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The first four papers in this issue constitute a partial report of a symposium on Ultrasound in Biology and Medicine held at the University of Illinois in May, 1952. The following 2 papers presented at this symposium are not as yet ready for publication and will appear in a later issue:

The Use of High Intensity Ultrasound in Experimental Neurology. P. D. WALL, D. TUCKER, F. J. FRY, AND W. H. MOSBERG, JR.
The Effects of Biological Tissues on 15-mc Pulsed Ultrasound. J. J. WILD AND J. M. REID.

Action of Ultrasound on Nerve Tissue—a Review*†

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This review is concerned with (1) certain physiological and structural changes produced in tissues of the central nervous system caused by high level ultrasound and (2) investigations into the physical mechanisms underlying these changes.

The cell bodies of neurons in the central nervous system are particularly susceptible to change by ultrasound. The effect of irradiation is immediately evident as a loss of function which may be reversible or irreversible depending on the dosage. Irreversible changes in function are accompanied by changes in the structure of the cell. The susceptibility of neurons studied so far is graded according to size, the larger neurons exhibiting a greater susceptibility. The dose of ultrasound can be adjusted to cause irreversible changes in neurons without causing any observable damage to the vascular and supporting components of the tissue. This selective and specific effect of ultrasound is being used as a tool in neuroanatomical studies now in progress and has considerable potential value in neurosurgery. The ultrasound alters the state of the nerve cells and, therefore, affords a basis for studying intracellular structure and function.

The physical basis for the ultrasonically produced biological effects has been investigated in part. The following aspects of temperature have been analyzed and rejected: (1) High average (space) level, (2) interface heating, (3) rapid time rate of change, (4) temperature changes resulting from cavitation, (5) heating at gas nuclei. The phenomenon of cavitation is also shown to play no essential direct role in producing the effects.

I. INTRODUCTION

THIS paper consists of a review of the principal results of research which have contributed to our present level of understanding of certain effects of ultrasound on tissues of the central nervous system and to our present state of knowledge regarding the physical mechanism involved in the production of these

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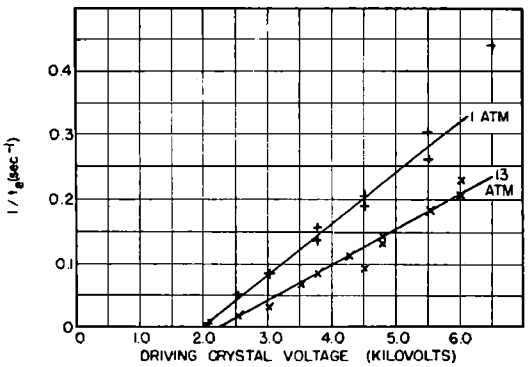

FIG. 1. Relations between driving crystal voltage, which is proportional to acoustic pressure amplitude, and reciprocal of the "minimum time for paralysis" for frogs cooled to 1°C and under hydrostatic pressures of one and thirteen atmospheres.

effects.[1–4] The extensive European work[5,6] on the action of ultrasound on tissue will not be discussed since the effects considered here appear to be limited to sound levels in excess of those used by most European investigators. A similar statement applies to the research reported by other investigators in this country.[7] Lehmann's research on the mechanism of action of ultrasound on tissue,[8] at sound levels used in therapy, demonstrates that the temperature factor is of primary importance.

It is convenient to subdivide the subject matter of this paper into two catagories: (1) changes in structure and function resulting from the action of ultrasound on the organism, and (2) the physical mechanisms involved in the production of these changes.

II. PHYSIOLOGICAL AND STRUCTURAL CHANGES

The frog, *Rana pipiens*, has served as a convenient biological test object for a number of the investigations on the effects of ultrasound on nerve tissue. When the lumbar enlargement of a frog spinal cord is irradiated with sound at a frequency of one megacycle and at a suitable‡ pressure amplitude, paralysis of the hind legs occurs. The duration of irradiation to produce paralysis must be greater than a minimum value which is dependent on the acoustic pressure amplitude. The paralysis is present immediately after the irradiation and is permanent. Experiments have been performed on frogs with the spinal cord exposed by laminectomy and on intact animals. In the latter the sound passed through the overlying skin, muscle and bone. Physiological salt solution is used as the coupling medium between the transducer and the animal. The results are the same whether the frog is initially at room temperature or at 1°C when irradiated. The biologic effects of ultrasound and the underlying mechanisms appear to be the same whether the frogs are subjected to 1 or 13 atmospheres pressure at the time of radiation. The quantitative relation between acoustic pressure amplitude and time of irradiation for paralysis is presented in Fig. 1, for a hydrostatic pressure of one atmosphere. We define a quantity, "the minimum time for paralysis," as the minimum period of irradiation which will result in paralysis of all frogs subjected to the radiation. As can be seen from the graph a straight line relationship exists between the reciprocal of this time and the driving crystal voltage which is proportional to the acoustic pressure amplitude. A similar relation between driving crystal voltage and reciprocal of paralysis time is obtained for frogs under a hydrostatic pressure of 13 atmospheres, as can be seen from Fig. 1.

It is possible to obtain summation of successive subparalytic exposures to produce paralysis. The time course of recovery of the spinal cord of a frog from the effects of a subparalytic exposure is indicated in Fig. 2. Frogs are exposed to a 5.4-second dose of radiation at an acoustic pressure amplitude which alone would produce paralysis in 7.8 seconds. At various times later these frogs are subjected to a second period of radiation at the same pressure amplitude. The minimum duration of the second exposure to produce paralysis is determined. The difference in the time interval for paralysis for a single exposure, and the duration required of a later exposure to produce paralysis is shown plotted as a function of the time interval between exposures. The data presented in the figure do not suggest a simple monotonic recovery process following exposure. However, the information obtained is insufficient to permit description of the recovery process in any detail.

In addition to the irreversible changes produced by ultrasound in the central nervous system of frogs which result in paralysis and anesthesia, we have obtained indications of reversible changes as follows (unpublished data): One branch of the sciatic nerve, the tibial, is excited electrically and the reflex discharge through the spinal cord is observed electrically on the peroneal branch of the sciatic. The cord is then subjected to a pulse of acoustic radiation with a pressure amplitude above the threshold value for paralysis. The reflex discharge can be completely suppressed by a pulse whose duration is less than that required for paralysis. When such is the case, essentially complete recovery ensues. It appears at present that by appropriate choice of the pulse length and the time interval between irradiations

[1] Fry, Wulff, Tucker, and Fry, J. Acoust. Soc. Am. **22**, 867 (1950).
[2] Fry, Tucker, Fry, and Wulff, J. Acoust. Soc. Am. **23**, 364 (1951).
[3] Wulff, Fry, Tucker, Fry, and Melton, Proc. Soc. Exptl. Biol. Med. **76**, 361 (1951).
[4] Wall, Fry, Stephens, Tucker, and Lettvin, Science **114**, 686 (1951).
[5] See, for example, the proceedings of the Erlangen and Rome conferences on Ultrasound for a general view of the field to 1949–1950. *Der Ultraschall in der Medizin* (S. Hirzel Verlag, Zurich, 1949). Supplements to volume VII, Ser. IX of Nuovo cimento, Rome (1950).
[6] H. Heyck, Schweiz. med. Woch. **82**, 97 (1952).
[7] Anderson, Wakim, Herrick, Bennett, and Krusen, Arch. Phys. Med. **32**, 71 (1951).
[8] J. F. K. Lehmann, J. Acoust. Soc. Am. **25**, 17 (1953).
‡ An amplitude above a threshold value, which is somewhat dependent on the temperature of the animal and the hydrostatic pressure of the environment.

this sequence of events can be repeated indefinitely or can be designed to result in permanent suppression, i.e., the frog is paralyzed, after any roughly prescribed number of repetitions.

In contrast to the effects just described isolated peripheral nerves (frog sciatic and crayfish leg nerves) are insensitive to sound as measured in terms of threshold to electrical stimulation and the shape and size of the action potential. This statement is made for sound at pressure amplitudes in the range used at present to effect the changes in the central nervous system. Even exposures several orders of magnitude greater than those used to produce major changes in the central nervous system produce only minor changes in peripheral nerve, which are readily explained by the slight heating that occurs.

The results of the histological studies of ultrasonically irradiated tissue of the central nervous system of cats, rats, and frogs are described in detail in another paper.[9] For the purpose of a general review and as a background for the discussion of the physical mechanism contained herein pertinent results are briefly described in this paper. From the histological work an order of susceptability to change by the acoustic radiation has been established for the various tissue components present in the central nervous system. The most sensitive elements are the large neurons in the spinal cord. It is possible to choose the dosage of radiation so that the large neurons in the irradiated region are affected, but the small neurons, glia cells, blood vessels and nerve fibers are left intact. As a specific example, when the dosage is adjusted so that only the large motor neurons of the lumbar enlargement of the frog spinal cord are damaged paralysis of the hind legs results. This occurs for irradiation times close to the minimum required for paralysis.

Heavier doses of radiation result in destruction of all nerve cells. Glia cells and supporting elements are next affected. Nerve fibers remain intact and the blood vessels are dilated but not broken at the highest acoustic pressures, about 15 atmospheres, used in the experiments on frogs. In a small percentage of the cats which have been irradiated there has been some blood vessel damage, but this may be associated with the high acoustic pressures, about 30 atmospheres, used in many of the experiments on cats up to the present time. It should also be noted that the changes produced in the tissue by the ultrasound begin to manifest themselves histologically from one to two hours after irradiation. The primary effect is not one of gross disruption of cellular or nuclear membranes.

Ultrasound doses which produce paralysis in frogs do no gross damage to the overlying skin, muscle and bone. All experiments on cats and rats have involved surgical removal of the bones overlying the areas to be irradiated. The bone was removed because of its high absorption for ultrasound at the frequency used.

The transducer used in irradiating cats and rats produces a beam which focuses at a distance of 6.4 cm from the transducer face. The intensity is down to 0.7 of the peak value in the radial direction 1.3 mm from the beam axis. The amplitude in the direction of the beam axis does not change rapidly. The duration, pressure amplitude, and position of the beam of acoustic radiation are accurately controlled. The shape of the lesion produced by the beam from this transducer is in the form of a long narrow cylinder. A multiple beam focusing transducer now under construction will enable us to produce lesions small in all three dimensions. As an outgrowth of the neuroanatomical studies we are now developing a multiple beam instrument for neurosurgical test.

III. PHYSICAL MECHANISMS

A. Temperature

Temperature changes which occur when ultrasound is propagated through tissue may play a role in producing biologic effects in the following ways: (1) The temperature of the tissue exceeds some value above which damage occurs, (2) high temperatures exist at interfaces in the tissue which could not be detected by a thermocouple as small as say 0.003 in. in diameter, (3) the time rate of change of temperature is too great, (4) temperature changes associated with cavitation are responsible, (5) heating at gas nuclei.

(1) Five independent arguments are presented against the possibility that the temperature of the tissue exceeds a critical value above which damage occurs.

(a) Experiments[1] on cooled frogs show that the ultrasound produces its effects on nerve cells when the temperature level in the cord is less than 20°C. Figure 3(a) shows a graph of the temperature change which takes place in a frog spinal cord as measured by a 0.003-in. diameter thermocouple, when the lumbar enlargement of the frog is subjected to a 4.3-second exposure of ultrasound at a level sufficient to produce paralysis with two exposures 4 minutes apart. A single exposure of sufficient duration to cause paralysis yields the temperature change indicated by Fig. 3(b).

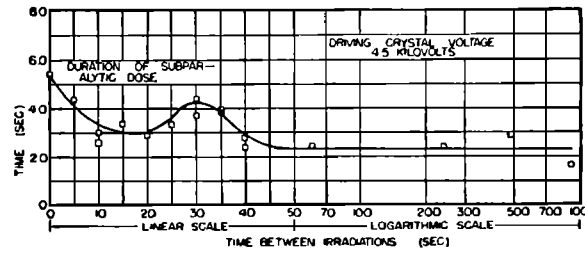

FIG. 2. Graphical indication of the time course of recovery of the nerve tissue of the lumbar enlargement of frog after a subparalytic dose of ultrasound. The experimentally determined minimum duration required of a second dose of radiation to produce paralysis, at various times after the initial period of irradiation, is subtracted from the duration required for a single dose (7.8 seconds) to yield the value plotted on the vertical scale.

[9] Wall, Tucker, Fry, and Mosberg (submitted for publication).

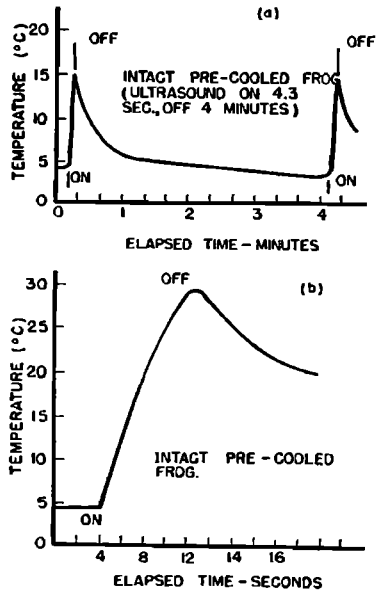

FIG. 3. Temperature changes in a frog spinal cord (lumbar enlargement) produced by ultrasound. (a) Temperature changes accompanying two 4.3-second exposures separated by a 4-minute interval. Paralysis is produced after the second exposure. (b) Temperature changes accompanying a single exposure of sufficient duration to produce paralysis.

A maximum of 30°C is attained. An intact frog immersed in a warm water bath to hold the cord temperature at 30°C does not exhibit impairment of motor functions after one half-hour.

(b) Summation[1] of the biological effects in motor neurons produced by sound pulses, separated by periods of time long compared to the interval required for the tissue to return to its initial temperature proves that paralysis (in the case of irradiation of the lumbar enlargement of a frog spinal cord) is not dependent on achieving any particular temperature level. Compare the two graphs in Fig. 3. The temperature levels attained are completely different in the two cases.

(c) On the basis of the experimentally determined relations between minimum paralysis time and acoustic pressure amplitude[2] (Fig. 1) it is readily seen that the absorption of a constant amount of acoustic energy by the tissue is not required to produce the effect.

(d) Thermocoagulation studies[10-12] on the cerebral cortex by other investigators have shown that, when the large cells are destroyed by heating, the small cells in the tissue are also destroyed. However, in our studies with ultrasound it is evident that the large cells are particularly sensitive to the acoustic radiation.[1,3,4]

(e) Preliminary studies at 500 kc have been made at a single value of the acoustic pressure amplitude. The minimum exposure time required to produce paralysis of the hind legs of frogs by irradiation over the lumbar region of the spinal cord has been determined. In order to obtain equal temperature levels in the cord at 500 kc and 1 mc in equal exposure times the ratio of the two intensities should be in the ratio of absorption coefficients. Such a ratio of intensities is not required for paralysis at the two frequencies.

(2) It has been shown on the basis of a theoretical analysis presented in one of our published papers[2] that the existence of high temperatures at interfaces in the cord is unlikely. The argument can be briefly summarized as follows. Assume that all sound absorbed in the tissue is absorbed at interfaces. Consider a volume of material consisting of cylindrical cells of diameter 10 microns. The temperature changes would be smaller for spherical cells or cells of either shape of smaller diameter. We assume that there is one absorbing interface per cell. Let the average temperature of the tissue differ from the average interface temperature by the amount ΔT. If the heat conductivity of the material at the interface is of the same order as the average value for the tissue, or if the interface is of zero thickness, the following relation for the temperature difference at equilibrium is obtained:

$$\Delta T = \mu I_0 L / KA, \qquad (1)$$

where μ is the average intensity absorption coefficient per unit path length of the tissue, i.e., the usual measured value, I_0 is the sound intensity, L is the cell diameter, A is the total area of the interfaces per unit volume, and K is the coefficient of heat conductivity. At a sound intensity of 50 watts/cm^2 and a value of μ of 0.4 per cm and using a value for the heat conductivity equal to that of water, the calculated value of the temperature difference is less than 0.001°C. Refinements of this argument to include, for example, such situations as interfaces of finite thickness of greatly different heat conductivity characteristics from the average for the tissue, do not change the general conclusion that localized heating at interfaces cannot be important in the mechanism.

(3) The possibility that subjecting the tissue to a time rate of change of temperature greater than a certain minimum value is sufficient to produce the effects is incompatible with the quantitative relations of Fig. 1. Neglecting conduction, which is justified during the initial part of the irradiation period, these relations yield the following functional form relating paralysis time, t_p, to time rate of change of temperature, dT/dt:

$$1/t_p = m(dT/dt)^{\frac{1}{2}} - b, \qquad (2)$$

where m and b are constants.

(4) The fourth possible manner in which temperature might produce biological effects is in association with cavitation, defined here to include both the process of cavity formation and collapse in the tissue (initiated at a point of low tensile strength) and the growth and collapse of a gas nucleus in the tissue under alternate tension and compression forces. It has been shown that the biological effects of ultrasound, on the tissue of the central nervous system are the same whether the exposure is made under a hydrostatic pressure of one atmosphere or under a pressure sufficiently high to eliminate tension forces in the biological materials during acoustic propagation.[2,4] This eliminates possible temperature changes associated with cavitation as important in the mechanism, since in the absence of tension forces cavitation does not take place. It is also noted that the quantitative relation between paralysis time and acoustic pressure amplitude is of the same form at the different pressures.

(5) The possibility of high temperatures produced during an acoustic disturbance at or near the surfaces

[10] W. S. McCulloch (private communication).
[11] Dusser de Barenne, Science 77, 546 (1933).
[12] Dusser de Barenne and H. M. Zimmerman, Arch. Neurol. Psychiat. Chicago, 33, 123 (1935).

of small gas nuclei, in the absence of tension forces, must also be considered in discussing the mechanism of the biological effects described in this paper. A mechanism based on the existence of such temperature changes is unlikely for the following reasons.

(a) Intracellular gas nuclei of diameters equal to or greater than about one-half of a micron would be detectable microscopically. However, there is no evidence for the existence of such nuclei. We assume then that if gas nuclei are present they are less than one half of a micron in diameter. Since the maximum temperature change occurs, in the imbedding material, at the surface of the bubble, and since the temperature rise increases with an increase in bubble radius we require a numerical estimate for the temperature change at the surface of a gas nucleus with a diameter equal to one half of a micron. The following relation, derived by Rosenberg,[13] will be used to obtain such an estimate. It is assumed that the gas filling the nucleus satisfies the conditions of a perfect gas and that the diameter of the nucleus is restricted to values small compared to the wavelength of the acoustic disturbance. For a steady state condition, which need only be considered here because the times required to produce the biological effects described in this paper are very long compared to a period of the acoustic disturbance, Rosenberg obtains the following result for the difference between the temperature, T_s, at the surface of the bubble and the temperature, T_0, which the imbedding medium approaches as one moves away from the bubble surface.

$$(T_s-T_0)=\left\{\frac{[(1+j)R\delta_2+1]\sinh(1+j)R\delta_1}{(1+j)[(K_1/K_2)R\delta_1\cosh(1+j)R\delta_1+R\delta_2\sinh(1+j)R\delta_1]+(1-K_1/K_2)\sinh(1+j)R\delta_1}-1\right\}\frac{b_1}{a_1}P_0e^{j\omega t}, \quad (3)$$

where

$$\delta_i=(\omega a_i/2)^{\frac{1}{2}}, \quad a_i=\rho C_{pi}/K_i, \quad b_1=-1/K_1.$$

Subscript 1 refers to the material within the bubble and subscript 2 refers to the surrounding material. The acoustic disturbance in the region of the bubble is represented by $P_0e^{j\omega t}$, where P_0 is the pressure amplitude. The symbols in the above formula are defined as follows: R, radius of bubble; K_i, coefficient of thermal conductivity; ρ_i, density; C_{pi}, heat capacity per unit mass.

If the inequality $R\delta_1\leq 1/3$ is satisfied relation (3) can be written in the approximate form

$$T_s-T_0=-\left\{\frac{(1/3)(1+j)^2(K_1/K_2)(\delta_1R)^2}{1+(1+j)\delta_2R}\right\}\frac{b_1}{a_1}P_0e^{j\omega t}. \quad (4)$$

The absolute value of T_s-T_0 for an air bubble of 1/2 micron in diameter in water and subjected to an acoustic disturbance at a frequency of one megacycle and at a pressure amplitude of 15 atmospheres is about 0.3°C. This computation thus indicates that the effect under discussion cannot be important in the mechanism of the effects of ultrasound on nerve tissue described herein.

(b) No evidence of the growth of gas nuclei is seen under the microscope in nerve tissue irradiated at a hydrostatic pressure of one atmosphere and fixed within two minutes after exposure. Vacuole formation (and probably tearing of fine fibers) would be expected if such gas nuclei exist and could grow under tension forces of the order of 15 atmospheres.

(c) It would be necessary to assume in addition to the existence of gas nuclei, either that the population of such gas nuclei per unit volume is greater in large nerve cell bodies than in small and is especially low in axons, or that cells of different sizes and axons contain constituents which vary tremendously in their susceptibility to damage by temperature change. Such a possibility as the latter, concerning the temperature sensitivity of cellular constituents, seems hard to reconcile with the experimental evidence referred to above on the sensitivity to heat of the cells of the cerebral cortex.

B. Cavitation

The possible role of cavitation which one might investigate, after the temperature factor in a study of the physical mechanism by which ultrasound produces the changes in the central nervous system under review here has already received some consideration in the above analysis of the temperature factor. Three further comments might be added, the first is a direct consequence of the experimental results obtained under a hydrostatic pressure sufficiently high to insure that no tension forces exist in the material during acoustic propagation. It is the observation that cavitation is absent in the experiments performed under the increased pressure and therefore could not enter into the mechanism in any way. The second is the experimental observation that no tearing, vacuole formation, or gross disruption which would be expected to accompany cavitation was seen in tissue irradiated at one atmosphere pressure and fixed immediately after exposure. The third follows from studies of the pathology of decompression sickness. These have shown that in the central nervous system necrosis is more common in the white matter than in the gray matter.[14] This is in contrast to the action of ultrasound. The cell bodies are much more susceptible to the action of the sound than the fibers.

IV. CONCLUDING NOTE

It is felt that we are still in the early stages of realizing the potentialities of high level ultrasound as a tool for quantitative research on the structure and function of living organisms. It is already established as a powerful method in investigations of the organization and activity of central nervous systems. Since the primary action is apparently not one of gross disruption of, for example, cellular or nuclear membranes, it may also develop into a method of studying intracellular organization. The relatively rapid time course of resulting physiological changes make it especially attractive in this regard. It is anticipated that a study of the effects as a function of frequency will provide essential information for the construction of a theory of the fundamental mechanism.

ACKNOWLEDGMENT

The following investigators contributed to the research reviewed in this paper: D. Tucker, F. J. Fry, P. D. Wall, and V. J. Wulff.

[13] M. D. Rosenberg, Tech. Memo. No. 25, Acoustics Research Laboratory, Harvard University.

[14] H. R. Catchpole and I. Gersh, Physiol. Rev. 27, 360 (1947).