Ascorbigen in Plant and Animal Tissues

WE have previously given evidence^{1,2} to show that cabbage contains ascorbic acid in a combined form from which ascorbic acid is released on heating. Chloroform extracts of dried cabbage, which were quite free from ascorbic acid as shown by the indophenol indicator, developed a marked dye-reducing value on heating. Treatment of cabbage with hydrogen sulphide in the hot and cold conditions also led to the same conclusion. Further, it was found by treatment with ascorbic acid oxidase, that 60–70 per cent of the indophenol reducing value of the chloroform extract of cabbage is to be attributed to combined ascorbic acid³. This work showed incidentally that, apart from combined ascorbic acid, which we called 'ascorbigen'⁴, cabbage contains some non-specific reducing substance also in a combined form.

Biological experiments with guinea pigs confirmed the view that 60–70 per cent of the indophenol-reducing value of the dried chloroform extract of cabbage was due to ascorbigen. The guinea pigs were fed on a scorbutic diet and when they were falling in weight one group was given supplements of ascorbigen and the other group was fed with equivalent doses of ascorbic acid (varying from 2 mgm. to 4 mgm. in terms of ascorbic acid). The rates of growth in the two groups during the next ten days were comparable. Similar results have just been reported by Reedman and McHenry⁵, who have given biological evidence for the presence of ascorbigen in potato. They have, however, fed the experimental guinea pigs with ascorbigen and ascorbic acid from the start of the experiment; but their results and ours are substantially in agreement. It has been possible to purify the ascorbigen of cabbage very considerably by extracting dried cabbage with chloroform, treating the dried chloroform extract with water, re-extracting with chloroform, again extracting with water and precipitating with alcohol. The substance is under further investigation.

We have observed the presence of ascorbigen and of some non-specific reducing substance in a combined form in the brain and liver tissues of the normally fed guinea pig. Similar results have also been obtained with urine by Mr. S. Bannerjee in this laboratory, which are confirmatory of the findings of Scarborough and Stewart⁶. It thus appears that the distribution of ascorbigen and some non-specific reducing substance in a combined form in plant and animal tissues is fairly general, though perhaps not universal.

In any estimation of ascorbic acid from the nutritional point of view, the foodstuff should be evaluated with reference to (1) free ascorbic acid, (2) dehydroascorbic acid, and (3) ascorbigen, as we pointed out elsewhere⁷. For this, a method has been developed involving treatment of the material with hydrogen sulphide in the hot condition, removing hydrogen sulphide in a current of carbon dioxide and treatment of an aliquot with ascorbic acid oxidase. This method appears to give the 'total' ascorbic acid of foodstuffs.

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² Guha and Pal, *NATURE*, 139, 844 (1937).

³ Guha and Sen-Gupta, *Science and Culture*, 3, 59 (1937).

⁴ Pal and Guha, *Proc. Biochem. Soc., Calcutta*, 3, 8 (1936–37).

⁵ Reedman and McHenry, *Biochem. J.*, 32, 85 (1938).

⁶ Scarborough and Stewart, *Biochem. J.*, 31, 2232 (1937).

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Action of Anol and its Derivatives on the Nipple of the Guinea Pig

DODDS and Lawson¹ have stated that anol will induce oestrus in rats. W. Jadassohn, Uehlinger and Margot² have investigated whether anol will cause enlargement of the nipples of guinea pigs by simple external application of the solution of this substance (nipple test with anol). The result was positive although not so strong as with oestrone. Later, Dodds and Lawson³ found that pure anol in not very large doses failed to induce oestrus. We reinvestigated the action of pure anol in the nipple test and found it to be completely negative. (A solution of the same anol prepared a few months later showed a positive nipple test.)

We have examined by means of the nipple test substances which may have been responsible for the activity of the 'active anol solutions', that is, derivatives of them.

The demethylation of anethol, by means of a Grignard reaction (Serini and Steinruck⁴) gave rise to an anol that contained polymerization products; this polymerized material after acetylation boiled at 180–210° under 0.5 mm. pressure. Similar experiments were also made with isoanol⁵ and its acetyl derivatives prepared by heating isoanethol with potassium hydroxide in a sealed tube under pressure (N. R. Campbell, E. C. Dodds and W. Lawson⁶).

All these preparations were active in the nipple test. However, the application of oestrone solution in a concentration of 0.003 γ per c.c. gives a definite effect, whereas these preparations in a concentration of 1 γ per c.c. were quite inactive; 10 γ per c.c. was weakly active and 60 γ per c.c. showed a strong effect.

So far as the nipple test is concerned, we must refer to our previous publications^{2,7,8}. For the present, however, we shall briefly recall its principle. We have developed a simple method of measuring the enlargement of the nipple (male guinea pig). We have found that external application of very small doses of female sex hormones produces such an enlargement. This method has proved to be specific and quantitative.

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⁴ *Naturwiss.*, 682 (1937).

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'Climbing Organs' in Insects

MANY insects possess adhesive organs (pulvilli, tarsal or tibial pads, etc.) which enable them to climb on perfectly clean glass. Presumably these organs function whenever the foot comes in contact with any surface, such as leaves or polished stones or the bodies of other insects, too smooth for the claws to grip. Miller has suggested¹ that the 'fossula spongiosa' of adult Reduviids may have been