DIETARY PROTEIN–ITS RELATIONSHIP TO VITAMIN B_6 REQUIREMENTS AND FUNCTION

J. E. Canham, E. M. Baker, R. S. Harding
H. E. Sauberlich and I. C. Plough
U.S. Army Medical Research and Nutrition Laboratory
Denver, Colo.

In the decade following the identification of vitamin B_6, it was recognized by many investigators that vitamin B_6 was intimately involved in amino acid metabolism. Lepkovsky and Nielson first reported increased urinary excretion of xanthurenic acid from B_6-deficient rats.[1] Other scientists established that the addition of specific amino acids to the diet of vitamin B_6-deficient animals would aggravate the deficiency state. Concomitantly, it was recognized that a high protein, vitamin B_6-deficient diet would hasten the onset and magnify the severity of B_6 deficiency in animals.[2] In infants receiving a diet providing a suboptimal intake of B_6, additional dietary casein aggravated central nervous system manifestations of B_6 deficiency, while a high carbohydrate diet alleviated these symptoms.[3] In a study conducted at our laboratory, Harding and colleagues[4] reported that human volunteers subsisting for 24 days on a packaged military ration providing 165 g of protein and 1.93 mg of vitamin B_6 per day developed a statistically significant elevation of xanthurenic acid excretion after a tryptophan load at the end of the period on the diet. No significant alteration in xanthurenic acid excretion after tryptophan loading was observed in the same subjects eating a similar ration that provided 164 g of protein and 2.76 mg of vitamin B_6 per day for 24 days.

Methods and Studies

In the past twelve years, staff members of this Laboratory have conducted multiple studies to determine the metabolic and clinical responses to vitamin B_6 deficiency and to ascertain the minimum vitamin B_6 requirement of the human. All studies have been conducted on young, adult males who were housed and maintained on a metabolic ward where the dietary intake was rigidly controlled and daily physical activity was controlled and constantly supervised. A variety of dietary regimens have been employed in an attempt to better define the interrelationships contributing to vitamin B_6 requirements and the influence of various nutrients upon the biochemical changes seen with the various levels of vitamin B_6 intake. Dietary protein is a parameter that has been varied in some of these studies. The daily intake has varied from 30 g to 165 g.

During the conduct of these studies, various parameters have been investigated, including hematological changes, blood vitamin B_6, plasma free amino acids, various transaminases, plasma proteins, serum lipids, the urinary excretion of oxalic acid, N-methylnicotinamide, riboflavin, sulfate, creatinine, nitrogen balance, amino acids, vitamin B_6 and 4-pyridoxic acid. The urinary excretion of xanthurenic acid and a limited number of the other metabolites of the kynurenine pathway, as well as metabolites of the serotonin pathway of tryptophan metabolism, have been studied before and after tryptophan loading. Serial electrocardiograms (ECG's) and electroencephalograms (EEG's) have been obtained during some studies. The effect of alanine loading on blood urea nitrogen was investigated during one study. Multiple other parameters, biochemical and clinical, were investigated depending upon the interest of the investigators. In this

report, emphasis will be placed upon the 24-hour urinary excretion of xanthurenic acid following a tryptophan load. In all instances, the tryptophan load was administered to subjects in a fasting state shortly after they arose in the morning. Xanthurenic acid was determined as described in the Manual for Nutrition Surveys of the ICNND.[5] Urinary 3-hydroxykynurenine was determined by the method of Brown,[6] and urinary levels of free vitamin B_6 were measured by a microbiological assay method using *Saccharomyces carlsbergensis*.[7]

Due to the brevity of this paper, only limited aspects of selected studies will be presented. Though the urinary excretion of xanthurenic acid after tryptophan loading is emphasized, the authors readily admit that this test does not provide an accurate picture of vitamin B_6 status in all instances. However, under the conditions of these investigations, the excretion of xanthurenic acid after tryptophan loading proved to be the most reliable of the biochemical tests performed for evaluation of the vitamin B_6 status of the subjects studied.

Rapidity of Onset of Biochemical Manifestations of B_6 Deficiency vs Protein Intake

In a test conducted at this Laboratory by E. M. Baker, I. C. Plough, and R. C. Powell, young, healthy, adult male test subjects were fed a completely synthetic diet consisting of vitamin-free casein, dextrose and coconut oil for seven weeks (Study 1). The diet was supplemented with sufficient vitamins and minerals to make it nutritionally adequate for all nutrients except vitamin B_6. Group A received a daily protein intake of 80 g, plus 3 g of DL-methionine, while Group B received 40 g of protein and 1 g of methionine per day. During the first week, (control period) 2 mg of pyridoxine hydrochloride were given per day. No vitamin B_6 supplement was provided during the next four weeks but during the recovery period (two weeks), 10 mg of pyridoxine were given per day. Xanthurenic acid excretion was determined in a control urine on the sixth day of each seven-day period and again on the seventh day after a 10 g DL-tryptophan load. FIGURE 1 discloses that by the end of one week of deficiency the high protein group (Group A) had a prompt rise in the net xanthurenic acid excretion following administration of the tryptophan load. By the end of four weeks of deficiency, the mean net xanthurenic acid excretion for the group was 430 mg per 24 hours. The low protein group (Group B) did not show a rise in the net xanthurenic acid excretion until the end of the second week of deficiency, following which there was a gradual but steady rise in net xanthurenic acid excretion until a total of 246 mg was measured on the last day of depletion. After only three days on the 10 mg supplement of pyridoxine, the xanthurenic acid excretion after a tryptophan load had fallen to normal values for both groups. During the depletion phase, there was a progressive fall in the levels of serum glutamic-oxaloacetic transaminase. These levels did not return to normal during the two-week recovery phase. The pyridoxic acid excretion reflected the dietary intake of vitamin B_6 but was not accurate due to the technique employed.[8] The levels of N-methylnicotinamide urinary excretion remained unchanged throughout the experiment.

In a subsequent study (Study 2), aspects of which have been reported in detail elsewhere,[9,10,11,12] 11 healthy young men were placed on a vitamin B_6-deficient formula diet, with 5 subjects receiving 30 g and 6 subjects receiving 100 g of protein per day. Prior to the study, all subjects were found to be healthy, historically free of convulsive seizures and had normal EEG's. For the purpose of this

study, a deficiency was defined as a net urinary excretion of xanthurenic acid of greater than 200 mg in the 24 hours following a 10 g DL-tryptophan load in at least 80% of the subjects of a group. Once a deficiency state was established for the group, supplementation with pyridoxine hydrochloride was begun. During the deficiency period, both 3-hydroxykynurenine and xanthurenic acid urinary excretion following a tryptophan load, rose.

FIGURE 2 discloses the results of EEG's taken throughout the study for the subjects on the high-protein diet. The average daily pyridoxine intake in the period intervening between each set of EEG's and the mean net xanthurenic acid excretion are also indicated. In this study, the rise in xanthurenic acid excretion did not become marked until after two weeks of depletion, but by the end of the third week of depletion, excretion had reached the criteria for deficiency as previously defined for this study. Five of the six individuals who started the study had abnormal EEG's at the end of the third week of depletion. There was a delay in return to normal of some of the EEG's during the period of vitamin B_6 supplementation. The individual with normal EEG's throughout the study consistently demonstrated the highest net excretion of xanthurenic acid and 3-hydroxykynurenine after tryptophan loading during the period of depletion.

FIGURE 3 discloses the results for the low-protein group. During the de-

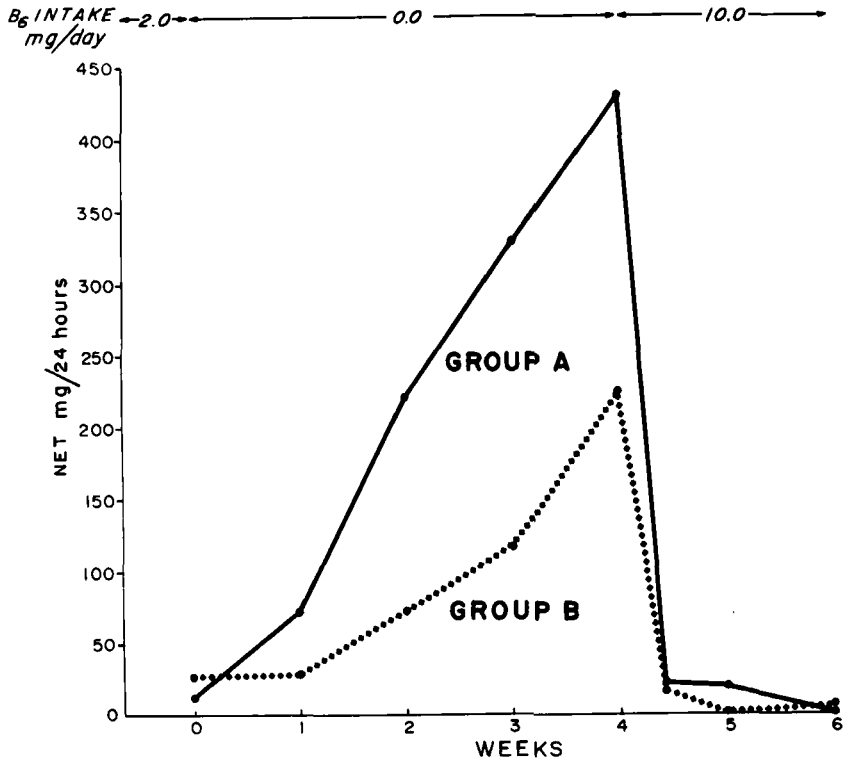

FIGURE 1. Net urinary excretion of xanthurenic acid during the 24 hours following administration of a 10 g DL-tryptophan load and level of vitamin B_6 intake while subjects of Group A and B were receiving a daily dietary protein intake of 80 g and 40 g, respectively.

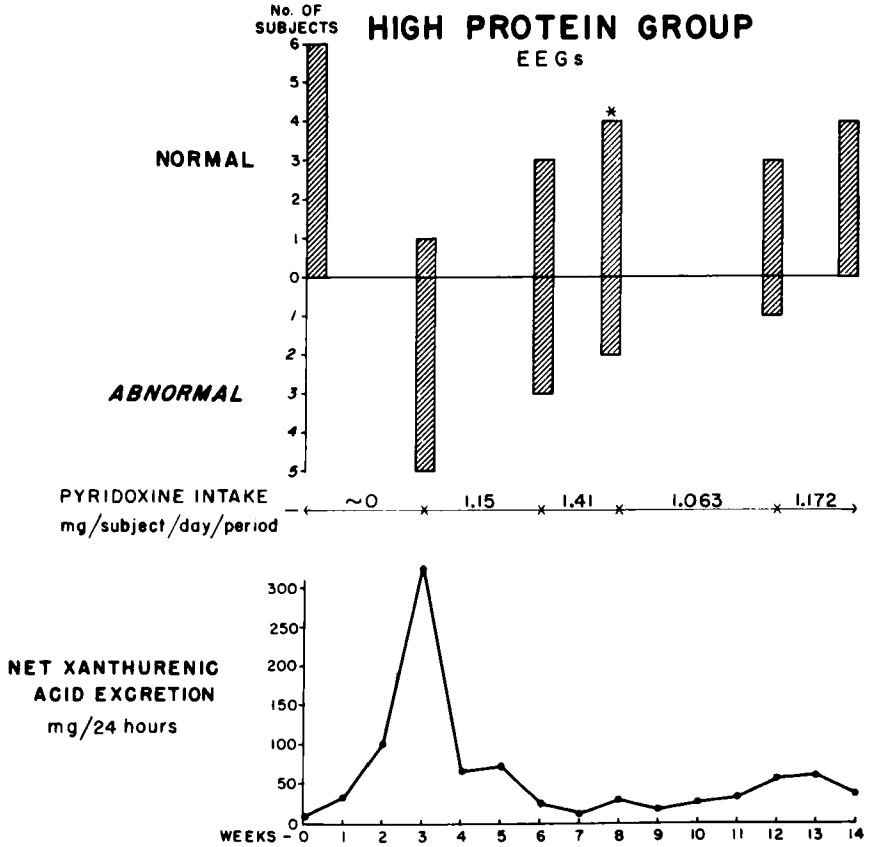

FIGURE 2. Net urinary excretion of xanthurenic acid following administration of a 10 g DL-tryptophan load in subjects receiving a high protein (100 g) diet. Level of pyridoxine intake represents average daily intake for periods between electroencephalograms (EEG's). The number of subjects with normal or abnormal EEG's is indicated. The asterisk indicates that one subject, with a previously abnormal EEG, had a normal EEG after five days on a normal diet and 150 mg of pyridoxine/day.

pletion period, the mean net xanthurenic acid excretion did not become elevated until the end of the third week, with only a gradual increase in xanthurenic acid excretion until after the fourth week of depletion. It is interesting to note the rise in net xanthurenic acid excretion near the end of the study. This appeared to be directly related to the addition of two grams of DL-methionine to the formula diet during the last two weeks of the study. The response of the 3-hydroxykynurenine during the first four weeks of depletion for this group was quite variable. Despite a relatively low mean net xanthurenic acid excretion, four of the five subjects had abnormal EEG's after three weeks of depletion. Again, the subject with the highest net 3-hydroxykynurenine (740 mg) and xanthurenic excretion (405 mg) at the end of the depletion period had normal EEG's throughout the study. The columns marked by the double asterisk indicate that one of the individuals with an abnormal EEG had been receiving a regular diet and 150 mg of pyridoxine per day after 6 1/2 weeks of depletion. His EEG's did not

revert to normal until approximately 30 days after institution of this regimen. The regimen had been instituted because of the impending completion of his contractural length of service.

It is unfortunate that EEG's were not obtained at shorter intervals during this study. Electroencephalograms were obtained prior to the study, at the end of the first three weeks, again at the end of six weeks of the study and when believed indicated throughout the rest of the study. More frequent EEG's may have provided better insight into the relationship of vitamin B_6 depletion, central nervous system B_6 deficiency, and the biochemical changes of the deficiency state.

It had been suggested prior to the study by some of our colleagues, that the subjects on the low-protein intake might have difficulty in obtaining nitrogen balance in the face of vitamin B_6 deficiency. Prior to the control period, the subjects had received approximately 125 g of protein/day from a standard hospital diet. On changing to the 30 g/day protein diet, all the subjects, as could be expected, were in a negative nitrogen balance for the control week, with an

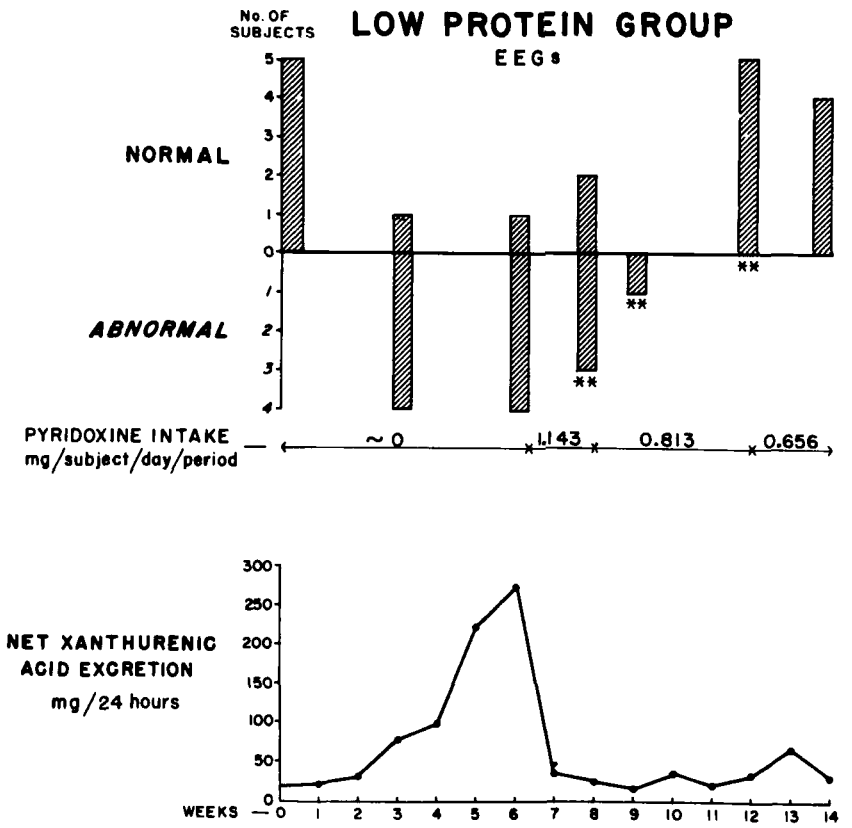

FIGURE 3. Net urinary excretion of xanthurenic acid following administration of a 10 g DL-tryptophan load in subjects receiving a low-protein (30 g) diet. Level of pyridoxine intake represents average daily intake for periods between EEG's. The number of subjects with normal or abnormal EEG's is indicated. The double asterisk indicates that one subject was receiving a normal diet and 150 mg of pyridoxine/day during the indicated period.

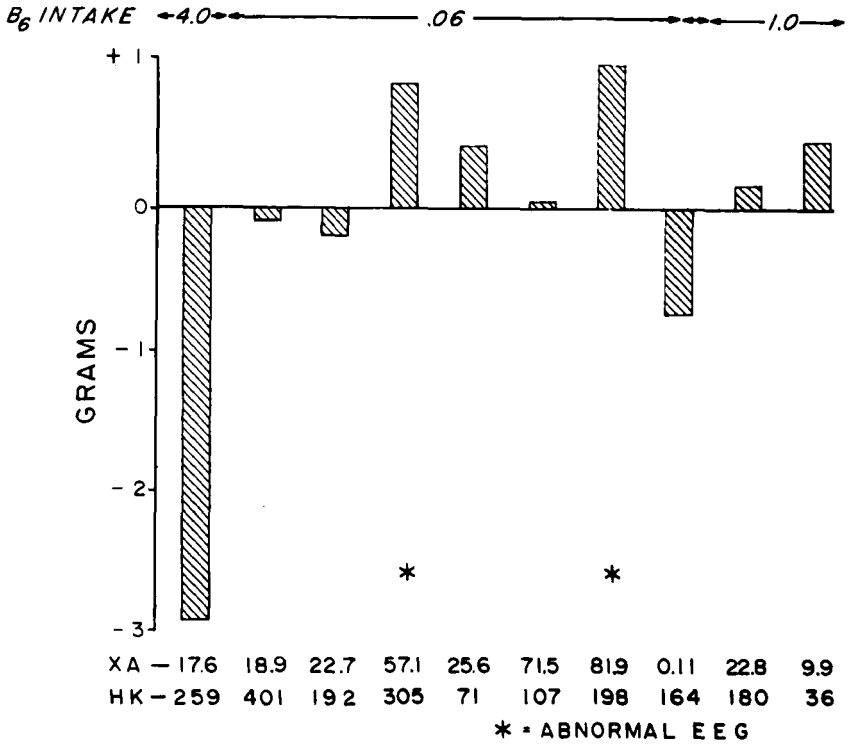

FIGURE 4. Nitrogen balance and level of vitamin B_6 intake of one subject receiving a low-protein (30 g/day) diet. Weekly values of net urinary excretion for this subject are indicated in milligrams/24 hours of xanthurenic acid (XA) and 3-hydroxykynurenine (HK) following administration of a 10 g DL-tryptophan load.

average loss of 1.34 g of nitrogen per man per day for that period. Thereafter, four of the five subjects obtained and maintained nitrogen balance throughout the duration of the study. The fifth individual (FIGURE 4), who had the largest lean body mass and greatest body weight (initially 79 kg), manifested greater negative nitrogen balance during the control period and slight negative nitrogen balance during the first two weeks of depletion. During the seventh week of depletion, his daily nitrogen loss averaged 0.76 g per day. Throughout the rest of the study, he remained in positive nitrogen balance. It is interesting that this individual had an abnormal EEG at the end of the third week and at the end of the sixth week of depletion, but his xanthurenic acid excretion never exceeded 81.9 mg throughout the depletion period. His 3-hydroxykynurenine excretion did promptly increase during the first week of depletion and was slightly elevated during the third week of depletion. Throughout the other weeks of the study, 3-hydroxykynurenine excretion was within normal limits.

This second study exemplifies the variability in subjects on a low-protein diet. All the subjects receiving a high-protein diet by the end of the third week of depletion had, xanthurenic acid excretions above 239 mg following a tryptophan load, while only two subjects in the low-protein group exceeded 100 mg at that point. Despite this, four of the five subjects on a low-protein diet had abnormal EEG's. The response of both groups to tryptophan loading as measured by

elevation of xanthurenic acid excretion was not as prompt as that observed during the first study, despite the similarities of the diets. One explanation for this might lie in the increased pyridoxine supplement that the subjects of the second study (4 mg) received during the control period as compared to the first study (2 mg). A second explanation may be the methionine supplement the subjects received throughout Study 1. Animals deficient in vitamin B_6 had increased severity and rapidity of the onset of deficiency when methionine was added to the diet.[13] Some evidence of this, as demonstrated by the excretion of xanthurenic acid and 3-hydroxykynurenine following a tryptophan load, was noted during the second study when 2 grams of methionine were added to the diets of the low-protein subjects during the last two weeks of the study.

Dr. H. Linkswiler and coworkers have conducted a series of similar studies [14-17] and have noted that the levels of hydroxykynurenine, xanthurenic acid, acetylkynurenine, kynurenic acid and quinolinic acid were increased in vitamin B_6-deficient subjects after a 2 g L-tryptophan load. In one study, subjects receiving a diet providing 0.16 mg of vitamin B_6 and 54 g of protein per day for 40 days were compared to a group of subjects receiving for 16 days a diet that provided 0.16 mg of vitamin B_6 and approximately 150 g of protein per day. There was a slow, gradual increase in excretion of tryptophan metabolites after a 2 g L-tryptophan load in the majority of the low-protein subjects. However, one subject failed to show any increase in the tryptophan metabolites throughout the 40 days

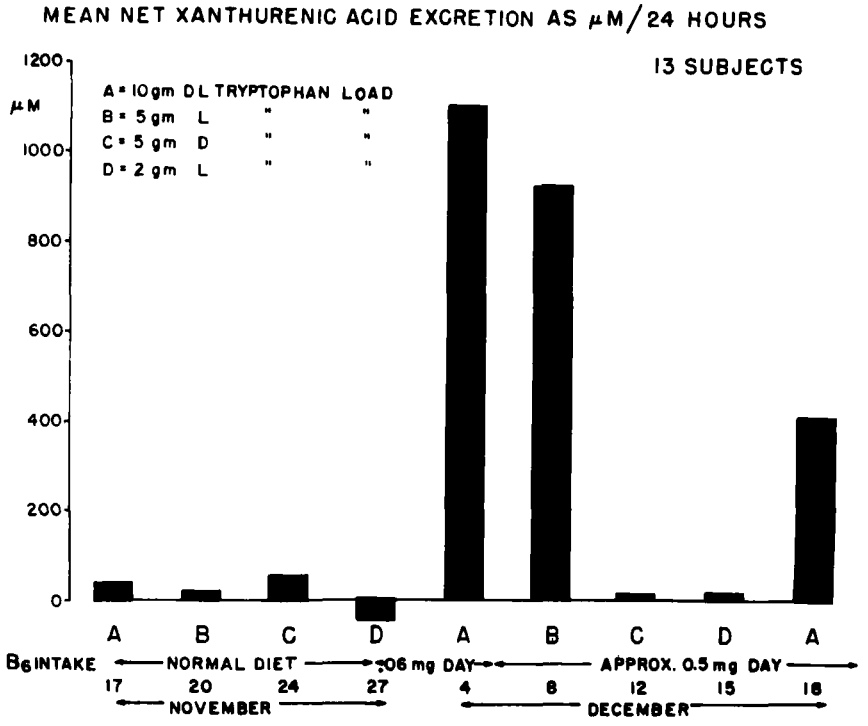

FIGURE 5. Net urinary excretion of xanthurenic acid following administration of various tryptophan loads, and level of vitamin B_6 intake.

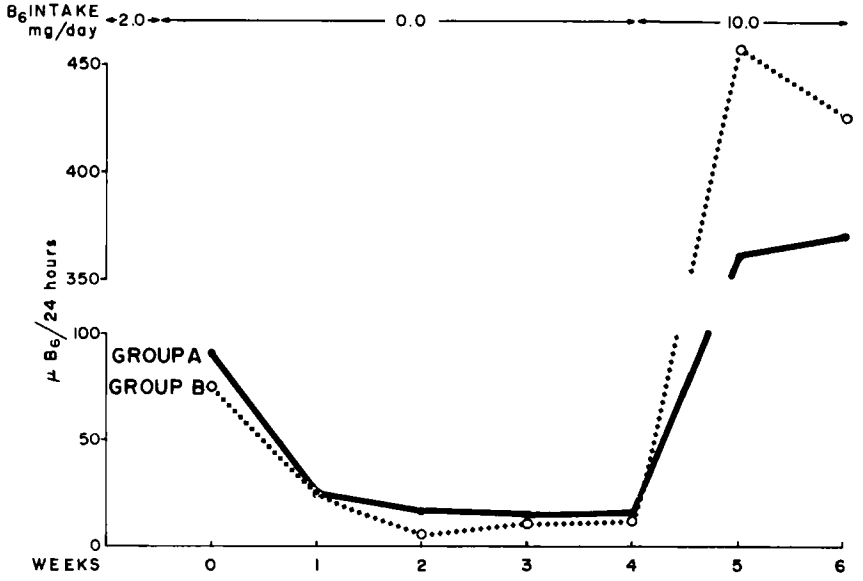

FIGURE 6. Urinary excretion (three-day means) of free vitamin B_6 and level of vitamin B_6 intake while subjects of Group A and Group B were receiving a daily dietary protein intake of 80 g and 40 g, respectively.

of depletion. Conversely, the high protein group demonstrated a fairly prompt increase in the excretion of the majority of the tryptophan metabolites after 6 days of deprivation and marked elevation after 14 days of deprivation.

Coursin has recommended that a standard dose of 2 g of L-tryptophan be utilized in adults for the tryptophan loading test.[18] However, he did not fully establish his reasons for arriving at this dose level. It was our feeling that possibly some of the minor observed differences between our studies and Linkswiler's could be explained by the difference in diet, the difference in dietary intake of vitamin B_6 during the depletion period, the fact that our subjects were on a controlled, required physical activity program throughout the studies (we have shown that the pattern of xanthurenic acid excretion after tryptophan loading is greater in the individual when he is physically active than when he is sedentary[19]) and the difference in the tryptophan load utilized in our studies. To evaluate this possibility, 13 normal adult males were placed upon a normal hospital diet for 12 days and received, in succession, loads consisting of 10 g of DL-tryptophan, 5 g of L-tryptophan, 5 g of D-tryptophan, and 2 g of L-tryptophan. They were then given a synthetic formula diet containing 0.06 mg of vitamin B_6 for eight days, followed by a diet containing normal or specially processed normal foods providing approximately 0.5 mg of vitamin B_6 per day. Net xanthurenic acid excretion after the various tryptophan loads was again evaluated. The net increase in xanthurenic acid excretion (FIGURE 5) following either a 10 g DL or 5 g L-tryptophan load in the deficient subjects was significantly greater than during the control period, but there was no statistically significant difference between the xanthurenic acid excretion following these two loads of the amino acid during the initial period of deficiency. There was no significant increase in xanthurenic acid excretion in the same subjects following a 2 g L-tryptophan

load. A 5 g D-tryptophan load neither increased the xanthurenic acid excretion nor interfered with the xanthurenic acid determination. This study suggests that the size of the dose of tryptophan load utilized to challenge the kynurenic pathway of tryptophan may be important in subjects receiving deficient or submarginal intakes of vitamin B_6.

Urinary Excretion of Free Vitamin B_6

The excretion of free vitamin B_6 was determined in the first study described above. FIGURE 6 discloses that on the deficient diet there was prompt fall of the free vitamin B_6 for both the high- and low-protein groups. When these groups were placed upon a 10 mg pyridoxine supplement per day, there was a prompt rise in the urinary vitamin B_6. It is of interest that during this period of supplementation the values for the low-protein group were considerably higher than for the high protein group. The values are expressed as the group mean urinary vitamin B_6 excretion per day but represent the average value excreted per day for the final three days of each seven-day period. It is of interest that, once the low point in excretion was reached at the end of the second week of depletion, the amount of free vitamin B_6 excreted for the rest of the depletion phase was of the same low magnitude. However, the net xanthurenic acid excretion observed from these subjects (FIGURE 1) continued to increase from the second week to the end of the depletion phase. This helps to illustrate the difficulty encountered in attempting to utilize the urinary levels of vitamin B_6 as an indication of the severity of the B_6 deficiency.

FIGURE 7 discloses the free vitamin B_6 urinary excretion during the second described study. During the control period and the initial three weeks of the study, when the subjects were receiving the same dietary intake of vitamin B_6,

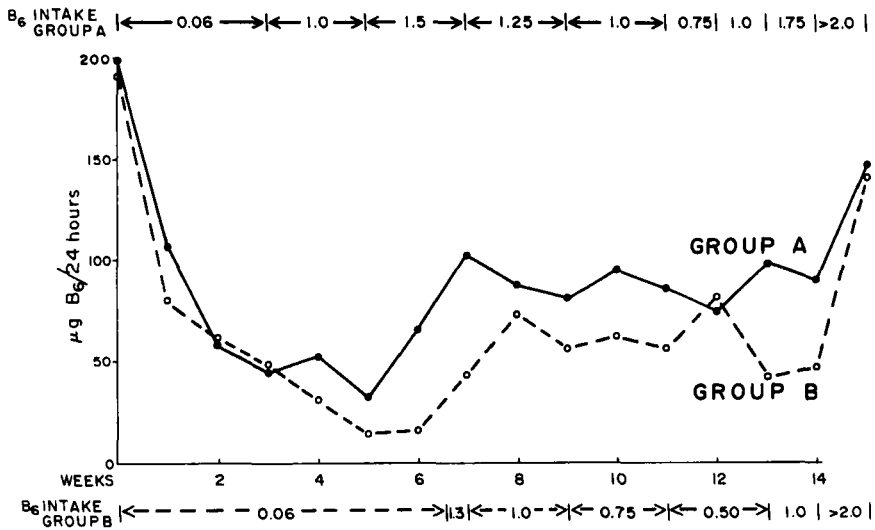

FIGURE 7. Urinary excretion of free vitamin B_6 with known intake of vitamin B_6 by two groups of subjects. Group A and Group B were receiving 100 g and 30 g of dietary protein, respectively. The vitamin B_6 content of the daily diet (0.06 mg) is not included in vitamin B_6 intake levels after the depletion phase.

the values for the high-protein and the low-protein groups were quite similar. By the end of five weeks of depletion, the urinary excretion of vitamin B_6 from the low-protein group appeared to have reached a "plateau." After dietary supplementation with pyridoxine hydrochloride was initiated, the excretion of the vitamin appeared to reflect the dietary intake of the supplement. There was no correlation between the level of protein intake and the level of urinary vitamin B_6 excretion. The above patterns of urinary excretion are quite similar to those reported by Linkswiler's group.[17] In one study, in which ^{14}C-labeled pyridoxine was administered to a subject maintained on a fixed daily intake of protein and 1.37 mg of vitamin B_6, the urinary metabolites of the vitamin were studied.[20] The biologically active metabolites of the vitamin (total vitamin B_6) represented only approximately 10% of the total metabolites present. It would be of interest to conduct a similar study in a vitamin B_6-depleted subject.

Relationship of Urinary and Plasma Free Amino Acids to Protein and Vitamin B_6 Intake

Many investigators have noted, in vitamin B_6-deficient animals, that the excretion of amino acids increased, that the level of protein intake was reflected in the amino acid excretion rate, that the plasma amino acid patterns were altered, and that interconversions of the amino acids were impaired. In Study 2, plasma was obtained for determination of free amino acids from the subjects in a fasting state, on the last day of (*a*) the control period, (*b*) the third depletion week for the high-protein group, (*c*) the sixth depletion week for the low-protein group and (*d*) the 14th week from both groups. Urine specimens were obtained for determination of the excretion of free amino acids on the next to the last day of (*a*) the control period, (*b*) the third week for the high-protein group, (*c*) the sixth week of the low-protein group and (*d*) the 14th week of both groups. The technical aspects of this work and the levels of the individual amino acids have been recorded elsewhere.[11]

TABLE 1 indicates the influence of deficiency and repletion on both the low-protein and the high-protein group for urinary and plasma free amino acids. It should be mentioned that repletion, as indicated in this Table, occurred when the subjects were still on a controlled protein intake and were still receiving graded amounts of pyridoxine hydrochloride. The possibility exists that a minimal deficiency of vitamin B_6 was present at the time these measurements were made. Of note is the marked increase in the total molar urinary excretion of free amino acids during both the deficiency and repletion stages of the study for both protein groups as compared with the control. Considering that there was a decrease in the total molar excretion of the essential amino acids during peak deficiency, the increase in the total molar excretion, at that time, was due solely to the increased excretion of nonessential amino acids. During the repletion phase, the excretion of essential amino acids was elevated for both the high- and low-protein groups. The marked increase in excretion of the essential amino acids for the low-protein group can be partially accounted for by the marked increase in methionine excretion. It was during this time that the low-protein group was receiving a daily two-gram methionine supplement. However, the high protein group, which did not receive the supplement, also demonstrated a marked increase in methionine, i.e., 313.6 as compared to a control value of 15.2 micromoles/gram of creatinine for the high-protein group and 706.7 as compared to 13.6 micromoles/gram of creatinine for the low-protein group.

Changes in plasma amino acids were not as dramatic. However, the molar values for the nonessential amino acids during deficiency and repletion were elevated for both the low-protein and the high-protein groups. During deficiency there was elevation of the essential amino acids for the subjects in the high-protein group. Alteration in the plasma levels and the urinary excretion of the various amino acids occurred during each phase of the study. In TABLE 2 are indicated the amino acids that showed a statistically significant change at the peak of deficiency when compared to the control values. The percentage of change from the control values is also indicated. For the most part, the significantly altered amino acids were different for the two levels of protein intake. However, the urinary excretion of tryptophan was decreased for both groups to approximately the same degree. Plasma levels of aspartic acid, serine and asparagine-glutamine were increased in both groups to approximately the same relative degree. It is felt that the changes indicated here reflect not only the difference in the protein intake but the influence of the deficiency upon the various B_6-dependent enzyme systems.

Relationship of Dietary Protein to Vitamin B_6 Requirements

Investigators have observed, in animals, that the requirement for the vitamin appears to be related to the protein content of the diet, and that the appearance and severity of a deficiency can be aggravated by increasing the protein content of the diet. We have observed in some of our studies that partial adaptation occurs in the human required to subsist on a submarginal intake of vitamin B_6.[19] This complicates the accurate assessment of the minimal requirement for the vitamin. In our studies (TABLE 3) where it was possible to predict the vitamin

TABLE 1

LEVELS OF FREE AMINO ACIDS OF HUMAN SUBJECTS ON TWO PROTEIN LEVELS DURING CONTROL AND WHILE ON A B_6-DEFICIENT DIET

A. URINARY EXCRETION	Millimoles of Free Amino Acids per gram of creatinine		
	Essential	Nonessential	Total
Low-Protein Group			
Control	0.448	1.479	1.927
Deficiency	0.316	2.626	2.942
Repletion	1.029	2.034	3.063
High-Protein Group			
Control	0.644	2.019	2.663
Deficiency	0.594	8.923	9.517
Repletion	0.747	2.731	3.478
B. PLASMA	**Micromoles of Free Amino Acids per 100 ml plasma**		
	Essential	Nonessential	Total
Low-Protein Group			
Control	97.0	218.6	315.6
Deficiency	89.9	303.3	393.2
Repletion	89.6	287.8	377.4
High-Protein Group			
Control	81.0	172.8	253.8
Deficiency	114.5	281.0	395.5
Repletion	87.3	241.1	328.4

TABLE 2

AMINO ACIDS SIGNIFICANTLY ALTERED BY B_6 DEFICIENCY IN HUMAN SUBJECTS ON TWO LEVELS OF PROTEIN INTAKE

A. URINARY EXCRETION	Amino Acid	Change	% of Control Value
Low-Protein Group			
Essential	Threonine	Increase	200.0
	Tryptophan	Decrease	24.7
	Lysine	Decrease	72.1
Nonessential	Proline	Decrease	10.3
	Citrulline	Decrease	5.7
	Glycine	Increase	393.0
	Alanine	Increase	118.4
High-Protein Group			
Essential	Tryptophan	Decrease	27.7
Nonessential	Ethanolamine	Decrease	85.5
B. PLASMA	**Amino Acid**	**Change**	**% of Control Value**
Low-Protein Group			
Essential	Phenylalanine	Decrease	61.2
Nonessential	Aspartic Acid	Increase	131.0
	Serine	Increase	177.2
	Glutamic Acid	Decrease	24.8
	Asparagine Glutamine	Increase	224.3
High-Protein Group			
Essential	Valine	Increase	192.7
	Leucine	Increase	145.1
Nonessential	Aspartic Acid	Increase	159.3
	Serine	Increase	184.8
	Proline	Increase	214.0
	Alanine	Increase	141.4
	Arginine	Increase	229.5
	Asparagine Glutamine	Increase	197.6

TABLE 3

RELATIONSHIP OF PROTEIN INTAKE AND VITAMIN B_6 REQUIREMENTS IN YOUNG ADULT MALE HUMANS

Dietary Protein Intake	Apparent B_6 Requirement		Comment
	Minimum per Day	Optimum per Day	
30 g/day	> 1.0 mg	1.25–1.5 mg	See Ref. 9 & 10
80 g/day	1.0 mg or less		I. C. Plough (Unpublished data). Subjects not initially depleted and observed for only three weeks.
100 g/day	1.3–1.5 mg	1.5–2.0 mg	Canham, Baker & Sauberlich (unpublished data).
100 g/day	1.5 mg	1.75–2.0 mg	See Ref. 9 & 10
165 g/day	> 1.93 and < 2.76 mg		See Ref. 4

B_6 requirements, as determined by the changes in the multiple tests performed, we observed a definite relationship between the daily protein intake and the vitamin B_6 requirement. Hence, for active young adult males subsisting on a daily intake of 30 g of protein the requirement for the vitamin appeared to be slightly in excess of 1 mg. On the other extreme, subjects receiving 165 g of protein per day appeared to require greater than 1.93 mg of vitamin B_6. There did appear to be greater biological variation in the biochemical tests performed on those individuals receiving a low protein intake than those receiving a normal or high protein intake.

Summary

In a series of studies which provided the opportunity to observe the influence of the level of protein intake on vitamin B_6 nutriture in young, adult males, the following conclusions appeared justifiable:

The rapidity of onset and severity of the biochemical manifestations of vitamin B_6 were directly related to the protein level of the diet. On a low protein intake the excretion of xanthurenic acid after tryptophan loading was not always an accurate reflection of the magnitude of a vitamin B_6 deficiency. The urinary excretion of free vitamin B_6 activity reflected the dietary intake of the vitamin and not the level of the protein intake. The level of protein intake altered the plasma and urinary amino acid patterns seen in vitamin B_6-deficient subjects. The requirement for vitamin B_6 in young adult males appeared to be directly related to the protein intake.

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References

1. LEPKOVSKY, S. & E. NIELSON. 1942. Green pigment-producing compound in urine of pyridoxine-deficient rats. J. Biol. Chem. **144**: 135.
2. CERECEDO, L. R. & J. R. FOY. 1944. Protein intake and pyridoxine deficiency in the rat. Arch. Biochem. **5**: 207.
3. COURSIN, D. B. 1955. Vitamin B_6 deficiency in infants. Amer. J. Dis. Child. **90**: 344.
4. HARDING, R. S., I. C. PLOUGH & T. E. FRIEDEMANN. 1959. The effect of storage on the vitamin B_6 content of a packaged Army ration with a note on the human requirement for the vitamin. J. Nutr. **68**: 323.
5. INTERDEPARTMENTAL COMMITTEE ON NUTRITION FOR NATIONAL DEFENSE – Manual for Nutrition Surveys, May 1957. Washington, D. C., U.S. Government Printing Office.
6. BROWN, R. R. 1958. The isolation and determination of urinary hydroxykynurenine. J. Biol. Chem. **227**: 649.
7. ATKIN, L., A. S. SCHULTZ, W. L. WILLIAMS & C. N. FRY. 1943. Yeast microbiological methods for determination of vitamins. Indust. Eng. Chem. **15**: 141.
8. HUFF, J. W. & W. A. PERLZWEIZ. 1944. A product of oxidative metabolism of pyridoxine, 2-methyl-3-hydroxyl-4-carboxy-5-hydroxymethylpyridine (4-pyridoxic acid). J. Biol. Chem. **155**: 345.
9. BAKER, E. M., J. E. CANHAM, W. T. NUNES, H. E. SAUBERLICH & M. E. MCDOWELL. 1964. Vitamin B_6 requirements for adult men. Amer. J. Clin. Nutr. **15**: 59.
10. SAUBERLICH, H. E. 1964. Human requirements for vitamin B_6. Vitamins & Hormones **22**: 807.
11. HARDING, R. S., H. E. SAUBERLICH & J. E. CANHAM. 1966. The free amino acids in the plasma and urine of human subjects on a vitamin B_6 deficient diet. *In*: Automation in Analytical Chemistry–Technicon Symposia, 1965. L. T. Skeggs, Jr., Ed. : 643–647. Mediad Inc. New York, N. Y.

12. RAICA, N., JR. & H. E. SAUBERLICH. 1964. Blood cell transaminase activity in human vitamin B_6 deficiency. Amer. J. Clin. Nutr. **15**: 67.
13. CERECEDO, L. R., J. R. FOY & E. C. DERENZO. 1948. Protein intake and vitamin B_6 deficiency in the rat. II. The effect of supplementing a low protein, pyridoxine-deficient diet with cystine or with methionine. Arch. Biochem. **17**: 397.
14. YESS, N., J. M. PRICE, R. R. BROWN, P. B. SWAN & H. LINKSWILER. 1964. Vitamin B_6 depletion in man: urinary excretion of tryptophan metabolites. J. Nutr. **84**: 229.
15. MILLER, L. T. & H. LINKSWILER. 1967. Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B_6 depletion. J. Nutr. **93**: 53.
16. KELSAY, J., L. T. MILLER & H. LINKSWILER. 1968. Effect of protein intake on the excretion of quinolinic acid and niacin metabolites by men during vitamin B_6 depletion. J. Nutr. **94**: 27.
17. KELSAY, J., A. BYSAL & H. LINKSWILER. 1968. Effect of vitamin B_6 depletion on the pyridoxal, pyridoxamine and pyridoxine content of the blood and urine of men. J. Nutr. **94**: 490.
18. COURSIN, D. B. 1964. Recommendations for standardization of the tryptophan load test. Amer. J. Clin. Nutr. **14**: 56.
19. CANHAM, J. E., E. M. BAKER, N. RAICA, JR. & H. E. SAUBERLICH. 1967. Vitamin B_6 requirement of adult men. *In*: Proceedings of the Seventh International Congress of Nutrition, Hamburg 1966, Vol. 5, Physiology and Biochemistry of Food Components : 558–562. Pergamon Press, Inc. Long Island City, N. Y.
20. TILLOTSON, J. A., H. E. SAUBERLICH, E. M. BAKER & J. E. CANHAM. 1967. Use of carbon^{14} labeled vitamins in human nutrition studies: pyridoxine. *In*: Proceedings of the Seventh International Congress of Nutrition, Hamburg 1966, Vol. 5, Physiology and Biochemistry of Food Components : 554–557. Pergamon Press, Inc. Long Island City, N. Y.