Meetings

Vitamin B_6

"Vitamin B_6 was first defined and delineated as a distinct entity in the vitamin B_2 complex 30 years ago (Gyorgy, 1934). Thirty years are often equated as the age period of one generation. Thus, one may say that the torch of the generation of 1934 is passing in 1964 to another new generation." These were the opening remarks of Gyorgy, the discoverer of vitamin B_6, as the first speaker at an International Symposium on Vitamin B_6 held in his honor in New York City on 23–24 July 1964. Gyorgy recounted the history of vitamin B_6 and the trials and tribulations which finally resulted in its isolation as a new and distinct vitamin. Invited speakers from this country and abroad then presented papers covering many aspects of current research related to vitamin B_6.

A wide variety of synthetic approaches to the preparation of pyridoxine were described by Osbond (Hoffmann-La Roche, Welwyn, England). In addition, methods were presented for the synthesis of C^{14}-labeled pyridoxine, pyridoxine-5-phosphate, pyridoxal, pyridoxal-5-phosphate, pyridoxamine, N^{15}-labeled pyridoxamine, and pyridoxamine-5-phosphate. The considerable amount of synthetic work which has beeen carried out during the last 25 years is a reflection of the biological importance of the vitamin B_6 group. The synthesis of pyridoxine from oxazoles offers a particularly flexible route for the preparation of labeled material for tracer work and for preparing analogs of pyridoxine and related substances.

The fact that pyridoxal phosphate is an essential constituent of glycogen phosphorylase places vitamin B_6 in an important role in carbohydrate metabolism. This relationship of vitamin B_6 to phosphorylase and related enzymes of glycogen metabolism was reviewed by Krebs (University of Washington). Phosphorylase *a* and phosphorylase *b* are composed of four and two subunits, respectively; one pyridoxal phosphate residue appears to be bound to each subunit. The binding of pyridoxal phosphate takes place between the aldehyde group and the E-amino group of a lysine residue. A free or potentially free aldehyde group is not essential for the activity of phosphorylase, since reduction of the enzyme with sodium borohydride does not destroy the activity. It may be that pyridoxal phosphate has a primary function as a particular structural unit in phosphorylase in contrast to its role in other vitamin B_6 enzymes. In fact, one-half of the vitamin B_6 content of the body can be accounted for in the phosphorylase of the muscle mass, so that this must be considered an important storage site for vitamin B_6. Comments of others pointed out that there is low blood glucose and glucose tolerance in vitamin B_6 deficiency, with a resultant sensitivity to insulin. More work should be done along the line of exercising B_6-deficient animals to bring out symptoms of deficiency related to carbohydrate metabolism.

Studies concerning the comparative properties of glutamic-oxaloacetic transaminases (GOT) from the mitochondrial and soluble fractions of mammalian tissues were described by Wada *et al.* (Osaka University, Japan). They also reported investigations which demonstrated that the pyridoxamine oxaloacetic transaminase of *Escherichia coli* is also the apo-protein of the mitochondrial GOT. This was also shown to be the situation in the case of rabbit liver. Confirmation was obtained by studies on crystalline GOT from both the mitochondrial and soluble fractions of beef liver. Further investigations showed that these two forms of GOT were distinct from each other in physical, chemical, kinetic, and immunochemical properties. Additional knowledge concerning the two GOT isoenzymes was obtained by isolating and studying the crystalline enzymes from both beef and pig heart. There is a lack of immunochemical distinction among GOT from the corresponding fractions of different tissues of a single species of animal. This may indicate that the corresponding enzymes from different tissues have a common protein structure, irrespective of the kind of tissue from which the enzyme was derived.

Norepinephrine, serotonin, and histamine are three physiologic regulators whose biosynthesis involves decarboxylation by pyridoxal phosphate containing enzymes. Udenfriend (National Institutes of Health, Bethesda, Maryland) discussed the decarboxylation reaction in the synthesis of each of these agents from the viewpoint as to whether it was rate limiting and thereby amenable to regulatory mechanisms. Only in the case of histamine, however, was the decarboxylation step rate limiting. The synthesis of the other two agents may be accomplished by a synthesizing particle composed of the necessary enzymes to effect the synthesis from the amino acid.

Braunstein (Academy of Sciences, U.S.S.R., Moscow) had submitted a paper for presentation at the symposium, but was unable to attend because of illness. His paper dealt with the binding and reactions of the vitamin B_6 coenzyme in the catalytic center of aspartate transaminase. With the availability in recent years of pure aspartate transaminase and other pyridoxal phosphate enzymes, more has been learned about the specificities and mechanisms of reactions catalyzed by different enzymes. The peptide sequence around the coenzyme-linked lysine residue, as well as the properties of the pyridoxal phosphate linkage in the catalytic center, has been elucidated to some extent. The methods of borohydride reduction, as well as inhibition analysis, have yielded valuable information in the study of pyridoxal phosphate enzymes. This paper, as well as the other papers in the morning session, was summarized by Snell (University of California), who also made some remarks on pyridoxal kinase. This enzyme, in which brain tissue is particularly rich, was found to be lower in concentration in aged persons. This was shown by a comparison of the pyridoxal kinase activity in the brain tissue of 120 samples from a state hospital and of a number of accident cases. In the latter case more than 50 percent of the samples had activities of over 500 units per gram, while the samples from the hospital tended to be lower, particularly in the aged.

The underlying cause of the outward symptoms of vitamin B_6 deficiency must be attributed to an impairment of the enzymes which depend on vitamin B_6 for their functioning. This biochemical pathology of vitamin B_6 deficiency was discussed by Wiss *et al.* (Hoffmann-La Roche, Basle, Switzerland). The degree of sensitivity of pyridoxine-dependent enzyme systems varies. Enzymes involved in the metabolism of the sulfur amino acids (for example, cysteinesulfinic acid decarboxylase) and tryptophan are very sensitive, while the various transaminases are less sensitive to depletion of vitamin B_6 deficiency. Pyridoxal phoscysteine or trytophan are therefore used to detect the earlier stages of vitamin-B_6 deficiency. Pyridoxal phosphate also appears to regulate the synthesis of apoenzyme. For example, complete reactivation of cysteinesulfinic acid decarboxylase by adding pyridoxal-5-phosphate in vitro is possible only after the first few days of vitamin B_6 depletion of the rat. This failure of reactivation indicates a loss of apoenzyme. Cerebral seizures are the most impressive symptoms of vitamin B_6 deficiency in human beings. Studies with rats indicate that correlations exist between cerebral symptoms produced by vitamin B_6 depletion, the reduction of the pyridoxal phosphate content in the brain, and the activity of its enzymes dependent on pyridoxal phosphate.

An interesting speculative approach was employed by Roberts *et al.* (City of Hope Medical Center, Duarte, California) in an attempt to construct a framework which might explain the role of vitamin B_6 and gamma-amino butyric acid in the control of neuronal excitability. Models were used to depict the synapse in the central nervous system. One of the requirements for the proper functioning of this synapse is a negative feed-back inhibitor liberated from the postsynaptic side which can control nerve impulse. In vitamin B_6 deficiency, one of the typical symptoms is abnormal electroencephalographs and in severe cases actual convulsive seizures. It may be possible that vitamin B_6 is involved in the synthesis of this negative feed-back inhibitor which could be gamma-amino butyric acid, necessary for proper nerve transmission regulation. Impairment of this synthesis would thus favor an excitatory state in the central nervous system and eventual nervous seizures. This speculative system should provide an effective guide for future research in neurochemistry.

Recent research in animals concerning vitamin B_6 and amino acid metabolism was described by Williams (University of California) and it is evident that all aspects of amino acid utilization and metabolism are affected by vitamin B_6. This explains why the vitamin B_6 requirement is related to the protein intake—the requirement for vitamin B_6 increasing with increasing levels of protein in the diet. From another aspect, a high intake of the vitamin can improve the ability of the animal to interconvert amino acids in order to utilize a low amino acid intake more efficiently. The need for vitamin B_6 in amino acid catabolism, however, becomes a dominant factor in high protein intakes. It appears, therefore, that the activation and synthesis of vitamin B_6-dependent enzymes is regulated to a certain extent by the intake of both protein and vitamin B_6.

There appears to be a definite relationship between vitamin B_6 deficiency and the endogenous production of oxalic acid. This relationship, which was discussed by Gershoff (Harvard School of Public Health), has been observed in several animal species and a recent survey in Thailand strongly suggests that the vesical calculi (composed of ammonium acid urate and calcium oxalate), which were observed in poor village boys, may be related to inadequate intake of vitamin B_6. In addition, data obtained in a recent nutrition survey in Burma suggest that there is a tendency towards oxaluria in groups of Burmese showing high excretion levels of urinary xanthurenic acid and low excretion levels of vitamin B_6. It is now possible to produce in rats urinary calcium oxalate calculi which are similar to those seen in man so that the effect of diet on the etiology of urinary stone formation can now be studied.

Axelrod *et al.* (University of Pittsburgh) presented an interesting discussion concerning the effect of pyridoxine on the immunological response. The basic role which pyridoxine plays in protein metabolism is further exemplified by the impairment of production of circulating antibodies as well as the state of delayed hypersensitivity which occurs in pyridoxine deficiency. The prolongation of skin homografts in pyridoxine-deficient rats is an illustration of the effect of this deficiency upon a delayed hypersensitivity reaction. This immunological inertness is of clinical value in the field of tissue transplantation. They described experiments in which tolerance to tissue homotransplants was achieved by administering ribosomes or RNA to newborn mice and microsomal RNA to adult pyridoxine-deficient rats. It is postulated that in pyridoxine deficiency the synthesis of messenger RNA is reduced so that a state of immunological inertness results.

Recent research concerning the effect of vitamin B_6 deficiency and various vitamin B_6 antimetabolites on transplantable tumors and lymphoid tissue was reviewed by Rosen (Roswell Institute, Buffalo). A variety of vitamin B_6 antimetabolites was tested against tumors in mice but none were active when the mice were fed laboratory diets containing adequate amounts of vitamin B_6. When a vitamin B_6 deficient diet was used, however, 4-desoxypyridoxine was active in suppressing the growth of neoplasms and also showed selective action against lymphoid tissue. When a vitamin B_6 deficiency was induced by dietary means in Swiss mice which had received transplants of sarcoma 180, complete tumor regression occurred. A complete reversal of this effect occurred, however, when 4-desoxypyridoxine was fed. This may indicate that the vitamin B_6 antimetabolite completely suppressed the generation of antibodies by the host animal. More potent vitamin B_6 antagonists that will be effective in diets containing normal levels of vitamin B_6 are needed before further significant progress can be made in this field.

Although vitamin B_6 deficiency symptoms can be produced in animals on laboratory diets, accounts of vitamin B_6 in textbooks on animal nutrition invariably state that a deficiency of this vitamin is not likely to occur because of its abundance in natural feedstuffs. The animal nutritionists' up-to-date view of the status of vitamin B_6 in farm animals was presented by Fuller (University of Georgia). Of the important economic animals, a limited amount of practical nutritional studies with vitamin B_6 have been conducted with poultry and swine. The ruminant animals do not require a dietary source of vitamin B_6 once the rumen is functioning. Most of the studies which have been carried out show that the average ration under average conditions supplies sufficient vitamin B_6 for normal growth and well being. There are indications, however, that under certain conditions of stress and when the animals are being pressed for maximum gain, vitamin B_6 may become a limiting factor.

In addition, due to the limited data available concerning the vitamin B_6 content of feedstuffs, it is not always possible to formulate a ration to insure adequate intake of vitamin B_6. It was Fuller's conclusion that "In order to insure the well-being of animals and to permit maximum expectation of economical production in farm animals, nutritionists should provide supplemental pyridoxine in feeds for non-ruminants of all species during the period of active growth and reproduction."

Greenberg (University of California) reported on an interesting series of nutritional experiments with rhesus monkeys whose diets were purified and deficient in vitamin B_6. The results of these experiments represent significant contributions to the major public health areas of arteriosclerosis, dental caries, and liver disease. The arteriosclerotic lesions which developed in the vitamin B_6 deficient animals were widespread and affected vessels of all caliber. The distribution of the arteriosclerotic lesions resembled that encountered in man. There also appears to be an effect on cholesterol metabolism in vitamin B_6 deficient monkeys because the feeding of cholesterol to the deficient animals causes a greater degree of hypercholesteremia than in control animals. Whether there is a relationship to unsaturated fatty acid metabolism in the development of the lesions is not definitely established. The development of dental caries in vitamin B_6 deficient monkeys was marked compared to control animals and amount to a fourfold increase. In addition to the caries and effect on tooth development, there were morphological changes in the gingivae, tongue, and jaws of considerable magnitude. Animals deficient in vitamin B_6 for a relatively short period have enlarged smooth, pale livers with fatty alterations, while prolonged deficiency produces scarring associated often with nodules.

Hillman (State University of New York) reviewed the evidence for a possible relationship between vitamin B_6 and dental caries. Although limited clinical trials have strongly suggested such a relationship, the evidence is as yet inconclusive. Pyridoxine lozenges have been given to adolescents (10- to 15-year age group) at dosage levels of 3 mg three times a day in several controlled studies. Two studies indicated significantly fewer cavities and a third study showed a lower but not statistically significant caries incidence. Hillman conducted an extensive clinical trial of vitamin B_6 involving 540 antepartum clinic patients in an urban community with a nonfluoridated water supply. Clinical and roentgenographic examination of the patients both initially and 6 weeks postpartum revealed significantly less incidence of caries in the patients. A pyridoxine lozenge program included an intake of 20 mg of pyridoxine per day. These favorable results highlight the need for further intensive exploration of the possible protective effect of vitamin B_6 against dental caries.

Our present knowledge of pyridoxine responsive anemia in human beings was reviewed by Harris *et al.* (Metropolitan Hospital, Cleveland). The "typical" clinical and laboratory manifestations of pyridoxine responsive anemia in an adult male patient who has been under observation for 9 years was described in some detail as the prototype of this condition. Basically the condition was described as a combination of severe, aregenerative, hypochromic-microcytic anemia, elevated serum iron, and low erythrocyte protoporphyrin which was unresponsive to various therapeutic agents and could be managed only by transfusions. After many experiments with various dosage levels of pyridoxine given either orally or intramuscularly, a dosage level of 50 mg of pyridoxine hydrochloride orally per day was found to give satisfactory management of the patient's hematological status. The authors then presented the analysis of 72 cases of pyridoxine responsive anemia in human beings. There was marked variability among these patients and only 25 of the 72 cases could be documented as "prototypic." The others showed variations in the characteristics of the anemia, in bone marrow findings, in tryptophan metabolism, and so forth, but all showed characteristic abnormalities in iron metabolism. Family histories in 25 percent of the cases suggest a genetically determined error of metabolism. The authors suggest that it would seem reasonable to try a trial of pyridoxine therapy in instances where etiologic mechanisms for the anemia cannot be defined.

The evidence for increased vitamin B_6 requirements in pregnancy and some other conditions and the vitamin B_6 metabolism in infants and children were discussed by Wachstein (Beth Israel Hospital, Passaic, New Jersey) and Coursin (St. Joseph Hospital, Lancaster, Pennsylvania), respectively. These areas have been rather fruitful in providing information concerning the occurrence and characteristics of borderline to actual vitamin B_6 deficiency states. Wachstein pointed out that the stress of pregnancy can increase the vitamin B_6 requirement, and indications are that supplemental vitamin B_6 may often be indicated for pregnant women, particularly in the third trimester. The tryptophan load test and the vitamin load test have both been used to bring out the increased need for vitamin B_6 in pregnancy. Coursin reviewed the actual cases of vitamin B_6 deficiency which have occurred in infants on hospital formulae and the cases of vitamin B_6 dependency which occur in children. One particular case of vitamin B_6 dependency in a young girl was described in some detail because from this particular case a great deal was learned about the nature of the nervous system disorders which are manifested in persons with abnormal vitamin B_6 dependency. These studies, as well as the cases of anemia described by Harris, have been of great value in increasing our knowledge of the function of vitamin B_6. It becomes apparent that since this vitamin is involved in so many important enzymatic reactions, the chances for genetic variations bringing about a whole range of degrees of vitamin B_6 dependency are much greater. It also suggests the possibility that many milder forms of dependency may be obscured or overlooked. Coursin summed up this situation as follows: "The infrequent episodes of convulsive seizures, mental retardation, anemia, oxalate stone formation, etc., that have been clearly shown to be B_6 related may represent the far end of a spectrum of degrees of severity of these entities. If this is true, then as we move toward the other end of the spectrum, we may encounter large numbers of patients in whom the degree of disability and present lack of appropriate diagnostic techniques may obscure the important contributing role of B_6."

Mueller (University of Colorado) reviewed the experimental work which has been undertaken to clarify the role of vitamin B_6 in fatty acid and cholesterol metabolism. For 14 years investigators have attempted to determine the exact nature of the relationship beween vitamin B_6 and fatty acids, but experimental studies with the rat, chicken, dog, and the monkey have failed to define the relationship clearly. There are definite indications that vitamin B_6 is involved in the conversion of lino-

leate to arachidonate but so far the animal studies have not conclusively proven this relationship. It may be that the use of an experimental animal in various stages of vitamin B_6 deficiency is too complicated a biological system to unravel the mystery, but work continues in this direction. There is evidence that pyridoxine deficiency is often associated with hypercholesteremia but here again there is very little information concerning the mechanism. Since there is no evidence of a direct effect on synthesis or degradation of cholesterol, pyridoxine may be influencing the transport of cholesterol by its effect on fatty acid metabolism or an alteration in the protein moiety of the lipoprotein required for cholesterol transport. A hypocholesteremic effect of vitamin B_6 in humans appears to be unproved. A human study, however, by Mueller and Iacono appears to have linked pyridoxine with fat metabolism and the results obtained are consistent with those found by the majority of investigators with animals. The site of action, however, still remains unclear.

Toepfer (U.S. Department of Agriculture, Beltsville, Maryland) and Storvick *et al.* (Oregon State University) covered the recent methods for analysis of vitamin B_6 in foods and the methods for determining vitamin B_6 in biological materials. Toepfer described analytical techniques using ion exchange resins for the separation of the three forms of vitamin B_6 followed by microbiological assay of each form separately against the proper standard. It was felt that this technique gave a more reliable estimate of the vitamin B_6 activity of a given foodstuff. Storvick reported her investigations on methodology for determining vitamin B_6 in biological materials. The rather wide variations in literature values for blood and tissue levels of vitamin B_6 in various species were found to be due primarily to difficulties in liberating the phosphorylated and protein-bound vitamin. Available methods involving acid hydrolysis or enzyme digestion require further development before they can be considered adequate. Problems encountered with microbiological, chemical, and enzymatic methods for measuring the liberated vitamin were discussed, as well as the fluorometric method for measuring 4-pyridoxic acid, the main urinary metabolite of vitamin B_6. Further development of methodology is required to permit reliable assessment of vitamin B_6 nutritional status and metabolic patterns.

The occurrence of vitamin B_6 deficiency in the human and the studies which have been conducted to arrive at an estimation of the vitamin B_6 requirement of the human were reviewed by Sauberlich (U.S. Army Medical Research and Nutrition Laboratory, Denver). Although the occurrence of vitamin B_6 deficiency in the human population definitely establishes a requirement for this vitamin, it gives little information concerning the minimum requirements. Recent studies in humans, however, have provided very good estimates of the vitamin B_6 requirement of man. Two recent nutrition surveys, one conducted in Burma and the other in Malaysia, have provided valuable information regarding the minimum requirements for vitamin B_6. In Burma the daily intake of vitamin B_6 averaged 1.70 mg per man per day with the highest being 2.10 mg. A considerable portion of these persons, however, reveal biochemical abnormalities related to the metabolism of vitamin B_6. In Malaysia intakes of vitamin B_6 range between 1.04 and 1.42 mg per man per day, but here again the biochemical abnormalities related to pyridoxine were noted. Recent studies conducted in Sauberlich's laboratory provide the best evidence to date of the human requirement for vitamin B_6. These studies were conducted with young, healthy, adult male subjects, ages 18 to 22 years, on liquid pyridoxine-free diets with high and low protein intakes. The most marked clinical manifestations observed during the deficiency period in the subjects were electroencephalographic abnormalities. In fact, one subject on the low protein intake had a grand mal convulsion during the 7th week of deficiency. As a result of these studies, the optimum daily vitamin B_6 diet for subjects on a high protein diet was found to be 1.75 to 2.0 mg per day and for subjects on a low protein diet 1.25 to 1.5 mg per day. The vitamin B_6 requirement for infants has been generally accepted to be approximately 0.40 mg per day. It is evident that besides the effects of protein intake on the vitamin B_6 requirement other factors such as stresses, sex, and age have an important bearing on vitamin B_6 requirements, but further studies in this area are definitely required.

The vitamin B_6 requirement and the methods for estimating it as well as the relationship of this requirement to actual amounts in the diet were reviewed by Borsook (California Institute of Technology). The literature suggests that the requirement of older people is greater than that of young adults. Estimation of requirements of vitamin B_6 based on energy expenditures is not feasible since pyridoxal phosphate is involved in many enzymatic reactions which release little energy. Individual requirements vary more than that of other vitamins and complicate definition of requirements. The tryptophan load test on young men indicates that 2.76 mg of vitamin B_6 is adequate for 90 percent of the population. It was pointed out that it is diffcult to fullfill the Recommended Dietary Allowance of 1.5 to 2.0 mg per day of vitamin B_6 for women and older men if the calorie intake is restricted to that recommended for their height, weight, and age. In addition, if the object of setting up a Recommended Daily Allowance is to prevent a vitamin B_6 deficiency state in 99.99 percent (10 per 100,000) of the population, then the data indicate that 1.5 to 2.0 mg are not enough. To fulfill this criterion, the requirement would be in the range of 2.5 to 7.0 mg. Borsook concluded that the case is strong for increasing the vitamin B_6 in the food supply. This could easily be done by adding vitamin B_6 to the present flour enrichment formula; all the reasons that led to the original enrichment formula apply to the inclusion of vitamin B_6.

This international symposium on vitamin B_6 was sponsored by Hoffmann-La Roche Inc., and the complete papers will appear in the forthcoming volume of *Vitamins and Hormones.*

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Forthcoming Events

November

5–7. **Nutrition Hygiene** Conf., Brno, Czechoslovakia (K. Halacka, Hygiene Section, Czechoslovak Medical Soc., Sokolska 31, Prague 2)

6–7. **Biochemistry**, 7th annual West Central States conf., State Univ. of Iowa, Iowa City. (G. F. Lata, Dept. of Biochemistry, State Univ. of Iowa, Iowa City)

6–7. Experimental Methodology and Applied Immunology in **Allergy Research**, symp., Erfurt, East Germany. (H. D. Faulhaber, Gesellschaft für Experimentelle Medizin der D.D.R., Littenstr. 78, Berlin C.2, East Germany)

6–7. Central Soc. for **Clinical Research**, Chicago, Ill. (J. F. Hammarsten, Ancker Hospital, St. Paul 1, Minn.)

7. International Acad. of **Oral Pathology**, 2nd conf., San Francisco, Calif.