

INTENSE ULTRASOUND—A NEW TOOL FOR NEUROLOGICAL RESEARCH.*

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A FOCUSED beam of ultrasound can be used to produce selective accurately localized lesions in central nervous systems which are quantitatively reproducible from one animal to another.

Discrete lesions can be produced without destruction of blood vessels. The extent of the destruction can be limited to nerve cell bodies only, nerve fibres traversing the irradiated region are left intact and functioning. By careful control of the dosage it is possible to effect a differential destruction of large nerve cells leaving the population of the small nerve cells in the same region essentially unchanged.

The lesion can be accurately localized by focusing a fine beam in the region to be affected. Beam widths of two or three millimetres are readily attained. We have used both a single beam focusing irradiator, which produces a lesion small in cross section but relatively long and a multiple beam instrument which is suitable for producing lesions small in all three dimensions.

The nature of the destruction depends on the intensity and duration of the exposure. Dosage studies made on a series of animals can be used as an accurate guide in choosing the conditions of irradiation for structural and functional studies.

The bibliography at the end of this paper can be consulted for detailed articles concerned with the subject matter of this paper (1-7).

We will illustrate the differential selectivity possible for affecting large cells only, by some results of a study currently in progress on the motor cortex of the monkey, *Macaca mulatta*. The conditions for irradiation and the results obtained in one experiment follow. The sound level and duration were chosen so that the large cells of the cortex would be destroyed but the smaller cells would be relatively unaffected. The sound was focused in a pattern of overlapping spots the majority being located in the hand and arm region of area 4 of the right cortex. The duration of the exposure was 4.0 seconds and the intensity was 70 watts/cm².

The animal was sacrificed one month after exposure. Histological examination showed that the majority of the Betz cells in the exposed area were destroyed while the smaller cells were relatively unaffected. Fig. 1 and 2 illustrate this differential destruction. Fig. 1 shows a section of the left motor

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cortex which was unirradiated. One notes the presence of the large Betz cells in this normal cortex. Fig. 2 shows the corresponding portion of the right motor cortex which was irradiated. The population of Betz cells in this irradiated cortex is greatly reduced while the population of the smaller cells is practically unaffected.



FIG. 1.—A section through the motor cortex (left) of *Macaca mulatta*.

Marchi studies of the spinal cord demonstrate degenerating fibres in the pyramidal tract but not in the ventro-medial tract. Fig. 3 shows Marchi preparations from three levels of the spinal cord. The upper section is taken from the cervical region, the middle from the thoracic, and the lower section from the lumbar region.

Studies currently in progress have as their objective the determination of possible functional and cortical electrical excitability changes in animals with such motor cortices. With this tool it thus appears possible to check by direct means the importance of the large pyramidal cells by removing them and leaving the remaining components of the tissue intact.

It should be noted here that the primary action of the sound is not the total

disruption of the nerve cell body. No change is observed histologically in nerve cells fixed immediately after irradiation and stained with toluidine blue. This is in contrast to functional or electrical changes which are apparent immediately after irradiation. For example, the paralysis of the hind legs of a frog following the irradiation of the unanaesthetized animal over the region of the lumbar enlargement is evident immediately after the irradiation. Lighter doses do not cause a delayed paralysis.

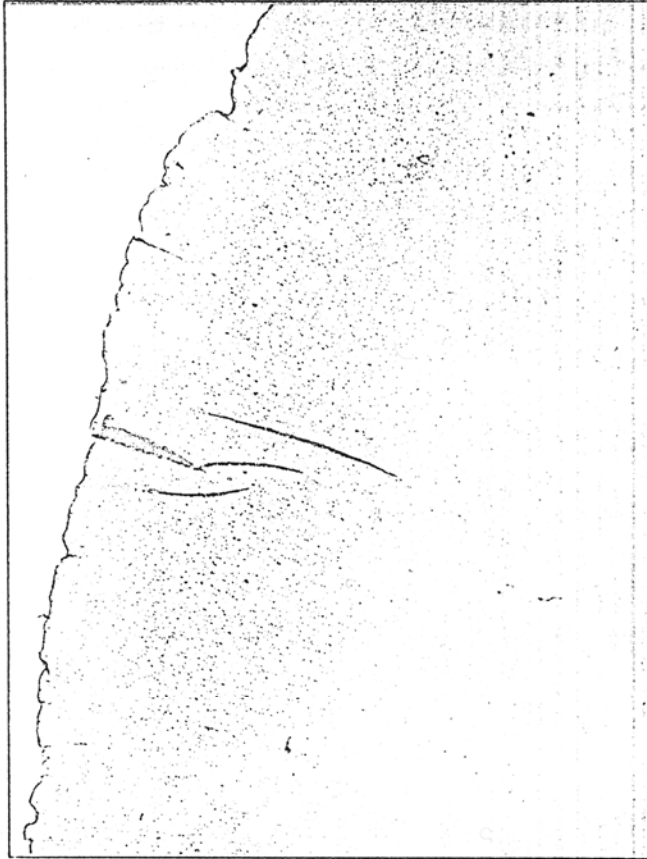


FIG. 2.—A section of ultrasonically irradiated right motor cortex from the animal of Fig. 1. This section corresponds to that of Fig. 1.

We can illustrate the possibilities of this technique for affecting deep structures by the results obtained in a study of the bulboreticular inhibitor formation of the cat. The sound passed through the cerebellum and the fourth ventricle before reaching the bulboreticular formation. A series of separate irradiations was carried out so that the focal point moved over the whole area in which the large cells of the bulboreticular formation lie. This region is so close to respiratory and other vital centres that almost all previous attempts to produce lesions in this area by electrolytic fulguration have resulted in the death of the animal due to swelling of the brain stem and interference with the blood supply.

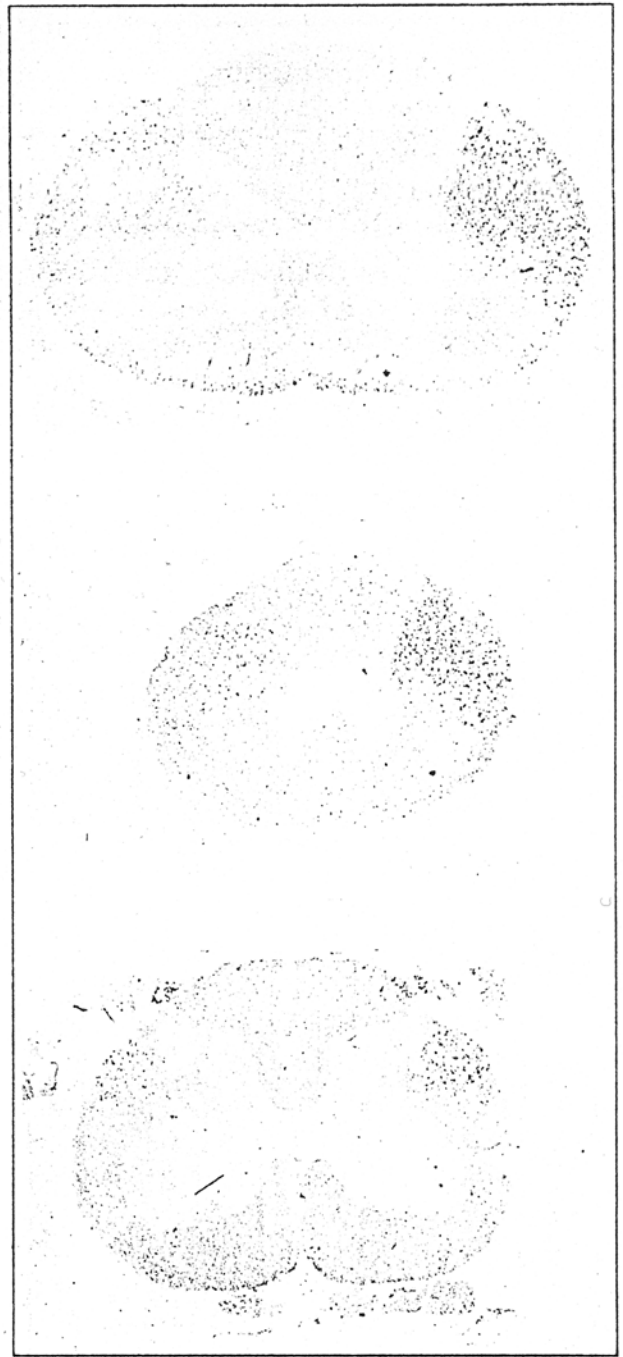


FIG. 3.—Marchi preparations from three levels in the spinal cord of the animal of Fig. 1 and 2.

None of the animals irradiated showed signs of respiratory disturbance or vascular collapse after the irradiation. The animals recovered rapidly and showed a striking and permanent abnormality of the motor system. The resting posture was with hyperextended fore limbs and slightly flexed hind limbs. On

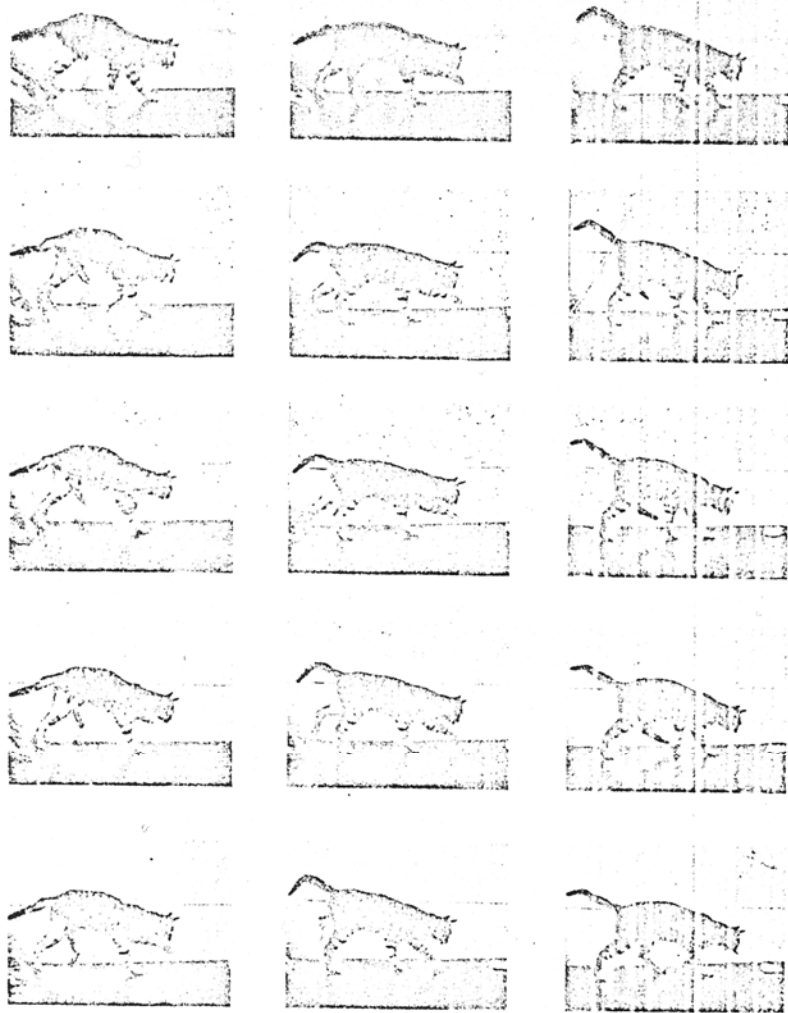


FIG. 4.—Typical walk of a cat with an ultrasonically produced lesion in the bulboreticular inhibitory formation.

walking the fore limbs showed a goose step and the hind limbs a wide base with circumduction. The fore limbs showed an exaggerated grasp reflex. In one animal followed over a period of two years no changes of this motor deficit have been seen.

Histological examination of the bulboreticular formation showed a depopulation of cells. The beam produced a lesion whose lateral borders were

sharply localized but, because of the long focal length, had the effect of irradiating cells for some distance above and below the region with sound above the threshold dosage. Consequently, the cells in the roof nuclei of the cerebellum were also damaged.

Further studies of the bulbo-reticular formation will be carried out with a multiple beam instrument in order to eliminate any damage to nearby cells in the cerebellum.

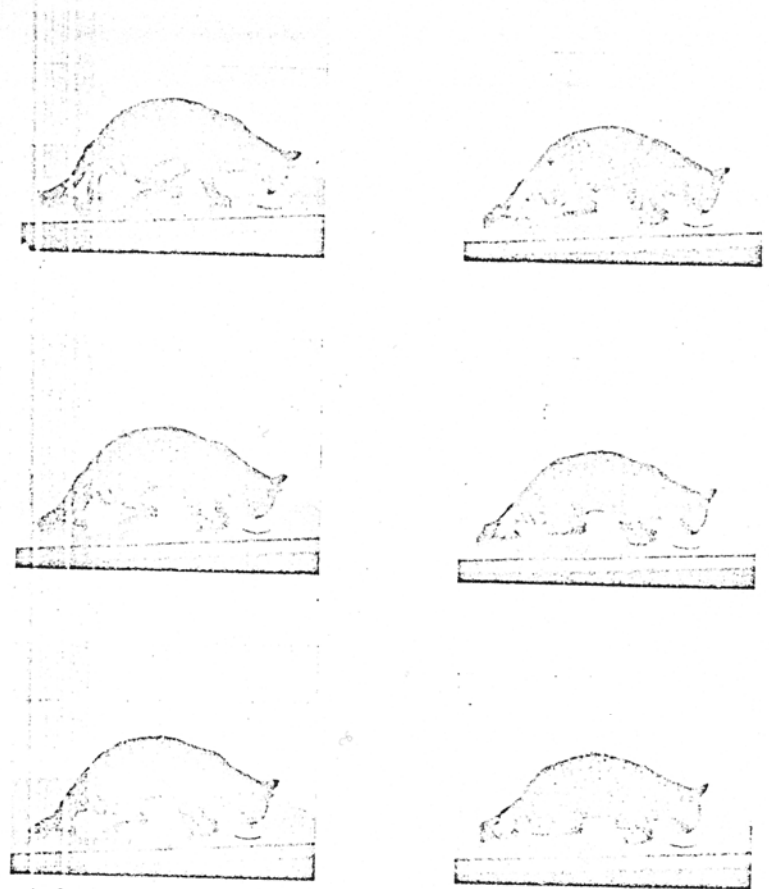


FIG. 5.—Awkward positioning of hind limbs in a cat with a lesion in the bulbo-reticular inhibitory formation produced by ultrasound.

Fig. 4 and 5 are illustrations of a cat with an ultrasonically produced lesion in the bulbo-reticular inhibitory formation. The pictures were taken approximately 5 months after irradiation. Fig. 4 shows the animal walking. The goose stepping is readily seen. In Fig. 5 the animal is shown drinking milk from a shallow dish. Awkward positioning of the hind limbs is illustrated in this figure.

Most of our work up to the present time has been accomplished at a sound frequency of 1.0 mc. using a single beam focusing irradiator. The sound is generated by a vibrating quartz crystal and focused by a lens. Transmission

The sound from the irradiator to the tissue is accomplished through physiological saline. This liquid must be degassed to prevent the formation of bubbles, which would interfere with the transmission of the acoustic energy. The bone overlying the region of the nerve tissue at which the sound is to enter the central nervous system must be removed in order to prevent overheating and to insure a well defined focused beam in the tissue.

The single beam type focusing irradiator produces a pencil-shaped lesion, that is, one which is small in cross-section but relatively long. This type of



FIG. 6.—Multibeam irradiator and partial view of experimental arrangement for producing lesions by ultrasound in the central nervous system.

lesion is not suitable for many neuroanatomical or neurophysiological studies. Consequently a multi-beam instrument has been developed and work has been started with this apparatus. The device consists essentially of four single beam focusing irradiators coupled together mechanically with a suitable number of adjustments to enable the beams to be brought into coincidence. Much smaller lesions can be produced with this multibeam arrangement than can be attained with the single beam apparatus.

Fig. 6 illustrates the multibeam instrument and a portion of the experimental arrangement for carrying out the irradiation. In practice the animal is supported in a standard Horsley-Clark apparatus partially visible in Fig. 7. The flanged cap, used to support the physiological saline, which acts as the transmitting medium for the sound, is tied to the skin of the animal. Fig. 8 shows a close-up view of the multibeam instrument positioned over the head of the animal. The cortex is exposed for irradiation. It is not necessary to open the dura unless a visual study of the cortex configuration is desired.

A study of the physical factors involved in the production of the physiological changes is important from the viewpoint of the fundamental mechanism of the action of the sound on the tissue. We will now indicate briefly the present status of our knowledge regarding these factors. The first factor which received consideration was temperature.

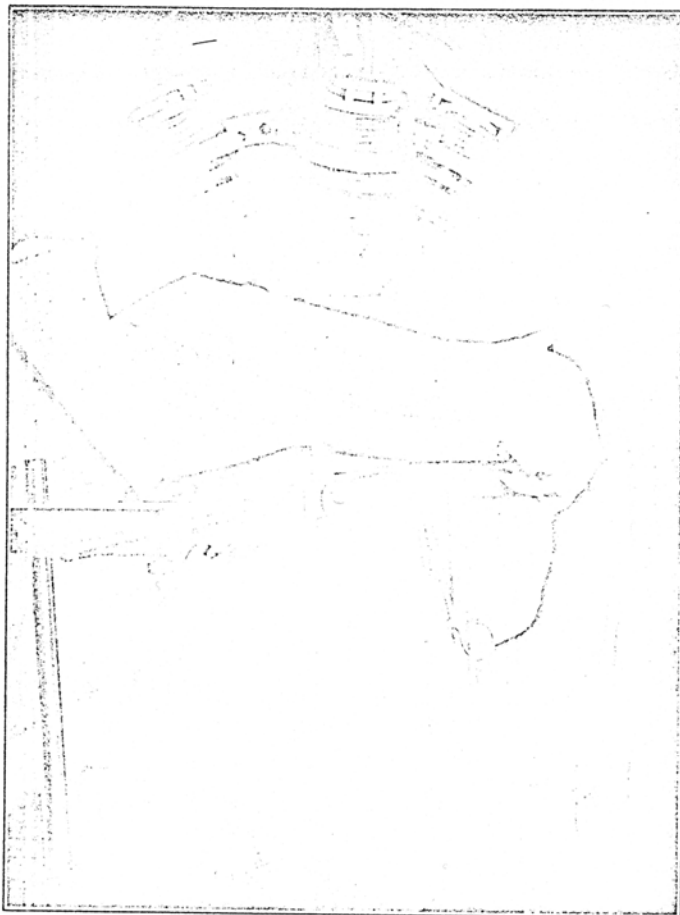


FIG. 7.—View of monkey supported in Horsley-Clark apparatus with multibeam irradiator in position.

Temperature changes which occur when ultrasound is propagated through tissue may play a rôle 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 are not detected by a thermocouple as small as say 0.003 in. in diameter; (3) temperature changes associated with cavitation are responsible; (4) heating at gas nuclei in the tissue is important.

Consider the various mechanisms in the order just mentioned.

(1) A number of independent arguments are presented against the possi-

bility that the temperature of the tissue exceeds a critical value above which damage occurs.

(a) Experiments 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 .

(b) Summation 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 is not dependent on achieving any particular temperature level.

(c) From experimental results obtained on frogs one can conclude on the basis of the experimentally determined linear relation between reciprocal of

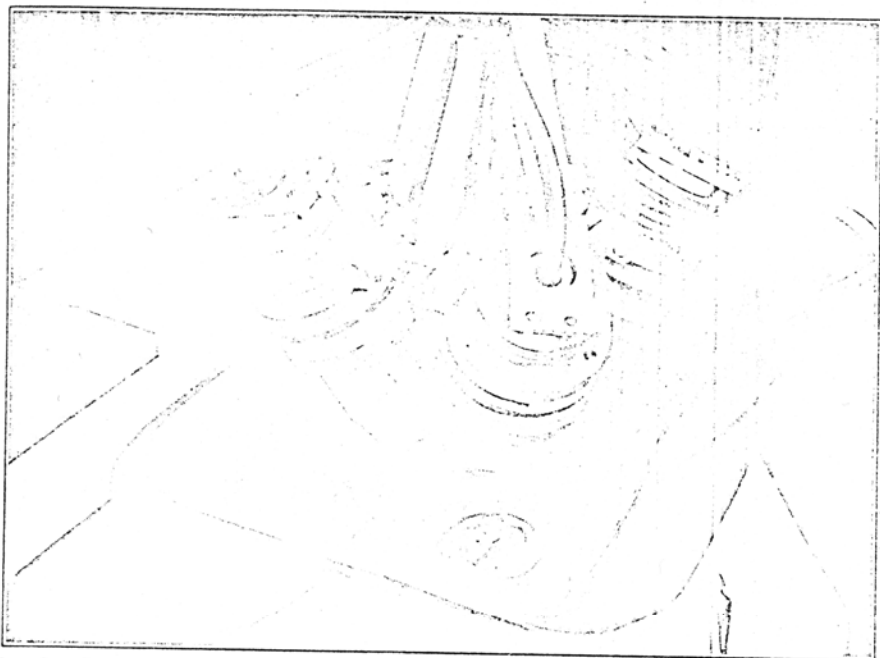


FIG. 8.—Close-up view of multibeam irradiator in position over the exposed cortex of the animal.

minimum paralysis time and acoustic pressure amplitude that the absorption of a constant amount of acoustic energy by the tissue is not required to produce the effect.

(d) Thermocoagulation studies on the cerebral cortex by McCulloch and others have shown that, when the large cells are destroyed by heating, the small cells in the tissue are also destroyed (8, 9). However, in our studies with ultrasound it is evident that the large cells are particularly sensitive to the acoustic radiation.

(2) Consider now the second possible manner in which temperature might be involved, that is through heating at interfaces. A relatively simple calculation proves that such an hypothesis is untenable. For example, we assume that all the sound which is absorbed, is absorbed at interfaces and consider a volume of material consisting of cells of diameter 10 microns. If we subject

such a material to a sound intensity as high as 100 watts/cm.², choosing as values for the physical parameters of the material those characteristic of nerve tissue we obtain for the maximum temperature rise at the interfaces a value of about 0.001° C.

(3) The third 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 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. 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.

(4) The possibility of high temperatures produced during an acoustic disturbance at or near the surfaces of small gas nuclei, in the absence of tension forces, must also be considered in discussing the mechanism of biological effects. A mechanism based on the existence of such temperature changes is unlikely for the following reasons.

(a) 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. 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 compute the temperature change at the surface of a gas nucleus with a diameter equal to one half of a micron on the basis of a formula derived by Rosenberg(10). The difference between the temperature at the surface of such a bubble and the average temperature of the medium for a sound intensity of 100 watts/cm.² and a frequency of 1.0 mc. is about 0.3° C. This computation indicates that the effect under discussion cannot be important in the mechanism of the effects of ultrasound on nerve tissue described here.

(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 fibres) 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.

The second physical factor to receive consideration as a possible participant

in the mechanism of the action of the sound on the tissue was cavitation, defined in the discussion of the temperature factor to include both the process of cavity formation and collapse and the growth and collapse of a gas nucleus in the tissue.

Since it is possible to produce by ultrasound the effects described in the central nervous system when the animal is under a hydrostatic pressure sufficiently high to prevent cavitation, then cavitation cannot enter into the mechanism. An independent argument follows from studies by Gersh and collaborators of the pathology of decompression sickness (11). These have shown that in the central nervous system necrosis is more common in the white matter than in the grey matter. This is in contrast to the action of ultrasound. The cell bodies are much more susceptible to the action of the sound than the fibres.

The physical factor now receiving consideration with respect to a possible fundamental rôle in the mechanism is viscous force action between the sub-microscopical structural components of the protoplasm and the surrounding fluid. It is expected that studies as a function of frequency and in standing wave fields will elucidate this mechanism.

In conclusion we note that by accurately controlled irradiation of the tissue of the central nervous system the experimental neurologist can selectively affect the neuron population to a desired extent leaving intact the blood supply and fibre tracts in the same region, thus completely eliminating the difficulties inherent in the older methods of producing lesions. Differential destruction of the large neurons can be accomplished making possible studies of their relative importance and function. Immediate physiological change is realized long with this selectivity. Lesions of any desired shape and depth in the central nervous system can be produced without affecting the intervening tissue.

Clearly the method has considerable potential value for neurosurgery. In addition to the characteristics just mentioned, it is noted in this connection that there are no long delayed deleterious effects. Animals have been observed up to a period of approximately two years after irradiation with no complications appearing. It is also noted that with this method of producing lesions a rapid recovery from the operation ensues. Usually within 24 hours after irradiation the animals are active.

SUMMARY.

A focused beam of high frequency sound can be used to produce both reversible and irreversible changes in central nervous systems. The focal region of the beam can be accurately positioned at any desired location in the tissue. Intervening tissue can be left unaffected, since the conditions of irradiation can be arranged so that the sound level is above the threshold required for producing the desired change only in the focal region. The neurons are affected most readily, the vascular system and supporting structure being left intact at dosages which affect drastically the neural components. Fibre tracts are functionally unaffected at dosages which cause major changes in the operation of the nervous system, indicating that the primary action of the sound takes place at the nerve cell body or synapse or both. It is possible grossly to affect

the large nerve cells in a region and yet leave the small cell population practically unchanged.

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