A Note on the possible Relationship between Ascorbic Acid and Glutathione *in vivo*

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In view of the fact that both glutathione and ascorbic acid belong to a redox system, it was thought that it might be of interest to determine the concentrations of both substances simultaneously in the same human subject during an extended 'saturation' test with ascorbic acid.

METHODS

Ascorbic acid in whole blood. Whole blood determinations were made by a slight modification of the method of Deeney, Mürdock & Rogan [1942]. Pure CO was used for conversion of the haemoglobin to carboxyhaemoglobin. 2 ml. of blood were diluted with 4 ml. of glass distilled H₂O and 4 ml. of 16% HPO₃ used for precipitation. It was found impossible to remove all the suspended material by centrifugation on account of the presence of the octanol. After centrifuging, the solution was passed through a 5 cm. No. 41 Whatman filter paper which readily removed the large suspended particles.

Plasma ascorbic acid. 4 ml. of plasma were treated with 16 ml. of 3% HPO₃ solution as soon as possible after withdrawal of the blood. After centrifugation, 5 ml. portions

of the clear supernatant fluid were titrated with a 0.01% solution of 2:6-dichlorophenolindophenol.

Glutathione in blood and plasma. The method of Woodward & Fry [1932] was employed. The analyses were carried out immediately after withdrawal of the blood and the appropriate corrections were made for the ascorbic acid content of the blood or plasma.

Two in-patients, W. (a male) and J. (a female) and one of us, V., were employed. No attempt at any kind of selection was made except that the third subject was chosen to give a range of initial plasma ascorbic acid values of 0.08, 0.26, 0.80 mg./100 ml.

A daily dose of 700 mg. of ascorbic acid for each 140 lb. of body weight was given as nearly as possible in two equal portions, one at the commencement of each 24-hr. collection of urine, the other approximately 12 hr. later. The dose was continued even when a 'state of saturation' had been reached. Blood samples were taken either at the end of the 24 hr. period, before the first dose of the subsequent 24 hr., or if no dose was given, at least 3 hr. after taking food. Blood samples in which the concentration of ascorbic acid was required were drawn into tubes containing 0.1 ml. of 20% potassium oxalate solution and 0.05 ml. of 5% KCN. The investigation was continued in subjects J. and V. after the administration of synthetic ascorbic acid had ceased for 3 and 7 days respectively.

Table 1. *Concentration of ascorbic acid and glutathione in the blood during and after an extended ascorbic acid saturation test*

Date	Ascorbic acid given orally mg./day	Ascorbic acid (mg./100 ml.) in		Reduced glutathione (mg./100 ml.) in			Oxidized glutathione (mg./100 ml.) in		Millimoles of	
		Whole blood	Blood plasma	Whole blood	Red cells	Blood plasma	Whole blood	Blood plasma	Ascorbic acid in blood plasma	Reduced glutathione in red cells
W. aet. 55. Male. Haematocrit 27.1%. Wt. 120 lb. ?Cirrhosis of liver										
30. x. 42	0	—	0.08	37.1	137	2.5	4.0	0	0.0045	4.46
1. xi. 42	600	—	—	—	—	—	—	—	—	—
4. xi. 42	600	—	0.88	35.5	131	2.8	5.2	0	0.0500	4.27
9. xi. 42	600	—	1.08	31.3	116	2.8	0	8.6	0.0613	3.78
23. xi. 42	600	—	1.07	30.7	113	3.7	3.1	2.1	0.0608	3.68
J. aet. 26. Female. Haematocrit 35.0%. Wt. 122 lb. Abdominal sinuses										
2. xi. 42	0	—	0.26	22.4	64	2.7	3.1	2.1	0.0147	2.08
10. xi. 42	600	—	—	—	—	—	—	—	—	—
11. xi. 42	600	—	0.98	22.4	64	1.5	4.9	5.5	0.0557	2.08
23. xi. 42	600	—	1.33	16.9	48	1.2	8.3	6.4	0.0756	1.56
27. xi. 42	0	—	—	—	—	—	—	—	—	—
30. xi. 42	0	—	1.12	18.4	53	0	0.9	6.1	0.0636	1.73
V. aet. 40. Male. Haematocrit 46.0%. Wt. 158 lb. Normal										
23. xi. 42	0	0.82	0.80	35.0	76	0	4.6	4.4	0.045	2.48
24. xi. 42	750	—	—	—	—	—	—	—	—	—
25. xi. 42	750	1.53	1.53	30.1	65	0	7.6	1.5	0.087	2.12
30. xi. 42	750	1.48	1.48	28.8	63	0	3.1	3.4	0.084	2.05
1. xii. 42	0	—	—	—	—	—	—	—	—	—
7. xii. 42	0	1.08	1.02	31.9	69	0	2.5	5.2	0.058	2.25

RESULTS

The data (Table 1) show: (1) In all three examples the maximum ascorbic acid level is associated with a decreased concentration of glutathione in the red blood corpuscles, viz. 18, 24, 26 % of the initial values in W., J., and V. respectively. (2) The plasma ascorbic acid content rises when the reduced glutathione in the red cells falls. Statistical analysis of the data by Mr H. T. Russell of the London School of Hygiene and Tropical Medicine showed that the values for plasma ascorbic acid and red cell reduced glutathione exhibited a negative correlation which is highly significant. (3) In the subject V., whole blood and plasma ascorbic acid varied equally in response to the test dose, although many authors find that the passage of ascorbic acid into the red blood corpuscle is slow [Borsook, Davenport, Jeffries & Warner, 1937].

DISCUSSION

Calculations from the data obtained show that the ratio of the fall in glutathione in the red blood corpuscles to the rise of the plasma ascorbic acid is approximately 10 mol. to 1 (variation 8–12). Further, in subjects J. and V. when the plasma ascorbic acid fell at the termination of the 'saturation' experiment, the rise in glutathione was of the same molecular order.

Possible relationships between ascorbic acid and glutathione and their interdependence as oxidation-reduction systems have been demonstrated by Hopkins & Morgan [1936] and by Crook & Hopkins [1938], who showed that reduced glutathione (GSH) prevented the oxidation of ascorbic acid *in vitro* even in the presence of its oxidase. Later Crook [1941], working with plant extracts, characterized a reductase which catalyzed the reduction of dehydro-ascorbic acid by GSH, and so was able to complete the system—plant substrate, dehydrogenase, GSH, reductase, dehydro-ascorbic acid and ascorbic acid oxidase—which allows the O_2 ultimately to bring about oxidation of the substrate. That dehydro-ascorbic acid is reduced in the body is well known [Johnson & Zilva, 1934], whilst Borsook *et al.* [1937] presented evidence that it was the GSH that was responsible for the reduction of dehydro-ascorbic acid in minced tissues. It is noteworthy that in order to obtain complete reduction a large molecular excess of GSH was required. Further evidence of the importance of the system

in the body, and a suggested role in the formation of bile pigments, is that put forward by Lemberg, Legge & Lockwood [1941], autoxidation of oxyhaemoglobin to choleglobin occurring in the presence of ascorbic acid in turn reduced by GSH. Schultze, Harrer & King [1939] found no H_2 transfer to occur in a system consisting of nicotine haemochromogen, ascorbic acid, co-enzyme, and glucose with glucose dehydrogenase. Oxidation of ascorbic acid in the presence of Cu is inhibited by GSH [Barron, Barron & Klemperer, 1936; Mawson, 1935].

The relationship of the ascorbic acid and GSH levels in the blood established by us is additional evidence for the interplay of these systems. There are also indications that the fall in the GSH is accompanied by a rise in the oxidized glutathione (GSSG). It is tentatively suggested that one of the possible reasons why scurvy takes such a long 'latent period' to become manifest on a scorbutic diet is that in part the GSH in the tissues may tend to counterbalance the functional diminution consequent on the lack of ascorbic acid. The difficulty of ascribing a role to ascorbic acid in respiratory catalysis, on the grounds that tissues deficient in ascorbic acid fail to show decreased respiration [Elvehjem, 1942], may also, at least in part, be removed.

Our results for the glutathione content of whole blood are comparable with those of Woodward & Fry [1932] and of Platt [1931], although the highest values obtained for subject J. are rather below the lowest normal limit given by any of these authors (25 and 30.7 mg./100 ml. respectively). J. showed some degree of anaemia (Hb 56 %), but the GSH content of the red cells is not in excess of that of V., a normal subject, a result which is not in agreement with the findings of Platt [1931] in anaemia, nor with our results in subject W. (Hb 52 %).

SUMMARY

1. The concentration of glutathione in the red blood corpuscles of the human being varies inversely with that of the plasma ascorbic acid. Calculations show that the molecular change is approximately 10 to 1.
2. A possible role of glutathione in the long 'latent period' for scurvy is suggested.

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Lysine Deficiency in Young Rats

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'Essential' amino-acids share with vitamins the property that they cannot be synthesized in the mammalian body and have therefore to be supplied in the diet. They differ from vitamins, however, in several other respects. Whilst vitamins are present only in small quantities in the tissues, amino-acids combined in proteins form a large part of the total mass of the body. The quantitative requirements for essential amino-acids are therefore considerably greater than those for vitamins. On the other hand, the body has a great store of amino-acids in its proteins, and the deleterious effects of a dietary deficiency of a particular essential amino-acid will therefore generally be less sudden and less marked, at least in the adult animal, than those of most vitamin deficiencies.

The clinical picture of a particular amino-acid deficiency will greatly depend on the biological function of the amino-acid concerned. If an amino-acid is needed only as building material for the synthesis of protein, a lack of that amino-acid in the diet will result only in an inhibition of protein synthesis and the effect will be more marked in the young animal than in the adult. The severity of the symptoms will depend on the quantitative requirements of the body relative to its stores of that amino-acid, and also on the extent of irreversible oxidation which occurs during the constant breaking-down and building-up of proteins in the mammalian body [Schoenheimer & Rittenberg, 1940]. This loss, which corresponds to the wear and tear quota of Rubner, may vary considerably for different amino-acids, and the effects of deficiencies on weight and nitrogen equilibrium may vary accordingly.

Some amino-acids, however, are apparently needed not only for the synthesis of proteins, but also for some specific metabolic purpose. For example, methionine acts as a donor of methyl groups and its absence from the diet produces specific symptoms peculiar to this amino-acid. Again, valine deficiency produces characteristic nervous symptoms [Rose & Eppstein, 1939] which suggest a special metabolic role for that amino-acid in the central nervous system. The deficiency or lack of such amino-acids will produce a clinical picture peculiar to the amino-acid concerned and will be superimposed on the general picture of deficiency common to all essential amino-acids which may be called a 'structural' deficiency.

This paper is concerned with lysine deficiency. Lysine was shown to be indispensable for the growth of young animals by Osborne & Mendel [1914; 1916], who also showed that animals could be kept alive for long periods and at fairly constant weight on diets containing very small amounts of lysine. Lack of lysine in young animals produces no specific symptoms apart from cessation of growth, and it has been claimed that it is not essential for maintenance in adults [Burroughs, Burroughs & Mitchell, 1940].

The animal kept at a constant weight by the lysine-deficient diet is, however, not static as regards the development of individual tissues and organs. Its body and tail lengths increase and so do the leg bones [Winters, Smith & Mendel, 1927], and many organs such as the kidneys and the eyes increase in weight, whilst others, like muscle, decrease [Lafon, 1939]. Male genital organs are not affected by lysine deficiency, but in females the