

in a shorter incubation period.

Discussion and summary. A relatively simple technic for the intraspinal inoculation of mice which lends itself to routine laboratory use has been described. For Lansing polio-myelitis virus, it seems to be a more sensitive method of detecting virus than the intracerebral technic. The quantitation of virus titers by this method is consistent between two different technicians and on re-

peated tests by one technician from any one set of virus dilutions. It can be applied to the routine testing of serums for Lansing antibodies. The chief advantage of the technic is the marked shortening of the incubation period of Lansing infection in mice as compared to that following intracerebral inoculation.

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Effects of Ultrasonic Vibrations on Nerve Tissues.* (18490)

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Numerous studies have already been published concerning the effects of ultrasound on living systems [Gregg(1), Harvey(2,3)]. Among these there are several kinds of experiments on the effects of ultrasound on nerve tissues, both excised(2) and *in situ* (4,5). Further, Lynn and Putnam(6) determined the effect of ultrasound on the brains of mammals, and demonstrated functional disturbances, neurone damage and even death of organisms.

The propagation of ultrasonic vibrations through living tissues is accompanied by a variety of physical factors such as: (1) heating caused by absorption of acoustic energy; (2) periodic pressure changes; (3) radiation pressure; (4) streaming or flow in viscous media; and, (5) high temperatures and pres-

ures associated with cavitation, defined as the formation of holes in liquid media. Any one or all of these factors may produce significant and measurable changes in the state of a living system. The experiments described above do not afford the opportunity to determine how ultrasound produces its effect. The experiments to be described were designed to determine whether tissue heating is a major factor contributing to the effect of ultrasound on nerve tissues.

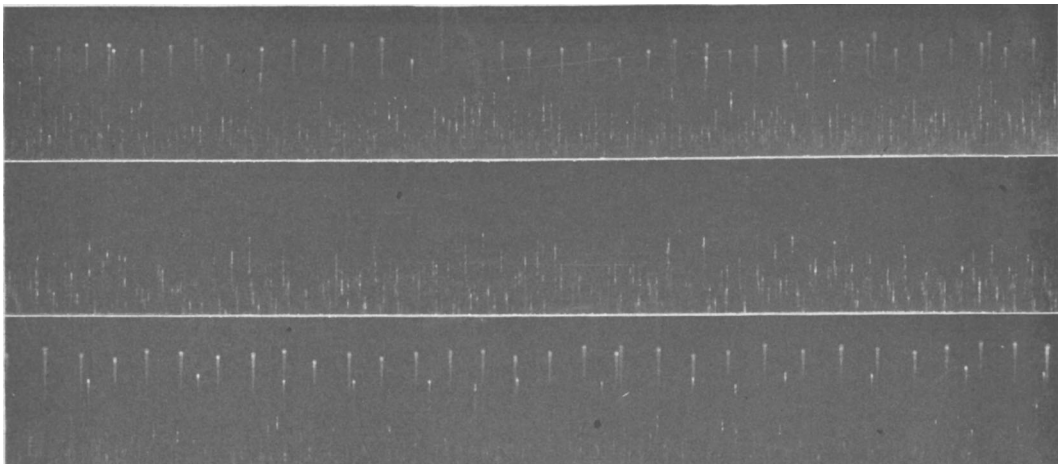
Materials and methods. The following types of preparations were employed:

(1) 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. The excised nerve cord was mounted in an electrode chamber in contact with 2 glass tubes filled with a salt solution. These salt bridges made contact with small calomel half cells which were, in turn, connected to the amplifier. The spike potentials were amplified by a condenser coupled amplifier and recorded by photographing the trace of a cathode ray tube.

(2) Intact frogs were placed vertically 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 so

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5. Parow-Souchon, E., *Z. f. ärztliche Fortbildung*, 1942, v39, 362.
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← 1.0 SEC.
FIG. 1.

Spontaneously occurring spike potentials recorded from the commissure between the first and second abdominal ganglia of an excised crayfish ventral nerve cord. Table I indicates the frequency of discharge (average values) as a function of the elapsed time during and after exposure to ultrasound. Records should be read from right to left.

as to fit directly over 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. The approximate center of the lumbar enlargement was estimated by adjusting the frog so that a line 12-14 mm behind the posterior edge of the tympanum bisected the hole in the board. The sound tank was filled with distilled water which had been boiled for 10 min. to drive off most of the gas.

(3) Temperature changes in the spinal cords of intact animals, in excised spinal columns containing the cord, and in the ganglia of excised crayfish ventral abdominal nerve cord were measured with constantan-copper thermocouples. For the crayfish ventral nerve cord, 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 ventral nerve cord of the crayfish was 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 approximately 12 mm behind the posterior edge of the tympanum. 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 by a condenser coupled amplifier and recorded by photographing the trace of a cathode ray beam. The chopping frequency was 10 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. The ultrasound used was at a frequency of about 1 mc and the maximum intensity was 35 watts/cm². The vibrations were propagated through water from the generating crystal surface to the preparation. For further details concerning the sound generator, see Fry, *et al.* (7).

Results. 1. *Effect of ultrasound on the ventral nerve cord.* Spontaneous activity recorded from the commissure between 2 adjacent ganglia (1 and 2) of the crayfish ventral abdominal nerve cord, immersed in a balanced

7. Fry, W. J., Wulff, V. J., Tucker, D., and Fry, F. J., *J. Acous. Soc. Am.*, 1950, v22, 867.

TABLE I.

Time sequence	Frequency
Control	7.8
Sound on 15"	12
Sound on 17"	7
Sound on 43"	0
Sound off 50"	5.3
Sound off 65"	6.7

salt solution, is illustrated in Record 1, Fig. 1. 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 2 ganglia and the commissure to ultrasound (~ 35 watts/cm²) produces a characteristic sequence of changes in the activity, a typical example of which is illustrated in records 1, 2, and 3 of Fig. 1 and tabulated in Table I. Note that after the sound is turned on, the frequency of the spike potentials at first increases, then decreases and is followed by total disappearance of the large spike potentials after 43 sec. exposure (Record 2). Twenty-five seconds after the ultrasound was turned off, the large spike potentials reappeared, first slowly, then more rapidly, finally reaching a stable frequency of 6.7 per sec (Record 3, Fig. 1). Subsequent treatment of the same preparation with ultrasound produced a similar sequence of events. Similar observations were made on 6 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.

2. *The effect of ultrasound on the frog spinal cord.* A. Physiological and morphological effects. Twelve intact normal frogs suspended under water at room temperature, 21°-25°C, and placed so that 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°-2°C. Ultrasound incident on the back over the lumbar enlarge-

ment 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. Stimulation of the sciatic nerves of a frog just after the production of paralysis with ultrasound resulted in muscular response. Similar stimulation of sciatic nerves one week after the paralyzing sound treatment produced no muscular response. Examination of histological preparations of sciatic nerves fixed and stained with osmic acid vapor 2 weeks after irradiation, revealed considerable degeneration of axones. A typical example is illustrated in Fig. 2, No. 2 (compare with control nerve, Fig. 2, No. 1). In all preparations examined, considerable degeneration of axones was evident, as well as degeneration of the region of the spinal cord exposed to the ultrasound.

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 (C₁₂H₉N₃S), (Fig. 2, No. 4). Note the ragged cell outlines and the very intense stain (compare with control, Fig. 2, No. 3). Normal motor neurons do not stain intensely and have smaller nuclei, usually centrally located. Examination of preparations made four and eight days after ultrasound treatment show neurons which stain intensely and exhibit peripherally located nuclei (Fig. 2, No. 5 and No. 6). These changes are evident in sections both above and below the degenerated regions. A reduction in population of neurons is evident. The eight-day-old lesion, Fig. 2, No. 6, exhibits a marked abnormal appearance. Lower motor neurons from the spinal cords of frogs exhibiting temporary paralysis of the hind legs indicate some abnormality (Fig. 2, No. 7) and those from a frog treated at low temperature for 5 sec. and exhibiting no paralysis of the hind legs (Fig. 2, No. 8) do not show any obvious abnormality.

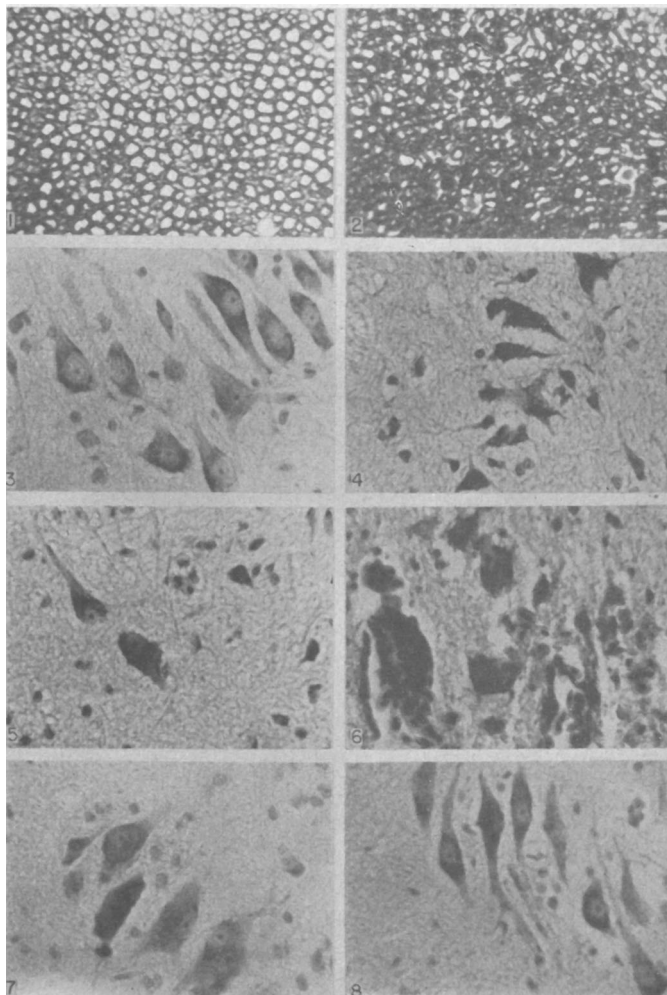


FIG. 2.
Photomicrographs of sections through sciatic nerves (1 and 2)
and spinal cords of frogs (3-8) showing neurones in the ventral
horns of the gray matter. $\times 175$.

B. Temperature changes produced by ultrasound. Changes in temperature of the spinal cord of intact frogs were measured during and following the period of ultrasound treatment. Ultrasound incident on frogs at $1^{\circ}\text{--}2^{\circ}\text{C}$ produced a sharp rise in temperature (Fig. 3, Graph 1), which reached a level between $25^{\circ}\text{--}30^{\circ}\text{C}$ at the end of the 7.3 sec. exposure. After the exposure, the spinal cord exhibits a decrease in temperature. Experiments on isolated spinal column preparations gave similar results. To determine the influence of temperature on the production of paralysis of the hind legs of frogs, 12 experi-

ments were performed using brief repetitive exposure to ultrasound. Frogs, cooled to $1^{\circ}\text{--}2^{\circ}\text{C}$, 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. 3, Graph 2. This exposure was followed by a 4 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. 3, Graph 2). Frogs subjected to a similar procedure (without insertion of the thermocouple) exhibited

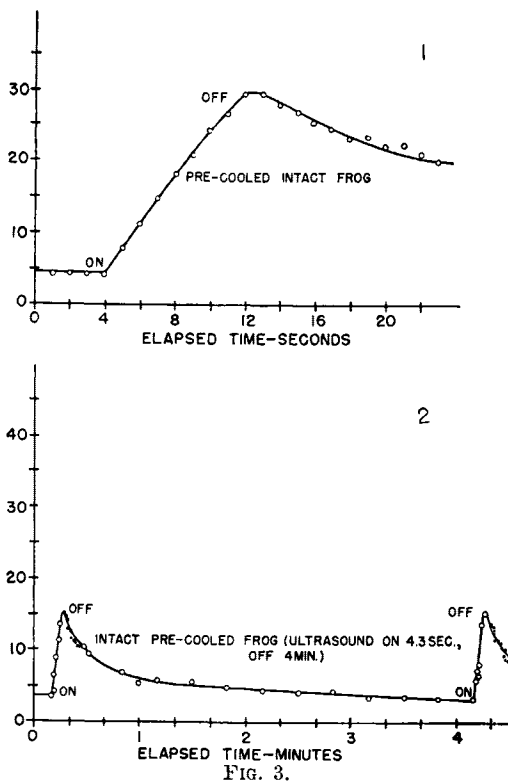


Fig. 3. Temperature changes as a function of time in the frog spinal cord during and after sound treatment.

permanent paralysis of the hind legs after the second exposure. 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 20 minutes and then raised to 38°C for 20 minutes showed no obvious abnormality in behavior. Frogs immersed in 40°C water for various periods up to 6 minutes showed a paralysis which gradually disappeared. Temperature measurements in the lumbar region of the spinal cord of frogs in 40°C water indicated a level of 40°C after 6 minutes.

Discussion and conclusions. Whenever ultrasound is transmitted through living tissues the existence of damaging temperature levels, produced by absorption of acoustic energy, must be ascertained. Ultrasound incident on ganglia of the crayfish ventral nerve cord containing spontaneously active neurones caused a reversible depression of the spontaneous activity. A maximal and rapid tem-

perature increase of 1°C was measured. Prosser(8) has shown that increasing the temperature 1°C between 26°-30°C may produce an increase in the frequency of discharge of single units of about 4-5 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.

Examination of the data pertaining to neurone damage in the frog indicates that paralysis can occur in the absence of high (35°-40°C) temperature levels. For instance, frogs pre-cooled to 1°-2°C and subjected to a 7.3 sec. dose of ultrasound never exhibit temperature levels in excess of 30°C (Fig. 3). Further, exposure of pre-cooled frogs to successive sub-paralytic doses (4.3 sec. exposure, followed by a four minute interval) show temperature maxima of 15°C (Fig. 3), and yet, paralysis occurred after two exposures. The initial rates of change of temperature measured in the intact frog spinal cord, are rather constant, 1.8°C/sec. It is possible that the rapid increase in temperature occurring in the spinal cord upon incidence of ultrasound may produce physiological changes even though the final level of temperature remains below 30°C. Since rates of temperature change of the order of 1.8°C/sec. could not be achieved except by ultrasound, it was not possible to assess this factor. Unless a rate of change of temperature of the order of 1.8°C/sec. exerts a specific effect leading to paralysis it is apparent that the temperature changes measured cannot account for the experimental results in all cases. It is concluded that ultrasound produces effects on the neurones of the spinal cord which are primarily dependent upon physical factors other than temperature, such as periodic pressure changes, particle acceleration, radiation pressure and temperature and pressure changes associated with cavitation.[†] The evaluation of some of these factors must be

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left to future research.

Summary. The excised crayfish ventral nerve cord exposed to ultrasound (~ 35 watts/cm², frequency 1 mc) 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 ex-

[†] Cavitation is a name applied to the phenomenon of formation of holes in liquid media.

hibited paralysis after 7.3 sec. exposure (at $1^{\circ}\text{--}2^{\circ}\text{C}$). Histological examination of the sciatic nerves showed extensive degeneration of nerves and examination of the spinal cord indicated destruction of the lower motor neurones. It was concluded that the effect of ultrasound on the systems studied is produced by physical factors other than temperature.

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Effect of Citrovorum Factor in Pernicious Anemia.* (18491)

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Although Addisonian pernicious anemia is therapeutically well controlled by the parental administration of vit. B₁₂, the exact mechanism of response, and particularly the relationship of vit. B₁₂ to pteroylglutamic acid (PGA), is still not well defined. Because of bacterial interrelationships of the citrovorum factor (CF) to vit. B₁₂, PGA, and thymidine, and because of the hematopoietic activity of vit. B₁₂, PGA, and thymine in pernicious anemia, it was decided to study the clinical and hematologic effect of CF in patients with pernicious anemia. CF(1) is a substance first obtained from liver extracts active against pernicious anemia, the unit of which has been defined as that amount of substance required for half maximal growth of *Leuconostoc citrovorum* 8081 in 72 hours on a basal medium. *L. citrovorum* shows some

growth response to thymidine(2-5) or to large amounts of PGA, but none to vit. B₁₂(4,5). CF can replace PGA as a growth factor in all organisms that have been tested. Acid hydrolysis destroys the citrovorum activity, leaving the PGA activity unchanged(6,7). CF can be obtained from liver and yeast extracts(1,5) and has been isolated from the urine of rats and humans given PGA(8,9). Its biological synthesis by rat liver slices is

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