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MONITORING CAVITATION AT 1.5 MHz.

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Ultrasound, in the megahertz frequency range, is being used increasingly for medical purposes at peak intensities (particularly in therapeutic applications) where the possibility of biological damage to tissues or circulating blood cells by cavitation must be considered a potential hazard. Cavitation is defined in this context as bubbles driven into non-linear oscillation in the sound field and which may be responsible for certain physical, chemical or biological effects. At low sonic amplitude, the process known as rectified diffusion may also take place to provide gas nuclei of appropriate size for cavitation to occur. The subsequent process has been referred to as "stable gaseous cavitation". No programme for the investigation of this phenomenon can proceed without some means for its monitoring, and the work reported here is the first step towards that goal.

The experimental arrangement is shown schematically in Figure 1. A 45 ml sample holder is suspended in the path of the beam, which may be focused, using a concave faced transducer, or plane wave, in either standing or travelling wave mode, using either an acoustic mirror or absorbing horn. The generator can supply either continuous or pulsed ultrasound over a wide range of frequencies, pulse-lengths and duty ratios, and at intensities well in excess of those encountered in

present medical applications. However, the work reported here is carried out at 1.5 MHz drive frequency only. The coupling medium is well-degassed and autoclaved circulating water maintained at a constant temperature (sample holder contents maintained to $\pm$ 1°C) throughout the experiment. In keeping with our concept of cavitation resulting from non-linear bubble oscillations, we have adopted the generation within the sample of a steady subharmonic signal, at half the drive frequency, as a criterion for the effect. It is essential, therefore, to monitor that no such signals are generated within the coupling medium itself. This is checked during each experiment over the range of intensities used. On occasions, subharmonic signals of short duration are picked up from the coupling medium, and these are attributed to stray bubbles in the system, as has been demonstrated by deliberately introducing bubbles into the field. Since these transient emissions are eliminated by the addition of surfactant to the water, this precaution is adopted throughout. The subharmonic signals are detected by a 2.5 cm diameter PZT transducer set in the side of the chamber, with its axis aligned orthogonally to the driving beam. The probe output is amplified (up to 70 db), filtered ($\sim$ 50 kHz band-width), and displayed on an oscilloscope.

Since the centre of the cylindrical (2.5 cm radius) sample holder is fixed at 3 cm from the transducer face, experiments are conducted in the near field. Acoustic output, averaged over the transducer area, is monitored with a radiation pressure balance under free-field conditions (large water tank, lined with acoustic absorber) and is found to follow closely the input power to the transducer, in the absence of cavitation in the tank (Figure 2).

With fresh tap water or aerated chemical solution in the sample holder, a subharmonic signal is detected only above a definite and repeatable threshold, and remains steady throughout the experiment. For pulsed fields (75 μsec pulses, 1 : 1 duty ratio) the subharmonic signal is similarly stable, with the same threshold, although it changes from a sinusoidal to saw-toothed behaviour. It was found impossible to generate the subharmonic with pulses shorter than 6 μsec, even at the highest powers available from the generator.

Sonochemical effects are often used as monitors of cavitation activity and two such effects were investigated to establish their association with subharmonic generation: the release of I_2 from KI solution in the presence of CCl_4 (starch test) and the oxidation of Fe^{++} to Fe^{+++} in ferrous ammonium sulphate solution. In both cases the end product was assayed with a Beckmann spectrophotometer. Typical results are shown in Figures 3 and 4. The thresholds for these effects are close to that for subharmonic generation, indicating that non-linear bubble activity is a prerequisite for their occurrence. In both cases, it is interesting to note that the threshold of the sonochemical effect rises from threshold to a peak at $\sim$ 1.5-2 W/cm^2, and then falls with increasing intensity. Such effects may be suitable, however, for quantitative monitoring of cavitation activity. These experiments were carried out with fully aerated samples, and further investigations are planned varying the gas content of the solutions.

Biological effects are often ascribed to cavitation, and two systems were selected for investigation.

Human lymphocytes were used in vitro, with the cells sonicated in suspension culture and assessed for damage at times after sonication. The end-point chosen is nuclear pyknosis (homogenisation and shrinkage of the nucleus) - a non-specific indicator of cell death. The results indicate a close similarity between the threshold for I_2 release from KI solution and the onset of lymphocyte killing.

Coliform bacteria are in essence an in vivo system since the bacteria grow in what can be considered a natural environment, and are discrete entities not parts of a larger system. Two tests were used: (i) the ability of the bacteria to form colonies after sonication, and (ii) the assessment of damage to bacterial DNA using tritium labelling in a hydroxylapatite chromatography system. It was found that there is a marked reduction in cell survival after sonication at levels well below the threshold for human lymphocyte killing. However, no damage to DNA was apparent, and this points to another mechanism of cell death, which will be the subject of further investigations. In no cases were bioeffects seen in the absence of subharmonic emissions. (These results are to be published more fully elsewhere)

Conclusions.

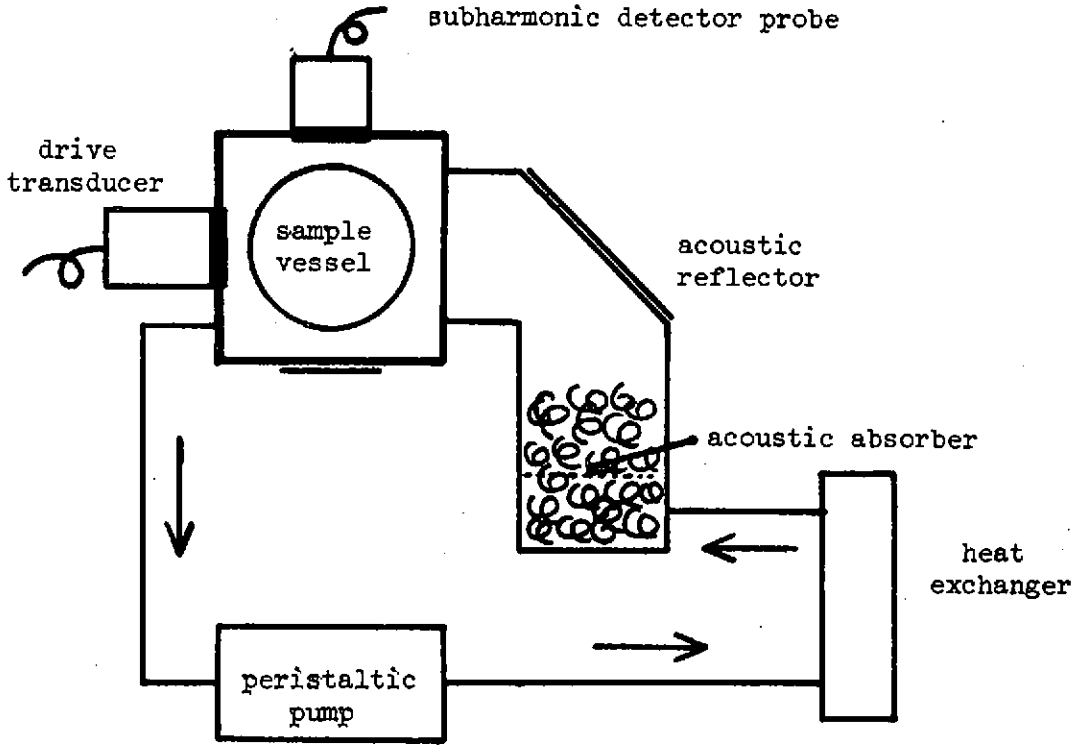
With the system described here, a definite and reproducible threshold for subharmonic excitation, at $\sim$ 0.4 W/cm^2, is found for gassy water. This compares well with the Eller-Flynn theoretically-derived threshold (Nyborg, personal communication). Subharmonic activity disappears in de-aerated water (for the intensities employed here) and transient emissions result when bubbles are introduced.

These experiments provide justification for the use of the subharmonic as an indicator of cavitation onset, even though this may be only stable non-linear bubble oscillation at these relatively low acoustic intensities.

An interesting correlation has been found between the subharmonic and sonochemical activity thresholds in the two systems studied, and this suggests a mechanism for these effects based on cavitation.

The bioeffects reported here occur only in association with subharmonic emission, but it is inconceivable that all biological consequences of ultrasound depend on cavitational activity. The search is being continued for a sensitive biological system which responds to sonic intensities below that for subharmonic generation.

Figure 1



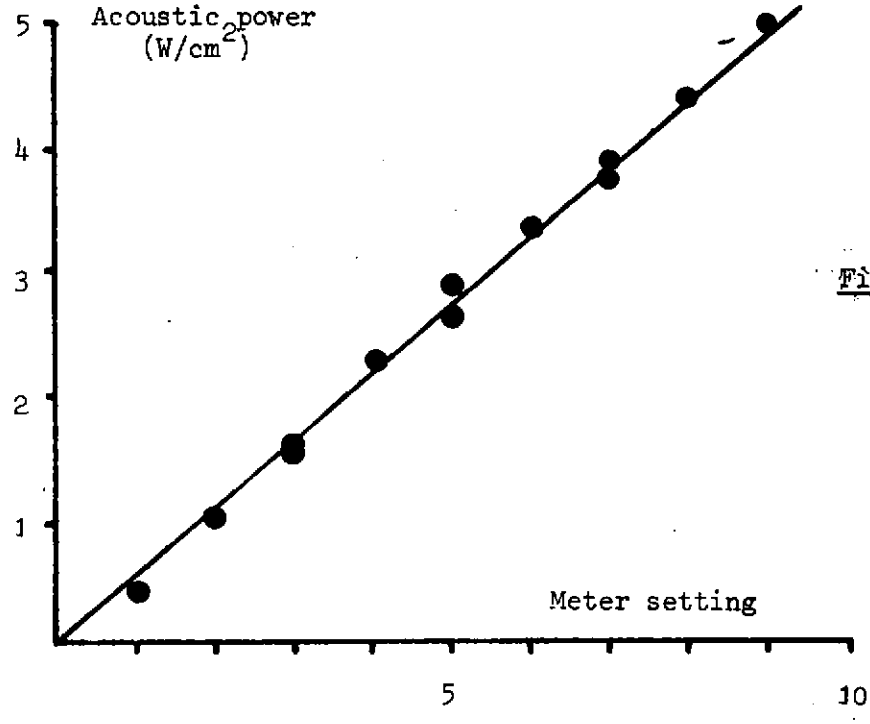


Figure 2

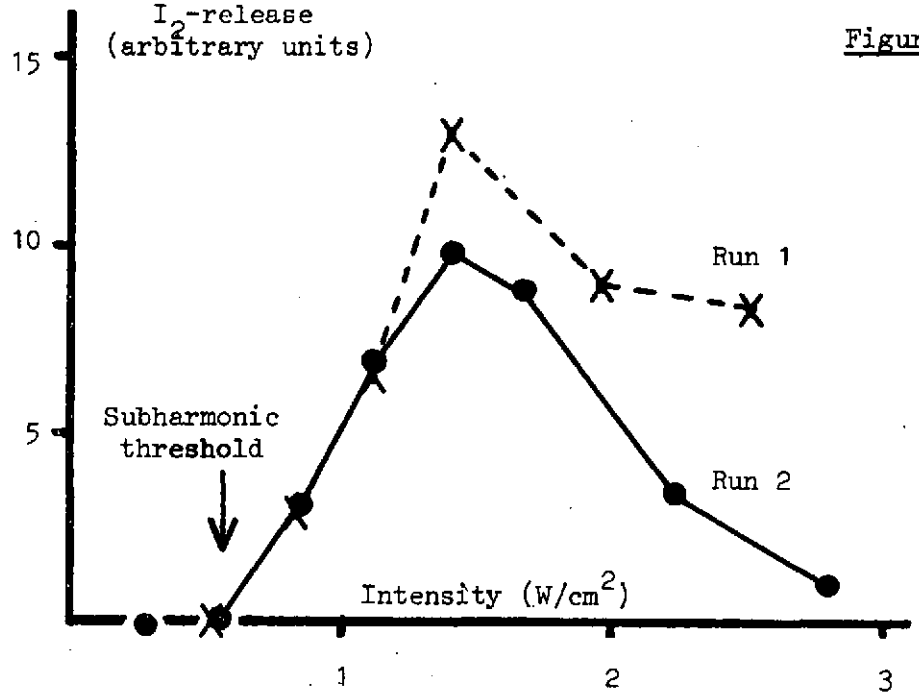


Figure 3

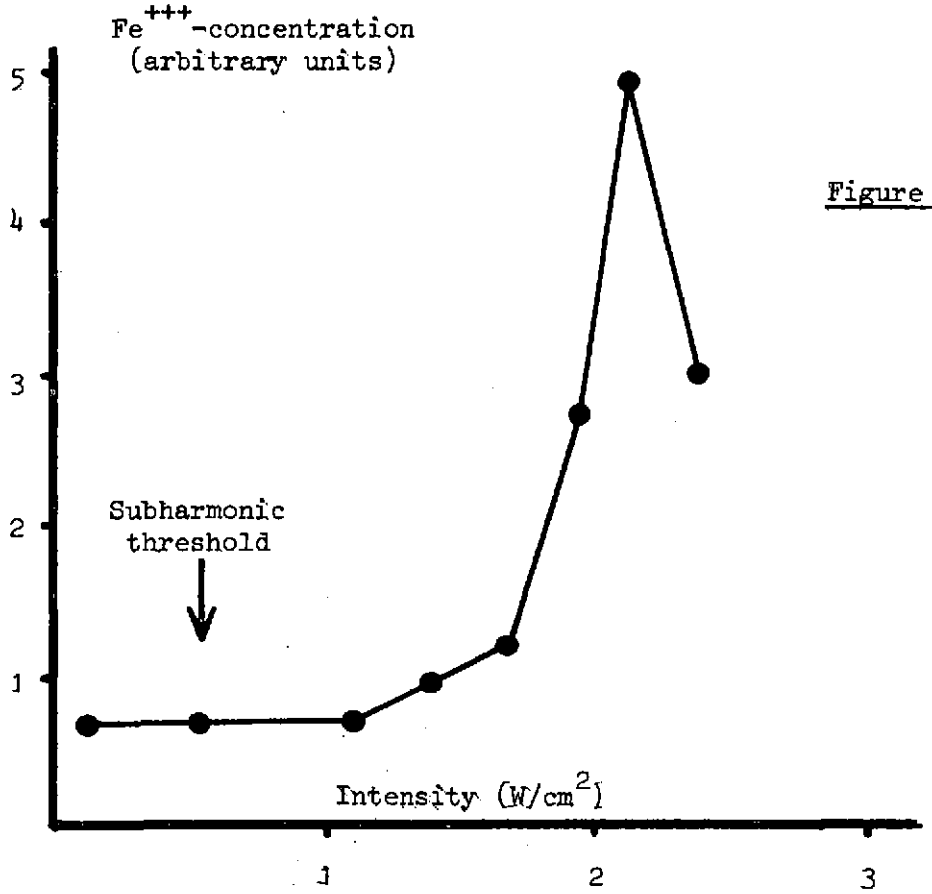


Figure 4

DISCUSSION

A question was asked about the relative sensitivities of the two sono-chemical tests described in the paper (i.e. liberation of iodine from potassium iodine solution and oxidation of ferrous iron to ferric). The Author replied that either could probably be used reliably as a quantitative test of transient cavitation activity.