Developments leading to the metabolic role of vitamin B_6[1]

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In the late 1920's my interest was focused on dermatological conditions in infants, with special reference to seborrhoid dermatitis and acrodynia. We tried to approach the problem by feeding various experimental rations to rats. One study was devoted to investigations of "egg white injury," which is produced in rats on a ration containing dry, uncooked egg white as the sole source of protein (1, 2). This work, covering the extended period from 1929 to 1940, led to the identification of the known yeast-growth promoting factor, biotin, with the egg white injury factor. In the later years of this study, the cooperation of Dr. Vincent du Vigneaud, Professor of Biochemistry at the Cornell School of Medicine was truly essential for success. Biotin has since been proven a very important vitamin for all living organisms, not only, as was originally assumed, for yeast alone (3).

The second, more intensive and more progressive, study dealt with the isolation of vitamin B_2. In 1927, the British Committee on Accessory Food Factors had distinguished two separate components of the vitamin B complex: *1*) vitamin B_1, the antineuritic factor; and *2*) vitamin B_2, the antipellagra factor. The same committee 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 (4) 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."

In 1929, we proceeded with the chemical isolation of the so-called vitamin B_2 in collaboration with Professor Richard Kuhn and Dr. Theodor Wagner-Jauregg. For assay purposes, we used the growth response of rats fed a purified supplemented diet, containing cod liver oil as the source of vitamin A and D and an alcoholic extract of wheat (5) as the source of vitamin B_1. Modern research workers, accustomed to microbiological tests that give an answer within 20 to 48 hr and enable 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 3 to 4 weeks, with a corresponding number of prepared experimental animals.

During the course of the isolation of "vitamin B_2," it was our collaborator Dr. Wagner-Jauregg who first noted that all concentrates prepared from cow's milk that proved to be active when used as a supplement to a vitamin B_1 concentrate such as an alcoholic extract from wheat (5), 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 (6). But the obvious working hypothesis, which identified vitamin B_2 with a yellow-green fluorescence, soon met serious difficulties. To the great disappointment and

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despair of the chemist, the concentrates became biologically inactive in the rat growth test as they were further purified, and became more highly colored. Here the biologist and animal experimentalist came to the rescue of the chemist (7). It was shown that, by supplementing the diet with a yeast concentrate (8) from which all colored material had been removed by charcoal absorption (the so-called Peters eluate), the biologic activity of the colored preparation was restored. Thus, what was at first sight disappointing, opened the gate to important new assaults on the problem of the vitamin B_2 complex. The pure crystalline, yellow compound first isolated in cooperation with Professor Richard Kuhn and Dr. Wagner-Jauregg from milk was originally named by our group "lactoflavin" (9–12). It has been identified chemically as a derivative of isoalloxazine with a ribose radical attached. Hence the present term, riboflavin.

Riboflavin was the first vitamin recognized as part of an enzyme system. Therefore, it was not only a vitamin but also a dietary pro-enzyme as a prosthetic group of the flavo enzymes. The yellow pigment isolated previously by Warburg and Christian (13) from their yellow enzyme was a photo-derivative of riboflavin and was inactive as a vitamin. Riboflavin bridged the gap between an essential nutrient and cell enzymes and cellular metabolism. In biochemical research it represented a special milestone. Today, with the general acceptance of this idea, it is not surprising that water-soluble vitamins have been found to be essential parts of enzyme systems.

Crystalline vitamin B_1 (thiamin) became available in 1933. The isolation of riboflavin in pure form gave us the key for a real breakthrough in the investigation of the vitamin B_6 problem. It was shown (7, 14) that after a few weeks on a diet free from the whole vitamin B complex and supplemented with required amounts of thiamin and riboflavin, young rats showed a reduced growth rate and developed a scaly symmetric dermatitis that was most pronounced on the peripheral parts of the body (paws, ears, snout). Inasmuch as the distribution of these cutaneous lesions somewhat resembled the skin lesions in human pellagra, the dermatitis seen in rats receiving a vitamin B-free diet supplmented only with pure thiamin and riboflavin was first called pellagra-like, "without prejudice as to their identity or non-identity with human pellagra" (7). This observation furnished exact proof for the conclusion that vitamin B_2 was not a single vitamin but a complex in itself, with riboflavin its first definitely established member. In order to avoid confusion with previously claimed and discussed in the literature, but not properly identified factors like vitamins B_3, B_4, B_5 (and Y), our "rat pellagra factor" was named vitamin B_6 (14).

Further investigations (15–17) led to the differentiation of riboflavin and vitamin B_6 from the specific pellagra preventive (P-P) factor of Goldberger and his associates, and definitely established the separate existence of these three members of the vitamin B complex. The designation "pellagra-like dermatitis" of vitamin B_6 deficiency has been changed to "rat acrodynia," again on the basis of the distribution of the cutaneous changes, without any reference to human acrodynia (15).

The isolation of pure crystalline vitamin B_6 was first reported by Lepkovsky (18) barely 4 years after recognition of this specific member of the vitamin B complex. Independently, but slightly later, several other groups (19–22) also reported the isolation of vitamin B_6. It is appropriate to recall the fine (in general, quite unusual) friendly gesture of Dr. Lepkovsky, who knowing my active interest in the isolation of vitamin B_6, advised me early in 1938 that he and Keresztesy were ready to submit independently their papers on the successful isolation of vitamin B_6 for publication. This enabled me, with some accelerated urgency, to make ready and submit my own publication in time.

Within a year, the exact chemical structure of vitamin B_6 as a pyridoxine derivative had been elucidated (23, 24). The term pyridoxine, proposed by us (25) for this compound, has received general acceptance.

At this stage of the historical development, microbiological research entered the scene. Credit is due to Snell and his associates (26) for first recognizing the existence of other

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forms of pyridoxine, i.e., pyridoxal and pyridoxamine, both (especially pyridoxal in its phosphorylated form) acting as coenzymes. It became customary (27) to speak of vitamin B_6 as a subgroup of the vitamin B_2 complex, with pyridoxine, pyridoxal, and pyridoxamine as its particular chemical representatives.

With all the rich history of vitamin B_6, approximately 20 years passed before its requirement by the human organism had been definitely established and recognized. At present we are facing an almost explosive interest in the metabolic role of vitamin B_6 in man. There are large numbers of findings of various types accumulating, but a clear, overall picture of all connections and of their role is still missing.

The role of vitamin B_6 in maintaining and regulating a normal physiological and neurological state has been discussed; apart from convulsions, the oldest metabolic observations on the role of vitamin B concern pregnancy (28). They originated from the demonstration of increased amounts of xanthurenic acid in the urine of vitamin B_6-deficient rats after oral administration of tryptophan (29). Xanthurenic acid, together with kynurenine, anthranilic acid, et cetera are the metabolites of tryptophan on the way to the formation of nicotinic acid (Fig. 1). Abnormally high excretion of xanthurenic acid and related metabolites of tryptophan was also found in pregnancy, beginning from the 12th to 14th week (30–33). This disturbance in the metabolism of tryptophan must be due to the deficiency or malfunction of not one but more B_6 enzymes involved in the enzymatic pathway of tryptophan to nicotinic acid.

This first metabolic study coincided with the work of enzymologists. The above findings were the not unexpected consequence of the statement of Braunstein, the famous Soviet biochemist: "Pyridoxal phosphate holds an exceptional place among the coenzymes with regard both to the unparalleled diversity of its catalytic function and to their paramount significance in biochemical transformations of amino acids and in integral pattern of nitrogen metabolism" (33).

The positive tryptophan load test in pregnancy was ascribed to a relative vitamin B_6 deficiency (28). Considering the more recent observations on similar derangement observed in women receiving contraceptive pills (34–37) and also the analytical findings on reduced pyridoxal phosphate (PLP) in the plasma of pregnant women and women on contraceptive pills, it appears to be more logical to consider an antagonism between hormones in pregnancy and in contraceptive pills to the coenzymes involved in the degradation of tryptophan, and (as discussed later) in other examples of metabolism of amino acids. With this assumption, the requirement of high doses of pyridoxine for the restoration of disturbed tryptophan metabolism (30 mg (!) of pyridoxine daily) (38) is in good accord.

The pyridoxal levels in the cord blood

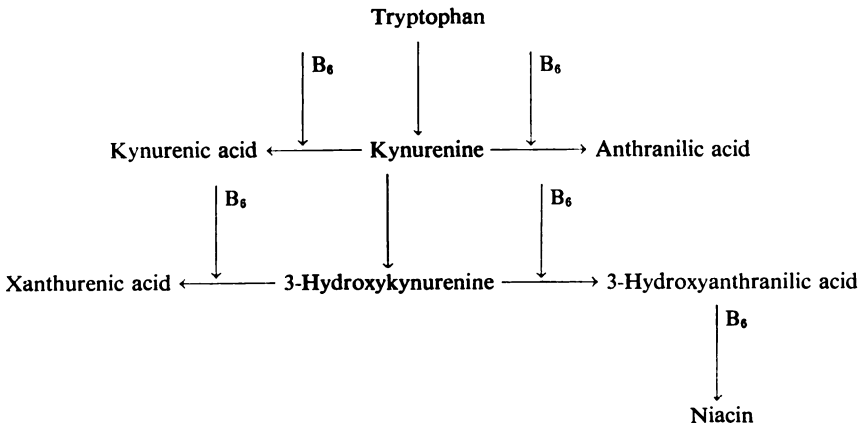

FIG. 1. Tryptophan–niacin pathway.

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after delivery is significantly higher than that in the maternal blood (39).

The irregular metabolites of tryptophan in a positive load test may have other clinical and metabolic consequences, such as migraine and depression of various grades in women receiving contraceptive pills (40) (requiring at least 50 mg pyridoxine daily compared with the usual daily recommended allowance of 2 mg and even in pregnancy only 2.5 mg). In animal experiments, tryptophan metabolic intermediates have been reported to be carcinogenic (41). Xanthurenic acid (XA) proved to be diabetogenic. High fat content in the diet increases the diabetogenic effect of XA, which first reduces the glutathione content of the blood, binds insulin, and may lead to chronic diabetes through exhaustion of the pancreatic β-cells (42, 43). Administration of pyridoxine will prevent all manifestations of the diabetogenic effect of XA, which in itself is analogous to that of alloxan in animals. Of course, complete destruction of the β-cells cannot be reversed by pyridoxine. The increased capacity of the liver and adipose tissue to synthesize fat (44), just as high dietary fat intake (45) in (absolute or relative) B_6 deficiency, may act as supporting factors in the development of diabetes. It is of special interest, not yet clarified, that XA in free form was invariably present in the urines of diabetic patients (45). These tryptophan intermediates can interfere also with gluconeogenesis (46) and energy metabolism (47).

The very common premenstrual edema and late massive edema of pregnancy is preventable or therapeutically influenced with high doses of pyridoxine (48) without the use of pharmaceutical antidiuretics.

Of even greater theoretical and practical importance than the disarranged B_6 enzymes in pregnancy or in women using oral contraceptives is a similar physiological disturbance of B_6-enzyme activity with increasing age in geriatrics. The levels of PLP in the blood plasma show gradual reduction in the aged, accompanied by a positive tryptophan load test. The serum–glutamic–oxalic acid transaminase (SGOT) was found also lower in old individuals than those of the young. Large doses of pyridoxine restored all metabolic changes mentioned to normal (49, 50). It has also been claimed that acute rheumatism, as well as osteoarthritis (the disease mainly of the aged), in its early stages may be beneficially influenced by 50 to 100 mg of pyridoxine daily (51).

In all the above clinical conditions, the amino acid load test was carried out with tryptophan. More recently, attention was directed toward methionine with expected changes in the transulfuration pathway observed in subjects kept on a low vitamin B_6 diet for an extended period of time. As a result of reduced activity of B_6-dependent cystathionase and cystathionine synthetase (Fig. 2), high excretion of cystathionine or homocysteine (homocystine), or both, was observed (52). It is of special pediatric interest that the enzyme cystathionase is absent in human fetal liver or in the liver of premature infants. In consequence, the fetus and the premature infant (at least at birth) are not capable of utilizing methionine and must depend on cystine as the essential sulfur-containing amino acid (53). In this connection, it must be called remarkable that human milk is the only source of animal protein with the lowest ratio methionine:cystine. Or, in other words, human milk is relatively rich in cystine, whereas cow's milk, meat, or any other animal protein have a great excess of methionine (two to five times more than cystine), which represents a special stress for the pregnant mother and thus, for the fetus and the premature infant, another glaring example of teleology.

Typical arteriosclerotic lesions were described more than 20 years ago (54) in B_6-deficient monkeys. More recently, accelerated arteriosclerosis has also been found in children in association with the inborn metabolic error of homocystinuria (55, 56). In cell cultures obtained from the skin of such individuals, an abnormal proteoglycan substance was found that is granular, aggregated, and flocculent (57). In the arterial wall, the normal fibrillar structure was altered in children with homocystinuria, showing the abnormal proteoglycan substance. The same changes were observed in rabbits after repeated injections of homocysteine. "Moreover, addition of cholesterol to the diet of

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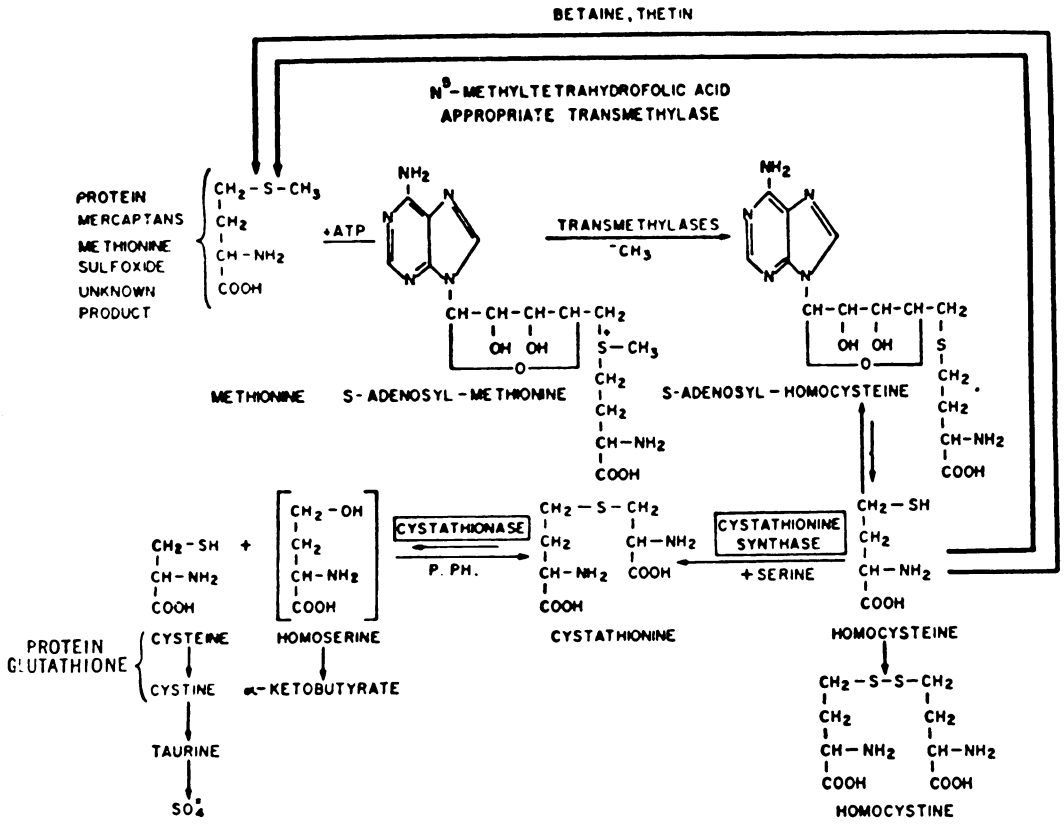

FIG. 2. Transulfuration pathway (53).

homocysteinemic rabbits resulted in deposition of lipids in the aortic lesions, suggesting that lipid accumulation is a complication of the primary vascular alteration" (58). In other words, hypercholesteremia is not the leading pathogenetic factor.

All this opens the way to further studies, bringing the high requirement for B_6 with increasing age and arteriosclerosis (plus other geriatric diseases) into proper focus.

In the past, a positive tryptophan load test (and this applies also to the most recently introduced methionine load test) was considered an interesting biochemical curiosity, without special medical value. With the recently acquired knowledge of the possible harmful effect of some abnormal metabolites formed in the course of these load tests, especially when of extended duration (such as in geriatrics or in women on contraceptive pills for years), important and urgent practical conclusions are in order. The present daily dietary allowance for B_6 should be changed from 2 to 2.5 mg to 25 mg/day as a precaution to prevent possible serious pathological conditions.

In summary, the history of vitamin B_6 is a further proof that success usually is preceded by trials, tribulations, and recurrent disappointment. The most helpful factor, apart from perseverance and timeliness of the line of research, is the deliberate recognition of a principle that is paramount in scientific research; it is often almost beyond our control, and touches closely on intuition. It is Walter B. Cannon's "serendipity."

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