XXXII. REVERSIBLE OXIDATION OF ASCORBIC ACID BY MEANS OF NORITE CHARCOAL.

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DURING the course of some work on the excretion of ascorbic acid in urine, using the method of measurement described by Birch *et al.* [1933], difficulty was sometimes experienced in obtaining sharp end-points when dealing with highly coloured specimens. In an endeavour to overcome this recourse was had to norite charcoal, and it was then found that although the urine was successfully decolorised, it no longer reduced the indophenol. That the ascorbic acid had been converted into the reversibly oxidised form, dehydroascorbic acid, by means of the norite is shown by the following experiments.

Exp. 1. 100 ml. of filtered orange juice had a titration value of 2.3 ml. per ml. with 0.05% indophenol solution. On shaking with 2.5 g. norite and allowing to stand for about 15 min. it was found on filtering that the reducing capacity had been entirely lost.

Exp. 2. 100 ml. filtered orange juice, on treatment with 0.4 g. norite, lost part of their reducing capacity in the first 10 min.; prolonged contact with the norite did not, however, lead to any further change in the titration value.

Exp. 3. All attempts to elute the vitamin from the norite having failed, the filtrate was examined. On passing hydrogen sulphide through the solution and then clearing it by treatment with carbon dioxide for 20 min., about 90% of the original titre was regained. This property and the stability of the solution so obtained are shown in Table I.

Table I. *Oxidation and subsequent regeneration of ascorbic acid in orange juice.*

Exp.	Mixture	Treatment with norite		Regeneration of ascorbic acid		
		Titration with 0.05% indophenol		Method	Titration with 0.05% indophenol	% recovery
		Before norite treatment	After norite treatment			
1	225 ml. to 10 g. norite	3.0	0.02	Reduced with H ₂ S and excess removed with CO ₂	2.6	87
2	200 ml. to 12 g. norite	2.6	0.01	(a) H ₂ S and CO ₂ as above	2.3	89
				(b) Saturated with CO ₂ . Kept on ice 4 days then treated with H ₂ S and CO ₂ as before	2.1	80
3	200 ml. to 12 g. norite	2.2	—	(a) H ₂ S and CO ₂	1.8	82
				(b) As 2 (b)—on ice 3 days	1.2	55
4	220 ml. to 10 g. norite	2.7	0.02	(a) H ₂ S and CO ₂	2.2	81
				(b) As 2 (b)—on ice 2 days	1.8	67
				(c) After 14 days on ice	0.6	—

Exp. 4. The effect of varying the amount of norite used is shown in Fig. 1. It will be seen that only for small quantities is the effect directly proportional to the amount taken.

Fig. 1. Effect of varying quantities of norite charcoal on conversion of ascorbic acid into dehydroascorbic acid.

Exp. 5. The relative values of different kinds of charcoal in bringing about this reaction were examined and it was found that, whereas bone and wood charcoal had little effect, blood charcoal was fairly active, though not as good as norite.

Very little has appeared in the literature regarding the oxidising powers of charcoal suspensions, but attention is drawn to the following papers.

Feigl [1921] has shown that many substances can be oxidised by treatment with blood charcoal, either in acid or alkaline solution. Warburg and Negelein [1921] have estimated quantitatively the oxidation of cysteine and other amino-acids when placed in contact with blood charcoal. Rideal and Wright [1925] describe the oxidising action of blood charcoal on certain dicarboxylic acids, various amino-acids *etc.* and state that substances with only one polar group, *e.g.* alcohol, formic acid and the higher fatty acids, are not attacked, but that those with adjacent polar groups, one of which may be a mobile hydrogen atom, are readily oxidised. This latter observation would account for the oxidation of ascorbic acid. Finally, Fürth and Kaunitz [1929] have shown that inactivation of the charcoal occurs during the process of oxidation of amino-acids, and that addition of fresh charcoal renews the oxidation. This finding, taken in conjunction with *Exp. 4* strongly suggests that the reactive oxygen is that occluded by the charcoal.

SUMMARY.

1. Norite charcoal rapidly converts ascorbic acid into dehydroascorbic acid, with very little loss by further oxidation to the irreversible stage. The dehydroascorbic acid prepared in this way remains fairly stable for several days if saturated with carbon dioxide and stored on ice.

2. Since with small quantities of norite the reaction is rapid, short-lived and proportional to the amount used, it is evidently not of the same type as that brought about by traces of iron or copper [Kellie and Zilva, 1935; Mawson, 1935]. Most probably it is a case of direct transference of oxygen occluded on the surface of the charcoal.

3. Attention is drawn to the simplicity of this reaction and its value where mild oxidation is required, unaccompanied by undesirable by-products.

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