

The isolation of vitamin C from lemon juice¹

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During my undergraduate training at Washington State College and graduate studies at the University of Pittsburgh, I developed an interest in vitamins but I did not have very much working experience with them. Hence, when the faculty at the University of Pittsburgh invited me to become a permanent member of the staff with a major responsibility to develop the senior and graduate studies in biochemistry, my acceptance was contingent upon having a year's leave for intensive study with Professor H. C. Sherman at Columbia University. Dr. Sherman was one of the world's great leaders 1) in emphasizing the relationships of food practices to health and 2) in making the study of vitamins and other essential nutrients a quantitative science. This view meant standard animals, standard care of the animals, and intense regard for the preservation of normal body tissues and functions. Among other things at Columbia University I had the experience of sharing quantitative studies with vitamins A, B₁, B₂, C, and D, and with marginal intakes of calcium and protein.

Upon returning to Pittsburgh we began a continued study of the chemical nature of vitamin C and the effects of deficient intakes. This decision was influenced in part by the experience of Alfred H. Hess in the New York City health department.

The death rate among children during the hot summer had reached one of the highest within the United States, but dropped sharply during the cool weather. After a hard fight he succeeded in establishing a city-wide requirement for pasteurization of milk sold within the city during the summer period. The death rate dropped dramatically during the first

year, but instead, there was a widespread occurrence of scurvy among the children. This new situation was caused by excessive exposure to copper in the equipment used, so another campaign was necessary to avoid copper exposure. In the following years there was a sustained low death rate and a low incidence of scurvy.

Two years after starting the vitamin C work at Pittsburgh there was an opportunity for a 3-month study with Professor Hopkins at Cambridge University. After a seminar report on our work in Pittsburgh he invited me to his office and inquired whether I thought it possible that vitamin C might be the same substance as the adrenal hormone that Dr. Szent-Gyorgyi had studied in their laboratory. I said: "Yes, of course, but we were told that it had been proved not to be the vitamin when you sent a sample to S. S. Zilva in London for testing." He replied, "That is true, but he never told us what specific tests he had made. His report was accepted as reliable, because he had been very active in vitamin C research for several years." Dr. Hopkins invited me to extend my stay in Cambridge to work on the problem, but I explained that I was obliged to return to Pittsburgh in December and that students there were working on the problem.

The first five students to work with me on the problem in parallel with teaching services and graduate courses all made substantial contributions toward identification of the vitamin, even though the time and facilities were extremely limited.

Our first and second publications with D. P. Grettie (1) and H. L. Sipple (2) respectively (1930) illustrated the high degree of separation that could

be made from material such as lemon juice, without an extreme loss in activity or apparent change in type of biological function. A paper in 1930, with R. B. McKinnis, who was well trained in physical chemistry, gave strong evidence that the vitamin was a low molecular weight organic acid, and without a basic nitrogen group (3).

The next two papers, with J. L. Svirbely (4) and F. L. Smith (5) in 1931, reported further progress in separating the vitamin but without complete success.

Dr. Svirbely at that point changed his appointment as a post-doctoral fellowship for enzyme studies in Germany, to study with academic groups in Hungary, his father's home country. This led him to joint study with Dr. Szent-Gyorgyi.

The sixth student, however, W. A. Waugh, who had been working in parallel with Svirbely during the past year, continued the program and succeeded in isolating small quantities of the vitamin in crystalline form and identified its characteristics as in agreement with those of the adrenal hormone, called "hexuronic acid" (6, 7). A small sample of the crystalline product was supplied to the University of Toronto for comparison with their tests of a new microbiological growth stimulant.

While we were preparing a manuscript for publication, however, the press carried a series of reports from the University of Oslo in Norway, by Dr. O. Rygh et al. (cited in ref 6) claiming isolation and synthesis of

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vitamin C, identified as a well-known alkaloid, a derivative of nornarcotine. Their technical journal was not available in Pittsburgh and a search for it caused a delay in our reply. On careful examination of the original papers, however, it was apparent that every one of the test groups of guinea pigs that did not develop scurvy had been fed a basal ration that contained vitamin C, as illustrated by a product called "dried sprouts."

A brief paper on our isolation and identification of the vitamin, including our comment on the Rygh papers, was then submitted to *Science* (6) and a much more detailed paper was submitted to the *Journal of Biological Chemistry* (7). A paper was also submitted for the spring meeting of the American Society of Biological Chemists, where our evidence and conclu-

sions were accepted with apparent enthusiasm. I was particularly pleased when Professor E. V. McCollum stepped from the front row to greet me with an outstretched hand, and said, "Congratulations, young man! You have finally gotten a vitamin for us." As a courtesy, Dr. E. C. Kendall at the Mayo Clinic supplied us with a sample of the "hexuronic acid" that he had isolated and studied as the adrenal hormone. It was identical with our product, both in laboratory tests and in protection from scurvy in guinea pigs (8). I asked him whether Dr. Szent-Gyorgyi, who had studied with him recently in relation to the preparation and functions of the hormone, had ever mentioned the possibility of the hormone being related to or identical with vitamin C, and he replied: "No, not once."

Often in cooperation with staff members in the schools of medicine and dentistry at the University of Pittsburgh, our continued studies of vitamin C included methods of analysis (9), degrees of tissue injury caused by dietary deficiency (10), the range of distribution in human tissues furnished with special care by the hospitals (11), and the risks of vitamin destruction during food processing (12).

Particularly rewarding beyond the immediate progress was the subsequent development of substantial support for graduate work at the University by the Buhl Foundation. This development permitted for the first time a steady growth in predoctoral and postdoctoral fellowships and the purchase of critically needed equipment and supplies. 33

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