SOME LIMITATIONS OF WARBURG'S THEORY OF THE ROLE OF IRON IN RESPIRATION

THE construction of models of biological processes has occasionally contributed greatly to the knowledge of the mechanisms of vital phenomena. However, enthusiasm over the successful construction of a model that in part duplicates the reaction of living protoplasm often obscures the fact that the duplication is only partial and misleads the investigator into undue dependence on deductive reasoning. Caution must be observed in accepting theories of the organism derived from the behavior of models, for in attempting to isolate a single process in this manner the controls and correlations that distinguish the living from the non-living are lost. Obviously, the value of any theory derived from the behavior of a model depends on the extent of resemblance between the behavior of the non-living system and the facts and characteristics of the process of the living organism which it purports to simulate.

An examination of the characteristics of biological oxidations shows that the comprehensive theory of Warburg of the mechanism of oxidations in the cell,[1] based largely upon the characteristics of his so-called models of respiration, is not free from the criticisms that have been levelled at other theories of biological processes similarly derived from models. On the contrary, the divergence between Warburg's theory and the actual facts is wide enough to justify regarding the theory as distinctly limited in application.

Briefly, Warburg has developed a theory of cellular oxidations in which iron in unknown combination with nitrogen is held to play the rôle of catalyzer. Molecular oxygen is said to enter into combination with the iron to form higher oxides of iron, and the iron-nitrogen is assigned the property of adsorbing and peculiarly loosening the bonds of amino acids in the cell. In the oxidation of carbohydrates phosphates replace nitrogen, and in the oxidation of fats the presence of the SH group is necessary. A transfer of active oxygen is thus effected, and the iron is returned to a lower oxide. Unfortunately for the theory in its present form, the facts of cellular oxidations which Warburg cites in support of his theory are in some important details disputable.

The theory demands, and Warburg has shown in the case of the unfertilized sea urchin egg,[2] that the oxygen absorption of disintegrated cells is equal to that of intact cells. This is contradictory to sound evidence[3] which shows that the chief energy-releasing oxidations in the cell are profoundly depressed by mechanical destruction of the cellular structure. This contradiction between the findings of Warburg and those of others shows that the experimental result which Warburg uses in support of his general theory is not a general but a particular case. The peculiar structure of the cell has thus been shown to be of great importance in its energetics. Warburg himself years ago[4] demonstrated the importance of the cell boundary in regulating oxidative metabolism when he showed that dilute sodium hydroxide accelerates the oxygen consumption of the sea urchin egg without entering the cell interior.

According to Warburg, anesthetics decrease oxidations in the cell because they are adsorbed by the iron-nitrogen, and are therefore described as general negative catalyzers of this reaction. However, there is abundant evidence[5] that dilute solutions of many anesthetics accelerate oxidative metabolism. The powerful action of the cyanides in depressing oxidative metabolism, Warburg asserts, strongly supports his theory. The validity of this evidence is open to question. Within certain limits the depression of oxidative metabolism in cyanide solutions increases with increasing concentration of the cyanide, but, after maximum depression characteristic of the concentration has been reached, continuous exposure does not result in further depression of oxidations. Warburg holds that the action of cyanide in depressing oxidations in protoplasm is due to its combination with iron, converting it into a form incapable of transferring oxygen. He regards the reaction between the cyanide and the iron as stoichiochemical, and he shows that the activity of an iron-nitrogen model in oxidizing an amino acid is depressed 97 per cent. by the addition of M/1000 HNC. According to this, we should expect very complete extinction of oxygen metabolism in the presence of strong cyanide. The expectation is not realized. It has been shown that M/1000 and M/2000 KNC have approximately the same effect on oxygen consumption in

[1] Warburg, O., 1921, *Biochem. Zeitschr.*, cxix, 134; 1923, *ibid.*, cxxxvi, 266; 1923, *ibid.*, cxlii, 518; SCIENCE, n. s., 1925, lxi, 575.

[2] Warburg, O., 1911, *Zeitschr. f. physiol. Chem.*, lxx, 413; 1914, *Arch. f. ges. Physiol.*, clviii, 189.

[3] Fletcher and Hopkins, 1907, *Jour. Physiol.* xxxv, 247; Harden and Maclean, 1911, *Jour. Physiol.*, xliii, 34; Batelli and Stern, 1914, *Biochem. Zeitschr.* lxvii, 443; Lund, E. J., 1921, *Amer. Jour. Physiol.*, lvii, 336. See also discussion in R. S. Lillie's book, "Protoplasmic Action and Nervous Action," Chicago, 1923, page 52, *et seq.*

[4] Warburg, O., 1910, *Zeitschr. f. physiol. Chem.*, cxvi, 305.

[5] Lillie, R. S., 1916, *Biol. Bull.*, xxx, 311 and references; Buchanan, J. W., 1923, *Jour. Exper. Zool.*, xxxviii, 331 and references.

Planaria dorotocephala, and although M/1000 KNC is lethal within a few hours, there still remains an oxygen consumption of about ten per cent. of the normal to be accounted for.[6] In Planaria agilis and in certain molluscan tissues the remaining oxygen consumption after maximum depression by cyanide is twenty per cent. of the normal.[7] Suitably diluted solutions of the cyanides appear to accelerate metabolism and in one case at least relatively strong solutions are required to depress cellular respiration.[8] It is also true that an oxidative enzyme has been isolated, the activity of which is not appreciably affected by cyanide.[9] The wide discrepancy between the theory and the observed facts is obvious. Cyanide is therefore not a "specific negative catalyst" in the strict sense that Warburg's theory requires.

A slight recovery of oxidative metabolism in the presence of cyanide has sometimes been noted. This, Warburg says, is due to the oxidation of the cyanide itself by the catalytic action of the iron-containing substance. Since the cyanide is said to render the catalyst incapable of transferring oxygen, a reasonable doubt arises that a catalyst can be instrumental in oxidizing a substance that has rendered it incapable of catalyzing an oxidative reaction.

The writer has recently studied the recovery period after depression by KNC more intimately than has been done heretofore.[10] When Planaria are removed from a solution of KNC in which their oxygen consumption has been depressed fifty per cent., the oxygen consumption rises above the normal during the first hour after removal from the cyanide. The extent of rise above normal is considerable, is independent of the duration of depression of KNC and persists, with gradual decrement, for at least six hours. To bring Warburg's theory into alignment with these facts it is necessary to assume that the quantity of active iron or of oxidizable materials in the cell increases during the period of depression. However, the fact that the extent of rise in rate of oxygen consumption above the normal is independent of the duration of depression renders these assumptions exceedingly improbable. A third assumption is possible, namely, that the catalyst, after being freed from the cyanide, becomes excessively active. Concerning this possibility there is no information available.

Why anhydrous conditions are necessary for the action of the iron catalyst in commercial nitrogen-fixing processes or why the reaction is strongly inhibited by minute quantities of carbon monoxide and other impurities is unknown, despite the vast amount of research that has been expended. The oxidative reactions in the living organism go on in a much more complicated system. Warburg's emphatic postulation regarding the action of cyanide on the system may be considered premature.

From the effects of cyanides on oxidation in living systems it is perfectly clear that the resemblance between Warburg's models and the oxidative mechanisms in the cell is distinctly limited. As Warburg states, the idea that iron is of importance in oxidative metabolism is not new. Warburg's results have yielded suggestions as to the possible nature of the rôle of iron. However, the universality of iron in biological oxidative mechanisms is not proven, and his theory as at present formulated is quite inadequate to explain many of the facts that are associated with changes in rate of oxidations in the living organism.

J. WILLIAM BUCHANAN
OSBORN ZOOLOGICAL LABORATORY,
YALE UNIVERSITY

6 Hyman, L. H., 1919, *Amer. Jour. Physiol.*, xlviii, 340.

7 Allen, G. D., 1919, *Amer. Jour. Physiol.*, xlviii, 93; Gray, J., 1924, Proc. Roy. Soc., Ser. B, xcv, 95.

8 Hyman, L. H., 1919, *Amer. Jour. Physiol.*, xlviii, 340; Townsend, 1901, Md. Agri. Exper. Sta. Bull., No. 75, 183; Lund, E. J., 1918, *Amer. Jour. Physiol.*, xiv, 365; 1921, *ibid.*, lvii, 336.

9 Dixon and Thurlow, 1925, *Biochem. Jour.*, xix, 672.

10 Buchanan, J. W., 1926, *Jour. Exper. Zool.*, xliv, 285.

SPECIAL ARTICLES

VISIBLE RADIATION FROM EXCITED NERVE FIBER: THE REDDISH BLUE ARCS AND THE REDDISH BLUE GLOW OF THE RETINA

THERE is a singular phenomenon—one of the countless interesting entoptic phenomena discovered by Purkinje[1]—which has remarkable consequences. In a perfectly dark room you give yourself a band of red light—any light of the spectrum, and white light as well, will give the phenomenon but it is rather more easy to obtain with red light. What you will see is not only the band of red light, but also stretching out from it on both sides big slightly reddish blue arcs—the bigger the further away you stand. They are not of the color of the rod pigment (visual purple), which is of a slightly bluish red. The angular size and the shape of these reddish blue arcs make

1 Purkinje: "Beobachtungen und Versuche zur Physiologie der Sinne," 1825, ii. 74. This rare work of Purkinje—so rare that Gertz reproduces the whole discussion which Purkinje gives of this phenomenon because so few of his readers will be able to see his book—has now been reproduced in Czecho-Slovakia: Purkinje, Johann Evangelista, *Opera Omnia*. Praze: C. Calve, 1919. My name for this phenomenon, "the reddish blue arcs" and "the reddish blue glow of the retina," has been very generally accepted.