Technical Papers

Changes Produced in the Central Nervous System by Ultrasound[1]

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Ultrasound will produce specific changes in the central nervous system and in specific nerve cells without obvious effects on surrounding tissue. The effects reported here have been studied primarily in the frog spinal cord, but the same phenomena have been seen in the rat spinal cord and in the cerebral cortex of the cat. The method of irradiation used, various physiological results obtained, and a detailed consideration of the physical factors involved have been discussed in previous papers (*1–3*). The effects described here were obtained with sound at a frequency of 1 Mc and at various acoustic pressure amplitudes. The media used to transmit the sound from the source to the tissue were physiological solutions. The volume and location of nerve tissue to be affected can be accurately controlled by focusing of the sound beam, and the nature and extent of the destruction depends on the pressure amplitude and duration of the exposure. Summation of the biological effects of short acoustic pulses occurs, so that it is possible to deliver a series of such pulses and produce biological effects without a serious coincident rise of temperature in the volume which is absorbing the sound energy. In frogs the ultrasound was incident on the spinal cord after passing through skin, muscle, and bone, but in mammals the spinal cord and cerebral cortex were exposed before irradiation because of the high absorption of ultrasound by bone.

When the lumbar region of the frog spinal cord has been subjected to large doses of ultrasound,[3] it is found immediately after exposure that the hind legs are completely anesthetic in addition to being paralyzed. Histological examination of these cords 2–7 days after treatment shows complete destruction of all nerve cells, of many glia cells, and a serious loss of supporting elements in the irradiated region, so that the cord loses its normal texture and becomes very soft. Many fibers remain apparently intact, and the blood vessels are dilated but not broken.

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[3] Large dose is ≥ 1.5 times the minimal dose required to produce permanent paralysis. See quantitative relations between minimum paralysis time and acoustic pressure amplitude given in (*3*).

In contrast to the effects of heavy dosage, an irradiation time close to the minimum required for paralysis results in complete permanent paralysis of the hind legs immediately following exposure, but sensation in the hind limbs remains. In these animals, the unparalyzed front legs react vigorously to stimulation of the skin of the hind legs even after decerebration. Histological studies of these cords show that the large cells in the anterior horn of the spinal cord have been severely damaged, leaving the small cells there and elsewhere in the cord intact. Two to 7 days after irradiation some of the large cells have fragmented, and the others show changes in the Nissl bodies, and pyknosis. Displacement and swelling of the nucleus occur; the nuclear membrane, however, remains intact in those cells that do not break up. The glia, blood vessels, and the fine fibers in the spinal cord do not appear affected histologically by this dosage of acoustic radiation.

Still smaller doses produce no obvious signs of paralysis or sensory change, although histological studies show that a large percentage of the anterior horn cells within a limited extent of the cord have been damaged; all other elements remain apparently normal.

These effects are not caused by the coincident temperature rise which accompanies ultrasonic propagation through the tissue for the following reasons:

1. Thermocoagulation studies on the cerebral cortex by Dusser de Barenne and colleagues have shown that when the large cells are destroyed by heating, the small cells in the tissue are also destroyed. However, from these studies with ultrasound, it is evident that the large cells are particularly sensitive to the acoustic radiation.

2. Experiments on cooled frogs show that the ultrasound produces its effects on the nerve cells when the temperature level in the cord is not greater than 20° C (*1*).

3. Summation of the biological effects 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 is not dependent upon achieving any particular temperature level in the spinal cord (*1*).

4. The experimentally determined relation between minimum paralysis time and acoustic pressure amplitude shows that (*a*) the absorption of a constant amount of acoustic energy by the nerve tissue is not required, and (*b*) the time rate of change of temperature is not a factor in producing paralysis (*3*).

5. The postulated existence of localized regions appreciably higher in temperature than the average temperature of the cell is without theoretical or experimental support (*3*).

Vacuolization does not enter into the mechanism, since histologically there was no evidence of tearing or vacuole formation in specimens fixed immediately after irradiation. In addition, the effects of ultrasound on frog spinal cords were similar whether the frogs were irradiated under 1 atmosphere pressure or under

a hydrostatic pressure sufficiently high to eliminate tension forces in the tissue which might initiate cavity formation (*3*).

It is now clear that a focused beam of ultrasound may be used to produce discrete lesions in the central nervous system without destruction of blood vessels or surrounding tissues. By careful control of the dosage, it is possible to destroy large cells only and leave small cells, fibers, blood vessels, and glia intact. Work is now in progress on the production of such selective lesions in order to determine the anatomical and physiological consequences.

References

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A Microbiological Assay Method for Microgram Quantities of Manganese in Biological Material

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Bentley, Snell, and Phillips (*1*) and MacLeod and Snell (*2*) have described microbiological assay methods for manganese involving acidimetric and turbidimetric measurement of growth response. The observation that filter paper disks, saturated with solutions of manganous ions, exhibit zones of apparent growth stimulation on agar plates seeded with various bacilli (*3*) suggested to us the possibility of a simple diffusion plate assay for this element. This growth stimulation is especially suitable as the basis for an assay, inasmuch as it appears to be a unique property of manganese (*3*). Moreover, the diameter of the stimulated zone was found to bear a linear relationship to the logarithm of the manganese concentration (Fig. 1).

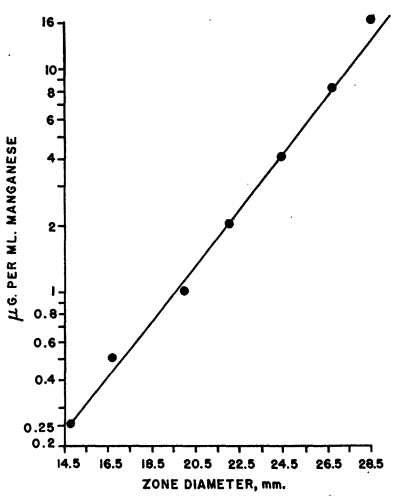

FIG. 1. Relationship between concentration of manganese and diameter of zone of stimulation. Diameter of paper disks was 13.5 mm.

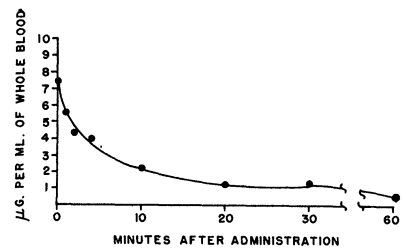

FIG. 2. Blood levels of manganese determined by diffusion plate assay following intravenous administration of 20 mg Mn as $MnCl_2$ to a 2-kg rabbit.

Using the Vincent and Vincent (*4*) assay method for penicillin as a model, the procedure finally developed was a filter paper disk assay employing a modified high-low technique, the high and low levels of manganese being 8 μg/ml and 2 μg/ml, respectively, in a 0.2% solution of Rochelle salt. The use of the latter as a complexing agent for manganese was adopted to avoid the possibility of binding or precipitation of the element during the assay. The standard solutions were conveniently obtained daily from a stock solution containing 80 μg Mn^{++}/ml, stored in a polyethylene container.

Three Petri dishes of nutrient soy agar, seeded with a suitable suspension of *B. subtilis* ATCC 6633, are used for each sample. On each of the plates is placed a disk saturated with the high standard, low standard, and duplicate test solutions, respectively, all solutions containing 0.2% Rochelle salt. Incubation of the plates and reading of the zone diameters are carried out as for any diffusion plate assay.

The mean of the three high and low standards are plotted on semilog paper with the zone diameter in millimeters as the abscissa, and the concentration of manganese in μg/ml as the ordinate; a straight line then is drawn through the two points. The mean value of the 6 test replicates is then referred to the curve to obtain the concentration of manganese in the test sample. This value, multiplied by the dilution factor used in preparation of the sample, yields the final result.

In order to define the applicability and limitations of the method, the determination of the blood levels of manganese following intravenous administration of manganese was attempted. As a preliminary test, it was found that whole rabbit blood contained no manganese detectable by this method, and, further, that added manganese could be completely recovered. Twenty mg of manganese as $MnCl_2$ was injected into the ear vein of a 2-kg rabbit. One-ml samples of blood were removed from the opposite ear vein at intervals and added to 9 ml of 0.2% Rochelle salt. After lysis of the red cells had ensued (approximately 1 hr at room temperature), the samples were assayed directly. The results obtained are shown graphically in Fig. 2.

The method described suffers from one severe limi-