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Effects of High Frequency Sound Waves on Protoplasm.

F. O. SCHMITT,* A. R. OLSON AND C. H. JOHNSON.
(Introduced by T. D. Beckwith.)

From the Department of Chemistry of the University of California.

The observations described in the following preliminary report were made by subjecting animal cells to the action of sound waves produced by a rapidly vibrating quartz crystal. The oscillating current was generated by a 250 watt tube connected in a modified Hartley circuit. The crystal lay between a heavy lower electrode and a light upper electrode of copper gauze in a crystallizing dish containing the dielectric liquid, usually xylene. The crystal vibrated at the rate of approximately 750,000 times per second.

Effects in Vitro. In this type of experiment the animals were placed in a test tube or similar vessel containing water and dipped into the dielectric over the vibrating crystal. Free-swimming *Triturus* larvae are rapidly killed, the gill filaments being torn away and areas of denuded surfaces exposed. Confirming the findings of Wood and Loomis,[1] the larger protozoa are killed by this method, while the smaller protozoa, being able to swim between the nodes of the stationary wave system set up in the water, escape unharmed. *Spirostema,* being relatively large, are quickly killed, *Paramecia* less rapidly, while the smaller protozoa are unaffected after many minutes. By means of a simple arrangement it was possible to observe the cells through the microscope while subjecting them to the sound waves. A drop containing the protozoa was placed on a microscopic slide and a cover glass laid over it. The slide was then laid across the oscillator dish. The sound waves were transmitted in this manner to the cells through the medium of the oscillator dish and the slide. Neglecting morphological details for the present, cell destruction is due to an actual disruption of the whole or of a part of the cell, to a gelation or coagulation of the protoplasm, and to a combination of the two effects. Short exposures of protozoa result in a sort of preliminary paralysis which rapidly goes over to death if the exposure is continued. In an effort to assign some cause for the death of protozoa in the absence of visible disruption of the cell, filtered solutions of crystallized egg albumin were subjected to the vibration. In a very few minutes the solution became turbid and the albumin precipitated out in fine shreds. That great pressures

* Fellow in Medicine of the National Research Council.

[1] Wood, R. W., and Loomis, A., *Phil. Mag.*, 1927, iv, 417.

may precipitate proteins in the absence of any great heating was noted by Bridgman.[2] We venture to suggest, on the basis of our experiments, that a similar denaturation of the proteins in protoplasm may be an important factor in the lethal effect of sound waves. This does not exclude a thermodynamic pressure-temperature coefficient.

Supersonic Micromanipulation. The searing effects of sound vibrations conducted along capillary tubes have been demonstrated by Wood and Loomis. We have attempted to devise a method of micromanipulation in which the end of the capillary tubing coming from the vibrating collector is pulled to a fine micropoint and, held in a micromanipulator, is directed at single cells under the microscope. Owing to the limited power at our disposal, it became necessary to find conditions under which maximum energy could be transferred to the collector. It was discovered that when a flat strip of glass, drawn to a thread at one end, was used, maximum heating of the thread resulted when the strip made a certain critical angle with the plane of the crystal. There appears to be a definite relationship between this angle and the velocities of sound in the dielectric liquid and the collector. This relationship offers an extremely simple method of studying the velocity of sound in various media at these frequencies; this phase of the subject is now under investigation in this laboratory.

Planarian worms touched even though lightly by the tip of such a vibrating needle are instantly burned, the effect manifesting itself in the form of a cut, which is produced at the point of contact. The edges of the cut appear to be smooth and the pigment recedes from the immediate neighborhood of the incision. If the shank of the needle be placed across the worm, immediate cutting takes place and the body of the worm is severed.

A difficulty is encountered when the attempt is made to insert the vibrating needle into single cells. Instantly when the needle is brought up into the hanging drop, although no motion of the needle itself is apparent, cells and suspended debris are rapidly rotated about the needle and are finally flung away from the vicinity. Under controlled conditions this effect is being utilized to cause general vibration in cells; conjugants, for example are rapidly separated with no apparent ill effects.

By modification of the attachment of the needle it became possible to insert the point into amebae and other free cells and to observe the effects on protoplasm. Full account of these observations is reserved for future communication but preliminary experiments

[2] Bridgman, P. W., *J. Biol. Chem.*, 1914, xix, 511.

indicate that insertion of the needle produces alteration of the viscosity of the protoplasm. Provided that the treatment is not too intense or too prolonged, effects appear to be reversible. More intense treatment results in local injury, and in extreme cases complete disorganization or even disruption of the cell.

Some time after this communication was submitted for publication, on April 28, our attention was called to a paper by Harvey and Loomis, published in *Nature* for April 21, entitled "High Frequency Sound Waves of Small Intensity and their Biological Effects." Although some of the observations therein described parallel closely results of some of our earlier, as yet unpublished experiments, we should like to point out that our method of affecting single cells differs distinctly from theirs; in our expriments, the supersonic effects are, for the most part, applied in definitely localized regions of cells by means of glass collectors pulled to micro-points. Data already in hand indicate that such localized disturbances may be followed by deep-set ontogenetic alterations.

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Effect of Liver Extract on Erythrocytes and Reticulocytes in Normal Individuals.

CHARLES H. WATKINS, RICHARD JOHNSON AND HILDING BERGLUND.

From the Medical Service of the University Hospital, Minneapolis.

The effect of liver extract in pernicious anemia may be analyzed from the point of view of the immediate reaction, as well as of the sustained reaction. The immediate reaction includes among other things 3 distinct morphological features: (1) the normoblastic response, (2) the reticulocyte response, and (3) the liberation of the mature erythrocytes.[1] It is a well established fact that the bone marrow in pernicious anemia is hyperplastic and contains large amounts of erythrocytes in various stages of development. In other words, there appears to be a difficulty in the maturation of the erythrocytes necessary for their discharge from the marrow into the circulation. This faulty maturation is in character probably morphological rather than chemical, for the cells in the marrow are completely filled with hemoglobin and products of hemoglobin have been deposited in the various tissues. Thus the hemosiderosis, in the classical interpretation of pernicious anemia looked upon as a proof of the hemolytic character of the disease, might indicate a destruction of erythrocytes in the foci where they were formed be-

[1] Watkins, Charles H., and Berglund, Hilding, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 206.