the effects of the above mentioned preparations were studied over periods of 30 days.

We may therefore conclude that all the various preparations of anterior pituitary substance which we used cause a rise in basal metabolism; however, the subcutaneous injection of acid or alkaline extracts produces the most rapid and greatest rise. Also the other preparations differ in the degree and the sharpness of the rise, and in the character of the curve following the period when they have reached the maximum point. The basal metabolism of all the animals, except those fed with Armour's anterior pituitary tablets, returns approximately to the normal level after some time, notwithstanding the continued administration of the various preparations during this period. These experiments furthermore suggest that some of these preparations may affect the basal metabolism, at least partly, through changes which they produce in the thyroid gland, while others affect it independently of such changes. We intend to study this question more directly in subsequent experiments.

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Induced Oxidations in Blood. Hemoglobin Destruction by Methylene Blue in Lactic Acid Peroxidation.

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We have previously reported experiments[1] which showed that the observations of Barron and Harrop[2, 3] that methylene blue added to blood increases the rate of oxygen consumption and decreases lactic acid production, may, in part, be accounted for by the oxidation of formed lactic acid. The incubation of washed dog erythrocytes in a solution containing added dl-lactate, in the presence but not in the absence of methylene blue, results in a disappearance of lactate.

Further study indicates that the lactic acid is oxidized to pyruvic acid, apparently quantitatively. That this oxidation is not mediated by the lactic "dehydrogenase" of the Wieland school is evi-

[1] Wendel, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 865.

[2] Harrop and Barron, *J. Exp. Med.*, 1928, xlviii, 207.

[3] Barron and Harrop, *J. Biol. Chem.*, 1928, lxxix, 65.

dent from the fact that molecular O_2 alone is incapable of effecting it; the dye is necessary. Experiments of Harned (unpublished) in this laboratory, indicating intermediate peroxide formation when leucomethylene blue undergoes oxidation, suggest that the mechanism by which the lactic acid is oxidized may be one of peroxidation. We find, however, that lactic acid is not affected by the hypothetical methylene blue peroxide alone, since shaking leucomethylene blue and buffered sodium lactate with O_2 fails to touch the lactate. Evidently another cell constituent is also essential.

A series of experiments with oxygenated dog erythrocytes (substantially free from sugar) in phosphate buffers containing added dl-lactate were carried out with the following determinations at intervals during 2 to 6 hours: O_2 content and capacity, CO_2 content, lactic acid and pyruvic acid. The following experiment illustrates the results obtained.

Experiment 128. Dog erythrocytes separated from serum and leucocytes by centrifugation, incubated 1.5 hours at 37°C, suspended in isotonic NaCl and phosphate buffer (pH 7.4) containing sodium dl-lactate and 0.005% methylene blue. Incubated with gentle shaking at 37°C. in closed filled tubes containing glass beads. Separate tubes analyzed at intervals. Results expressed in mMols per liter of mixture.

Hours:		0	2	4	6
Lactic acid.	Content	9.9	8.6	7.65	7.2
	Decrease	—	1.3	2.25	2.7
Pyruvic acid:	Content	0.7	2.5	3.7	4.6
	Increase	—	1.8	3.0	3.9
Oxygen:	Content	11.8	9.0	7.5	6.8
	Decrease	—	2.8	4.3	5.0
	Capacity	11.8	9.2	7.7	6.8
	—Decrease	—	2.6	4.1	5.0
O_2 required for oxidation of lactic to pyruvic acid:		—	0.65	1.12	1.35
O_2 required to oxidize hemoglobin to methemoglobin.		—	0.65	1.02	1.25
O_2 to other substances:		—	1.5	2.16	2.4

The results of these experiments appear to indicate a close relation between the hemoglobin destruction, as measured by loss of O_2 capacity and the oxidation of lactic acid to pyruvic acid. If it be supposed that hemoglobin be oxidized to methemoglobin, the O_2 required in all cases is approximately the same as required in the lactic acid oxidation. The sum of these fractions leaves at least one half of the O_2 for other oxidations. The CO_2 production varies, the total R.Q. of the process being 0.5 to 0.8.

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.