activity. (3) Hexuronic acid after various repurification processes maintained its original activity. (4) The hexuronic acid contents of numerous natural sources were then determined chemically (by the method described below) and were found to account precisely for their known antiscorbutic activities. (5) In the case of several materials it was also possible to estimate the hexuronic acid content by intensity of absorption at 265 mμ (carried out by W. J. Dann), and this result was in further agreement with the biological or chemical tests. (6) The destruction of hexuronic acid by heat and aeration as measured chemically was found to proceed at a rate similar to that of vitamin C activity. (7) In the guinea pig the antiscorbutic activity of the suprarenal (or liver) was lost with the development of scurvy and this coincided with the disappearance of hexuronic acid. (8) With other species, the rat or dog, which are able to synthesise their own vitamin when none is provided in the diet, the antiscorbutic activity and the hexuronic acid both remain unaffected.

A rapid micro-chemical method has been devised for estimating the hexuronic acid content of foods, involving preliminary extraction with trichloracetic acid and titration against the oxidation-reduction indicator, 2, 6–dichlorophenolindophenol in acid solution. Certain naturally occurring reducing reagents, which were found to react when the reducing capacity of foodstuffs was determined according to the Tillmans technique in more neutral solution, did not interfere by this method (special precautions, however, being necessary in the presence of adrenaline). Sensitivity is about 1 part in 30; an amount of vitamin C represented by 0·03 c.c. of orange juice suffices for an accurate assay; and the determination requires only a few minutes to carry out. Results coincide with those obtained biologically. Values below are expressed as the 'minimal protective day dose' for guinea pig (figures in brackets being the determined or reputed values as measured biologically):

Cabbage 0·9 (1); watercress 1·3 (1); lemon juice 1·5, 1·5 (1·5); orange juice, several types 1·2, 1·5, 1·9 (1·5); grape fruit juice 1·4, 1·5 (1·5–2); pineapple juice 3 (2–3); imported tomato, juice of 4·3 (3–5); banana 6 (5–10); potato 6 (6–10); rhubarb 15 (12); carrots 32 (10–35); grapes 30 or above (> 20, 40); imported peach, juice of > 60 (–).

Horse-radish 0·6 (–).

Apples:—Bramley's seedling, cortex 5·5 (3–5), peel 1·2 (1); Newton, cortex 17 (10), peel 3·7 (3); Blenheim orange, cortex 29, peel 2·7; Edward VII, cortex 53 (> 20), peel 7·5 (2 ?); Cox's orange, cortex 56 (> 20), peel 10 (–).

Ox suprarenal cortex 0·5 (0·5); ox liver 1·3 (< 3); cows' milk 47 (20–60); "Ostermalt" 3·3 (–).

The exact antiscorbutic activity of hexuronic acid has been determined by several alternative methods (curative, tooth structure and preventive): 1·0 c.c. orange juice = 0·5 ± 0·15 mgm. hexuronic acid (or best values = 0·6 mgm.). We recommend its adoption as an international standard.

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The conclusion having been reached that vitamin C is identical with hexuronic acid, it becomes of obvious importance in the first place to see how this can be reconciled with Zilva's contention[1] that there is a lack of parallelism between antiscorbutic activity and reducing capacity—one of the most characteristic properties of hexuronic acid. Fig. 1 summarises all the data given by Zilva, *mean* values for antiscorbutic potencies being plotted at each level of reducing capacity. Bearing in mind

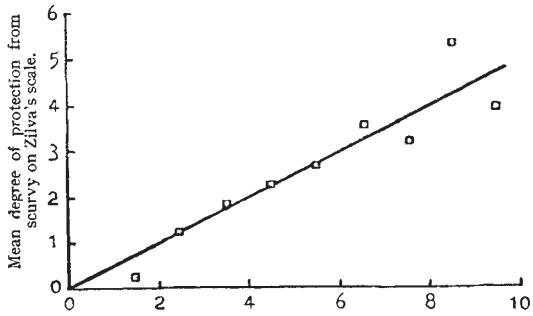

Fig. 1. Relation between reducing capacity and antiscorbutic activity.

the unavoidable variation in biological response between individual experimental animals, it becomes evident that Zilva's data, once they are analysed in this way, appear in fact to afford good evidence of a close quantitative relation between reducing capacity and antiscorbutic activity in lemon juice fractions, and therefore are not at variance with the hypothesis that hexuronic acid and vitamin C are identical.

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Feb. 10.

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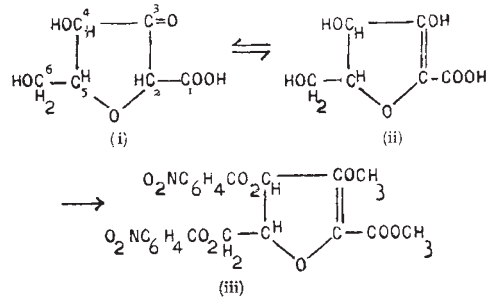
[1] Zilva, *Biochem. J.*, **26**, 1624; 1932.

Constitution of Vitamin C

In our first communication[1] we proposed the formulation of vitamin C as a furane- or cyclopentane-derivative. In consideration of further investigations which we have made, we now consider the furane type of structure (i and ii) well established. This conclusion rests upon the following observations; neither the dimethylvitamin nor the di(nitrobenzoyl)-dimethylvitamin (iii) contains a carbonyl group. The



double bond in (iii), which does not react with bromine or with permanganate, is attacked by ozone to give a neutral product, melting point 162°, which does not react with ketone reagents. Its composition is