9389 P

Effect of Hydrogen Peroxide on Methemoglobin.

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Barnard and Gowen[1] reported that the addition of hydrogen peroxide to methemoglobin solutions resulted in the formation of oxyhemoglobin. This change was considered to be in harmony with that deduced from the relative redox potentials of the hydrogen peroxide-oxygen system and the methemoglobin-oxyhemoglobin system.

Keilin and Hartree[2] subsequently showed that the addition of hydrogen peroxide to methemoglobin solutions led to the formation of a substance which they characterize as a methemoglobin peroxide compound.

Because of the discrepancy between the results of Barnard and Gowen and those of Keilin and Hartree the question was reinvestigated. It was found that the observations of the latter investigators could be confirmed when the methemoglobin used was prepared by the oxidation of hemoglobin with ferricyanide. However when methemoglobin is prepared by the oxidation of hemoglobin with quinhydrone the addition to it of hydrogen peroxide results in the formation of oxyhemoglobin. That this latter compound was actually oxyhemoglobin and not a substance with similar absorption bands was demonstrated by treatment with carbon monoxide when CO-hemoglobin resulted.

In a typical experiment, a sample of a 0.5 millimolar oxyhemoglobin solution (molarity in this case refers to iron molarity) is divided into two portions. Each of these portions is placed in the chamber of a double spectrophotometer vessel at the collimator of a Hillger spectrophotometer. The telescope slit of the spectrophotometer is opened to its maximum extent. The photometer is adjusted so that these two solutions cast spectra of corresponding intensities, one above the other—the absorption bands are of course identical since they emanate from identical solutions.

To the contents of the upper chamber, a small quantity of powdered quinhydrone is added, sufficient to change the upper spectrum to that of methemoglobin (about 3 milligrams suffices for the usual

[1] Barnard, R. D., and Gowen, G. H., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 521.

[2] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc. B*, 1935, **117**, 1.

contents of one chamber). There are now two distinct spectra, one of methemoglobin above and one of oxyhemoglobin below.

When a drop of 3% hydrogen peroxide solution is instilled into the methemoglobin solution in the upper chamber, the upper spectrum immediately reverts to that of oxyhemoglobin, identical in intensity and in coincidence of bands with the spectrum of unaltered oxyhemoglobin below.

The passage of illuminating gas through both chambers causes a change of both spectra to that of carbon monoxide hemoglobin and again these bands are identical in position and intensity.

The experiment proves that the compound formed, in this instance, by the reaction of hydrogen peroxide on methemoglobin is actually oxyhemoglobin and not a compound with superficial similarity of absorption bands.

The opinion is advanced that it was a mixture of oxyhemoglobin with the methemoglobin-peroxide compound that led Haurowitz[3] to the statement that the reaction product of methemoglobin and peroxide resembled in its spectroscopic appearance NO-hemoglobin.

9390 P

The Comb of the Baby Chick as a Test for the Male Sex Hormones.

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This report describes a method of utilizing the 6-day chick in the bio-assay of the male sex hormone, of androsterone particularly. Much of this study was occupied in eliminating pitfalls which obscured assay. Ruzicka[1] first suggested the chick as a test object, utilizing external application to the crest region as described by Fussgänger.[2] The latter claimed that external application of an extract containing only 1/50 of a capon unit gave a reaction as great as 50 times this amount, given intramuscularly to the capon.

The white leghorn responds best. Chicks younger than 6 days show a high mortality. Ten days' treatment has been found the best.

3 Haurowitz, F., *Z. Physiol. Chem.*, 1931, **198**, 9.

* Joseph Brettauer Research Fellow in Gynecology.

1 Ruzicka, M. L., *Bull. Soc. Chimique de France*, 1935, **5**, t. 2, 1497.

2 Fussgänger, R., *Mediz. u. chem. Abteil. aus den med. chem. Forschungstätten der I. G. Farbenindustrie Act. Ges.*, 1934, **2**, 1934.