Our tentative hypothesis of the mechanism of the process is as follows: Some reducing substance (inductor) present in the corpuscles is oxidized by methylene blue. The leucomethylene blue forms a peroxide-like compound with oxyhemoglobin. This complex peroxide effects the oxidation of lactic to pyruvic acid, and is itself thereby converted to methemoglobin, methylene blue and $O^{=}$, somewhat as follows:

Inductor + 4 MB → Oxidized inductor + 4 MBH_2 (leuco dye)
4 MBH_2 + $Hb(FeO_2)_4$ → $(MBH_2)_4$ $Hb(FeO_2)_4$ (complex peroxide)
$(MBH_2)_4$ $Hb(FeO_2)_4$ + 2 lactate → 4 MB + $Hb(Fe^{+++})_4$ + 2 pyruvate + 6 H_2O + 2 $O^{=}$.

Features included in this scheme: (1) An inductor (possibly unsaturated fatty acid or other easily oxidizable substance), the oxidation of which by methylene blue forms some CO_2 and the leuco-dye. (2) The reversible catalytic action of the dye. (3) The non-reversible catalytic action of the hemoglobin. (4) The stoichiometric relation between lactic acid oxidized and hemoglobin destroyed. (5) The sum of the O_2 required by these two processes is one half of the total O_2 consumption.

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The Mechanism of the Lethal Effects of Ultrasonic Radiation.

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That the lethal effects of ultrasonic waves on protozoa and other single cells could be traced to the cavitation of dissolved gas, has recently been discovered by Johnson.[1] Any gas suffices, apparently; there is no specificity of cavitated oxygen as was found for acceleration of chemical reactions by Schmitt, Johnson and Olson.[2] We have repeated the experiments of Johnson and are able to confirm his results fully. The present communication extends these observations and examines the mechanism of the lethal effect. A large number of microorganisms were tested, including various types of protozoa, rotifers, copepods, Daphnia, etc. In addition to these a number of eggs and embryos of frogs and snails were treated. About

[1] Johnson, C. H., *J. Physiol.*, 1929, lxvii, 356.

[2] Schmitt, F. O., Johnson, C. H., and Olson, A. R., *J. Am. Chem. Soc.*, 1929, li, 370.

10 cc. of the fluid containing the material to be tested was placed in a glass tube and radiated at atmospheric pressure, and at pressures of 60-80 lb. per square inch. After the minimum lethal dose for those radiated at atmospheric pressure was determined, it was found that an exposure many times this dosage had no effect upon cells radiated under 60-80 lb. pressure. Radiation of frog embryos yielded results which seem to indicate that radiation affects the head region rather preferentially, and that there is a definite selective interference in organogenesis. A study of these effects will be reported later.

To test the possibility that the function of the cavitated bubbles is to reflect the waves from their surfaces and thus tend to concentrate the radiation locally, two types of experiments were performed. In the first, cells were radiated in a suspension of infusorial earth, the suspended particles ranging from very small to relatively coarse. In the second, paramecia were allowed to ingest Chinese black which packed the food vacuoles with solid granules. Radiation of the cells in either case, with pressure and without, gave no results which would lend support to the theory that such an artificial increase of surface, inside or outside the cell, aided in producing the lethal effects concerned. It seems, therefore, that the lethal effect is produced at the surface of the cavitated gas bubbles. Johnson is of the opinion that the effect on cells is external rather than internal, and to his reasons for so believing, we may add the fact that, owing to the greater viscosity of the protoplasm, there will be considerably less tendency for cavitation inside the cell than outside. Thus, for example, when cells are radiated in a viscous medium, the minimal lethal dose is greatly increased. This retarding effect of viscosity is observable also in the promotion of chemical reactions by ultrasonic radiation.

To test the view that the lethal effect is due to a chemical or physical change at the plasma membrane, Spirogyra filaments were treated with a radiation strength insufficient to rupture the cellulose wall, or to cause visible disorganization. The strands were stained with neutral red, and then placed in a mixture of NaOH, NaCl, and $CaCl_2$. Radiated material gave evidence of a much more rapid penetration of the alkali than unradiated controls. Although adequate temperature control is difficult there seems little doubt but that the radiation alters the membrane so as to make it more permeable.

In further testing this view, another interesting and suggestive experiment was performed. Artificial cells were made by mixing a chloroform solution of lecithin with a protein solution according to

the directions of Harvey.[3] After the chloroform has diffused out of the "cells", an aqueous mixture of lecithin surrounded by a denaturized protein membrane presumably remains. Radiation of these "cells" gave very curious results. Sufficiently long exposures, of course, destroy the "cells". A dose of 10 seconds, however, sufficing to kill all paramecia present, results only in the accumulation of a bubble due to the cavitation of air inside the "cell"; successively longer radiations cause the appearance of protrusions, which in reality, are myelin forms resulting from the escape of lecithin into the water through ruptures in the cell wall. Microscopic examination occasionally reveals minute breaks in the membrane. That radiation may almost instantly break certain unstable emulsions, such as oils in water, and cause a separation of phases, has been demonstrated by Schmitt.[4] This seems highly significant in view of the Clowes theory of the emulsion structure of the membrane. It has also been discovered[4] that types of chemical reactions other than oxidations may be promoted by radiation in the presence of water and cavitated gas. Thus the hydrolysis of carbon tetrachloride and other halogens requires only cavitation; for this, pure nitrogen suffices.

The membranes of artificial cells may not be ruptured even though cavitation has taken place on the inside and the outside by radiation strong enough to kill all protozoa present; one seldom, if ever observes a cavitated bubble inside of radiated protozoa; very energetic chemical reactions may be promoted by radiation, when cavitation is permitted, even in the absence of oxygen; unstable emulsions may be instantly cracked by the radiation. These facts render plausible our tentative position that the lethal effect is due to a rupture of the plasma membrane by a chemical or a physical chemical effect produced by cavitation in the water immediately surrounding the cell.

[3] Harvey, E. N., "Laboratory Directions in General Physiology," 1913, 31.

[4] Schmitt, F. O., in press.