The History of Vitamin B_6

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HISTORICAL reminiscence implies for the participant of the event in question the interplay of unavoidable, subjective, personal considerations. It is difficult, well-nigh impossible, for the active participant to observe a completely detached, objective attitude. On the other hand, it is equally dangerous for anybody not having been actively engaged in the particular historical development to reconstruct *a posteriori* the events as they occurred.

The discovery of vitamin B_6 has to be reconstructed as in any historical process, in the light of knowledge available at the time of the discovery, and not with convenient "hindsight." It has been part of one of the most intriguing chapters in the rapid development of vitamin research: the unravelling of the vitamin B complex.

By the end of 1932 only two separate components of the vitamin B complex had been distinguished as needed by the rat: (a) vitamin B_1, the antineuritic factor; and (b) vitamin B_2 (or vitamin G), the antipellagra factor. The separate existence of a third factor called vitamin B_4, absence of which was said[1,2] to be associated with symptoms of nerve lesions, such as disturbances of co-ordination and ataxia, had not been generally accepted. Even less credit had been given to the claim that there were two more special factors, B_3 and B_5, as needed by pigeons.[3] Chick and Copping[4] had postulated the existence of a separate growth-promoting factor, called by them Factor Y, but neither its specific biologic effect nor its relation to the vitamin B complex had been investigated.

The British Committee on Accessory Food Factors[5] in 1927 defined vitamin B_2 as "the more heat stable, water-soluble dietary factor, recently described and named P-P ("pellagra-preventive") factor by Goldberger, Wheeler, Lillie, and Rogers (1926) and found necessary for maintenance of growth and health and prevention of characteristic skin lesions in rats, and considered by the latter workers to be concerned in the prevention of human pellagra."

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EARLY STUDIES

The first way-station on the road to the solution of the puzzling vitamin B complex was reached by the isolation of riboflavin, originally obtained in 1933 from milk and called lactoflavin, as the result of a co-operative study of Richard Kuhn, Th. Wagner-Jauregg, and myself.

For the production of vitamin B_2 deficiency in rats, we first used a ration originally devised by Bourquin and Sherman[6] which employed an alcoholic extract of wheat as the source of vitamin B_1. Pure vitamin B_1 was not available at that time. On the diet of Bourquin and Sherman, the weight curves of young rats soon flattened out or showed a decline. Addition of crude extracts of yeast, rice bran, liver, or of milk concentrates (or whey), in all of which vitamin B_1 was destroyed by autoclaving, restored normal growth.[7]

Modern research workers, accustomed to microbiologic tests which give an answer in 24 to 40 hours, and which permit the simultaneous assays of scores of test substances, should be impressed by the fact that each assay for vitamin B_2 in rats required a testing period of three to four weeks, with a corresponding number of prepared experimental animals.

Doctor Wagner-Jauregg first noted that all concentrates which proved to be active in the animal assays were colored and showed an intensive green-yellow fluorescence, in direct proportion to their biological effect. Exposure to visible light destroyed the growth-promoting activity of these concentrates.[8] The obvious working hypothesis, which identified vitamin

B_2 with a yellow-green pigment, soon met serious difficulties when concentrates that were further purified and more highly colored proved to be biologically inactive in the rat growth test. Here the biologist, or animal experimentalist, came to the rescue of the chemist. It was shown that by supplementing the diet with a yeast concentrate from which all colored material had been removed by adsorption, the biologic activity of the colored preparation was restored. Thus, it was proved that vitamin B_2 is not a single substance but that it may be separated into at least two components, one of which was characteristically a pigment: riboflavin.

The question arose whether riboflavin, which to some extent fell under the definition of vitamin B_2 as given above, had any relation to human pellagra, or to its experimental counterpart, canine black-tongue. In co-operation with Birch and L. J. Harris,[9] we were able to show, independent of and simultaneously with the Wisconsin group under Elvehjem,[10] that riboflavin was different from the specific pellagra-preventive factor (P-P) of Goldberger and his associates. Elvehjem and his colleagues[11] later identified nicotinic acid (amide) with the antipellagra factor, adding another chapter to the fascinating story of the unraveling of the vitamin B_2 complex. Inasmuch as riboflavin was the first member of the vitamin B_2 complex isolated and identified, it is not surprising that it is still often called vitamin B_2, without reference to the comprehensive character of the original term "vitamin B_2."

ACRODYNIA

A satisfactory supplement to promote further growth in rats receiving pure, crystalline vitamin B_1 and riboflavin was found to be the so-called vitamin B_4 concentrate obtained according to Peters and his collaborators[1,2] by adsorption of a yeast concentrate on charcoal and subsequent elution with alcohol acidified with hydrochloric acid. In young rats kept on a semi-synthetic diet with added vitamin B_1 and riboflavin, but without any further supplement, severe cutaneous lesions soon developed, which were characterized by edema, redness, scaliness of the paws, snout, nose, and ears, i.e., of the most distal parts of the body. Correspondingly we called the condition rat acrodynia without any prejudice as to whether this condition is in its causation and pathogenesis analogous to a human condition.

As a matter of fact, at present it may be stated without any reservation that the usual human acrodynia is not based on a deficiency of any constituent of the vitamin B_2 complex. On the other hand, during the starvation period in Italy after the recent war, Professor Frontali (Rome) observed young infants with typical cutaneous manifestations which responded to treatment with the same factor of the vitamin B_2 complex which prevents and cures rat acrodynia.

The dermatologic condition of rat acrodynia as a state of specific dietary deficiency has not been described previously. The yeast concentrate of the so-called vitamin B_4 factor, prepared according to Peters and his associates,[1,2] had in our experience a definite curative and preventive effect on rat acrodynia. Inasmuch as vitamin B_4 deficiency was supposedly characterized exclusively by nerve lesions,[2] rat acrodynia must have been due to the deficiency of another constituent of yeast, also present in the yeast concentrate of Peters and his associates. The even less well defined factors, B_3 and B_5, claimed to be required by the pigeon, have been disregarded and the factor curing rat acrodynia has been named vitamin B_6[12] and as such delineated from all other constituents of the vitamin B_2 complex.

It may be added that later Peters and his associates[13] revoked the existence of vitamin B_4 and explained their previous observations on this deficiency by simultaneous hypovitaminosis of vitamin B_1 and riboflavin.

The clinical picture of experimental vitamin B_6 deficiency changed according to species studied, as well as the age of the animals and also to extraneous dietary conditions. In our original studies, we relied chiefly on the cutaneous manifestations as the easily recognizable, specific sign of vitamin B_6 deficiency. In collaboration with Birch,[14] we further observed that fat with a high percentage of unsaturated fatty acids, at that time also called vitamin F, prevented and beneficially influenced these

cutaneous manifestations of vitamin B_6 deficiency. Later Birch[15] advanced the idea that the essential fatty acids were necessary for the utilization of vitamin B_6 and, in return, vitamin B_6 was required for the utilization of the essential fatty acids.

Among other manifestations of vitamin B_6 deficiency, it was early recognized that in young animals, such as rats, pigs,[16–18] and dogs [19] epileptiform convulsions may appear. Recently these experimental observations had led to the recognition of vitamin B_6 deficiency in young infants, as will be discussed later.

In dogs[20] and in pigs[16,21] lacking vitamin B_6, a microcytic anemia developed. It has been claimed[22] that in rhesus monkeys chronic vitamin B_6 deficiency leads to arteriosclerotic changes.

All these abnormal conditions responded promptly and specifically to vitamin B_6 medication. In many instances vitamin B_6 deficiency in animals became apparent only by nonspecific retardation of growth, and most authors used the retardation of growth as the criterion of vitamin B_6 deficiency. Under well-controlled experimental conditions the growth assay has proved to be useful, in spite of its unspecific character. It deserves to be pointed out that in the experimental animal vitamin B_6 added to a semisynthetic diet supplemented only with vitamin B_1 and riboflavin will prevent or cure the specific acrodynia and the epileptiform convulsions, as well as the microcytic anemia, but will not promote growth, or—in curative experiments—only for a very short period, after which the growth will again decline. This observation led Jukes and Lepkovsky[23] to the recognition of pantothenic acid, first called "filtrate factor," having been found in the filtrate of yeast extract after charcoal adsorption. The addition of pantothenic acid is required for the promotion of growth, in addition to vitamin B_1, riboflavin, and vitamin B_6. Thus, another member has been added to the rapidly increasing number of constituents of the vitamin B_2 complex.

PYRIDOXINE

The chemical nature of vitamin B_6 has been studied in collaboration with Birch[15] on crude concentrates of the vitamin. The isolation of pure crystalline vitamin B_6 was first reported by Lepkovsky[24] in 1938, barely four years after recognition of this specific member of the vitamin B_2 complex. Independently, but slightly later, several other groups[25–28] also reported the isolation of vitamin B_6. Within a year Harris and Folkers[29] and Kuhn with his associates[30] showed that vitamin B_6 was a pyridine derivative, specifically 3-hydroxyl-4, 5-dihydroxymethyl-2-methyl-pyridine. The term *pyridoxine*, proposed by us[31] for this compound has received general acceptance.

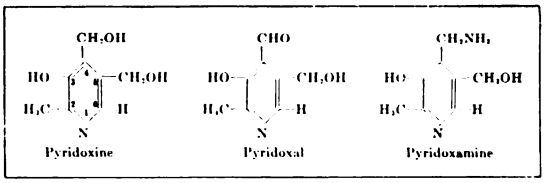

Figure 1

At this stage of the historical development, microbiological research entered the scene. Credit is due to Snell and his associates[32] for first recognizing the existence of other forms of pyridoxine as the result of the comparison of microbiologic assays on extracts of natural materials with the values based on chemical and animal assay. Snell[33,34] further observed that autoclaving of pyridoxine with the assay medium or amino acids greatly increased the activity of pyridoxine for the test organism *Streptococcus faecalis R.* This increased activity was assigned to the aminated product of pyridoxine called pyridoxamine. Through mild oxidation the formyl derivative of pyridoxine, pyridoxal, was obtained (Fig. 1).

Pyridoxine, pyridoxal, and pyridoxamine may occur naturally in free or in several conjugated or "bound" forms.[35] The latter include pyridoxal phosphate and pyridoxamine phosphate and their combinations with protein and perhaps other unidentified conjugates. The fact that pyridoxine occurs in bound form in many natural products has also been observed by Birch and myself.[14]

Pyridoxal, pyridoxamine, and pyridoxine are as a rule equal in activity for animals[36,37]; in many instances, however, pyridoxal and pyridoxamine show slightly less activity than

pyridoxine. In contrast, for many micro-organisms the three forms show very different activities.[36]

In natural food products, pyridoxal and pyridoxamine and the corresponding conjugates are in excess of pyridoxine. Pyridoxine is more resistant to heat than the other natural forms of vitamin B_6.[38,39] This observation recently became of great practical significance, in relation to the convulsive disorders seen in infants receiving autoclaved liquid milk preparations, as will be discussed later in detail during this Symposium.

It became customary[40] to speak of vitamin B_6 as a sub-*group* of the vitamin B_2 complex, with pyridoxine, pyridoxal, pydridoxamine, etc. as its particular chemical representatives.

FUNCTION OF VITAMIN B_6

A large number of investigations and publications in the years closely following the discovery of vitamin B_6 were concerned with the metabolic activity of this vitamin, both *in vivo* and *in vitro*. As first shown for riboflavin[41] and later confirmed for all other members of the vitamin B_2 complex, it was to be expected that vitamin B_6 as another member of the vitamin B_2 complex should act not only as a vitamin but also as a proenzyme. Pyridoxine, pyridoxal, pyridoxamine, and their respective phosphates owe their vitamin activity to the ability of the organism to convert them into the enzymatically active form, i.e., pyridoxal-5-phosphate.

Vitamin B_6 was found to participate in a wide variety of enzyme systems, in the intracellular and extracellular metabolic utilization and transformation of amino acids. The most important relevant reactions are decarboxylation and transamination. In consequence, it was to be expected that deficiency of vitamin B_6 would manifest itself metabolically in some aberration of amino acid metabolism. One of the first such pathologic pathways was found in the utilization of tryptophan even in—what may be called—relative vitamin B_6 deficiency. In such conditions, as first shown by Lepkovsky and his associates,[42] an extra load of tryptophan will increase the urinary excretion of xanthurenic acid.

With all this rich history of vitamin B_6, it took approximately 20 years until its requirement by the human organism could be definitely established and recognized. From this point of view, vitamin B_6 is one of the newer vitamins as stated in the title of this symposium. For those of us who are both physicians and experimentalists, the need of man for vitamin B_6 has not come as a surprise. Vitamin B_6 is required by all animals studied and by a very large number of micro-organisms. Further, it has proved to be one of the key constituents of important enzyme systems. Under these circumstances, and in analogy to other similar nutrients, it appeared to be permissible *a priori* to extrapolate, and to extend the vitamin character of vitamin B_6 to man. Be that as it may, for one who witnessed the whole story of vitamin B_6, it is gratifying to say that vitamin B_6 has come of age.

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