CCXXXI. THE INFLUENCE OF VITAMIN C ON INTRACELLULAR ENZYME ACTION.

By ARNULF PURR.

From the Cancer Research Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

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The discovery of glutathione by Hopkins [1921], and the recognition that it is the natural activator of certain intracellular proteolytic enzymes in animals as well as in plants [Waldschmidt-Leitz *et al.*, 1930; Grassmann *et al.* 1930; 1931], has established a relationship between these enzymes and the oxidation-reduction processes in the cell. In the ascorbic acid (vitamin C), isolated and crystallised from suprarenal gland, oranges and cabbage, Szent-Györgyi [1928; 1932; 1933] has recently found another constituent of the cell which assists in determining the oxidation-reduction potential and which may be reversibly oxidised or reduced.

Ascorbic acid in the organism is not limited solely to antiscorbutic action but has a still more comprehensive rôle. By its reversible oxidation and reduction, it is indirectly related to cell respiration and is also a determining factor in establishing the equilibrium between SH and SS compounds. The vitamin assumes a protective rôle against the oxidation of SH compounds in the organism[1]. The physiological significance of these two substances (glutathione and ascorbic acid) as catalysts of oxidation-reduction processes has been considerably increased through the discovery that the activity of certain intracellular enzymes is dependent on the presence of definite oxidation-reduction potentials.

Arginase is one of the most important enzymes of intermediary protein metabolism, whose activity, according to Waldschmidt-Leitz *et al.* [1933] and Edlbacher *et al.* [1925; 1927; 1932; 1933] is dependent upon oxidation-reduction potentials. This enzyme is also activated by the system, ascorbic acid *plus* iron as is shown by the following experiment.

Example. 0·25 cc. glycerol-liver-suspension (1:10), 4·5 cc. water, 10 cc. 1 % arginine carbonate, 5 cc. 0·1 *N* glycine buffer (p_H 9·5), incubated 60 minutes at 30°; addition, 20 mg. cysteine-HCl, previously neutralised, or 20 mg. crystalline ascorbic acid (prepared from oranges), or 0·5 cc. 0·1 *N* $FeSO_4$; incubated 60 minutes, p_H 7.

Addition	Arginase activity (cc. 0·02 *N* NH_3)
None	7·0
Iron	8·0
Cysteine-iron	15·0
Ascorbic acid (crystal pulp)	12·0
Ascorbic acid-iron	16·0

[1] This may explain the high concentration of sulphydryl compounds in the suprarenal gland.

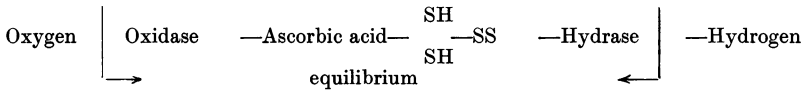
It is known that intracellular proteolytic enzymes of the cathepsin type require the presence of sulphydryl compounds for their activation. Ascorbic acid, by virtue of its oxidation-reduction potential, also apparently regulates the relation of SH to SS as is shown by these experiments. The decomposition of proteins is thus dependent on the presence of vitamin C. The conception, therefore, of a regulation of the intracellular protein metabolism by the interaction of these two substances, glutathione and ascorbic acid, in the sense of hydrolysis on the one hand and of synthesis on the other, possesses a physiological significance. It remains to be determined [*cf.* Grassmann *et al.* 1931] whether sulphydryl compounds alone are responsible for the activation of the catheptic enzyme systems.

Example. 5 cc. carcinoma-glycerol suspension (1:10), 0·40 g. gelatin, incubated 24 hours at 30°, p_H 4·0, total volume 25 cc. Results given are for 10 cc. of the mixture.

Additions	NH_2 increase (cc. 0·05 *N* KOH)
None	0·75
Cysteine (20 mg.)	1·70
Ascorbic acid (20 mg. crystal pulp)	1·60

There is scarcely a doubt that, in addition to the catheptic enzymes and arginase, other intracellular enzymes require the participation of these two substances for their activation. Among the enzyme groups which we are investigating from this point of view in our laboratories, methylglyoxalase is of particular interest. If it should turn out that the action of this enzyme is related to the oxidation-reduction potential in the cell, a new explanation would be found for the excess glycolysis in anaerobically developing cells, *e.g.* in malignant tumours.

These findings, by which for the first time a relationship is established between a vitamin and the intracellular enzymes of metabolism, are of particular significance for the question of the cause of autolysis in dying cells, which plays such an important part in malignant tumours. The much disputed question of the formation and occurrence of sulphydryl compounds in living and dying cells is perhaps plausibly explained by the following working hypothesis for respiration.



The solution of this question in connection with the activation relationships of intracellular enzyme systems, particularly those of the carbohydrate-degrading enzymes in healthy muscle and malignant tumours, will be the object of further experiments.

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