From Vitamin C to Vitamin P.

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MY first real interest in Biochemistry was in the function of the adrenal cortext. At that time we only knew, that this internal secretory gland was essential for life. Without this organ life failed. Furthermore, we knew, that patients, suffering from the deficiency of the gland, turned brown, before dying. Bananas and pears do the same. The pigmentation of dying vegetable tissues has been shown by Palladin, the great Russian plant biochemist, to be due to a disturbance in oxidation-reduction equilibrium. So I hoped, oxidation-reduction processes will explain to me the function of the adrenal cortex. But we knew too little about oxidation systems too. So I set out to study first animal, and later vegetable, oxidation systems. The first systems analysed, that of succinic oxidation in muscle, and the polyphenoloxidase in potatoes gave me no clue, but while analysing the peroxydase systems in turnips, I found there was a substance present, which was capable of inhibiting the formation of melanoid pigments. This substance was a strong reducing agent, which reduced immediately oxidised phenols, before they could undergo further modification and form pigments. I isolated the substance and made its first approximate analysis, establishing its empirical formula and some of its more important characteristics.

It was a great excitement, when I found the same substance in relatively big quantities in the adrenal cortex.

The "isolation" and "identification" of this substance was not quite as easy, as writing these words down. It was not a simple matter and it needed not only involved technical equipment but also money and in the laboratory of the Physiological Institute of Groningen (Holland), where I was working at that time, none was available. Further researches on the isolation and identification were made possible by a friendly invitation by Professor F. G. Hopkins to join him at Cambridge, and by a generous grant from the Rockefeller Foundation.

Having established the main characteristics of this fascinating new substance I wanted to know more about it, especially its exact steric configuration, before attacking the problem of its biological activity. The trouble was however, that I had too little of it, only a few milligrams and I could make no more, because, of the labile nature of the substance. The only suitable material for large-scale preparations was the adrenal gland, which was not available in Europe in sufficient quantity. Prof. A. Krogh of Copenhagen tried to help me by sending me adrenal glands from Denmark to Cambridge by air. The material, however, deteriorated during transit and was therefore, useless.

Once more, international co-operation rendered fresh progress possible. Prof. E. C. Kendall, of the Mayo Foundation (Rochester, Minn, U.S.A.), invited me to Rochester and the rich resources of the Mayo Foundation together with the large quantity of material of the big American slaughter houses became available. The glands were packed in dry ice and shipped in this hard frozen condition to Rochester where the material was worked up; I was able to prepare as much as 25 g. of the substance. One exciting experience I had, was a clinical trial on Addison patients with adrenal insufficiency. The patients failed to get better, but they bleached out!

Returning from the States I shared my substance with Professor Haworth at Birmingham, who was deeply interested in it. His long-standing experience with carbohydrates, enabled him to investigate the steric configuration of the molecule. The substance unfortunately proved to be insufficient for the work and there was no chance of preparing it again.

After settling down to a more quiet life in my own country, in Szeged, Hungary, I found an opportunity, to put to the test an old suspicion of mine, (for investigating which my earlier roaming life was unsuited): whether the substance, which I had in my hands for five years, was not identical with the long sought Vitamin C. I started the work in collaboration with an young American, Svirbely, in the autumn of 1932. In November, we had the first definite answer. The animals treated with our substance, which we used to call "Hexuronic acid" all lived, while the controls all died. Owing, however, to defective diet (we had difficulty in securing milk powder), the weight curves were not satisfactory. So we repeated the whole experiment, before we

published our result in March of the next year. Meanwhile, also King and Waugh at Pittsburg isolated from lemon juice, a crystalline substance which was antiscorbutically active and which resembled our preparation of hexuronic acid. Also Tillmans in Germany found at about the same time, a close parallel between the reducing power and vitamin content of plant juices, which made the identity of hexuronic acid and Vitamin C probable.

Our substance was thus, according to its newly discovered activity, rebaptised now, and called "Ascorbic acid". By its vitamin nature the substance acquired increased importance and it was the more regrettable to have none of it. This difficulty was solved by an unforeseen discovery.

Szeged, the city in which my Laboratory is situated, lies in the middle of the Hungarian paprica—red pepper, *Capsicum anuum*—fields. I once tested paprica for its Vitamin C content and found it a rich storehouse of Vitamin C. Large-scale preparation gave good yields and in two consecutive years I could prepare about 4 kg. of ascorbic acid, providing all laboratories of the world which were wanting to work on this substance with ample material. This work was not without results. Professor Haworth at Birmingham soon established the steric configuration of the substance and its synthesis was effected both by Reichstein at Zurich and by Haworth.

In this way the most mysterious vitamin, which so long resisted analysis, succumbed to laboratory investigation. At present, it is produced synthetically on a big scale at a very low price, so that it is available for all those who are in need of it; and all this in the incredibly short time of hardly two years. This is what international collaboration and understanding can do.

Further research showed ascorbic acid to be an essential part of our diet. I myself went back to my old line of research, *viz.*, oxidation, which led me to Vitamin C. I forgot ascorbic acid and the ascorbic acid people forgot me.

Only in the last months have old reminiscences begun to awake again. As I mentioned at the beginning, I was led to the discovery of ascorbic acid by the analysis of the peroxydase system. At that time I also found, that peroxydase + peroxide oxidised ascorbic acid reversibly. This reaction occurred only, if there was an aromatic substance present, which induced the reaction. Peroxydase had no direct effect on ascorbic acid; it however oxidised aromatic substances to quinols, which, in their turn, oxidised ascorbic acid. I wondered which aromatic substance was playing this rôle in the plant, especially in lemons. I was led to a very peculiar new substance, which seemed to belong to the widely spread group of vegetable benzo-pyran dyes, the flavons. I suspected that this substance might have a vitamin nature too. Only there was no animal test for its study. So I put the substance aside in the hope, that at some later date I might find one such. Now nature seems to have given us a suitable test in the form of certain human pathological conditions, such as the Hæmorrhagic Diathesis (vascular type). My friend, St. Rusznyák, Professor of Medicine, has told me, that he had some very good cures of such conditions with paprika, but the effect was not due to the ascorbic acid present. To what was it due then? The same effects could be obtained with lemon juice. We set out to investigate the question and to find the substance responsible for this activity. And in the end we found it. It was nothing else than my old friend, the flavone, carrying the reaction between peroxydase and ascorbic acid. The crystals of the substance had the same therapeutic effect, as the whole pepper. They cured in a striking way disorders of the permeability of the capillary wall. So we gave the name "Vitamin P," to this flavone and we are hopeful, that in its ability, to reduce human suffering, this new substance will be no less important than ascorbic acid.

If the vitamin character of this substance be firmly established, this will also mean that the great group of vegetable dyes, the flavones, which seem to play such an important rôle in plant biochemistry, also function in the animal organism. So the substance will form a new chemical link between plant and animal physiology and may bear new evidence for the big chemical unity of living matter.

The researches on ascorbic acid described in this article serve to bring out certain features characteristic of modern research. Thus, work on some fundamental problems yields results quite unsought and opens up vistas quite unsuspected; rapid advance is dependent on international collaboration and in modern research investigations of microquantities of substances should proceed side by side with the preparation of materials on a large scale using tons of raw material.