

DISCUSSION

CRYSTALLIZED VITAMIN C AND HEXURONIC ACID

THE well-merited award to Albert von Szent-Györgyi of the 1937 Nobel Prize in Physiology and Medicine "for his discovery of the biological processes

of oxidation with special regard to vitamin C and the fumaric acid catalyses" is erroneously interpreted in many quarters solely as an award for the identification of vitamin C. Reports on crystalline vitamin C are sufficiently clear that no such interpretation should

be made, but numerous careless or erroneous statements have appeared in scientific journals, reviews and books and in lay publications, which tend to obscure the priority of Dr. C. G. King and W. A. Waugh, of the University of Pittsburgh, in establishing the identity of vitamin C with a pure crystalline substance.

In 1927 Szent-Györgyi¹ made the first statement concerning a reducing substance which he had not yet isolated in crystalline form. He designated it C_{XII}, an apparently fortuitous use of the letter "C" and with no implication that it was related to vitamin C.

Again in 1927 Szent-Györgyi stated:²

To exclude any anticipation of function and chemical structure, the substance giving this reduction was named by its protocoll number "C_{XII}" being the twelfth substance prepared and examined in my work on tissue oxidation and the function of the adrenal cortex. Having been enabled to resume this work at the Biochemical Laboratory, Cambridge, the substance has been further investigated and finally isolated in crystals, which on recrystallization showed a constant melting point.

And

Preliminary experiments tend to show that the substance is not devoid of biological activity. My earlier experiments, showing that C_{XII} is not the hormone of the adrenal cortex, seem in the light of Banting's and Rogoff and Stewart's recent work to be inadequate. Analysis of the biological significance and chemical constitution has been started.

Zilva³ on May 7, 1932, wrote:

Ever since Prof. A. Szent-Györgyi suggested the possible identity of hexuronic acid⁴ with the "reducing principle" present in all active antiscorbutic solutions . . .

The paper⁴ to which Zilva refers contains the following sole reference to vitamin C:

The reducing properties of plant juice have repeatedly attracted attention, especially from students of vitamin C. Bezssonoff has applied Folin's phosphomolybdic acid reagent. The reducing substances of lemon juice have been made the object of a thorough study by Zilva, who established interesting relations between vitamin C and the reducing properties of the plant juice. The main reagent employed by Zilva was phenolindophenol. Indophenol blue is readily reduced by the hexuronic acid, so that it is probable that it was this substance which has been studied by Zilva.

The title of Zilva's paper⁵ (received for publication on August 25, 1932), "The Non-Specificity of the

Phenolindophenol Reducing Capacity of Lemon Juice and Its Fractions as a Measure of Their Antiscorbutic Activity," succinctly reveals that Zilva did not believe that the reducing substance with which he had dealt in a series of investigations was identical with vitamin C but a substance associated with it. Since Szent-Györgyi, as quoted above, believed "that it is probable that it was this substance which was studied by Zilva," then hexuronic acid was identified by Szent-Györgyi in 1928 with a substance associated with vitamin C but not vitamin C itself.

Tillmans, Hirsch and Hirsch⁶ in January, 1932, stated (in a note added at proofreading) in regard to Szent-Györgyi's 1928 paper:

Die Frage der etwaigen Übereinstimmung dieser Substanz [hexuronic acid] mit dem vitamin C wird von Szent-Györgyi nicht aufgeworfen. Er ist vielmehr der Ansicht, dass dieser Stoff in der Oxidationsmechanismus des Peroxydase-Systems eingeschaltet ist.

This note expresses an independent opinion that Szent-Györgyi did not consider hexuronic acid to be vitamin C.

McKinnis and King⁷ in 1930 established the acidic nature of vitamin C, and in discussing the acidity said:

It is also of interest in relation to the close association of vitamin C with the actively reducing uronic acid isolated by Szent-Györgyi.⁴

That the idea was current among King's students that vitamin C was a uronic acid is further evidenced by the statement in 1931 by Smith and King.⁸

In view of the fact that the active preparations were always characterized by a high content of strongly reducing substances and acidic material analogous to the "hexuronic acid" isolated by Szent-Györgyi, it was thought desirable to find out whether the use of a strongly reducing, weak acid, such as formic, would prove advantageous in protecting the concentrates.

In a review of the work on vitamin C during 1932, Harris⁹ contributes a parenthetical statement of significance:

Early in 1932 Szent-Györgyi, in collaboration with Svirbely (who, it may be recalled, had lately been associated with King in work on vitamin C concentration at the University of Pittsburgh), announced . . .

On April 1, 1932, as the culmination of a series of studies concerned directly with vitamin C, King and Waugh¹⁰ announced the isolation of vitamin C in crystalline form. They said:

¹ *Biochem. Zeits.*, 181: 433, 1927.

² *Nature*, 119: 782, 1927.

³ *Nature*, 129: 690, 1932.

⁴ *Biochem. Jour.*, 22: 1337, 1928.

⁵ *Biochem. Jour.*, 26: 1625, 1932.

⁶ *Zeits. Untersuch. Lebensm.*, 63: 1, 1932.

⁷ *Jour. Biol. Chem.*, 87: 615, 1930.

⁸ *Jour. Biol. Chem.*, 94: 491, 1931.

⁹ *Ann. Rev. of Biochem.*, 2: 264, 1933.

¹⁰ *SCIENCE*, 75: 357, 1932.

The recrystallized substance corresponds in chemical and physical properties to a hexuronic acid, and is apparently identical with the hexuronic acid described by Szent-Györgyi and reported as a reducing factor in adrenal cortex, cabbage and other sources. Feeding approximately 0.5 mg. daily protects growing guinea pigs from scurvy and permits normal vitality in the animals when on a vitamin C free diet. A detailed account of the experimental work will be published in the near future, but this involves only a few steps beyond the work previously published.

On April 16, 1932, Svirbely and Szent-Györgyi¹¹ announced that they had, by means of 1 mg of hexuronic acid daily, protected guinea pigs from scurvy in an experiment lasting 56 days. Because of the unavailability of milk powder for making the basal diet this test was unfortunately marred by loss of weight of all animals. A current experiment with a satisfactory diet was in progress, and three animals which had received 1 mg of hexuronic acid for 55 days were found to be free of scurvy. However, it was not until May 7, 1932, that Svirbely and Szent-Györgyi¹² stated that hexuronic acid is vitamin C. They said:

This allows us to conclude that vitamin C is a single substance and identical with hexuronic acid.

Simultaneously with our previous note, C. G. King and W. A. Waugh¹⁰ reported that they have obtained, from lemon juice, crystals which showed antiscorbutic activity and were apparently similar in chemical and physical properties to hexuronic acid. The duration of the test period was not stated, and apparently no chemical analysis was made. Until this is done, the nature of their product remains in doubt.

The results of Waugh and King¹³ and those of Svirbely and Szent-Györgyi¹⁴ were published in full in 1932. Subsequent studies by others have shown that vitamin C had indeed been identified.

The following facts in chronological order are evident from the above analysis of the statements appearing in the literature:

(1) Szent-Györgyi first isolated "hexuronic acid" but viewed it only as a reducing substance associated with vitamin C (1928).

(2) McKinnis and King first published a positive suggestion that hexuronic acid is vitamin C (1930).

(3) King and his students carried out a sustained study of vitamin C resulting in the isolation of the active substance in the crystalline form and first stated that the crystalline substance was apparently identical with Szent-Györgyi's hexuronic acid (April 1, 1932).

(4) Fifteen days after King and Waugh published, Svirbely and Szent-Györgyi gave their first evidence that hexuronic acid has anti-scorbutic properties, but it was not until May 7, 1932, that they stated that "Vitamin C is a single substance and identical with hexuronic acid."¹²

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¹¹ *Nature*, 129: 576, 1932.

¹² *Nature*, 129: 690, 1932.

¹³ *Jour. Biol. Chem.*, 97: 325, 1932.

¹⁴ *Biochem. Jour.*, 26: 865, 1932.