A STUDY OF ULTRASONIC THRESHOLD DOSAGES FOR THE MAMMALIAN CENTRAL NERVOUS SYSTEM

BY

JAMES EARL LOHNES
B.S.E.E., University of Illinois, 1971

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Electrical Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 1973

Urbana, Illinois

ACKNOWLEDGMENT

The author wishes to express his grateful appreciation to his advisor, Professor Floyd Dunn, for his patience, guidance, and encouragement throughout this study. The assistance of Professor Frank Fry, Mr. Joe Cobb, Mr. Robert Noyes, and Mrs. Kay Bailey in completing this research study is also appreciated.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The potential uses of ultrasound in medicine are varied. First, ultrasonic techniques are used to visualize soft tissue for diagnostic purposes [1]. Second, ultrasound is used as a therapeutic agent. Treatment methods include heat-producing diathermy techniques and methods for the modification of tissue [1]. Knowledge of the mechanisms by which ultrasound interacts with biological tissue can enhance the usefulness of the applications mentioned above. Previous investigations have shown the existence of a time-intensity threshold for irreversible damage to mammalian neural tissue [2, 3, 4]. The purpose of the present study was to investigate in considerable detail the fine structure in this region, particularly in the region of higher acoustic intensities ($I_s \geq 1000$ W/cm^2). For purposes of this study, the threshold region will be described as the dosage parameters, i.e., specification of both the time of the ultrasonic pulse and the peak acoustic intensity at the site in question, with other parameters specified as the experimental conditions. Thus a positive dosage condition specifies parameters to produce a lesion seen histologically, while a negative dosage condition specifies parameters which result in no lesion appearing. The time of initial appearance, histologically, of lesions produced using ultrasound, and the rate of growth of the lesion are also treated in this study. The following sections deal with a description of the instrumentation used and the experimental methods employed, presentation and analysis of results, and a summary of the findings.

CHAPTER II

DESCRIPTION OF EQUIPMENT

Temperature control of the experimental animal and of the de-gassed saline coupling (sound transmission) solution is accomplished with the system shown schematically in Figure 1.

Temperature control of the experimental animal begins prior to pre-irradiation surgery with a rectal probe inserted into the animal and two disc-type probes attached to the bellypan supporting the animal. The three probes through a resistive network in the control selector provide a control signal to the proportional controller (YSI Model 72). The controller drives a 1000 Watt heater which controls the temperature of the water flowing through the bellypan. The temperature-controlled water flows through tubing located on the interior of the belly pan and through a reservoir to pre-heat the incoming water. The system maintains approximately $\pm$0.1 degrees C. animal temperature control except at the site of skull removal where a slight temperature gradient exists at the surface. After the pre-irradiation surgery, is completed, a small opening is made in the skull, directly posterior to the tentorium, to receive a needle-type brain temperature probe after the headpan is mounted and filled with the saline coupling solution. The needle probe output, through the resistive network in the control selector controls the brain temperature.

Thermal regulation of the saline coupling solution minimizes temperature gradients at the brain-solution interface. A single temperature probe is mounted on the headpan, in the saline solution, and an

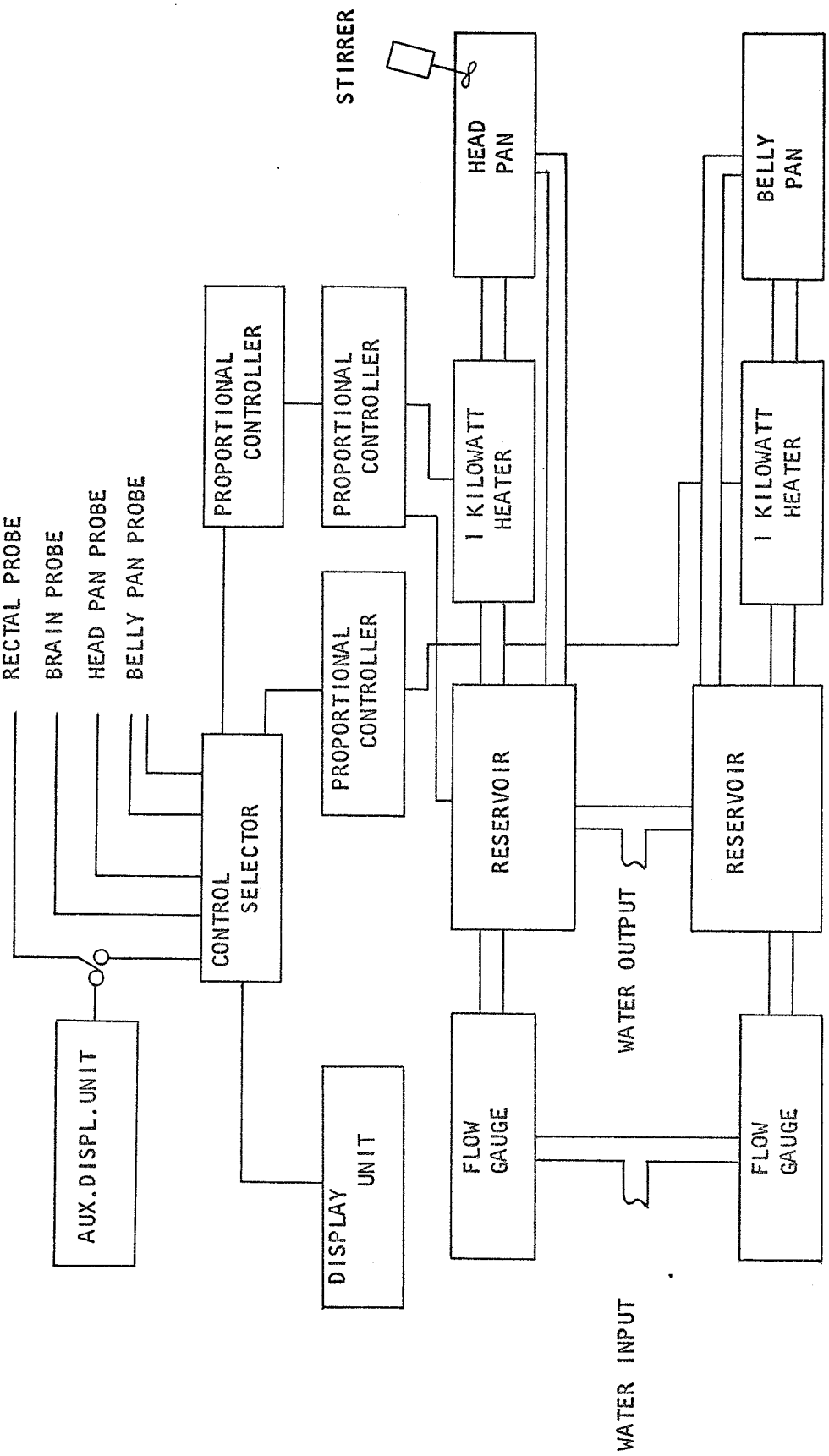

Figure 1. Temperature Control System

electrical stirrer is used to eliminate temperature gradients in the saline solution.

Prior to each irradiation pulse, the brain temperature (T_{brn}) and the temperature of the saline solution (T_{bth}) are recorded from a tele-thermometer (YSI Model 5773). The maximum variation in these temperatures during an irradiation period is 0.4 degrees C.

The transducer assembly used in this study, shown schematically in Figure 2, contains an x-cut, air-backed quartz crystal whose output is coupled through an oil layer to a Lucite lens. The crystal has a resonant frequency of 1.01 MHz; and for this study, the transducer was operated at its fundamental frequency and the third and ninth harmonic frequencies. The lens focuses the ultrasound as can be seen in Figures 3, 4, and 5.

The electrical circuitry employed to excite the transducer is shown in Figure 6. The oscillator is a crystal-controlled vaccum-tube type and the desired pulse width (t_u), generated in the timing circuitry gates the output amplifier in the oscillator. Attenuators in the oscillator circuit control input signal level to the tuned power amplifiers, a 50 W. driver amplifier, a 2 KW. driver amplifier, and a 200 KW. final amplifier as shown. The voltage level applied to the transducer (V_{st}) is set by a variable capacitor (C_s) in the matching network and stabilized during each pulse, calibration and irradiation, by a feedback loop beginning with a capacitive probe in the matching network operating through the stability network and the supply voltage circuits. The impedance matching network serves to match the output of the amplifier impedance to that of the transducer or dummy load.

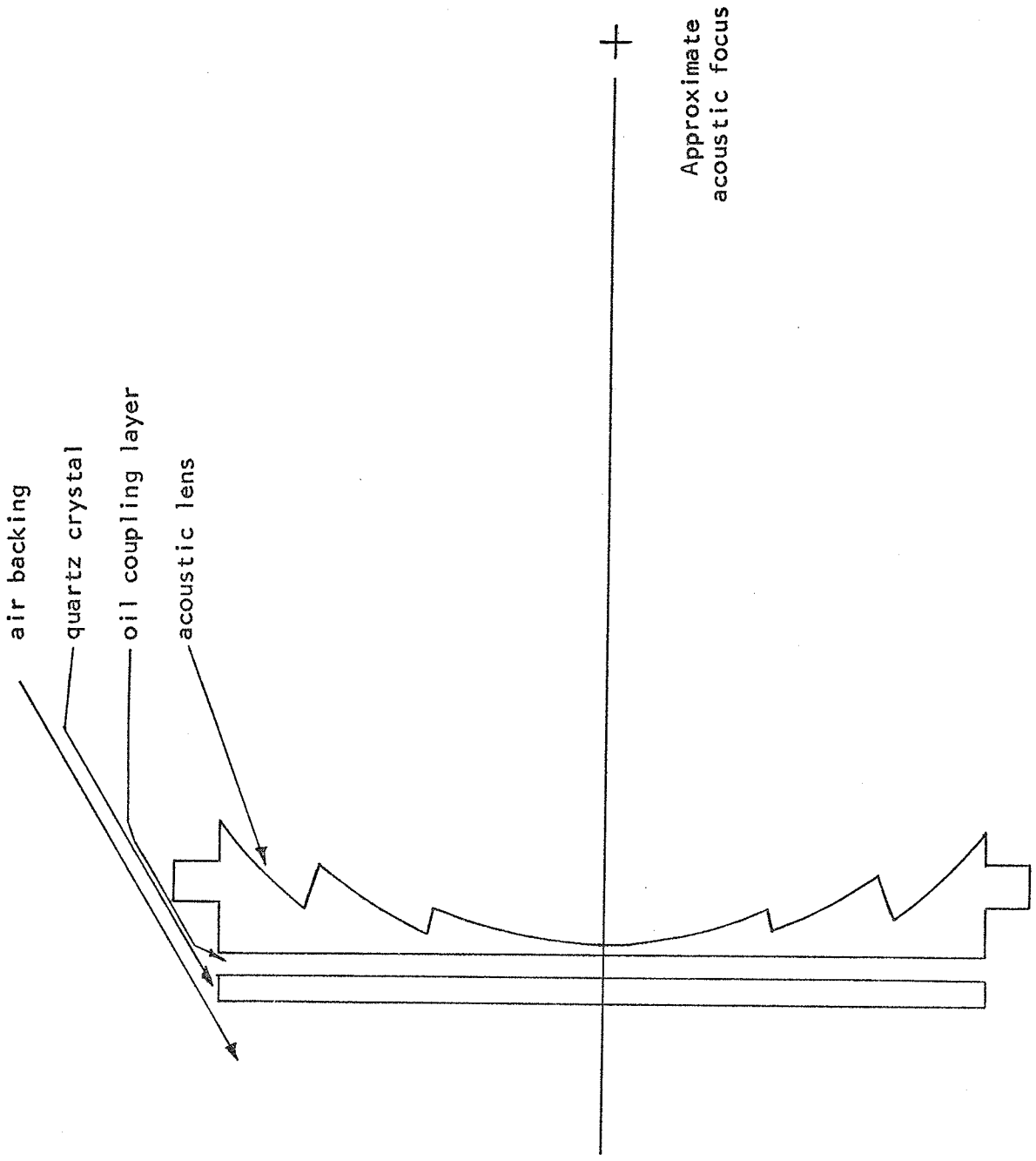

Figure 2. Transducer assembly diagram

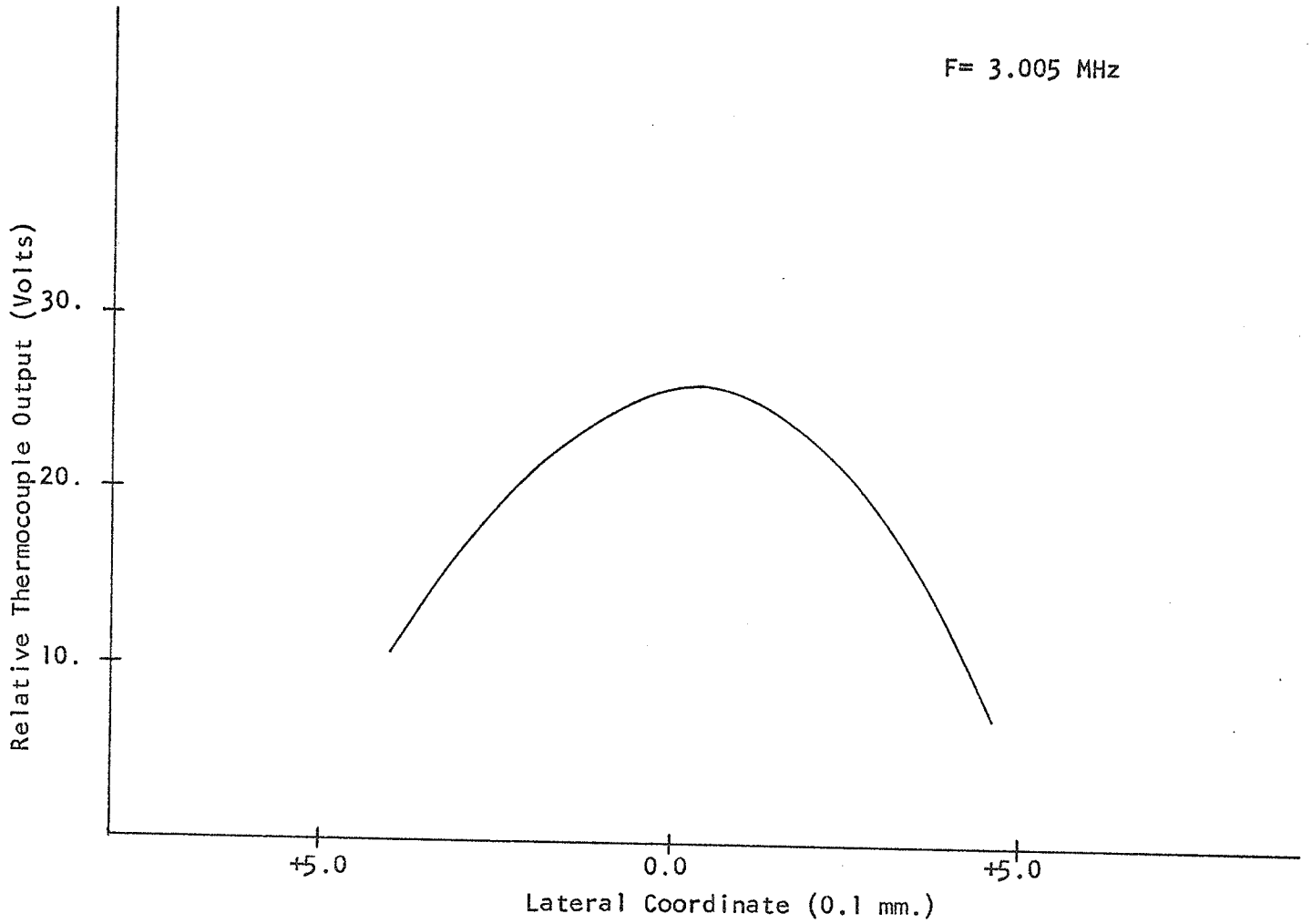

Figure 3. Relationship between thermocouple probe output and transducer assembly lateral coordinate for calibration

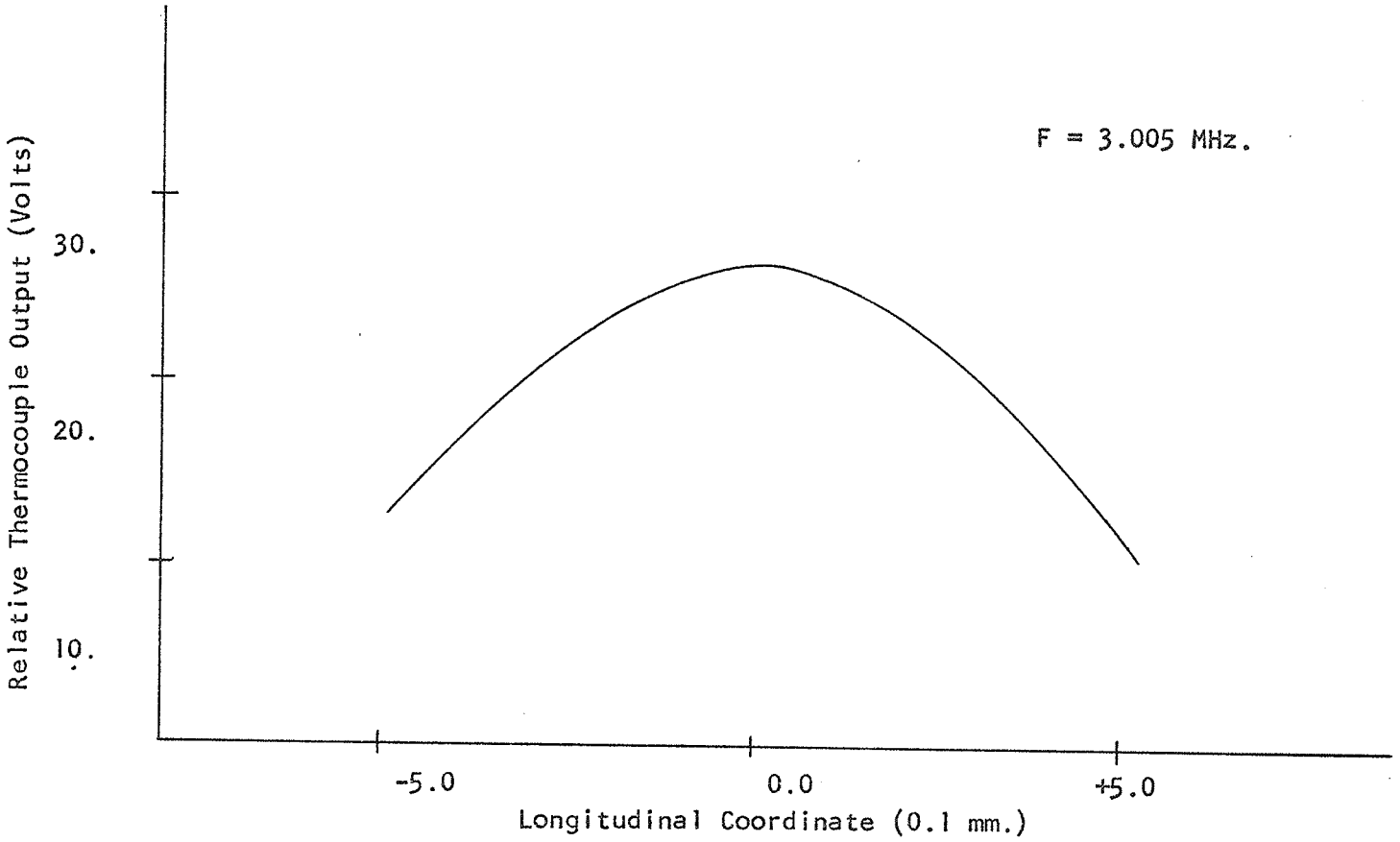

Figure 4. Relationship between thermocouple probe output and transducer assembly longitudinal coordinate for calibration

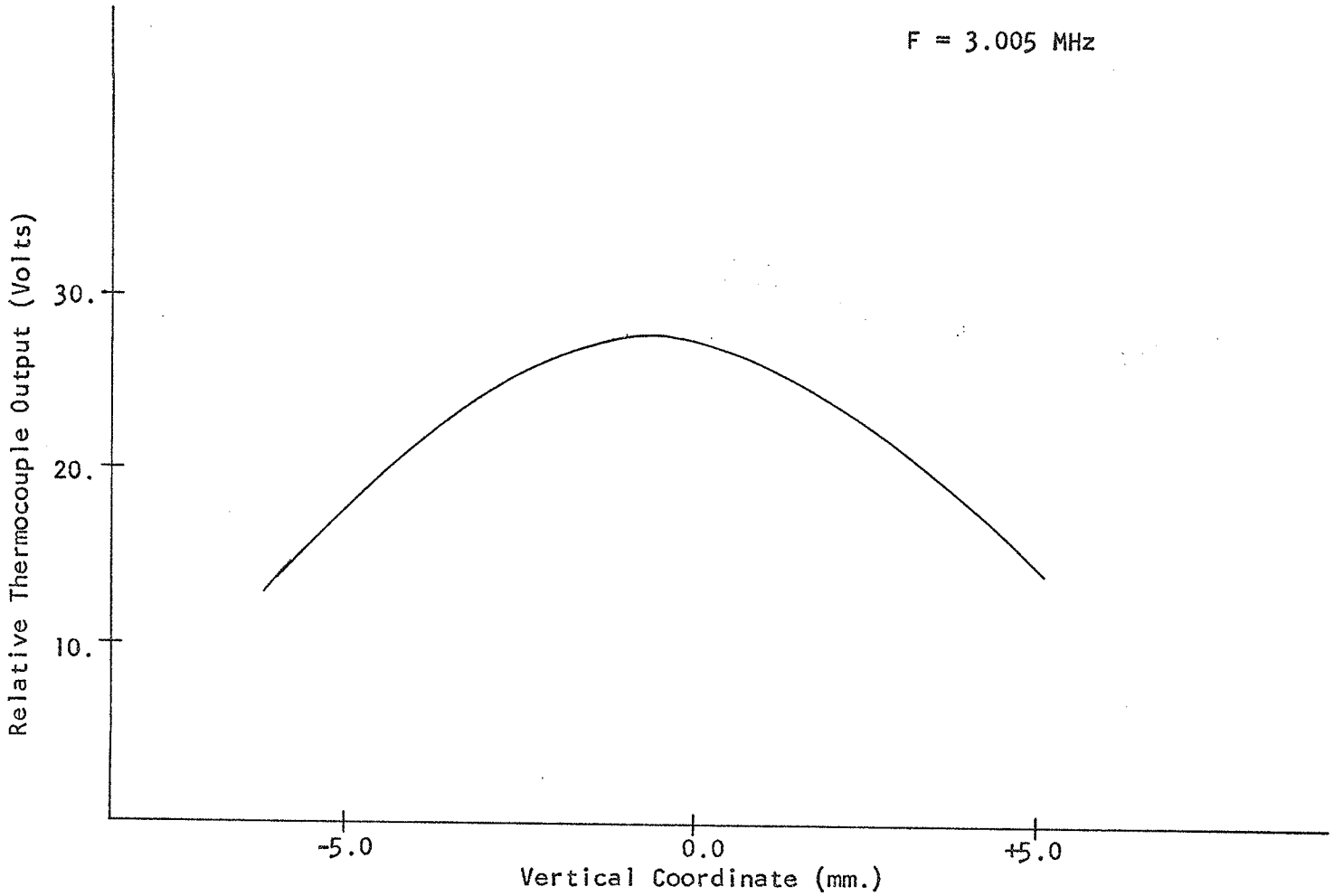

Figure 5. Relationship between thermocouple probe output and transducer assembly vertical coordinate for calibration

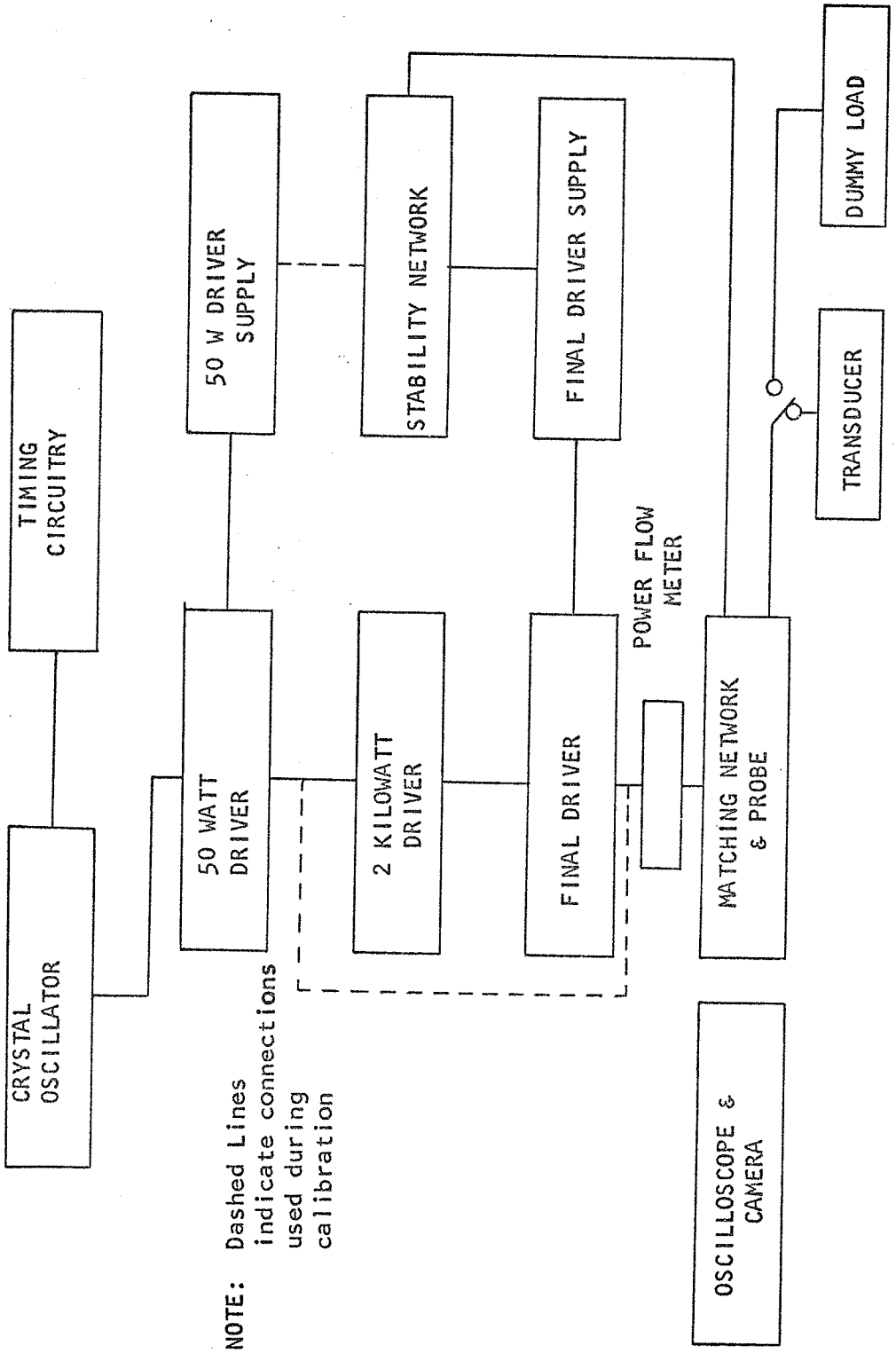

Figure 6. Block diagram of electronic system used for irradiation

The system has been operated at frequencies of 1, 3, and 9 MHz, with pulse times ranging from 400 μsec. to 5 sec. and corresponding peak acoustic intensity levels (I_s) ranging from $1.5 \cdot 10^4$ W./cm.^{2} to 200 W./cm.^{2}, respectively.

Changing the frequency of operation involves component changes in the oscillator, the amplifiers, and the matching network and retuning of the system. The timing circuitry provides for manual and semi-automatic modes of operation. In the semi-automatic mode, irradiation pulses are initiated at preset intervals, e.g., 10 millisecond pulses are delivered at two minute intervals during fast fixation experiments.

Calibration of the transducer assembly and electronic circuitry is carried out using the method of acoustic force on a small steel sphere as described in Fry and Fry [6, 7] and standard electronic calibration procedures. Since the transducer assembly is used at a temperature of 37.0° C. for irradiations, all calibration procedures are also carried out at the same temperature. First, the relation between the voltage applied to the transducer (V_{st}) and the capacitance of the variable capacitor (C_s) in the matching network is determined using precision resistors and a precision voltage generator in a comparison technique that gives values accurate to within 5.0%. The V_{st} - C_s data is used to evaluate the equation

$$C_s = C_o + A\ V_{st}$$

where A is a constant for the specific calibration and C_o is a reference value. A table of V_{st} vs C_s values is prepared to enable selection of appropriate C_s values, prior to irradiation.

The next step in the procedure is the determination of the sensitivity of the thermocouple probe expressed as acoustic intensity per unit deflection of the galvanometer of the magnetic oscillograph used to record the probe output. I_{cal} is found by observing the output of the thermocouple probe for known deflections of a stainless steel sphere, subsequently placed in the identical position of the same acoustic field from which sound field determinations are obtained from radiation force calculations. A PDP-8 computer is used to reduce the data. This procedure is carried out for each frequency used and typical values of probe sensitivity are given in Table 1.

TABLE 1

THERMOCOUPLE PROBE SENSITIVITIES

Freq. (MH_z)	Probe No.	I_{cal} W./cm.^{2}/cm.
1	44	13.96
3	44	5.37
9	34	1.52

With the probe sensitivity and the V_{st} vs C_s relation known, the transducer sensitivity (V_{cal}) is obtained. For this purpose, the thermocouple probe is mounted in the calibration tank containing 36 liters of degassed water at 37.0° C. and containing an acoustic absorption chamber to eliminate standing waves. The transducer assembly is supported on a machine carriage over the tank and introduced into the acoustic coupling fluid. Any air bubbles trapped on the lens surface are removed. Electrical voltage pulses of 0.1 sec. duration are applied to the transducer, whose

output is monitored by the thermocouple probe, the latter's acoustic output observed on an oscilloscope as shown in Figure 7, and the position of the transducer assembly is recorded from the scales on the machine carriage. A typical set of data is shown in Figures 3, 4, and 5 where relative thermocouple probe output is plotted against the three orthogonal coordinates of the transducer assembly, vertical, lateral, and longitudinal. With the beam plot completed, the transducer assembly is positioned such that the peak acoustic output falls on the same point as the thermocouple probe. The output of the thermocouple probe is then connected to the magnetic oscillograph (Hathaway Model 10). With 1.0 sec. voltage pulses applied to the transducer, oscillograms (recorded on Kodak Linograph Paper) of the thermocouple probe output are obtained. Ten oscillograms are taken in this way; two at each of five different voltage settings. The recorded data is reduced using the PDP-8 computer to obtain the transducer voltage sensitivity (V_{cal}). V_{cal} is determined from one to six hours prior to each animal irradiation, and a graph of V_{cal} versus time is shown in Figure 8 illustrating variations over an appreciable period of time.

Accurate location of irradiation sites in the neural tissue of the experimental animal requires mechanical supporting methods for both the animal and the transducer assembly and a method for spatial orientation of the focal region of the acoustic beam to the specific irradiation site in the brain.

The animal is supported by a stereotaxic apparatus and a belly pan, which also provides some of the temperature control. The stereotaxic

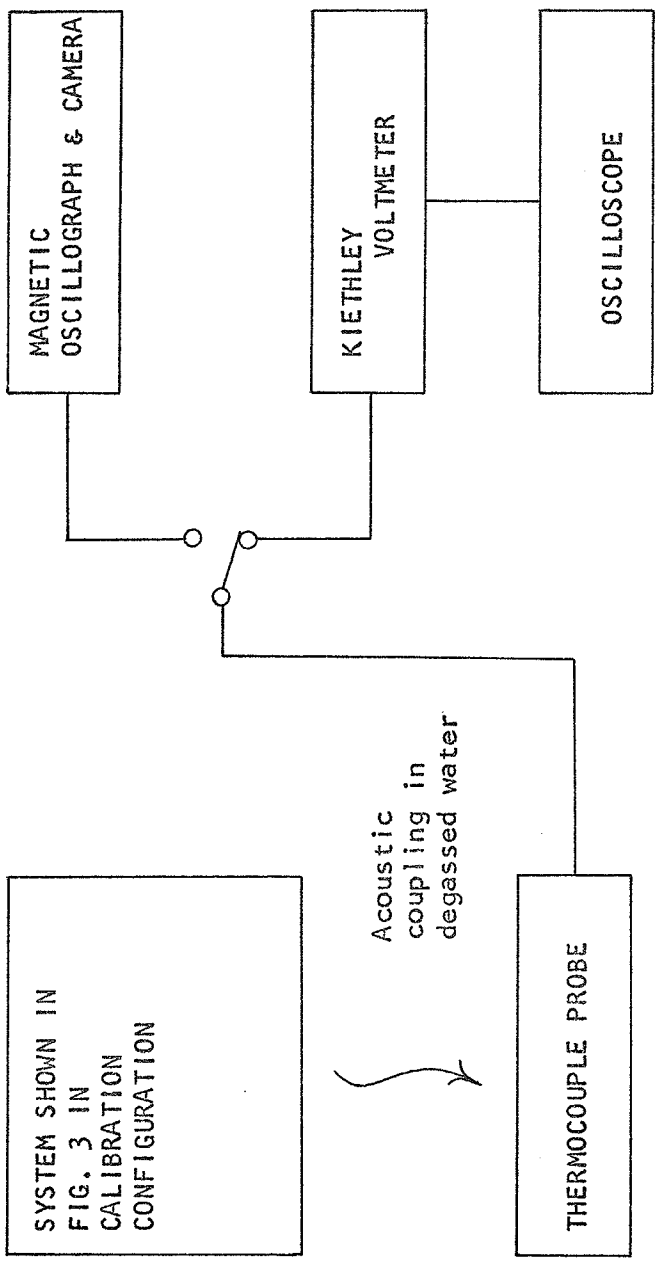

Figure 7. Block diagram of electronic system used for calibration

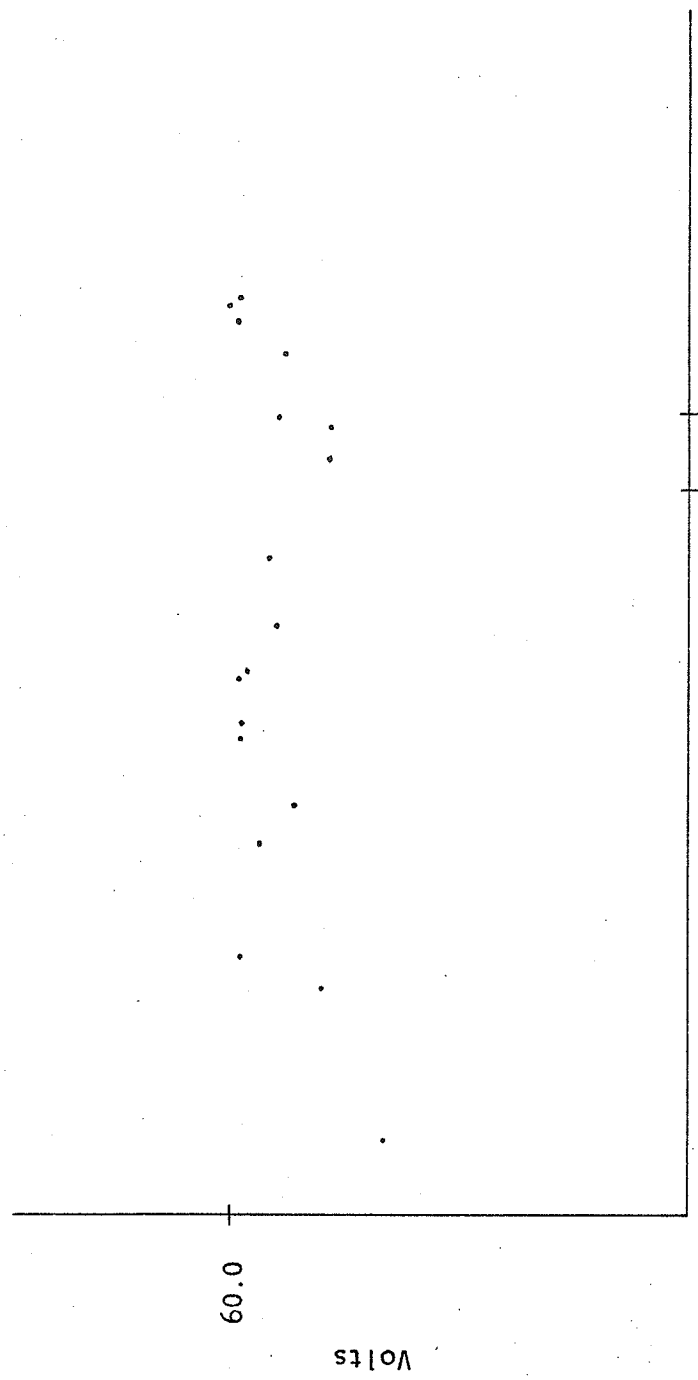

Figure 8. Variation of calibration voltage with time

apparatus supports the animal's head via support bars placed in the ear canals, over the infraorbital ridge, and up against the animal's incisor teeth. This method locates the ear bar zero plane [9] relative to a known point on the stereotaxic apparatus.

The transducer assembly is supported by a machine carriage having three degrees of freedom with a positional accuracy of 0.5 mm. in each of the three orthogonal planes.

Orientation of the focal volume, produced by the transducer, to the reference point of the animal's brain is accomplished by first placing the transducer assembly in the calibration tank and aligning it so the thermocouple probe is at the point of peak acoustic output. Next, a mechanical pointer is attached to the transducer assembly. The pointer, adjustable relative to the transducer assembly, is positioned to have the same spatial coordinates as the thermocouple junction, except for the correction to the vertical coordinate necessitated by the depth of the thermocouple junction in the bubble of oil of the probe. The transducer assembly is then mounted in the transducer support assembly and, using the carriage, the tip of the pointer is aligned with the ear bar zero reference point on the stereotaxic apparatus. The readings from the carriage of the transducer support assembly (V_{ebz}, Lat_{ebz}, $Long_{ebz}$) are recorded for use in irradiation site selection.

CHAPTER III

EXPERIMENTAL METHODS

A. Animal Preparation

The experimental animals, cats obtained from a commercial supplier, are prepared for irradiation as described here with modifications to procedures for the fast fixation experiment described in Section F. Animals are anesthetized with a dosage (dosage in cubic centimeters equals animal's weight in pounds times 0.2 cc./pound plus 0.3 cc.) of Diabutal[1] according to their weight via an intraperitoneal injection. Hair is removed from the top of the head, the stomach, and the insides of the hindlegs of the anesthetized animal to facilitate exposing the animal's brain, temperature control, and access to veins for maintenance of the level of anesthesia, respectively. The animal is then placed in the stereotaxic apparatus and temperature control is established using the rectal probe and two bellypan probes as sensors. The surgical procedures employed are non-sterile, since the animal will be sacrificed within twenty-four hours. An initial incision is made along the midline of the skull beginning over the frontal bone and extending approximately eight centimeters posterior. With the skin reflected, the temporalis and occipitalis muscles are reflected to expose approximately twenty square centimeters of skull bone, including the posterior portion of the frontal sinus. A trepine is used to place six holes in the skull, two over the frontal sinuses and four

[1]Diabutal Sodium Solution, Diamond Laboratories, DesMoines, Iowa.

directly over the brain, two on each side of the midline. Removal of the remaining bone is accomplished by separating the dura mater from the bone with an elevator and removing the bone with rongeurs. The dura mater is kept warm and moist with warm physiological saline solution and hemorrhage is controlled with bonewax and Surgicel.[2]

B. Pre-irradiation Procedures

The pre-irradiation procedures begin after surgery is completed and include measurement of the cortical surface vertical coordinate (V_{cor}) for each irradiation site, calculation of the acoustic intensity required to produce I_s at the irradiation site based on the V_{cor} measurements and absorption, installation and filling of the headpan, and establishment of temperature control of the degassed physiological saline solution in the headpan.

The determination of V_{cor} is accomplished by setting the lateral and logitudinal coordinates of the transducer assembly to the irradiation site coordinates. With the stereotaxic apparatus, supporting the cat, mounted on the irradiation stand, the transducer assembly, with pointer attached, is lowered until the tip of the pointer just touches the surface of the dura mater directly above the irradiation site. The V_{cor} reading from the machine carriage and the irradiation site vertical coordinate (V_s) give the on-axis tissue depth (d) by the equation

$$d = V_s - V_{cor} \qquad (1)$$

With the depth at each irradiation site (eight to forty sites per brain) determined, the voltage (V_{st}) required to produce the desired accoustic

---

[2]Surgicel, Oxidised Regenerated Cellulose, Johnson and Johnson, New Brunswick, N. J.

intensity (I_s) at the site is obtained from the following equations which include the acoustic intensity (I_r) prior to transversing the distance d, through media having an acoustic intensity absorption coefficient μ, to the radiation site.

$$I_r = I_s e^{\mu d} \qquad (2)$$

Values of μ used in these computations are given in Table 2.

TABLE 2

ACOUSTIC INTENSITY ABSORPTION VALUES

F (MHz)	μ (cm.$^{-1}$)
1.000	0.2
3.005	0.6
9.015	1.8

With I_r from (2) and the ratio $V_{cal}/(I_{cal})^{\frac{1}{2}}$ from the calibration procedure, the required value of V_{st} is given by

$$V_{st} = (I_r/I_{cal})^{\frac{1}{2}} V_{cal} \qquad (3)$$

The required capicitance setting (C_s) is read from a table of V_{st} vs C_s values.

The headpan is held in place by a bracket on the stereotaxic apparatus and positioned so that the lip of the lower opening is parallel with the exposed brain tissue. The reflected skin is then pulled over and around the lip of the headpan and held in place with a wire tourniquet to form a water-tight seal. The reflected temporalis muscles are sutured to small posts inside the headpan to remove them from the path of the acoustic

waves. The brain needle temperature probe is inserted in the small hole directly behind the tentorium and secured to the headpan to prevent movement. After the stirrer is installed, the headpan is filled with nine liters of degassed physiological saline solution previously heated to 37.0 degrees C. in an oven, the headpan temperature probe is mounted to monitor the saline solution temperature, and temperature control of the saline solution is initiated. The transducer assembly, with pointer removed, is then introduced into the acoustic coupling fluid and air bubbles trapped on the lens surface are removed.

The irradiation sites are selected using the Jasper-Martin Atlas and knowledge of the acoustic beam shape by first noting that sites can be in gray matter entirely, in white matter entirely, at gray matter-white matter interfaces, or at tissue-ventricular interfaces. Tissue bone interfaces aren't selected due to the differences in the acoustic impedance between bone and neural tissue. Sites must also be selected so that the focal volume of one site does not overlap on the focal volume of another site. It should be noted that sites may be placed closer together at higher irradiation frequencies when the focal volume is smaller. A vertical sketch of an irradiation array of sixteen sites is shown in Figure 9. Pulses are applied to the sites in the order indicated by the numbers directly below the sites to allow approximately four minutes to insure that the brain temperature is 37.0 degrees C.

C. Irradiation

When the temperatures of the brain and the saline solution are stabilized near 37.0 degrees C., the selected sites are irradiated at two

minute intervals. During each two minute interval, the oscillogram of V_{st} from the previous irradiation is indexed with the irradiation pulse number and scaling information when necessary, the site coordinates (V_s, L_s, and F_s) for the next site are set on the machine carriage, necessary changes in t_u and C_s are made, and the temperatures T_{bth} and T_{brn} are recorded. After the final irradiation pulse, the transducer assembly is removed, the saline solution is pumped out of the headpan, the headpan is removed, the incision on the head is closed with sutures or wound clips to prevent possible mechanical damage to the brain as the animal awakes and to keep the neural tissue as warm as possible, and the animal is placed in a crib, with the rectal probe left installed to monitor body temperature.

D. Animal Sacrifice and Histology

Twenty-four hours after irradiation, the animal is sacrificed by cannulating the systemic aorta through the left ventricle of the heart. The animal is then perfused with 900 cc. of physiological saline solution (pH = 7.1 $\pm$ 0.1), and the tissue is fixed by perfusion with a 10% Formalin solution. Times for each of these sacrifice procedures are listed in Table 3.

TABLE 3

SACRIFICE PROCEDURE TIMES

Procedure	Time (Minutes)
Cannulation	2.5
Saline Perfusion	2.0 - 4.0
Formalin Perfusion	60.

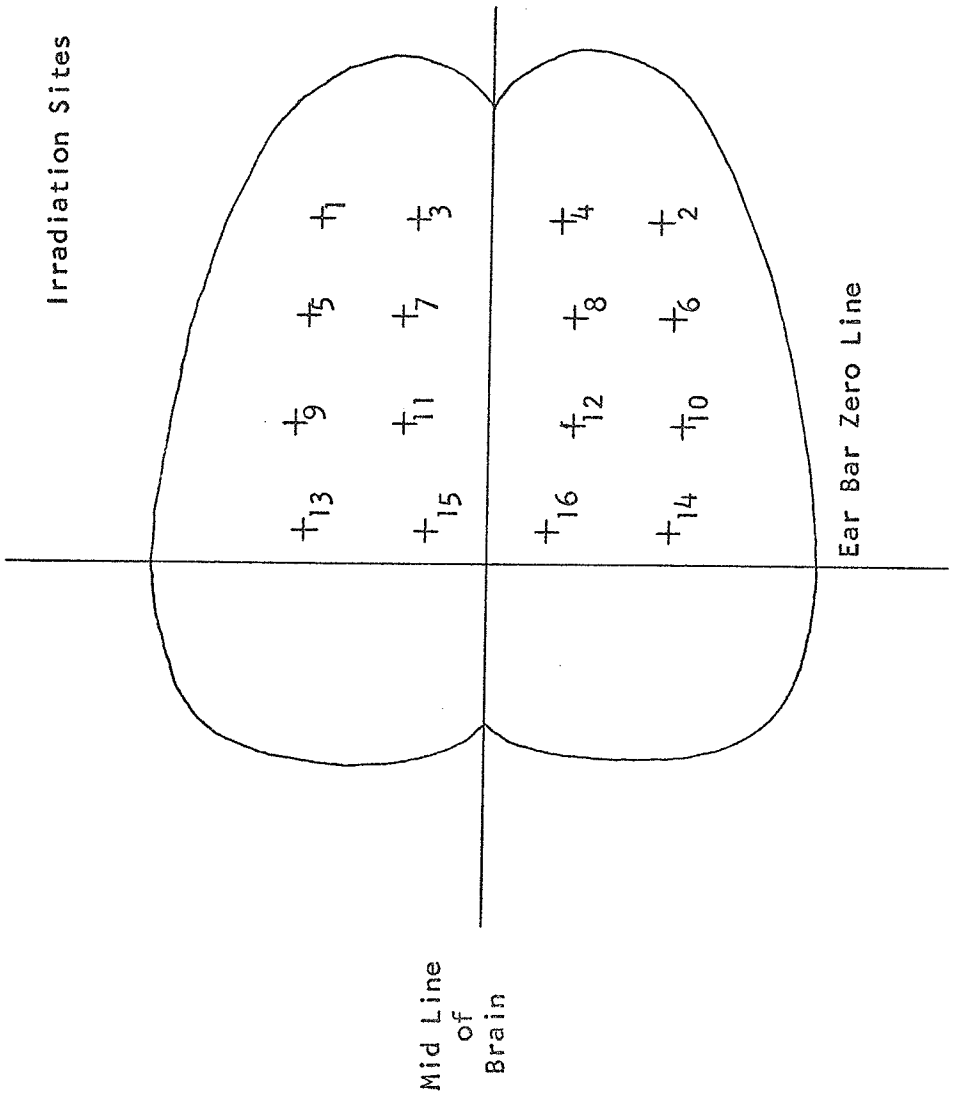

Figure 9. Typical irradiation array in vertical view

The head including the brain is then removed and stored in 500 cc. of a 10% Formalin solution.

Since each brain contains from eight to forty irradiation sites, each having possibly different dosage conditions, the histological procedures must not lose the spatial orientation of the irradiation sites. The first step in histology is to insert a locator pin parallel to the midline of the brain and on the left side. The next step involves embedding the tissue in paraffin using the Ethanol-Methyl Benzoate method [9]. After the brain is embedded, a microtome is used to section serially the brain into approximately 2600 sections each with a thickness of 10 . The sections are stored in boxes indexed so that frontal/longitudinal integrity is maintained. For initial analysis, every tenth section is mounted using a water bath procedure [9]. The glass slides used for mounting are indexed with the animal number and the slide number (SN). The remaining sections are stored in darkness until initial analysis is completed and either more sections are needed for a more detailed analysis or the remaining sections can be discarded, not containing any useful information. After the paraffin is removed, the mounted sections are stained with a weil Stain. The stained sections are then cover-slipped for microscopic analysis.

E. Histological Analysis

The histological analysis of the lesions produced by the ultrasound using light microscopy includes correlation of the section (slide) numbers with the coordinates of the Jasper-Marson Atlas [8], microscopic

examination of each irradiation site for evidence of tissue alteration, and determination of the extent of such tissue alteration in terms of volume and/or cross-sectional area measurements.

Correlation of the slide numbers to the Jasper-Marson Atlas coordinates is accomplished using a lateral schematic diagram of the brain and locating four points on the slides, including the most anterior and the most posterior portions of the N. medialis (M_d) and the Corpus Mamillare (MM_n). The posterior point of the Md and a point in the area of the MM_n, found by selecting the point corresponding to the F 7.0 plane in the MM_n, can be used to define the F 7.0 plane through brain since the serial sections are cut parallel to the midline.

When the lesion is located, the sections posterior and anterior to the slide containing the lesion are inspected to determine the longitudinal lesion limits. Volume and cross-sectional area measurements are made, using the light microscope and a scaled eye-piece, by taking measurements (described below) on each slide where the lesion appears and approximating the dimensions for the tissue between the sections examined.

For cross-sectional area measurements, the most lateral points of the lesion on both sides of the midline of the lesion are identified and the distance between these two points is recorded. After the lateral lesion dimension is measured on each slide exhibiting the lesion, the lesion is sketched graphically as shown in Figure 10. The cross-sectional area of the lesion is taken as the sum of the areas of the rectangles as seen in the example of Figure 10.

Both the vertical and lateral lesion dimensions are measured to approximate the volume of altered tissue, and the total volume of altered

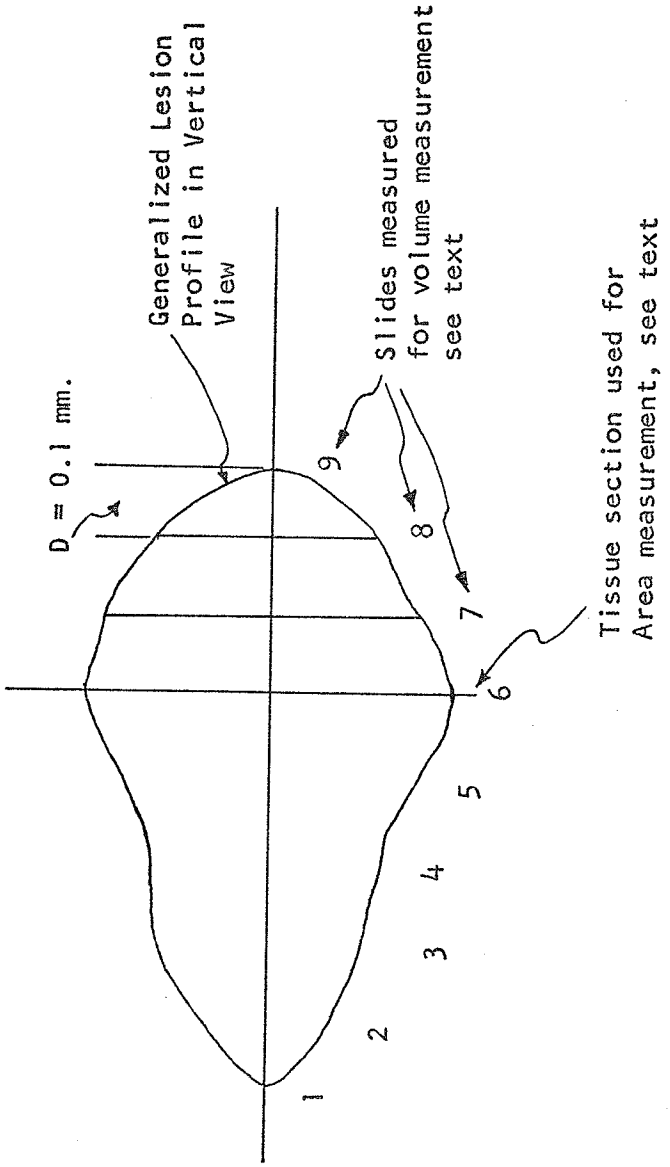

Figure 10. Diagram for determination of lesion area

tissue is taken as the sum of the volumes of the individual sections.

F. Fast Fixation Methods

The fast fixation experiments are designed to yield information about the initial appearance of the lesion and its rate of growth, and it is therefore necessary to be able to specify the amount of time taken to fix the tissue relative to the last irradiation pulse and to have accurate data concerning the temporal as well as spatial orientation of the irradiation sites. Animal preparation is similar to that for a high intensity threshold animal except that the neck and upper chest portions of the animal are shaved and cleansed.

Pre-irradiation surgical techniques for fast fixation animals include first making two injections (0.5 cc. each) of Xylocaine, a local anesthetic, one on each side of the midline on the ventral side of the neck. This anesthetic blocks the presso-receptors in that area and facilitates cannulation by blocking the response of constriction of the internal carotid artery. An incision is then made on the ventral side of the neck approximately seven centimeters along the midling beginning one centimeter posterior to the transverse vein and extending posteriorly. With the skin reflected, the transverse vein is separated from the surrounding tissue and a suture is threaded around the vein in order that just before the fixation procedure begins, the veing can be opened to vent the brain tissue to be perfused into a collector pan below the animal, rather than to pump the 1.5 liters of perfusion solution into the animal which would cause a great increase in fluid level in the cardiac system. Next, a tracheotomy is performed, and the animal is placed on positive respiration

with a gas mixture of 60% Oxygen and 40% atmospheric gases. The internal carotid artery is then exposed and cannulated. The cannula contains Heparinized biostatic saline solution (0.1% Heparin) that is pumped into the artery at a rate of 3 cc./hr. From this point, the pre-irradiation techniques follow those for the high intensity threshold animals. The ultrasonic irradiation pulses are automatically applied at two minute intervals by the timing circuitry and the output of the 'A' trace of the oscilloscope displaying V_{st} is fed into a strip chart recorder so that each irradiation pulse time and the time that fixation is initiated can be recorded. Fixation is initiated two minutes after the final irradiation pulse, by first using the suture to extend the transverse vein and then bisecting the vein. Next, the mechanical pump used to deliver the Heparinized biostatic saline solution into the artery is disconnected and hand-held 50 cc. syringes are used first to perfuse the brain with 150-200 cc. of saline solution and then with 1000 cc. of 10% Formalin solution. After fixation, the ultrasonically irradiated brain undergoes the same histological procedures described previously. Since the same positive dosage conditions are used for each irradiation site, analysis of the serial sections is modified to include a determination of the first appearance of damaged tissue and a measurement of the volume of each lesion present.

CHAPTER IV

RESULTS

For the purpose of discussion of these results, a threshold lesion is defined as a lesion with specified dosage conditions determined from the highest negative exposure conditions (i.e., the highest dosage for which a lesion has been found experimentally not to occur) and the lowest positive exposure conditions (i.e., the lowest dosage for which a lesion has been found experimentally to occur). Figure 11 shows two threshold points established from the highest negative and lowest positive dosages, by taking the geometric mean of the respective exposure times, for the acoustic intensities of exposure held constant. The threshold line is a best fit to the threshold lesion points and the threshold region is defined as the region between the set of highest negative and lowest positive dosage points used to determine the threshold lesion points. The threshold lesion points and the threshold region can be refined by irradiating additional animals, as seen in the example for 100 W./cm.^{2} in Figure 11. A graph of the threshold lines for the acoustic frequencies of 1, 3, and 9 MHz. are shown in Figure 12 where acoustic intensity delivered to the lesion site (I_s) is plotted against the time of the ultrasound pulse (t_u). Similar data from other researchers [2, 3, 4] is also presented for comparison purposes with the respective frequencies as noted in Figure 13.

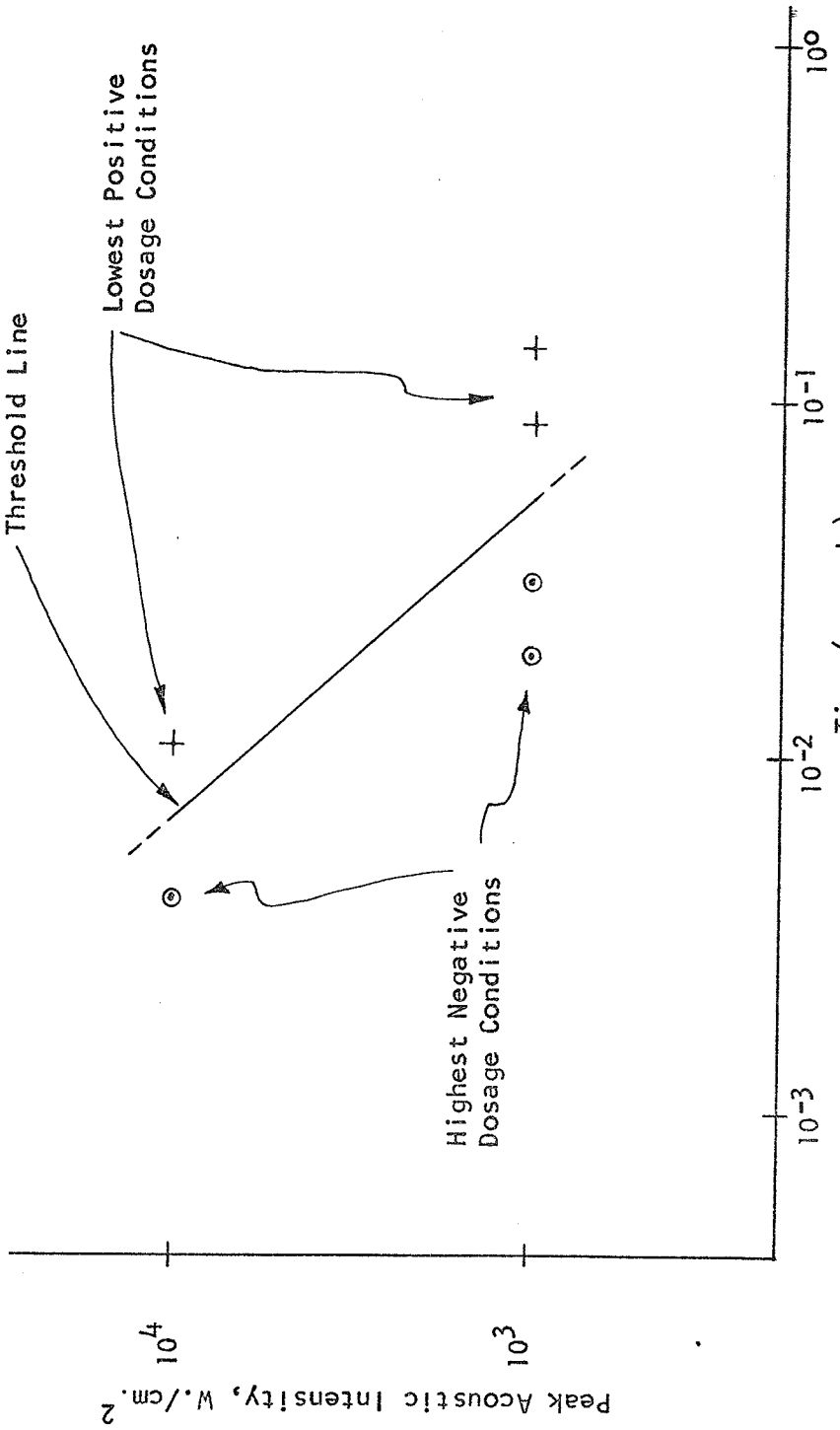

Figure 11. Determination of threshold

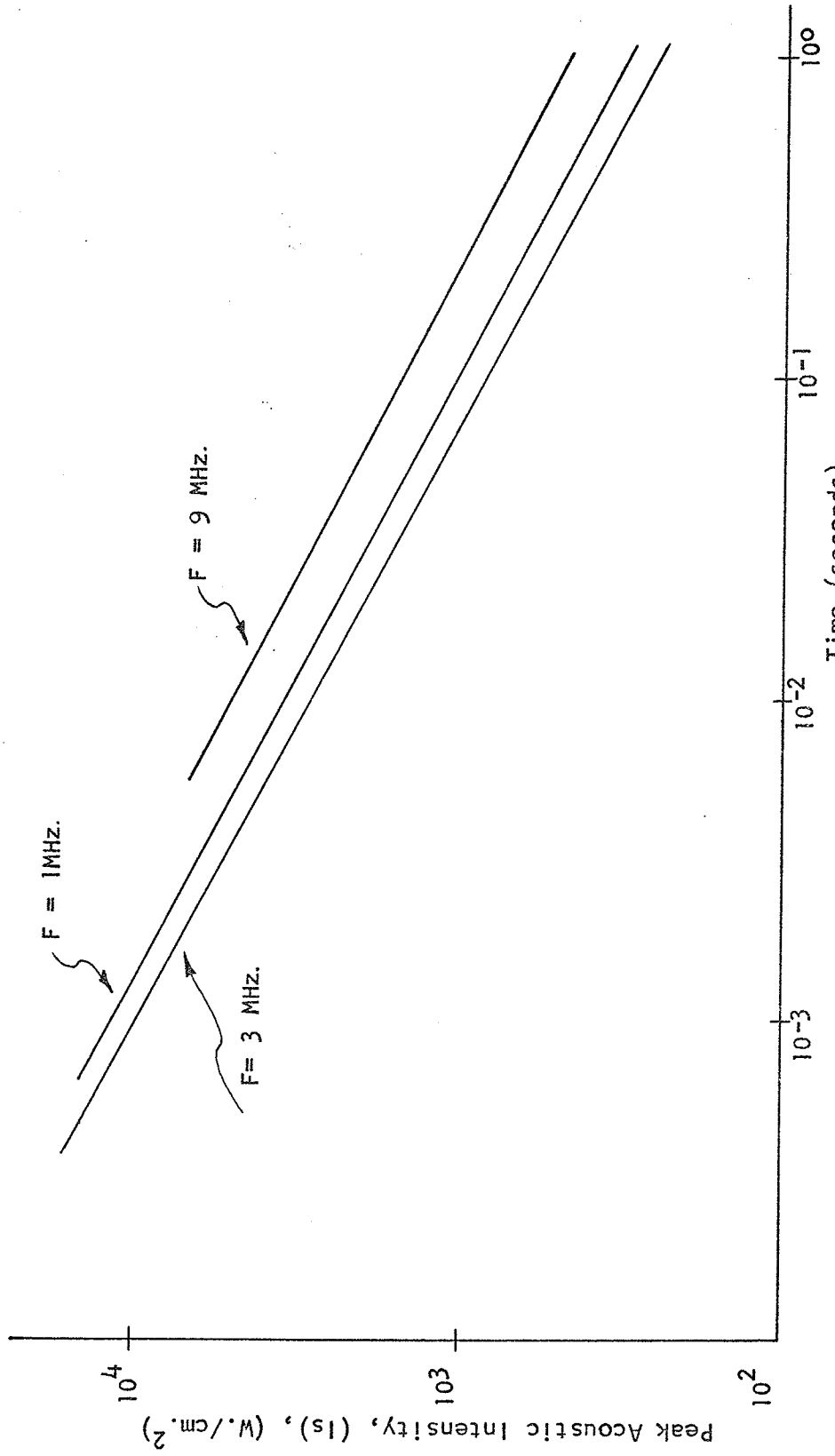

Figure 12. Relationship between peak acoustic intensity and time of ultrasound pulse

For the fast fixation experiments, the irradiation time was selected to be 50% to 100% greater than the lowest positive dosage condition previously found for the twenty-four survival animals. The initial appearance of the lesion was determined by examining the array of irradiation sites in each experimental animal and noting the first appearance of a lesion at the light microscope level. It was found that a lesion produced using an acoustic intensity (I_s) of 1000 W./cm.^{2} at a frequency of 3 MHz first appears at four minutes after ultrasonic exposure and a lesion produced using an acoustic intensity of 750 W./cm.^{2} at a frequency of 9 MHz first appears at ten minutes after ultrasonic exposure.

Using the method described in Chapter III, the volumes of the lesions appearing in each fast fixation animal were determined. These volumes were compared to the volume of a lesion produced using the same dosage conditions in a twenty-four hour survival animal. This comparison is shown in Figure 14 where the ratio of the lesion volume at twenty-four hours to the lesion volume at the fast-fixation times is plotted against the fixation time for the lesion.

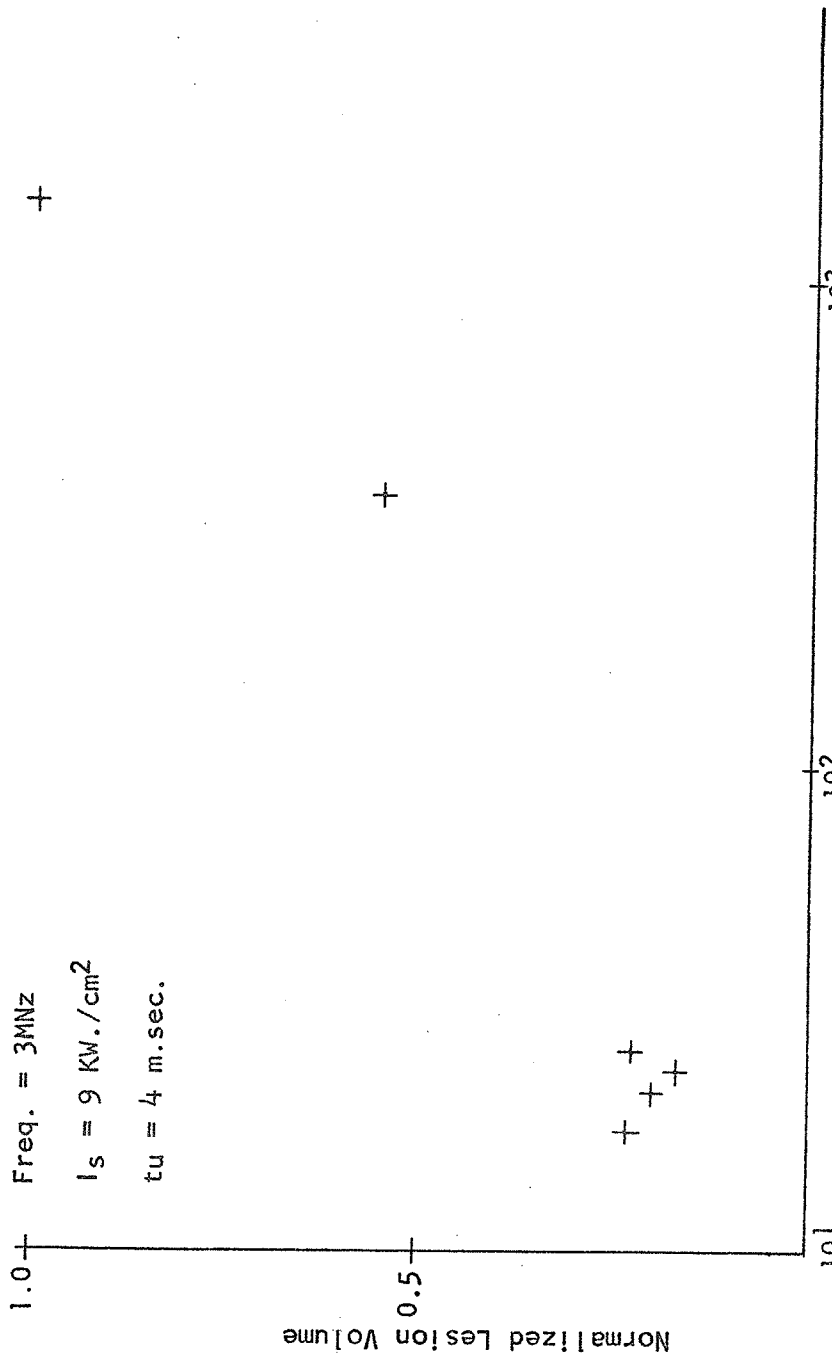

Figure 14. Relationship of normalized lesion volume to fixation time

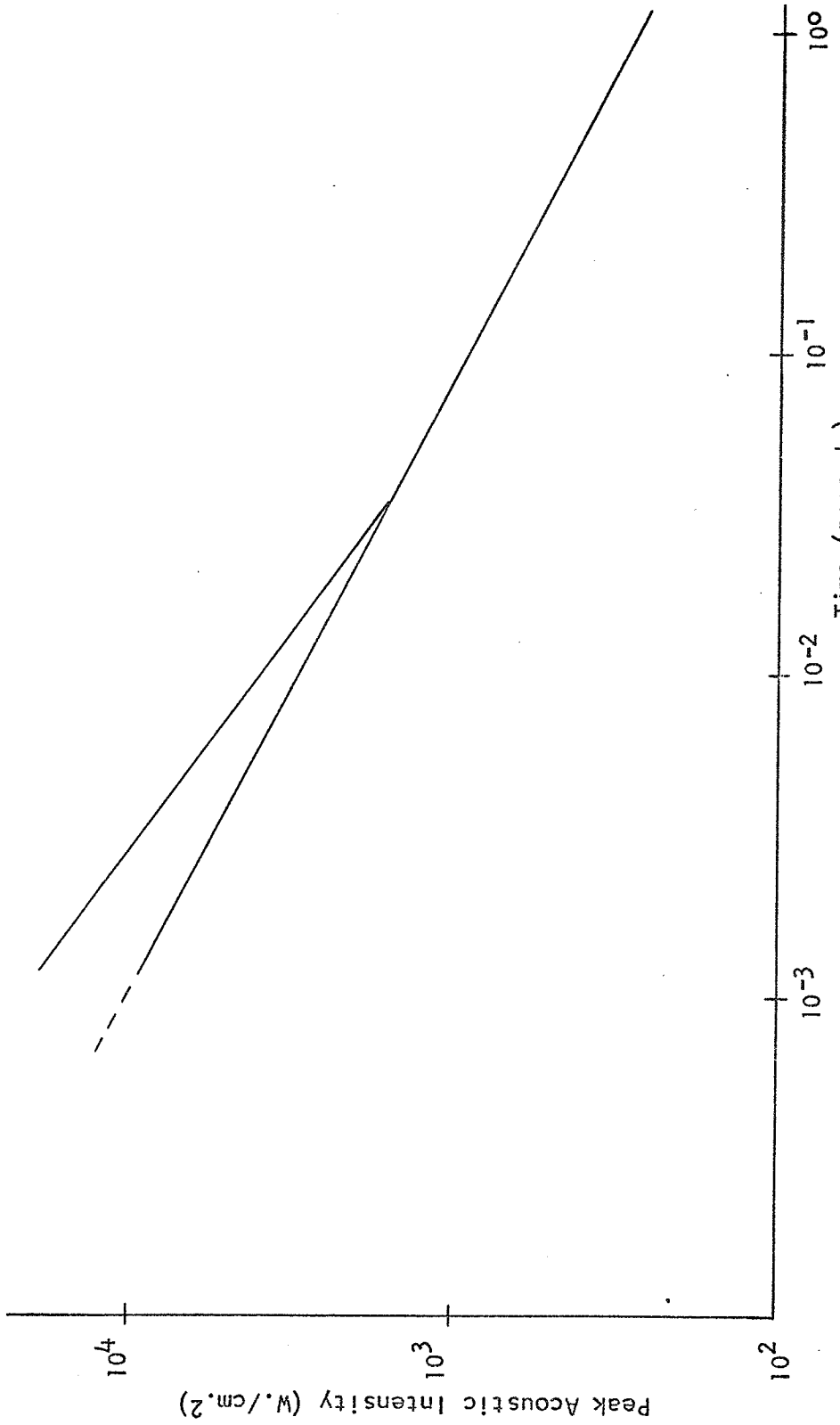

Figure 13. Acoustic intensity verus single-pulse time duration to produce threshold lesions in white matter of the mammalian brain (Fry, Kossoff, Eggleton, and Dunn, 1970)

CHAPTER V

DISCUSSION OF RESULTS

As seen in Figures 12 and 13 the slope of the thresholds at 1, 3, and 9 MHz determined in this study are the same as previously reported [2, 3, 4] with the exception of the difference at 3 MHz above an peak acoustic intensity of 4000 W./cm.^{2} This difference may be attributed to the more detailed study (i.e., higher number of threshold points above the peak acoustic intensity level of 4000 W./cm.^{2}) just concluded.

The fast-fixation experiment demonstrates (1) that fast-fixation of ultrasonically irradiated neural tissue is possible, (2) that the data obtained by light microscopy techniques yields information concerning the first appearance of gross tissue alteration, but (3) other techniques of observing initial tissue alteration produced by focused ultrasound may yield more information concerning the mechanisms by which the alteration occurs. Electron microscopy is one method for studying details of the tissue ultrastructure and the changes in ultrastructure due to ultrasonic irradiation. A passive technique for investigating the changes in tissue temperature due to ultrasonic irradiation would yield additional information concerning tissue alteration due to localized heating of the tissue.

CHAPTER VI

SUMMARY

1. Threshold determinations for structural alterations in the adult cat brain by ultrasound and fast fixation of treated tissue were carried out on approximately 150 animals.

2. Thresholds were established at acoustic frequencies of 1, 3, and 9 MHz over the acoustic intensity range 200 to 15000 W./cm.^{2}

3. From the fast fixation experiments it was found that a lesion produced using an acoustic intensity (I_s) of 1000 W./cm.^{2} at a frequency of 3 MHz. first appears at four minutes after ultrasonic exposure, while at 9 MHz., a lesion produced using an acoustic intensity of 750 W./cm.^{2} appears no sooner than ten minutes.

4. The threshold data shows that while the slope of each log I_s - log t_u curve appears to be the same for all frequencies, the threshold levels vary somewhat with frequency.

5. It is felt that additional absorption data essential for determining the acoustic intensity at the lesion site and that electron micrographic studies of the lesion will yield important information regarding the initial physiological events following ultrasonic exposure.

LIST OF REFERENCES

1. F. J. Fry, R. F. Helmburger, L. V. Gibbons, and R. C. Eggleton, "Ultrasound for Visualization and Modification of Brain Tissue," IEEE Journal of Sonics and Ultrasonics, Vol. SU-17, No. 3, July 1970.

2. F. J. Fry, G. Kossoff, R. C. Eggleton, and F. Dunn, "Threshold Ultrasonic Dosages for Structural Changes in the Mammalian Brain," J. Acout. Soc. Amer., 48, No. 6 (Pt. 2), pp. 1413-1417, December 1970.

3. L. Basauri and P P Lele, "A Simple Method for Production of Trackless Focal Lesions with Focused Ultrasound," J. Physiol. 160 513-534 (1962).

4. J. Pond, "A Study of the Biological Action of Focused Mechanical Waves (Focused Ultrasound)," Ph.D. thesis, Univ. of London (1968).

5. W. J. Fry and F. Dunn, "Ultrasound: Analysis and Experimental Methods in Biological Research," in Physical Techniques in Biological Research, Vol. 4, ed. (1962) Academic Press, New York.

6. W. J. Fry and R. B Fry, "Determination of Absolute Sound Levels and Acoustic Absorption Coefficients by Thermocouple Probes-Theory," J. Acoust. Soc. Amer., 26, No. 3, pp. 294-310, May 1954.

7. W. J. Fry and R. B. Fry, "Determination of Absolute Sound Levels and Acoustic Absorption Coefficients by Thermocouple Probes -- Experiment," J. Acoust. Soc. Amer., 26, No. 3, pp. 311-317, May 1954.

8. H. H. Jasper and C. Ajmone-Marsan, (1960). "A Stereotaxic Atlas of the Diencephalon of the Cat", The National Research Council of Canada, Ottawa 2, Canada.

9. Joe Pankau, private communication, Mercy Hospital, Champaign, Illinois, 61820.