Physical Factors Involved in Ultrasonically Induced Changes in Living Systems: II. Amplitude Duration Relations and the Effect of Hydrostatic Pressure for Nerve Tissue*

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The results of experiments with frogs under a hydrostatic pressure demonstrate that cavitation is not an important factor in the mechanism of production of paralysis of the hind legs of frog by ultrasonic (frequency one megacycle) irradiation over the lumbar enlargement region of the spinal cord. Experimental results indicate that a linear relation exists between the reciprocal of the minimum exposure time for paralysis and the acoustic amplitude. This result is readily described in terms of a one factor rate process. On the basis of this experimentally determined relation, it is shown that time rate of change of temperature cannot be correlated with the observations. It is concluded on the basis of a theoretical calculation that absorption of ultrasound at interfaces in the spinal cord does not result in minute hot regions.

Further work on summation of subparalytic doses, spaced apart at various time intervals, indicates that the recovery process following exposure to a subparalytic dose of ultrasonic radiation may not be a monotonic function of time.

INTRODUCTION

IN the first paper of this series concerned with the physical factors involved in ultrasonically induced changes in nerve tissue, we presented experimental data which demonstrated the existence of non-temperature effects.[1] Observations have been made, during ultrasonic irradiation, on the random activity of the ventral nerve cord of crayfish and on the reflex discharge of the frog spinal cord.[1,2] Under appropriate experimental conditions, permanent paralysis of the hind limbs of frog is observed after irradiation.[1]

In this paper, we are concerned with the possible role of cavitation as a factor in the mechanism producing paralysis of the hind legs of frogs. For this purpose, observations were made on paralysis in frogs under a hydrostatic pressure sufficiently high to suppress all cavitation.

In addition, the time required for paralysis (for a single irradiation) has been determined for various acoustic amplitudes. These data were obtained both at atmospheric pressure and under a hydrostatic pressure sufficient to suppress all cavitation up to an acoustic pressure amplitude of 13 atmospheres. The results indicate that rapid time rate of change of temperature of the tissues of the spinal cord under ultrasonic irradiation is not an important factor in the production of the paralysis. Arguments are also presented which indicate that minute local hot regions cannot exist in the tissue which is under irradiation.

A further study of the summation process for subparalytic doses has been accomplished.

DESCRIPTION OF APPARATUS AND EXPERIMENTAL PROCEDURE

The experiments described in this paper were all performed at an ultrasonic frequency of 0.98 mc. The acoustic pressure amplitude was continuously variable from zero to about 15 atmospheres. The absolute sound level and field distribution were determined by the method described in a previous paper.[1] The electronic part of the ultrasonic generator is identical with that used in earlier experiments.

However, in order to irradiate the frogs in an environment under a hydrostatic pressure, it was necessary to design a different sound chamber from that used for previous experiments. The essential design features of the new system are illustrated in Fig. 1. There are three ports of entry to the stainless steel chamber, each bolting into position. The port A gives access to the left-hand section B which is isolated from the rest of the chamber so that the gas composition in this part is independent of the composition in the other section. Since B contains the sound head C which requires high voltage for its operation, it is necessary, for stability and safety, to introduce a dry inert atmosphere. Port D is used to introduce the sound absorber E which is filled with castor oil and is capped on its left-hand side by a $\frac{1}{32}$-in. sheet of "ρc" rubber. Under normal oper-
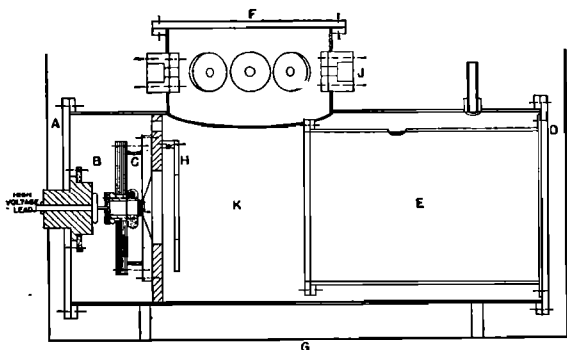

FIG. 1. Sound chamber for pressure studies.

ating conditions, port F is used for introducing the object to be irradiated into the chamber.

The entire pressure chamber is enclosed in a container G supporting a bath which furnishes temperature control for the system.

Electrical lead-ins are provided through a radial array J of pressure tight seals.

In practice, the sequence of events for a run is as follows. The bath in container G is brought to the desired temperature, and degassed water is introduced into the region K through F. When the degassed water has attained the desired temperature, the specimen is introduced on support H which accurately positions it in the sound field. After a sufficient time has elapsed for temperature equilibrium of the specimen and bath to be attained (which may be quite short since the specimen can be brought to the test temperature in an external bath), the desired gas compositions are simultaneously introduced into both sections of the pressure chamber under controlled conditions. There never exists more than a few pounds per square inch pressure difference. This control is necessary for mechanical protection of the crystal-crystal holder assembly L. The specimen is irradiated, and the pressures are then simultaneously released.

The sound projector used in this arrangement is similar to those described previously.[3]

The rate of compression is adjusted so that a pressure change from 1 atmosphere to 13 atmospheres was accomplished in about one to two minutes. Decompression is accomplished in an equal period of time. The frogs were irradiated on the dorsal surface over the region of the lumbar enlargement. The coupling medium between the frog and the projector is water. The ultrasonic beam width at half-amplitude is approximately 5 mm. The frogs were rigidly supported and accurately positioned in the sound field. The average weight of the frogs used in these experiments was about 23 grams. The range of weights was approximately 15 to 32 grams.

The irradiation procedure usually consisted in setting the sound level to some given value, and then subjecting frogs to this constant level for increasing lengths of time. In this way, the maximum time for which "no" frogs are paralyzed and the minimum time for which "all" frogs are paralyzed can be determined. The criterion of "all" or "none" is arbitrarily specified by observations on from three to five frogs. Such a criterion leads to results which can be duplicated.

EXPERIMENTAL RESULTS

The results of the experiments on the relation between the sound amplitude and the time required for paralysis are shown graphically in Fig. 2 and Fig. 3. The results obtained with the frogs at atmospheric pressure, at a temperature between 0° and 1°C and coupled to the

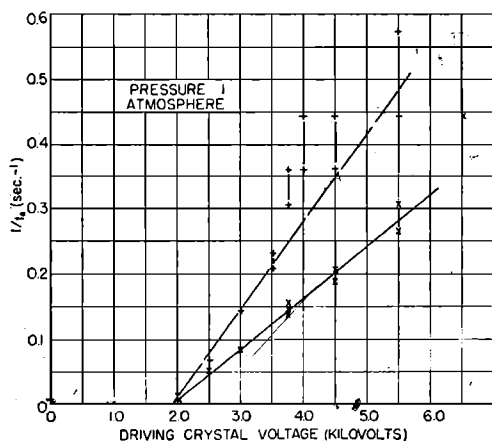[3] F. J. Fry, Rev. Sci. Instr. 21, 940–941 (1950.)

FIG. 2. Reciprocal of minimum irradiation time for paralysis and maximum irradiation time for no paralysis as a function of relative sound amplitude for frogs at atmospheric pressure and 0°–1°C.

sound source by degassed water, are presented in Fig. 2. The rms driving crystal voltage, which is proportional to the acoustic amplitude, is plotted along the horizontal. A voltage of 5.5 kilovolts corresponds to a free field pressure amplitude of about 12 atmospheres. The reciprocal of the time is plotted along the vertical.

The ratio of the acoustic pressure amplitude within the cord to the free field pressure amplitude was determined by direct probe measurements in several cords. A probe whose sensitive element consists of a small insulated crystal was used for these measurements. The average value of this ratio for a set of random positions of the probe in the lumbar enlargement region of the cord was computed for each cord. These averages for different cords agree closely. The measurements indicate that the average acoustic pressure amplitude in the cord is 0.8 of the free field pressure amplitude.

The solid line of the graph designates the relation between the sound amplitude and the minimum time for which "all" frogs are paralyzed. The dashed line is the relation between the sound amplitude and the
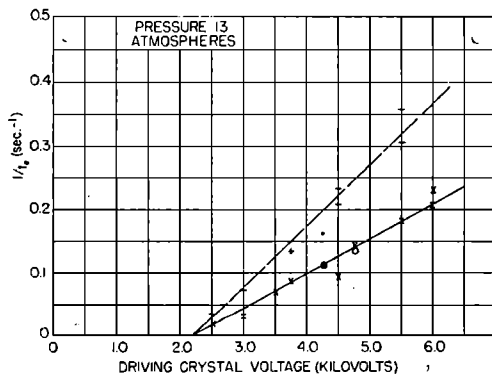

FIG. 3. Reciprocal of minimum irradiation time for paralysis and maximum irradiation time for no paralysis as a function of relative sound amplitude for frogs at 13 atmospheres pressure and 0°–1°C.
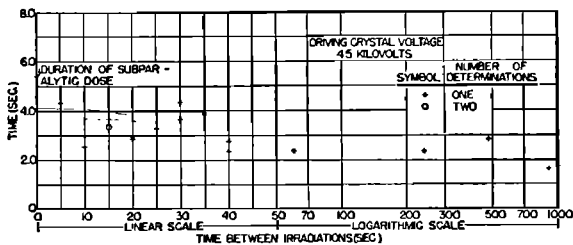
FIG. 4. Difference between the duration of a single irradiation for paralysis and the duration required of a second irradiation to produce paralysis when the first irradiation is subparalytic as a function of the time between the first and second irradiations. The sound amplitude is equal for all irradiations.

maximum time for which "no" frogs are paralyzed. The solid vertical lines connecting two points are not an indication of variability in the experimental results, but rather the result of the fact that a step timer was used to control the duration of the sound and that timer settings, corresponding to durations intermediate between the points indicated on the graph, were not possible. In general, the minimum step in irradiation time was 10 percent if this was not less than 0.5 second.

With the frog under a hydrostatic pressure of 13 atmospheres and a temperature of 0° to 1°C, the results illustrated in Fig. 3 were obtained. As in the previous case, two sets of data are shown, one corresponding to the maximum time for no paralysis and the other corresponding to the minimum time for paralysis. Most of the determinations were made with the frog's head in pure oxygen. However, two determinations were made under pure nitrogen. These are indicated by the circles on the lower curves.

Observations on about 500 frogs were required to obtain the data for Figs. 2 and 3.

Results of previous experimental work on summation of successive subparalytic doses to produce paralysis were presented in reference 1. These previous studies were confined to a fixed period of four minutes between irradiations. A more complete picture of the decay process which follows an exposure of the frog to a subparalytic dose of ultrasound at one acoustic amplitude and dose duration is indicated by the results illustrated in Fig. 4. The sound level is held constant at a pressure amplitude of 10 atmospheres, which corresponds to a driving crystal voltage of 4.5 kilovolts. This level corresponds to a paralysis time of 7.8 seconds for a single irradiation. The duration of the initial dose is 5.4 seconds. The frogs were under a hydrostatic pressure of 13 atmospheres and at a temperature of 0° to 1°C. On this graph, the difference between the required duration of a single irradiation for paralysis and the duration required of a second irradiation to produce paralysis, when the first irradiation is subparalytic, is plotted as a function of the time between irradiations. The plotted points correspond to irradiation times for which paralysis is obtained. An irradiation time shorter by 0.4 second did not produce paralysis.

Because of the complex appearance of the relationship, as indicated graphically, more than one determination was made at some of the time intervals between irradiations. The symbols used in plotting indicate the number of times a particular point was determined by independent experiments. These results indicate that the over-all decay process, determined as described above and following exposure of the frog to a subparalytic dose of ultrasound, is not monotonic for at least some conditions of irradiation. It is possible that the decay process is monotonic for shorter subparalytic doses or for different acoustic amplitudes.

A curve is not drawn through the points of Fig. 4, since it is felt that many more data would be necessary to specify a reasonably quantitative relationship. The graph is included in the paper simply to illustrate the complexity of the aftereffects of a subparalytic exposure. Irradiation of from 12 to 15 frogs is required for the determination of one point.

DISCUSSION

(1) Cavitation

One of the purposes of this study was the investigation of the possible role of cavitation as the agent responsible for the observed paralysis of the frogs when ultrasound is incident on the dorsal surface over the region of the lumbar enlargement. That such is not the case is shown by the results of the experiments in which a hydrostatic pressure sufficient to completely prevent cavitation at acoustic pressure amplitudes equal to or less than 13 atmospheres was used (see Fig. 3). This pressure amplitude corresponds to a driving crystal voltage of 5.7 kilovolts. Experimentally, paralysis was obtained at voltages as low as 2.5 kilovolts.

It is of interest to note that other experimental observations indicate that frog tissues will withstand considerable tension before cavitation occurs.[1] It is inferred that cavitation probably does not occur in the frog spinal cord at the highest driving crystal voltage, 6.0 kilovolts, indicated on the graph of Fig. 3. At atmospheric pressure, cavitation may cause a deviation from linearity at the higher sound levels, as suggested by the results of Fig. 2.

An interesting result of this investigation is the linear relations, presented graphically in Figs. 2 and 3, between the reciprocal of the minimum irradiation time for paralysis ("all" frogs paralyzed under a single irradiation) and the relative acoustic amplitude, and also between the reciprocal of the maximum irradiation time for no paralysis ("no" frogs paralyzed under a single irradiation) and the relative acoustic amplitude. The form of the relationship can be expressed mathematically as

$$1/t_e = m(A - A_0), \qquad (1)$$

where t_e is the exposure time, and A is the relative acoustic amplitude (the voltage across the projector).

A_0 is the value of A at the intersection of the curve and the horizontal axis. This relationship is valid under 1 atmosphere pressure of air and under 13 atmospheres pressure of O_2. The existence of a definite threshold (acoustic amplitude below which no paralysis can occur, irrespective of the duration of irradiation, and above which paralysis always occurs if the period of irradiation is sufficiently long) is established. This threshold increases slightly with pressure (14 percent for a pressure change from 1 to 13 atmospheres). The slope of the line, which designates the relation between the reciprocal of the time for paralysis and the relative acoustic amplitude, is smaller at the higher pressure, i.e., the time for paralysis under pressure is always greater than the time for paralysis under 1 atmosphere for equal acoustic amplitudes. That the environment of pure oxygen (13 atmospheres) does not account for these differences is demonstrated by the fact that when the frog is immersed in an environment of pure nitrogen (13 atmospheres), the time for paralysis is unchanged from that obtained for an environment of pure oxygen. This was checked at two different sound levels, as indicated by the two points designated by circles in Fig. 3.

(2) Temperature

a. Time Rate of Change of Temperature

Results reported in reference 1 showed that the paralysis was not produced by excessive temperatures. This was proved by direct measurement of the temperatures of the cord under irradiation and by experiments demonstrating summation. It has been suggested that the time rate of change of the temperature may be a factor. That this is not the case can be seen from the following. Consider two relative sound levels near threshold A_1 and A_2 such that

$$(A_2 - A_0) = n(A_1 - A_0), \qquad (2)$$

where n is any arbitrary positive number. Then, from Eq. (1), letting t_e designate the paralysis time, we have $t_{e2} = t_{e1}/n$. Now the intensity of the sound is proportional to A^2, and the time rate of change of temperature is proportional to the intensity. It is clearly possible to choose A_1 and A_2 in such a fashion that the difference between their squares is as small as we wish and yet preserve the relation (2). We can thus make the difference in the rates of change of temperature in the two cases as small as we wish and yet the times for paralysis differ by the factor n. This indicates that the time rate of change of temperature is not a contributing factor in the mechanism determining paralysis.

b. Minute High Temperature Regions

It has also been suggested that another way in which the temperature factor might enter into the mechanism

of the effects produced by ultrasound on tissue is through localized heating at interfaces. It is felt, on the basis of the following discussion, that local heating is not important in producing the results which we have observed on nerve tissue.

As reported previously,[1] we observed no appreciable change in the electrical threshold in excised frog sciatic nerves subjected to ultrasound at 1.0 megacycle up to intensities of the order of 35 watts/cm². The change in average temperature of the sciatic nerve in the sound field is about 2°C. This indicates that the temperature at the interface across which the nerve demarcation potential exists is not appreciably different from the average temperature of the preparation.

When an excised ventral nerve cord of the crayfish is irradiated with ultrasound, it is first observed[1] that the electrical activity of a single neuron increases and is then depressed. The increase in activity can be accounted for by the rise in average temperature of the preparation, as measured by a small thermocouple.[4] This suggests that the temperature at the interfaces in close proximity to the active neuron does not differ much from the average temperature of the portion of the nerve cord under ultrasonic irradiation.

It is possible to calculate an upper limit for the difference between the temperature of the interfaces and the average temperature of the tissue under ultrasonic irradiation. We assume that all sound absorbed is absorbed at interfaces and consider a unit volume of material filled with cells of a cylindrical shape of average diameter 10μ. Calculations based on spherical cells would differ by only a small factor from those based on cylindrical cells, and the calculated temperature difference would be smaller. If we choose a smaller size cell, the calculated temperature differences would be smaller. We assume that there is one absorbing interface per cell. If the difference in temperature between the interfaces and the average temperature of the tissue is ΔT, then the heat H conducted from the interfaces per second per unit volume is given by

$$H = KA(\Delta T/L), \qquad (3)$$

where A is the total area of the interfaces per unit volume, $\Delta T/L$ is the temperature gradient, and K is the coefficient of heat conductivity. The time rate at which heat is absorbed from the sound wave in a slab of unit area and thickness Δx (Δx along the direction of propagation) is given by

$$\Delta H_a = \alpha I_0 \Delta x, \qquad (4)$$

where I_0 is the incident sound intensity, and α is the intensity absorption coefficient per unit path length. At equilibrium, $H\Delta x = \Delta H_a$ if we neglect convection which would act only to further decrease the temperature difference. Expressions (3) and (4) yield the

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

following equation for the temperature difference:

$$\Delta T = \alpha I_0 L / KA. \tag{5}$$

To obtain a numerical estimate for ΔT, we assume that the heat conductivity of the tissue is equal to that of water, and the absorption coefficient α is equal to 0.4 per cm. This value of α is greater than that calculated from data given in reference 1. We insert the radius of the cells for L, i.e., 5μ. At a sound intensity of 50 watts/cm^2, the calculated value of the difference between the temperature at the interfaces and the average temperature of the tissue is, roughly, $6(10)^{-4}$ degree C. Even if only 1 percent of the cells have membranes which absorb the sound, the temperature difference would still be less than 0.1°C. It thus appears that temperature differences between the interfaces in the nerve tissues and the average temperature of the tissue are not important in producing the observed results.

c. Summation

The experimental results on summation plotted in Fig. 4 indicate that more than one process is important in determining the rate of change with time of the aftereffects of a subparalytic dose of radiation. Many more experimental data are necessary before a complete picture of the summation process can be presented.

d. One Factor Theory

The linear relations obtained experimentally between the reciprocal of the time for paralysis and the acoustic amplitude can be described in terms of a rate process as follows. We assume that a factor x in the spinal cord tissue satisfies the relation

$$dx/dt = K(A - A_0), \tag{6}$$

which has the solution

$$x = K(A - A_0)t + b. \tag{7}$$

The quantity A is proportional to the acoustic amplitude; for example, it may be the driving crystal voltage. A_0 is the value of this quantity at threshold. The quantity K is a proportionality constant which is dependent on the hydrostatic pressure and the base

temperature of the cord† and may be dependent on the acoustic frequency.

We let x_0 designate the value of the factor x before ultrasonic irradiation and let x_p be the value necessary for paralysis. We assume that there is negligible decay in the factor during irradiation.

We obtain from Eq. (7) the result

$$1/t_p = [K/(x_p - x_0)](A - A_0), \tag{8}$$

where t_p is the time for paralysis under a single irradiation. This is the experimentally observed relation (1), both at atmospheric pressure and at 13 atmospheres pressure. We let $m = K/(x_p - x_0)$ and observe that m is a function of hydrostatic pressure, base temperature, and ultrasonic frequency.

Further study of the mechanism of the effects of ultrasound on nerve will involve a determination of the dependence on acoustic frequency. Experiments will be designed to differentiate between the roles of the periodic variables; pressure, particle velocity, and particle acceleration.

SUMMARY

By applying a hydrostatic pressure sufficient to prevent cavitation, it is shown that the primary physical factor involved in the mechanism producing permanent changes in nerve tissue, as manifested by paralysis of the hind limbs of frogs, is not cavitation. (It has been previously shown that the average temperatures produced by absorption of the sound in the tissue cannot account for the observed results.)

It is determined experimentally that a linear relation exists between the reciprocal of the minimum time for paralysis ("all" frogs paralyzed) and the acoustic amplitude. The existence of a definite threshold is established. A linear relation also exists between the reciprocal of the maximum time for no paralysis and the acoustic amplitude. An argument is formulated which shows that the "time rate of change of temperature" is not important in the mechanism of paralysis.

Calculation shows that interface absorption of the ultrasound in the spinal cord cannot result in local hot regions. The single-dose paralysis results are described in terms of a one factor rate process.

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† In reference 1 it is shown that the time required for paralysis is shorter when the base temperature of the frog is higher.