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Reactions of Hydrogen Selenide with Hemoglobin Derivatives.

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Keilin[1] reported the formation of a compound between hydrogen sulfide and ferrihemoglobin which appears to come into the category of a class of ferrihemoglobin compounds, the existence of which was predicted by Gamgee[2] and termed "hemoferrides" by Barnard.[3] Because of the close chemical, physical and toxicologic similarities of hydrogen sulfide to hydrogen selenide, we have studied the effects of the latter on various hemoglobin derivatives. The analogy between the two gases appears to extend to their behavior toward hemoglobin solutions, thus, treatment of ferrihemoglobin solutions with hydrogen selenide results in the formation of a compound which is spectroscopically similar to hydrogen sulfide ferrihemoglobin whereas the ferrihemochromogens are reduced by hydrogen selenide to the corresponding ferrohemochromogens.

Experimental. I. Preparation of Solutions. 1. *Ferrihemoglobin.* Fresh erythrocyte suspensions (rat, dog, and human) of known oxygen capacity were hemolyzed with ether-saturated water and diluted to a concentration of 0.1 millimolar in terms of iron molarity. An equivalent of potassium ferricyanide was added and the solution kept at room temperature for an hour before use. Complete oxidation to ferrihemoglobin was confirmed by spectroscopic examination.

2. *Ferrihemochromogens.* 63 mg of crystalline ferriheme chloride was dissolved in each of the following solvents: (a) 5% aqueous pyridine to form *pyridine ferrihemochromogen hydroxide.* (b) 26% ammonia water to form *ammonia ferrihemochromogen hydroxide.* (c) 2% sodium cyanide to form *cyanoferrihemochromogen cyanide.*

3. *Ferrihemes.* 63 mg of crystalline ferriheme chloride was dissolved in (a) 1% sodium carbonate to give *sodium ferriheme hydroxide* and (b) 3% triethanolamine to form *triethanolamine ferriheme hydroxide* solution. All ferrihemochromogen and ferriheme

[1] Keilin, D., *Proc. Soc. B.*, 1933, **113**, 393.

[2] Gamgee, A., in Schafer, E. A., *A Textbook of Physiology*, Edinburgh, 1898, 242.

[3] Barnard, R. D., *J. Biol. Chem.*, 1937, **120**, 177.

solutions were 0.2 millimolar, a concentration that is well suited for spectroscopic work.

II. *Treatment of Samples with Hydrogen Selenide.** 5 cc of the sample to be studied was placed in each chamber of a double spectroscopy vessel. The outlet tube of the hydrogen selenide generator was immersed in one of the chambers and the gas ejected through the solution at the rate of 3 bubbles per second for 10 seconds. The saturated sample was then examined through a Hilger constant deviation angle spectrometer along with the control sample which had not been subjected to the action of the gas.

The spectroscopic observations were also controlled by an examination of colloidal aqueous selenium, sodium polyselenide solutions and the reaction product of hydrogen selenide on potassium ferricyanide solution. All of these products are red in color but show no definite absorption bands in the visible spectrum.

Results. Hydrogen selenide immediately converts ferrihemoglobin solution into one of a salmon red color. The change is apparent in less than 2 seconds by the method of saturation employed. The solution shows a single broad absorption band between 5400 Å and 5200Å.

On treatment with a drop of saturated sodium hydrosulphite solution this band disappears, to be replaced by that of hemoglobin. Since ferrihemoglobin is the direct precursor of hemoglobin (Conant[4]), this may be taken as evidence of the reversibility of the reaction between hydrogen selenide and ferrihemoglobin. Hydrogen selenide appears to be without effect on pyridine ferrihemochromogen hydroxide insofar as compound formation is concerned. The solution turns red after 10 seconds of saturation but spectroscopically it cannot be distinguished from the original substance. Cyanoferrihemochromogen cyanide and ammonia ferrihemochromogen hydroxide are immediately reduced by hydrogen selenide to the corresponding ferrohemochromogens and, on exposure to air, these latter are soon reoxidized to the original ferrihemochromogens. The

* Hydrogen selenide was prepared by hydrolysis of aluminum selenide. 4 g of electrolytic aluminum and 10 g of powdered black selenium are intimately mixed in a mortar and transferred to a pyrex test tube over a small pellet of metallic sodium. The tube is heated over a Bunsen flame until the contents fulgurate. The upper portion of the contents is removed discarding the sodium selenide which remains in the bottom. About 5 g of granulated aluminum selenide is placed in a small wash bottle, the inlet tube of which is connected to a tank of nitrogen. When the latter is released it carries over to the outlet an appreciable (and dangerous) concentration of hydrogen selenide which is liberated by the small quantity of moisture present.

4 Conant, J. B., *J. Biol. Chem.*, 1923, **57**, 401.

cyanide and ammonia solutions are strongly alkaline; the pyridine solution is not; the effect in the strongly alkaline solutions is probably that of alkali selenide and is entirely comparable to the effect of alkali sulfide on the same solutions.

Passage of hydrogen selenide through the sodium ferriheme hydroxide solutions causes a change from the greenish color of these solutions to salmon red. The absorption band of the alkaline ferriheme disappears and is replaced by a single band in the yellow centering at 5620Å. Since alkaline ferriheme is irreducible by the sulfides in the absence of suitable nitrogenous "hemochromogen formers," (Bertin-Sans and deMontessier[5]), and since there is no evidence of the characteristic hemochromogen doublet in the reaction product of hydrogen selenide and alkaline ferriheme, the latter probably represents a hemoferride.

From some studies not as yet published, we have found that solutions of hematin in triethanolamine are ferrihemes, the nitrogen of the amine being incapable of coördination with the iron. The results in this study confirm this conclusion. Triethanolamine solutions of hematin behave toward hydrogen selenide in a manner similar to sodium ferriheme hydroxide; the band in the red is replaced by one in the yellow green. This latter band is not so well defined as that appearing in the sodium carbonate solutions of hematin and may possibly represent a slight tendency toward the formation of a hemochromogen.

Hydrogen selenide is without effect on the spectroscopic bands of either oxyhemoglobin or alkaline hematoporphyrin. On solutions of methemoglobin in 0.5% acetic acid, hydrogen selenide reacts with the formation of a greenish pigment similar in appearance to the decomposition products which arise from hemoglobin by the prolonged action of sulfide and described by Hoppe-Seyler[6] and Araki.[7]

[5] Bertin-Sans, H., and deMontessier, J., *Compt. rend. Acad.*, 1892, **114**, 923.

[6] Hoppe-Seyler, F., *Zentralbl. f. med. Wissensch.*, 1863, **28**.

[7] Araki, T., *Z. physiol. Chem.*, 1890, **14**, 405.