14340

O-Benzoic Sulphinide (Saccharin) Ferridehemoglobin.

ROBERT D. BARNARD.* (Introduced by Charles A. Doan.)

From the Division of Medical Research, Department of Medicine, Ohio State University, Columbus, Ohio.

In discussing the reactions of nitrite with blood pigment, Gamgee[1] claimed priority on the discovery of the class of ferrihemoglobin derivatives typified by the cyanmethemoglobin of Kobert.[2] Smith and Wolf[3] discovered the azide and Haurewitz,[4] the fluoride derivatives. On the basis of its potentiometric behavior, Barnard[5] characterized alkaline ferrihemoglobin as the hydroxide of this class and predicted the ability of ferrihemoglobin to form polar valent compounds with many anions. Keilin[6] described the hydrosulfide of ferrihemoglobin and in 1937, Barnard[7] designated the class of derivatives as "hemoferrides" to emphasize their binary nature, *i.e.*, they were salts of hydro-acids, exclusively.

On the basis of the demonstration of the nitrile oxide structure of fulminic acid and the fulminates by Pauling and Hendricks,[8] Barnard and Neitzel[9] prepared a fulminate-ferrihemoglobin compound. Hydrogen selenide derivatives of ferrihemoglobin and ferriheme were also prepared.[10] Combinations between thiocyanate and ferrihemoglobin and between cyanate and ferrihemoglobin were shown to exist by Jung[11] and Hecht[12] respectively. Thiocyanic and cyanic acids are now known to be hydroacids.[13] A cyanamide derivative of ferriheme[14] and ferrihemoglobin[15] have also been prepared. In the description of the cyanamide compounds, the terms ferrideheme (in place of hemoferride), ferridehemochromogen and ferridehemoglobin were introduced in order to adapt to these substances, the system of nomenclature proposed by Pauling and Coryell[16] for the blood pigment derivatives.

It is assumed that the ferridehemoglobin engendering combination between ferrihemoglobin and hydro-acid anions is conditioned by the comparatively small ionic diameter of the latter. Of the relatively limited class of acids which are not oxy-acids, the majority do combine with ferrihemoglobin. Potentiality for this type of combination seems to be restricted to the smaller members of the class; the halides with the exception of the smallest, fluoride, do not so combine. In those instances where the rule interdicting ferrideheme formation with oxyacid anions appears to be excepted, as in the cases of hydroxyl, peroxide, and nitrite ion; the first two have ionic mobilities comparable with those of hydro-acid anions and the last may be coordinated as nitro ion. It has been suggested that the small ionic diameter of hydro-acid anions permits their penetration of the interstices of the porphyrin ring and the formation of polar covalent bonds with the iron.[15]

* On active duty, Medical Corps, Army of the United States.

1 Gamgee, A., in Schafer, E. A., *A Textbook of Physiology*, Edinburgh, 1898, 242.

2 Kobert, R., *Pflüger's Arch.*, 1900, **82**, 603.

3 Smith, L., and Wolf, C. G. L., *J. Med. Research*, 1904, **12**, 451.

4 Haurewitz, F., *Z. physiol. Chem.*, 1924, **68**, 138.

5 Barnard, R. D., *J. Gen. Physiol.*, 1933, **16**, 657.

6 Keilin, D., *Proc. Roy. Soc. London, B*, 1933, **113**, 393.

7 Barnard, R. D., *J. Biol. Chem.*, 1937, **120**, 177.

8 Pauling, L., and Hendricks, S. B., *J. Am. Chem. Soc.*, 1926, **48**, 1926.

9 Barnard, R. D., and Neitzel, W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 171.

10 Barnard, R. D., and Neitzel, W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 462.

11 Jung, F., *Biochem. Z.*, 1940, **304**, 37.

12 Hecht, G., *Biochem. Z.*, 1940, **305**, 290.

13 Linhard, M., and Betz, K., *Ber.*, 1940, **73B**, 177.

14 Barnard, R. D., *J. Lab. and Clin. Med.*, 1942, **27**, 774.

15 Barnard, R. D., *J. Am. Pharm. Assn.*, in press.

16 Pauling, L., and Coryell, C. D., *Proc. Nat. Acad. Science*, 1936, **22**, 159.

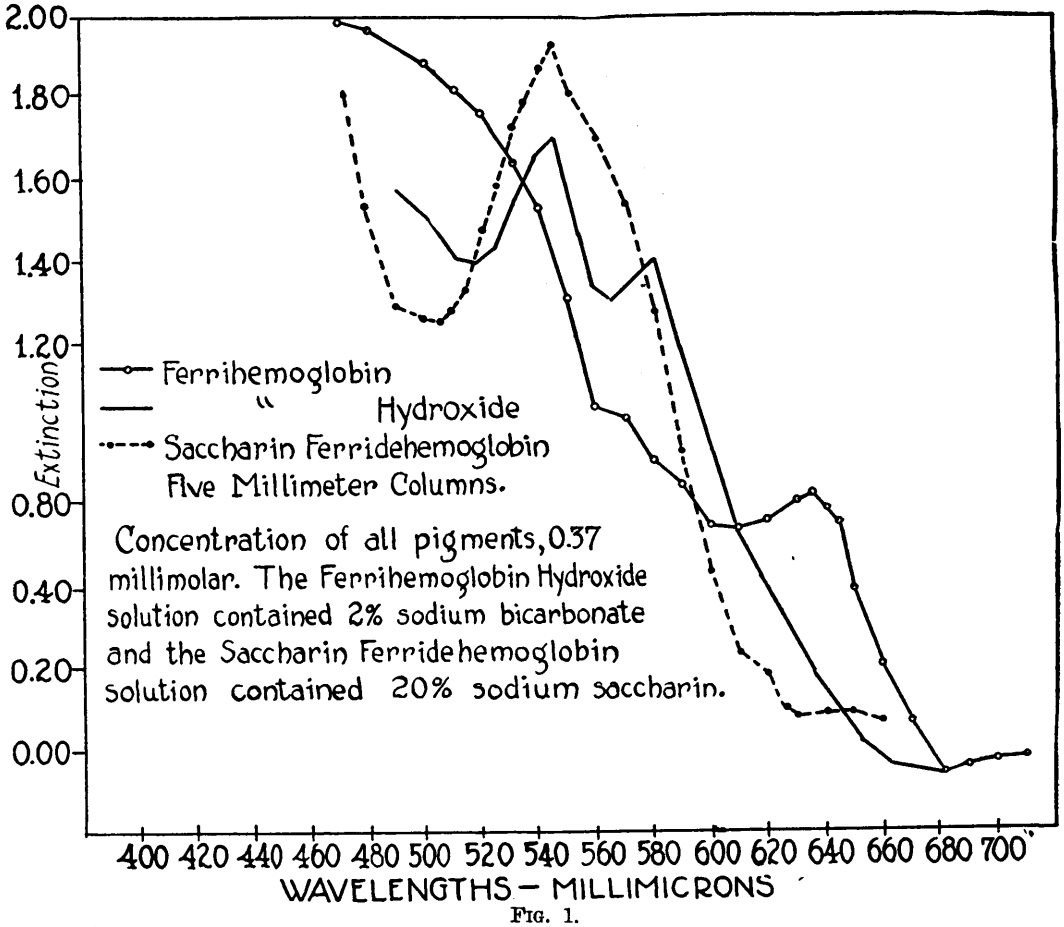

FIG. 1.
Spectrophotometric absorption curves of ferrihemoglobin, ferrihemoglobin hydroxide and saccharin ferridehemoglobin.

Whether or not this hypothesis is the correct one, its utility is beyond question for it has led to the prediction of the existence of several members of a class of blood pigment derivatives. An additional member, a compound of ferrihemoglobin with o-benzoic sulphinide (saccharin) is the subject of this report.

When soluble saccharin is added to a solution of ferrihemoglobin, the color of the latter changes to a bright red. This change is not complete until there is present in the solution a relative molar ratio of five hundred of saccharin and is not apparent until this ratio is over one hundred. If the ferrihemoglobin solution has been buffered at pH 6 so that all of the pigment is in the acid form, a visible color change is produced by 10 molar equivalents of saccharin but the latter also induces rapid denaturation and precipitation at this pH so that spectrophotometry on such acid specimens is out of the question. The curves shown were therefore obtained at pH 7.4.

The color of the solution of the saccharin-ferrihemoglobin reaction product bears a superficial resemblance to that of ferrihemoglobin hydroxide but the formation of the latter substance in the former solution was ruled out by the absence of any change in pH of the ferrihemoglobin solutions on the addition of soluble saccharin as well as by the distinction in the respective spectrophotometric curves. Any possible role which salt effect of the high concentrations of sodium saccharin employed might have exerted in the production of the color change, was ruled out by half saturation of control samples of ferrihemoglobin with dextrose, sodium chloride and ammonium sulfate, in which instances the

molarity of such solutions was about twice that existing in the saccharin-ferrihemoglobin solution. No visible or spectroscopically detectable alteration was produced.

On the basis of the facts that soluble saccharin is the sodium salt of a hydro-acid, o-benzoic sulphinide; that it reacts with ferrihemoglobin in solution to produce a colored entity which is similar in its spectroscopic picture to that of the class of blood pigment derivatives termed ferridehemoglobins, the saccharin derivative is designated as saccharin- or o-benzoic sulphinide ferridehemoglobin.

Like certain other ferridehemoglobin formers (fluoride, fulminate, and cyanate) saccharin does not form ferridehemochromogens. The apparently high degree of dissociation of saccharin ferridehemoglobin which would seem to be indicated by the relatively high concentrations of saccharin required for the complete conversion of ferrihemoglobin is similar to that exhibited by the ferridehemoglobins of cyanamide, cyanate, and thiocyanate while it differs markedly from that observed in the cases of the ferridehemoglobins formed by the highly toxic anions, azide, cyanide, and fluoride. There appears to be some parallelism in the degree of association of hydro-acid anions with ferrihemoglobin and their inhibitory effect on the blood catalase. In this connection it is found that saturation with soluble saccharin is without appreciable effect on the catalase activity of whole blood, plasma or hemoglobin solutions. From the high concentrations of soluble saccharin found necessary for the completion of its reaction with the prosthetic nucleus of ferrihemoglobin, it is doubtful if this reaction is of any toxicologic importance.

14341

The Anti-Bacterial Action of the Xanthine Oxidase System.*†

DAVID E. GREEN AND RUTH PAULI. (Introduced by M. Heidelberger.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City.

Notatin, an anti-bacterial substance isolated from *Penicillium notatum,* has been shown by Raistrick and coworkers[1] and others[2,3] to be a flavoprotein which catalyses the aerobic oxidation of glucose to gluconic acid with production of H_2O_2. According to these authors H_2O_2 is the actual anti-bacterial agent and the function of the flavoprotein-glucose-oxygen system is merely that of providing a continuous albeit minute supply of H_2O_2. It follows that any flavoprotein system which gives rise to H_2O_2 should have at least qualitatively the same anti-bacterial action as notatin. We have tested this hypothesis with the flavoprotein from milk which catalyses the oxidation of hypoxanthine to uric acid, and the results clearly indicate that there is little to choose between the flavoproteins from cow's milk and *Penicillium notatum* respectively as far as anti-bacterial action is concerned. The sole difference is that notatin is a somewhat more stable enzyme.

Experimental. Milk flavoprotein was prepared in purified form by the method of

* This investigation was aided by grants from the Research Corporation, Nutrition Foundation, Rockefeller Foundation, and Lederle Laboratories.

† Since our article was submitted for publication on June 25, 1943, there has appeared in *Science* for September 10, 1943, an article by Lipmann and Owen who made observations comparable to those which we are reporting.

1 Coulthard, C. E., Short, W. F., Michaelis, R., Sykes, G., Skrimshire, G. E. H., Standfast, A. F. B., Birkinshaw, J. H., and Raistrick, H., *Nature,* 1942, **150**, 634.

2 Van Bruggen, J. T., Reithel, F. J., Cain, C. K., Katzman, P. A., Doisy, E. A., Muir, R. D., Roberts, E. C., Gaby, W. L., Homan, D. M., and Jones, L. R., *J. Biol. Chem.,* 1943, **148**, 365.

3 Kocholaty, W., *Science,* 1943, **97**, 186.