at various intensities.

(a) (+) background; (A) 0.5 W cm$^{-2}$; (O) 1.0 W cm$^{-2}$

(b) (+) background; (□) 0.25 W cm$^{-2}$

Every third point is shown on these graphs and figures 4, 5 and 8. This is sufficient to indicate the forms of the spectra, even on this log scale. Error bars are not shown as they are less than the size of the symbols; this also applies to figures 4-6 and 8. The crossing over of curves at very small pulse heights is the combined effect of pulse pile-up and saturation in the photomultiplier tube.

A shift in the mean pulse height to a higher value. The increases continued with intensity up to 3 W cm$^{-2}$, the highest intensity measured.

At 0.25 W cm$^{-2}$ (see figure 2(b)), there was a slight, but not insignificant, increase in the recorded count rate over a range of pulse heights, so the threshold for cavitation-induced sonoluminescence, under the conditions described, lies between 0.1 and 0.25 W cm$^{-2}$. With our standing wave system, 0.25 W cm$^{-2}$ corresponds to a pressure amplitude of 126–140 kPa at an antinode, and 33–48 kPa at a node. We have attempted to demonstrate that this is primarily a threshold and not simply a limit on the sensitivity of the detection system. Figure 2(b) can be used to find the percentage increase in light output over background at 0.25 W cm$^{-2}$ for different pulse heights. The most sensitive pulse height for detection of sonoluminescence is 1.0. Figure 3 shows the percentage increase in sonoluminescence at this pulse height for the full range of powers investigated. Above the detection limit, light output rises extremely rapidly with increasing intensity.

When distilled or degassed water was used, the threshold for sonoluminescence was found to be higher, and for a given set of conditions the spectra were more similar to the background spectrum (figure 4). The results of measurements of partial pressures of O$_2$ and CO$_2$ for the various types of water are shown in table 1.

3.3 Assay of light output

Neutral density filters, increasing in value by 0.2, were inserted between the tank of freshly drawn tap water and the photomultiplier tube. At 1 W cm$^{-2}$ a neutral density
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Figure 3. Percentage increase in count rate for different intensities of ultrasound at a pulse height of 1.0. The curve shows a sigmoid shape, which frequently characterises a threshold phenomenon. If the intensity is plotted on a logarithmic scale, it is clear that the threshold lies between 0.1 and 0.25 W cm$^{-2}$.

Figure 4. Spectra obtained by insonating (△) freshly drawn tap water; (□) old tap water; (○) degassed water; (+) distilled water, with 1 MHz, continuous wave ultrasound of intensity 1 W cm$^{-2}$. (×) is a background spectrum.

Table 1. Partial pressures of oxygen and carbon dioxide for various samples of water

<table>
<thead>
<tr>
<th>Sample</th>
<th>$P_{O_2}$ (kPa)</th>
<th>$P_{CO_2}$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly drawn tap water</td>
<td>17.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Old tap water (left to stand for a few days)</td>
<td>19.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Distilled water</td>
<td>24.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Degassed water</td>
<td>11.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>
filter of value 2.0 was the minimum that produced a spectrum indistinguishable from background. Thus by attenuating 99 in every 100 photons, the limit of detection of this system has been reached. When filters were inserted between the beta light and the photomultiplier, a neutral density filter of value 5.0 was required, indicating that the sonoluminescence was 1000 times less intense than the photon flux from this source. Hence the photon flux from sonoluminescence will be of the order of $5 \times 10^5$ photons per second.

3.4. Effect of temperature

Figure 5 shows that increasing the temperature to 37 °C always produced more sonoluminescence for 1 MHz continuous wave ultrasound at 1 W cm$^{-2}$. The same effect was observed at 0.5 W cm$^{-2}$, and also with 1:4 pulsed ultrasound. The background count is greater at the higher temperature, an effect that is primarily due to warming of the photomultiplier, but inspection of the curves at a pulse height where the background is negligible at both temperatures shows that the increase in sonoluminescence is real.

![Graph showing the effect of temperature on sonoluminescence spectra.](image)

Figure 5. The effect of temperature on the sonoluminescence spectra. Samples were insonated with 1 MHz continuous wave ultrasound at 1 W cm$^{-2}$. (+) background at 22 °C; (×) background at 37 °C; (○) with ultrasound at 22 °C; (A) with ultrasound at 37 °C.

Figure 6 is an expanded version of the results at 37 °C when 1:4 pulsed ultrasound was used. This spectral curve contains several peaks, which correspond exactly to readings taken at 37.9 °C, subsequent readings being at successively lower temperatures until the next heating (see § 2). This is further confirmation that light output is greater at higher temperatures and shows that the effect can be observed even when the change in temperature is small.
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Figure 6. Expanded version of part of the sonoluminescence spectrum from pulsed ultrasound (duty cycle 1:4) at 1 W cm\(^{-2}\) at 37 °C. All the points are shown. The vertical arrows indicate readings taken at 37.9 °C, with subsequent readings (to the right) being taken at lower temperatures until the next arrowed point where the water was reheated to 37.9 °C. Background has been subtracted from all readings.

The variation of sonoluminescence with temperature for pulse heights below 0.5 is not amenable to testing with the above methodology because of saturation of the photomultiplier. The temperature variation was therefore also tested simultaneously over the entire range of pulse heights, by replacing the photomultiplier and counting system with the EMI 9781B photomultiplier and nanoammeter. Sonoluminescence produced in the water bath was carried to the photomultiplier tube by a light guide, so temperature variations in the bath did not affect the photomultiplier.

The results show an increase of sonoluminescence with temperature in the range 22–45 °C (figure 7), for ultrasound intensities of 1 and 3 W cm\(^{-2}\). In all cases a constant dark current of 0.11 nA has been subtracted.

Figure 7. Total photomultiplier current as a function of temperature. lower curve; 1 W cm\(^{-2}\), upper curve; 3 W cm\(^{-2}\).
3.5. Effect of duty cycle

The effect of changing the duty cycle was investigated, keeping the frequency fixed at 1 MHz and the spatial average temporal peak intensity fixed at 1 W cm$^{-2}$. The continuous wave beam would be expected to give the highest count rate for all pulse heights, with the 1:2 duty cycle next, followed by the 1:4, then the 1:7, and the count rate should be in the ratio 1:1/3:1/5:1/8. This is indeed observed for the smaller pulse heights, but as the discriminator level is set higher, the continuous wave spectrum falls off faster than the others (figure 8(a)). At very large pulses, the 1:7 duty cycle produces most light, followed by 1:4, 1:2, and continuous wave ultrasound.

When degassed water was insonated, the curves still crossed over but at lower light levels (figure 8(b)). If an agar gel was used instead of freshly drawn tap water, no crossing over of the four curves was seen, and the continuous wave ultrasound always

![Figure 8. The effect of duty cycle on the sonoluminescence spectra. Samples were insonated with 1 MHz ultrasound at 1 W cm$^{-2}$ (x) continuous wave; (+) pulsed ultrasound of duty cycle 1:2; (O) pulsed ultrasound of duty cycle 1:4; (A) pulsed ultrasound of duty cycle 1:7. (a) results from freshly drawn tap water; (b) results from degassed water; (c) results from 1.875% agar gel.](image)
produced most light (figure 8(c)). When using agar the insonated medium contained less gas, because preparation of the gel involved boiling, but it was also more viscous. Note that because of the log scale in figure 8, the differences in light output are quite large and were consistently reproducible.

4. Discussion

4.1. Threshold measurements

When tap water was insonated with 1 MHz continuous wave ultrasound, light output was a function of ultrasound power output (figure 2(a)). If the ultrasonic power was gradually reduced, the minimum intensity at which sonoluminescence was just discernible was about 0.25 W cm\(^{-2}\) (figure 2(b)). No sonoluminescence was ever detected at intensities below 0.1 W cm\(^{-2}\). The steep rise in sonoluminescent output with intensity in the most sensitive part of the detector range suggests that this is a threshold for our system for practical purposes.

4.2. Gas content

Freshly drawn tap water always gave more sonoluminescence than either degassed tap water or distilled water (figure 4). There are few measurements reported in the literature on the gaseous condition of liquids during sonoluminescence experiments. The data in table 1 and figure 4 indicate that in our work the partial pressures of both oxygen and carbon dioxide may affect light output. Comparison of the first three sets of figures suggests that dissolved CO\(_2\) is an important source of gas required for bubble growth prior to unstable collapse. Although distilled water showed a higher PO\(_2\) than degassed water, it should be noted that the process of distillation is likely to remove a number of potential nucleation sites as well as dissolved gas.

4.3. Light output measurements

At 1 W cm\(^{-2}\) and 1 MHz, the photon flux from sonoluminescence was of the order of 5 \times 10^5 photons per second. The region of water under investigation had a volume of about 20-25 cm\(^3\), so the photon flux was 2 \times 10^4 photons cm\(^{-3}\) s\(^{-1}\). Assuming that each free radical pair produced one photon when it recombined, this is also an estimate of the number of free radicals produced per cm\(^3\). It is of interest to note that background radiation produced free radicals as a result of ionisation at a rate of approximately 10^4 ion pairs cm\(^{-3}\) s\(^{-1}\). Using different insonation conditions, Carmichael et al (1986) have reported free radical concentrations from sonoluminescence comparable to those produced by 10 \mu G) of \(^{60}\)Co gamma radiation. We have shown (Leighton et al 1988) that sonoluminescence and hence presumably the free radicals can be highly localised at the pressure antinodes.

4.4. Temperature

Results in figures 5, 6 and 7 show conclusively, using two independent systems, that sonoluminescence increased with temperature in the range 22-45 °C for our experimental system. Such a conclusion is consistent with an early observation of Blake (1949), who showed that cavitation thresholds decreased with temperature. We are aware that there are a number of reports in the literature suggesting that over most,
if not all, of the temperature range studied here, sonoluminescence falls with temperature (Sehgal et al. 1980, Chendke and Fogler 1985, Jarman 1959, Iernetti 1972). However, all these workers used frequencies lower than MHz, most liquids other than water and some found an increase in sonoluminescence with temperature over part of the temperature range they studied. Furthermore, other factors likely to affect the amount of sonoluminescence were not always well controlled. The results in figures 6 and 7, which were repeated on several occasions, appear to show conclusively that sonoluminescence increases with temperature.

4.5. Duty cycle

For large pulse heights, continuous wave ultrasound produces least light in water, whilst the 1:7 duty cycle produces most, even though the ultrasound is on for the shortest time. This effect is not seen in agar, where continuous wave ultrasound produces the most light for all pulse heights.

Pulsed enhancement of the effects of stable cavitation has been reported by Hill et al. (1969), and Ciaravino et al. (1981) reported pulsed enhancement of unstable cavitation. These workers were using different experimental conditions and different regimens, and their explanations, and those of Flynn and Church (1984) cannot entirely explain our results. The transient excitation theory proposed by Leighton et al. (1988) will cause pulsed enhancement of sonoluminescence. However, this cannot explain all the features of figures 8 (a), (b) and (c), particularly the crossing over phenomenon. The explanation we favour is based on the clustering of small bubbles at the pressure antinodes as a result of Bjerknes forces (Walton and Reynolds 1984). This creates local degassing and acoustic impedance mismatch, thereby inhibiting cavitation. The bubbles migrate away from the pressure antinodes during the off-time and some regassing of the medium occurs. They return when the sound is switched on again. Theoretical calculations (Leighton et al. 1988) show that this explanation is consistent with the time scale of bubble migration required to explain the results of figure 8. The crossing over effect did not occur in agar where bubble migration would be greatly reduced. In future work the effect of pulsing will be studied in agar solutions of different concentration. The anomalous behaviour of pulsed ultrasound might not occur in body tissues, but the behaviour of blood, which is of intermediate viscosity, cannot be predicted without further experiments.

Experiments are also in progress, using the conditions known to cause sonoluminescence in water, to study the viability of mammalian cells when insonated in aqueous media and in agar, and so search for direct evidence of sonoluminescence in biological tissues.

Acknowledgements

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STUDIES OF THE CAVITATIONAL EFFECTS OF CLINICAL ULTRASOUND BY SONOLUMINESCENCE.

3. Cavitation from pulses of a few microseconds in length.

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Short Title: Cavitational Effects of Clinical Ultrasound.
Abstract.

Sonoluminescence can readily be seen when aerated water is insonated with continuous wave therapeutic ultrasound at room temperature but is not easily observed when short pulses of diagnostic ultrasound are used. In this work an ultrasound generator, operating in the region of 1 MHz and capable of producing pulses of different length and repetition rate, was used for insonation. The pulse repetition frequency of the ultrasound was fixed at 1 kHz since this is characteristic of diagnostic machines, and a series of thresholds for sonoluminescence was obtained for two transducers, one therapeutic and one diagnostic, as the number of cycles in each pulse was varied.

Sonoluminescence was observed for pulses of a few cycles, but the threshold for onset of sonoluminescence increased sharply with decreasing pulse length. It was possible to generate the powers required for sonoluminescence at pulse durations of more than 20 cycles per pulse, which is uncomfortably close to the diagnostic situation and well within some regimes for Doppler ultrasound. Indeed the reason that sonoluminescence was not observed for even shorter pulses may have been that the output power from the generator used fell off sharply as the number of cycles per pulse decreased below 20.
1. Introduction.

Transient or stable cavitation of gas-filled bubbles may occur in a liquid that is subjected to high intensity ultrasound. During a transient collapse, the gas in the bubbles reaches temperatures at which free radicals are formed (Edmonds and Sancier, 1983). On recombination, these free radicals produce sonoluminescence (Walton and Reynolds, 1984), and thus the production of light from a medium, on insonation, indicates a potential biological hazard.

Sonoluminescence is readily seen in our experimental system when a tank of water is insonated with continuous wave therapeutic ultrasound (Pickworth et al, in press), and is primarily associated with the establishment of a standing wave pattern (Leighton et al, in press). It is also observed with pulsed physiotherapeutic ultrasound, where the shortest pulses are typically 2 ms, and a standing wave field is set up in water whenever a reflection occurs. We wish to know whether cavitation can occur with the same experimental arrangement under diagnostic ultrasound conditions where the pulse length may be too short for a standing wave system to be set up, but other cavitation mechanisms may be operating e.g. non-linear distortion causing shock excitation (Bacon 1984, Duck and Starritt 1984). Crum and Fowlkes (1986) have observed luminescence flashes characteristic of violent cavitation from ultrasonic pulses as short as one cycle at a frequency of 1 MHz, implying that there is a mechanism for cavitation when standing waves are not present. However they used duty cycles of between 1:3 and 1:20, i.e. pulse repetition frequencies of between 250 and 50 kHz, whereas in the diagnostic situation, duty cycles are more typically 1:1000 (Duck et al 1985). As discussed in several earlier papers (Hill et al 1969, Ciaravino et al 1981, Pickworth et al in press), pulsed enhancement of cavitation can occur if the length of the
The present work involves an alternative approach to that of Crum and Fowlkes. The frequency of the ultrasound from the generator was set at about 1 MHz to provide comparisons with previous work, and the pulse repetition frequency was fixed at 1 kHz. The ultrasound intensity (I), and the number of cycles in each pulse (N) were then varied and for each value of N, the threshold value of I for sonoluminescence was found. Sets of threshold values for two transducers have been obtained and possible implications for the diagnostic use of ultrasound are discussed.


2.1 The Signal Generator.

The ultrasound generator system was built at the University of Surrey and comprises three separate modules; a signal oscillator and control module, a power supply providing stabilised H.T. and E.H.T. voltages, and an ultrasonic power output unit with tuning and amplitude controls.

The high frequency pulse generator provides a continuous train of pulses which feeds the power output stage via a 50 Ω line driver. An additional feed controls a count down timer stage which in turn provides a gating signal to the mode control switch. Pulsed or continuous wave modes are provided. The output of the mode control switch feeds the control input of the power output stage.

In pulsed mode, the counter stage is initiated by a trigger pulse from a rate oscillator. When the counters reach zero another pulse is
generated, thus the initiating trigger pulse and the "count complete" pulse define the exact number of pulses within the pulse envelope. The two pulses toggle a bistable which controls the output power amplifier.

The output stage was complicated by the techniques used to generate the variable frequency drive and pulse counting and gating circuitry for the pulsed mode. The grid of the power output valve is driven by rectangular pulses which, due to the band pass characteristics of the tuned circuit loading the anode, produce a sine wave output signal. The quality of the sine waves produced will depend on the "Q" of the tuned circuit loading the output. A high "Q" tuned circuit gives good waveforms but produces a slow build-up and decay envelope in the pulsed mode.

To enhance the build-up of the pulse, the output valve is overdriven by modulating the cathode with a decaying pulse at the start of each pulse. The decay of the pulse is damped by applying a short circuit to the tuned circuit by way of a hydrogen thyratron which, when fired, absorbs the energy in the tuned circuit.

The mode control stage gates the ultrasonic oscillator to produce the required high frequency pulse burst. This is amplified and buffered by a unity gain push-pull stage before feeding the grid of the output valve. Amplitude control is achieved by varying the H.T. voltage of the amplifying stage. The signal across the transducer is capacitatively attenuated and rectified to provide a peak RF voltage detector.

Electrical tuning was achieved using plug-in ferrite cored fixed inductors and an additional variable capacitor in parallel with the transducer for fine tuning control. The design enables the generator to
be robust enough to survive being driven at maximum output into a mismatched load.

Thus the properties of the signal generator are such that it can produce;

i) a variable intensity output, from zero to the maximum limit for the transducer,

ii) a sound wave of frequency approximately 1 MHz tunable over a narrow range,

iii) continuous wave or pulsed ultrasound,

iv) in pulsed mode, any number of complete cycles between 1 and 99,

v) a pulse repetition rate of between 100 Hz and 1 kHz.

2.2 The Transducers.

The signal generator was used to drive two different transducers. One was a physiotherapeutic transducer of the type designed for use with a Therasonic 1030 generator (Electro-Medical Supplies). It had an effective radiating area of 440 mm$^2$. To drive this transducer, the frequency of the generator was set to 1.09 MHz, the exact resonant frequency of this transducer. The second transducer was a diagnostic transducer NE 4161 (Nuclear Enterprises), with an effective radiating area of 299 mm$^2$ and designed to operate at 1.5 MHz. Since this frequency could not be obtained with our generator, a calibrated needle hydrophone (Dapco NP10-3) and oscilloscope were used to find the maximum value of the spatial peak temporal peak intensity. This was obtained with a driving frequency of 0.97 MHz.

The needle hydrophone was also used to determine the temporal shapes of the pulse produced by the two transducers. The appearance of the pulse
as measured by the hydrophone was recorded on an oscilloscope so that build-up and decay times could be studied.

2.3 Calibration of Output.

Two methods were used to calibrate the ultrasound output. Firstly, for each transducer, the position of the spatial peak was found using the needle hydrophone. This was 10 cm from each transducer. The value of the peak negative pressure was then found at this point as the peak RF power output was varied. The spatial average, temporal average intensity was found from radiation pressure measurements using a calibrated force balance (Anson et al, in preparation). For these measurements a transducer-target distance of 10 cm which corresponded to the position of the focus of the diagnostic transducer, was selected.

Since the pulse repetition frequency of the generator is known, a value for the spatial average pulse average intensity can be found from the relation

\[ I_{sapa} = I_{sata} \times \frac{1000}{N} \]  

We shall equate \( I_{sapa} \) to \( I_{satp} \). Due to build-up and decay times these are only approximations which are better at 99 cycles per pulse than at 10 cycles per pulse. \( I_{satp} \) is the intensity figure given on most therapeutic units.

2.4 Experimental Set-up.

The experimental arrangement is shown in figure 1 and has been described in detail elsewhere (Pickworth et al, in press). Essentially the transducer insonates a tank of water, and sonoluminescence is
detected by a photomultiplier tube (RCA 8575R) coupled to a pulse height analyser. For the present set of experiments, a limited range of pulse heights was selected from the middle of the spectrum i.e. large enough to be above the region of electronic noise and pulse pile-up but not so high that the background count has become insignificant.

The presence or absence of sonoluminescence was investigated for both transducers using a fixed pulse repetition frequency but the number of cycles in each pulse varied between 1 and 99, and the peak output from the generator varied between 0 and 300 V. Observations were made as the pulse length was both increased and decreased to its final value at fixed intensity, and as the intensity was increased and decreased to its final value at fixed pulse length.

Light pulses were counted for thirty seconds and sonoluminescence was assumed to have occurred if a series of counts was significantly greater than the background over the selected pulse height range.

3. Results.

3.1 Pulse Shapes.

The temporal shapes of the pulses produced by the two transducers are shown in figure 2. N is defined as the number of complete cycles within the time that the generator is counting out the pulse duration. Thus N includes the pulse build-up time but not the decay time as indicated in figure 2. Both pulses reached 95% of their maximum amplitude within a few microseconds (8-9 μs for the therapeutic transducer, 6-7 μs for the diagnostic transducer). From this point until the pulse from the generator is terminated and the hydrogen thyratron fired, there is only
around 5% variation in the maximum and minimum pressure values. So far the two pulses are very similar. However with the therapeutic transducer the decay time significantly lengthens the effective pulse duration. The acoustic pressure has only decreased to about 80% of its maximum value 10 us after the end of the generator's pulse, and only after about 17 us has there been a significant reduction. Thus at short pulses the effective pulse length will be much greater than the measured pulse length with this transducer. In contrast, with the diagnostic transducer the pulse stops almost instantaneously (figure 2). The slow build-up now causes greater inaccuracies than the decay period, and any cavitational effect will be underestimated.

3.2 Calibration.

The hydrophone was used to convert scale readings on the RF power output meter to spatial peak temporal peak negative pressure amplitudes using a hydrophone calibration factor of 2.25±0.05 kPa/mV. Results are shown in figures 3 and 4.

Spatial average temporal average intensities were measured using the force balance for different numbers of cycles per pulse and results are shown in figures 5 and 6. From these figures and equation 1, values of $I_{sapa}$ may be calculated. Table 1 shows results for the diagnostic transducer and similar results were obtained for the therapeutic transducer. The errors quoted include the systematic error introduced by pulse build-up and decay and this can be seen to be small. The error due to pulse shape was somewhat greater for the therapeutic transducer.
3.3 Observations of Sonoluminescence.

The background count for the middle of the selected range of pulse heights was 950±200 in thirty seconds. Sonoluminescence was assumed to be present either when the count obtained from the insonated water was greater than the average background count plus three standard deviations at this pulse height (1550 for the quoted background), or when the count was consistently between one and three standard deviations above background at three or more pulse heights.

The results obtained using the therapeutic transducer are summarised in figure 7. On a graph of RF power output from the generator against the number of cycles in each pulse, three regions can be seen. In the upper region, sonoluminescence could always be detected. In the lower region, sonoluminescence did not occur, or was at a level too faint to be recorded as significant (detection limit about 5×10^5 photons per second). There is also a broad middle region where sonoluminescence occurred sometimes, while at other times it did not.

The principle feature of the graph is that as the number of cycles per pulse, N, is decreased, the ultrasound intensity, I, required to generate cavitation is greater. With the powers that could be generated with the present system, the smallest value of N for which sonoluminescence could be observed was N=7. In particular no sonoluminescence could be observed for N=1. The maximum voltage output that could be imparted to the diagnostic transducer by the generator was about 220 V, which corresponded to an intensity (sata, see figure 6) of 0.18 W cm⁻² at 99 cycles per pulse. The fact that this transducer was not being driven at its resonant frequency meant that it was not possible to get as much power out of it as out of the
therapeutic transducer. The results for the diagnostic transducer are shown in figure 8. This graph has the same form as figure 7, but there are some differences. The minimum value of N at which sonoluminescence can be observed is now 20, and the band of uncertainty is narrower.

In both figures 7 and 8 the appearance or non-appearance of sonoluminescence in the middle region is in part random, although it is influenced by whether the output is being turned up or down to reach the value specified. As shown in figure 9, for the diagnostic transducer, if the power is being increased to its final value, or if the number of cycles per pulse is being increased to its final value, sonoluminescence is more likely to be absent, whereas if the power is first turned up to its maximum value before being turned down to its final value, or if the number of cycles per pulse is decreased from 99 to its final value, sonoluminescence, once initiated, is sustained at the lower values. Noted that figures 7 and 8 show the boundaries between the three regions and not experimental points.

4. Discussion.

This work shows that it is possible to record sonoluminescence from water on insonation with pulses of a few microseconds duration when relatively long time intervals between pulses are used. In general, the results with the two types of pulse are similar. The threshold for sonoluminescence detection remains virtually unchanged between 60 and 99 cycles per pulse. For the therapeutic transducer the threshold power output is 70-130 V (figure 7), corresponding to an intensity (sata) at 99 cycles per pulse of between 0.03 and 0.07 W cm$^{-2}$ (figure 5), while for the diagnostic transducer the threshold power output is 135-155 V (figure 8), corresponding to an intensity of 0.06-0.08 W cm$^{-2}$ at 99.
cycles per pulse (figure 6). The corresponding peak negative pressures are 330-660 kPa for the therapeutic transducer (figure 3) and 270-330 kPa for the diagnostic transducer (figure 4). If the threshold is expressed as a pulse average (table 1) it is of the same order of magnitude as that found for therapeutic ultrasound which utilises long pulses of 2000 cycles, or continuous wave ultrasound.

In the regions of uncertainty in figures 7 and 8, the appearance of sonoluminescence depends partly on whether N and/or I is being decreased or increased in order to reach the values at which measurement is to be made. If N or I is decreased towards its final value, sonoluminescence is more likely to be present, indicating that it is more easily sustained than initiated.

When the number of cycles in the pulse becomes very small the peak RF output required for sonoluminescence rises sharply. For the diagnostic transducer this rise is at N=20. For the therapy transducer the sharp rise appears to be for much shorter pulses (N=10). However because of the long decay time for the therapy transducer (figure 2) the effective pulse length is about 10-15 cycles longer than the recorded value of N. Thus the steep rise in peak RF output occurs when the pulse contains less than about 20 cycles for each transducer. Using the data in figures 5 and 6 it may be shown that the sharp rise in RF power required to induce sonoluminescence as the pulse length is reduced occurs at an ultrasound intensity (sata) of about 0.02-0.03 W cm⁻² for each transducer.

Crum and Fowlkes (1986) mention a ringing down effect in their pulses. Their acoustic pressure waveform was not a single cycle (see figure 2). However, they point out that their pulse closely resembles the scanning
mode pulse of a diagnostic ultrasound system, and that it is similar to
that used by Flynn (1982) in his theoretical studies of the
cavitational effects of pulses of one microsecond duration. A
diagnostic scanner will produce a very short pulse but it is still not
a single cycle. All work in which pulses of 1 or 2 cycles are used is
subject to practical limitations on pulse shape. It may then also be of
importance whether the pulse is positive- or negative-going first.

It has been suggested that sonoluminescence is associated with standing
waves, and Leighton et al (in press) give a convincing demonstration
that strong sonoluminescence depends on the presence of a high standing
wave content. Observation of sonoluminescence at low values of N
implies that any light must have come from the bottom of the water tank
if standing waves are a prerequisite for sonoluminescence. For example,
with a pulse of 20 cycles there can only be standing waves within
13.6 mm of the bottom of the tank (the wavelength of the ultrasound
being 1.36 mm). We have examined the insonated tank with an image
intensifier: as either N or I is decreased, bands of sonoluminescence
(see Leighton et al) gradually fade away, but they are never stronger
near the reflecting surface. The implication is that there is some
sonoluminescence associated with travelling waves.

Flynn (1982) has calculated that microsecond pulses of ultrasound can
generate transient cavitation in water, although the peak intensities
required are 10-30 W cm^{-2}. In our work, a practical limit to the lowest
value of N at which sonoluminescence could be recorded was set by the
power that could be achieved with the generator. Extrapolation of
figures 7 and 8 to one or two cycles per pulse is difficult but the
results do not contradict Flynn's calculation. Some diagnostic systems
now in clinical use generate microsecond length pulses of ultrasound
with temporal peak intensities in excess of 100 W cm\(^{-2}\) (Carstensen and Flynn 1982) and with peak negative pressures of 2 MPa (Duck et al 1985) whereas in our work the maximum values of peak negative pressure are 1.4 MPa for the therapeutic transducer and 480 kPa for the diagnostic. For Doppler equipment, pulse durations of 20 or 30 cycles per pulse are not uncommon and pulses of 90 cycles are possible (Duck et al 1987). Pulse repetition frequencies are between 1 and 10 kHz, peak negative acoustic pressures can again be anything up to 2 MPa, and peak intensities may be as much as 300 W cm\(^{-2}\). These figures indicate that cavitation could occur under conditions encountered with diagnostic and Doppler ultrasound in aqueous media.

In conclusion, we have demonstrated that sonoluminescence can occur when the time interval between pulses is relatively long. At short pulse lengths, the ultrasonic intensity required to initiate sonoluminescence is high and the light output is low. The output characteristics of diagnostic imaging and Doppler equipment should be such that the peak intensity and the pulse duration are at their lowest practicable value, so that the chance of sonoluminescence occurring is minimised.

Acknowledgements.

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Average values of Isapa for the diagnostic transducer over the range of pulse lengths. The pulse lengths used to obtain these averages were N=10, 20, 40, 60, 80 and 99.

<table>
<thead>
<tr>
<th>Pk RF o/p /V</th>
<th>Isapa ± S.D. /W cm⁻²</th>
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<tr>
<td>60</td>
<td>0.17 ± 0.02</td>
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<tr>
<td>70</td>
<td>0.22 ± 0.05</td>
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<td>80</td>
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<td>220</td>
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References.


Figure Captions.

Figure 1. Schematic diagram of apparatus.

Figure 2. Temporal pulse shapes of the therapeutic transducer (top), the diagnostic transducer (middle) and the diagnostic generator and transducer used by Crum and Fowlkes (1986) (bottom), at their respective spatial peaks.

Figure 3. Peak negative hydrophone pressure against scale reading of RF voltage output for the therapeutic transducer.

Figure 4. Peak negative hydrophone pressure against scale reading of RF voltage output for the diagnostic transducer.

Figure 5. Spatial average temporal average intensity against scale reading of RF output for the therapeutic transducer for several pulse lengths; 

(× × ×) 99 cycles per pulse, 
(+ + +) 80 cycles per pulse,  
(○ ○ ○) 60 cycles per pulse,  
(△ △ △) 40 cycles per pulse, 
(□ □ □) 20 cycles per pulse, 
(· · ·) 10 cycles per pulse.

Figure 6. Spatial average temporal average intensity against scale reading of RF output for the diagnostic transducer for several pulse lengths. Symbols refer to the same pulse lengths as in figure 5.

Figure 7. Peak RF output against the number of cycles per pulse for the therapeutic transducer, showing three distinct regions. The shaded area
represents settings where sonoluminescence sometimes occurs.

Figure 8. Peak RF output against the number of cycles per pulse for the diagnostic transducer, the shaded area again representing the region of uncertainty.

Figure 9. Expanded section of figure 8 showing how the appearance of sonoluminescence depends on whether I or N is being turned up or down;

(△) sonoluminescence present, I or N being increased,
(▼) sonoluminescence absent, I or N being increased,
(▽) sonoluminescence present, I or N being decreased,
(▼) sonoluminescence absent, I or N being decreased.
Fig. 1
Fig. 2

(a) Acoustic pressure

8-9 cycles

N

17 cycles

Off

(b) Acoustic pressure

(c) Acoustic pressure

Off

Fig. 2
Fig. 3

Peak negative hydrophone pressure / kPa

Peak RF output / V
Fig. 4

Peak negative hydrophone pressure /kPa

Peak RF output /V
Fig. 5
Fig. 6
Fig. 7
Sonoluminescence always observed in this region

Sonoluminescence never observed in this region

Fig. 8

Peak RF output /V

number of cycles in each pulse, N.
Sonoluminescence always observed in this region

Sonoluminescence never observed in this region

Peak RF output /V

number of cycles in each pulse, N