Some Metabolic Effects of Vitamin B_6 in Vivo

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THIS discussion of biochemical changes in the vitamin B_6-deprived rat commences with reference to a simple set of observations with which we are all familiar. Within a few days after the start of the deprivation the rat shows a decreased appetite, consequent to which there is a progressively more evident failure to increase body weight. A control, receiving the vitamin but pair-fed with the deprived rat, exhibits no loss of appetite and gains weight slowly, so that after several weeks there is a significant difference in weight between the deprived and the control rats. Carcass analyses have shown that the difference in weight is accounted for entirely by difference in content of fat and water; the protein contents are nearly equal.[1]

This set of observations is not unique for vitamin B_6 deprivation but is shown in nearly all nutrient deficiencies. In the case of vitamin B_6, we have no explanation for the decrease in appetite. For some years the anorexia in thiamine-deprived rats has been explained by the use of early observations of gastric hypotonus, observations which could not be duplicated in some subsequent studies.

An elevation in fasting blood sugar or a prolonged rise after food ingestion could explain the decrease in appetite. Neither of these changes has been observed so far. In fact, a decreased fasting blood sugar has been reported from our laboratory.[2]

Although the vitamin B_6-deprived rat has less body weight and less fat than the pair-fed control, the oxygen consumption per unit of body weight is equal.[3] The consumption per animal is greater for the control than for the deprived rat because of the difference in body weight. The data for oxygen consumption have failed to explain the difference in body weight. Studies on food absorption in several laboratories[4,5] have been similarly fruitless in explaining the body weight difference, since no failure in absorption was evident in the deprived animal.

PROTEIN AND FAT METABOLISM

We have not seen any failure in protein maintenance or synthesis in vitamin B_6-deprived rats. The blood proteins are not decreased;[5] the total protein content in the carcass is not lessened, although the deprived animal is in somewhat less positive nitrogen balance.[5] A negative nitrogen balance has not been observed even in severe deficiency. Neither does the deprived rat show any lessening in the ability to regenerate liver protein after partial hepatectomy.[6] We have concluded that vitamin B_6 deficiency in the rat does not impair protein maintenance or protein synthesis. It should be noted that the less positive nitrogen balance is due to an increased excretion of urea and that liver slices show an increased rate of urea formation.[7]

Reference has been made to the difference in fat content between the deprived rat and the pair-fed control. No real difference in the spectrum of fatty acids has been seen other than some increase in the proportion of highly unsaturated fatty acids in the deficient animal.[8] The proportion of phospholipids is also increased. Studies with C^{14}-labeled glucose suggest that the deprived rat's ability to synthesize fat from carbohydrate is not impaired.

CARBOHYDRATE METABOLISM

Dr. J. R. Beaton has recently reported his observations on the effects of vitamin B_6 depri-

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vation on carbohydrate metabolism. These observations are summarized briefly. As early as five days after omitting the vitamin from the diet, rats show a reduction in fasting blood sugar, and the discrepancy in blood sugar between deprived and control rats is accentuated as the deficiency progresses.[2] The fasting blood levels of pyruvic and lactic acids are also decreased. The amount of hepatic glycogen is diminished, as is the lactic acid dehydrogenase activity of liver homogenates. Beaton also found significant elevations in inorganic phosphorus and glutathione in blood and livers from deprived rats.[9] No significant difference was found in glucose utilization by muscle, nor in liver cytochrome oxidase activity. Liver aldolase activity, however, was definitely decreased. The plasma alkaline phosphatase activity was markedly increased in deprived rats.[10] All of these findings led to the conclusion that vitamin B_6-deprived rats exhibit alterations in carbohydrate metabolism.

ENZYMATIC ACTIVITY

It is widely accepted that vitamin B_6 acts as a coenzyme for one or more transaminases, for amino acid decarboxylase, and presumably in the dehydration of hydroxyamino acids and in the desulfhydration of sulfur amino acids. A recent review[11] contains the statement that "Pyridoxal-5-phosphoric acid can be considered as almost generally required for enzymatic reactions involving the nonoxidative degradation and interconversion of free amino acids." This explanation of the function of vitamin B_6 is generally accepted but, unfortunately, there are still questions regarding biochemical changes in the intact animal. Does this function explain the various biochemical alterations which have been observed in the intact animal? Does the accepted function explain the dermatitis found in the deprived rat? Does this explain the production of atherosclerosis in the deprived monkey?

From these questions stem a secondary set of problems: Is the evidence regarding the relation of vitamin B_6 to transamination so clear-cut that it must be accepted? What purposes are served by transamination in the metabolic processes of the intact animal? Furthermore, how many of the reported biochemical abnormalities are specific for vitamin B_6 deficiency?

In a study[1] of the chronological development of biochemical changes in the vitamin B_6-deprived rat, we found that transaminase activity in the liver did not decrease as the deficiency developed but remained fairly constant. The transaminase activity in the livers of control rats increased and seemed to keep pace with body weight. If we had had no transaminase estimations available except those at the end of the experiment, we would have concluded, probably, that transaminase activity had decreased in the deficient rat. Several other discordant notes may be added. The total vitamin B_6 content of the liver attains a minimal level comparatively early in deprivation. Administration of deoxypyridoxine does not lessen transaminase activity in the liver but actually causes an increase.[12]

TRANSAMINASE

We have recently extended observations on the alanine-glutamic and aspartic-glutamic transaminase activities in rat liver, and in parallel experiments have compared the effects of several B-vitamin deficiencies. Separate observations were made on male and female rats. In each deficiency the selected time of measurement was when the deprived animals had reached a plateau in body weight. Starting with young rats, there was an initial period of weight increase. Following an impairment of appetite, body weight was constant for several days. Deficient animals and corresponding pair-fed controls were killed for enzyme and other studies. This arrangement was followed in the hope that the various deficiencies would be comparable in degree at that period. It should be noted that rats deprived of various vitamins reached the chosen stage at different intervals.

In the case of alanine-glutamic transaminase, deficient male rats showed the failure to increase which had been noted previously, but the deficient females had a real decrease. The data for this enzyme are given in Table I. Rats deprived of any one of four other B vitamins (thiamine, riboflavin, pantothenic acid,

TABLE I

Hepatic Alanine-Glutamic Transaminase in Deficiencies of Several B Vitamins

Status	Pyruvate (μl/g/hr) Males	Females	Mean
Initial	40	48	44
Thiamine-deficient	58	63	60
Riboflavin-deficient	88	56	72
Vitamin B_6-deficient	38	34	36
Pantothenate-deficient	60	64	62
Biotin-deficient	65	63	64
Average paired controls	52	51	52
Ad lib controls	52	65	58

or biotin) showed increases in alanine-glutamic transaminase, the most marked increase being evident in riboflavin deprivation.

Values for aspartic-glutamic transaminase showed a lesser change (Table II). Vitamin

TABLE II

Hepatic Aspartic-Glutamic Transaminase in Deficiencies of Several B Vitamins

Status	Pyruvate (μl/g/hr) Males	Females	Mean
Initial	124	94	109
Thiamine-deficient	126	121	123
Riboflavin-deficient	139	130	135
Vitamin B_6-deficient	101	99	100
Pantothenate-deficient	117	104	111
Biotin-deficient	135	129	132
Averages of paired controls	123	112	118
Ad lib controls	92	125	109

B_6-deficient male rats had a slight decrease, while rats deprived of either riboflavin or biotin exhibited increases in this enzyme. It had been reported previously[13] that aspartic-glutamic transaminase was insensitive to changes in vitamin B_6 nutriture.

Contrasting the deficiencies of five separate B vitamins, it is evident that alanine-glutamic transaminase either decreases or fails to increase in vitamin B_6 lack. In four other deprivations the hepatic activity of this enzyme is increased. This difference provides support for the conclusion that vitamin B_6 deficiency has an opposite effect on alanine-glutamic transaminase from that found in four other deficiencies (thiamine, riboflavin, pantothenic acid, and biotin). Observations on aspartic-glutamic transaminase show the two opposite trends, but not so definitely.

TABLE III

Ratios of Alanine-Glutamic Transaminase to Body Weight in Several B-Vitamin Deficiencies

Vitamin		Deficient	Pair-fed control
Vitamin B_6	Males	0.19	0.21
	Females	0.21	0.36
Thiamine	Males	0.31	0.21
	Females	0.45	0.30
Riboflavin	Males	0.49	0.28
	Females	0.36	0.31
Pantothenic acid	Males	0.27	0.18
	Females	0.37	0.26
Biotin	Males	0.26	0.22
	Females	0.33	0.23

In a previous report[13] from our laboratory a sex difference in transaminase activities was noted. Some differences in the sexes were also found in the more recent observations. From work on hamsters, Shwartzman and Hift[14] concluded that hepatic transaminase activity increases with age. In one experiment we noted a confirming observation. However, we have had the impression that there is a relation between the amount of hepatic transaminase activity and body weight, at least in normal animals. If such were the case, the ratio of transaminase activity to body weight should be fairly uniform. A deviation from such a normal ratio could be a better indication of a change in transaminase activity than the absolute activity. At least, this ratio would allow for a possible correlation of transaminase with body weight. The ratios for group averages in the recent observations are shown in Table III. Two conclusions may be drawn from these ratios: (1) some sex difference is evident; (2) transaminase alterations in vitamin B_6-deficient rats are different from alterations in individual deficiencies of four other B vitamins. The observations, then, are in accord with the generally accepted view that vitamin B_6 is related to transamination.

SPECIFICITY

Before we can consider how the involvement in transamination could explain any of the various biochemical alterations seen in vitamin B_6-deprived rats, it is necessary to decide whether various biochemical changes

are specific for this deficiency. There is no point in attempting to correlate a function with observed alterations unless the function and the alterations are specifically caused by the nutritional deficiency.

It has been noted that the rat deprived of vitamin B_6 shows a lessening in appetite, a failure to gain weight, and a decrease in fat storage (or at least less fat storage than a pair-fed control). None of these effects of the deprivation is specific for a lack of vitamin B_6. All of them have been observed in rats deprived of almost any nutrient. Recently we have made observations on several biochemical aspects of five B-vitamin deficiencies. At the particular stage of insufficiency used we could find no evidence that changes in fasting blood sugar, in fasting blood urea, or in liver glycogen were characteristic for any one of the deficiencies. While many other aspects need to be studied, it should be noted: (1) that B-vitamin and possibly other nutrient deficiencies present a common picture with regard to some biochemical alterations; (2) that the only alteration observed by us so far to be different in vitamin B_6 deficiency from other B-vitamin deprivations concerns alanine-glutamic transaminase.

DISCUSSION

There are, however, discordant aspects with regard to the relation of vitamin B_6 to transamination. Hepatic transaminase activity does not keep pace with the total vitamin B_6 content of the liver and tissues. This could be explained by conjecturing that the enzyme activity keeps pace with the amount of pyridoxal, but not with the quantities of other vitamin B_6 compounds. Such a conjecture would imply a lack of convertibility even in a stage of deprivation. Rats given deoxypyridoxine and deprived of the vitamin develop dermatitis rapidly but show an increased transaminase activity in liver homogenates. Either an impairment in transamination has no relation to the dermatitis, or else transamination is not the principal role of the vitamin. Changes in appetite, in carbohydrate metabolites, and in body composition may not be specific for vitamin B_6 deficiency, but they are clearly evident before a decrease in transaminase can be detected.

If we assume, as is commonly done, that the role of pyridoxal as the coenzyme for transamination is the main function of the vitamin, it is useful at the present stage of our knowledge to consider the purposes which may be served by the vitamin acting in transamination in the intact animal. It may be that transamination is a pathway in amino acid conversion to carbohydrate and, through carbohydrate, to fat. A decrease in transaminase activity would lessen this type of amino acid degradation and force greater amounts of amino acids to be catabolized in other ways, for example, by deamination. In this way we could explain the increased production of urea, and, possibly, an unusual pathway for the degradation of tryptophan. If transamination is one of several customary methods for handling some carbohydrate metabolites, we could partially explain the presence of an increased amount of pyruvic acid and perhaps of lactic acid, both of which have been found in augmented amounts in the blood of fasting, deprived rats.

In a discussion of biochemical changes in the intact animal two admissions are necessary at present. I have presented a picture showing a lack of essential information and of confusion. A partial account of biochemical lesions is available, but the number of these alterations which are really specific for vitamin B_6 deficiency is not known. The lessons to be drawn are obvious.

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