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The Discovery and Chemistry of Vitamin C

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This gathering, to sharpen and deepen our appreciation of a great Scottish scientist, is made especially significant by meeting within the halls of his Alma Mater. We, as guests of the University of Edinburgh, count it a privilege to share in paying tribute to a renowned alumnus. And in like spirit, we share the hope that sons in future years will honour both themselves and James Lind by seeing as clearly and labouring as diligently as did he (Lind, 1753).

As a biochemist who has had a measure of fellowship with other students of vitamin C in relation to human health, comparative physiology, and food processing, I could not escape (even if I wanted to) a sense of inspiration and deepened friendships from being here.

Other speakers will review the early literature on scurvy, but beyond the reach of manuscripts, one point is intriguing to a student of nature: did the loss of one or more genes that permit biological synthesis of the antiscorbutic vitamin, without disrupting other structures essential to survival, occur only once, or more often in nature? Assuming a common heritage of man and other surviving primates, can it be that we, among all the higher forms of animals and plants, share the single successful experiment with guinea-pigs, in the process of evolution?

From the records of primitive man's wide knowledge of the necessity of antiscorbutic foods (e.g. the American Indian, as reported by Cartier (Biggar, 1924)),

it is clear that scurvy challenged the right to survival whenever man relied upon foods held in storage.

But it is to Lind's great credit that medical scientists adopted in principle two very important concepts—first, the value of control groups in nutrition research, and second, a reliable basis for the prevention of scurvy. The masterful way in which Lind reviewed and interpreted the literature of his day pertaining to scurvy, and designed experiments to find the truth, makes us charitable toward his lingering belief that moist salt air had much to do with the disease.

Unfortunately, neither McCollum (who was accidentally cured from scurvy when his mother gave him raw apple to stop his crying when a very small child), nor Funk (who isolated nicotinic acid while looking for vitamin B_1), worked with vitamin C. Otherwise vitamin C by all historical rights would have been 'vitamin A' and vitamin A would have been 'vitamin C'.

Despite the excellent report on protective foods by Kramer (1721, 1737), and Budd's (1841) clear suggestion that chemists would soon isolate the antiscorbutic substance ('essential element') from natural products, a key required for progress was not forged until Holst & Frölich (1907, 1912) reported their use of the guinea-pig as a means of measuring the nutrient that vanished so easily.

Studies of the chemical nature of vitamin C, guided by frequent animal assays, were continued through more than two decades by Zilva and his associates at the Lister Institute (Daubney & Zilva, 1926, Harden & Zilva, 1918; Hoyle & Zilva, 1927; Zilva, 1924*a*,*b*, 1925, 1927, 1928, 1929, 1930). Then a second group took up the trail under the guidance of Nikolai Bezssonoff (1921, 1931) in France. Both groups succeeded in preparing syrupy concentrates, based primarily on selective precipitation with lead acetate and fractional extraction with organic solvents. Sugars were removed by fermentation.

Later it became evident that many investigators had abandoned or failed to publish their work for various reasons. The two great hazards to progress were the instability of the vitamin and the difficulty of guiding the required chemical steps by biological assays.

For example, Karl Link at the University of Wisconsin prepared several grams of crude calcium ascorbate during the nineteen-twenties, while studying the reduction of nitrates in sprouting oats. But the work was carried no further when the Dean refused a research grant of a few hundred dollars to support bio-assays of the product. Dr E. B. Vedder, in the office of the Surgeon General, U. S. Army, obtained crude crystals of the vitamin during the nineteen-twenties (Vedder & Lawson, 1927) but he was transferred to another post before the work could be finished, so his completed isolation was delayed until late in 1932. Admiral Richard Byrd's enormous supply of powdered lemon juice taken on his Antarctic expedition (1933–5) was the remainder of a carload lot that the Eli Lilly Company had set aside after unsuccessful attempts to isolate the vitamin.

During a post-doctorate year (1926–7) with Professor Sherman at Columbia University, where special emphasis was placed on making vitamin bio-assays accurately, I decided to undertake the characterization of vitamin C as a part of

the graduate student training at the University of Pittsburgh. We followed three basic practices: (a) to assay all final fractions from each new series of laboratory tests, (b) to regulate very carefully the pH values of all solutions, and (c) to avoid, in so far as possible, exposure to copper and air.

While completing their thesis work (1927–9), H. L. Sipple (Sipple & King, 1930) and D. P. Grettie (Grettie & King, 1929) obtained increased yields with higher activity than had been obtained before, and in less time. Assay periods were shortened to 8 weeks instead of 90 days. The active material always behaved as a rapidly diffusible anion, even during electro-dialysis in a direct current (McKinnis & King, 1930). In agreement with Zilva, the molecular weight was found to be about that of glucose. There was no evidence of separation into more than one component at any time.

The results of these investigations were presented at the spring meeting of the American Chemical Society in 1929. Upon hearing the papers, Dr. E. C. Kendall mentioned that our material seemed to resemble the 'hexuronic acid' that had just been reported by Szent-Györgyi from Hopkins's laboratory at Cambridge University. The paper did not give sufficient detail to permit duplication in our experience or in the experience of two other laboratories. At Cambridge University that fall I reported our work in a seminar paper. Professor Hopkins somewhat excitedly invited me to his office after the seminar and asked whether I would venture a guess concerning the chemical identity of the vitamin. I replied that its properties and occurrence so far as known corresponded with the 'hexuronic acid' isolated in his laboratories. He explained at some length that L. J. Harris had called attention to the similarities between the acid and Zilva's preparations at their seminar during the preceding year and that he had sent a sample to Zilva in London. He was disturbed because Zilva had never reported the evidence of his tests, but had only told them that the product was not vitamin C. He mentioned, too, with his characteristic humour at a later time, that they made assays of different varieties of citrus fruits at Cambridge, as a return courtesy to the company that had generously given them large quantities of oranges for the preparation of hexuronic acid.

The next two students, F. L. Smith (Smith & King 1931) and J. L. Svirbely (Svirbely & King 1931) simplified the procedures further and reached an approaching constant activity in the summer of 1931, in the range of $\pm$ 1 mg/day, but without obtaining regular crystalline preparations. A remaining student, W. A. Waugh*, succeeded in obtaining crystalline preparations fairly regularly and with constant assay activity in the range of 0·5 mg/day in September 1931. Conscious of the relatively high intake required and the great number of earlier vitamin claims that had been in error because of contaminated crystals, we decided to re-check the procedures through another lead precipitation from aqueous alcohol in which the free

* Mr Waugh's previous research director had encouraged him to work with me, but had urged him not to work on vitamin C for his thesis because it was 'an obviously impossible task'. My own major professor had advised me, in 1918, that it would probably be unwise to take up a study of vitamins, because they would be of little interest or significance in America or Europe where food supplies were so good. Said he, 'If you were going to China or the Philippines, they would have some interest for a university man'.

acid was very soluble. The confirmatory assays (8 weeks after starting the tests) were just being completed and a journal paper was being prepared, when press reports told of the discoveries of Ottar Rygh (Rygh, Rygh & Laland, 1932) at the University of Oslo, in which he claimed to have isolated the antiscorbutic vitamin as methylnornarcotine, from four different sources and to have confirmed its identity by synthesis and assay (more active than our preparations). His claims were shocking, because they were in complete disagreement with our relatively consistent findings through a period of 4 years. We therefore carried our preparations through an additional recrystallization beyond the lead precipitation from alcohol and again repeated the assays without finding any basis for doubting our earlier conclusions (including negative results with methylnornarcotine).

Rygh's technical papers first became available during the re-test period. It was evident that he probably had made a gross error by introducing the real vitamin into his basal ration (either in 'boiled orange juice' or in 'an extract of sprouted grain') for all tests in which his animals had survived. There were other glaring inconsistencies.

We then submitted our paper for the spring meeting of the American Society of Biological Chemists (Waugh & King, 1932*a*,*b*) and sent another manuscript to *Science* (King & Waugh, 1932). A few weeks later in March, I received a letter from Dr. Svirbely (who had gone to Hungary to study with Szent-Györgyi in the fall of 1931*), in which he mentioned that they were just finishing their first assay in which animals grew satisfactorily and were protected from scurvy when given 1 mg/day of their crystalline 'hexuronic acid'. They were sending a report of the assay to *Nature* (Svirbely & Szent-Györgyi, 1932*a*). Other papers soon followed (Svirbely & Szent Györgyi, 1932*b*). Their later assays and several reports from other laboratories were all in essential agreement on the minimum protective level of 0·5 mg/day.

Perhaps the following were the most crucial of the subsequent independent findings: Tillmans in Germany, who had noted the correlation between reducing action and antiscorbutic value, succeeded independently in crystallizing the vitamin (Tillmans & Hirsch, 1932). E. K. Nelson (1932) repeated our procedure with identical results and—of special interest in retrospect—went on to identify inositol (later shown to be itself a vitamin) as the colourless material in lemon juice that caused us and others so much trouble in the final crystallization steps. E. C. Kendall then sent us a sample of hexuronic acid which he had prepared by new and very different procedures, from adrenal glands. This product being identical with ours in chemical and biological tests, there seemed to be no room for doubt of the identity of the vitamin (Waugh & King, 1932*c*). Vedder (1932) reported an original isolation of the vitamin also in 1932, and Harris, Mills & Innes (1932)

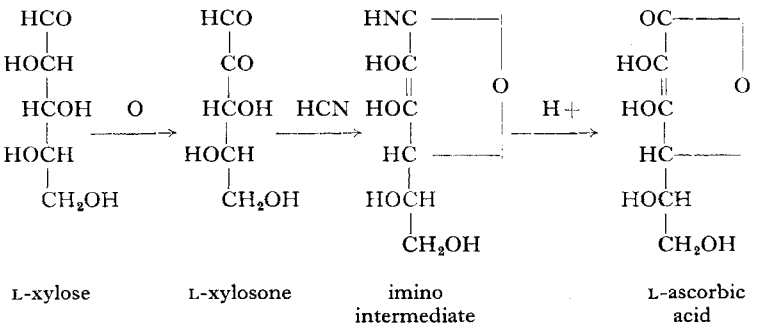
* Svirbely had initially planned to spend a post-doctorate year with Professor Wieland, but changed his plans, in part because his Hungarian parents encouraged him to work in Hungary, with Professor Szent-Györgyi. After arrival in the fall of 1931, he obtained permission to assay the 'hexuronic acid' obtained earlier from adrenal glands by Szent-Györgyi. However, he had a series of difficulties in getting an assay completed because no milk powder was available for the rations and none of the test groups showed a satisfactory response. Another sample of the vitamin had been 'resting' in the laboratories at Birmingham University for about 2 years, but it likewise suddenly became 'activated'.

confirmed the antiscorbutic value of hexuronic acid. Szent-Györgyi and his group then did a crucial test in preparing an acetone derivative and found that the recovered product had not changed in activity. They made another major contribution in finding a way to prepare large quantities of the vitamin from paprikas (Svirbely & Szent-Györgyi, 1933; Vargha, 1932), which permitted study of its structure by a number of chemists in Europe.

Organic chemists made rapid progress in establishing the molecular structure of the vitamin. While these investigations were under way, but before the structure was known, Szent-Györgyi & Haworth (1933) proposed the name ascorbic acid, referred to by a British colleague as 'a kind of scurvy name'. Among those who contributed in major degree to establishing the structure of ascorbic acid were Haworth and his associates, (Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith & Stacey, 1933; Haworth, Hirst & Smith, 1934) who first suggested its structure, chiefly on the basis of identifying the derived L-threonic acid. They postulated the correct enolic lactone form on the basis of reversible oxidation products and the methyl ethers at positions 2 and 3 and synthesized the substance.

Other chemists who made important contributions in the structural work included Karrer (Karrer, Salomon, Schöpp & Morf, 1933; Karrer, Schwarzenbach & Schöpp, 1933; Karrer, Schöpp & Zehender, 1933); Micheel & Kraft (1933*a-c*) and Reichstein (Reichstein, Grüssner & Oppenauer, 1933; Reichstein & Grüssner, 1934). The last named was especially successful in developing methods of synthesis that confirmed the structure of the vitamin on the basis of its preparation from xylose (I) and sorbose (II). The latter set the stage for its commercial manufacture from glucose at low cost. Although an extensive number of syntheses have been published, the dominant steps for industrial synthesis have been from glucose, through sorbitol, sorbose, acetone sorbose, 2-ketogulonic acid and ring closure to the enolic lactone:

(I)



(Reichstein *et al.* 1933; Ault *et al.* 1933).

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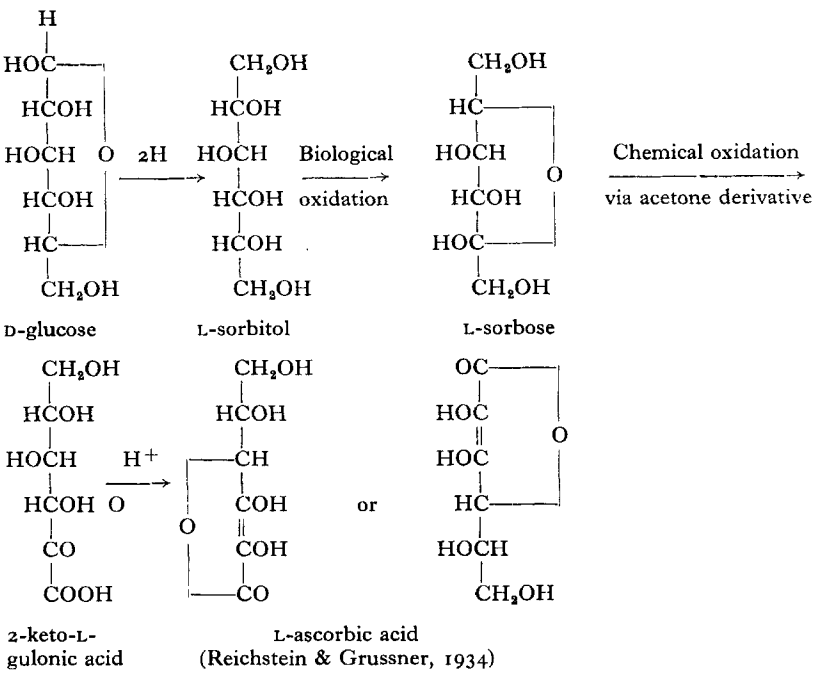
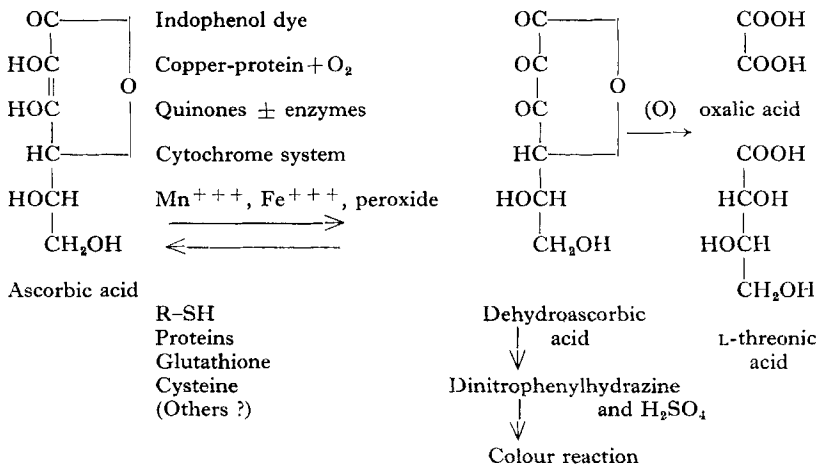
(II)



Reactions by which the vitamin is chiefly characterized in laboratory tests and, so far as known, in plant and animal cells, are outlined below (III).

(III)



The reduced form of the vitamin reacts rapidly with so many oxygen carriers present in all living cells that there is no reason for doubting the broad significance of ascorbic acid in regulating the reactions (including several enzymes) in almost all the higher plants and animals. A remarkable feature of its role is that a deficiency results in acceleration of the total respiratory exchange (King, 1936, 1939). In several plants copper is linked with proteins to form enzymes that are relatively

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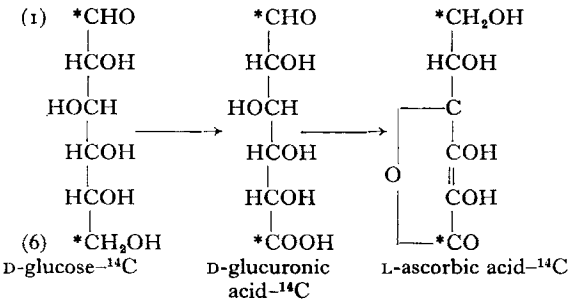
specific for ascorbic acid as a substrate in reacting with molecular oxygen (Stotz, Harrer & King, 1937; Dodds, 1948; Dawson, 1950). Additional plant enzymes of similar composition can oxidize the vitamin via the formation of quinones from natural phenols (Dawson, 1950).

Dehydroascorbic acid is unstable in neutral or alkaline solutions, but when it is fed to or injected into guinea-pigs utilization is nearly complete because of reduction by sulphydryl groups. The instability of this reversibly oxidized product appears to account in part for the relatively high intake requirement. A small but significant quantity of ^{14}C from labeled ascorbic acid appears as oxalate in the urine of guinea-pigs and rats (Burns, Burch & King, 1951).

The reactions by which the vitamin is formed in plants and animals have not been established in full detail, but it is clear on the basis of tests with glucose-^{14}C that there is a direct conversion from glucose (Jackel, Mosbach, Burns & King, 1950; Horowitz & King, 1953). Glucose labelled uniformly, or specifically in positions 1 or 6, gives rise to ascorbic acid (in rats) with the ^{14}C atoms chiefly in the respective initial positions (IV).

(IV)



Isherwood and associates have recently developed strong evidence for a similar conversion in plants (Isherwood, Chen & Mapson, 1953).

The conversion yield from labeled glucuronic acid to ascorbic acid was higher than from glucose—indicative, at least, that the former is an intermediate in the normal pathway. Another interesting point demonstrated with glucose-^{14}C was the failure of guinea-pig embryonic tissues to form any detectable quantity of ^{14}C-ascorbic acid—a hypothesis long in dispute but previously difficult to prove or disprove.

There has been no evidence, thus far, that appreciable quantities of ^{14}C-labeled ascorbic acid are incorporated into the tissue constituents that it controls, such as tyrosine (Sealock & Goodland, 1951, 1952; La Du & Greenberg, 1953), collagen, chondroitic acid, or cholesterol.

A new aspect of the chemical role of ascorbic acid came to light in recent months, in relation to cholesterol and steroid synthesis (Becker, Burch, Solomon, Venkitasubramanian & King, 1953). By the time guinea-pigs are only slightly scorbutic, their adrenal glands incorporate acetate-1-^{14}C into cholesterol at a rate well above normal. By the time severe scurvy is established the acceleration reaches roughly 300 to 600% (Table 1). In the liver, arteries, lungs and heart, the changes

are less marked, but they are in the same direction, compared with controls. We have not had an opportunity, yet, to identify the specific enzyme changes that account for the disturbed metabolism. However, this new lead opens a number of interesting areas for exploration.

Table 1. *Conversion of acetate-1-^{14}C to cholesterol in guinea-pigs*

(Specific activities in cts/min/mg. Each figure represents a mean for three or more animals)

	Adrenals		Liver	
Treatment	Experimental animals	Control animals, pair-fed	Experimental animals	Control animals, pair-fed
Normal chow diet, *ad lib.*	100	—	80	—
Depletion, 15–18 days	170	150	75	80
Severe scurvy	600	195	145	90

The question of whether ascorbic acid enters characteristically into labile complexes within living cells has been studied by several investigators (e. g. King, 1936, 1939; Sealock & Goodland, 1951, 1952). This area of work will very likely lead to correlation with specific enzyme systems on a broad basis. Unfortunately time does not permit a review of this fascinating and speculative kind of work, in the present paper. On this and other points, the author regrets the inescapable limitations in citing the work of many fellow scientists whose work merits high praise and more generous treatment.

In summary, among the characteristics of Doctor James Lind, whose studies of scurvy have become classic in the history of medicine and nutrition, we can be especially grateful for the following:

(1) A compelling sense of meeting opportunities to be of service to his fellow men,

(2) clarity of reasoning from step to step through a maze of complex and conflicting reports, and

(3) rare discernment in planning and interpreting his own experiments.

In place of death and the scourge of a disease that had plagued mankind since long before the dawn of history, he gave to his generation and ours the delights of orange juice for breakfast, lemonade on hot summer days, fresh salads at dinner, and a new, higher concept of normal health.

He freed men's bodies to travel the high seas in full vigour. But perhaps of greater importance, he set their minds at liberty to travel more freely in the realm of truth.

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