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On the Nature of the Oxygen Linkage in Oxyhemoglobin.

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In order to test Haldane's hypothesis that oxygen exists in oxyhemoglobin as a peroxide compound, an attempt was made to identify hydrogen peroxide in solutions of the blood pigment which had been denatured by acid. If oxyhemoglobin is an iron peroxide, we should expect it to behave as do other metallic peroxides, by reacting with acids to form hydrogen peroxide. Inasmuch as the acid hematin resulting from such denaturation has little, if any, catalase activity, any hydrogen peroxide formed should persist in solution.

Solutions of 10 millimolar human oxyhemoglobin were treated with 8 volumes of .04 molar sulphuric acid and then precipitated with 5 volumes of 2% phosphovanadic acid, the latter serving the double purpose of precipitating the protein and of indicating the presence of any peroxide that might be liberated. In every instance the test was negative. Using chromic acid in acetone solution as a test for hydrogen peroxide, none of the latter could be detected in the ordinary Folin-Wu filtrate from blood. The absence of hydrogen peroxide from acid denatured oxyhemoglobin is deducible from the fact that solutions of benzidine base in acetic acid will not be oxidized to benzidine blue by blood, unless peroxide is added to the system.

The phosphovanadic acid test described above is sensitive to 1 part of hydrogen peroxide in 200,000. The final dilution attained by the test solution, assuming that all the oxyhemoglobin yielded an equivalent of peroxide, would be 1 millimolar, or 1 part in 30,000.

For this reason the author is inclined to discard the peroxide formula for oxyhemoglobin and to suggest the ozonic linkage. According to this theory, the electronic formula for oxyhemoglobin may be compared with its homologue, ozone:



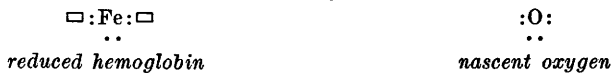
Carbon monoxide hemoglobin may be represented with its homologue, carbon dioxide, as:



and nitric oxide hemoglobin with nitrogen dioxide:



It is interesting that molecular oxygen, carbon monoxide and nitric oxide each combine with nascent oxygen, under appropriate conditions, to form the homologues listed. Nascent oxygen could therefore be considered a homologue of reduced hemoglobin:



Inasmuch as ozone, carbon dioxide and nitrogen dioxide are hydrated to form acids, it is suggested that the increased acidity of hemoglobin, attendant on its oxygenation or carbonylation, is due to hydration of the resulting compound with the formation of one or 2 additional acidic groups.

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Active Immunization in Human Cases with the Polysaccharide of Pneumococcus Type I.

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Tillett and Francis^{1, 2} showed that patients suffering with pneumonia who were injected intracutaneously with small amounts of pneumococcus polysaccharide of the homologous and heterologous types, showed after some time antibodies not only against the infecting type of organism but against the other types. In explanation they suggested that probably the injections of the heterologous polysaccharides produced antibodies.

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¹ Tillett, W. S., and Francis, T., *J. Exp. Med.*, 1929, **50**, 687.

² Francis, T., and Tillett, W. S., *J. Exp. Med.*, 1930, **52**, 573.