

ULTRASONIC BIOLOGICAL EFFECT EXPOSURE SYSTEM

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ABSTRACT. An ultrasonic biological effect exposure system was developed to provide experimental data which could be used in assessing the biological risk from exposure to ultrasonic energy. The system can reproduce the ultrasonic signals of medical diagnostic and therapeutic equipment at comparable or greater intensities. Signal synthesis is at low electrical powers (<0 dBm) with linear amplification of the signal to the desired level by a high power, broadband amplifier. A minicomputer controls the exposure time and the net electrical power to the ultrasonic transducer assembly through an interface to a programmable frequency synthesizer. The net electrical power is monitored by a dual directional coupler which, in turn, is monitored by the minicomputer with an r.f. digital voltmeter. The ultrasonic transducer assembly is mounted at one end of a temperature controlled, water or saline filled exposure tank. A castor oil load at the far end of the exposure tank serves as an ultrasonic absorber to minimize standing waves. The total ultrasonic power is measured by the buoyant float technique which is used to determine the ultrasonic transducer assembly efficiency of converting the net electrical power into ultrasonic power. A scanning hydrophone technique is used to obtain either axial or three dimensional perspective plots of the ultrasonic field. The exposure system can support two general types of experiments, *viz.*, small animal exposures such as 25 to 40 gram mice and liquid suspension exposures.

INTRODUCTION

Even though the use of ultrasound continues to increase (Erikson *et al.*, 1974), it is not possible yet to develop a reliable assessment of the risk associated with exposure to ultrasound (O'Brien *et al.*, 1972). One major reason is that insufficient qualitative and quantitative information exists with respect to dose-effect relationships and interaction mechanisms (Reid and Sikov, 1972).

An ultrasonic biological effect exposure system has been developed. This system can be used to develop experimental data which, in turn, can be used to aid in the assessment of the biological risk from exposure to ultrasonic energy. The minimum criterion for the development of the system was to reproduce the exposure conditions of ultrasonic emitting devices which are used in the healing arts on humans. The typical therapeutic frequency range is from 0.1 to 10 MHz with spatially averaged intensities as high as 4 W/cm² (Lehman and Guy, 1972) which could result in an on-axis intensity as high as 16 W/cm². The typical frequency range for diagnostic, pulse-echo, equipment is from 1 to 30 MHz with peak intensity levels of the microsecond pulses as high as 100 W/cm² (Hill, 1969). Time average intensities are much lower owing to duty cycles between 0.05 to 0.2 percent or less. Ultrasonic Doppler monitoring devices operate in a frequency range from 0.5 to 15 MHz and have had intensities reported to be as high as 0.3 W/cm² (Rooney, 1973). Concomitant with an exposure capability is a measurement capability. The measurement aspects of the system must be such that whatever exposure conditions are utilized, they are accurately quantifiable. The approach decided upon was one of synthesizing the desired electronic wave shape at low (<0 dBm) power levels and linearly amplifying the signal to the required level before it is supplied to the ultrasonic transducer.

EXPOSURE EQUIPMENT

The exposure equipment consists of the electronic support for the production of the electrical signal supplied to the ultrasonic transducer assembly and the exposure tank. The electronic support is capable of providing a known net electrical power to the

ultrasonic transducer assembly. Given the efficiency of converting the net electrical power to ultrasonic power, which will be described later, the electronic support can control the ultrasonic power. The exposure tank is designed to support two general types of exposures, small animals and liquids.

ELECTRONIC SUPPORT

The electronic support consists of the minicomputer and interface, the synthesizer, the double balanced mixer and pulse generator, the amplifier, the dual directional coupler and relay box and the voltmeter, all shown schematically in Figure 1. The electronic equipment used in this system is listed in Table I. The output level, frequency and modulation are the three major programmable functions of the synthesizer, but since the majority of exposures are performed at the same frequency and modulation, only

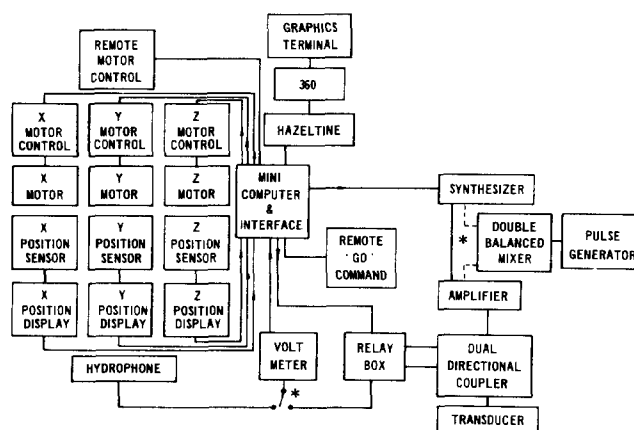


Figure 1: Schematic diagram of the electronic equipment required for the ultrasonic biological effect exposure system. The arrows directed outward from the minicomputer and interface indicate a control function and those inward directed indicate a monitor function. The asterisk indicates a cable change required.

TABLE I

Equipment list of the electronic hardware shown in Figure 1.

Minicomputer	Texas Instruments 960A with Silent 700ASR Electronic Data Terminal Houston, Texas
Synthesizer	RF 808 Signal Generator RF Communications Rochester, New York
Double Balanced Mixer	HP 10534A Hewlett-Packard Palo Alto, California
Pulse Generator	Datapulse 117 Pulse Generator Systron-Donner Culver City, California
Amplifier LO	Model 3100L Electronic Navigation Industries Rochester, New York
HI	Model 660 Amplifier Research Souderton, Pennsylvania
Dual Directional Coupler	Model 6077 Reaction Instruments McLean, Virginia
Relay Box	Amphenol Coaxial Relay model 320-010931-3
Voltmeter	Model 3403C True RMS Hewlett-Packard Palo Alto, California
Motor Control and Motor	Buffered Translator model BTR 103RT and SLO-SYN motor type M 112-FJ25 Superior Electric Bristol, Connecticut
Position Sensor and Position Display	Acu-Rite-5 Micro-Line Jamestown, New York

the output level is under computer control. The level is variable from -127 dBm to +33 dBm (0dBm = 1mW into 50Ω) and all impedances are 50Ω unless otherwise indicated. The frequency range of the synthesizer is variable from 50 kHz to 80 MHz in 1 kHz steps. Its output can be pulse modulated to obtain the microsecond pulse conditions used in diagnostic ultrasonic devices with the double balance mixer operated as a pulse modulator in conjunction with the pulse generator. For continuous wave exposures (pulse widths greater than about one second), the output of the synthesizer is directly connected to the amplifier.

Two high power, broadband amplifiers are in use, one designated "LO" and the other "HI". The choice of the amplifier is a function of the maximum intensity desired for the particular experimental set-up. The LO amplifier has a 100 watt (50 dBm) maximum output over the frequency range from 250 kHz to 105 MHz at a

nominal input of 0 dBm. The HI amplifier has a maximum continuous wave output of at least 1000 watts (60 dBm) and a maximum pulsed output of at least 4000 watts (66 dBm) at a maximum pulse width of 8 milliseconds and a maximum duty cycle of 25% at a nominal input of 0 dBm over the frequency range from 100 kHz to 200 MHz.

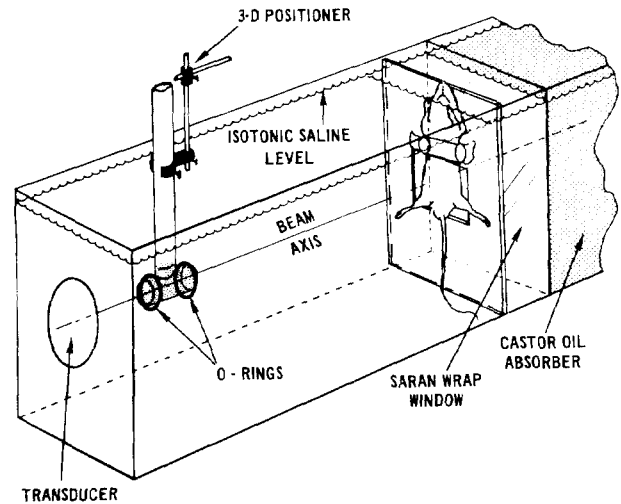


Figure 2: Schematic view of the ultrasonic exposure tank demonstrating the positioning of the Pyrex(R) vessel which is attached to the 3-D positioner and the positioning of the mouse mounting board with mouse. The distances from the transducer are determined by the specific experimental conditions.

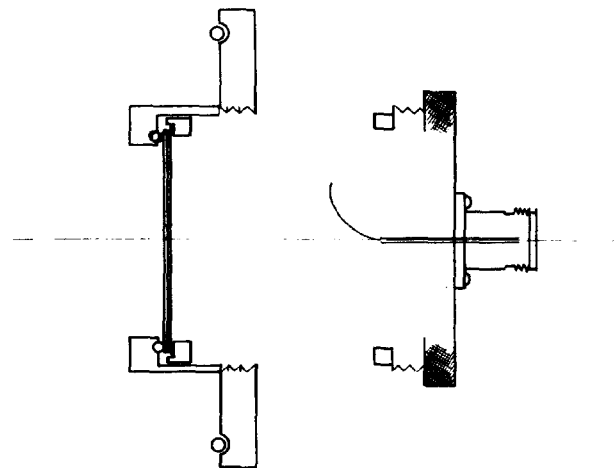


Figure 3: Schematic, cross sectional view of the ultrasonic transducer assembly. The stainless steel assembly supports a ceramic disk such that the air backed side has a gold foil positioned between the disk and the support ring. The screw-in backplate compresses the ring against the gold foil and disk. The front surface of the disk, in turn, compresses an O-ring to provide, simultaneously, a liquid seal and contact with the stainless steel.

The output of the high power, broadband amplifier is connected to the ultrasonic transducer assembly through a dual directional coupler. The relay box which consists of a coaxial relay is under computer control and permits both the forward and reverse electrical power to be monitored by the voltmeter which permits automatic determination of the net electrical power delivered to the ultrasonic transducer assembly.

EXPOSURE TANK

The exposure tank, shown schematically in Figure 2, is constructed of one inch thick clear acrylic sheet (Plexiglas^(R)) to permit, simultaneously, the necessary thermal insulation and visibility. The visibility is required during experimentation to position and observe animals and specimens and during calibration to make certain observations and measurements. The overall inside dimensions of the exposure tank are 15 inches long, 7 inches wide and 8 inches deep with the top open. The ultrasonic transducer assembly is located on one end wall of the tank and an ultrasonic load at the opposite end. An acoustic Saran Wrap^(R) window, 0.0006 in. thick, located 12 inches from the wall which contains the transducer assembly separates the isotonic saline and castor oil. An aluminum right angle is positioned on-axis on the end wall in the castor oil to divert the ultrasound at right angles in order to maximize the path length to at least 20 cm before returning to the isotonic saline.

Figure 3 schematically represents a cross sectional view of the ultrasonic transducer assembly. The stainless steel assembly is constructed to permit variation in the thickness of the PZT-4^(R) ceramic disks (Vernitron Piezoelectric Division, Bedford, Ohio) from 0.4 inches to 0.02 inches which represents a fundamental thickness mode from 200 kHz to 4 MHz, respectively. The assembly presently in use supports a 1.5 inch diameter disk although 1.0, 2.0 and 2.5 inch diameter assemblies have also been constructed. The circular areas of the four sized disks which contact the isotonic saline have diameters of 0.75, 1.25, 1.75 and 2.25 inches. The air backed side of the disk has a 0.001 inch thick gold foil positioned between the disk and the teflon ring. A 0.01 inch groove is machined in the teflon ring to position the disk and gold foil in the center. The gold foil minimizes high current densities in the disk at the point where the ½ inch wide copper sheet contacts the back surface. The screw-in back plate compresses the teflon ring against the disk which in turn compresses the O-ring against the stainless steel housing. The O-ring groove is cut such that upon compression, the front surface of the disk contacts the stainless steel and the O-ring provides a liquid seal.

The exposure tank can support two general types of experiments, *viz*, mouse exposures using the mouse mounting boards and liquid suspension exposures using the Pyrex^(R) vessels as shown schematically in Figure 2. Pairs of vertical slots are located along the side walls at one inch increments to support the mouse mounting boards. When the ¼ inch thick Plexiglas^(R) board, which measures 6½ inches wide and 7 inches high, is positioned in the vertical slots, a square 2 inch hole in the board has its center coincident with the ultrasonic beam axis.

Ultrasonic exposures of liquids and liquid suspensions can be performed in cylindrical Pyrex^(R) glass vessels. Various inside diameters and lengths are in use, depending upon the volume requirements of the experiment. Access to the vessel is through a stem which is mounted at 90° to the vessel axis and which also serves to support the vessel in the ultrasonic beam. Both ends of the vessel are covered with Saran

Wrap^(R) and held taut by two O-ring supports. The vessel is positioned in the exposure tank in such a manner that its main axis is coincident with the ultrasonic beam axis and at the desired distance from the transducer.

The temperature of the degassed isotonic saline and castor oil is controlled by circulating water from a temperature controlled bath through ¼ inch copper tubing which is positioned along the side walls of the exposure tank. The temperature of the reservoir is controlled with an on-off temperature controller (Model 71A Yellow Springs Instrument Company, Yellow Springs, Ohio).

Degassed isotonic saline is prepared by adding distilled water to 108 grams of reagent grade NaCl to a volume of 12 liters (0.9 percent) and the mixture is placed under laboratory vacuum (20 to 60 mm Hg) for a minimum of 3 hours at 30°C.

MEASUREMENT EQUIPMENT

Three techniques are used to measure the ultrasonic field parameters. The bouyant float technique is used to determine total ultrasonic power and the suspended ball technique is used as a point by point technique to measure intensity. Both the bouyant float and suspended ball techniques are absolute, that is, the measured displacements can be related through derived mathematical expressions to the ultrasonic field parameters of total power and intensity, respectively. The scanning hydrophone technique is used to obtain either axial (on-axis) or planar (three dimensional perspective) plots of the relative pressure or intensity.

BOUYANT FLOAT TECHNIQUE

The bouyant float technique is used to measure the radiation force of a downward directed ultrasonic beam by measuring the displacement of a float bouyant between isotonic saline and carbon tetrachloride. The float shown in Figure 4 is designed with a totally reflecting, inverted conically shaped target such that the incident angle of the ultrasonic energy onto the target is 31° (Stewart *et al*, 1973). The inverted conical shape permits the float to be self centering when the ultrasonic power is incident upon the target. The float has a mean density just slightly greater than isotonic saline. Thus, when the float stem is immersed into the carbon tetrachloride (density = 1.52 gm/cc), a position of neutral bouyancy is obtained.

The chamber in which the bouyant float measurements are made has been designed to minimize reflections upon the target assembly. The inner, cylindrical tank (8 inches in diameter and 24 inches in depth) is fabricated from ¼" polypropylene with a one inch wide Plexiglas^(R) window running the 24 inch height to view the float, and is surrounded by castor oil. The beaker at the bottom of the cylinder contains the carbon tetrachloride. Pulse-echo measurements (Panametrics Pulser/Receiver 5050PR and Automation Industries 2.25 MHz transducer) inside the polypropylene tank yielded a maximum ultrasonic power reflection coefficient of 4% at normal incidence. Power measurements are performed with the target at least 3.5 inches below the transducer to allow for a minimum of two reflections of the ultrasonic energy from the polypropylene wall.

SUSPENDED BALL TECHNIQUE

The measurement of radiation force exerted on a steel ball mounted on a bifilar suspension was first reported by Fox and Griffing (1949). The present technique consists of a steel ball glued with a minute amount of Silastic to a Nylon filament, assumed to be massless. The assembly is suspended vertically into

Poisson's ratio: 0.2962
 Density: 7.84 gm/cc

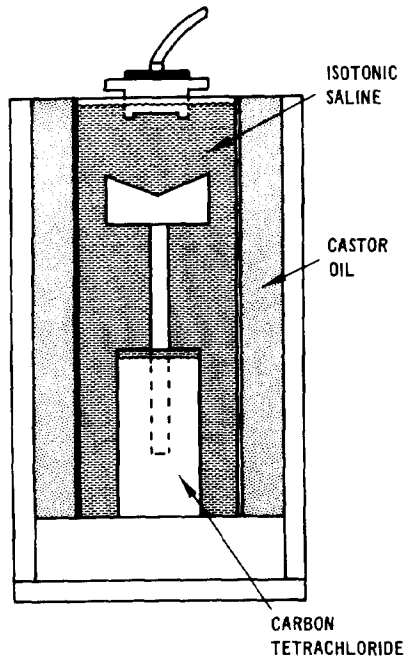


Figure 4: Schematic view of the bouyant float technique float and chamber with the ultrasonic transducer assembly positioned at the top. The float is shown with its stem submersed into the carbon tetrachloride at a position of neutral bouyancy.

the isotonic saline. When the ultrasonic energy is applied, the horizontal displacement, d , of the suspended ball is measured with a cathetometer (Graetner model M 1236-44, Chicago, Illinois). For small angular deflections, the radiation force, F_r , is determined by

$$F_r = \frac{dm_b g}{l} \quad (1)$$

where m_b is the bouyant mass of the ball, g is the gravitational constant and l is the suspension length (Fry and Dunn, 1962, Dunn and Fry, 1972). The relationship between the radiation force and the ultrasonic intensity is given by

$$I = \frac{F_r c}{\pi a^2 Y} \quad (2)$$

where c is speed of sound of the isotonic saline, a is the ball radius and Y is the "acoustic radiation force function" (Hasegarva and Yosioka, 1969) which depends upon the elastic properties of the ball, the density of the isotonic saline and the ratio of the ball size to the wavelength in the saline. Three suspended ball assemblies (fabricated at the Bioacoustics Research Laboratory, University of Illinois, Urbana, Illinois) are employed and consist of different ball sizes, viz., radii of 0.794 mm, 1.191 mm and 1.588 mm. The balls are grade 10 440C stainless steel (Winsted Precision Ball, Winsted, Conn.). The Y factor was calculated for the three size balls on the Bioacoustics Research Laboratory PDP 8 using the Hasegarva and Yosioka (1969) theory and the following elastic properties of the balls:

Compressional velocity: 5854 m/s
 Shear velocity: 3150 m/s

At a fluid velocity of 1533 m/s and a density of 1.00 gm/cm, Y calculates to be 0.887, 0.827 and 0.921, respectively. For example, a 1 cm horizontal deflection, the intensities would calculate to be 1.11 W/cm², 1.79 W/cm² and 2.15 W/cm² for a 11 cm suspension length.

SCANNING HYDROPHONE TECHNIQUE

Figures 5 and 6, which will be discussed in detail, show the types of plots which are produced from the data taken with the scanning hydrophone technique, which consists of the hydrophone, voltmeter and computerized millbase positioning system. The electronic hardware is shown schematically in Figure 1. This technique uses the hydrophone fixed in space while the exposure tank is moved.

The piezoelectric element of the hydrophone is a PZT 5H (Model 1-1010-5H, Vernitron Piezoelectric Division, Bedford, Ohio) ceramic cylinder, 1.6 mm by 1.6 mm by 0.25 mm. It is positioned coaxially on the end of a 1.6 mm diameter stainless steel tube and attached with conducting epoxy such that the outer wall of the ceramic cylinder is in electrical contact with the tube and the inner wall is in electrical contact with a wire. The wire is insulated from and passes through the tube to a type BNC connector. Insulating epoxy covers the opposite end of the ceramic cylinder to prevent the isotonic saline from shorting the inner to the outer conductors. The calculated radial, length and thickness fundamental resonant frequencies are approximately 0.8 MHz, 1.3 MHz and 8 MHz and coupling between radial and length modes can be assumed (Kinsler and Frey, 1962).

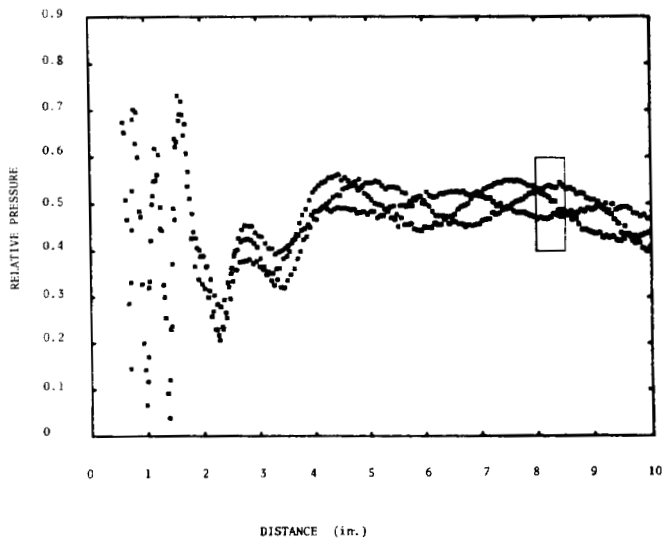
The hydrophone is rigidly mounted to the head of a Bridgeport Milling Machine frame (Model 31951, Bridgeport Machines Co., Bridgeport, Conn.) with the piezoelectric element submersed in the exposure tank which is placed on the milling machine table. Three synchronous stepping motors control the horizontal (x-axis and y-axis) and vertical (z-axis through a 10:1 gear reducer) positions of the table. Each motor is controlled from the minicomputer through a buffered translator which converts the computer commands into the appropriate motor pulses and automatically accelerates and decelerates the motor at a proper rate. Non contact, optical sensing position controls and displays for the three dimensions permit position feedback control and thus eliminates incorrect positioning due to thread backlash.

USE AND DISCUSSION

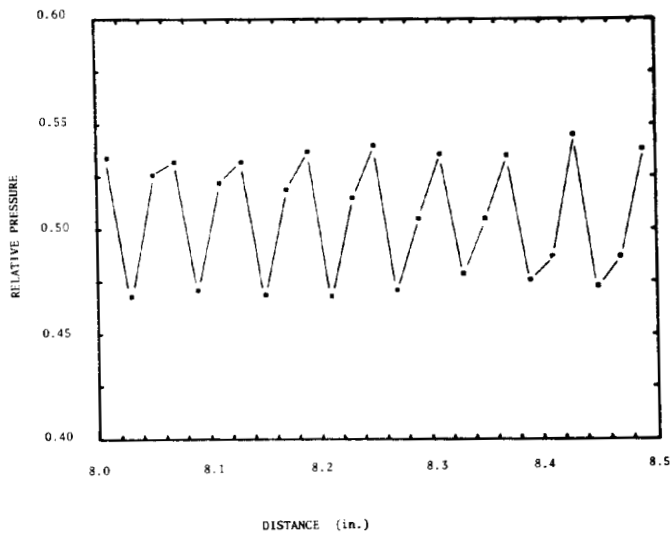
The procedure by which specimens are exposed consist of three steps, viz., ultrasonic calibration, equipment calibration and specimen exposure. The ultrasonic calibration determines the ultrasonic transducer assembly efficiency and the intensity distribution at the site where the specimen is positioned. The equipment calibration determines the minicomputer signals to the synthesizer to obtain the required net electrical power and hence the specified intensity.

ULTRASONIC CALIBRATION

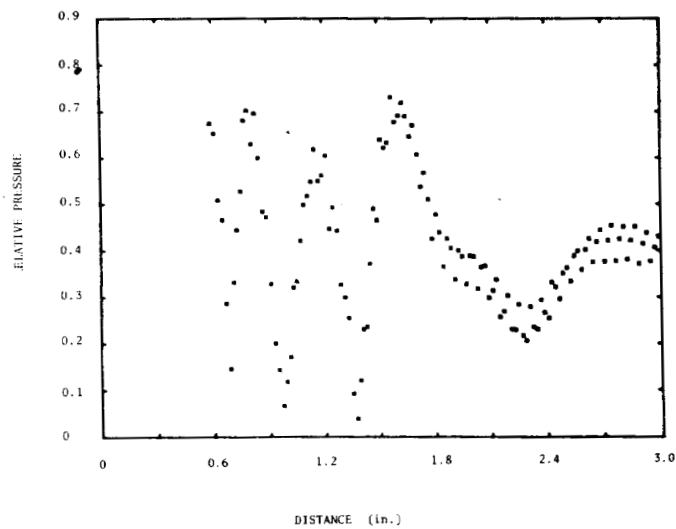
The ultrasonic transducer assembly is removed from the exposure tank and positioned above the bouyant float tank, as shown schematically in Figure 4, to measure the total ultrasonic power. The bouyant float calibration procedure is used to determine the efficiency of converting the net electrical power supplied to the transducer assembly into ultrasonic power. This is accomplished by simultaneously, measuring the float displacement and net electrical power supplied to the ultrasonic transducer assembly. From the float displacement, the ultrasonic power is



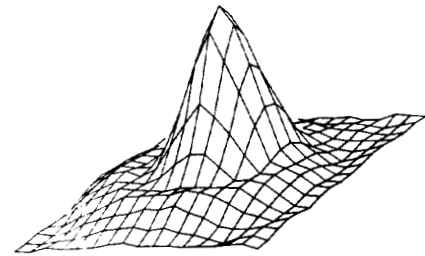
5A



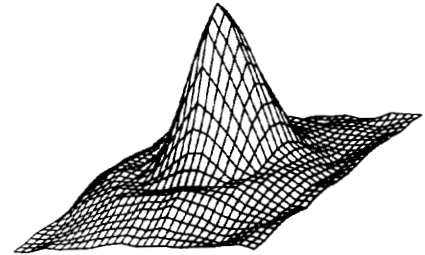
5B



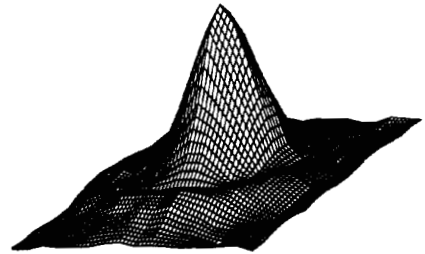
5C



6A



6B



6C

Figure 5 (Left): On-axis scan of the relative pressure versus distance from the transducer surface of the 1.5 inch ultrasonic transducer assembly. 5A is the complete scan and 5B and 5C are expanded views of the data.

Figure 6 (Above): Three dimensional perspective view of the relative pressure in the plane normal to the beam axis and 9.5 inches from the transducer surface of the 1.5 inch ultrasonic transducer assembly. 6A is the actual data, 6B is a single interpolation and 6C is a double interpolation.

calculated.

As an example, the total force, F , in newtons, exerted on this target in isotonic saline at 30°C ($c=1520\text{ m/s}$) per total ultrasonic power, P , in watts is given by

$$\frac{F}{P} = \frac{2 \cos^2 \theta}{c} = 0.000967 \text{ N/W} \quad (3)$$

where θ is the incident angle of 31° (Hueter and Bolt, 1955). The float is calibrated to determine the vertical displacement per unit force by measuring the float displacement from 1.000 gm and 2.000 gm brass weights (density = 8.4 gm/cc) which, for example, at 1.0 in. per gm (air) yields 1135 inches per kilogram (bouyant mass). Dividing by the gravitational constant (9.8 m/sec^2) and multiplying by equation 1 yields 0.112 inches per watt or for every inch of displacement, the ultrasonic power has changed by 8.93 watts.

The transducer assembly is replaced into the exposure tank and a three dimensional perspective plot is generated in the plane normal to the beam axis, at the site where the specimen is to be positioned, and at a known total ultrasonic power. From the perspective plot and total power, the point by point intensity distribution is mathematically obtained.

To generate field plots, a Scan Program is implemented on the minicomputer. The program causes the millbase to scan along a single axis between any desired ranges at any increment or to scan in either of the three planes of a rectangular coordinate system over any desired size of a rectangular plane at any increment. The scan consists of placing the hydrophone probe with the axis of the ceramic cylinder normal to the ultrasonic beam axis. The probe is connected to the voltmeter. The minicomputer then positions the millbase table, records the voltage from the voltmeter and the coordinates of the millbase table on a magnetic tape cassette, moves the millbase table to the next position and continues until the required area or line has been traversed.

The next step is to transfer the data recorded on the magnetic tape into the FDA time-sharing IBM 360 computer system, CALL/OS, from where the data may be analyzed and plotted. The minicomputer lacks the necessary software support for direct communication with CALL/OS so the data is first transmitted via telephone lines to a Hazeltine 2000 CRT terminal with magnetic tape cassette recorder. The data is recorded onto the Hazeltine cassette and then transmitted from cassette through the terminal into CALL/OS.

Depending upon whether a plane or axial scan was performed, it is possible to obtain an x-y type plot, as shown in Figure 5, or a perspective three dimensional plot as shown in Figure 6. An x-y plotting package and a perspective plotting package have been developed under CALL/OS. Both packages drive the Tektronix 4013 Graphics Terminal with a Tektronix 4610 hard copy printer in real time which provides for the production of plots in a relatively short period of time.

At first glance, the on-axis plot shown in Figure 5A appears to be three separate curves. The ultrasonic transducer assembly operates at a frequency of 1 MHz and the diameter of the transducer surface exposed to the saline is 1.25 inches. Therefore, a^2/λ is 6.53 in. The plot is generated by stepping at increments of 0.02 in. which is almost one-third of a wavelength (0.0598 in.). An expanded view in Figure

5B shows that a standing wave exists and the standing wave ratio is 1.17. From this the impedance of the castor oil can be calculated, assuming that the isotonic saline impedance is 1.52×10^6 rayls, to be 1.78×10^6 rayls. The ultrasound power transmission coefficient calculates to be 99.4% (Kinsler and Frey, 1962) indicating the fraction of power being transmitted into the castor oil load.

Figure 6 is generated under the same exposure conditions as Figure 5. The plane is located 9.5 in. from the transducer surface and is normal to the beam axis. This is the distance at which the mouse exposures occur. The probe scanned from -1.2 to 1.2 inches in both orthogonal axes in 0.150 in. increments. The half-power intensity width is 0.52 in. in both orthogonal axes. The interpolations in Figures 6B and 6C are produced by generating a data point within the center of each area which is the average of the four surrounding pressure data points.

The suspended ball technique may provide an independent check of the on-axis intensity, for example, which is calculated from equation (2).

EQUIPMENT CALIBRATION AND EXPOSURE

With the ultrasonic field calibrated, that is when the ultrasonic intensity distribution is determinable from the net electrical power supplied to the ultrasonic transducer assembly, the Ultrasonic Control Program is implemented on the minicomputer. The program permits the experimenter to specify exposure criteria for up to 32 separate exposures. Should more than 32 exposures be required, the program is executed again. The input parameters include (1) the HI or LO power amplifier, (2) the ultrasonic transducer assembly efficiency parameter, (3) the diameter of the transducer surface in contact with the isotonic saline, (4) a spatially averaged intensity for each exposure, determined by dividing the ultrasonic power by the transducer surface in contact with the isotonic saline and (5) the duration of each exposure.

The voltage signal from the minicomputer to the synthesizer determines the net electrical power supplied to the ultrasonic transducer assemblies. The front controls of the synthesizer and the amplifier gain are variable, necessitating prior to each group of exposures implementation of the Calibration Program within the Ultrasound Control Program. The Calibration Program determines the minicomputer voltage signal to the synthesizer required to obtain specific net electrical powers which in turn yield the specified spatially averaged intensities. From empirically derived equations, the minicomputer voltage signal is approximated and applied to the synthesizer. The forward and reverse signals from the dual directional coupler are automatically monitored and a spatially averaged intensity is calculated. The voltage signal to the synthesizer is adjusted up or down appropriately and new readings are obtained. This continues until the calculated intensity is within 1% for each of the specified intensities. Following this calibration, the Ultrasound Control Program prompts preparation of each specimen to be exposed and accordingly irradiates. Exposure initiation is controlled by the experimenter permitting adequate time to change specimens.

Abdominal exposures of 25 to 40 gram male and pregnant female mice represent the predominant use of the system. The mice are weighed on a top loading scale (Model 2255 Sartorius), and anesthetized with veterinarian sodium pentobarbital diluted to 10% with 0.9% sodium chloride administered intraperitoneally according to Table II. The total abdominal surface

TABLE II

Veterinarian Sodium Pentobarbital diluted to 10% with 0.9% sodium chloride anesthesia dosage for adult mice. Mice will remain anesthetized about 45 to 75 minutes.

MOUSE WEIGHT (grams)	DOSE (cc)	MOUSE WEIGHT (grams)	DOSE (cc)
10	0.11	31	0.38
11	0.12	32	0.40
12	0.13	33	0.41
13	0.15	34	0.42
14	0.16	35	0.44
15	0.17	36	0.46
16	0.18	37	0.47
17	0.20	38	0.48
18	0.21	39	0.49
19	0.22	40	0.50
20	0.23	41	0.51
21	0.24	42	0.52
22	0.26	43	0.53
23	0.28	44	0.54
24	0.29	45	0.55
25	0.30	46	0.56
26	0.32	47	0.57
27	0.33	48	0.58
28	0.34	49	0.59
29	0.36	50	0.60
30	0.37		

is shaved with a small animal clipper (Model A2, Oster, Milwaukee, Wisconsin) using a size 40 head and, in some experiments, the ears are punched to code the animal to the exposure condition. Five to 10 minutes after injection, the animals can be positioned in a spread eagle manner, ventral side against board, on the mouse mounting board with the umbilical region centered in the square hole. An elastic strap positioned across the upper back area securely holds the upper part of the mouse and tape secures the hind limbs and tail. The mouse mounting board is then positioned in the exposure tank at a distance of 9.5 inches from the transducer surface with the abdominal wall facing the transducer, as shown in Figure 2. The abdominal wall is carefully wiped free of visible bubbles and the programmed exposure commences with the pressing of the remote "GO" button. At the completion of the exposure time, the mouse is removed from the exposure tank, released from the board, wiped with a towel and placed in a cage. The procedure can be performed such that the experimenter is unaware of exposure condition.

Figure 2 also shows the positioning of the Pyrex^(R) vessel within the exposure tank. An example of its use is in studying the recalcification time following exposure of platelet rich plasma (Williams et al., 1974a) and other associated phenomenon (Williams et al., 1974b).

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DISCLAIMER

The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health, Education, and Welfare, Public Health Service.

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