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A SIMPLE METHOD FOR PRODUCTION OF TRACKLESS FOCAL LESIONS WITH FOCUSED ULTRASOUND: PHYSICAL FACTORS

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A convenient method for destroying preselected structures or regions of the central nervous system of experimental animals in a consistent manner without any undesired damage to surrounding neural tissue has thus far not been available. Most of the techniques used for this purpose (e.g. mechanical, thermal, chemical, electrical—d.c., a.c. or RF—X-rays and the use of radioactive materials) not only leave an undesirable track of trauma extending from the meninges through the intervening parts of the nervous system to the desired target, but also the size, the shape and the position of the resultant lesions cannot be controlled adequately for a meaningful analysis of the anatomical and physiological consequences (Carpenter & Whittier, 1952). Specialized methods like multiple-beam ultrasonic irradiation (Fry & Fry, 1960) or the use of high-energy, high-velocity particles (Tobias, Van Dyke, Simpson, Anger, Huff & Koneff, 1954; Larsson, Leksell, Rexed, Sourander, Mair & Anderson, 1958), demanding as it does the skilful use of highly complex and expensive equipment by specialists, are not likely to become generally available to investigators in basic neural sciences, even when their potentialities and limitations have been established in a statistical manner.

However, a relatively simple method for production of trackless focal lesions by use of a single beam of focused ultrasound has been described by Ballantine and his co-workers (Hueter, Ballantine & Cotter, 1956; Cosman & Hueter, 1959). The preliminary results obtained with the use of this technique (Ballantine, Hueter, Nauta & Sosa, 1956; Ballantine, Bell & Manlapaz, 1960) showed this method to be of sufficient promise to justify a statistical study of the results of irradiation after suitable modification of the equipment, calibration procedures and the techniques.

This paper will deal with the principles, equipment and calibration procedures used in this study. A subsequent paper will describe the technique and present the results obtained by its application to the brain of the cat in a statistical manner.

EQUIPMENT

General considerations

The lesions are produced by the application of high-intensity focused ultrasound to the desired structure in such a manner that the effects in the surrounding tissues are subliminal. Ultrasound is generated by application of radiofrequency (RF) power to a piezo-electric plate transducer. The emergent plane wave of ultrasound is focused by refraction after passing through a planoconcave plastic lens in contact with the transducer. The substance to be irradiated is coupled to the lens by a relatively loss-free medium (degassed water or saline solution) contained in a suitably shaped airtight applicator. As the design of the equipment is determined by the frequency of sound to be used, the characteristics of the piezo-electric transducer and the numerical aperture of the focusing system selected, these factors will now be briefly considered.

Choice of frequency. On theoretical grounds, for a fine graded control over the volume of the lesion produced, the increased resolution obtainable with shorter wave-lengths (higher frequencies) of sound would seem to be desirable. But since the attenuation of sound in its passage through media (including biological tissues) increases with frequency, the acoustical output and power requirements, and consequently the size of the equipment needed, also increase. A reasonable compromise seems to lie in the region of 2·0–3·0 Mc/s (λ about 0·05 cm).

Choice of transducer. Ceramic piezo-electric transducers were rejected, owing to the temperature-dependence of their acoustical power output which precludes accuracy in calibration, and their comparatively poor long-term calibration stability. An X-cut quartz plate ('X' is the electrical axis of the crystal) with fundamental resonance of 900 kc/s is about 3·2 mm thick and adequately withstands mechanical strains of clamping, coupling and usage. Such a crystal plate driven at 2·7 Mc/s (3rd harmonic of 900 kc/s) has been found to produce lesions adequately small for neurological investigation of the central nervous system of experimental animals (Basauri & Lele, 1962). For reasons of flexibility, cost and availability quartz plate-plastic lens combination is used in preference to a concave (pre-focused) quartz transducer, in spite of the theoretically higher power output of the latter. Moreover, the power output available with the use of plastic lenses has been found to be more than adequate for any probable requirements in experimental animals (Basauri & Lele, 1962).

Design of the lens. According to diffraction theory, about 84 % of the energy irradiated from a single focusing transducer flows through an ellipsoidal focal region with the lateral diameter of the main lobe of the diffraction pattern $d_f = 1{\cdot}2\ F\lambda/r$, where F is the focal length, r the effective transducer radius and λ the wave-length. The ratio of the length to the diameter of the focal region is inversely related to the solid angle of irradiation. Furthermore, within practicable limits the larger this solid angle, the lower the flux density immediately above and below the focal region and, therefore, the less the likelihood of subliminal damage outside the focal region when a substance is irradiated. A large aperture would,

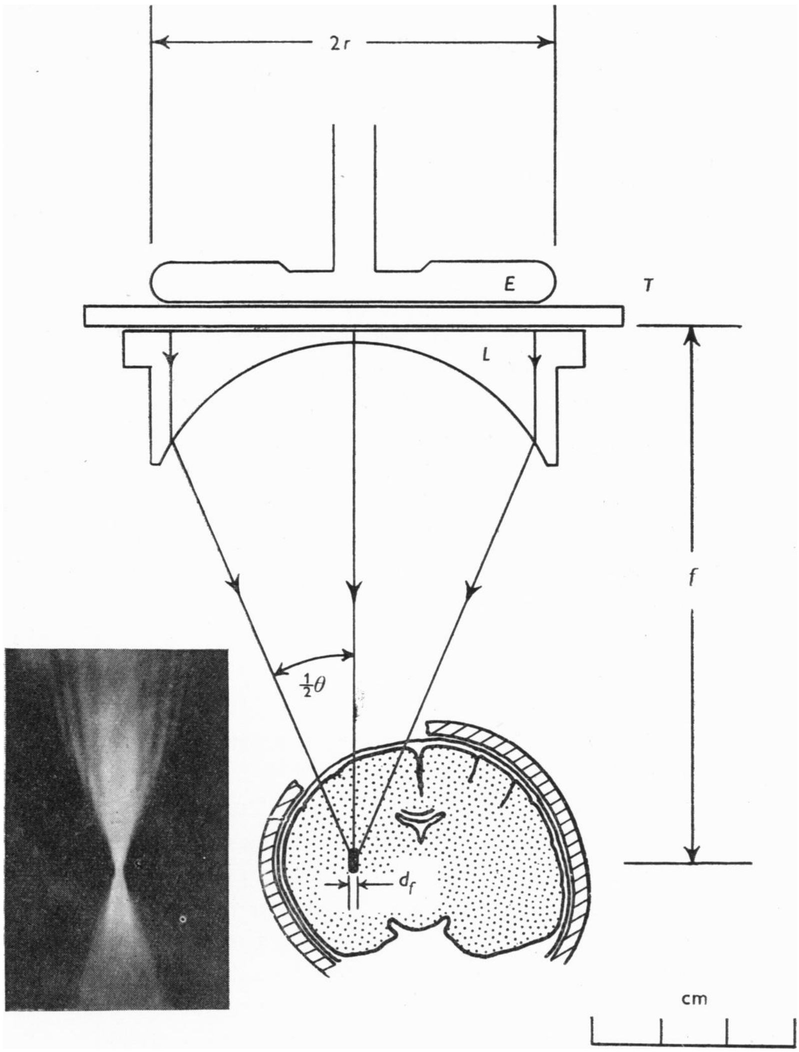

Fig. 1. Factors governing design of the focusing system. $2r = 6{\cdot}0$ cm. At 2·7 Mc/s in degassed water at 37° C, $f = 8{\cdot}0$ cm, $\theta = 42°$, diameter of the main lobe of diffraction lobe, $d_f = 0{\cdot}16$ cm. Inset shows Schlieren photograph of the focused beam in water. Note that, because of low transmission of sound by bone, at the frequency used, portions of skull have to be removed to provide access to the brain.

therefore, tend to produce discrete, globular lesions. The choice of the focal length of the lens is determined by the maximum depth at which lesions may need to be placed. The solid angle of irradiation and thus the maximum diameter of the crystal transducer that can be used effectively are restricted by the maximum available diameter of the surface of the medium containing the target. The numerical aperture of the focal beam, i.e. the ratio of the focal length of the lens to the diameter of the transducer, is thus governed by the dimensions of the medium containing the target. On these considerations for use in the brain of the cat, optimum dimensions are found to be $F = 8{\cdot}0$ cm and $r = 3{\cdot}0$ cm. With these dimensions the numerical aperture $F/2r = 1{\cdot}33$, the angle $\theta = 42°$, $d_f = 0{\cdot}16$ cm and area of the main focal lobe $a_f = 0{\cdot}02$ cm^2 (see Fig. 1).

Generator. The radiofrequency (RF) generator is a tuned plate oscillator designed for good calibration stability (Cosman & Hueter, 1959), and provides adequate output to drive the transducer at odd harmonically related frequencies from 900 kc/s to 4·5 Mc/s. The use of triode power oscillator permits E_{RF} (RF voltage) to be a direct function of $E_{d.c.}$ (plate voltage) when other conditions such as the acoustic load and the oscillator tuning are fixed. Plate voltage $E_{d.c.}$ is applied to the oscillator while it is cut off and, therefore, it can be set accurately to correspond to the desired acoustical output. Continuous irradiation or intermittent irradiation in short-duration bursts (pulses), with pre-set pulse durations of 0·005–2·0 sec in 29 steps and pulse intervals of 0·1–10·0 sec in steps of 0·1 sec, is possible. For pulses longer than 2·0 sec, operation by an external timer is necessary. Radiation can be controlled remotely or triggered externally. It can be cut off automatically by a pre-set pulse counter. Synchronizing pulses are available to trigger oscilloscope sweeps or stimulator.

The connecting flexible coaxial cable and the crystal are part of the output tank of the generator. An oscillator tuning control allows micrometer adjustment of tuning necessary for peaking on the sharply resonant crystal load. The cathode current in the output tank is monitored on a d.c. milliammeter and recorded on a pen recorder as the potential difference across a 1 Ω resistor in series with the oscillator cathode. This tracing provides a permanent record of the radiation dosage, i.e. the level of the power output, the duration of each pulse, the interval between pulses and the total number of pulses. For economy, the irradiation control on the generator is coupled to the paper drive control of the pen recorder. A built-in delay ensures that the paper begins to run 3 sec before the onset of irradiation and continues to run until 3 sec after irradiation ceases. An audio-oscillator synchronous with the RF oscillator provides an audible signal during irradiation.

Irradiation head. The irradiation head (Fig. 2) is essentially a watertight housing to clamp and energize an air-backed 900 kc/s X-cut quartz crystal plate, the exposed surface of which is silvered and held at earth potential. The other surface is coupled by a spring-loaded high-voltage electrode to the coaxial output cable from the generator. The design of the backing electrode and the inside contours of the housing permit electrical excitation of the largest possible area of the crystal without arc-over to the housing, and thus yield the maximum possible numerical aperture for the transducer-lens system. A concentric collar integral with the upper part of the housing permits the irradiation head to be mounted easily but securely on a straight rigid post. The lower part of the housing is threaded to carry a lens retainer ring and applicator cones which are concentric with it. In the plane of the lower surface of the transducer it also provides a bearing surface for a retractable and detachable pointer, the tip of which when protruded indicates the location of the centre of the focal region of the system, and is used to facilitate rapid positioning.

The lens is machined from a plastic (polystyrene or rexolite) and is planoconcave with a focal length of 80 mm at 37° C when coupled to degassed water or saline solution. It is attached to the free silvered surface of the transducer by a uniform thin film of degassed castor oil and held in place by a retainer ring. The curvatures for lenses of different focal lengths can be computed by geometrical optics if the refractive index is known. The refractive index $Mp-w = C_p/C_w$ when C_p is the velocity of sound of the frequency used in plastic and C_w is its velocity in degassed water at 37° C. Approximate values for C_p and C_w at 2·7 Mc/s and 37° C are 2400 and 1500 m/sec respectively.

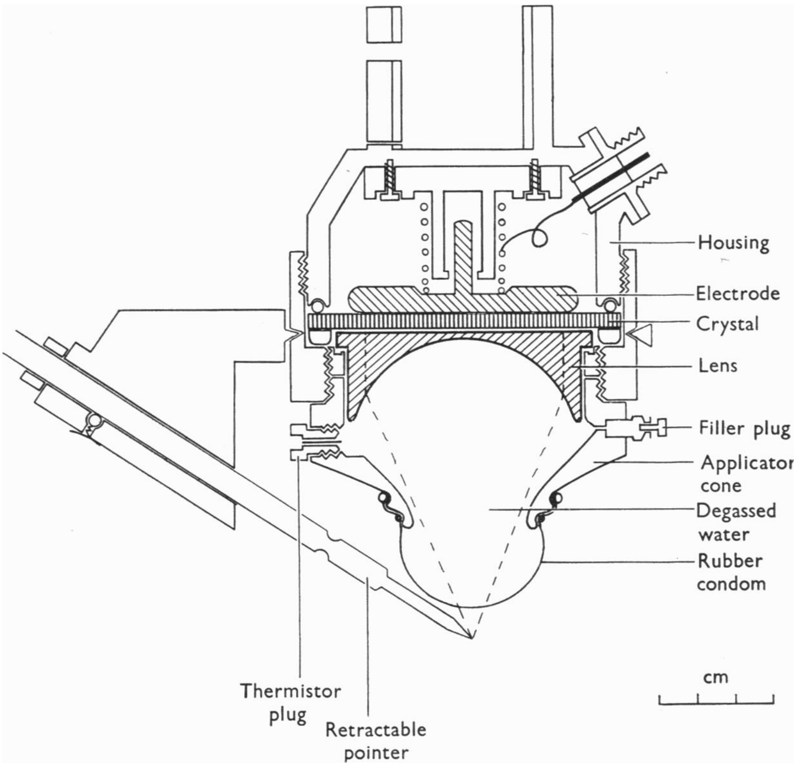

Fig. 2. Diagram of assembled irradiation head. See text.

The applicators are sections of conical containers open at both ends and are machined from transparent, alcohol-resistant polystyrene or rexolite. Near the wider end the wall of each applicator has a round aperture into which a watertight plug carrying a calibrated thermistor is inserted to monitor the temperature of the system. Depending on the depth of the selected target from the surface, the suitable applicator is used. It is filled completely with degassed water (see below) to provide a loss-free transmitting medium for the sound and the open end is sealed with a rubber condom. The assembled irradiation head can be sterilized by gas (20 % ethylene oxide and 80 % CO_2 for 6 hr) or by immersion in alcohol-base sterilizing fluids for 12–24 hr.

The presence of dissolved air, even in minute quantities, in the coupling medium is detrimental to reproducible results. Hence, a supply of consistently well degassed fluids is prepared daily by boiling distilled water or normal saline for not less than 1 hr. Osmoticity of the saline is restored to the normal by addition of the requisite quantity of degassed distilled water. The degassed fluids are stored under a layer of degassed mineral oil to exclude contact with air, and siphoned off for use. The containers of degassed water and saline as well as the irradiation head are maintained at 37° C by immersion in a thermostatically controlled water-bath.

For irradiation, the head is attached to a post which is secured perpendicular to two planes of a rectilinear stereotaxic apparatus. The post is concentric with the irradiation head so that the position of the focal spot does not shift when the post is rotated on its long axis. The irradiation head can be positioned accurately to 0·01 mm in each of the three mutually perpendicular rectilinear planes relative to the stereotaxic apparatus. When setting the equipment, care is taken to ensure that movements of the head in each plane are independent of those in the other two planes.

CALIBRATION

Instrumentation and general considerations

An ultrasonic field may be specified by stating the frequency, intensity and geometry. In the system described above, drifts in the frequency are small and for the intended applications inconsequential.

The intensity of an ultrasonic field is specified in terms of energy (Joules) passing a unit area in unit time. For measurement of the intensity microphonic, mechanical and thermometric devices are commonly used. At frequencies above 1 Mc/s, microphonic probes (piezo-electric, magnetostrictive, mechano-electric, capacitative) which measure the alternating pressure are unreliable for measurement of all but the very low intensities, owing to cavitation effects resulting from imperfect wetting of the probe surface (Hueter *et al.* 1956). Mechanical devices measure the unidirectional (radiation) pressure, which is proportional to the intensity of the ultrasonic field. With these instruments the gross measurement of the intensity of a parallel beam of ultrasound is independent of frequency up to about 3 Mc/s and is accurate to $\pm 5\,\%$ (Hueter & Bolt, 1955; Carlin, 1960). Thermometric (Fry & Fry, 1954) and calorimetric (Wiederhielm, 1956) devices measure the heat generated when ultrasonic energy is dissipated in a medium, e.g. castor oil or water. The rise of temperature produced by short pulses of ultrasound at low intensity levels is taken to be a measure of the power or intensity of the ultrasonic radiation.

The geometry of an ultrasonic field can be visualized by the Schlieren technique (Fig. 1, inset). Measurement of the distribution of energy in this field is theoretically possible with a sensitive transducer, the receiving surface of which is small enough to resolve the field adequately without disturbing it in any way. Thermocouples are generally used because of their small size (minimum field distortion and maximum resolution), calibration stability (unlike the thermistor, being unaffected by temperature gradients in excess of 10° C/sec as with pulsed ultrasonic irradiation), lack of hysteresis and insensitivity to RF fields.

However, accurate measurement of energy distribution within the tissue irradiated with ultrasound is an ideal at present unattainable, since the insertion of measuring devices of physical characteristics (acoustical impedance, acoustical absorption coefficient, thermal capacity and conductivity) different from those of the tissue distorts the configuration normally obtained. Attempts have, therefore, been made to delineate the intensity configuration of the ultrasonic field in an absorbing medium (castor oil) and to correlate it subsequently with the lesions resulting from the irradiation of the brain of the cat (Hueter *et al.* 1956). It has been shown by Hueter (1956) that radiation pressure, particle velocity and intensity are interrelated, and any of the three may be used to describe the ultrasonic conditions of the focal point of a single focusing transducer.

The intensity configuration in the region of convergence of multiple beams of ultrasound as determined with a thermocouple appears to be similar to but not coextensive with that delineated by piezo-electric probes (Fry & Fry, 1954). Direct measurement of radiation pressure at increasing distances from the 'focal point' in three mutually perpendicular planes related to the axis of focused ultrasonic irradiation was, therefore, attempted with a mechano-electronic transducer probe (Fig. 5*A*). The energy profiles obtained by using the thermocouples and the mechano-electronic transducer were found to be coextensive within the limits of experimental error.

The profile of energy distribution in any particular horizontal plane perpendicular to the main axis of radiation (lateral 'scan', Fig. 3) is constant for a particular focusing system and frequency, being determined by the characteristics of the lens. The width of the lateral scan in the middle horizontal plane represents the diameter of the main lobe of the diffraction pattern (as measured with an instrument too large to resolve the field adequately) and can be used for comparison of different focusing systems. Since 84 % of the total ultrasonic energy passes through this area, it is possible to derive a figure of 'average focal intensity' ($I_{av.f.}$) for given irradiation parameters to permit correlation of observed effects to the ultrasonic dosage. But, as the energy distribution in the main lobe is not uniform, the biological

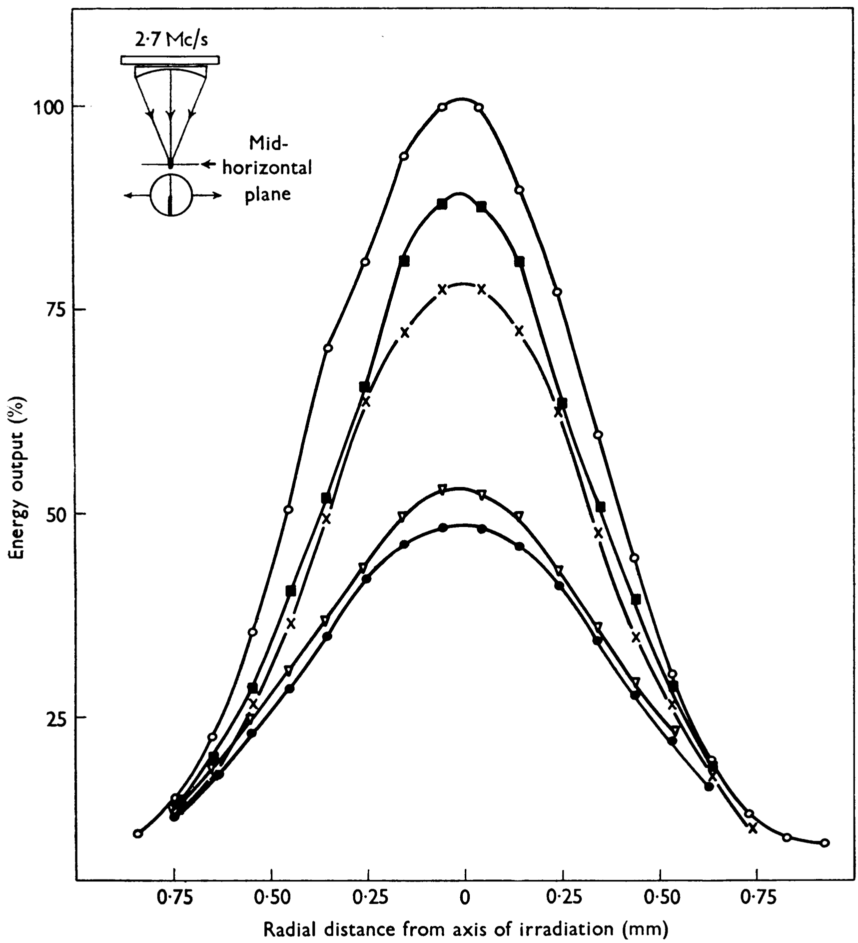

Fig. 3. Lateral 'scan' of energy distribution in the focal region at 2·7 Mc/s in water at 37° C. Axial distance from mid-horizontal focal plane: ○ zero; ■ 1·5 mm above; × 1·5 mm below; ▽ 3·0 mm above; ● 3·0 mm below.

effects following ultrasonic irradiation may not necessarily be related to the average focal intensity in a direct or simple manner. Comparison of the lateral 'scan' (Fig. 3) with the axial 'scan' (Fig. 4) indicates that the focal region is elongated, diameter:length ratio (sphericity) in degassed water being about 0·114 (also see Fig. 1 inset).

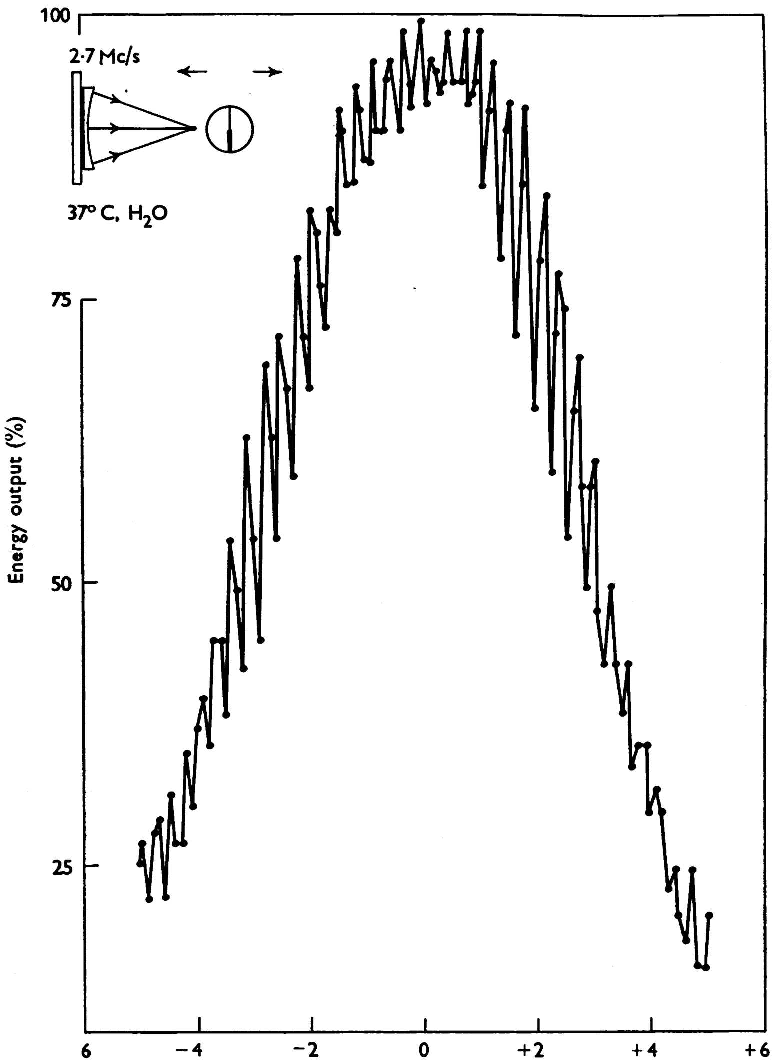

Fig. 4. Axial 'scan' of energy distribution in the focal region at 2·7 Mc/s in water at 37° C. The oscillations in this curve are due to changes in acoustical loading of the crystal resulting from the formation of standing waves with changing distance between crystal and thermocouple. They are not seen when this distance is held constant, as in curves of Fig. 3. The distance between the adjacent peaks is dependent upon the wave-length of the ultrasonic irradiation. The magnitude of these oscillations can be reduced by tuning for maximum resonance.

Intensity measurement in the focal region

Intensity of focused ultrasonic fields at low power levels has customarily been derived from measurements of ΔT (T = temperature), allowing for viscosity, specific heat, thermal conductivity and acoustical absorption coefficient of castor oil at the frequency and (since all these factors are temperature-dependent) the base temperature at which the measurements were made. The power output thus arrived at was then plotted against the square of the crystal-driving voltage. The d.c. plate voltage (for capacity of the particular length of the cable, other output tank adjustments and oscillator tuning) for the desired output was then computed by extrapolation (Hueter, 1956). In practice, because the viscosity, volume and acoustical absorption of castor oil change with the rise in temperature resulting from dissipation of ultrasonic energy in it, and because standing waves result from the reflexion of ultrasound from the thermocouple wire, this method of intensity calibration has been found to be both time-consuming and unsatisfactory for routine use. Over prolonged periods of use, the stretching of the thermocouple wire by the downward radiation pressure renders the thermo-electric calibration and the spatial location of the thermojunction in castor oil and, therefore, the acoustical calibration (as well as its relation to the external positioning devices) inaccurate. A spring-loaded thermocouple designed to compensate automatically for these slow changes was found to be unreliable because of spurious results due to its vibration at its resonant frequency during irradiation with ultrasound.

Furthermore, accurate measurement of intensity of an ultrasonic field with the thermocouple or other available devices is possible only at relatively low power levels; above 100 W/cm^2 streaming phenomena occurring in the boundary layer of the thermocouple wire lead to turbulence and formation of cavitation nuclei (Hueter, 1957). Extrapolation of the results thus obtained to the higher intensities generally used (400–1200 W/cm^2) assumes linearity of transmission characteristics throughout the transmitting media from the crystal to the focal region, which is an assumption which may not be valid under some experimental conditions.

Measurement of radiation pressure of the focused ultrasonic beam (Fig. 5), on the other hand, is direct, rapid and free from frequency and temperature-dependence and is obtained under the existing conditions of use (output tank adjustments, transmission losses in lens and coupling media). The measuring devices measure the actual total acoustical output from the irradiation head. Their calibration can be easily checked at three points on the scale by loading the pan while completely immersed in degassed, distilled water with 0·67, 2·01 and 3·35 g weights (when submerged in water) to read 10, 30 and 50 W respectively (Hueter & Bolt, 1955). They maintain the calibration satisfactorily over prolonged periods and with an accuracy of about 5 %. The sensitivity with which the measurements can be made is adequate to ensure proper setting of the oscillator tuning and to detect any unusual losses of energy occurring in the transmission path or fluctuations in the plate voltage ($E_{d.c.}$). Presence of air in the ultrasonic field or alteration in the microscopic film of castor oil holding the lens on to the transducer crystal is readily detected, owing to the decreased energy output. Furthermore, since the intensity measurements can be made at energy levels the same as or even higher than those actually used, this method of calibration can detect any incipient break-down in the system, e.g. presence of dissolved gas in the coupling media leading to cavitation phenomena and possible arc-over from the electrode to the transducer housing due to excessive humidity within the irradiation head or to other factors. The mechanical radiation pressure gauge (Fig. 5*B*), being commercially available and rugged, is preferred to the more sensitive mechano-electronic transducer.

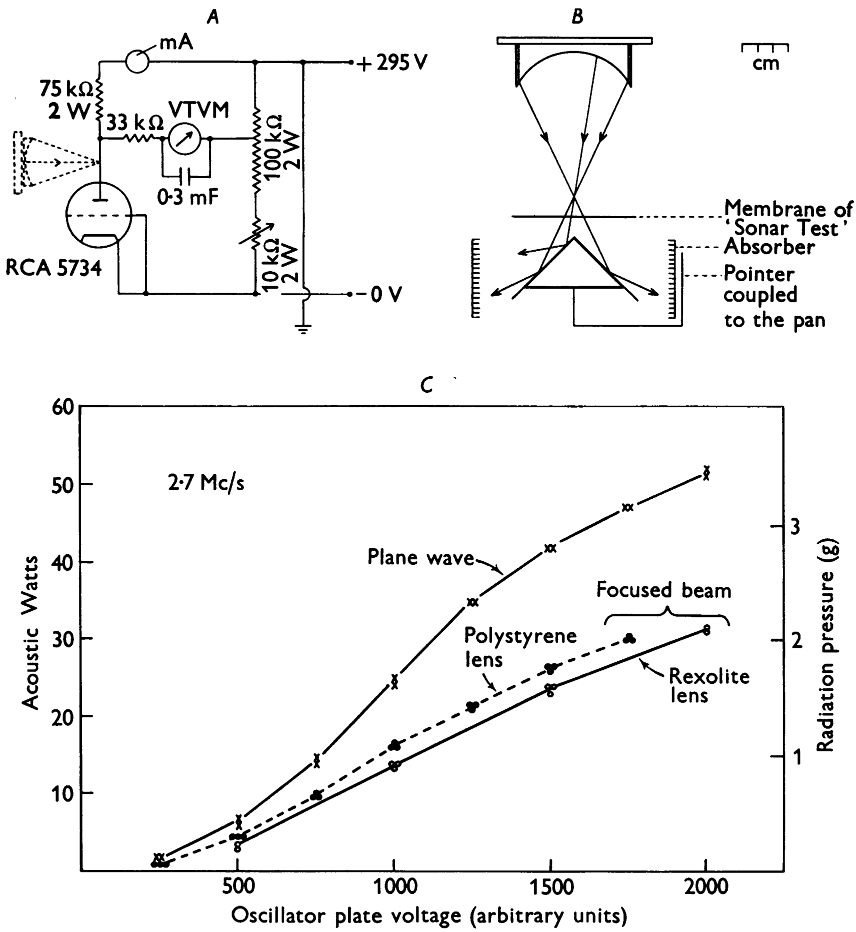

Fig. 5. *A*, Circuit diagram of electrical connexions of mechano-electronic transducer RCA 5734. VTVM, vacuum tube voltmeter.

B, Use of mechanical radiation pressure gauge for measurement of intensity of focused ultrasound. Note that the cross-section of the ultrasonic beam where it passes through the membrane of the 'Sonar Test' (Dallons 'Sonar Test', Dallons Laboratories, Inc., 5066 Santa Monica Blvd., Los Angeles 29, California, U.S.A.) is larger than 1 cm and all the ultrasonic energy impinges upon the pan and is absorbed after reflexion.

C, Relation between oscillator plate voltage and ultrasonic output as measured with the 'Sonar Test', 2·7 Mc/s.

Calibration procedure

The unidirectional (radiation) pressure of an ultrasonic field is proportional to its intensity. The intensity (acoustical output) of the focused ultrasonic beam is measured under actual conditions of use with a mechanical radiation pressure gauge (Fig. 5*B*, *C*) and corrected for the angle of convergence.

The average focal intensity

$$I_{\text{av.f.}} = \frac{0{\cdot}84\,W}{a_f}\Big/\text{cm}^2,$$

where W is the total acoustical output in watts from irradiation head and a_f is the area of the main focal lobe; and since in the focusing system used $a_f = 0{\cdot}02\ \text{cm}^2$, $I_{\text{av.f.}} = 42$ W.

The coefficient of absorption of ultrasound in water or saline is of the order of $3{\cdot}4 \times 10^{-5}\ \text{cm}^{-1}$ (Hueter & Bolt, 1955) and changes little between 20 and 40° C. Hence, for short transmission lengths the effect of temperature changes on the intensity measurements is negligible. But velocity of sound (in solids and liquids) is temperature-dependent and temperature coefficient of the focal length of the lens is found to be about $+0{\cdot}25$ mm/1° C.

The profile of energy distribution within the focal region is studied by scanning the ultrasonic field with a fine thermocouple suspended in degassed castor oil (Fry & Fry, 1954). The focusing characteristics of the lens determined by this or other methods, at a given temperature, are found to remain unchanged (within the limits of resolution of the method) over a period of time and in spite of minor damage (e.g. scratching) incidental to extensive use. The thermocouple, therefore, is used only to check the focusing characteristics of the lens by lateral and axial scans and to measure the focal length and align the zero of the stereotaxic apparatus (Horsley-Clark zero) and the pointer tip with the focal point.

Thus, by actually setting the ultrasonic output to the level desired before irradiation, it is possible to reproduce irradiation conditions accurately. This, however, does not necessarily mean that the desired dosage will be administered to the target during irradiation, e.g. presence of bone or air in the acoustical field will significantly reduce the energy reaching the target, owing to the formation of standing waves. Introduction of intensity-measuring devices into the medium (or tissue) for the purpose of calibration would not only distort the ultrasonic field normally obtained, but also would defeat the *raison d'être* of this method by creating a traumatic track in the substance. A ceramic piezo-electric detector (frequency response 20–20,000 c/s) incorporated in the wall of the conical applicator, out of the path of ultrasonic field, was used to monitor the radiation pressure during irradiation. The integrated output of the detector was found to have a linear relationship to the radiation pressure and to reveal changes in the acoustical output and load. However, since the power output of an acoustical transducer is dependent on the acoustical load which it 'sees', recordings of cathode current in the output tank of the oscillator also were found to have a linear relationship to the acoustical output and to reflect changes of acoustical load of the transducer. Cathode current recordings are, therefore, made routinely at every irradiation and the use of a piezo-electric detector has been discontinued as being superfluous.

FUNCTIONAL TESTS

The reproducibility of biological effects of ultrasonic irradiation will depend on the accuracy with which a given dosage can be repeatedly delivered to the target and the homogeneity and stability (physical and physiological) of the medium. The dosage actually reaching a given target depends upon proper acoustical couplings between the lens and the medium. Furthermore, various degrees of tissue selectivity to ultrasonic irradiation have been described for different neural elements (Wall, Fry, Stephens, Tucker & Lettvin, 1951; Fry, Barnard, Fry & Brennan, 1955; Hueter *et al.* 1956; Ballantine *et al.* 1956). Therefore, in order rapidly to test the proper functioning of the equipment and to establish the repetitive stability of the acoustical output, a relatively homogeneous medium in which the effects of irradiation could readily be studied was sought. Ice, gelatine and agar, although being in aqueous phase and hence probably similar to brain, were rejected because of complexity in making rapid, accurate measurement or of the transitory nature of the results. Irradiation of the lens oculi of the cat resulted in readily apparent and reproducible lesions but could not be used for the contemplated studies owing to difficulties in procuring adequate supplies and preservation of specimens. Liver, adipose tissue and the vitreous body of the cat, paraffin waxes of different melting points, thermosetting plastics and laminates of cellulose acetate were found to be unsatisfactory; but the superficial destruction resulting from irradiation of the surface of transparent thermoplastic polymers, e.g. polystyrene, rexolite and methyl methacrylate (acrylic resin, lucite, plexiglas or Perspex), for a fixed duration was found (also independently by R. Warwick, personal communication) to be roughly proportional to the intensity of the ultrasonic field. A systematic investigation of the effects of focused ultrasonic irradiation on and within plastics was, therefore, undertaken. Transparent, clear plastics were obtained as bars $\frac{3}{4}$ in. (18 mm) square and 18 in. (45 cm) long, with all surfaces buffed and polished. In each bar the effect of ultrasonic irradiation, alternately from opposite surfaces, was studied at forty-four different sites 1 cm apart.

The superficial destruction occurring with irradiation at the surface was found to provide a rough and ready method for determination of the approximate focal length of the system in the absence of a thermocouple, but the extent of the damage was difficult to measure accurately. However, when it was irradiated with an adequate dosage with the focal region aimed below the surface, a structural alteration was seen to develop within the plastic and gradually to expand in all directions until it broke through the surface and produced an irregularity. In many instances a brownish discoloration formed the core of the deformity. Within this core highly refractile globular or elongated 'bubbles' were often seen. With a lower dosage the alteration was entirely within the plastic. It was faintly visible if viewed by transillumination with plane-diffracted light and appeared to be a localized change in refractive index. It could, however,

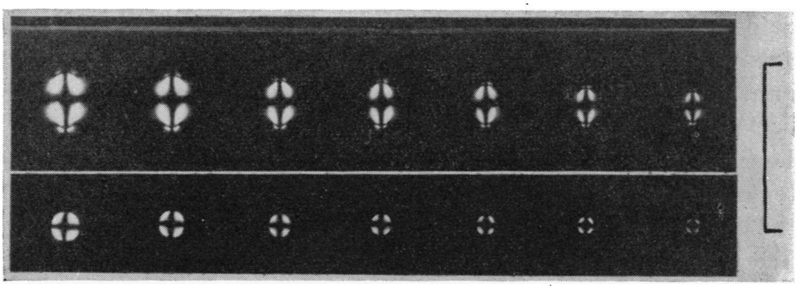

Fig. 6. Photograph of a series of 'lesions' each made with a single pulse in a strain-free methacrylate bar. The pulse duration was increased in steps from left to right. The two rows show the two views of lesions at right angle to (top) and in the axis of irradiation (bottom) respectively. Calibration = 1·0 cm.

be visualized easily as an interference pattern when viewed between a polarizer and an analyser. Plastics are normally singly refracting, but when subjected to mechanical stress they become doubly refracting and the strained regions are seen as coloured interference patterns.

Ultrasonic irradiation was seen to produce discrete areas of structural change (lesions) lying within the substance showing marked generalized stress. The shape of the lesions was variable and the core of discoloration and the 'bubbles' referred to above were also seen in the larger lesions.

In strain-free methacrylate bars (rendered strain-free by annealing thermally before irradiation), however, the lesions when made are found to be of a regular shape; ovoid when viewed at right angles, and circular when viewed in the axis of irradiation (Fig. 6). Each lesion appears to be a radially symmetrical and uniform stress as evidenced by the planes of polarization seen as the dark cross. This cross was found to rotate with the polarizing planes but not on rotation of the lesion. Furthermore, when a cylinder of plastic was irradiated from the plane surface the resultant lesion was seen to be an egg-shaped globule approximating a prolate spheroid.

Repetitive stability. In order to determine the reproducibility of the lesion size at different intensities of irradiation, ten lesions at each of the five dosage parameters shown in Table 1 were made in strain-free methacrylate bars, in random order. The temperature of the irradiation head and the plastic bars was kept constant and the focal region of the ultrasonic beam was aimed 7·5 mm below the surface of the plastic. The lesions were photographed in both axes at a magnification of $\times 2$ in polarized light and measurements of length and diameter of each lesion were made by projection. The volume of each lesion was calculated by assuming it to be a prolate spheroid whose volume $V = \frac{4}{3}\pi\,(\frac{1}{2}l) \times (\frac{1}{2}d)^2$ when l and d are the major and minor axes respectively. The results are presented in Table 1 and indicate that, except at the lowest intensity used, the reproducibility of lesion size is not influenced by the intensity level of radiation.

TABLE 1. Effect of intensity on reproducibility of lesion size with one ultrasonic pulse in methacrylate

$I_{av.f.}$ (W/cm^2) 42 ×	10	15	20	25	30
Pulse duration (sec)	1·00	0·50	0·40	0·25	0·20
No. of observations	10	10	10	10	10
Mean volume of lesion (mm^3)	4·44	2·84	4·36	3·47	6·07
S.D.	0·320	0·053	0·100	0·137	0·142
Coefficient of variation	0·072	0·019	0·023	0·039	0·023

The experiment was repeated at one dosage level with methacrylate bars from different production batches. The results were compared by application of the t test (Fisher, 1946). No statistically significant differences ($P > 0·5$) were detected in the volumes of lesions made (with the same dosage) in methacrylate bars from different batches. The influence of lesion size on reproducibility was also examined in a similar manner. Smallest lesions were found to be more variable in size than larger ones, but the range or variability was no greater than that obtained at different intensities. In view of the comparatively low coefficient of variation (Table 1) of the lesions it was decided that 3–5 replicates would constitute a statistically adequate sample for functional tests.

Test for linearity of calibration. Three sets of lesions were made each at (42 ×) 5, 10, 15, 20, 25 and 30 W/cm^2 average focal intensity (see p. 504) and single pulses of increasing pulse durations. Temperatures of the irradiation head and the methacrylate bars were kept constant and the focal region of the ultrasonic beam aimed 7·5 mm below the surface of the bar. In every series at each intensity it was found that irradiation at pulse durations below a certain minimum did not result in any permanent lesions. With longer pulse durations the resultant lesions were progressively larger prolate spheroids until at a certain pulse duration they became 'acorn'-shaped, being wider in the upper equatorial plane, and showed discoloration and 'bubbles' in the long axis. All lesions of regular shape were photographed and the length (l), diameter (d) and depth were measured and the volume of each lesion calculated. At each intensity

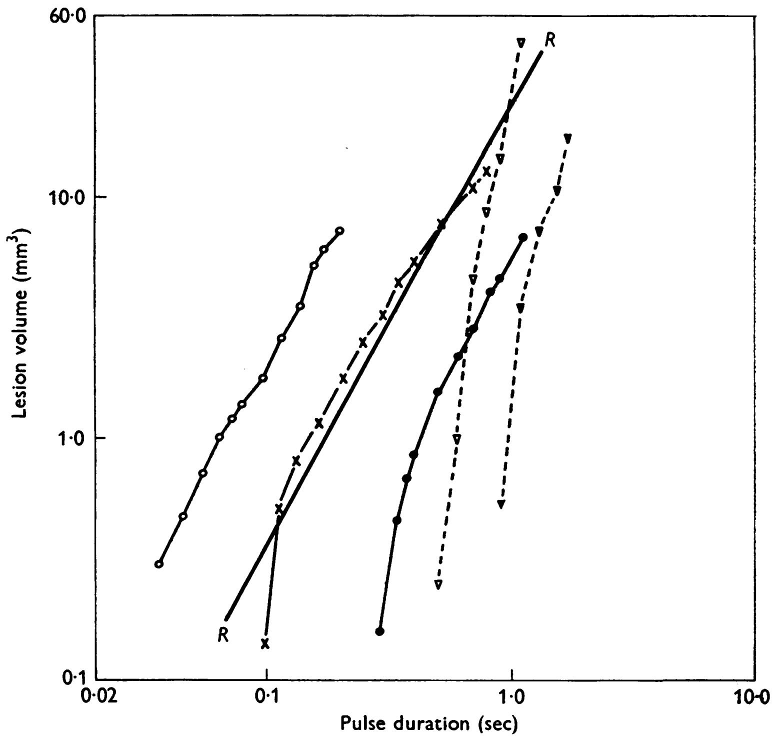

Fig. 7. Relation between pulse duration (log. scale) and lesion volume (log. scale) at different intensities and frequencies. Each lesion was made with a single pulse. Calculated regression line R–R is shown for 42×20 W/cm² average focal intensity at 2·7 Mc/s; regression coefficient 1·825; correlation coefficient 0·997; $P < 0{\cdot}001$. Regression lines for other intensities were parallel to the one shown. At 2·7 Mc/s, $I_{\text{av.f.}}$ 42×10 ●; 42×20 ×; 42×30 ○; at 0·9 Mc/s, $I_{\text{av.f.}}$ $4{\cdot}6\times40$ ▽; $4{\cdot}6\times30$ ▼.

level the length and the diameter of the lesion were found to be linear functions of the logarithm of the duration of irradiation. The relation between the log. pulse duration and log. volume of lesions at the three different intensity levels at 2·7 Mc/s shown in Fig. 7 is seen to be linear except at the threshold duration. Similar dosage–lesion-size relationship was found to obtain when the brain of the cat was similarly irradiated

(Basauri & Lele, 1962). It was indeed found possible to evaluate rapidly the probable biological effects of different dosage factors, frequencies, focal lengths and transducers by using methacrylate as a test medium (Lele, 1962).

Effect of output tank adjustments. Optimum setting of the oscillator tuning, controlling as it does the frequency of the generator, is essential in order to obtain maximal acoustical output at a particular oscillator-plate voltage setting. In the foregoing tests the desired level of ultrasonic intensity was obtained by adjusting the oscillator tuning for the maximum ultrasonic output and then changing the oscillator-plate voltage until the required radiation pressure was indicated on the radiation pressure gauge. Thus, all the relations between ultrasonic dosage and lesion size, described above, were obtained with the oscillator tuning set at the optimum value and, therefore, at a frequency very nearly 2·7 Mc/s. To study the effects of any frequency drifts due to improper setting of the tuning control or changes in the acoustical loading, the following experiments were performed.

TABLE 2. Effect of oscillator tuning setting on lesion size

$I_{av.f.}$ 42 × 20 W/cm², pulse duration 0·3 sec, single pulse

Tuning set at	$E_{d.c.}$ (V)	l (mm)	d (mm)	Volume (mm³)
66·5 (optimum)	1425	3·05	1·54	3·80
58·0	1600	3·05	1·54	3·80
73·0	1500	3·05	1·53	3·75

Five lesions were made each with a single pulse at 42 × 20 W average focal intensity and 0·3 sec pulse duration with optimum tuning as hereto. The oscillator tuning was then deliberately shifted from the optimum and the resultant drop in acoustical output compensated for by raising the oscillator-plate voltage. Five more lesions were made. The procedure was repeated with the tuning shifted in the other direction.

The results are presented in Table 2. No statistically significant difference was detected in the three sets of lesions. Small drifts in the frequency of oscillation, occurring as a result of changes in acoustical loading of the transducer, are thus seen to have a negligible effect (if any) on ultrasonic dosage–lesion-volume relationships.

Effect of using an odd harmonically related frequency. Series of lesions were made with increasing pulse durations and average focal intensities of approximately (4·64 ×) 20, 30 and 40 W respectively at 0·9 Mc/s frequency. It may be pointed out that at this frequency the diameter of the main lobe of diffraction being 0·48 cm (since λ 0·9 Mc/s $=$ 3 × λ 2·7 Mc/s) the area of the main focal lobe is 0·181 cm². The average focal intensities are only about one tenth (0·11) of those at 2·7 Mc/s for the same measured (total) radiation pressure of ultrasound.

The log. volume of lesions in Fig. 7 is seen to be a linear regression of log. pulse duration for each intensity, as in lesions made at 2·7 Mc/s. The slope of the regression line, however, is steeper and finely graded control is, therefore, not possible, although the reproducibility was found to be as good as that at 2·7 Mc/s. The size of the smallest lesion that was permanent was 0·30 mm^3 as compared with 0·15 mm^3 made at 2·7 Mc/s. The lesion made with single pulses at 0·9 Mc/s was found to be more spherical than lesions of comparable size made at 2·7 Mc/s, the smallest lesions being almost spherical. The ultrasonic energy necessary to produce equivalent volumes of lesion is found to be a little less at 0·9 Mc/s than at 2·7 Mc/s.

GENERAL OBSERVATIONS

In the course of more than 7000 irradiations performed during this investigation it was found that there was a consistent relation between the oscillator-plate voltage ($E_{\text{d.c.}}$) setting and the ultrasonic output as measured with the radiation pressure gauge; provided of course that output tank adjustments, the cable length and the acoustical loading remained unaltered. However, routine pre-setting of the ultrasonic output to the desired level by using the radiation pressure gauge was found to be necessary for the following reasons:

(1) At a certain critical length of the loose rubber condom sealing the lower end of the applicator cone (Fig. 2) the measured radiation pressure was found to be only about 50 % of the expected level. This was corrected by readjusting the length of condom by a few millimetres. This phenomenon was observed about ten times and in every instance was reflected in the cathode current recordings. It occurred only when the rubber membrane lay in the focal region and, therefore, it is obvious that it could not occur during irradiation of a medium since the focal region would always be below the medium.

(2) With prolonged and extensive use, the radiation pressure at a particular plate-voltage setting ($E_{\text{d.c}}$) showed a progressive fall, starting about a week after the lens had been coupled to the transducer. The maximum diminution occurred in about 15 days and was of the order of 20 % of the expected output, but the energy profile in the focal region remained unaltered. This condition is attributed to occurrence of physico-chemical changes in the layer of castor oil coupling the lens to the transducer and was corrected by renewing it.

The focusing characteristics of the lens as determined by 'lateral scans' were found to remain unchanged even after prolonged and extensive use and in spite of minor damage such as scratching of the surface. Recordings of the oscillator cathode current during irradiation of the plastic provided valuable clues as to the coupling conditions under which the irradiation was performed. In four instances (out of more than 7000 irradiations in this study) the cathode current recordings showed abnormalities together with absence of 'lesion' development in the plastic. Examination of the conditions of coupling in each of these instances revealed the presence of air in the film of degassed water coupling the condom to the plastic.

SUMMARY

1. A simple and stable but flexible system of generating a single beam of focused ultrasound for production of trackless focal lesions in the central nervous system of the experimental animal is described.

2. Calibration of the intensity of ultrasonic irradiation is made by direct measurement of the radiation pressure of the focused beam.

3. Adequate irradiation of strain-free methacrylate produces permanent egg-shaped lesions within the plastic. These are easily visualized by polarized light and are invaluable for making rapid functional tests of the equipment.

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