tions, lymphocytic infiltration, young fibroblasts and new connective tissue containing many small capillaries. Near the upper end of the tarsus, the layer of infiltrated inflammatory cells was thicker and contained plasma cells and histiocytes. The lids of the uninoculated eye showed a similar picture. The centers of the follicles contained groups of epithelioid cells.**

5336

Oxidation of Lactate by Methemoglobin in Erythrocytes with Regeneration of Hemoglobin.

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In earlier communications[1] we reported the oxidation of lactic to pyruvic acid and hemoglobin to methemoglobin by normal dog erythrocytes in the presence of 0.005% methylene blue. Pyruvic acid is not further oxidized. Because the oxygen partition seemed to indicate coupled reactions, it was tentatively suggested that the mechanism was one of peroxidation. Further studies, in which the stochiometric relationships observed with sugar-free cells were not obtained in presence of glucose, led us to question the explanation first offered.

Warburg, Kubowitz and Christian[2] explain methylene blue catalysis in red blood cells by the following chain of reactions: (a) oxidation of hemoglobin to methemoglobin by methylene blue; (b) oxidation of carbohydrate (or derivative) by methemoglobin with regeneration of hemoglobin; (c) oxidation of leuco-methylene blue by O_2. (A detailed paper by Warburg *et al*[3] which reached us while writing this report appears to substantiate their earlier interpretation.)

If methemoglobin is the agent responsible for methylene blue catalysis in red blood cells, it should be possible to oxidize lactic acid with methemoglobinized cells in the absence of O_2. And if no

** We are indebted to Dr. Harvey D. Lamb for the histological report on this specimen.

1 Wendel, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 865; 1930, **27**, 624; *J. Biol. Chem.*, 1930, **87**, p. xx.

2 Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **221**, 494.

3 Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **227**, 245.

other oxidations occur, an equivalent amount of hemoglobin should be formed, as follows:

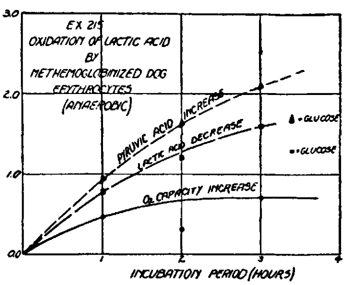
I. $2\ CH_3 \cdot CHOH \cdot COOH + MetHb(Fe^{+++})_4 \rightarrow 2\ CH_3 \cdot CO \cdot COOH + Hb(Fe^{++})_4 + 4H^+$.

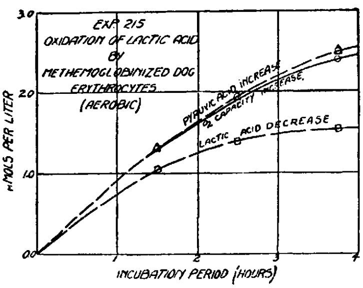
and on exposure to oxygen,

II. $Hb(Fe^{++})_4 + 4O_2 \rightarrow Hb(FeO_2)_4$

The equations say that for each mol of pyruvic acid formed 2 mols of O_2 capacity are created. (This would be the minimum ratio; if other oxidations occur simultaneously, a larger reduction of methemoglobin should be found.) Furthermore the increase in O_2 capacity should be the same whether reaction I. occurs in presence or absence of O_2.

Experiment: Dog erythrocytes, treated with amyl nitrite until all hemoglobin is converted to methemoglobin (as measured by O_2 capacity), washed twice with saline to remove excess nitrite, were suspended in isotonic Na lactate-phosphate-chloride solution (pH 7.47), and incubated (37.5°) with rocking in wide bottom flasks (aerobic) and also in closed filled tubes (anaerobic). At intervals samples were analyzed for lactic acid, pyruvic acid and O_2 capacity.





The figures illustrate the results obtained. The rate of pyruvic acid formation is the same aerobically and anaerobically. The increase in O_2 capacity is greater aerobically than anaerobically; but in neither case is it enough to account for the pyruvic acid formed. (The difference between pyruvic acid increase and lactic acid decrease is probably due to progressive decomposition of a hexose-phosphoric ester with formation of lactic acid.) With both glucose and lactate present, the rate of methemoglobin reduction is increased and lactate oxidation *reduced,* indicating preferential oxidation of glucose. This behavior probably indicates the manner in which methemoglobin, formed in red cells by drugs or from other causes, is in the living animal reconverted to hemoglobin, and may also account for the apparent difficulty in producing methemoglobin in blood of some species.

The discrepancy between methemoglobin reduced (increase of O_2 capacity) and lactate oxidized means either that the nitrite-"methemoglobin" is not methemoglobin (Hartridge[4]) and has much higher capacity as an oxidant, or that other oxidants are involved beside methemoglobin. The question is being investigated.

Methemoglobin in simple buffer solution (in absence of cells) fails to attack lactic acid, and methemoglobin dissolved in buffer solution, in which normal hemoglobin-containing, glycolytically active cells are suspended, likewise fails to oxidize lactate. We may infer, therefore, that methemoglobin is effective in causing this oxidation only when present within the cell where lactate activation occurs.

5337

Ovarian and Anterior Pituitary Hormones from the Pregnant Monkey.

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The hormones referred to are (1) the ovarian hormone (theelin, oestrin, folliculin) and (2) the active principle of the anterior lobe of the hypophysis which stimulates development of ovarian follicles and culminates in ovulation. The simplest and most decisive test for the former is the full oestrous growth reaction of the epithelium of the genital tract of the ovariectomized adult rat determined by the characteristic changes in the cell content of vaginal smear preparations. One of the best tests for the second active substance is the reaction of the normal immature rat which results in the attainment of puberty including the first ovulation. This is accomplished by stimulation of rapid growth in the ovarian follicles which in turn produce the follicular hormone which causes rapid growth in the genital organs.

It has been demonstrated repeatedly that the human placenta contains large amounts of folliculin, the total increasing with the growth of the placenta as pregnancy progresses. Positive tests have been obtained from fetal membranes of the cow, sheep and horse but negative tests so far from zonular placentas of the cat and dog. The urine of pregnant women and cows furnishes an abundant

[4] Hartridge, *J. Physiol.*, 1920-21, **54**, 253.