Physical Factors Involved in Ultrasonically Induced Changes in Living Systems: I. Identification of Non-Temperature Effects*

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The results of the first step in a systematic investigation of the mechanism of the action of ultrasound on tissue are reported. The temperature changes resulting from absorption of acoustic energy were determined while irradiation was in progress.

Experimental evidence is presented which demonstrates the existence of non-temperature effects in various nerve tissue preparations.

INTRODUCTION

THIS is the first of a series of reports concerned with the physical factors involved in changes produced in the physiological characteristics of tissues by ultrasound. It appears desirable to consider briefly those changes which take place in a medium in which any ultrasound field exists and which appear to require consideration for understanding the biological effects produced by ultrasound. Accordingly, we will first discuss changes in physical variables which may be of general importance. Then, as the first step in a systematic program of investigation, we will discuss the results of a series of experiments which demonstrate that temperature change, caused by absorption of acoustic energy, cannot account for the observed changes which occur in certain nerve tissue preparations exposed to ultrasound.

Numerous reports have appeared in the literature concerned with the effects of ultrasound on biological material.[1,2] With respect to its effect on nerve tissue, Harvey[3] has reported some work on excised frog peripheral nerve; Pohlman, Richter, and Parow[4] and Parow[5] have presented results obtained on human subjects with peripheral nerve disorders; and Lynn and Putnam[6] have observed effects on the brains of mammals.

GENERAL DISCUSSION

The changes in physical variables which occur in a medium in which a sound field exists and which appear to require consideration to understand the possible effects manifested in tissues are: changes of temperature and pressure; forces resulting from radiation pressure and viscosity; and cavitation and its concomitants.

A. Temperature Changes in the Absence of Cavitation

The changes in temperature which occur in a liquid medium propagating a periodic acoustic disturbance of constant amplitude in the absence of cavitation are of two types; a periodic temperature change and a monotonic temperature change caused by absorption of acoustic energy. However, it is quite readily shown that the amplitude of the periodic changes is small even for high intensity sound and would, therefore, be of minor significance in producing biological effects. For example, for a sound wave in water with a pressure amplitude of 10 atmos., the amplitude of the periodic temperature change is only of the order of 0.01°C.

However, the magnitude of the temperature changes which result from the absorption of acoustic energy are much greater than the periodic changes for the media with which we are concerned. They are great enough to produce changes in the functional characteristics of living systems and, therefore, must be considered in relation to the effects produced by ultrasound.

Consider a plane wave traveling in the positive direction of the x-axis. We can write the following expression for the intensity, I,

$$I=I_0e^{-\alpha x}, \tag{1}$$

where α is the intensity absorption coefficient. The energy absorbed per unit volume per second at the position x_0 can be expressed as $\alpha I(x_0)$. This energy manifests itself as a change in temperature. Acoustic absorption coefficients of some of the biological materials of interest in this investigation are of such magnitude that time rates of temperature rise up to 50°C/sec. have been observed under radiation intensities of the order of 30 watts/cm². (The ultrasonic frequency was 0.98 Mc.) Under continuous irradiation, the temperature increases and approaches an equilibrium value which is determined by the processes of conduction and radiation if there is no mass movement. Both of these processes account for transportation of energy because of temperature gradients. At the time when irradiation is initiated, there is no temperature gradient as the result of sound absorption. It is, therefore, possible to evaluate the absorption coefficient of

* This research was supported by Contract N6ori-71, Task XXI with the Physiology Branch of the ONR.

[1] E. E. Gregg, Jr., *Medical Physics* (Year Book Publishers, Inc., Chicago, 1944), pp. 91–96.

[2] E. N. Harvey, Biol. Bull. 59, 306–325 (1930).

[3] E. N. Harvey, Am. J. Physiol. 91, 284–290 (1929).

[4] R. R. Pohlman, R. Richter, and E. Parow, Deutsche Med. Wochenschr. 65, 251–254 (1939).

[5] E. Parow, Zeits. f. ärtzliche fortbildung. 39, 362–366 (1942).

[6] J. S. Lynn and T. J. Putnam, Am. J. Pathol. 20, 637–649 (1944).

the material by experimental determination of the shape of the heating curve at the time of initiation of irradiation. Specifically, we can relate the slope of this curve at this instant of time to the acoustic absorption coefficient as follows:

$$(dT/dt)_0=(\alpha/\rho C_p)I, \qquad (2)$$

where I is the sound intensity at the location under consideration, C_p is the heat capacity of the material at constant pressure, and ρ is the density. This method of determining the coefficient α is extremely useful when the dimensions of the material are small. A fine wire thermocouple can be used to indicate the temperature changes. The results of experiments carried out during this investigation are indicated in Fig. 1. The sound intensity at the thermocouple will depend on the reflection coefficient at the water interface and the thickness of the material through which the sound has traveled. Assuming a reflection coefficient of zero, a density of 1 g/cc, and a heat capacity of 1 cal./g/deg. C, a value for α of 0.2 is obtained for nerve and muscle. This value may be compared with absorption coefficients obtained by Hüter:[7] 0.35 for heart muscle; 0.57 for tongue muscle, sound propagation normal to fiber direction; 0.25 for tongue muscle, sound propagated in the direction of muscle fibers.

Various mechanisms are involved in the determination of acoustic absorption and are certainly of interest with respect to biological media. Their relative importance is dependent on the sound frequency and the structure of the medium.[8] For pure non-metallic liquids, it appears at present that acoustic absorption is caused principally by viscous damping. For many liquids, the dilatational viscosity is of much greater importance in this respect than the shear viscosity.[9,10] However, in solutions, absorption of sound energy may result from the fact that equilibrium is not maintained between the different chemical species as the pressure changes because of the presence of an acoustic disturbance. Water solutions of $MgSO_4$ show such anomalous behavior.[11]

In addition to these two mechanisms which are certainly of importance in understanding sound attenuation in biological material, there are other possibilities which arise from the fact that such material is not a homogeneous liquid. Hüter[7] has shown for various bovine tissues that the absorption coefficients as a function of frequency are approximately linear relations over the range 1.5 to 4.5 Mc. The mechanisms discussed above do not account for such a dependence. It has been suggested that plastic flow and viscous slip at grain boundaries (elastic hysteresis) might account for such a relation in metals.[8,12] Consideration of similar

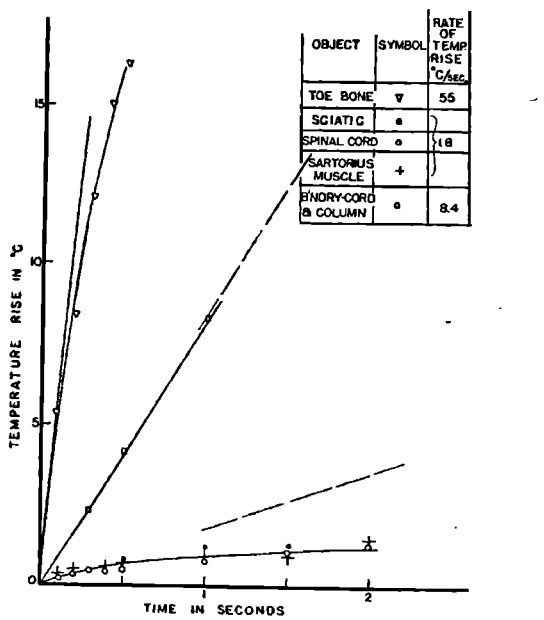

FIG. 1. Temperature rise as a function of time in various tissues under ultrasonic irradiation at a frequency of 980 kc and an intensity ~35 w/cm².

mechanisms in biological systems would appear to be of interest.

B. Pressure Changes in the Absence of Cavitation

A second variable of interest in any consideration of the mechanisms of the effect of ultrasound on biological material is the pressure. For a traveling wave, the sound pressure amplitude, P, is related to the intensity, I, by

$$P^2=2\rho VI, \qquad (3)$$

where V is the velocity of sound in the medium and ρ is its density.[13] At the maximum intensity used in the experiments reported upon later in this paper (~35 w/cm²), the corresponding sound pressure amplitude from (3) is about 10.0 atmos. A static hydrostatic pressure of 10 atmos. has a negligible effect on the electrical activity of nerve axons.[14] A similar situation exists with respect to the effect on gel sol transformations.[15,16] Stresses of this magnitude varying at the rate of $(10)^6$ cycles per second may, of course, act somewhat differently. Definite evidence has been obtained by Freundlich and Gillings and others which shows that in the absence of cavitation and after elimination of temperature change as a possibility, one still obtains major changes in the structural viscosity of some colloidal solutions.[17]

[7] Th. Hüter, Naturwiss. **35**, 285–287 (1948).
[8] C. Kittel, Reports on progress in physics **9**, 205–247 (1948).
[9] G. Eckart, Phys. Rev. **73**, 68–76 (1948).
[10] L. N. Liebermann, Phys. Rev. **75**, 1415–1422 (1949).
[11] L. N. Liebermann, Phys. Rev. **76**, 1520–1524 (1949).
[12] W. P. Mason and H. J. McSkimin, J. Acous. Soc. Am. **19**, 464–473 (1947).
[13] P. M. Morse, *Vibration and Sound* (McGraw-Hill Book Company, Inc., New York, 1948).
[14] H. Grundfest, Cold Spring Harbor Symposia on Quantitative Biology **4**, 179–187 (1936).
[15] D. A. Marsland and D. E. S. Brown, J. Cell. Comp. Physiol. **8**, 167–178 (1936).
[16] D. A. Marsland and D. E. S. Brown, Anat. Rec. **75**, 141 (1939).
[17] H. Freundlich and D. W. Gillings, Trans. Faraday Soc. **34**, 649–660 (1938).

In addition to the forces resulting from periodic pressure changes, there are two other forces which require consideration from the viewpoint of possible biological effects. The first of these is the so-called radiation pressure which manifests itself as a unidirectional force at an acoustically reflecting interface. This force causes migration or movement of reflecting particles in a sound field. In a traveling wave field, the force on a rigid sphere, large compared to the wave-length of the sound, is given by the expression[18]

$$F=(I/V)\pi r^2 Y. \qquad (4)$$

The quantity Y is close to unity if $(2\pi r)/\lambda > 10$. For a sound intensity $\sim$35 w/cm^2, we obtain a force of $\sim 2\pi r^2$ g (r is the radius expressed in cm). For rigid spheres, small compared to the wave-length, King[19] has shown that in a traveling wave field, the forces are of a much smaller order of magnitude than in a standing wave field.

In order to determine the order of magnitude of the rate of migration to be expected in a standing wave field, we consider rigid spherical particles of small enough size that the inertia forces can be neglected compared to the viscous forces. The condition which must be satisfied in order to insure this is $vr \ll \eta/\rho$, where v is the velocity and r is the radius of the particle, η is the coefficient of viscosity and ρ is the density of the fluid medium.[20] The viscous force is given by the expression $f=6\pi\eta rv$. The formulas of King show then that the velocity of such particles in a standing wave field is independent of their size. For such a field of stored energy per unit volume equal to that for a traveling wave of intensity $\sim$35 w/cm^2 and for a frequency of 1 mc, one obtains for the maximum velocity of migration

$$v=[3(10)^3/\eta]\ \text{cm/sec},$$

where the velocity v is subject to the above restriction. For a viscosity coefficient of the order of 1000 poises, one obtains a velocity of the order of 3 cm/sec.

In the experiments reported upon later in this paper, the standing wave ratio was of the order of five percent. We can thus divide the total sound field into two parts, a pure traveling wave and a standing wave of amplitude about 1/20 the traveling wave amplitude. The traveling wave component does not contribute appreciably to migration of small particles through the mechanism of radiation pressure. The standing wave component would, however, yield a velocity of migration at the maximum sound intensity ($\sim$35 w/cm^2) of the order of 1/400 the value given by the above formula.

The second system of forces is a result of the fact that the medium has viscosity. Eckart[9] has shown that the viscous forces account for the flow of a homogenous liquid in a sound field. He has also shown that the velocity of streaming involves only the ratio of the bulk and shear viscosity coefficients.

These two systems of forces may act to produce movement and reorientation of cell contents which could cause changes in biologic functions.

D. Physical Factors Associated with Cavitation

Cavitation is present in many experiments involving high intensity sound in liquid media. It is, therefore, necessary to consider the possible role of cavitation and its concomitants in discussions of mechanisms of the effects of ultrasonic irradiation on biological materials. In the experiments to be described later, no cavitation was present in the medium surrounding the test object.

A detailed understanding of the effect produced by cavitation involves a study of conditions promoting formation, growth, and collapse of cavities in liquid media. In this discussion we will review only briefly some of the features of this phenomenon which have been studied by other investigators and which appear to be of importance in considerations of the biological effects produced by intense ultrasound. We are interested both in cavities which contain only relatively small amounts of gas (the dynamics of which are not dependent appreciably on the diffusion of gas through the liquid) and in those where the diffusion of gas into the cavity from the surrounding liquid is an important factor in the growth process.[21]

Harvey and co-workers[22,23] have demonstrated that cavities result from growth of gas nuclei, and in the absence of such gas nuclei, cavity or bubble formation cannot occur even when the water is under considerable tension. The formation of such bubbles is accompanied by pressure and temperature changes. Knapp and Hollander[24] studied the history of individual bubbles and from their data on the velocity of bubble collapse, maximum pressures of several thousand atmos. may be calculated. The pressures produced by cavitation in an ultrasonic field at a frequency of 1.0 Mc are probably much lower than this because of the relatively short time between pressure reversals. The bubbles studied by Knapp and Hollander spent 0.001 sec. in the low pressure field. In a sound field of 1 Mc, the bubble would grow for only 1/1000 of this period before compression took place. It has been suggested that bubbles grow in a step-like manner and Harvey has suggested a possible mechanism for such growth. The pressure variations accompanying this type of bubble

[18] F. E. Fox, J. Acous. Soc. Am. 12, 147–149 (1940).
[19] L. V. King, Proc. Roy. Soc. London 147A, 212–240 (1934).
[20] H. Lamb, *Hydrodynamics* (Cambridge University Press, London, 1932).
[21] For a comprehensive discussion of various aspects of cavitation, see F. G. Blake, Tech. Memo. No. 12 (1949), Acous. Res. Lab., Harvard University.
[22] Harvey, Barnes, McElroy, Whiteley, Pease, and Cooper, J. Cell. Comp. Physiol. 24, 1–22 (1944).
[23] Harvey, Whiteley, McElroy, Pease, and Barnes, J. Cell. Comp. Physiol. 24, 23–34 (1944).
[24] R. T. Knapp and A. Hollander, Trans. A.S.M.E. 70, 419–431 (1948).

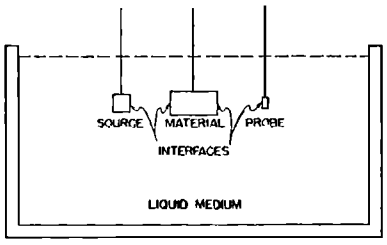

FIG. 2. Experimental arrangement for detecting the presence of cavitation.

formation are certainly smaller than the above except for the possibility discussed by Smith and others.[25,26]

Temperatures at or near the surface of collapsing bubbles were obtained indirectly by Marinesco[27] who immersed powdered explosives in liquids which do not wet them and subjected the suspension to ultrasound. The known detonation temperatures of the explosives enabled him to obtain an approximate idea of the temperature. In a sound field of intensity 20 w/cm^2 and at a frequency of 1 Mc, he obtained values up to 230°C.

Much research with ultrasound has been concerned with differentiating between effects which appear to require cavitation and those that do not. For example, Freundlich and co-workers[17,28] and others[29] have demonstrated that (1) emulsification of non-metallic systems by ultrasound (200 kc, intensity unknown) requires cavitation; (2) liquefaction of thixotropic gels by ultrasound requires cavitation (not caused by temperature rise since the gels studied do not soften on heating); (3) reduction of the structural viscosity by ultrasonic radiation requires cavitation for some materials. For other materials, the changes may be greater when cavitation is present but it is not required.

It has been shown that the disruptive effects of ultrasound on single cellular organisms are in many cases associated with cavitation[30] and it has also been reported that disruptive effects occur in the absence of cavitation.[2] The acceleration of chemical reactions in an ultrasound field has also been related to cavitation.[26] The splitting of the macromolecule haemocyanin has been reported.[31] The irreversible reduction of the molecular weight of macromolecules by ultrasound can take place in the absence of cavitation.[32]

The arrangement indicated in Fig. 2 is a convenient method for detection of cavitation either in the interior of media or at interfaces between them. It consists of a sound source and a pick-up probe and means for suspending the test object between them. In the absence of cavitation, the voltage measured across the pick-up probe is a linear function of the driving voltage across the source. However, if cavitation occurs, the probe voltage readings will fall below the values indicated by this linear relation and fluctuating values will result. The minimum intensity for cavitation is the point at

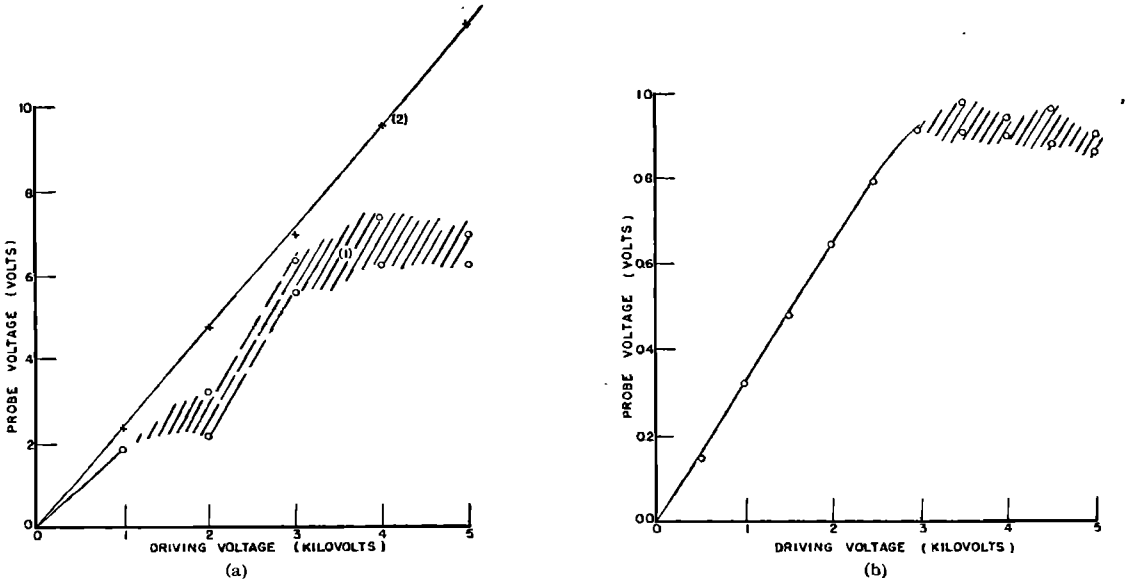

FIG. 3. (a) Sound probe voltage as a function of driving crystal voltage for degassed distilled water and for tap water. (b) Sound transmission through a frog immersed in degassed water.

[25] F. D. Smith, Phil. Mag. **19**, 1147–1151 (1935).
[26] W. T. Richards, Rev. Mod. Phys. **11**, 36 (1939).
[27] N. Marinesco, Comptes Rendus **201**, 1187–1189 (1935).
[28] H. Freundlich and K. Sollner, Trans. Faraday Soc. **32**, 966–970 (1936).
[29] C. Bondy and D. Sollner, Trans. Faraday Soc. **31**, 835–843 (1935).
[30] F. O. Schmitt and B. Uhlemeyer, Proc. Soc. Exp. Biol. Med. **27**, 626 (1930).
[31] S. Brohult, Nature **140**, 805 (1937).
[32] See for example, H. Mark, J. Acous. Soc. Am. **16**, 183–187 (1945).

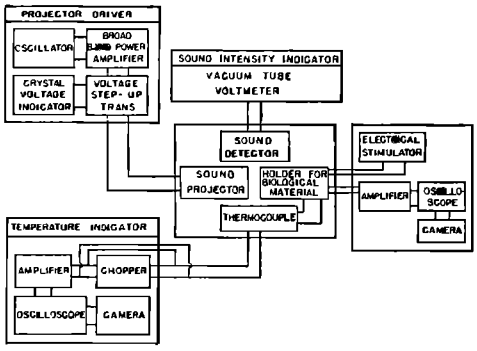

FIG. 4. Block diagram of the experimental setup.

which deviation from a straight line occurs. Curve 1 of Fig. 3a indicates that cavitation occurred in tap water at a crystal driving voltage of the order of 1.0 kv. (X-cut quartz crystal operating in thickness mode at 1.0 Mc.) Curve 2, of Fig. 3a is obtained when degassed water (boiled) is the coupling medium between the source and the probe. There is no indication of cavitation below 5.0 kv. The insertion of a frog between generating and pick-up crystal (all in previously boiled distilled water) yielded the curve of Fig. 3b, indicating that cavitation has occurred above 3 kv.

II. EFFECTS OF ULTRASOUND ON NERVE TISSUE (NON-TEMPERATURE EFFECTS)

As the first step in a systematic program to determine the mechanism of the effects of ultrasound on nerve tissue, it was felt desirable to evaluate the temperature factor associated with the ultrasonic propagation. The following experimental results are presented as evidence to show that there exist reversible and irreversible effects produced in nerve tissue by ultrasound which are not the result of temperature changes.

A. Materials and Methods

1. Ultrasonic Generation and Detection

A schematic diagram of the instrumentation is given in Fig. 4. A detailed description of all of the apparatus indicated in this diagram will not be given in this paper.[33]

The ultrasonic frequency used in the following experiments was between 975 and 980 kc. The source was an X-cut quartz crystal, one-inch diameter, vibrating in thickness mode. Because quantitative results with respect to sound intensity were desired, a voltage measuring arrangement was incorporated into the electrical generator which drives the crystal, so that the voltage across the crystal was known and could be set at any desired level. The intensity of the acoustic radiation is proportional to the square of this measured voltage.

The measurement of bio-electric potentials and other relatively small voltages in the immediate neighborhood of the sound generator made complete electrical shielding necessary. The face of the generator crystal was at ground potential. To achieve consistent and quantitative measurements, it was required that there be no continuously changing geometry in the sound field, such as a variable liquid surface. This was accomplished by arranging the crystal holder and sound tank so that propagation took place in a horizontal direction. For the work presented here, it was desirable to utilize a traveling wave field. This insures uniformity of irradiation of small samples by eliminating critical positioning in a standing wave field.

An important feature of the sound generator used in these studies is the possibility of either continuous or pulsed operation. In addition to continuous variation in amplitude, one has available a range of pulse durations from 0.5 msec. to continuous operation, and a repetition rate variable from 500 cycles per sec. to as low a frequency as desired. In addition, it is possible to obtain delay or precession times from 100 μsec. to 1.0 sec. relative to a pulse generator which is used to apply electrical stimuli to nerve tissues.

The crystal holder is illustrated in Fig. 5, upper. It will be described in detail elsewhere.[34]

The crystal probe is illustrated in Fig. 5, lower. It consists of a small insulated piezoelectric crystal mounted on the end of a hypodermic needle. In practice, it is supported and moved about by a coordinate system which permits accurate measurement of field distribution. Horizontal and vertical field distributions are given in Fig. 6 for a plane $2\frac{5}{8}$ in. from the crystal face. This corresponds to the approximate position of materials under observation.

In these studies, the relative sound intensity was determined by means of the voltage generated by the crystal probe inserted in the sound field and by the

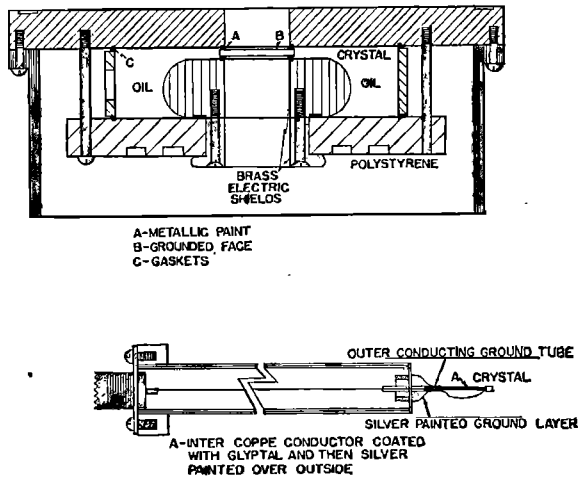

FIG. 5. (Upper) Detailed diagram of the sound projector. (Lower) The acoustic probe.

[33] Tech. Report I—ONR Contract N6ori-71, Task XXI, University of Illinois (1949).

[34] F. J. Fry, Rev. Sci. Inst. 21, 940 (1950).

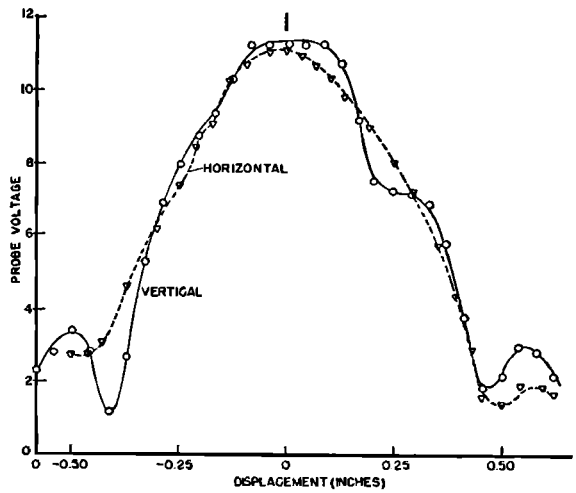

FIG. 6. Horizontal and vertical beam pattern distributions.

driving crystal voltage. The absolute sound intensity was determined by the method described by Fox and Griffing.[35] The measured free field sound intensity at the positions used in the experiments at maximum crystal voltage (5500) is between 30 and 40 w/cm². The uncertainty is due partially to the variation in the position of the test object in the sound tank. A calculated value of the intensity based on the assumption of a pure thickness mode yields a value of 10 w/cm². In this calculation, account was taken of the energy loss in the crystal holder. The high values of the measured sound intensity may be attributed to focusing at a distance of about two inches in front of the crystal. Measured intensity values as a function of distance from the crystal face show a maximum in this region.

2. *Types of Preparations*

(a). Fresh preparations of sciatic nerves from Rana pipiens were employed in these studies. The excised nerves were bathed continuously in cold-blooded vertebrate Ringer's solution at room temperature, 21° to 25°C.

(b). Walking leg nerves of the crayfish were prepared after the method of Welsh and Gordon.[36] This nerve contains two large motorneurons, one to the flexor of the dactyl and the second to the extensor. After excision, these nerves were continuously bathed in van Harreveld's solution at room temperature, 21° to 25°C.

(c). The ventral abdominal nerve cord of the crayfish was dissected out in its entirety and immersed in van Harreveld's solution. The sixth abdominal ganglion was always removed and either part or all of the remaining cord was used.

(d). The electrode chamber consisted of a stainless steel shell containing four glass tubes filled with Ringer's solution. These salt bridges made contact with small calomel half-cells or with coils of silver-silver chloride wire. The salt bridges terminated in small jets against which the nerve preparation was fixed. This chamber was always used in the vertical position. The nerve was immersed in physiological salt solution. It was found undesirable to use plastic nerve holders because of the large temperature change induced in the plastic by ultrasound.

(e). Whole frogs were placed in the path of the sound beam by mounting the animal (dorsal surface down) as firmly as possible on a piece of plywood cut to fit the sound tank. A 2.0-cm hole, centered in front of the crystal, served to admit the sound. The frogs were oriented on the board so that the region of the lumbar enlargement was approximately centered in the aperture. Intact frogs were employed for the most part. The sound tank was filled with distilled water which had been boiled for 10 minutes to drive off most of the gas.

(f). Temperature changes in the spinal cords of intact animals, in excised spinal columns containing the cord, in excised sciatic nerves of the frog, and in the ganglia of excised crayfish ventral abdominal nerve cord were measured with constantan-copper thermocouples. For the sciatic nerve and crayfish ventral nerve cords, a soldered junction made of 0.013-mm copper and 0.038-mm constantan was used. For the spinal cord of the frog, a soldered junction of 0.25-mm copper and constantan was employed. The sciatic nerve and the ventral nerve cord of the crayfish were threaded over the junction and the ultrasound was incident on the preparation in the region of the junction. The thermocouple in the spinal cord was introduced laterally through the foramina of the column through which the peripheral nerves pass. The foramina selected were in the region exposed to the ultrasound. The frogs used in making the temperature measurements were not utilized for any other purpose.

The thermocouple output was interrupted by a mechanical chopper, amplified, and recorded by photographing the trace of a cathode ray beam. The chopping frequency was 10 cycles per sec. A sudden temperature change, produced by thrusting the junction into a cold water bath, produced a deflection reaching 0.7 of maximum in 0.030 sec. This measurement was made without the chopper.

RESULTS

A. Effect of Ultrasound on the Ventral Nerve Cord

Spontaneous activity recorded from the commissure between two adjacent ganglia (1 and 2) of the crayfish ventral abdominal nerve cord, immersed in physiological salt solution is illustrated in Record 1, Fig. 7. Superimposed on a low level background activity is a series of periodically occurring spike potentials at an average frequency of 7.8 per sec. Exposure of the two ganglia

[35] F. E. Fox and V. Griffing, J. Acous. Soc. Am. **21**, 352–359 (1949).

[36] J. H. Welsh and H. T. Gordon, J. Cell. Comp. Physiol. **30**, 147–171 (1947).

and the commissure to ultrasound (~35 w/cm²) produces a characteristic sequence of changes in the activity, a typical example of which is illustrated in Records 2, 3, and 4 of Fig. 7. Note that the frequency of the spike potentials at first increases (Record 2, center), then decreases (Record 2, left), and is followed by total disappearance of the large spike potentials after 43.5-sec. exposure to the sound. Twenty-five seconds after the ultrasound was turned off, the large spike potentials reappeared, first slowly, then more rapidly (Record 5, Fig. 7), finally reaching a stable frequency of 6.7 per sec. (Record 6, Fig. 7). Subsequent treatment of the same preparation with ultrasound produced a similar sequence of events. Similar observations were made on six other preparations.

Measurements of temperature changes in the ganglion of a preparation similar to the above, under identical conditions, indicated a maximal rise of 1°C.

B. Effect of Ultrasound on Peripheral Nerves

Prolonged application of ultrasound to excised frog sciatic nerves suspended in Ringer's solution and in contact with salt bridges, produced no detectable changes in wave form, magnitude of the spike potential, and excitability. The nerves were exposed to ultrasound in the region of the stimulating electrodes, in the region of the recording electrodes, and in the intermediate region. Similar experiments on the excised leg nerves of crayfish gave negative results. Temperature changes in excised frog sciatic nerves recorded from the region of the incident ultrasound indicated a rise of 2°C.

C. Physiological and Morphological Changes Produced by the Action of Ultrasound on the Frog Spinal Cord

Twelve intact normal frogs, suspended under water at room temperature, 21° to 25°C, and placed so that

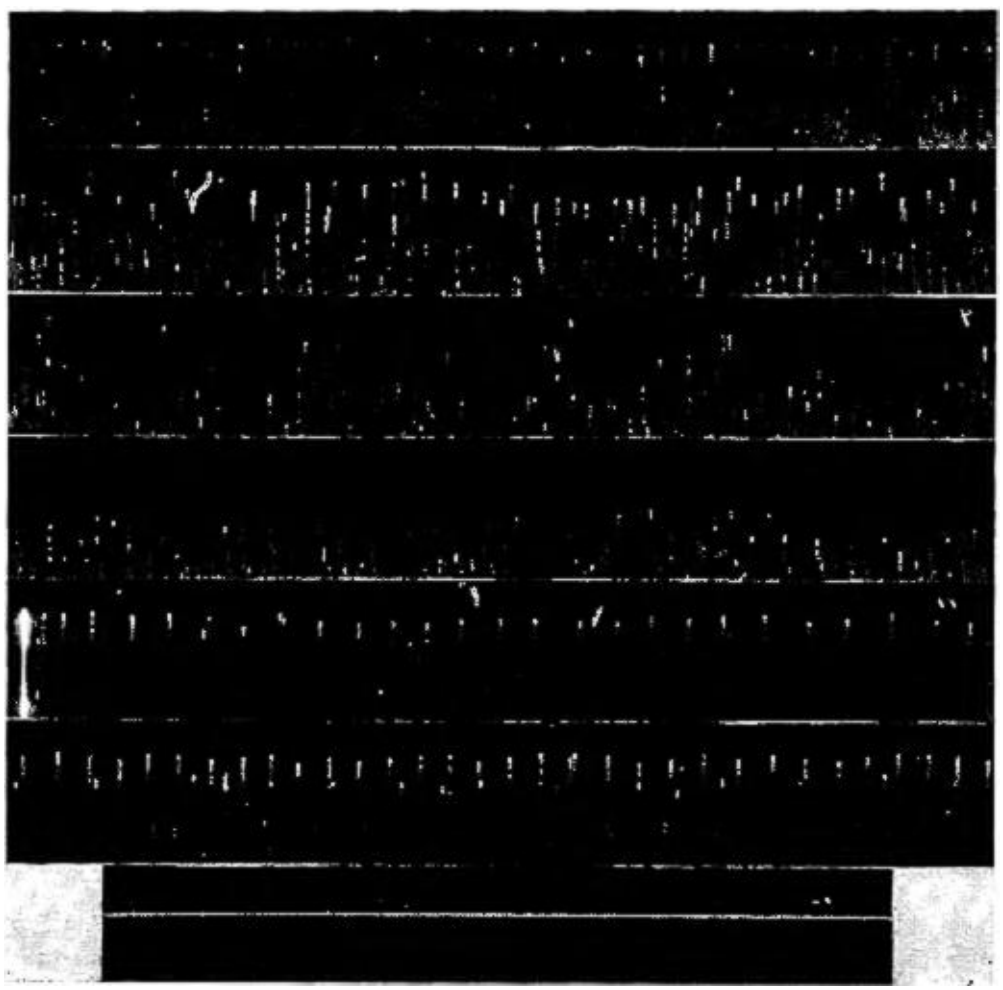

FIG. 7. The effect of ultrasound on the frequency of discharge of spontaneously occurring spikes in the excised crayfish ventral nerve cord.

the sound beam was incident on the center of the back over the lumbar enlargement, showed complete paralysis of the hind legs with exposures of 4.3-sec. duration. Shorter exposures to sound either produced no paralysis or a temporary partial paralysis which disappeared after a variable time interval.

Experiments similar to the above were performed with frogs cooled and maintained at 1° to 2°C. Ultrasound incident on the back over the lumbar enlargement of these cooled frogs produced paralysis of the hind limbs after exposures of 7.3 sec. The paralysis was permanent in all of 50 frogs so treated. Exposures of shorter duration produced no paralysis or a temporary paralysis which disappeared.

Examination of histological preparations of sciatic nerves obtained one week after complete paralysis, fixed and stained with osmic acid vapor, revealed considerable degeneration of axones. A typical example is illustrated in Fig. 8, No. 2 (compare with control nerve, Fig. 8, No. 1). In all preparations examined, considerable degeneration of axones was evident.

Gross examination of the spinal cord of frogs immediately after exposure to a paralyzing dose of ultrasound revealed a loss of surface configuration and a definite change in appearance of the white matter in six frogs examined. Examination of the cord one and two weeks after ultrasound treatment indicated marked degeneration of the cord tissues in the treated region. In some cases, this degeneration was indicated by a constriction in the cord, the superficial white matter appearing intact, and in others, the cord was completely divided in two.

Histological examination of the spinal cords of ultrasound treated frogs revealed marked abnormality of the large motor neurons of the ventral horn of the gray matter. These abnormalities are evident in spinal cords dissected out and fixed 20 minutes after treatment and stained with thionine ($C_{12}H_9N_3S$) (Fig. 8, No. 4). Note the ragged cell outlines, the very intense stain, and enlarged nuclei (compare with control, Fig. 8, No. 3).

D. Temperature Changes Produced by Ultrasound

1. Spinal Cord of the Frog

Changes in temperature of the spinal cord of intact frogs were measured during and following the period of ultrasound treatment. In experiments at room temperature, a rapid rise occurs with onset of ultrasound, usually reaching a maximum between 40° and 50°C at the end of the exposure, and, thereafter, exhibiting a typical decline in temperature (Fig. 9, Graph 1). Temperature measurements on frogs at 1° to 2°C indicate again a sharp increase with onset of ultrasound (Fig. 9, Graph 2), which usually reached a level between 25° to 30°C at the end of the 7.3-sec. exposure. After the

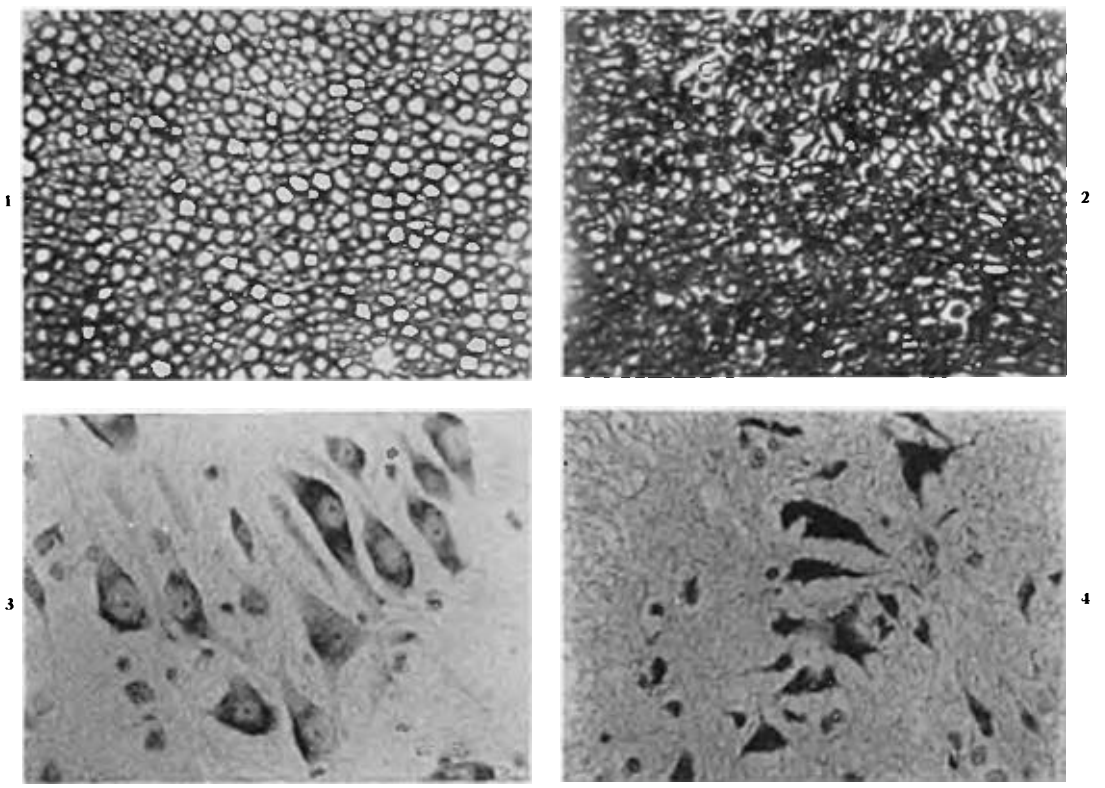

FIG. 8. Photomicrographs of sections through sciatic nerves (1 and 2) and spinal cords of frogs (3 and 4). Magnification 350×. (1) and (3) are controls. (2) and (4) illustrate the results of a damaging exposure to ultrasound.

exposure, the spinal cord exhibits a decrease in temperature. Experiments on isolated spinal column and cord preparations gave similar results (Fig. 9, Graph 3).

To determine the influence of temperature on the production of paralysis of the hind legs of frogs, twelve experiments were performed using brief repetitive pulses of ultrasound. Frogs, cooled to 1° to 2°C, were exposed to ultrasound for 4.3 sec. This is a sub-paralytic dose. The temperature change (max. temp. 15°C) produced by this exposure is indicated in Fig. 9, Graph 4. This exposure was followed by a four-minute interval to permit the cord temperature to return to the previous level. Then the frog was subjected to a second 4.3-sec. dose of ultrasound, which produced a temperature change similar to the first (Fig. 9, Graph 4). Frogs subjected to a similar procedure (without insertion of the thermocouple) exhibited permanent paralysis of the hind legs after the second exposure.

In six experiments in which a 3.3-sec. exposure followed by a four-minute cooling-off period was used, paralysis was produced after the sixth exposure. A third experiment, in which an exposure of 2.8 sec. and a four-minute interval was used, produced paralysis after 50 exposures. Another set of experiments utilizing sound pulses of shorter duration were performed with frogs at room temperature, 22° to 24°C. Sound pulses of 0.08-sec. duration delivered at a rate of two per sec. produced a rapid rise in temperature, reaching equilibrium at about 36°C after four minutes of treatment. Five frogs so treated were permanently paralyzed after three minutes. Sound pulses 0.01-sec. duration delivered at a rate of 20 per sec. produced an equilibrium temperature of 40°C after four minutes. Five frogs treated in this manner were not paralyzed even after ten minutes.

The effect of temperature on the spinal cord was assessed in the absence of ultrasound. The posterior half of frogs immersed in water baths at 35°C for twenty minutes and then raised to 38°C for twenty minutes showed no obvious abnormality in behavior. Frogs immersed in 40°C water for various periods up to six minutes showed a paralysis which gradually disappeared. Temperature measurements in the lumbar region of the spinal cord of frogs immersed in 40°C water indicated a level of 40°C after six minutes.

2. *Bone and Muscle*

The initial rate of rise of temperature of the frog spinal cord appeared to be constant for different preparations exposed to equal sound intensities, regardless of the starting temperature level. This suggested a similar series of measurements for frog bone and muscle. The rate of change of temperature as a function of time after beginning of exposure to ultrasound of ~35 w/cm² is shown in Fig. 1. Note that the curves fall off with time, indicating flow of heat from the tissue into the environment. An approximate value for the initial rate of change of temperature of nerve and muscle is 1.8°C/sec., while for bone it is 55°C/sec.

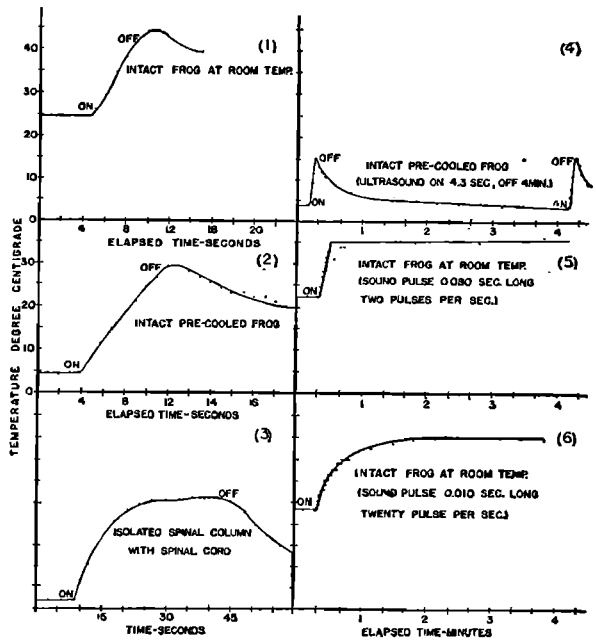

FIG. 9. Temperature changes in frog spinal cord during and after ultrasonic irradiation as a function of time for various experimental conditions.

DISCUSSION

A. Biological Effects Produced by Ultrasound

The temperature changes produced by absorption of acoustic energy may be of a magnitude sufficient to produce changes in the functional characteristics of the living systems studied. Ultrasound incident on ganglia of the crayfish ventral nerve cord containing spontaneously active neurones caused a reversible depression of this spontaneous activity. A maximal and rapid temperature increase of 1°C was measured. Prosser[37] has shown that increasing the temperature 1°C between 26° to 30°C may produce an increase in the frequency of discharge of single units of about 4 to 5 per sec. The effect of ultrasound in depressing the frequency of discharge is in a direction opposite to the effect of the temperature change. It is concluded, therefore, that the effect of ultrasound on these spontaneously active neurones is mediated by physical factors other than the simultaneously occurring but slight temperature change.

The marked increase in temperature of the spinal cords of frogs exposed to ultrasound suggests, at first glance, that the paralysis may be caused by heat. Further examination of the data, however, indicates that paralysis can occur in the absence of high (35° to 40°C) temperature levels. Observations on frogs cooled to 1°C and subjected to pulses of sound separated by four-minute intervals demonstrate summation when the maximum temperature did not rise above 15°C.

[37] C. L. Prosser, J. Gen. Physiol. 19, 65–73 (1935).

SUMMARY

A general discussion of the changes in physical variables which accompany a high intensity ultrasonic disturbance in liquid media and which appear important in understanding the effects of such disturbances on tissue is presented. As the first step in a systematic investigation, the role of the temperature changes produced in an ultrasound field is investigated experimentally.

The results indicated that ultrasound (~35 w/cm², frequency 1 mc) was without effect on excitability, wave form of the spike potential, or propagation velocity of excised peripheral nerve, even after prolonged exposures. The excised crayfish ventral nerve cord exposed to ultrasound exhibited a reduction of spontaneous activity after several seconds exposure and recovered its original activity about one minute after the ultrasound was turned off. Frogs positioned so that ultrasound was incident, on the dorsal surface over the lumbar enlargement of the spinal cord exhibited paralysis of the hind legs after 4.3-sec. exposure (at room temperature) and exhibited paralysis after 7.3-sec. exposure (at 1° to 2°C). Histological examination of the sciatic nerves showed extensive degeneration of nerves and examination of the spinal cord showed marked pathology of the lower motor neurones.

Temperature measurements indicated that peripheral nerve and crayfish ventral nerve cord exhibited a maximal rise of 1° to 2°C. The spinal cord of intact frogs exhibited temperature increases of the order of 25°C. By using frogs cooled to 1°C and reducing the ultrasound exposure to two 4.3-sec. pulses interrupted by four-minute cooling-off periods, it was demonstrated that temperature rises did not exceed 15°C and that paralysis of the hind legs occurred during the second 4.3-sec. exposure. Similar experiments on frogs (room temperature) indicated paralysis upon exposure to ultrasound pulses of 0.080 sec. delivered at a rate of 2.0 per sec. and no paralysis upon exposure to sound pulses of 0.010 sec. delivered at a rate of 20 per sec., yet, the latter procedure produced a higher cord temperature than the former.

It was concluded that the effect of ultrasound on the system studied is produced by physical factors other than temperature. Of these factors, cavitation is the one most easily controlled and will be investigated in the future.

A method is presented for obtaining acoustic absorption coefficients by measuring the initial rate of change of temperature in various test objects.

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An Ultrasonic Underwater "Point Source" Probe

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A conically shaped piezoelectric probe of ammonium dihydrogen phosphate is described. Its sensitivity over a frequency range from 1235 to 1280 kc was obtained by means of a reciprocity calibration. The average value is about 0.12 microvolt per dyne per square centimeter. Since the measured pattern of the probe as a receiver was the same at various points in the acoustic field, it may be concluded that the probe is a "point source."

ONE of the inherent limitations on the accuracy of any measurement is the effect of the measuring instrument on what is being measured. Accordingly, a receiver should be small in order to minimize distortion of the acoustic field it is endeavoring to measure. Where there are large pressure gradients or sharp curvature of the wave fronts, a small receiver is also dictated. A point source indeed is the real desideratum. The response of a finite crystal in such a neighborhood is actually an integration of the response of various points on the crystal face to acoustic signals differing in intensity and phase. Even in a neighborhood where a constant amplitude plane wave field exists, the response of a finite crystal might not be a measure of the intensity, because of varying sensitivity of different points of the surface of the crystal.

High sensitivity is obviously a desirable characteristic of a receiver because it decreases the lower limit of acoustic intensity that can be accurately and reliably measured and, at the same time, increases the accuracy of measurements of higher intensities.

A receiver used as a probe should preferably be nondirectional. Moreover, it would be preferable for the sensitivity to be constant over the interesting frequency range or, at worst, should have a slowly varying characteristic independent of temperature. Since there is

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