LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Can the Iron in Peroxidase be Replaced by Manganese?

In 1940¹ we demonstrated that pure horse radish peroxidase can be split reversibly into protohæmin and a colourless protein component. The original activity could be restored by mixing protohæmin and the protein. Later², we tested different iron-porphyrins for their ability to replace protohæmin. Only deuterohæmin and mesohæmin gave activity, both lower than protohæmin. Hæmatohæmin and many others were inactive. A graph, already published³, shows that after one mol of hæmin per mol of protein is added, further addition of hæmin does not change the activity.

Gjessing and Sumner³ cleaved impure peroxidase preparations according to our method, and investigated the activity resulting from addition to the protein part of (1) Cu-, Co-, Mn- and Ni-protoporphyrins; and (2) Fe-meso- and Fe-hematoporphyrin.

Copper, cobalt and nickel gave no activity. Some activity (28.4 per cent) was observed in one single test with the manganese protoporphyrin, whereas remarkably enough higher and lower additions gave practically no activity. Contrary to our experience, Gjessing and Sumner's protein preparations always gave strongly decreasing activity when the hæmins were added in increasing excess. We therefore considered it of interest to see if their positive result with Mn-protoporphyrin could be confirmed. The question is important, because iron is the only metal hitherto found in peroxidases, catalases and cytochromes.

We further know from our own experience that a positive peroxidase reaction in one single test is far from conclusive, since it may sometimes be caused by unknown impurities.

We prepared Mn-protoporphyrin by heating protoporphyrin in pyridine + acetic acid + chloroform with manganese acetate. It seems never to have been crystallized (we did not succeed in crystallizing it), so it was precipitated with water or ether, washed with water and dried. Mn found = 8.2 per cent, calc. 8.9 per cent. Absorption bands in pyridine: 593 and 572–542 m μ .

Pure horse radish peroxidase was split with acetone-hydrochloric acid in ice. The protein component contained 1.8 per cent of the original activity, that was restored to 80 per cent by the addition of 100 or 200 per cent of the quantity of iron-hæmin split off. Addition of 25, 50, 100, 200 or 400 per cent manganese hæmin gave no increase over the blank value tested both one and twenty-four hours after the addition. We thus feel justified in stating that manganese-hæmin is entirely incapable of giving any activity with peroxidase protein, though a spectroscopic change showed that a reaction, probably a coupling, occurred between Mn-protoporphyrin and protein. The light absorption, measured in a Zeiss photometer, increased during the reaction in the blue (*S* 47) and yellow (*S* 57) regions.

It should finally be pointed out that contrary to our experience Gjessing and Sumner found Fe-hematoporphyrin to be active (max. 76 per cent) and Fe-mesoporphyrin to be even more active than Fe-protoporphyrin. The discrepancy is probably

due to the fact that Gjessing and Sumner worked with impure preparations.

HUGO THEORELL.

Medical Nobel Institute,
Department of Biochemistry,
Stockholm.
Sept. 4.

¹ Theorell, H., *Ark. Kemi, Min. och Geol.* (Stockholm), **14** B, No. 20 (1940).

² Theorell, H., Bergström, S., and Åkeson, Å., *ibid.*, **16** A, No. 13 (1943).

³ Gjessing, E. C., and Sumner, J. B., *Arch. Biochem.*, **1**, 1 (1942).

Vitamin C Peroxidase Activity

ENZYMATIC oxidation of vitamin C, first recorded in 1921¹ and made more specific since 1928 by Szent-Györgyi, has given rise to the hypothesis that the vitamin acts as a constituent of dehydrogenating enzymes. However, so far as we know, direct evidence of the enzymatic activity of ascorbic acid has not been given. Our results show that vitamin C, acting as a peroxidase or as an antioxidant, can increase or reduce the already known peroxidase action of ionized copper² and iron³. The experiments now reported consist in a comparison between the extinction coefficients of solutions, *A*, that contain an oxidizable substrate, ascorbic acid, hydrogen peroxide, copper sulphate and ferric chloride dissolved in 10 c.c. water, and solutions, *T*, devoided of vitamin, but similar to *A* in every other respect.

The freshly prepared ferric chloride solution contains 9 mgm. citric acid to each 10 mgm. iron. The measurements of the extinction coefficients were carried out in the Pulfrich Stufenphotometer, 10 minutes after the starting of the reaction, and refer to a layer of 20 mm. The extinction coefficients in the spectral region of wave-length 470 m μ of the solutions *A* and *T* will be designed respectively by E_A^{470} and E_T^{470} , and the difference ($E_A^{470} - E_T^{470}$) and the ratio E_A^{470}/E_T^{470} by E_{AA}^{470} and *V*. E_{AA}^{470} is positive or negative and *V* greater or less than 1 according as the vitamin promotes or checks the oxidation; the molar (atomic for Cu and Fe) concentrations are expressed in 10⁻⁴ gm. per litre.

Action of the vitamin on phenol. Standard solution: phenol 2 mgm. (21 m.); ascorbic acid, 1 mgm. (6 m.); hydrogen peroxide, 3.1 mgm. (91 m.); Cu, 0.48 mgm. (8 a.); Fe, 0.01 mgm. (0.2 a.). (See Table 1.)

Solutions *A* are brown-yellow, except for solutions 2 and 5 which take a pink tint turning to brown. Solutions *T* are colourless or yellow.

Cresol (1) and tyrosin (2). Standard solution: (1) phenol is substituted by 2.29 mgm. (21 m.) cresol; (2) tyrosin 2 mgm. (11 m.); ascorbic acid 2 mgm. (11 m.); hydrogen peroxide 6.2 mgm. (182 m.) Cu 0.48 mgm. (8 a.); Fe 0.02 mgm. (0.4 a.). (See Table 2.)

Solutions *A* of *ortho*-, *para*- and *meta*-cresol take respectively a brown-orange, brown-yellow and pink coloration. Solutions *T* are colourless or pale yellow.

Benzidin: Standard solution: benzidin 2 mgm. (11 m.); ascorbic acid 1 mgm. (6 m.); hydrogen peroxide 3.1 mgm. (91 m.); Cu 0.48 mgm. (8 a.); Fe 0.01 mgm. (0.2 a.); acetic acid 10 mgm. per 1 mgm. of benzidin. (See Table 3.)

Solutions *A* develop immediately a brown reddish coloration. Solutions *T* first give a faint blue tint turning to reddish-brown.

According to colorimetric measurements of the red tinted chloroform extracts of solutions *A*₁ and *T*₁,