

## Identification of Vitamin C\*

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ABOUT a year ago vitamin C seemed to be the most elusive of vitamins, apparently defying all attempts at isolation and identification. To-day this substance can be bought at a relatively low price, neatly sealed in tubes, in the form of beautiful white crystals. It is even used as a reagent for reducing gold and silver in microscopical technique. Also its chemical constitution is cleared up to a great extent.

In spite of this, however, the development of our knowledge has not been so dramatic, and these results have been the outcome of laborious work extending over more than ten years. This vitamin was crystallised more than five years ago and was, so far as we know now, the first vitamin ever isolated in the crystalline form.

The development in our knowledge of vitamin C has taken place in four distinct steps. The first step was the observation of 'hexuronic acid' in 1927 and its subsequent isolation and partial identification at Cambridge in 1928. This observation and isolation in itself was the result of many years extensive studies on oxidation systems of plants and animals. I was led to these studies by the belief that the adrenal gland was in some way involved in oxidation processes. Before this function of the gland could be understood, more had to be known about oxidation itself. Thus oxidation systems were examined one by one, until a new oxidation system was brought to light in which the central rôle seemed to be played by an interesting substance with very striking chemical properties. This substance has been isolated and found to be a monocarboxy acid corresponding to the formula  $C_6H_8O_6$ . This formula, together with some qualitative reactions, suggested a relation with the hexuronic acid series. The substance was thus called 'hexuronic acid' and the oxidation system the 'hexoxidase system'. More recent results, however, obtained in Prof. Karrer's and Prof. Haworth's laboratories, do not bear out this relation to uronic acids and, I have proposed, in association with Prof. Haworth, to call it henceforth 'ascorbic acid'.

The most remarkable chemical property of this acid is its very high reducing power, unique among purely carbon compounds. This reducing power is the more remarkable, since the oxidation of the acid itself is reversible. It is in fact this reversible oxidisability by which the substance exerts its biological activity. It is oxidised and reduced alternately, giving off and taking up two atoms of hydrogen, thus acting as a hydrogen carrier between different parts of its oxidation system.

This same substance has been found in relatively large quantities in the adrenal cortex, of which it forms definitely a specific constituent.

The second step to the establishment of the chemical nature of vitamin C was the discovery of the antiscorbutic properties of ascorbic acid. The striking similarity in chemical properties and distribution of the acid and the vitamin has been evident since the discovery of the former. It was not, however, until the autumn of 1931 that, in collaboration with J. L. Svirbely at the Institute of Medical Chemistry at the University Szeged, I was able to find the necessary external condition for the test of the antiscorbutic activity.

Meanwhile evidence was accumulating that one of the reducing substances of plant juice and the vitamin were identical. Tillmans in Germany pointed out in his extensive studies the very close parallelism of both, not only in distribution, but also under different experimental conditions. In the end he arrived at the conclusion that the main reducing factor and the vitamin were identical. In the United States, C. G. King and his collaborators emphasised the reducing power and acidic nature of active fractions, while S. S. Zilva in England has also found many close parallelisms between reduction and antiscorbutic activity. This author, however, also found differences which have led him to believe that these factors are not identical.

Our first test, undertaken by J. L. Svirbely in the autumn of 1931, clearly brought out the antiscorbutic activity of 'ascorbic acid'. We were able to show, that not only had this acid a strong antiscorbutic activity, but also that the antiscorbutic activity of lemon juice corresponded to the activity of the quantity of ascorbic acid which it contained. Our results were not announced until March 1932, by which time the experiments were repeated three times with identical results.

At this time, March 1932, W. A. Waugh and C. G. King obtained crystals from lemon juice, which they thought to be identical with hexuronic acid and which seemed to have some action on the vitality of animals. Two months later the same authors were also able to present evidence for this identity and the antiscorbutic activity.

Our own experiments were checked at our request by S. S. Zilva and L. J. Harris and were confirmed. This evidence may be summarised by saying that all the preparations of ascorbic acid tested had strong antiscorbutic activity. The investigation had now arrived at a most difficult point. The question arose, whether the activity of the preparations was due to the ascorbic acid itself or to a contamination of the preparations with some more potent substance. The daily protective dose of ascorbic acid is 0.5-1.0 mgm. Should the crystals have contained 0.1 per cent of a substance, acting (as other vitamins) in doses of 1 $\gamma$ , their activity could be explained by this contamination. Our own substance employed in the test as well as the preparations of Waugh

\*Substance of a lecture given at the University Club, Szeged, on December 15.

and King had a melting point  $10^{\circ}$  lower than that of pure ascorbic acid, and thus certainly contained more than 0.1 per cent impurity. This question, whether the activity is due to a definite substance or to contaminations, applies to all research on vitamins and is usually one of the most difficult problems to solve.

There is, however, much circumstantial evidence in favour of the identity of vitamin C and ascorbic acid. The parallelism in distribution and behaviour under experimental conditions, and the quantitative correspondence of the antiscorbutic activity of plant juice and its ascorbic acid content, point in this direction. Harris has shown that this quantitative correspondence holds also for the adrenal cortex with its high ascorbic acid content. Svirbely has shown that the ascorbic acid disappears from the gland very readily on vitamin-free diet, thus demonstrating the inability of the animal to build up this substance. Svirbely has also shown that our ascorbic acid, prepared from the adrenal gland, retained its antiscorbutic activity after five recrystallisations. Work on purely chemical lines alone could give direct and definite evidence of the identity of the vitamin and ascorbic acid. Unfortunately, the great scarcity of the material rendered such work impossible. Fresh adrenal glands, a convenient source for preparations, were not available in the necessary quantity, and all our efforts to prepare ascorbic acid from lemons, oranges, tomatoes, or cabbages on a large scale have failed.

In the third step in our work these difficulties were overcome and the way opened for direct chemical work, providing definite evidence for the identity of vitamin C and ascorbic acid. This progress we owe to the discovery that local varieties of Hungarian red pepper (*Capsicum annuum*) contain the acid not only in relatively large quantities but also under conditions which make its isolation exceedingly simple. It is due to this fact that during the last few months Svirbely and I have been able to prepare a full pound of crystalline ascorbic acid for chemical studies. Unhampered thus by the lack of material, we could set out with L. v. Vargha to study derivatives of ascorbic acid, an investigation which has led to the preparation of a mono-acetone derivative. This substance seemed to be almost ideally suited for our purpose. It forms

big, beautiful crystals with great ease and is stable in anhydrous solution, from which it can be recrystallised without difficulty. After due recrystallisation the original acid can be recovered unchanged by simply boiling it in water for a few minutes. Since the acetone derivative does not hydrolyse in cold water immediately, it was found in the antiscorbutic experiment to be but moderately active. The ascorbic acid, recovered from the recrystallised derivative, was fully active, a definite evidence for the identity of ascorbic acid and the vitamin.

The last and perhaps the most difficult step towards the definite chemical identification of the vitamin is the establishment of the chemical constitution of ascorbic acid. The possibility of preparing ascorbic acid in quantity has also opened the way for the chemical analysis. It was a great privilege for my American collaborator Svirbely and myself to be allowed to supply material to Prof. Haworth at Birmingham and Prof. Karrer at Zurich for this analysis.

The work of the laboratories at Zurich and Birmingham has clearly brought out that ascorbic acid is, confirming my original findings, a simple substance, consisting of 6 carbon, 8 hydrogen and 6 oxygen atoms; it contains one carboxy group, but is, however, not a member of the uronic acid series, and thus is not a hexuronic acid. Two of the carbon atoms are present as C=O groups forming a keto or aldehyde group with strong enolisation. One carbon is present as  $\text{CH}_2$ . The remaining two carbon and oxygen atoms are present as alcoholic groups. In this way the essential features of the molecule are cleared up and there remains only to ascertain the relative position of these groups.

It is hoped that the fact that the vitamin is a specific constituent of the adrenal cortex, from which it disappears on vitamin-free diet, will open up many fascinating problems to research and may contribute to the deeper understanding of the relation of hormones and vitamins.

There is one aspect of these investigations on which I look with pride and gratitude. This work has been made possible only by the closest international collaboration. It has been helped by Dutch, English and American hospitality and generosity. At present it is aided by the Josiah Macy, Jr. Foundation.

### Geology in the Life of a Nation

THE Indian Science Congress held its twentieth annual meeting this year at Patna under the presidency of Dr. L. L. Fermor, Director of the Geological Survey of India. In his presidential address Dr. Fermor refers to some matters of general interest in the history of the Indian Science Congress, which is modelled on the lines of the British Association, and, like that body, meets annually at different towns.

The initial meeting that led to the formation

of the Congress was held in 1912 in the rooms of the Asiatic Society of Bengal and that Society has since taken the general responsibility for the organisation of the Congress year after year. Until 1931 the Congress had no permanent organisation, but a constitution was then adopted whereby it did become a continuous organisation under the title of the Indian Science Congress Association with a roll of permanent members—though the close association with the Asiatic Society of Bengal