DUNN, FLOYD

DETERMINATION OF ULTRASONIC DOSAGE RELATIONS FOR THE MAMMALIAN CENTRAL NERVOUS SYSTEM.

DETERMINATION OF ULTRASONIC DOSAGE RELATIONS FOR THE MAMMALIAN CENTRAL NERVOUS SYSTEM

BY

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B.S., University of Illinois, 1949
M.S., University of Illinois, 1951

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ELECTRICAL ENGINEERING
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1956

URBANA, ILLINOIS

UNIVERSITY OF ILLINOIS

THE GRADUATE COLLEGE

May 16, 1956

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Floyd Dunn

ENTITLED Determination of Ultrasonic Dosage Relations for the Mammalian Central Nervous System

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy in Electrical Engineering

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ACKNOWLEDGEMENT

The author is pleased to express his indebtedness to Professor William J. Fry who suggested the problem and offered many helpful suggestions and criticisms as well as continued guidance during the course of this work.

The author also wishes to acknowledge the assistance and cooperation of the members of the staff of the Bioacoustics Laboratory.

I. INTRODUCTION

The use of ultrasound in the fields of biology and medicine has increased greatly during the past decade. Ultrasound has been used clinically as a diathermy procedure[1,11,30,37] in the treatment of superficial cancers[44] and arthritis[2]. It has also been employed in dental drilling[12,39] and several attempts have been made to use it in the localization of tumors[16,31]. Diagnosis of malignancy has also been attempted[40]. As a research tool, ultrasound has been used in biological investigations encompassing the fields of muscle physiology[9,23,25,26,27,28], bacteriology[33,41,46], virology[42,46], enzymology[38], and hematology[3]. Much of the early work was performed without due consideration for the physical aspects of the problem and the proper design of the apparatus. In many cases the investigators were highly skilled in the biological and medical aspects of the problem, but their research background was of such a nature that they were unable to cope with the acoustical aspects. As a result, the biological portion of the problem was handled with the most advanced techniques available, while the physical portion was of doubtful quality. Consequently, many of the results obtained were of a dubious nature. The more recent applications of ultrasound as a research tool in biology include the fields of neuroanatomy[5] and neurophysiology[4,10,36].

There is strong evidence which demonstrates that ultrasound, when properly controlled, can produce unique results in biological systems[4,5,24]. For example, the high level ultrasonic method for producing selective, accurately localized alterations in brain tissue by focussing the sound energy

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1. Superscripts refer to numbers in the Bibliography.

in the region to be affected now constitutes a unique tool for neurological research and human neurosurgery. Extensive histological studies, on monkeys and cats, have demonstrated that accurately localized selective changes can be produced in a chosen region in the brain without destroying tissue between the chosen region and the area of entry of the sound energy into the brain. It has also been shown that the neural components of both white and gray matter can be destroyed without interrupting blood vessels traversing the region of alteration, and nerve fiber tracts can be interrupted without destroying neighboring or surrounding cell body regions.

If the full potentialities of the ultrasonic methods, e.g., the method of producing selective changes in brain tissue, are to be realized, it is essential that precision instrumentation and techniques be available to the investigator and that the physical mechanism of the action of the sound on the tissue be understood. A comprehensive understanding of the physical mechanism involved in the action of ultrasound on biological materials will enable physical and engineering scientists to design proper instruments for specific research and clinical applications. As a result, it is quite probable that approaches to solutions of current problems will be more readily apparent and that problems which have heretofore defied solution by other methods will yield to the ultrasonic approach.

The work described and the results presented in this dissertation relate to the initiation of an elaborate series of experiments which has been designed to yield information regarding the fundamental physical mechanisms involved in the irradiation of biological materials with ultrasound. The purpose of this thesis is to demonstrate that it is possible to realize accurately reproducible results on a suitably prepared and precisely irradiated

biological specimen. The following is a brief historical account and summary of the work preceding the present study. Earlier work by Fry[18,20] and associates, concerned with investigating the physical mechanism by which high level ultrasound (intensities in the range from 20 to 1,000 watts/cm^2) effects the biological system, dealt with the possible role of temperature change and cavitation. In this work, the spinal cords of cooled frogs were irradiated with ultrasound in the region of the lumber enlargement and under suitable conditions motor paralysis was produced. This work indicated that increased temperature produced by the acoustic energy is not the fundamental physical factor which causes the biological alteration. This was shown by inserting thermocouples in the tissue and observing that the maximum temperature rise during irradiation did not reach a damaging temperature level. That temperature change is not the fundamental physical factor is also supported by experiments in which repetitive exposures were applied to the animal. It was shown that the effect of such repetitive exposures, which individually do not produce paralysis, can sum to produce an irreversible change. With this type of procedure, the temperature rise in the tissue was allowed to return to almost zero between exposures and the maximum temperature rise in the tissue was considerably less than that of a single exposure capable of producing paralysis. In this early work, it was also shown that cavitation, which might be caused by tension forces produced in the media at high sound levels, does not contribute to the physical mechanism. This was accomplished by irradiating the animals under a hydrostatic pressure sufficiently high to prevent tension forces from occurring in the tissue.

Other physical factors accompanying intense high frequency ultrasound must now be considered. These physical factors include high alternating

acoustic pressure, acceleration and particle velocity. In order to investigate this problem further and to isolate the primary physical factor(s), it is important that ultrasonic dosage studies be accomplished over a wide range of frequencies, acoustic pressures and particle velocities, base temperatures, and hydrostatic pressures of the preparation. As indicated above, it is the purpose of the present thesis to demonstrate that these fundamental investigations are both feasible and practical.

The subject used in the present study is an intact mouse approximately 24 hours after birth. The spinal cords of the animals are irradiated with ultrasound with the center of the beam positioned at the third lumbar vertebra. The functional endpoint observed is motor paralysis of the hind legs. The results indicate that a narrow and well defined threshold region exists such that at dosages less than those exhibited by the threshold region the probability of producing a paraplegic animal is very small while at dosages greater than threshold, the probability is very great. Within the threshold region there is a sigmoidal distribution between paralyzed and unparalysed animals. At a single frequency, the relationship between the reciprocal of the exposure time and the square root of the acoustic intensity is linear over a wide dosage region. (The content of this paragraph will be made more precise in the subsequent text.)

A discussion of the mechanical and electrical instrumentation necessary to obtain the desired results is presented first (Section II). The apparatus was designed specifically for this investigation, constructed under the supervision of individuals with considerable experience in the field of bioacoustics, and carefully examined and tested previous to the initiation of the experiments described herein. Section III discusses the biological preparation and procedure. These two sections are developed in some detail

since the reproducibility and accuracy of the results is largely due to the proper design of the instrumentation and the careful planning and performance of the procedure. Section IV presents the results obtained with over 1,000 animals. These results are discussed in Section V. Section VI contains concluding remarks based upon results presented in this dissertation.

II. MECHANICAL AND ELECTRONIC INSTRUMENTATION

As indicated in the introduction, the quality of the results obtained from the experiments discussed in this dissertation are greatly dependent upon the precision of the design and construction of the mechanical and electronic equipment. For this reason, each major component of the apparatus is discussed in some detail in this section and its characteristics and the associated experimental proceedure are presented. When it appears necessary for clarity, further discussion of the apparatus is included in the succeeding sections.

Figure 1 shows an overall block diagram of the electronic and mechanical instrumentation. The acoustic energy is produced by an x-cut quartz crystal 1½" in diameter. The quartz crystal assembly, which is of the type described by Fry[17], is mounted in one end-wall of the essentially cylindrical sound tank. The opposite end of the tank contains a sound absorption chamber which is filled with castor oil and closed off on the end nearest the quartz crystal with a pc-rubber diaphragm. The sound absorption chamber, by preventing reflections, assures an essentially travelling wave field. The sound irradiation chamber, i.e., the region between the wall containing the quartz crystal assembly and the sound absorption chamber, is filled with degassed 0.9% saline, the acoustic transmitting medium. At the high sound levels employed in this study, efficient transfer of the acoustic energy can only be accomplished by transmission through either a liquid or a solid. The saline solution was chosen in preference to other solutions, e.g., distilled water, so that both intact and laminectomized animals can be irradiated under similar acoustical conditions. In order to prevent cavitation during irradiation, this solution is degassed by boiling for ten minutes and then rapidly

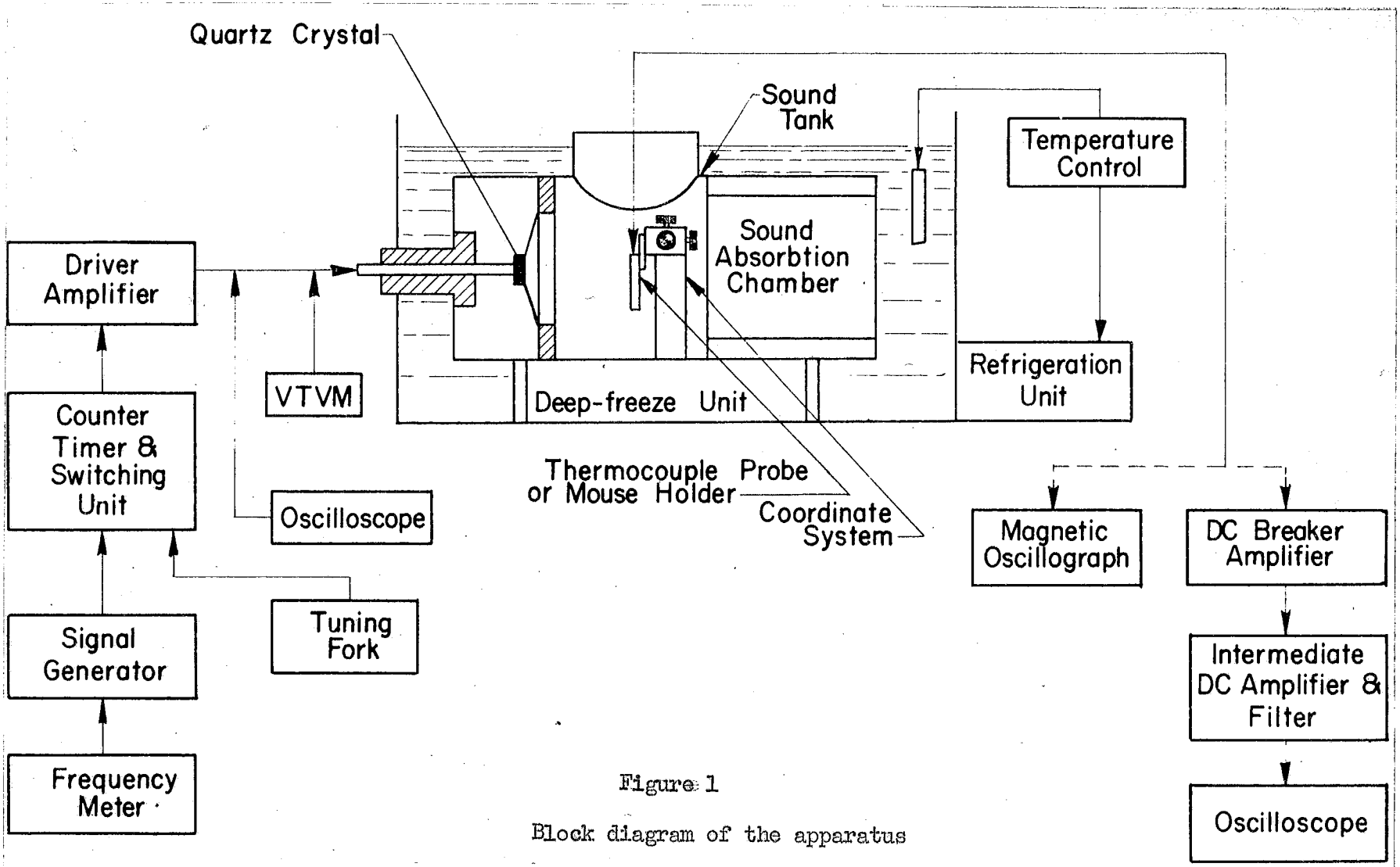

Figure 1

Block diagram of the apparatus

cooled. The sound tank is immersed in a deep-freeze unit, which in conjunction with a temperature control mechanism, maintains the proper temperature of the saline to ± 0.1°C. The heat exchange liquid in the deep-freeze unit is ethylene glycol.

In the course of the experimental work described in this dissertation, it is necessary that two instruments be placed in the irradiation chamber in time sequence in such a fashion that specific portions of these instruments will be positioned in the identical portion of the sound field. These two instruments are the thermocouple probe [21,22], for determining the characteristics of the acoustic field, and the mouse-holder. To facilitate the positioning of these instruments, a coordinate positioning system[19], capable of continuous variation in three orthogonal directions, is rigidly mounted in the irradiation chamber. An accurately machined tongue is used to attach either the thermocouple probe or the mouse-holder to the coordinate system.

Two independent electronic systems are required for this type of investigation. One is used to drive the crystal transducer and the other is used for observing the temperature change experienced by the thermocouple probe. A Ferris Signal Generator, model 22-A, deriving power from a regulated line, furnishes the excitation for a power amplifier. The frequency of the signal generator is monitored by a Signal Corps model BC-221 frequency meter. The frequency meter is checked periodically against station WWV and is capable of measuring frequency to 10 cps in the neighborhood of one mc/s. The low level portion of the power amplifier which operates Class A, drives a Class AB amplifier which in turn drives a Class AB_2 power amplifier which furnishes the power for the crystal transducer. The various amplifier stages are needed to obtain the necessary voltage and power gain and stability. The driver-amplifier block of Figure 1 represents all the amplifier stages. The driver

amplifier is capable of supplying approximately 8,000 volts RMS to the 1½ inch one mc/s quartz crystal. The timing and switching circuit, which is interposed between the power amplifier and the signal generator for controlling the duration of the acoustic signals, is supplied by a 1,000 cycle vacuum tube tuning fork which is the timing standard. The 1,000 cycle signal is converted into positive trigger pulses by pulse shaping circuits. The number of the positive trigger pulses, which the timer will accept before activating the switching circuit to terminate excitation to the power amplifier, is preset by control knobs on the timer control panel. The timer and counter circuits utilize glow-transfer tubes. By means of appropriate circuitry, a gate is provided which operates the electronic switching unit to deliver an rf pulse into the low level amplifier for the duration of the preset value of the timer. With this arrangement, the time duration of the acoustic pulse can be set-up in interval multiples of one millisecond. The rise and decay times of the acoustic pulse are of the order of ten microseconds.

A Hewlitt Packard model 410-A vacuum tube voltmeter is used in conjunction with a capacitive divider to measure the voltage across the crystal transducer for time durations of one second or greater. For time durations as short as one second, the period of oscillation of the voltmeter movement is short enough so that accurate values of the voltage can be read. To measure the crystal voltage at the shorter time durations, a Tektronix Model 514 oscilloscope is first calibrated against the voltmeter. The vertical deflection of the calibrated oscilloscope is then used to meter the crystal voltage. This scheme can be used for time durations as short as 5 milliseconds.

The thermocouple probe[21,22] serves a two-fold purpose in these experiments. It is used (1) to calibrate the vacuum tube voltmeter (and the

oscilloscope when necessary) such that each deflection of the meter can be converted to absolute sound levels at the position in the field where the junction of the thermocouple was placed and (2) to determine the relative spatial distribution of the sound field. In the first case a one second pulse is used and in the second case it is convenient to use a 0.1 sec. pulse. In determining the acoustic level of the field (particle velocity, pressure, and intensity) a Hathaway S-14 magnetic oscillograph is used. A camera is provided so that both visual and photographic observations can be made. For determining the spatial characteristics of the acoustic field, DC amplifiers are used in conjunction with an oscilloscope which has a low frequency cut-off in the neighborhood of 2 cps. The thermocouple probe signal is fed into a Perkin-Elmer DC amplifier which converts a low level DC signal into AC by means of a motor driven synchronous breaker arrangement, the AC signal is amplified to a specified level and then converted back to a DC signal by another breaker arrangement.

The thermocouple probe has been thoroughly described by Fry and Fry[21,22]. Its essential composition is a small thermocouple imbedded in an acoustic absorbing medium which closely matches the density and sound velocity of the medium in which the acoustic characteristics are to be determined. The thermocouple probe used in these studies is shown in Figure 2 with the tongue attached. Figure 3 is a view looking into the irradiation chamber showing the thermocouple probe and tongue assembly mounted on the coordinate positioning system. The thermocouple is composed of 0.003 in. iron and constantan wire. The wire is etched down to approximately 0.0005 in. at the junction. The acoustic absorption medium is degassed castor oil. The probe functions as follows: The transducer[17] which produces the acoustic field is excited to

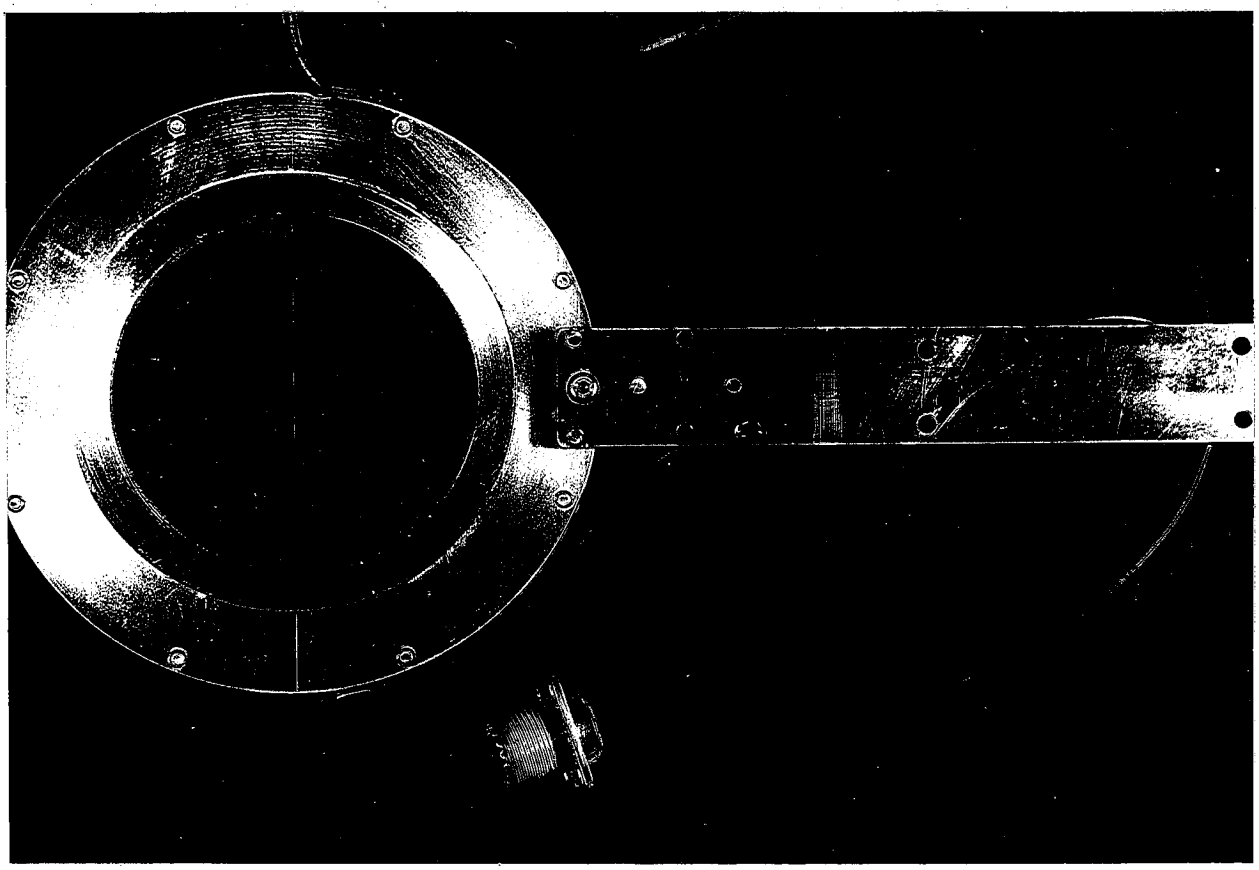

Figure 2

Thermocouple probe and tongue assembly.

generate a sound pulse with a rectangular envelope. The initial time rate of change of the temperature at the thermocouple junction is observed. From a knowledge of the time rate of temperature rise at the junction, the absorption coefficient of the probe absorption medium and its heat capacity per unit volume at the temperature at which the measurements are made, the intensity of the acoustic field at the thermocouple junction can be calculated for a plane traveling wave field. A probe of this type is very desirable for observing the fine structure of a high frequency acoustic field. For example, in the experiments described in this dissertation, the frequency is 982 kc/s

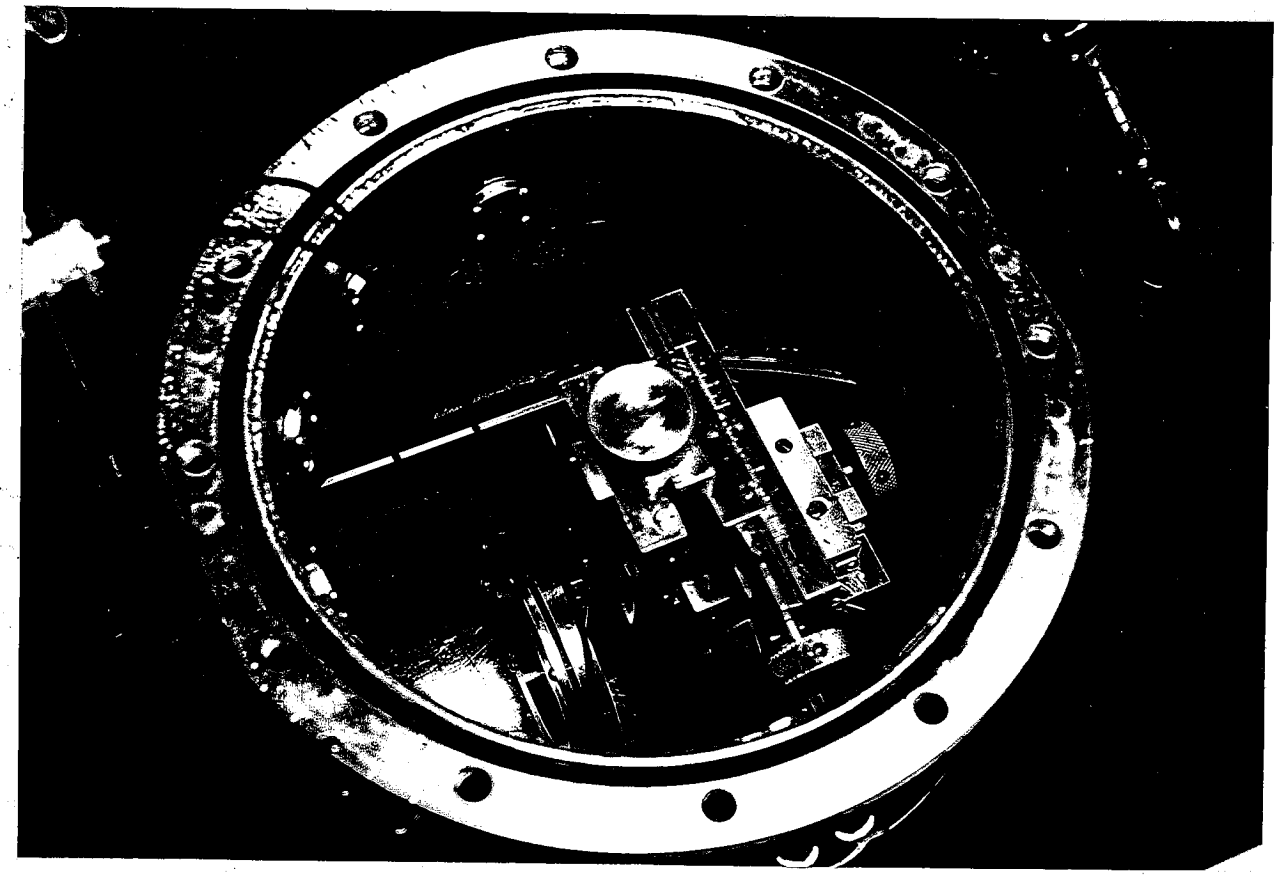

Figure 3

A view looking into the irradiation chamber. The thermocouple probe and tongue assembly is shown mounted on the coordinate positioning system.

and the sound velocity in the saline at 10 °C. is 1439 m/sec which gives a wave length of 1.46 mm. The junction of the thermocouple probe is approximately 0.001 in. or 0.025 mm., which is sufficiently small such that disturbance of the field due to its presence is negligible.

Since data more accurate than ±10% are not available for the absorption coefficient of castor oil[44], the procedure adopted for using the thermocouple probe is the following: The probe is calibrated against an acoustic radiation pressure detector[15], in a particular laboratory arrangement, which

utilities a small steel sphere. The radiation pressure detector constitutes an absolute determination of the acoustic intensity. The calibration (or sensitivity) of the probe is then used to determine the characteristics of the acoustic field in the irradiation chamber. The error in the calibration of the probe is of the order of 2.5%. The reproducibility of all the necessary measurements, including the probe calibration, is quite adequate to satisfy the stringent requirements necessary for this investigation as will be seen in a later section of this dissertation.

As indicated above, for the purpose of determining the spatial characteristics of the acoustic field, the crystal transducer is excited to produce 0.1 sec. sound pulses. The thermocouple probe, which is mounted on the coordinate positioning system in the irradiation chamber, is moved about and the deflection of the oscilloscope is observed. The transverse and vertical acoustic field patterns, obtained by this method, are shown in Figure 4. The thermocouple probe is positioned approximately 10 cm. from the quartz crystal for all the work described in this thesis. The field patterns indicate that the spatial distribution is very nearly that of a plane wave. For this investigation, it is most desirable that the spatial distribution of the acoustic field characteristics be very nearly uniform over the region to be irradiated, e.g., in this study, over an appreciable portion of the lumbar enlargement of the mouse spinal cord. In Figure 4 it is seen that at 5% below the peak acoustic intensity, the vertical beam width is 2.6 mm. The mouse is oriented in the holder such that the axis of the cord is in the vertical direction. At 10% down, the beam width is 7.1 mm. As will be seen in a following section, from a consideration of the dimensions of the lumbar enlargement in the mouse cord, nearly four vertebral segments of the cord are irradiated with an acoustic intensity variation of not more than 5%.

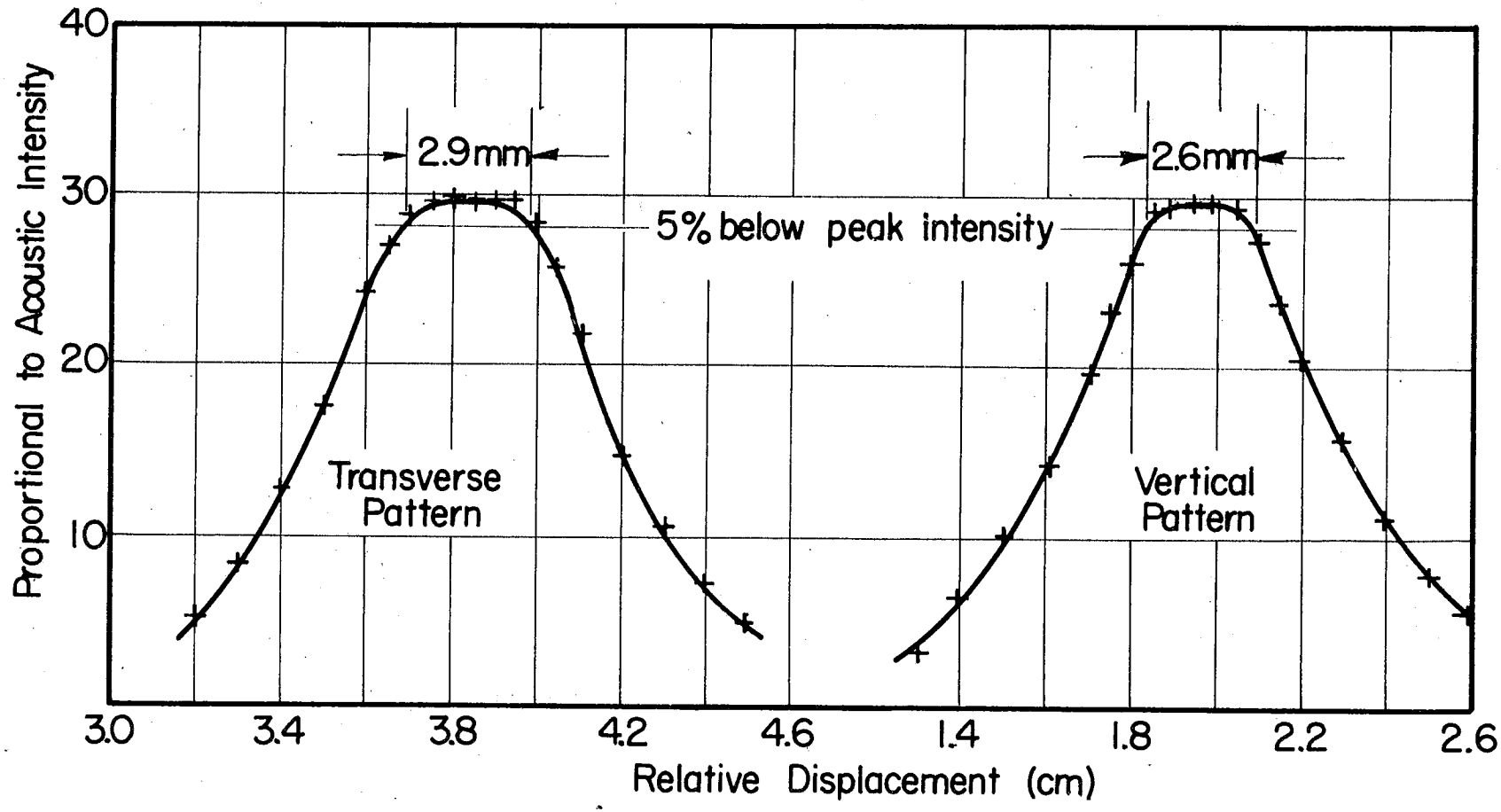

Figure 4

The transverse and vertical field patterns developed by the quartz crystal transducer. The spinal cord of the mouse is positioned along the vertical direction.

For the purpose of calibrating the vacuum tube voltmeter (and oscilloscope) such that the meter deflection can be readily converted to quantitative values of the acoustic field characteristics at the junction of the thermocouple, the electrical energy developed by the thermocouple, as a consequence of its presence in the sound field, is fed to the magnetic oscillograph. The quartz crystal transducer is excited to produce a one second sound pulse of rectangular envelope. The voltage applied to the quartz crystal is successively set to increasing values and the deflection of the magnetic oscillograph is observed simultaneously with the deflection of the vacuum tube voltmeter. Figure 5 shows a typical example of such a calibration curve. Once the calibration curve is obtained, the acoustic field characteristics can be computed by a simple arithmetical operation. For example, the sensitivity of the thermocouple probe shown in Figure 5 is 12.30 watts/cm^2/cm. deflection on Galvanometer #6 of the magnetic oscillograph. This was determined by calibrating the probe against the radiation pressure detector. Expressing the sensitivity of the probe in terms of deflection of the galvanometer is done for convenience only. The probe sensitivity can be expressed in terms of the open circuit voltage developed at its terminals for a specified acoustic intensity at the junction. The relation between the galvanometer deflection and the square of the crystal driving voltage (proportional to the vacuum tube voltmeter deflection) is linear at low driving voltages. As the driving voltage increases the relation deviates from linearity due to the fact that thermal conduction processes become important[22]. At high sound levels, acoustic flow phenomena will also contribute to the non-linearity of the thermocouple response. From Figure 5 it is seen that the relation is linear to a galvanometer deflection of 0.8 cm. and a voltmeter deflection of 56 volts. It then follows that a voltmeter deflection

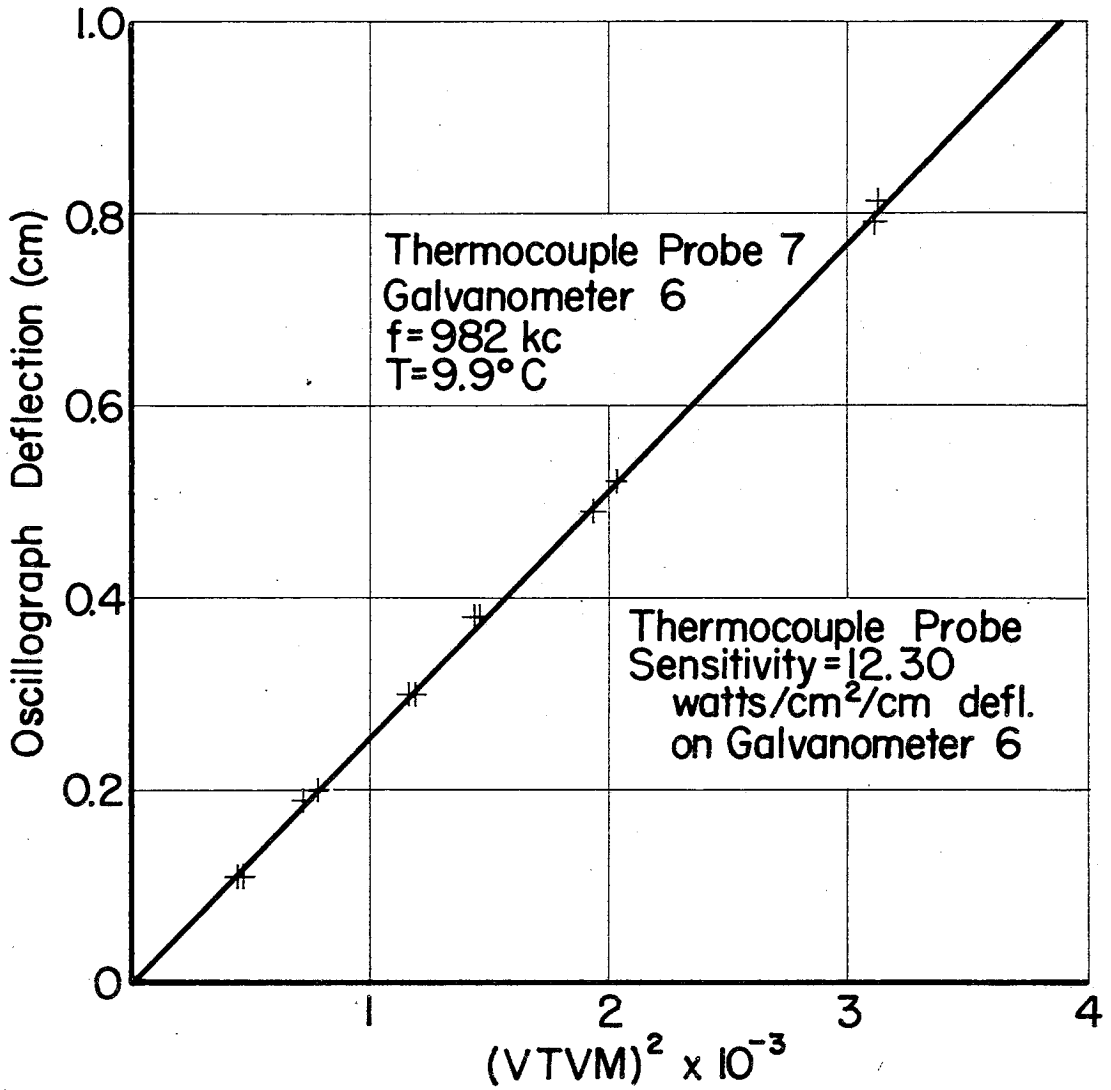

Figure 5

Calibration curve of Acoustic Field

, for example, 200 volts corresponds to an acoustic intensity of 126 watts/cm^2. nce the acoustic field developed by the quartz crystal is, to a close approx-mation, a plane wave field, the particle velocity and pressure amplitude, spectively, can be readily computed from the following relations[32],

$$I = \frac{p^2}{2 \rho c} = \frac{\rho c v^2}{2} ,$$

ere I is the acoustic intensity, p is the pressure amplitude, v is the rticle velocity amplitude, ρ is the density of the medium and c is the ee field sound velocity in the medium.

The instrument which supports the animal during the acoustic ir-diation procedure, the mouse-holder, is designed such that it can be rigidly ttached to the tongue. A cross-hair attachment can also be placed on the ngue. The intersection of the cross-hairs has been previously positioned, ith the aid of a microscope, such that when the cross-hair attachment is ssembled to the tongue and placed on the coordinate system, it is in the dentical position previously occupied by the junction of the thermocouple obe. Thus, when the mouse-holder, the tongue and the cross-hair attachment re assembled, the intersection of the cross-hairs locates the position where e region of the mouse to be irradiated, should be placed. This is readily ccomplished with the aid of an optical arrangement. Figure 6 is a close-p view of the mouse-holder, the tongue and the cross-hair attachment assembled.

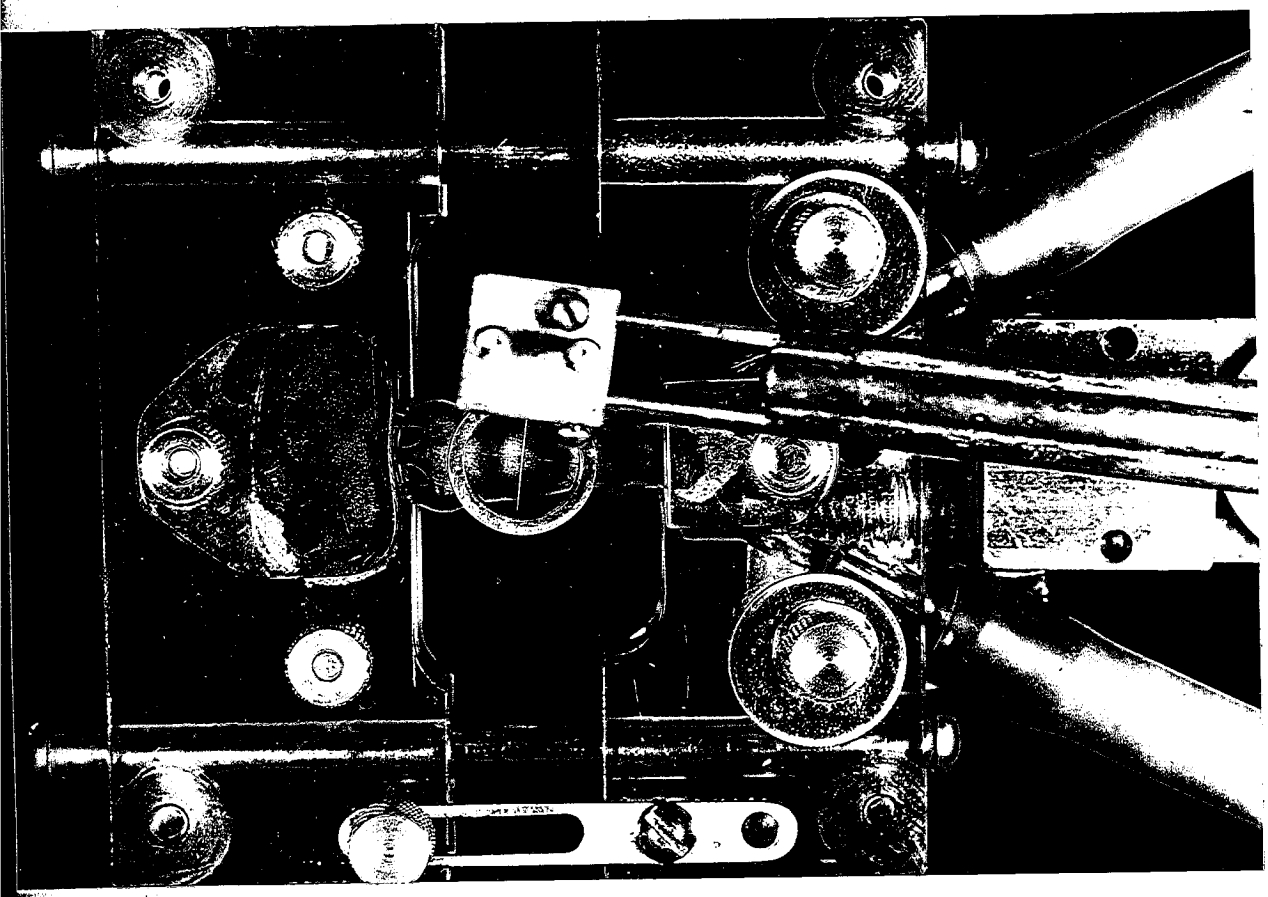

Figure 6

A close-up view showing the complete assembly of the mouse-holder (containing a specimen), the tongue and the cross-hair attachment. The two knurled bolts on the right permit adjustment of the mouse-holder such that nearly any part of the mouse can be positioned under the intersection of the cross-hairs.

III. DISCUSSION OF THE PREPARATION AND PROCEDURE

The subject chosen for this study is a young intact mouse (strain), approximately 24 hours after birth. The animals range in weight from to 1.4 grams.

The reasons for choosing the young mouse as the biological test imen for this extensive dosage study are as follows:

(1) It is essentially poikilothermic[29,43], that is, it possesses tually no temperature control mechanism, so that it can be carried through ersible temperature cycles, with its temperature being readily reduced to rly 0°C., without producing either permanent physiological or morphological nges. The young mouse can therefore be utilized in experiments in which temperature of the animal can be held at any value in the range from 0°C. t least 37°C. without thermal damage.

(2) Ossification is not complete. As determined by standard stain- techniques, the tissue overlying the dorsal side of the cord is non- eous tissue, while that over the lateral and ventral sides shows a slight ree of ossification. Thus, acoustic absorption in the region surrounding spinal cord is low, and surgery need not be performed in preparing the mal for irradiation.

(3) The animal is small in size so that it is possible to irradiate desired region with a nearly uniform acoustic field with a single controlled rasonic pulse.

(4) For a study of this type it is necessary to have a large number animals available, continuously supplied. This is readily accomplished by eeding mice in the laboratory.

A specific change in motor function (paralysis of the hind legs)

ves as a convenient and unambiguous end point for an ultrasonically induced ect in the irradiated animals. The lumbar enlargement of the spinal cord chosen as the region to be irradiated with the ultrasonic energy and pa- sis of the hind legs then serves as the functional endpoint. This region he cord contains a high density of the neurons and fibers associated with femoral, sciatic and obturator nerves. Thus, alteration of motor neurons he lumbar enlargement produces functional changes in the hind limbs of the al. These functional changes are easily detected, for example, by ob- ing the response to electrical stimulation of the skin of the animal in foot region of the hind limb. In the case of no alteration by ultrasound, animal displays a strong reflexive action to stimulation by violently ing the limb away from the origin of the stimulus. In the case where the al cord has been altered in such a fashion as to produce motor paralysis he hind limbs, very strong stimuli will not produce a reflexive action of hind limbs.

The sensory neurons (cells plus processes) of the lumbar region also be altered by the acoustic energy. In such a case, electrical stim- tion of the hind limbs may not produce a motor reflex action. It then omes necessary to stimulate the animal in a region in which the sensory vous system is intact. However, in the work reported here, the sensory vous system of the hind limbs remained intact in all cases where the ir- iated animals were subsequently found to be unparalyzed and in approximately of the cases where paralysis was produced. In the latter cases, it was nd that electrical stimulation of all other surface regions did not pro- e a motor reflexive action of the hind limbs. Although the lack of sensory sponse was observed in this work, correlation between the lack of sensory sponse and ultrasonic dosage was not the subject of this study. The electrical

ulation of the hind limbs and other surface regions has been found to a very convenient and unambiguous method of observing motor functional nges in the animal.

When an intense light beam is passed through the animal from the tral side, the vertebrae from the first lumbar through the sacral and dal regions can be seen clearly, when viewed from the dorsal side. The rum and the sacral vertebrae were therefore taken as convenient reference i for locating other skeletal structures of the young mouse. Thus any tebra in this region can be identified by first identifying the sacrum and sacral vertebrae and then counting the vertebral segments until the desired is located.

During the irradiation of the mouse, the axis of the acoustic beam centered on the third lumbar vertebral segment. This region was determined acoustic means as follows: An arbitrary, but adequate, ultrasonic dosage produce paralysis in the hind limbs of the mice was chosen. The mice e divided into groups, each group being irradiated with the axis of the rasonic beam positioned at a different vertebral segment in the lumbar gion. The number of paralyzed animals in each group was recorded. The rasonic dosage was then reduced, i.e., the time duration of exposure was creased while the acoustic intensity was unchanged. An approximately equal mber of animals was then irradiated in groups with the axis of the acoustic am positioned at the various lumbar segments at this latter ultrasonic sage. This sequence of events was continued for a number of different ultra- nic dosages (each succeeding dosage level being less than the previous one). was found that paralysis of the hind limbs was most easily affected when axis of the beam was centered on the third lumbar vertebra.

This result was also corroborated by histological examination of

mouse spinal cord in that the region of the third lumbar vertebra is the oximate center of the lumbar enlargement which contains the high density of motor neurons. The histological study also indicated that the spinal ds of the animals used in this investigation very nearly fill the vertebral l. Therefore, there is very nearly a one-to-one spatial correspondence een the vertebral segments and the cord segments.

The ensuing discussion pertains to the procedure utilized in pre- ing the animal previous to, and following, the acoustic irradiation. The e is first examined by the electrical stimulation method described above nsure that a specimen possessing normal motor facility is utilized. The l is then cooled to render it dormant so that it can be properly posi- ed in the mouse-holder and to insure that it will remain in that position l it is placed in the sound tank and irradiated. When the mouse is suf- ently cooled, it is placed in the mouse-holder which holds the head, the limbs and the tail firmly. The animal is then fully extended to reduce ible lateral movement. Figure 7 is a close-up view showing a mouse mounted the mouse-holder. The mouse-holder, the tongue and the cross-hair attach- are then assembled, as shown in Figure 6, and placed over an intense, light source. The mouse-holder is then adjusted to place the center of third lumbar vertebra under the intersection of the cross-hairs. The ss-hair attachment is then removed and the mouse-holder is ready to be ed in the sound tank. Figure 8 shows the assembly of the mouse-holder tongue.

The mouse-holder assembly is then placed in the sound tank, which filled with degassed 0.9% saline. Several minutes are permitted to elapse ore irradiation in order that the animal can reach temperature equilibrium ch is realized, as checked by measurement with imbedded thermocouples, in

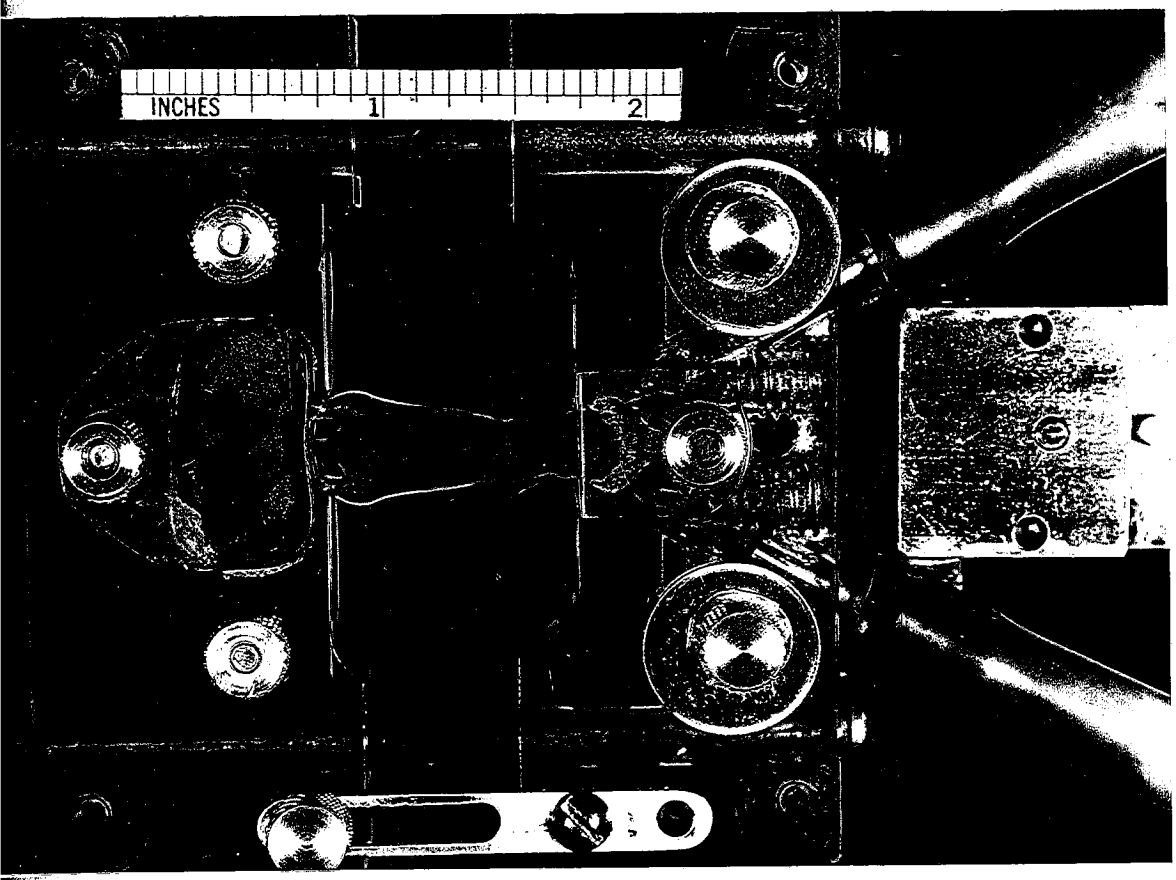

Figure 7

A close-up view showing a mouse mounted in the mouse-holder.

eriod of time. When the mouse reaches temperature equilibrium, a single ic pulse of rectangular envelope (having rise and decay time of approx- y ten microseconds), predetermined intensity (plane wave case), and uration is then initiated. After the cessation of the sound, the mouse- assembly is removed from the sound tank, the cross-hair attachment is in position and the assembly is again placed over the intense, cool source to determine whether or not the position of the animal has moved respect to the intersection of the cross-hairs. In cases where such s are observed, the animals are discarded from the compilation of the s. After checking for a possible change in position of the animal in the

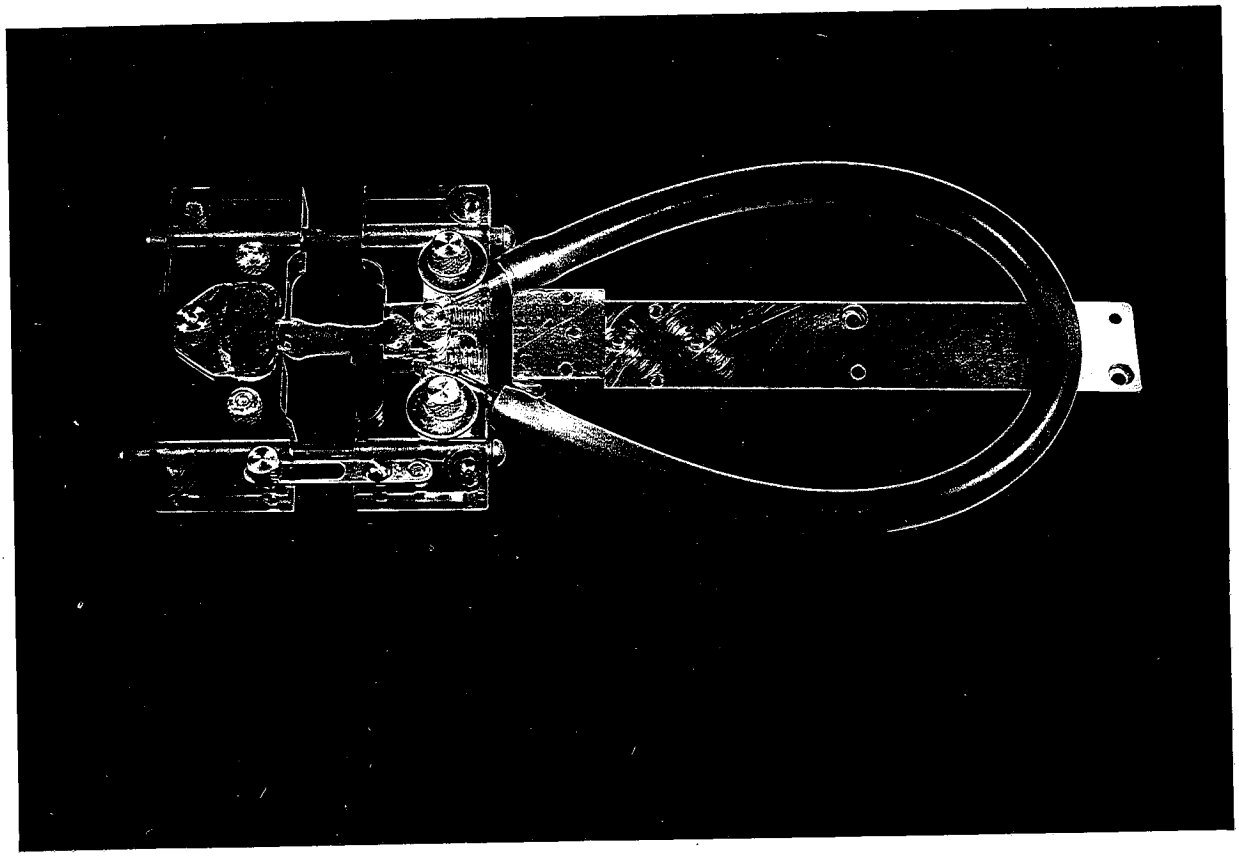

Figure 8

An over-all view of the mouse-holder and tongue assembly. The rubber tube provides oxygen for the animal to breathe when the assembly is submerged in the acoustic transmitting liquid of the sound tank.

mouse-holder, the mouse is removed from the holder and rapidly warmed to room temperature. The animals are examined for paralysis or overt movements, by the electrical stimulation method outlined above approximately 15 minutes after exposure and again after 6 hours.

Concerning the accuracy of placement of the third lumbar vertebra with respect to the axis of the sound beam, the following statements can be made: (1) The accuracy of the machined parts is ± 0.002 in. or ± 0.05 mm. (2) The position of the axis of the beam can be determined to ± 0.1 mm.

The accuracy of locating the center of the third lumbar vertebra, which pproximately 0.6 mm. in length, is ±0.1 mm. Thus, the overall uncertainty he position of the center of the third lumbar vertebra with respect to the of the sound field is ±0.25 mm. Since the beam width at 95% of the peak sity is 2.6 mm., it appears that the overall accuracy of positioning the l in the sound field is adequate. Also, in the lumber region, the verte- segments are 0.67 mm. long, measured from corresponding edges. Thus, y four vertebral segments of the cord are irradiated with an acoustic sity variation of not more than 5%.

Since the young mice are essentially poikilothermic, their temperature ilibrium value will be slightly greater than that of the environment, the ise amount being a function of their age and the temperature of the sur- ings[43]. For the animals and the temperature considered here—one day ice and a saline temperature of 10°C.—the equilibrium temperature of the e is approximately 0.2°C. greater than that of the saline bath.

IV. RESULTS

In this dissertation, the term ultrasonic dosage is specified by g values for a set of acoustic variables which uniquely define the field ions, the exposure time, the temperature and the hydrostatic pressure. e plane wave of temporally rectangular envelope is used, the frequency ensity are sufficient to define the field conditions. Other field les such as the pressure amplitude, particle displacement, velocity and ration amplitudes can be readily computed.

By analogy with the previous work by Fry[18,20] et al which utilized the dosage data was examined for a threshold region (precisely defined associated with the paralysis of the hind limbs of the young mice. In ng, a large number of animals were irradiated with a variety of ultra- dosages. When the date were plotted on a coordinate system for which dinate is the reciprocal of the exposure time and the abscissa is the root of the acoustic intensity, the following qualitative observations be made: First, three distinct regions became apparent (see Figure 9). animals irradiated in the region A of lesser dosages, i.e., the region latively low acoustic intensities and relatively short exposure times, small fraction were paralyzed. Conversely, of the animals irradiated region B of relatively greater dosages, nearly all were paralyzed. In gion C, between these two dosage regions (A and B), the animals were ibuted between paralyzed and unparalyzed. Second, the boundaries (this et qualitative concept can be made precise as shown below) of region C yed an approximately linear relationship between the reciprocal of the re time and the square root of the acoustic intensity. This empirically ed relationship, of course, was the original reason[18,20] for the choice

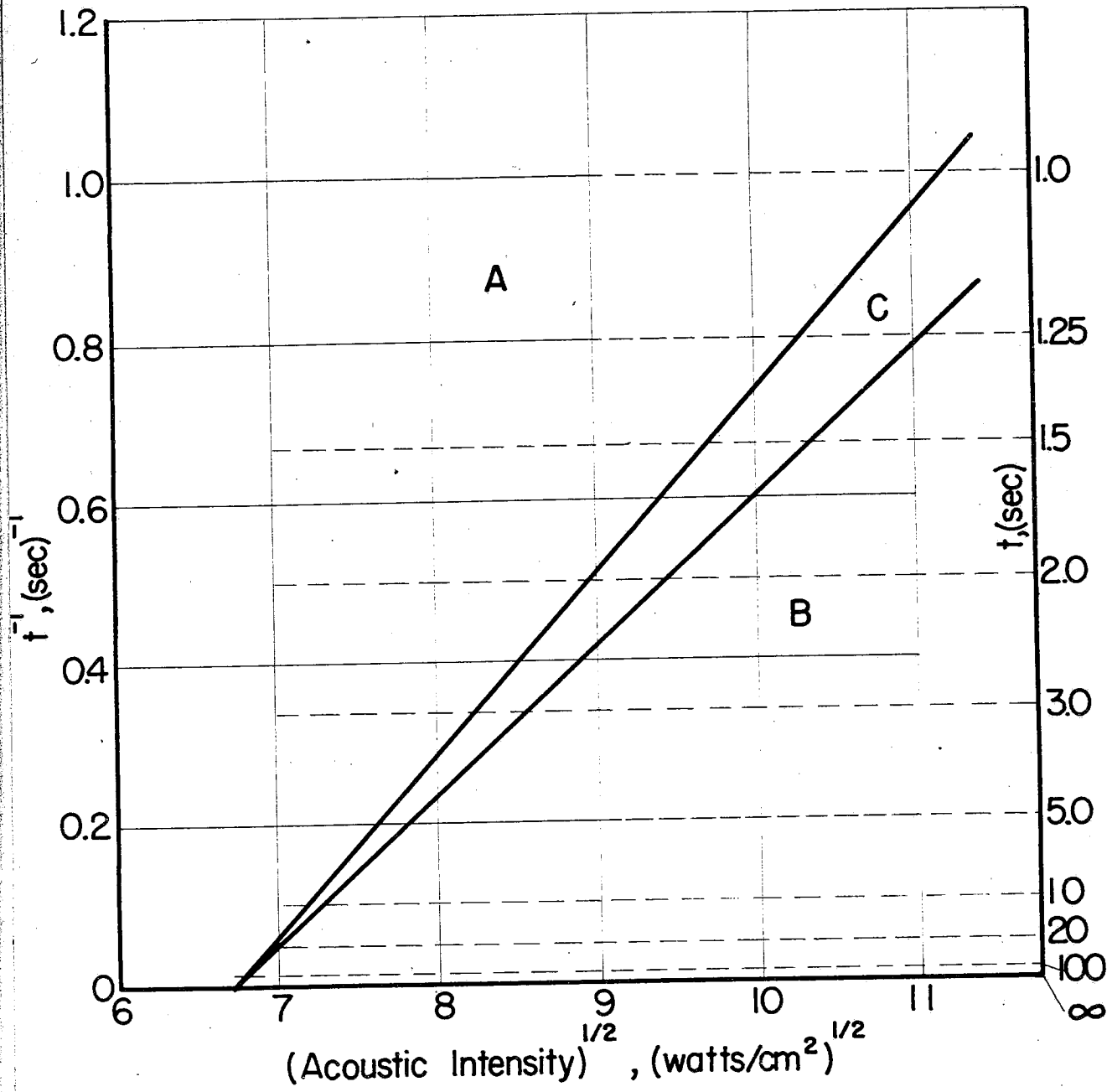

Figure 9

First approximation of threshold region.

of these coordinates. Third, the boundary lines of region 6 differed by less than 20%, indicating that the threshold region is relatively narrow.

With this approximate concept of a threshold region at hand, the following plan was used to obtain the remaining data. A large number of animals were irradiated at a particular acoustic intensity at approximately 10 to 15 different exposure times. The exposure times were chosen such that they overlapped by a considerable amount the approximate threshold region and their reciprocals were somewhat evenly distributed within this range. The total range was then divided into five equal intervals of the reciprocal of the exposure time and the percentage of the mice paralyzed in each interval was calculated. The percentage of mice paralyzed is plotted as a function of the reciprocal of the exposure time. The value for the percentage is plotted graphically at the midpoint of the interval. The plotted points of Figure 10 are obtained in this fashion. Each point represents approximately 20 animals. The sigmoid curve shown in Figure 10 is obtained by treating the data by the method of probit analysis[14]. The standard deviation is approximately 3%. A χ^2 value for three degrees of freedom is 0.78, which, being less than three, is sufficiently small to be attributed to random fluctuations about the curve. The curve thus obtained by statistical treatment of the data is a good representation of the relationship between the reciprocal of the exposure time (at a specified acoustic intensity) and paralysis of the hind legs of young mice under the indicated conditions of treatment. Figures 11 and 12 are additional examples illustrating results of the method of treating the experimental data. From these curves, the value of the reciprocal of the exposure time can be obtained for any percentage of animals paralyzed. The values for 10, 50 and 90 percent of the mice paralyzed are indicated on the figures.

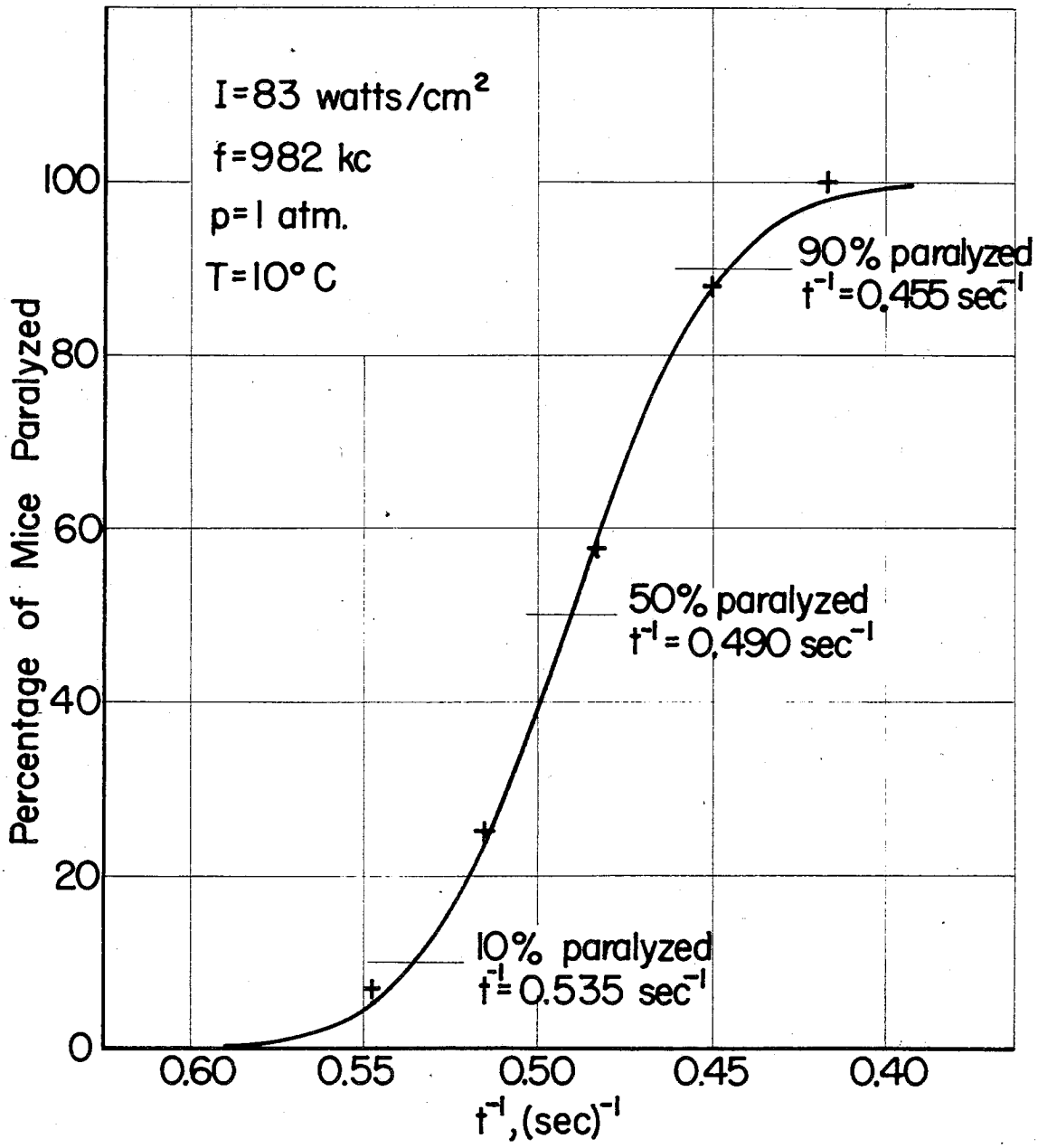

Figure 10

Sigmoidal distribution of percentage of mice paralyzed as a function of the reciprocal of the duration of exposure at an accoustic intensity of 83 watts/cm^2.

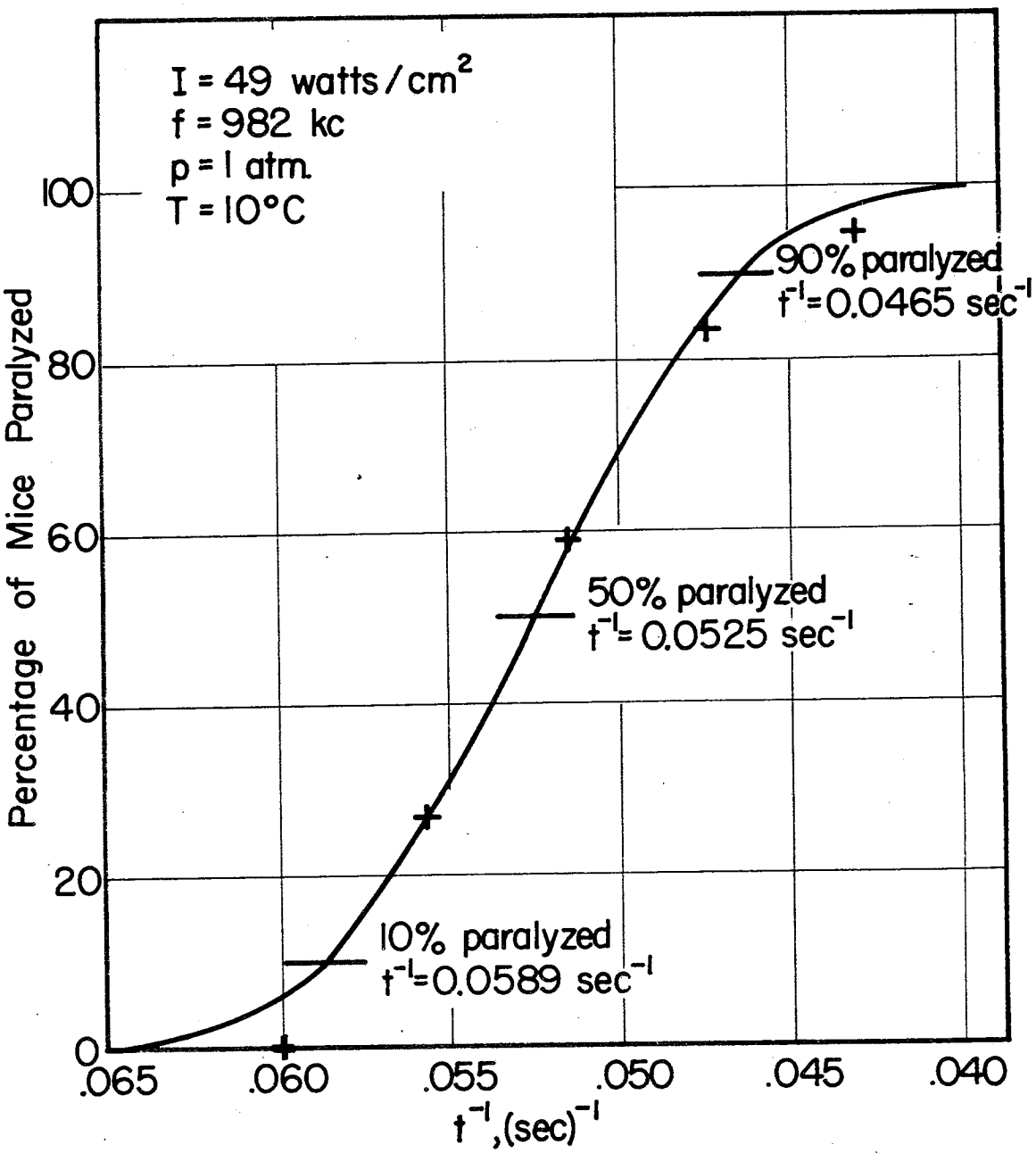

Figure 11

Sigmoidal distribution of percentage of mice paralyzed as a function of the reciprocal of the duration of exposure at an acoustic intensity of 49 watts/cm^2.

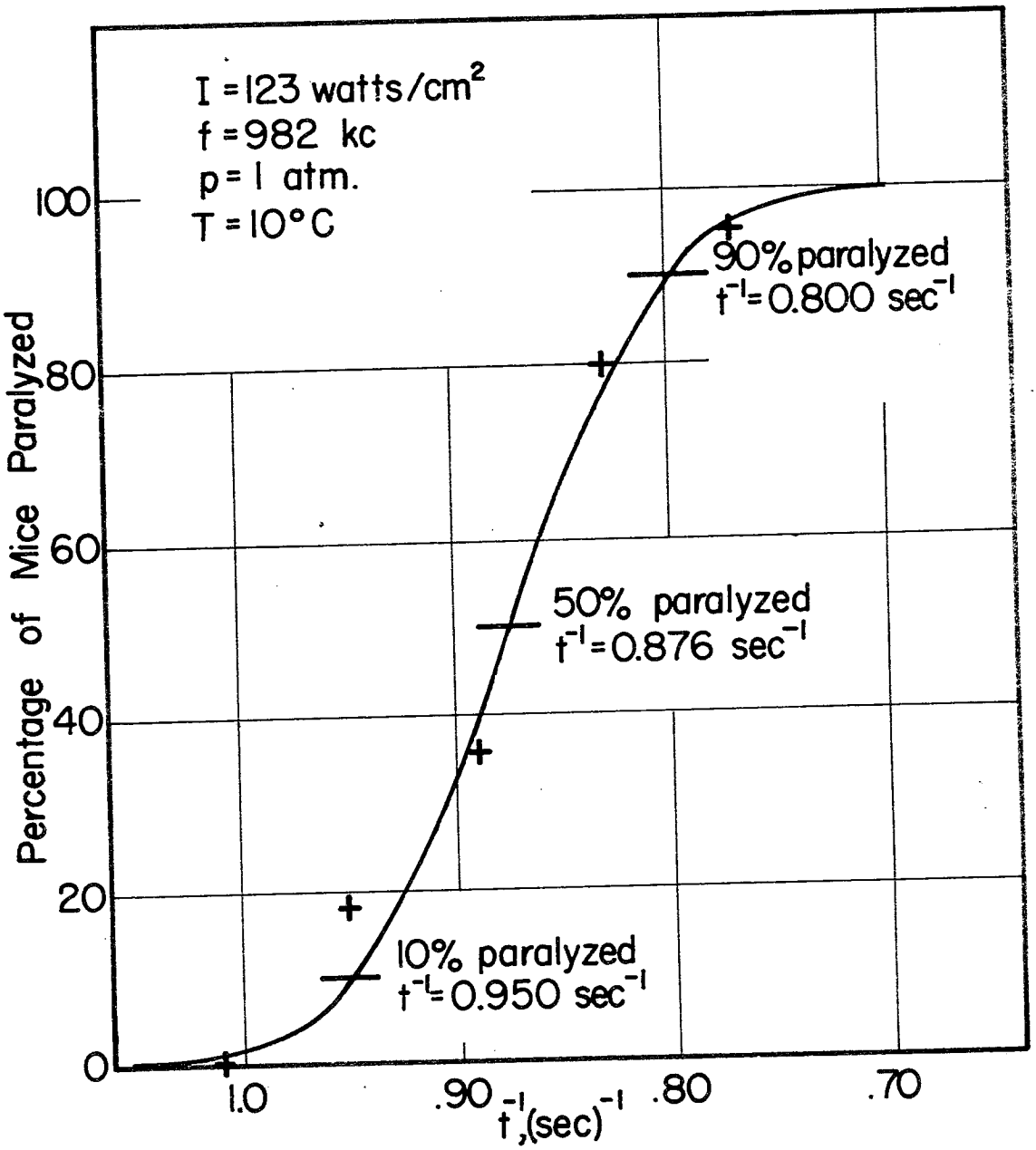

Figure 12

Sigmoidal distribution of percentage of mice paralyzed as a function of the reciprocal of the duration of exposure at an acoustic intensity of 123 watts/cm^2.

A more precise definition of the threshold region can now be given: a large number of animals are irradiated with identical values for the acoustic field variables for various periods of time, and the percentage of animals paralyzed at each duration of exposure is plotted as a function of the reciprocal of the time duration of exposure, a sigmoid curve is obtained. The threshold range at the chosen values for the acoustic field variables is arbitrarily defined as the range of time durations of exposure from 10% of the animals paralyzed to 90% of the animals paralyzed. The collection of these threshold ranges for various values of a specific acoustic variable defines the threshold region for that variable. The threshold region determines an infinite number of "threshold relations." Three threshold relations are conveniently defined for discussion and future analysis. These relations are defined as the reciprocal of the exposure time as a function of an appropriate acoustic field variable for 10%, 50% and 90% of the animals paralyzed.

Figure 13 exhibits the threshold region (with the square root of the intensity as the acoustic field variable) at a frequency of 982 kc/s, a hydrostatic pressure of one atmosphere, and a base temperature of 10° C. The values of the plotted points are shown in Table I. The ordinate is the reciprocal of the exposure time and the abscissa is the square root of the acoustic intensity. Time and intensities are also indicated on the coordinate axes for convenience. The plotted points are obtained from the sigmoid curves of Figures 10, 11, and 12 and similar ones obtained at other values for the acoustic intensity.

In the course of this study, it appeared desirable to know the temperature rises in the spinal cords of the mice as a result of exposure to the acoustic energy. Consequently, temperature measurements were made by imbedding small calibrated thermocouples in the cords of the animals and ob-

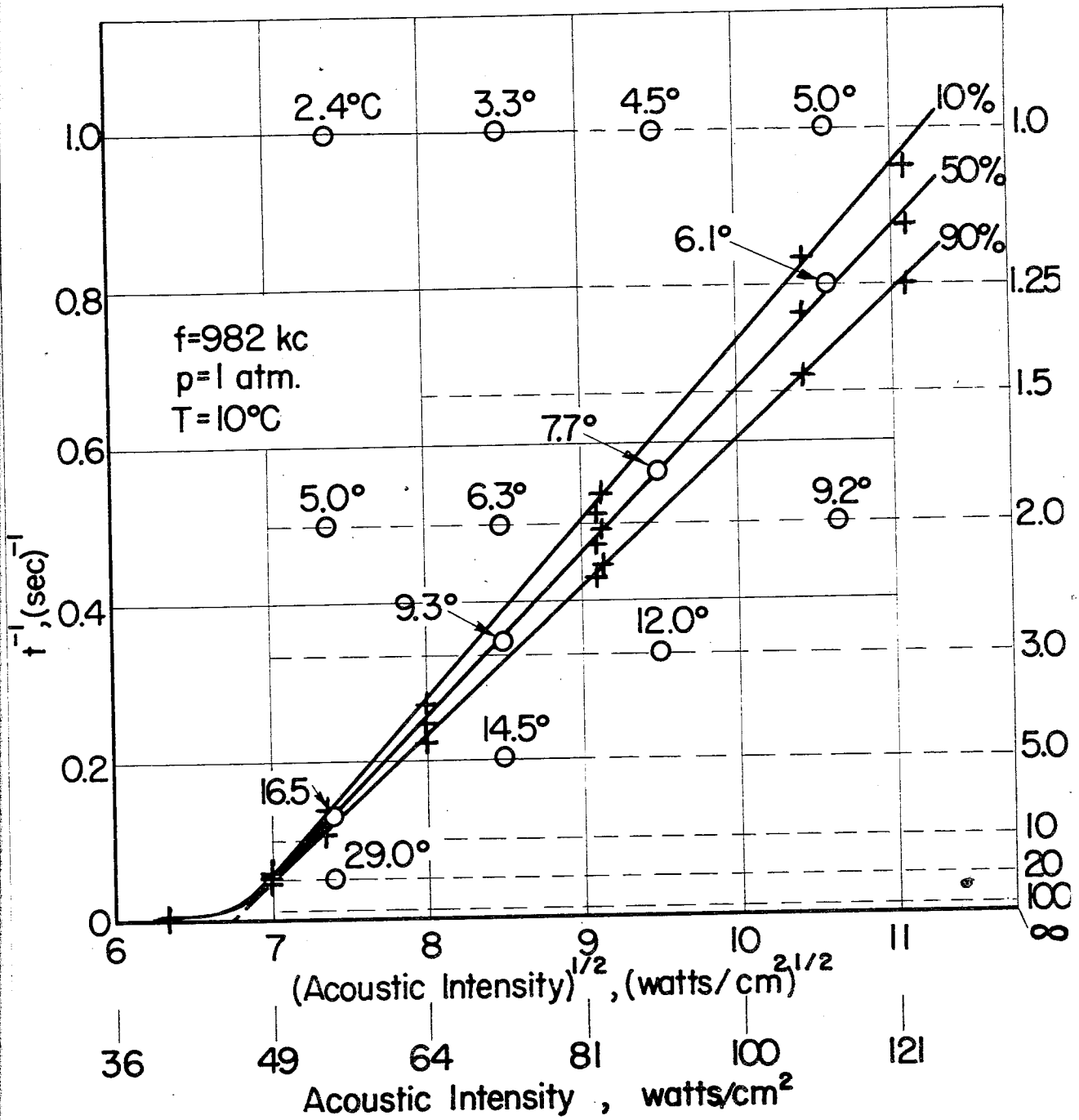

Figure 13

Threshold region for paralysis of the hind legs of mice under ultrasonic irradiation. The indicated temperature rises (in degrees Centigrade) were measured by imbedded thermocouples in the spinal cords of irradiated mice. Time and intensity are also indicated on the coordinate axes.

Table I. Threshold region values for 10%, 50% and 90% of the animals paralyzed.

I(watts/cm^2)	$I^{\frac{1}{2}}$	t_{90}^{-1}(sec)$^{-1}$	t_{50}^{-1}(sec)$^{-1}$	t_{10}^{-1}(sec)$^{-1}$
40.1	6.33^b	0.00138	0.00153	0.00167
49.0	7.00^b	0.0465	0.0525	0.0589
54.0	7.35^a	0.108	0.121	0.136
64.0	8.00^a	0.221	0.247	0.271
82.8	9.10^a	0.430	0.472	0.510
83.5	9.14^b	0.445	0.490	0.535
109.0	10.44^a	0.684	0.764	0.838
123.2	11.10^b	0.800	0.876	0.950

-serving the thermal e.m.f. developed by the thermocouple as a result of its presence in the acoustic field. The precise position of the thermocouple, with respect to the anatomical structure of the animal, was determined after the animal was sacrificed. The soft tissues were cleared in a 1% aqueous solution of KOH and the bones were stained with Alizarin Red. The specimen was then viewed under a microscope and the position of the thermocouple junction was accurately located with respect to the vertebral structure. As a result of the technique which was used, the position of the thermocouple junction could not be known until approximately one week after irradiation of the animal. For a number of specimens, the thermocouple junctions were found placed in regions outside the vertebral columns and thus were of little interest for this study. The temperature rises observed in these extraneous regions were less than those observed in the cord. The greatest temperature rises were observed on the ventral side of the cord adjacent to the spinal column. These values are plotted in Figure 13 at the proper coordinates. Table II shows the results of the temperature rise measurements for dosages within the threshold region. Other temperature rise values exterior to the threshold region are also shown in Figure 13.

Table II. Measured temperature rises for dosages within the threshold region.

t(sec)	I(watts/cm^2)	$I^{\frac{1}{2}}$	$\Delta T(^{\circ}C)$
7.70	54	7.4	16.5
2.88	71	8.5	9.3
1.80	90	9.5	7.7
1.25	112	10.6	6.1

The data of Figure 13 represents results obtained from experiments on 1048 animals.

V. DISCUSSION

As indicated previously, the purpose of this study is to demonstrate the feasibility of realizing accurately reproducible results on a suitably prepared and precisely irradiated biological specimen. The degree to which this goal has been achieved can, perhaps be best evaluated by considering the accuracies of the various component measurements and the overall reproducibility. The component measurements which must be considered are: (1) the determination of the absolute sensitivity of the thermocouple probe, (2) the calibration of the sound field in which the animals are irradiated, (3) the sigmoidal distribution of the percentage of mice paralyzed as a function of the reciprocal of the duration of exposure at a single acoustic intensity, (4) the collection of these distributions which define the threshold region for paralysis, (5) the measurement of the ultrasonically produced temperature rises in the spinal cord, and (6) the accuracy of positioning the animal in the sound field.

Items (1) and (2) above determine the uncertainty in the absolute acoustic intensity at which the mice are irradiated. An analysis of the procedure used in determining the sensitivity of the thermocouple probe based on the method of least squares[47] has yielded an uncertainty of ±2.5%. A similar analysis of the daily calibration of the sound field in the irradiation chamber has yielded an uncertainty of ±1%. Thus, the overall uncertainty in determining the acoustic intensity at which the animals are irradiated is ±3.5%. The above uncertainties are the calculated standard deviations (determined by the method of least squares) of the observed points from the straight line of best fit.

The procedure adopted for irradiating the animals in order to establish the threshold range at a particular value of the acoustic intensity has been

discussed in the previous section (Figures 10, 11, 12). It was indicated that the method of probit analysis[14] was used to treat the data statistically. The analysis yielded a standard deviation of the experimentally determined points from the sigmoidal curve of best fit of ±3%. The χ^2 value obtained was sufficiently small to be attributed to random fluctuations about the sigmoid curve.

Figure 13 displays the threshold region, for the conditions of these experiments, as defined in the previous section. The plotted points of Figure 13 are the abscissas corresponding to the ordinates at 10%, 50% and 90% of the animals paralyzed for the particular sigmoid curve. The threshold relations exhibit a "linear portion" which extends from approximately 48 watts/cm^2 (25 seconds time duration of irradiation) to 125 watts/cm^2 (1.1 seconds duration). At the low intensity extremity of the linear portion, the threshold relations deviate from linearity. The plotted points of the linear range (Figure 13) have been analyzed statistically by the method of least squares. The analysis yielded a value for the standard deviation of the points from the straight line of best fit of ±1.3%. An analysis performed to determine the degree of non-linearity of the "linear portion" of the threshold relations yielded a quadratic coefficient of such magnitude that the deviation (non-linear case) from the statistically determined linear curve was considerably less than the standard deviation (linear case). In the "linear portion" of the threshold region, the relation between the reciprocal of the irradiation time and the square root of the acoustic intensity is thus, to a high degree of accuracy, a linear relationship. For this linear portion, the straight lines of best fit for 10%, 50% and 90% of the animals paralyzed are, respectively,

$$(2)\quad t_{10}^{-1} = (0.2212)\, I^{\frac{1}{2}} - 1.4921,$$

$$(3)\quad t_{50}^{-1} = (0.2042)\, I^{\frac{1}{2}} - 1.3808,$$

(4) $t_{90}^{-1} = (0.1854)\ I^{\frac{1}{2}} - 1.2250.$

The width of the threshold region, $\left[\frac{t_{10}^{-1} - t_{90}^{-1}}{t_{50}^{-1}}\right]_{I=I_0} \times 100$, as determined from the above relations is 18%. The intercepts at $t^{-1} = 0$ for the 10%, 50% and 90% lines are, respectively, $I_{10}^{\frac{1}{2}} = 6.75$, $I_{50}^{\frac{1}{2}} = 6.76$, and $I_{90}^{\frac{1}{2}} = 6.77$.

The degree of reproducibility can also be discussed by comparing the results obtained from two independent determinations of the threshold region. After approximately half of the points of Figure 13 had been determined, the thermocouple probe was damaged by accident. Before the work described here could be continued, it was necessary to repair the probe and recalibrate it against the radiation detector. When this procedure was completed, it was thought wise first to redetermine a point on the dosage curve. Referring to Figure 13 and Table I, the original point and the redetermined point occur at acoustic intensities of 82.8 watts/cm^2 and 83.5 watts/cm^2, respectively. In Table I, the points indicated by the superscript a are those determined before the accident and the points indicated by the superscript b are those determined after the accident. The straight lines of best fit determined from each of these sets of data yield values for the slopes and intercepts which differ from those of equations 2, 3, and 4 by less than 2%.

Concerning other aspects of the measurements, the following may be noted: The time duration of the irradiation is known to ±one millisecond. For the shortest irradiation times, say one second, this is an uncertainty of ±0.1%. The frequency is known to 10 cps, or an uncertainty of ±0.001%. The measurement of the temperature, both the temperature of the liquid in the irradiation chamber, the base temperature of the animal, and the temperature rise in the spinal cord, is known to ±0.1° C.

The accuracy of placement of the third lumbar vertebra with respect to the axis of the sound beam and the distribution of the acoustic energy over

the desired portion of the spinal cord has already been discussed in Section III. There it was stated that the accuracy of positioning the center of the third lumbar vertebra with respect to the axis of the acoustic field is ±0.25 mm. In the lumbar region, the vertebral segments are 0.67 mm. long, measured from corresponding edges. Since the beam width at 95% of the peak intensity is 2.6 mm., nearly four vertebral segments of the cord are irradiated with an intensity variation of not more than 5%. The position of the ultrasonically produced lesion in the spinal cord can be demonstrated by histologically prepared tissue sections. Figure 14 shows a sagittal section through the ventral horn of a mouse cord prepared by the Bodian silver technique[7,8]. The animal was irradiated with 40.2 watts/cm^2 for 620.112 seconds. The animal was found to be paralyzed and fell in the 90% paralysis region of the sigmoid curve associated with this dosage. The animal was sacrificed approximately five minutes after the termination of the exposure. The darkened area in Figure 14 is the lesion. (Extensive histological studies are planned as part of the proposed dosage study. However, only a few specimens have been prepared to date.)

Referring to Figure 13, the narrow width (at a single value of the acoustic field variable) of the threshold region, 18%, is strong evidence that high level ultrasonic irradiation experiments involving biological systems can be designed and performed to yield a minimum of scatter in the resulting data. That this is indeed a narrow range of distribution in tolerance of the mice to the ultrasonic energy can be seen by comparing these results with those of other experiments involving biological systems. For example, the evaluation of an insecticide[6,35] in which successive groups of insects are exposed to different concentrations of the insecticide for a constant time and, after a suitable time interval, are scored for the number of dead and alive specimens, may offer an interesting basis for comparison. These experiments have been carried out extensively with a variety of insects, insecticides and experimental

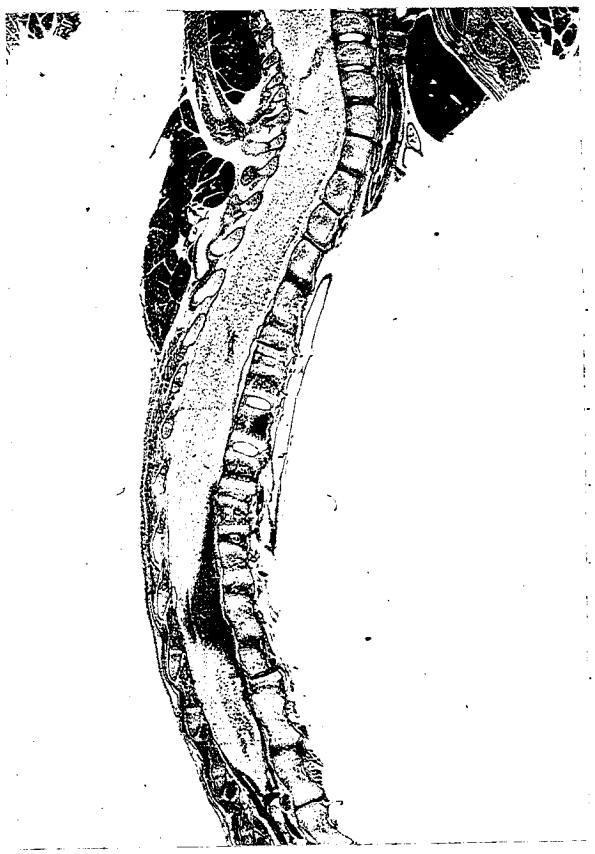

Figure 14.

A sagittal section through the ventral horn of a mouse cord. The darkened area is an ultrasonically induced lesion.

conditions. The work appears to have been performed in a careful and meticulous fashion and the data has been examined and treated by individuals acquainted with contemporary statistical methods. The statistical parameters indicate that the random errors are relatively small. Yet, the spread between points where 10% and 90% of the insects are killed is of the order of 100% to 200%. While the experimental preparation and procedure of these insecticide studies are certainly different from the studies presented in this dissertation, they do indicate the quality of the results obtained in the presented work. It must also be recalled that all of the 18% width of the threshold region (of the mouse data) cannot be attributed entirely to the animals, since on the basis

of past experience it has been found that results can be improved by careful reexamination of the experimental method and by identification of procedures which should be amended. The uncertainty in the physical measurements may contribute approximately ±1.5% to the total width. The narrow threshold width obtained in these studies is further evidence that a good choice of specimen was made.

Finally, a statement should be made concerning the possible presence of cavitation in these experiments. In the initial period of this work, i.e., prior to the establishment of the procedures described in Section III, cavitation was observed during the irradiation of mice which had thermocouples inbedded in their spinal cords for the observation of the temperature rise produced by the acoustic energy. In these cases, the cavitation occurred at the interface between the thermocouple wires and the surrounding media. Figure 15 is a photograph of a galvanometer record which shows the type of response obtained both with and without cavitation present. Cavitation occurs after 8.27 sec. of irradiation. In the absence of cavitation, the temperature rise versus time curve has the characteristic shape described by Fry and Fry[21], i.e., there is an initial rapid rise due to viscous forces and a subsequent linear rise due to acoustic absorption in the vicinity of the thermocouple. With the onset of cavitation, the galvanometer response becomes very erratic with the curve rising to rather high values and presumably high temperatures are developed. Referring to Figure 15, after 8.27 seconds of acoustic irradiation at 71 watts/cm^2, the temperature rise was 22° C. The maximum rate of rise during this time was 10.0° C./sec. which occurred at the beginning of the irradiation period and which was caused by the viscosity effect. During the initial cavitation blast, the temperature rose 13.4° C. in 0.17 second, for a total rate of 79° C./sec.

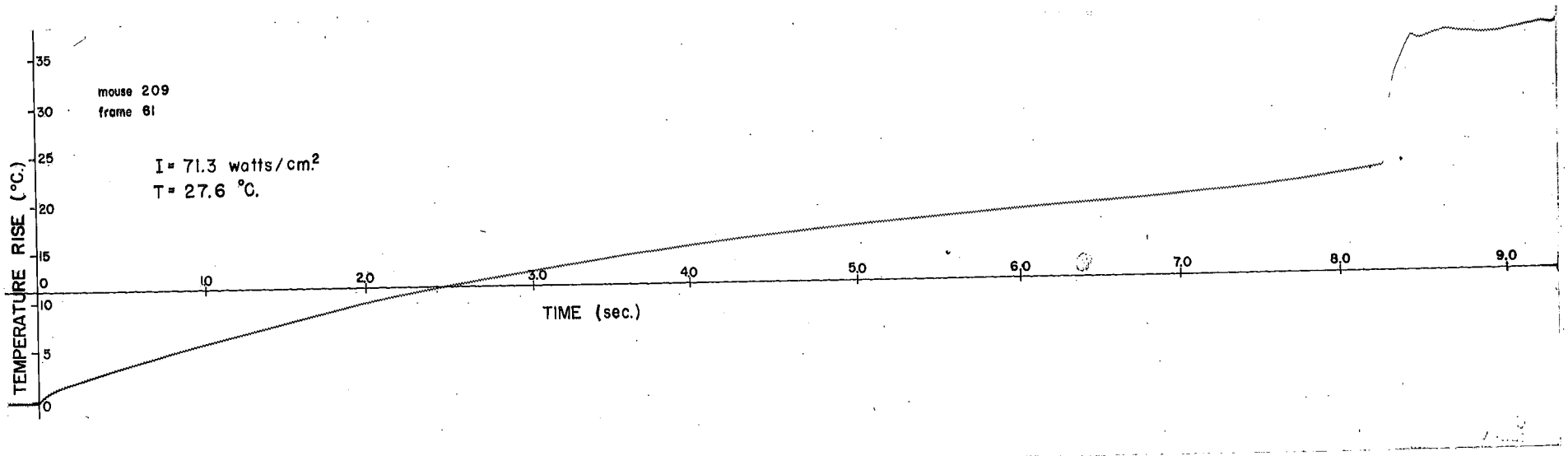

Figure 13

Photograph of a galvanometer recording of the response of a small thermocouple imbedded in a mouse ear during irradiation with ultraviolet. The record shows the response obtained both with and without ventilation present.

In the experimental procedure, the vacuum tube voltmeter, which meters a fraction of the voltage impressed across the quartz crystal transducer (see Figure 1), is always observed. When cavitation occurs, a reaction is produced on the crystal as a result of reflection of sound from the cavitating region. The reaction is reflected back into the electronic circuit and is readily observed. The results presented in this study were performed in the absence of any phenomena suggestive of the presence of cavitation. That cavitation was not present at the sound levels employed in this study is not surprising in view of the work of Fry[16] et al and Esche[13]. Also, the histological examination of the tissue does not show tearing, vacuole formation or gross disruption which would be expected to accompany cavitation in tissue irradiated at one atmosphere and sacrificed immediately after exposure.

VI CONCLUSIONS

The results presented in this dissertation demonstrate the possibility of obtaining accurately reproducible data on a suitably prepared and precisely irradiated biological specimen in which changes have been induced by intense ultrasound. The work reported here lays the foundation for a comprehensive dosage investigation concerned with determining the physical mechanism of the effect of ultrasound on nerve tissue. The results reported indicate that a dosage study of sufficient accuracy can be accomplished to elucidate the physical mechanism.

The planned dosage study will be carried out for wide ranges of environmental conditions of the preparation (temperature and hydrostatic pressure) and over a wide range of acoustic frequencies. The results reported here were obtained by irradiating the lumbar enlargement of the spinal cords of young mice 20 to 28 hours after birth. Paralysis of the hind legs was the functional endpoint used as an indicator of the ultrasonically induced changes. The body temperature of these young mice can be readily reduced to any value down to 0° C. For the specific work reported here, the animals were held at 10° C. and at hydrostatic pressure of one atmosphere. The ultrasonic frequency was 982 kc/sec which was a convenient starting point since previous work[4,5,23] on the production of ultrasonic lesions in the brains of adult animals has been accomplished at this frequency.

In order to obtain data which will permit interpretation in terms of fundamental physical mechanisms, it is essential that the ultrasonic dosage, over the region of the tissue controlling the function which serves as an indicator of the ultrasonic effect, be accurately known and that variations over the region be kept to a minimum. In this study, it was possible to keep the

acoustic intensity variations over the region of the spinal cord of interest to less than 5%. This required accurate positioning of the appropriate region of the spinal cord with respect to the axis of the sound beam. In these experiments the uncertainty of the position was ± 0.25 mm.

In order to compare dosage results at different frequencies, it is necessary that the absolute value of the acoustic intensity be accurately known. In the experiments reported here, the intensity is known to an accuracy of ± 3.5%.

In order to describe the results on these animals following exposure to the sound, it is convenient to define a threshold region of paralysis as follows: If a large number of animals are irradiated with identical values for the acoustic field variables for various periods of time, and the percentage of animals paralyzed at each duration of exposure is plotted as a function of the reciprocal of the time duration of exposure, a sigmoid curve is obtained. The threshold range at the chosen values for the acoustic field variables is arbitrarily defined as the range of reciprocal time duration of exposure from 10% of the animals paralyzed to 90% of the animals paralyzed. The collection of these threshold ranges for various values of a specific acoustic variable defines the threshold region for that variable. The threshold region determines an infinite number of "threshold relations". Three threshold relations are conveniently defined as the reciprocals of the exposure time as a function of an appropriate acoustic field variable for 10%, 50% and 90% of the mice paralyzed. The boundaries of the threshold region are arbitrarily taken as the relations for 10% and 90% of the mice paralyzed. The overall relative accuracy of the data (for the frequency used in this work) is indicated by the uncertainty in the slopes of these boundaries, viz., ±1.5%.

The threshold relation for 50% of the mice paralyzed (Figure 13)

displays a linear portion which extends from approximately 47 watts/cm^2 and 25 seconds time duration of irradiation to 125 watts/cm^2 and 1.1 seconds duration and a non-linear portion which extends below 47 watts/cm^2 and 25 seconds time duration. The shape of the threshold region suggests that several different processes may be involved in producing changes in the central nervous system in the dosage range of investigation. The temperatures developed at the long exposure times in the non-linear region suggest that a thermal process may be important in the production of paralysis of the hind legs of the animals in this region. In the linear portion, the maximum temperatures developed in the cord are considerably less than the normal temperature of the adult animal, viz., approximately 36° C. Hence, a thermal process may be considered unimportant in the linear region. The measured temperature rises displayed in Figure 13 corroborate the results of Fry[20] et al in that biological alterations were obtained in the absence of damaging temperature levels. The actual elucidation of the physical mechanism of the action of the acoustic energy on the nerve tissue will have to be delayed until further studies (of the type described here) are performed for a wide range of variation of the physical parameters.

It is felt that the accuracy, reproducibility and type of results which have been obtained in this study clearly demonstrate the feasibility of accomplishing a dosage study which will be fruitful in furnishing information concerning the various fundamental physical mechanisms involved in the production of changes by ultrasound in biological systems.

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