A search for sonoluminescence *in vivo* in the human cheek

T.G. Leighton*, M.J.W. Pickworth, J. Tudor† and P.P. Dendy****

* Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, UK; ** Department of Medical Physics, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK and † Department of Radiology, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

Received 28 June 1989; revised 8 September 1989

In a previous experiment, sonoluminescence was observed in aerated water, especially at the pressure antinodes in the standing-wave field of a physiotherapeutic ultrasound device (Therasonic 1030). Mammalian cells *in vitro* showed growth inhibition when placed at the pressure antinodes but not at adjacent pressure nodes. In the light of these results, we looked for sonoluminescence *in vivo* when a similar standing-wave field was set up. To detect luminescence, a light guide was held against the inner surface of the human cheek. This would channel any luminescence photons to a cooled, red-sensitive photomultiplier which would quantify the light. Direct insonation of the cheek produced no detectable luminescence. Similarly when a water bag was placed against the outer surface of the cheek, and the latter was insonated through the bag, no luminescence was detected. Sonoluminescence from the water bag was, however, detected when the bag was placed against the inner surface of the cheek, showing that absorption of sound by the cheek tissue was not preventing cavitation. Further analysis showed that if cavitation had been occurring in the cheek without detection using the system employed, then the resulting sonoluminescence would have to be at most 0.025 times as intense as that produced by an equivalent volume of aerated water.

Keywords: sonoluminescence; cavitation; human cheek

Introduction

When ultrasound is passed through a liquid, bubbles in that liquid may be forced into non-linear volume pulsations. When the bubble volume is a minimum, the gas inside the bubble is adiabatically compressed and may reach temperatures of several thousand degrees kelvin, and several hundred atmospheres pressure. These hostile conditions can generate free radicals within the bubble. The radiative recombination of the radicals produces sonoluminescence. Because free radicals represent a potential biological hazard, the appearance of sonoluminescence is cause for concern[1].

Leighton *et al.*[1] set up a partial standing-wave acoustic field in aerated water at 22°C and 37°C, and sonoluminescence was seen to occur at the pressure antinodes. The sound was generated by a standard physiotherapeutic unit. A similar field was then used[2] to insonate mouse mammary tumour EMT/Ca/VJAC cells (EMT6) *in vitro*. After insonation, the subsequent growth of the cells was measured. Cells that had been placed at pressure antinodes showed significant growth inhibition when compared with cells that had been at pressure nodes (which did not show any significant growth differences when compared with control cells that had not been insonated).

Because both sonoluminescence and cell growth inhibition occurred at the pressure antinodes but not at the nodes in two such similar acoustic fields (generated by clinical equipment), and because the presence of sonoluminescence indicates the generation of free radicals and therefore a potential biological hazard[1], it was necessary to search for sonoluminescence produced *in vivo* by a similar ultrasonic field.

Materials and methods

The human cheek was chosen for insonation for four reasons. Firstly, it attenuates light passing through it to a lesser extent than other regions, because it is only about 6 mm thick. At 700 nm wavelength 8% intensity is transmitted, and at 600 nm, 4% intensity is transmitted[3]. Secondly, because it is thin, acoustic attenuation as 1 MHz sound passes through the cheek is only about 35% in intensity. Thirdly, because the attenuation is low, and because there is a considerable acoustic impedance mismatch at the air–flesh (or air–light guide) interface at the end remote from the transducer, a partial standing-wave system will be set up. Fourthly, if the light sensor is held against the inner surface of the cheek, the chances of detecting sonoluminescence are improved. This is because this moist, living tissue would be a better site for cavitation than the dry, dead skin that covers most regions of the body.

The optical detection system is shown in *Figure 1*. Sonoluminescent photons would be collected by the light guide and channelled to the red-sensitive photomultiplier (EMI 9658R), which was operated at −20 C to reduce

0041-624X/90/030181-04

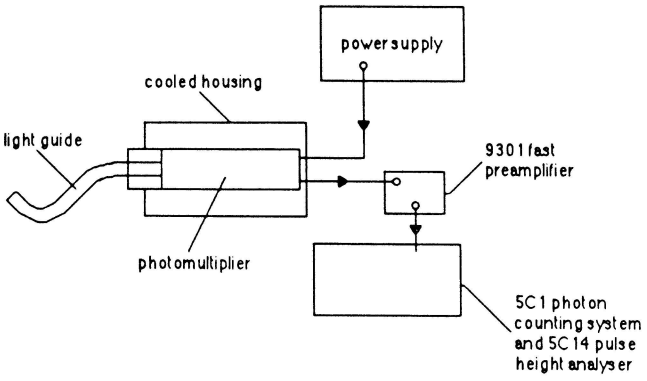

Figure 1 The light detection system

the noise level inherent in the tube. The output from the photomultiplier was quantified by a 5C1 photon counting system and 5C14 pulse height analyser (EG&G Brookdeal Electronics Ltd). The background count was 60.5 ± 19.0 counts per second (c.p.s.). For different experimental arrangements detailed below, the count rates taken during insonation of the cheek were recorded and the means calculated. These were then compared with the background count rate using the null hypothesis and the Student's *t*-distribution to test for significance. Significance at the 5% level or less is used as the criterion for having detected sonoluminescence.

The experimental arrangements employed are shown in *Figure 2*. A beam of 1 MHz continuous wave (c.w.) ultrasound is generated at up to 2 W cm^{-2} by a Therasonic 1030 (Electro Medical Supplies). Above this level the volunteers experienced discomfort. For further details of beam calibration see Pickworth *et al.*[4].

Initially the transducer was held against the outer surface of the cheek (*Figure 2a*) with coupling gel used to ensure good contact. For the experiment illustrated in *Figure 2b* a sealed bag of aerated water was insonated and the light guide was used perpendicular to the acoustic axis, to detect sonoluminescence. (This was so that the lower surface of the bag rested on the table, thereby taking a flat form to allow planar reflections.) The water bag was sometimes inserted in the system shown in *Figure 2a*, either between cheek and transducer (*Figure 2d*) or cheek and light guide (*Figure 2e*). In *Figure 2c* the arrangement is as for *Figure 2d* but with a glass plate (1.6 mm thick) replacing the cheek. To check that during cavitational conditions in the water bag, the ultrasound intensity was not markedly reduced, a needle hydrophone (Dapco

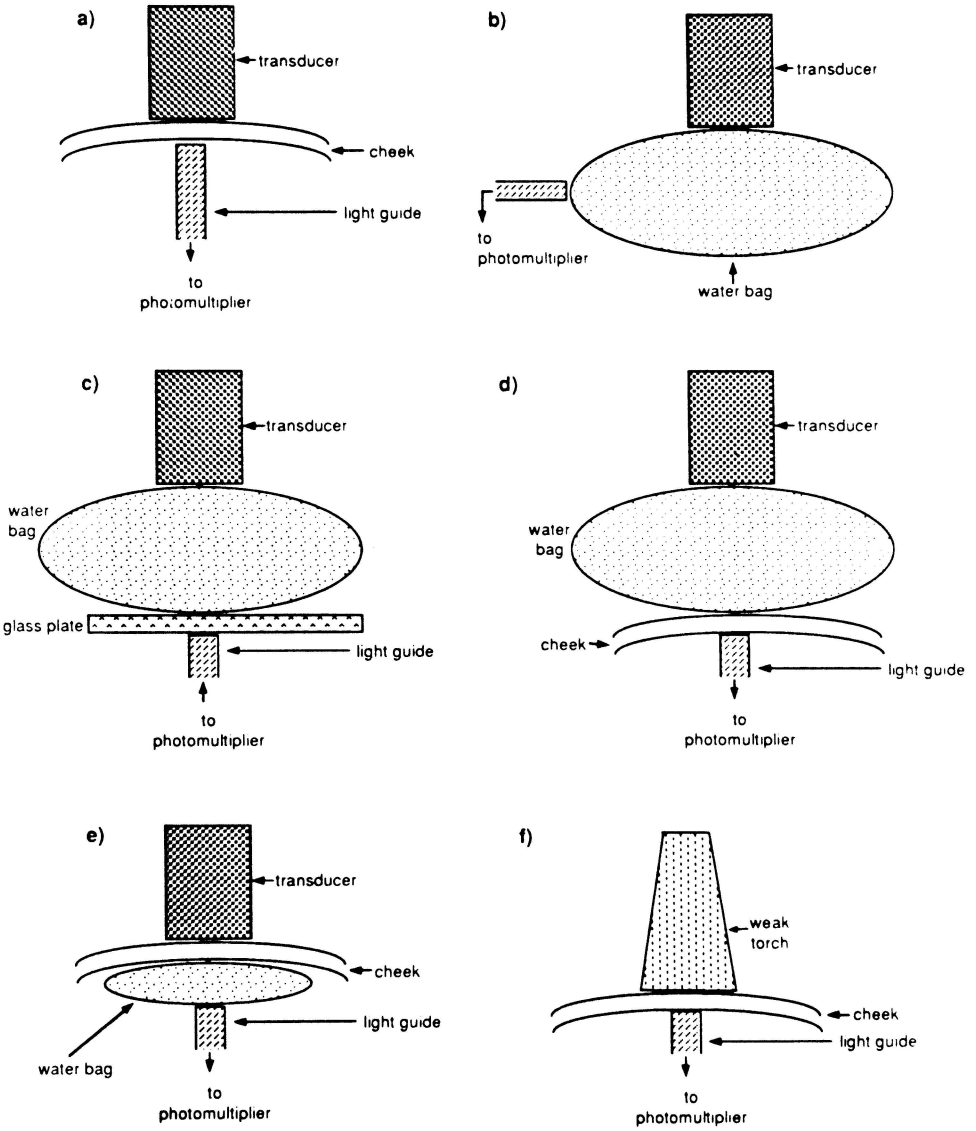

Figure 2 The various geometries of the experiments, showing the relative placements of the transducer, light guide, water bag, glass plate and cheek (the top edge of which in the figure corresponds to the outer surface of the cheek). Not to scale

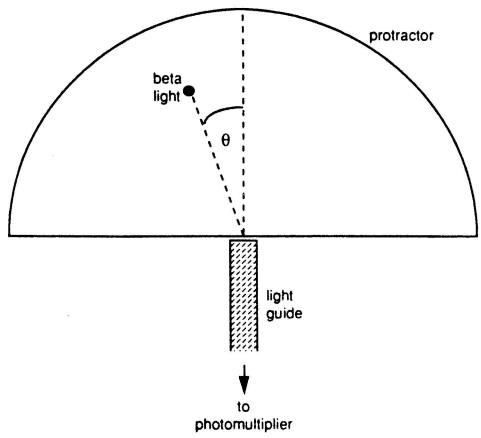

Figure 3 The apparatus employed to determine the acceptance characteristics of the light guide

NP10-3) was inserted between the water bag and tissue surface. Attenuation of the ultrasound beam was negligible. Finally, a weak red torch was shone against the outside of the cheek and the photon count noted (*Figure 2f*). This series of arrangements allowed various deductions to be made about the processes involved. During insonation, the transducer was massaged over the water bag or the cheek, mimicking its clinical use.

To determine the input characteristics of the light guide, a standard light source (a beta light) was moved in a measured arc about the mouth of the optical fibre, whilst the photomultiplier count was noted (*Figure 3*).

Results

No sonoluminescence was detected in the experiment shown in *Figure 2a*. To check that the system would produce luminescence from aerated water (as had been assumed from the results of Leighton *et al.*[1]), a water sample was insonated (*Figure 2b*) and luminescence was indeed detected. However, it was only detected at distances greater than 3 mm from the transducer face-plate. This suggests that a stand-off of at least this distance is required with this system. Therefore a water bag providing a (6 ± 2) mm offset was inserted between the transducer and the cheek, using acoustic coupling gel at both bag interfaces.

Firstly however, an optically transparent glass plate was substituted for the cheek (*Figure 2c*), to quantify the count rate for luminescence which this optical system and geometry measure from the water bag alone. The bag was 9 mm thick, to correspond to a 3 mm non-luminescent offset, and 6 mm of luminescing material. A rocking motion of the transducer drastically increased the luminescence to a count rate of 1930 ± 360 c.p.s. After transducer motion had ceased, the luminescence fell to 560 ± 60 c.p.s. The increase in sonoluminescence that results from the motion of the transducer is a result of transient excitation of the bubbles[1,5,6].

Having quantified the luminescence from water, the arrangement shown in *Figure 2d* was investigated, yielding a negative result. To rule out excessive acoustic attenuation as the cause for this, the water bag was used within the cheek (*Figure 2e*). Sonoluminescence was detected, though from the results of the experiments illustrated in *Figure 2a* and *2c*, this clearly originated through cavitation in the aerated water contained within the bag. The results of experiments with geometries shown in *Figure 2a* to *2e* are summarized in *Table 1*.

Two further experiments were performed to characterize details of the optical detection system. The experiment shown in *Figure 3* demonstrated that 75% of the light collected by the light guide originates within a cone of half-angle $\theta = 10°$. The apparatus shown in *Figure 2f* was used to find the attenuation of light within the cheek. In a darkened room, a weak torch was used to illuminate the outer surface of the cheek at normal incidence, and the photomultiplier count rate was measured. Then the cheek was replaced by neutral density filters, inserted between torch and light guide until the count rate was that measured through the cheek. This occurred for ND 3 filters, therefore 6 mm of cheek effectively stop 999 out of every 1000 photons. It can be deduced that the count rate is attenuated by 90% for every 2 mm of cheek tissue through which the sound propagates.

Discussion and conclusions

The experiments were unable to detect sonoluminescence from the human cheek. Because it was demonstrated that the system was capable of producing and detecting luminescence from aerated water, this could be because there was no sonoluminescence, or because the level of luminescence was such that after passing through the tissue, the light was attenuated to an intensity below the level of detection of this system. Therefore it was possible to calculate the upper limit on the amount of sonoluminescence that could have occurred *in vivo* in this experiment. To do this, two facts had to be determined: the attenuation of light with distance within the cheek, and the acceptance characteristics of the light guide. Then, using this data, the proportion of the light generated by such a standing-wave field in tissue that would reach the light guide could be calculated.

The light intensity is attenuated by 90% for every

Table 1 The count rates and statistical significances of the observations

Geometry	Number of observations	Mean (c.p.s.)	Standard deviation (c.p.s.)	Test of statistical significance
Background	40	60.5	19.0	
Figure 2a	18	57.8	15.0	Not significant: $p > 25\%$
Figure 2b	6	323	250	
Figure 2c (transducer rocked)	14	1930	360	
Figure 2c (transducer stationary after rocking)	11	560	60	
Figure 2d	4	56.5	13	Not significant: $p > 25\%$
Figure 2e	10	88	32	Significant: $p < 0.1\%$

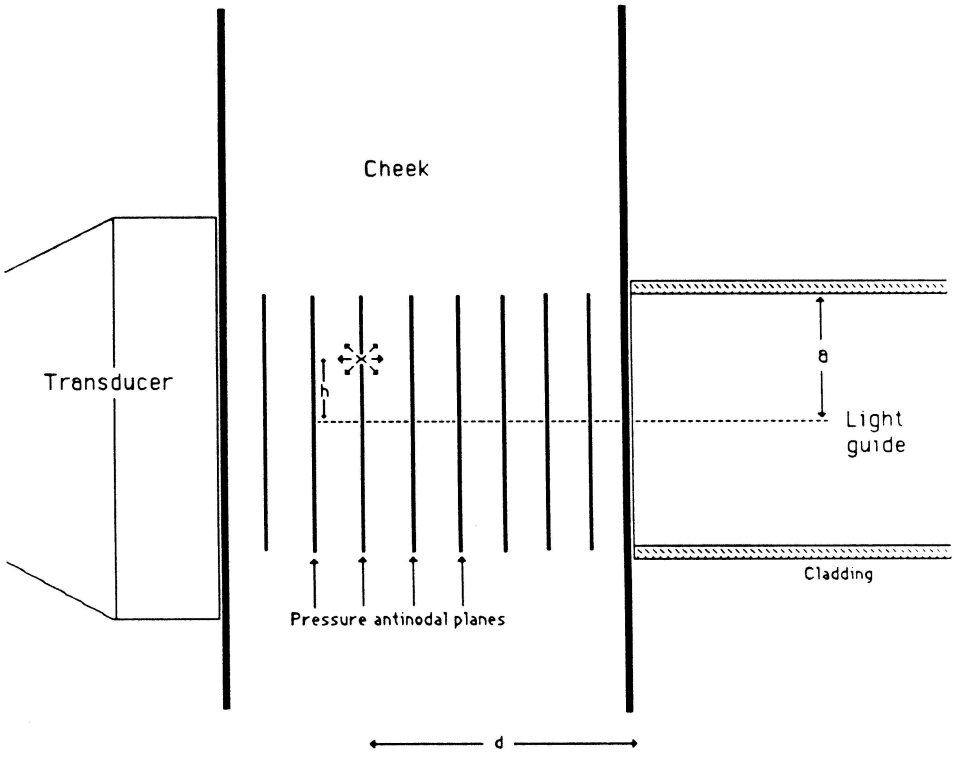

Figure 4 The geometry of the antinodal light sources with respect to the mouth of the light guide (of radius *a*). A sonoluminescent photon originates from point X, distance *h* from the optic axis of the guide, in a pressure antinode that is distance *d* from the guide (cf. text)

2 mm of tissue, and 75% of the light collected by the guide originates from the region within a half angle of 10 . Using these two results, a calculation was made based on antinodal light sources in a medium which attenuates light as a known function of the distance propagated (*Figure 4*). For a given antinode (distance *d* from the front of the light guide), the proportion of photons originating from a point source X (a distance *h* off-axis) that are collected by the guide is calculated. A computer program was written to integrate this proportion along a given antinode (which is assumed to be of length $2a$, where a is the radius of the light guide), and then over all antinodes, for the 6 mm depth of the cheek. The computation was done twice, once for water and once for cheek tissue, to find the collection efficiencies of each.

The calculation revealed that the efficiency of the light guide from water is 15%, whilst from the cheek it is only 2.9% (the reduction being due mainly to the opacity of tissue). The sonoluminescent count rate from water was 1930 ± 360 c.p.s. Therefore if exactly the same level of sonoluminescence were to occur within cheek tissue, we would expect a count rate of about 400 c.p.s.

Using the Student's t-distribution to compare means, the smallest mean increase in c.p.s. that would have been statistically significant at the 5% level with the degrees of freedom and standard deviations of our experiments, would be about 10. Therefore we conclude that, using the statistical criterion we have adopted, sonoluminescence in the cheek is less than 0.025 of that from a comparable volume of water insonated under similar conditions.

In conclusion, the conditions which optimize the likelihood of cavitation are an intensity of ultrasound above the required threshold[4], long wave trains[7], a high standing wave ratio[1], plenty of potential for bubble formation[8], and the ability for free bubble migration[9]. Of these, the lack of suitable nucleation centres is the most likely cause for the reduction of sonoluminescence in the cheek compared to that from aerated water. In addition, the increased viscous drags encountered in tissue compared to water will hinder free bubble migration[4,9]

Acknowledgements

T.G.L. wishes to thank the SERC and Magdalene College, Cambridge, for Research Fellowships. M.J.W.P. acknowledges the support of the East Anglian Regional Health Authority Committee for Locally Organised Research.

References

1 **Leighton, T.G., Pickworth, M.J.W., Walton, A.J. and Dendy, P.P.** 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 *Phys Med Biol* (1988) **33** 1239–1248

2 **Pickworth, M.J.W., Dendy, P.P., Twentyman, P.R. and Leighton, T.G.** Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 4. The effect of therapeutic ultrasound on cells in monolayer culture in a standing wave field. *Phys Med Biol* (1989) **34** 1553–1560

3 **Cartwright, C.H.** Infra-red transmission of the flesh, *J Opt Soc Am* (1930) **20** 81–84

4 **Pickworth, M.J.W., Dendy, P.P., Leighton, T.G. and Walton, A.J.** 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 *Phys Med Biol* (1988) **33** 1249–1260

5 **Leighton, T.G.** The transient excitation of insonated bubbles *Ultrasonics* (1989) **27** 50–53

6 **Leighton, T.G.** The high-speed photography of transient excitation *Ultrasonics* (1989) **27** 370–373

7 **Pickworth, M.J.W., Dendy, P.P., Leighton, T.G., Worpe, E. and Chivers, R.C.** Studies of the cavitational effects of clinical ultrasound by sonoluminescence: 3. Cavitation from pulses of a few microseconds in length *Phys Med Biol* (1989) **34** 1139–1151

8 **Apfel, R.E.** Acoustic cavitation *Methods in Experimental Physics* (Ed. P.D. Edmonds) Academic Press Vol. 19 (1982)

9 **Leighton, T.G., Pickworth, M.J.W., Walton, A.J. and Dendy, P.P.** The pulse enhancement of unstable cavitation by mechanisms of bubble migration *Proc Inst Acoust* (1989) **11** 461–469