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Ann-Gau Nancy Wang   for the degree of   Master of Science   .

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Title: The Effect of Pyridoxine Supplementation on Erythrocyte

Aminotransferase Activity in Man

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    The effect of pyridoxine (PN) supplementation on the activities
of erythrocyte alanine aminotransferase (EAlaAT) and aspartate amino-
transferase (EAspAT) was observed in five men, aged 22 to 25 years.
The subjects received a constant diet containing 1.34 mg of vitamin
B-6 Monday through Friday of each week during this five-week study.
Starting on day 6 of week 1, the subjects were given orally 5 mg PN
daily, except on Tuesday and Thursday of each week when they were given
either no PN or 2 mg of vitamin B-6 in the form of crystalline PN or
as food. Basal and pyridoxal phosphate (PLP)-stimulated EAlaAT and
EAspAT activities were determined weekly. Both basal and PLP-
stimulated activities of the two enzymes increased after only three
days of PN supplementation and continued to increase throughout the
four weeks of PN supplementation; percent stimulation by PLP added in
vitro decreased concomitantly. It is suggested that the binding of PLP
to erythrocyte apoaminotransferases may be another reservoir for
vitamin B-6.

The Effect of Pyridoxine Supplementation on Erythrocyte
Aminotransferase Activity in Man

by

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INTRODUCTION

Taking vitamins and other nutrient supplements is common in the United States. The extent of vitamin usage was reviewed recently by English and Carl (1981). Most of the people taking vitamins on a regular basis or to treat an acute disorder do so without a physician's prescription (Bootman and Wertheimer, 1980; English and Carl, 1981).

In cases of nutritional deficiencies supplementary nutrients are obviously beneficial to health. Excessive use, however, even of some water-soluble vitamins, can produce clinical symptoms, provide nutritional imbalance, or interfere with diagnostic tests (Herbert, 1980). Thus, research on the effect of taking vitamins in excess of physiological needs is needed.

No clinical symptoms have been reported from taking excessive vitamin B-6. There have been no reports of deleterious effects associated with daily ingestion of large doses (0.2 to 1.0 g/day) of vitamin B-6. Little is known, however, on the effect of excessive vitamin B-6 intake on the metabolism of that vitamin in the body.

In this present study, the effect of small doses of vitamin B-6 was examined in five apparently healthy young men. The activities of erythrocyte alanine aminotransferase (glutamic-pyruvic transaminase, EC 2.6.1.2) (EAlaAT) and erythrocyte aspartate aminotransferase (glutamic-oxaloacetic transaminase, EC 2.6.1.1) (EAspAT), two pyridoxal phosphate (PLP)-dependent enzymes, were measured weekly in the

subjects before and during the four weeks they received 5 mg of pyri-doxine daily. Basal activity and stimulation by PLP added in vitro of these erythrocyte aminotransferases were tested.

In the research reported in this thesis, the activities of the erythrocyte aminotransferase were compared to plasma vitamin B-6 con-centration. The effect of exercise on erythrocyte aminotransferase activities was also measured.

REVIEW OF LITERATURE

Background on Vitamin B-6

In 1934, György recognized that vitamin B-6 can prevent skin lesion in rats ("rat acrodynia" produced by a "purified supplemental diet"). Since then, the essentiality of vitamin B-6 in metabolism has been established (György, 1971).

There are three free forms of vitamin B-6: pyridoxal (PL), pyridoxine (PN), and pyridoxamine (PM), which are equally effective in animal nutrition (Sauberlich and Canham, 1980). Vitamin B-6 occurs in animal products largely as PL and PM, while in vegetable products PN is the prevalent form. The three free forms as well as their respective phosphorylated forms are interconvertible (Fig. 1). The major metabolite of vitamin B-6 is 4-pyridoxic acid, which is excreted in urine.

Pyridoxal phosphate (PLP), the main active or coenzyme form of vitamin B-6, is involved in many different kinds of enzyme reactions, which are almost entirely associated with amino acid metabolism (Sauberlich and Canham, 1980). The reactions catalyzed by these PLP-dependent enzymes include transamination, racemization, decarboxylation, desulfhydration, and dehydration (Sauberlich, 1968).

In addition to its coenzyme role, PLP is also a structural or conformational factor in glycogen phosphorylase. PLP plays an indirect role in lipid metabolism. PLP is also essential in the conversion of tryptophan to niacin; the synthesis of the porphyrin ring in hemoglobin

pyridoxine   PL kinase   →   pyridoxine phosphate

phosphatase

PN dehydrogenase

4-pyridoxic acid   alde-hyde oxidase ←   pyridoxal   PL kinase   →   pyridoxal phosphate

phosphatase

PNP oxidase

PNP oxidase   aminotransferase   PNP oxidase   amino-transferase

pyridoxamine   PL kinase   →   pyridoxamine phosphate

phosphatase
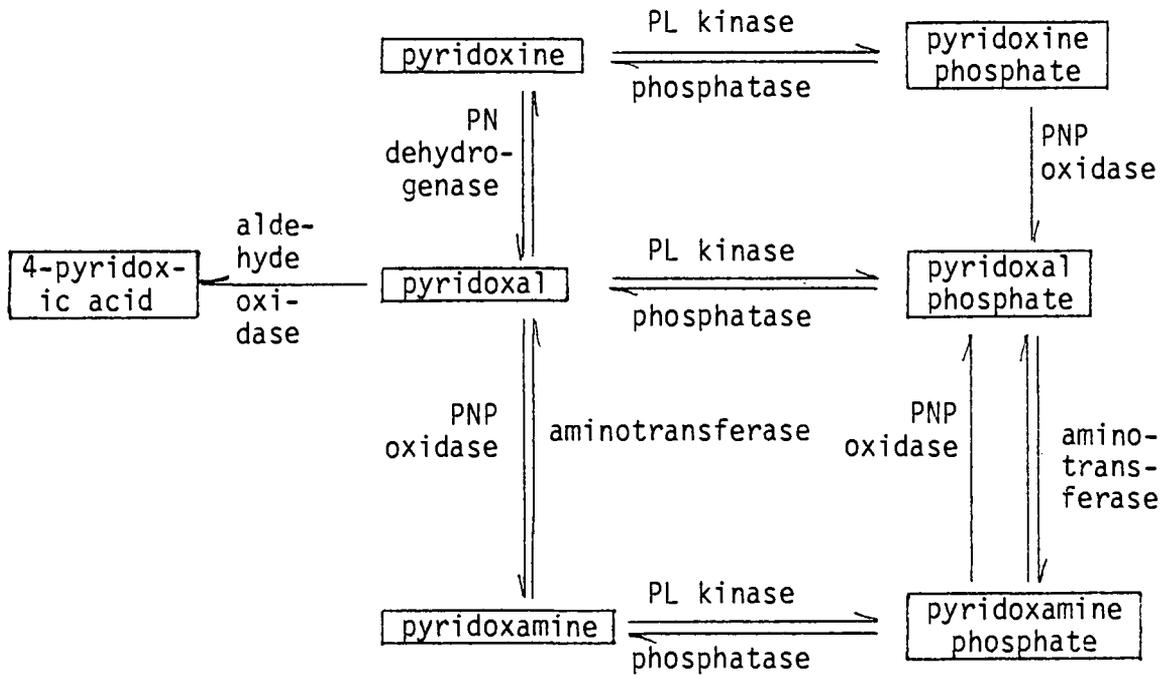
FIGURE 1. Interconversion of vitamin B-6. Adapted from Sauberlich and Danham (1980) and Contractor and Shane (1970).

(Sauberlich, 1968); and the synthesis and/or metabolism of the neuro-transmitters gamma-aminobutyric acid, serotonin, 3,4-dihydroxyphenyla-lanine (DOPA) and norepinephrine. Vitamin B-6 also plays an essential role in the development and maintenance of immunity (Axelrod, 1971).

Aminotransferase

Aminotransferases, a major group of PLP-dependent enzymes, cata-lyze the reversible transfer of the α-amino group of an amino acid to an α-keto acid. PLP and PMP function in aminotransferase reaction in a Schiff's base mechanism (Snell and Dimari, 1970). Alanine aminotrans-ferase (AlaAT) and aspartate aminotransferase (AspAT), which are widely distributed in human tissue (Wroblewski, 1956; Snell and Dimari, 1970), are two commonly measured enzymes for clinical purposes. Erythrocyte alanine and aspartate aminotransferase (EAlaAT and EAspAT, respective-ly) activities are used as an indicator of vitamin B-6 status. Serum and plasma aminotransferases are measured in the diagnosis of heart and liver damage. The reactions catalyzed by these two aminotrans-ferases are presented in Figure 2.

Effect of Vitamin B-6 Depletion and Repletion on Erythrocyte Aminotransferase Activity

Since erythrocyte aminotransferase activity varies widely among normal and vitamin B-6 deficient subjects, Raica and Sauberlich (1964) and Cinnamon and Beaton (1970) suggested that erythrocyte aminotrans-ferase activity (basal activity) alone is not a valid measurement of vitamin B-6 status. Both groups suggested that the measurement of basal erythrocyte aminotransferase activity combined with the in vitro

alanine + α-ketoglutarate $\underset{\longleftarrow}{\overset{\text{AlaAT}}{\rightleftharpoons}}$ pyruvate + glutamate

aspartate + α-ketoglutarate $\underset{\longleftarrow}{\overset{\text{AspAT}}{\rightleftharpoons}}$ oxaloacetate + glutamate
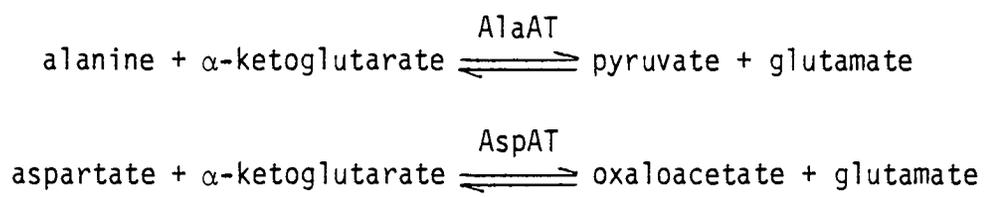
FIGURE 2.   The reactions catalyzed by AlaAT
            and AspAT.

stimulation by PLP, appears to be a better indication of vitamin B-6 status.

Under normal conditions, the erythrocyte aminotransferases are not saturated with the coenzyme, PLP. In vitamin B-6 deficiency, these enzymes lose some of their PLP, becoming more unsaturated. Consequently, the basal activity decreases in vitamin B-6 deficiency because the proportion of holoenzyme decreases and that of apoenzyme increases. To determine the proportion of apoaminotransferase and holoenzyme, the activity of the enzyme is measured with (stimulated) and without (basal) the in vitro addition of PLP to the assay medium. This is known as the aminotransferase stimulation test. In vitamin B-6 deficient persons, due to the loss of PLP from the aminotransferases, the percent increase in activity due to PLP stimulation will be much greater than that in normal well-nourished persons.

Raica and Sauberlich (1964) reported that in vitamin B-6 depleted subjects, EAspAT basal activity declined and the percent stimulation by PLP added in vitro increased concomitantly. When subjects were subsequently repleted with vitamin B-6, the basal activity and percent stimulation of EAspAT returned to predepletion levels. Cinnamon and Beaton (1970), who measured both EAlaAT and EAspAT activities in male subjects depleted of vitamin B-6, obtained results similar to those observed by Raica and Sauberlich for both erythrocyte aminotransferases. In addition, Cinnamon and Beaton observed that the activities of both of these erythrocyte aminotransferases reflected vitamin B-6 depletion as well as urinary xanthurenic acid excretion after tryprophan loading and at about the same time basis. But, on the other hand, basal

erythrocyte aminotransferase activities did not return to normal until three to four weeks of 2 mg of PN supplementation daily, whereas xanthurenic acid excretion returned to normal after one to two days of 2 mg PN daily. Cinnamon and Beaton thus suggested that erythrocyte aminotransferase activities are both sensitive and specific, and reflect long-term vitamin B-6 status. In another experiment, Brown et al. (1975) also reported decreased basal activities and increased percent stimulation of both erythrocyte aminotransferases in oral contraceptive-using women and women depleted of vitamin B-6. The aminotransferase activities were gradually restored to normal after vitamin B-6 supplementation to the subjects.

Cinnamon and Beaton observed that EAlaAT and EAspAT activities were stimulated 15 to 29% by the in vitro addition of PLP in three normal subjects prior to vitamin B-6 depletion. Cheney et al. (1965) had also studied EAlaAT and EAspAT activities in a group of healthy young adult men and women; EAspAT activity was stimulated an average of 80%, whereas the EAlaAT activity was stimulated 25% by the added PLP. Woodring and Storvick (1970), who experimented with a group of normal women, reported that the in vitro stimulation of EAlaAT by PLP did not exceed 15%. Sauberlich et al. (1972) indicated that in vitro addition of PLP to normal erythrocytes seldom stimulated EAlaAT more than 25%, or EAspAT more than 50%. Because EAlaAT and EAspAT assay procedures are not standardized, each laboratory needs to establish its own range of normal values for activity and percent stimulation.

The Effect of Pyridoxine Supplementation on Erythrocyte Aminotransferase Activity

Vitamin B-6 supplementation corrects the abnormal erythrocyte aminotransferase activity in vitamin B-6 deficient persons (Raica and Sauberlich, 1964; Cinnamon and Beaton, 1970; Brown et al., 1975). The supplement of vitamin B-6 provides coenzyme in vivo to the erythrocyte apoaminotransferases so that the amount of holoenzyme increases. (A discussion on the conversion of vitamin B-6 to PLP in the erythrocyte is in the following section.) Consequently, in vitamin B-6 supplemented persons EAlaAT and EAspAT basal activities increase, and percent stimulation by PLP decrease. Jacobs et al. (1968) observed a decrease in EAlaAT basal activity with increasing age in men and women, which suggested that older subjects may have a chronic deficiency of vitamin B-6. A daily vitamin B-6 supplement of 10 mg for six weeks elevated both basal and stimulated activities (activities with PLP added in vitro) of both aminotransferases. In young subjects, prolonged vitamin B-6 supplementation will also produce elevated basal erythrocyte aminotransferase activity (Jacobs et al., 1968; Woodring and Storvick, 1970; Rose et al., 1973).

Erythrocyte Aminotransferase Activity in Mild Vitamin B-6 Deficiency

Some researchers report that the percent stimulation of erythrocyte aminotransferases with PLP added in vitro is not sensitive enough to measure mild vitamin B-6 deficiency. Shane and Contractor (1975) assessed vitamin B-6 status in pregnant women and oral contraceptive users before and after they received supplementary vitamin

B-6. They compared the EAspAT percent stimulation test with that of whole blood PLP. They found that in the absence of PN supplementation, there was a significant correlation between percent in vitro stimulation of erythrocyte aminotransferases and blood PLP only in pregnant women and none in the other women. In the majority of the pregnant subjects studied by Lumeng et al. (1976), no significant correlation between plasma PLP levels, and EAlaAT and EAspAT percent stimulation values were observed. Thus, Shane and Contractor and Lumeng et al. suggested that the erythrocyte aminotransferase activation test is a poor indicator of vitamin B-6 status in pregnant and oral contraceptive-using women. Miller et al. (1981) in a study dealing with pre-school children also came to the same conclusion.

The Relationship Between Erythrocyte Vitamin B-6 and Vitamin B-6 in Blood

Transport and Metabolism of Vitamin B-6 in Blood

The erythrocytes play an important role in the transport and metabolism of vitamin B-6 (Snell and Haskell, 1971; Anderson et al., 1971; Lumeng and Li, 1974; Lumeng, Brashear and Li, 1974). Erythrocytes contain all enzymes needed for the interconversion of vitamin B-6 compounds. Erythrocyte contains kinase for phosphorylation of the free vitamin B-6 (Hamfelt, 1967; Lumeng and Li, 1974; Anderson et al., 1971), PNP oxidase for the formation of PLP from PNP or PMP (Lumeng and Li, 1974), and a membrane-bound phosphatase (Lumeng and Li, 1974; Anderson et al., 1971) for controlling the level of PLP in the red cell.

PN and PM taken up by red cells are phosphorylated by PL kinase to PNP and PMP which are converted to PLP by PNP oxidase. PLP in plasma is bound tightly to albumin, and in the red cell PLP is tightly bound to hemoglobin (Lumeng et al., 1974; Anderson et al., 1974), which prevents the PLP from crossing the red cell membrane. Thus red cell vitamin B-6 metabolism cannot contribute to plasma PLP levels. Plasma PLP is derived from the liver (Lumeng et al., 1974). PLP in red cells can be dephosphorylated to PL, a form which can cross the red cell membrane, and be released to the plasma, so that PL can be available for tissue uptake (Anderson et al., 1971). The distribution of PL between plasma and red cells is governed by competing protein binders (Anderson et al., 1974). Figure 3 gives a summary of reactions involved in the metabolism of vitamin B-6 and the transport of vitamin B-6 in and out of the erythrocyte.

PLP Level Between Plasma and Red Cells

The level of plasma PLP, which is derived from the liver, correlates well with tissue levels of vitamin B-6 and with vitamin B-6 intake (Lumeng, Ryan and Li, 1978). Erythrocyte PLP, however, is derived entirely from red cell metabolism of nonphosphorylated vitamin B-6 (see above). Under normal conditions, PLP in whole blood is approximately equally distributed between plasma and erythrocyte (Bhagavan, Coleman and Coursin, 1975) and plasma PLP levels correlate well with erythrocyte levels (Hamfelt, 1967). In persons given large doses of PN, however, erythrocyte PLP levels increase faster than plasma levels. Bhagavan et al. (1975) noted a red cell to plasma PLP

PLASMA   RED CELL

albumin - PLP   PLP-Hemoglobin

                PLP   PLP

PL-albumin ⇌ PL ═══════════⟶ PL ⇌ Hemoglobin-PL

        PN ═══════════⟶ PN ⟶ PNP ──────────

        PM ═══════════⟶ PM ⟶ PMP ──────────
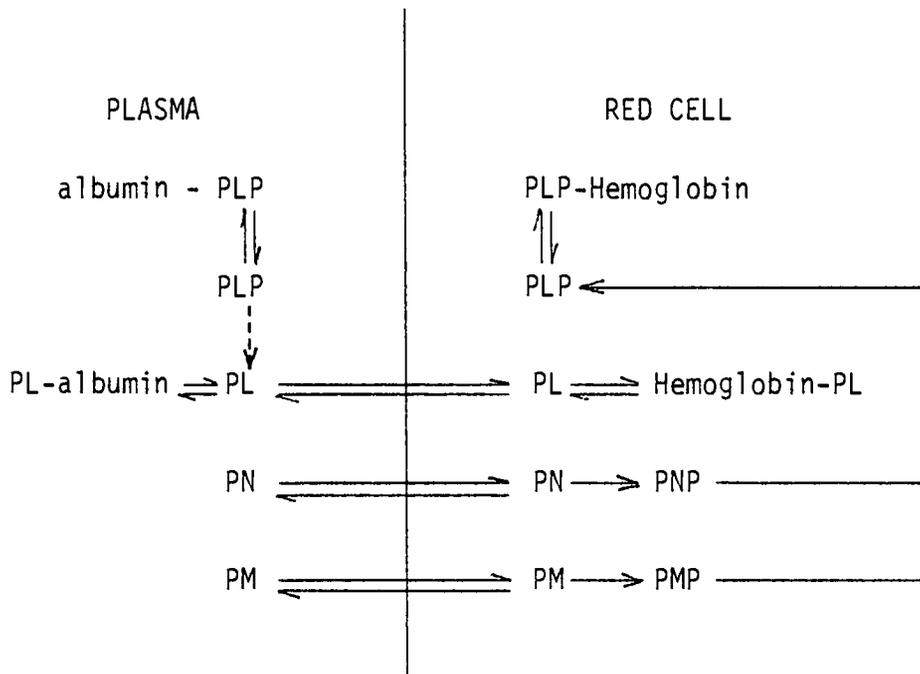
FIGURE 3.   Summary of metabolism and transport of vitamin
            B-6 in blood (from Shane, 1978).

ratio of up to 50 to 1 after the chronic administration of 1 g/day of
PN to control, hyperactive patients and patients with Downs' syndrome.
Lumeng et al. (1974), on the other hand, reported that the oral ad-
ministration of 25-50 mg PN to man daily increased the plasma PLP con-
centration to a maximal plateau of 150-180 ng/ml within four days.
These authors did not measure red blood cell PLP and aminotransferase
activity.

Widespread Use of Vitamin Supplements

Vitamin use is common these days. Research by Bootman and
Wertheimer (1980) studied the vitamin usage in a sample of university
students. For the question "whether any member of the family consumes
vitamin supplements on either a daily basis or an 'as needed' basis
for acute disorders," 56.6% of the respondents answered "yes." Most
of these were not prescribed by a physician. Miller et al. (1981)
noted that 26% of 276 Oregon preschool children took a vitamin supple-
ment containing one mg PN   or more. Houghton (1982) found that 64%
of the 65 freshman women at Oregon State University whom she had
studied took vitamin supplements. English and Carl (1981) interviewed
60 patients and their physicians and reported 67% of the patients used
a nutritional supplement on a regular daily basis or had used them to
treat an illness. These same authors reviewed the widespread use of
vitamin supplements in this country.

Safety of Vitamin B-6 Supplementation

The safety margin of each vitamin taken as an everyday supplement

is important to establish. There have been no reports of deleterious effects associated with daily oral ingestion of large doses of vitamin B-6 (0.2 to 1 gm/day) (American Academy of Pediatrics, 1966) so that the 2° or 5 mg of pyridoxine daily given to the subjects in our study was safe.

Influence of Exercise on Plasma Vitamin B-6

Wozenski (1977) observed abnormally high plasma vitamin B-6 levels in a subject who had been loaded with 0.5 mg of PN. Upon questioning the subject answered that he had been running shortly before the blood was drawn. Wozenski subsequently showed that plasma vitamin B-6 and PLP levels were increased by acute exercise.

MATERIAL AND METHODS

This research was ancillary to an investigation on the bioavailability of vitamin B-6 in subjects who were saturated with this vitamin. Results of the research on bioavailability of vitamin B-6 will be presented elsewhere.

Subject Selection

Five apparently healthy young men between the ages of 22 and 25 years served as subjects. Their descriptive data are presented in Table 1. They were free from any known disease.

Experimental Protocol

This study, which lasted five weeks (April 21 to May 24, 1980), was approved by the Human Subjects Committee at Oregon State University. Before participating in this investigation, the subjects signed an informed consent form approved by this committee.

During the study, the subjects were fed a constant diet consisting of three meals containing a total of 1.34 mg of vitamin B-6 (Table 2), Monday through Friday of each week. They were given breakfast on Saturday mornings. The meals for the remainder of Saturday and all day Sunday were self-selected. Each person participating in the study was not allowed to consume any other food or drinks on the days when the three meals were provided. Due to the effect of alcohol on the metabolism of vitamin B-6 (Walsh et al., 1966), the subjects were

TABLE 1. Descriptive Data of Subjects[1]

No.	Age (yrs.)	Height (cm)	Weight (kg)
1	22	189.0	86.6
2	25	172.5	84.3
3	22	180.3	94.5
4	23	181.5	83.0
5	25	176.0	70.0

[1]Subjects were recruited by advertisement placed on bulletin boards around the campus. Health of the subjects was determined by questionnaire.

TABLE 2. Constant Diet[1]

	g
Breakfast	
Orange juice, frozen reconstituted	230
Bread, white enriched	50
Applesauce, canned	75
Crispy Rice[2]	30
Milk, nonfat, reconstituted	240
Margarine	variable[3]
Honey	variable[3]
Lunch	
Cheese, cheddar	35
Bread, white enriched	50
Peaches, canned, solids	100
syrup	20
Carrots, raw	100
Pickles, sliced dill	30
Milk, nonfat, reconstituted	240
Cookies	45
Dinner	
Rice casserole (weights before cooking)	
Beef, ground	120
Rice	45
Tomato juice, canned	150
Mixed vegetables, dehydrated	4
Salt	¼ tsp.
Green beans, canned, solids	100
juice	10
Pears, canned, solids	100
syrup	20
Milk, nonfat, reconstituted	240
Bread, white enriched	25
Ice cream, vanilla	100

[1]Contained 1.34 mg of vitamin B-6 as determined by using Saccharomyces uvarum as the assay organism (AOAC 1980).

[2]Ralston-Purina Company, St. Louis, Missouri.

[3]In quantities to maintain weight.

allowed no alcoholic beverages Monday through Friday. On the weekends, the subjects' consumption of alcoholic beverages was limited to 16 oz. of beer or 8 oz. of wine.

No strenuous exercise was permitted; the maximum allowed was the running of not more than one mile/day or its equivalent. The subjects reported their exercise and consumption of coffee, tea and carbonated beverages on forms provided daily. On the days that meals were not provided, each person kept an accurate record of the type and amount of foods he consumed.

Starting on day 6 (Saturday) of week 1, the subjects were given daily (except on each Tuesday and Thursday of the remaining four weeks) 5 mg of pyridoxine[1] (PN) orally at breakfast. On Sundays, the sub-jects came in to the laboratory to take their PN supplements. During the following weeks, the subjects received no PN supplement on the Tuesday (April 29) and Thursday (May 1) of week 2; 2 mg of PN on the Tuesday (May 6) and Thursday (May 8) of week 3; 500 gms banana (con-tained 1.51 mg B-6)[2] on Tuesday (May 13) and 333 gms of filberts (con-tained 2.12 mg B-6) on Thursday (May 15) of week 4; and 333 gms soy-beans (contained 1.51 mg B-6) on Tuesday (May 20) and 666 gms beef (contained 1.56 mg B-6) on Thursday (May 22) of week 5. The prepara-tion and administration of these foods will be given elsewhere. No

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[1]Prepared as pyridoxine HCl, taking into account the difference in molecular weights.

[2]The vitamin B-6 content of those selected foods were determined by using Saccharomyces uvarum as the assay organism (AOAC 1980). These assays were performed by H. Kabirmeidanshah.

PN was administered on the last two days of study (May 23 and 24).

Blood was collected before breakfast on Monday (day 1 of study) and Friday of the first week; on Wednesdays and Fridays of the remaining four weeks; and on the last day of the study (Saturday, May 24). Blood (20 ml) was collected by a registered medical technologist from the subject's antecubital vein into heparinized Vacutainer tubes.

Exercise Experiment

On the Friday of the first week (April 25, 1980) and of the last week (May 23, 1980), the subjects participated in an exercise experiment. The purpose of this study was to assess the effect of exercise on the metabolism of vitamin B-6 in subjects saturated with the vitamin. The exercise event consisted of the following:

1. Collect 20 ml of blood from fasting subjects before breakfast.

2. Collect blood four hours later, before beginning the exercise. The exercise consists of pedalling a bicycle ergometer for 21 minutes. The work required to pedal the bicycle was increased at the end of seven minutes and then again at the end of fourteen minutes. The amount of work was set depending on the subjects' heart rates which were monitored throughout the exercise period. In no case was their heart rate allowed to exceed 175 beats per minute.

3. Before lunch, collect blood at the end of exercise and after 30 minutes.

4. Collect 20 ml blood two hours after exercise event.

Laboratory Analyses

Erythrocyte AspAT (EAspAT) was determined by a slight modification of the procedure of Woodring and Storvick (1970). To determine EAspAT in hemolysates (diluted 1:10), aspartate was used as substrate and the product, oxaloacetate, was converted to pyruvate by reacting with aniline citrate. EAlaAT was determined as the method of Woodring and Storvick (1970). The basal activities of the two enzymes were determined without the addition of pyridoxal phosphate (PLP) to the assay medium; in vitro stimulation was measured by the addition of 100 µg PLP.

The basal and stimulated activities of EAlaAT and EAspAT were expressed as µg pyruvate/mg Hb/hour. The percent stimulation of each enzyme with added PLP was also calculated. The formula was:

$$\frac{\text{EAlaAT (or EAspAT) with PLP} - \text{EAlaAT (or EAspAT) without PLP}}{\text{EAlaAT (or EAspAT) without PLP}} \times 100$$

EAlaAT activity was assayed on the day blood was drawn, except week 1 (Monday, day 1) when it was assayed after five days. Red cells hemolysates (1:10) for EAspAT were prepared and forzen on the day blood was drawn (Wednesday). EAspAT activities were measured within one week. Preliminary experiments in our laboratory showed that EAlaAT and EAspAT activity was stable for at least two weeks when the sample were frozen. Dang (1976) reported no change in serum AspAT after two months of frozen storage. Beutler (1975) found less than 10 percent loss of EAspAT activity in whole blood stored with anticoagulant for 20 days at 4°C. Hemoglobin was determined by a standard method.

Statistical Analyses

The data were statistically analyzed by analysis of variance (ANOVA) for determining the effect of vitamin B-6 supplementation on erythrocyte aminotransferase activities by using statistical interactive programming system (SIPS). The significance of weekly changes of enzyme activities was tested by the paired t-test. The erythrocyte aminotransferase activity changes vs. time throughout the supplementation period were analyzed by linear regression analysis.

RESULTS

Table 3 presents the effect of PN supplementation on the activities
of EAlaAT and EAspAT. As determined by analysis of variance, PN sup-
plementation has a significant effect on the basal activity, PLP-
stimulated activity and percent stimulation of both enzymes. Values
for P of 0.05 or less were regarded as significant.

EAlaAT

After only three days, from week 1 to week 2, of 5 mg of PN daily,
EAlaAT basal (without PLP) and PLP-stimulated (PLP added in vitro)
activities increased and the percent stimulation decreased. After the
subjects had been supplemented with PN for four weeks, both basal and
PLP-stimulated EAlaAT activities more than doubled. Concomitantly, the
percent stimulation of EAlaAT with PLP added in vitro decreased approxi-
mately 50% (Table 3). All of these changes were statistically signi-
ficant. In spite of the great variation in EAlaAT activity among the
subjects, both basal and PLP-stimulated EAlaAT activities rose weekly
in each subject during the four weeks of PN supplementation (Fig. 4).
Although the percent stimulation of EAlaAT decreased weekly with PN
supplementation (Table 3), it can be observed from Figure 4 that the
increase due to adding PLP to the assay medium (shown by the open
bars), was relatively constant in each subject from week to week during
the four-week period of PN supplementation (Table 4).

As determined by the paired t-test, both basal and PLP-stimulated
EAlaAT activities were significantly increased after three days of PN

TABLE 3. Effect of PN Supplementation on Basal Activity, PLP-stimulated Activity and Percent Stimulation of EAlaAT and EAspAT (Mean ± S.D.).

	Week				
	1	2	3	4	5
	No PN supplement	PN supplementation[1,2]			
EAlaAT					
basal activity[3,4]	0.60±0.30	0.82±0.37	1.06±0.44	1.28±0.53	1.52±0.60
PLP-stimulated activity[3,5]	0.76±0.38	1.00±0.44	1.26±0.52	1.48±0.59	1.69±0.67
percent stimulation[6]	28.20±8.30	21.36±5.90	16.40±4.60	13.30±3.60	11.10±3.10
EAspAT					
basal activity	15.50±3.20	17.40±2.50	19.60±1.90	21.60±1.50	23.60±1.80
PLP-stimulated activity	27.80±5.70	30.00±4.30	32.70±3.30	35.10±2.60	37.80±3.40
percent stimulation	80.00±12.20	72.10±10.40	65.90±7.00	62.80±7.80	59.50±7.30

[1]Starting day 6 of week 1 until day 6 of week 5, the subjects received daily, except on Tuesdays and Thursdays, 5 mg of PN at breakfast. The subjects received no supplement on the Tuesday and Thursday of week 2, 2 mg PN on the Tuesday and Thursday of week 3, and some specific food containing approximately 2 mg vitamin B-6 on Tuesdays and Thursdays of week 4 and week 5.

[2]PN had a significant effect on EAlaAT and EAspAT activities and percent stimulation ($p \leqslant 0.05$).

[3]Measured in unit of μg pyruvate/mg Hb/hr.

[4]No PLP added to assay medium.

[5]PLP added to assay medium.

[6]Calculated by: $\dfrac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(or EAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}} \times 100$

FIGURE 4.   Effect of PN supplementation on EAlaAT basal activity and
PLP-stimulated activity. Hatched bars represent basal
activity (no added PLP) and open bars represent PLP-
stimulated activity; additional activity due to PLP added
in vitro to assay mixture.

Starting day 6 of week 1 until day 6 of week 5, the sub-
jects received daily, except on Tuesdays and Thursdays, 5 mg
of PN at breakfast. The subjects received no supplement
on the Tuesday and Thursday of week 2, 2 mg PN on the Tues-
day and Thursday of week 3, and some specific food contain-
ing approximately 2 mg of vitamin B-6 on Tuesdays and
Thursdays of week 4 and week 5.

PN supplementation had a significant effect on the basal
and stimulated activities of EAlaAT ($p \leqslant 0.05$).

FIGURE 4.

TABLE 4. The Percent Increase in Basal and PLP-stimulated Activities
of EAlaAT and EAspAT Compared to Initial Values.

	Week			
	1 to 2	1 to 3	1 to 4	1 to 5
EAlaAT basal activity[1]	36.7	76.7	113.3	153.3
PLP-stimulated activity[2]	31.6	65.8	94.7	122.4
EAspAT basal activity	12.3	26.5	39.4	52.3
PLP-stimulated activity	7.9	17.6	26.3	36.0

[1]No PLP added to assay medium.

[2]PLP added to assay medium.

supplementation (from week 1 to week 2)(Table 5). Basal EAlaAT activity increased significantly from week to week thereafter. PLP-stimulated activity, although it also increased progressively, was significantly increased from week to week only during the first three weeks of supplementation. Although the weekly decrease in percent stimulation was significant during the first two weeks of PN supplementation, the weekly decrease during the last two weeks was not significant.

EAspAT

The EAspAT activities, both basal and PLP-stimulated, also increased and percent stimulation decreased after three days of PN supplementation. This trend continued throughout the period of PN supplementation. The mean basal and PLP-stimulated EAapAT activities increased about 50% and 35%, respectively, after four weeks of PN supplementation; the percent stimulation of EAspAT with added PLP in vitro dropped by one-fourth. All of these changes were statistically significant. Although the PN supplements elevated EAspAT activities in each subject, there were, as with EAlaAT, great interindividual variations in EAspAT activity (Fig. 5). The weekly increase in activity from PLP added in vitro (shown by the open bars) was also relatively constant from week to week in each subject (Fig. 5).

From the determination of paired t-test (Table 5), EAspAT basal activities increased significantly after three days of PN supplementation and during each successive week thereafter. The PLP-stimulated EAspAT activities also increased every week, but this increase was not

TABLE 5. Paired t-test Values for the Significance of Weekly Changes in the Basal Activity, PLP-stimulated Activity and Percent Stimulation for Both EAlaAT and EAspAT.

Enzyme		Week			
		1 to 2	2 to 3	3 to 4	4 to 5
EAlaAT	basal activity[1]	2.28*[2]	2.32*	2.28*	2.36*
	PLP-stimulated activity[3]	2.23*	2.34*	2.19*	1.92
	percent stimulation[4]	-3.74*	-2.72*	-1.68	-1.20
EAspAT	basal activity	2.28*	2.53*	2.26*	2.40*
	PLP-stimulated activity	1.31	1.69	1.44	1.66
	percent stimulation	-4.32*	-3.44*	-1.66	-1.84

[1]No PLP added to assay medium.

[2]* significant at $p \leq 0.05$ level.

[3]PLP added to assay medium.

[4]$\dfrac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(orEAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}}$ X 100 .

FIGURE 5.   Effect of PN supplementation on EAspAT basal activity and
PLP-stimulated activity. Hatched bars represent basal
activity (no added PLP) and open bars represent PLP-
stimulated activity; additional activity due to PLP added
in vitro to assay mixture.

Starting day 6 of week 1 until day 6 of week 5, the sub-
jects received daily, except on Tuesdays and Thursdays, 5 mg
of PN at breakfast. The subjects received no supplement
on the Tuesday and Thursday of week 2, 2 mg PN on the Tues-
day and Thursday of week 3, and some specific food contain-
ing approximately 2 mg of vitamin B-6 on Tuesdays and
Thursdays of week 4 and week 5.

PN supplementation had a significant effect on the basal
and stimulated activities of EAspAT ($p \leqslant 0.05$).

FIGURE 5.

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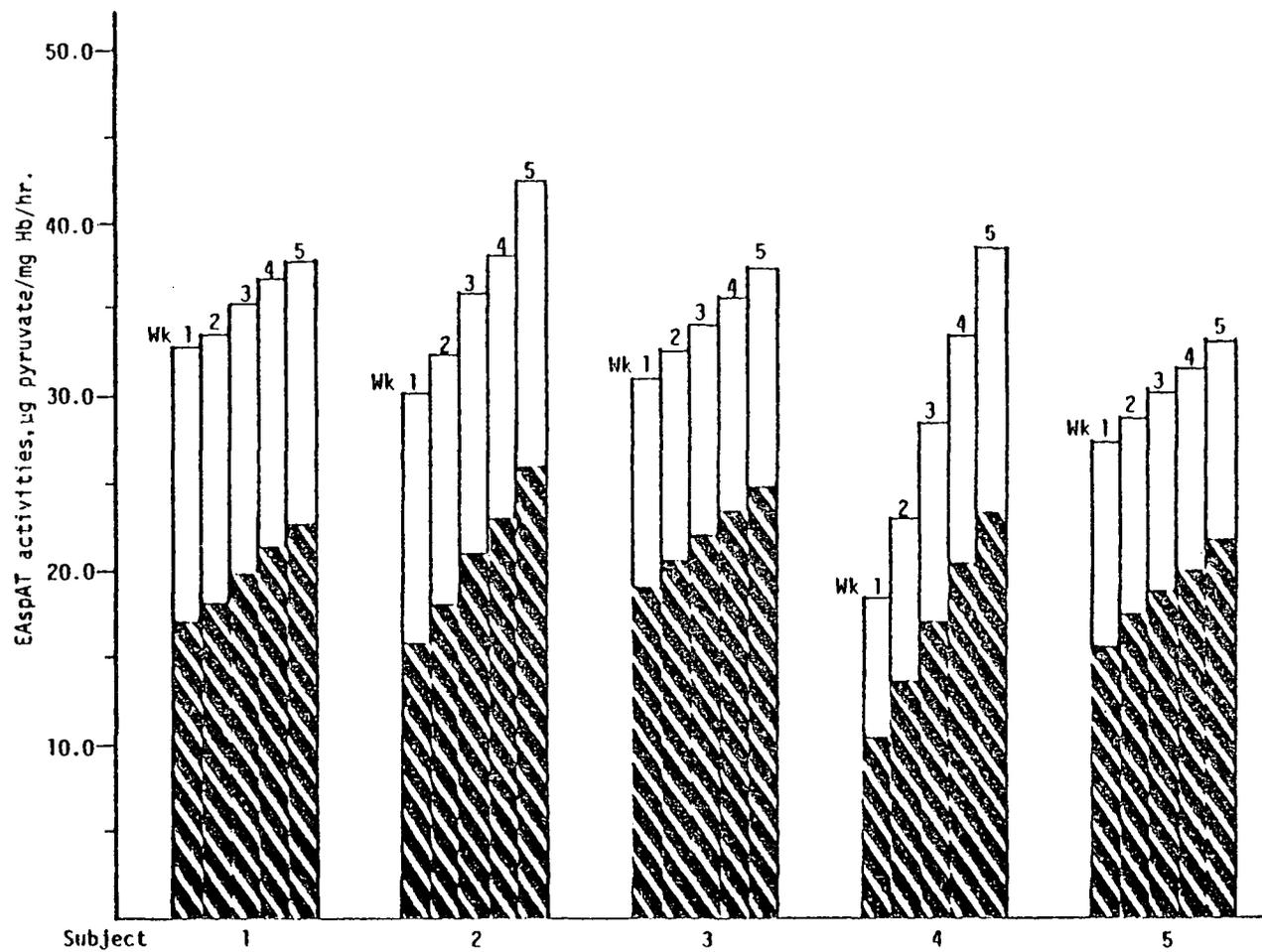
statistically significant (Table 5). The percent stimulation values for EAspAT had the same trend as that of EAlaAT, which dropped sharply during the first two weeks of supplementation (statistically significant), and decreased more slowly during the last two weeks.

Regression Analysis

For both enzymes, basal activity and PLP-stimulated activity had significant linear correlations with length of PN supplementation. In addition, the percent stimulation with PLP added in vitro was linearly correlated with the log of supplementation time. The "r" values and the regression line equations are presented in Table 6.

Exercise Experiment

The blood of subjects 1, 3 and 5 from the exercise experiment was analyzed for EAlaAT. Selection of these subjects was arbitrary. Analyses were limited to these subjects because of the large number of samples which needed to be assayed on the day blood was drawn. The results were presented in Table 7. The overall EAlaAT activities increased from week 1 to week 5 due to PN supplementation, but exercise had no significant effect on the activity of this enzyme. Although there was a decrease in percent stimulation from fast to pre-exercise blood sample, it was not statistically significant.

TABLE 6. Equation and "r" Values for the Correlation between Aminotransferase Activities or Percent Stimulation (y) and Supplementation Period (X).

	Equation for regression lines	"r" values
EAlaAT basal activity[1] PLP-stimulated activity[2] percent stimulation[3]	$y = 0.3636 + 0.2308X$ $y = 0.5432 + 0.2320X$ $\log(y) = 7.0185 - 0.4698X$	0.62 0.57 0.79
EAspAT basal activity PLP-stimulated activity percent stimulation	$y = 13.424 + 2.040X$ $y = 25.190 + 2.498X$ $\log(y) = 4.4239 - 0.0726X$	0.82 0.70 0.64

[1] No PLP added to assay medium.

[2] PLP added to assay medium.

[3] $\dfrac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(orEAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}}$ X 100.

Week[1]		Fast[2]	Exercise[3]			
			Pre.	Post	+30	+2 hr.
1	basal activity[4],[5]	0.68±0.40	0.70±0.40	0.72±0.40	0.68±0.40	0.68±0.40
	PLP-stimulated activity[4],[6]	0.84±0.40	0.84±0.40	0.86±0.40	0.82±0.40	0.82±0.40
	percent stimulation[7]	24.50±7.50	23.70±7.30	21.80±7.30	23.50±6.50	24.00±7.50
5	basal activity	1.48±0.78	1.30±0.58	1.44±0.62	1.28±0.60	1.30±0.30
	PLP-stimulated activity	1.56±0.82	1.34±0.60	1.52±0.60	1.34±0.62	1.38±0.30
	percent stimulation	7.10±2.70	5.40±1.80	4.90±2.10	6.00±3.10	6.40±2.30

TABLE 7. The Effect of PN Supplementation and Exercise on EAlaAT Basal Activity, PLP-stimulated Activity and Percent Stimulation (Mean ± S.D.).

[1]Week of study. Week 1, no PN supplement was given; week 5, subjects had received four weeks of PN supplement.

[2]Fasting blood collected before breakfast.

[3]Four hours after breakfast, the subjects had pre-exercise blood drawn, then started exercise experiment. The exercise consisted of pedalling a bicycle ergometer for 21 minutes. The work required to pedal the bicycle was increased every 7 minutes. Before lunch, blood was collected at the end of exercise (post) and after 30 minutes (+30). Two hours after exercise, another blood sample was collected (+2 hr). Exercise had no significant effect on EAlaAT activity ($p \leqslant 0.05$ regarded as significant).

[4]Measure in unit of µg pyruvate/mg Hb/hr.

[5]No PLP added to assay medium.

[6]PLP added to assay medium.

[7]Calculated by: $\dfrac{\text{EAlaAT(orEAspAT) with PLP} - \text{EAlaAT(orEAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}} \times 100$.

DISCUSSION

Effect of Vitamin B-6 Supplementation on Erythrocyte
Aminotransferase Activities

The results of this present study show that even a relatively small supplement of PN can have a significant influence on erythrocyte aminotransferase activities within a very short period of time. Statistically significant increases in basal and PLP-stimulated EAlaAT and EAspAT activities, and decreases in percent stimulation of both enzymes, were observed after only three days of 5 mg PN daily (Table 5). These impressive results are similar to those observed by Krishnaswamy (1971) who reported a 13% increase in basal EAspAT activity of his high socio-economic group after eight to ten days of 5 to 10 mg of vitamin B-6 daily, and a 43% increase in his low socio-economic group.

In the present study, the basal activities after four weeks of PN supplementation were increased by 150% and 50% of the initial levels of EAlaAT and EAspAT, respectively. The PLP-stimulated activities increased 120% and 35%, respectively, for EAlaAT and EAspAT. These activities (basal and PLP-stimulated) for both enzymes during the PN supplementation period increased at a relatively constant rate from week to week (Table 4, Figs. 4, 5). The percent stimulation by PLP added in vitro was, on the other hand, decreased (Table 3) during PN supplementation.

Jacobs et al. (1968) also observed significant increase in basal and PLP-stimulated activities, and a decrease in percent stimulation for both EAlaAT and EAspAT in a group of older subjects who had

received 10 mg of PN daily for six weeks. When a group of younger subjects was supplemented with 20 mg of PN daily for two weeks, Jacobs et al. observed similar, but less dramatic changes. Rose et al. (1973), using a larger PN supplement, also reported comparable results. Rose et al. pointed out that the correlation between the initial EAlaAT levels and those reached after PN supplementation implied a constant proportional rise in enzyme activity throughout the whole range of pretreatment values. They observed about 100% and 50% increases in basal EAlaAT and EAspAT activities, respectively, after four weeks of 40 mg PN supplementation daily in one group of subjects; another group of subjects who received the same amount of PN daily for eight weeks had basal values that increased 195% and 110%, respectively, approximately twice that of the group that received PN for four weeks.

Compared to the normal values for erythrocyte aminotransferase activities in young males from our laboratory (Leklem et al., 1980), subjects in the present study initially had lower EAlaAT basal activity and higher EAlaAT percent stimulation values, suggesting that subjects in our study were in the lower part of the normal range for erythrocyte aminotransferase activities. In addition, the initial plasma total vitamin B-6 levels of these subjects, determined by Wang (1982), were also slightly lower than the normal values observed by Leklem et al. Wang reported a plasma vitamin B-6 mean value of 4.63 ± 0.58 nmoles/100 ml in our subjects, while Leklem et al. had a value of 6.50 ± 1.18 nmoles/ 100 ml. Although the subjects did not receive any vitamin B-6 supplement during the first week of the study, they were consuming a constant diet

which was adequate with vitamin B-6 as well as with other nutrients; the significant difference between week 1 and week 2 thus resulted from the adequate dietary vitamin B-6 intake as well as three days of 5 mg/day PN supplement.

Comparison between EAlaAT and EAspAT

Our data also indicate that EAlaAT is more responsive to PN supplementation than EAspAT, which is in agreement with other investigators. Cavill and Jacobs (1967) reported a higher percent stimulation of EAlaAT than of EAspAT in a group of patients with iron-deficiency anemia. Cinnamon and Beaton (1970) also suggested that, from the response of aminotransferases to PLP stimulation in vitro, EAlaAT is a more sensitive measurement of vitamin B-6 status than EAspAT in man. Cavill and Jacobs (1967) suggested that AspAT has a greater affinity for PLP than AlaAT. That may be the reason why in the slightly vitamin B-6 deficient person, a lowered basal activity was not observed in AspAT, but in AlaAT. Lumeng et al. (1978), experimenting with rats, found that EAspAT activity tended to reach a constant maximal value when pyridoxine intake was increased from 24 to 100 µg/day for nine weeks, while EAlaAT continued to increase. They thus suggested that EAlaAT activity will more accurately reflect vitamin B-6 intake than EAspAT activity.

The Possible Mechanism for Erythrocyte Aminotransferase Activity Changes Following Vitamin B-6 Supplementation

Under normal conditions, the red cell aminotransferases are not

saturated with the coenzyme, PLP. When PN is taken up by erythrocytes, it is phosphorylated and subsequently converted to PLP (Anderson et al., 1971). Thus, the PN supplement increased the PLP content of the erythrocytes in the subjects of this present study. This PLP was then bound to apoaminotransferase, increasing the saturation of this enzyme with coenzyme. This was reflected by the increased basal aminotransferase activities and decreased percent stimulation in vitro by PLP after PN supplementation. In the present study, as well as the one by Jacobs et al. (1968), the PLP-stimulated activities of both EAlaAT and EAspAT by PLP added in vitro also increased throughout the supplementation period. This increase of PLP-stimulated aminotransferase activity, which represents the total enzyme (holo- and apoenzyme), suggests that the vitamin B-6 supplementation not only increased the degree of erythrocyte aminotransferase saturation with coenzyme, but also increased the activity of the enzyme.

The increased aminotransferase activities by PN supplementation is most likely due to enzyme stabilization. Since erythrocyte aminotransferase activity increased after a short term of PN supplementation, Rose et al. (1973) and Brown et al. (1975) suggested that this increase in activity resulted from enzyme stabilization with a reduced rate of degradation because the mature erythrocyte has lost its complement of RNA and its ability to synthesize protein. (Shane (1978) reported that the protein binding of PLP in the body, by means of a Schiff's base complex, protects PLP to a large degree from the action of phosphatases which convert PLP to PL.)

According to our data, the extent of increase in PLP-stimulated

activities for both erythrocyte aminotransferase was less than that in the basal activities (Table 4), which means the apoenzyme increase was not parallel to that of the saturated enzyme increase. In other words, the elevated enzyme activities resulting from the increase of total enzyme (apoenzyme plus holoenzyme) was not as much as from that of holoenzyme. Greengard and co-workers suggested that coenzyme levels in vivo may also influence the amount of protein moiety of appropriate enzyme systems (Greengard and Gordon, 1963; Greengard, 1964). Since new red blood cells are formed every day, the ones formed during the PN supplementation period may have possessed a higher level of apoamino- transferases. An increased level of PLP available during hematopoiesis may have stimulated increased production of aminotransferases. Con- sidering the low turnover rate (approximately 23%) of red cells in the short period of this study, however, this contribution is not the main reason for the increased PLP-stimulated activity of EAlaAT and EAspAT. Also, the decrease in the percent stimulation for both enzymes seemed to level off gradually (Table 5): The increases were greater at the beginning than at the end of the study (significant in the first two weeks but not in the last two weeks), which makes us think that the in- crease of total enzyme (apoenzyme plus holoenzyme) is limited. This phenomenon suggests that enzyme stabilization is a more likely explana- tion for the increased enzyme activity during the period of PN supple- mentation. We suggest that enzyme induction and enzyme stabilization both existed in our study, but that enzyme stabilization was the more effective and reasonable factor, at least for erythrocyte aminotrans- ferases. The effect of PN supplementation on erythrocyte

aminotransferase activity should be observed over a longer period of time, at least four months for complete red cell turnover. Rose et al. (1973) observed that the activities of EAlaAT and EAspAT in subjects receiving 40 mg of PN daily for eight weeks were twice those of subjects who received this same level of PN for four weeks.

Correlation Between Plasma Total Vitamin B-6 and Erythrocyte Aminotransferase Activity

Under normal conditions, PLP in whole blood is approximately equally distributed between plasma and erythrocytes. After PN loading, however, the erythrocyte PLP levels increased faster than plasma levels (Bhagavan et al., 1975). Lumeng et al. (1974) observed that plasma PLP reached a maximal plateau shortly after oral supplementation with 25-50 mg of PN had started (within four days). In our experiment, the plasma total vitamin B-6 reached a plateau at the end of the third week of PN supplementation (Fig. 6), reflecting our lower level of PN supplementation. Although we did not determine erythrocyte PLP levels in our study, we believe, from the increasing erythrocyte aminotransferase activities, that the erythrocyte PLP was also increasing throughout the PN supplementation period. We propose that erythrocyte aminotransferases may serve as a reservoir for PLP. Veitch et al. (1976) suggested that PLP stored in muscle is bound mainly to glycogen phosphorylase; in liver, it is stored bound to phosphorylase and aminotransferases. In view of the increased basal and PLP-stimulated activities of erythrocyte aminotransferases observed in the present study, it is plausible that the erythrocyte aminotransferase may also bind excess PLP. In

FIGURE 6. The effect of PN supplementation on the mean basal and PLP-
            stimulated activities of EAlaAT (represented by ———— and
            -----, respectively), of EAspAT (represented by ===== and
            =====, respectively), and on the concentration of plasma
            vitamin B-6 (□-□-□-□). During week 1 the subjects received
            no PN supplement. Starting on day 6 of week 1, the sub-
            jects received 5 mg PN daily, except on Tuesday and Thurs-
            day of each week, during the remaining four weeks of this
            study. The lines representing basal and PLP-stimulated
            activities for both aminotransferases were drawn from re-
            gression equations.

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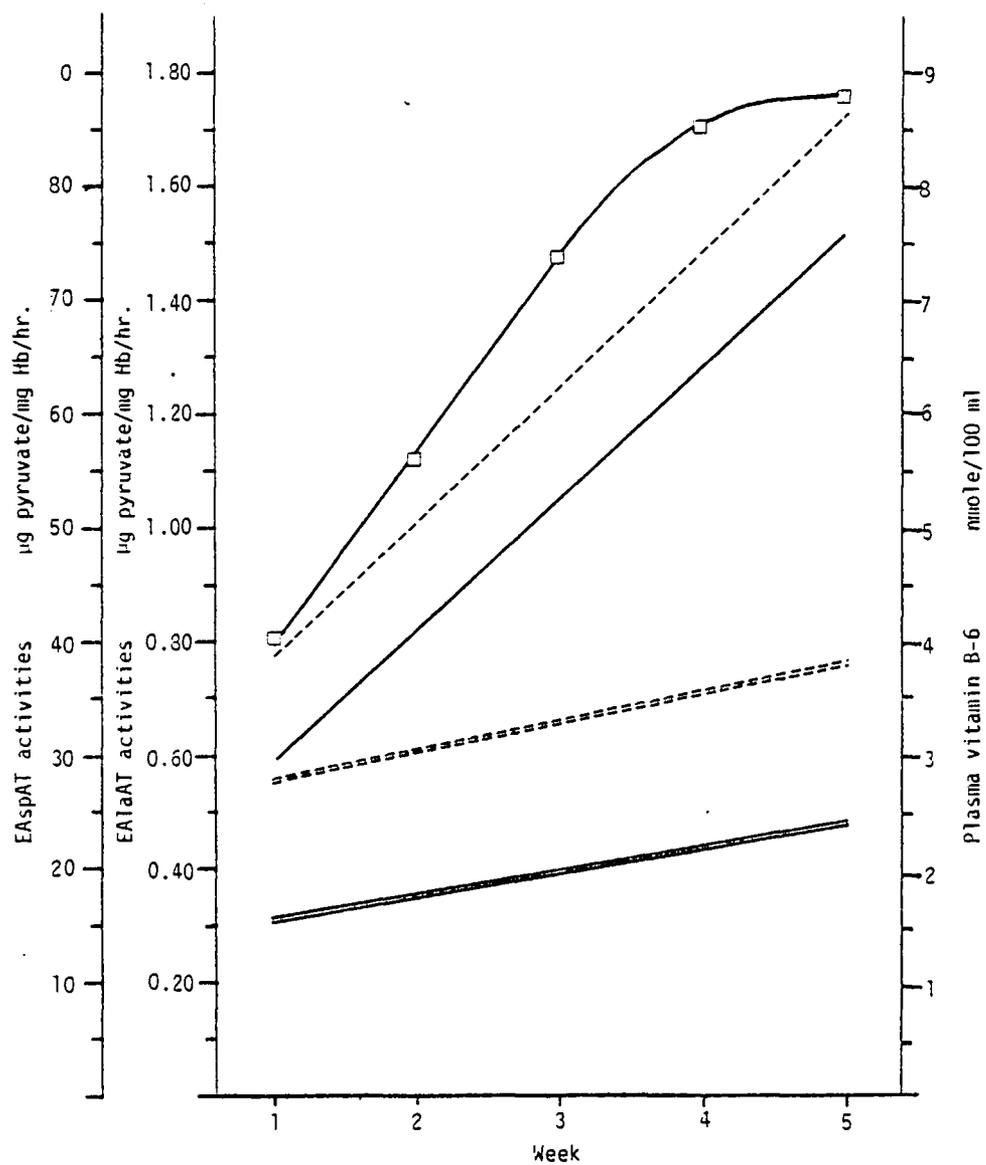
FIGURE 6.

other words, erythrocyte aminotransferases may be another PLP-binding protein in blood, in addition to albumin and hemoglobin. We suggest that PLP levels and aminotransferase activities in erythrocytes be tested in subjects who are receiving supplementary PN over an extended period of time. A follow-up study on these vitamin B-6 measurements in these same subjects during deprivation of vitamin B-6 would give further information on the site of erythrocyte aminotransferases as a PLP or vitamin B-6 pool in the body.

SUMMARY AND CONCLUSIONS

The effect of PN supplementation on the activities of EAlaAT and EAspAT was examined in five men, 22 to 25 years of age. The subjects received a constant diet containing 1.34 mg of vitamin B-6 daily, Monday through Friday of each week, during the five weeks of this investigation. Starting on day 6 of the first week, the subjects were given orally 5 mg of PN daily except on Tuesday and Thursday of each week during the remaining four weeks. On these two days, the subjects received either no supplementary PN or 2 mg of vitamin B-6 in the form of crystalline PN or as food. Basal and PLP-stimulated EAlaAT and EAspAT activities, as well as plasma vitamin B-6, were determined weekly.

Both basal and PLP-stimulated activities of the two aminotransferases increased after only three days of PN supplementation and continued to increase throughout the four-week period of supplementation. The percent stimulation of both enzymes by PLP added in vitro to the assay medium decreased concomitantly. EAlaAT was more responsive to PN supplementation than EAspAT. Plasma vitamin B-6 increased during the first three weeks of PN supplementation and then reached a plateau. Short-term exercise had no significant effect on EAlaAT activity.

The increased basal and PLP-stimulated activities of EAlaAT and EAspAT are most likely to be due to stabilization of these two enzymes by their coenzyme, PLP. Induction of these two aminotransferases by PLP may also contribute to the increased activity of these enzymes

in response to oral PN supplementation. We propose that the binding of PLP to erythrocyte apoaminotransferases may serve as a reservoir for vitamin B-6 in the body.

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APPENDICES

APPENDIX 1. Effect of vitamin B-6 supplementation on EAlaAT basal activity, PLP-stimulated activity and percent stimulation.

Subject		←—No PN—→	←——————————— PN supplementation[1] ——————————→			
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5
1	basal activity[2,3]	1.10	1.40	1.68	1.96	2.20
	PLP-stimulated activity[2,4]	1.40	1.66	1.94	2.16	2.40
	percent stimulation[5]	27.30	18.60	15.50	10.20	9.10
2	basal activity	0.42	0.64	0.90	1.16	1.42
	PLP-stimulated activity	0.50	0.74	1.00	1.26	1.52
	percent stimulation	19.00	14.30	11.10	9.40	7.00
3	basal activity	0.51	0.74	0.92	1.08	1.34
	PLP-stimulated activity	0.70	0.96	1.12	1.22	1.50
	percent stimulation	39.40	28.80	20.00	14.60	11.90
4	basal activity	0.61	0.92	1.28	1.64	1.98
	PLP-stimulated activty	0.80	1.61	1.56	1.94	2.28
	percent stimulation	33.30	26.10	21.90	18.30	15.20
5	basal activity	0.36	0.42	0.50	0.58	0.66
	PLP-stimulated activity	0.44	0.50	0.58	0.66	0.74
	percent stimulation	22.20	19.00	16.00	13.80	12.10

[1] Starting day 6 of week 1 until day 6 of week 5, the subjects received daily, except on Tuesdays and Thursdays, 5 mg of PN at breakfast. The subjects received no supplement on the Tuesday and Thursday of week 2, 2 mg PN on the Tuesday and Thursday of week 3, and some specific food containing approximately 2 mg vitamin B-6 on Tuesdays and Thursdays of week 4 and week 5.

[2] Measured in unit of µg pyruvate/mg Hb/hr.

[3] No PLP added to assay medium.

[4] PLP added to assay medium.

[5] Calculated by: $\frac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(or EAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}}$ X 100.

APPENDIX 2. Effect of vitamin B-6 supplementation on EAspAT basal activity, stimulated activity and percent stimulation.

Subject		← No PN →	PN supplementation[1]			
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5
1	basal activity[2],[3]	17.0	18.1	19.8	21.4	22.5
	PLP-stimulated activity[2],[4]	32.6	33.5	35.2	36.8	37.6
	percent stimulation[5]	92.0	85.0	77.8	71.9	67.2
2	basal activity	15.6	18.0	20.8	22.8	26.0
	PLP-stimulated activity	30.0	32.3	35.8	38.1	42.6
	percent stimulation	91.9	79.4	72.1	67.4	63.8
3	basal activity	19.0	20.5	21.9	23.3	24.7
	PLP-stimulated activity	31.0	32.5	34.1	35.5	37.2
	percent stimulation	63.2	58.5	55.7	52.4	50.6
4	basal activity	10.4	13.5	17.0	20.3	23.3
	PLP-stimulated activity	18.3	23.0	28.5	33.5	38.5
	percent stimulation	76.7	70.4	67.6	65.0	63.1
	basal activity	15.4	17.1	18.6	20.0	21.6
	PLP-stimulated activity	27.3	28.6	30.1	31.5	33.0
	percent stimulation	76.1	67.3	62.1	57.5	52.8

[1]Starting day 6 of week 1 until day 6 of week 5, the subjects received daily, except on Tuesdays and Thursdays, 5 mg of PN at breakfast. The subjects received no supplement on the Tuesday and Thursday of week 2, 2 mg PN on the Tuesday and Thursday of week 3, and some specific food containing approximately 2 mg vitamin B-6 on Tuesdays and Thursdays of week 4 and week 5.

[2]Measured in unit of μg pyruvate/mg Hb/hr.

[3]No PLP added to assay medium.

[4]PLP added to assay medium.

[5]Calculated by: $\dfrac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(or EAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}} \times 100.$

APPENDIX 3.   The effect of exercise on EAlaAT basal activity, stimulated activity and percent stimulation.

Week[1]	Sub-ject		Fast[2]	Exercise[3]			
				Pre.	Post	+30	+2 hr.
1	1	basal activity[4] [5]	1.06	1.14	1.18	1.08	1.04
		PLP-stimulated activity[4] [6]	1.24	1.32	1.36	1.26	1.20
		percent stimulation[7]	16.70	16.30	14.80	15.80	16.50
	3	basal activity	0.62	0.58	0.60	0.60	0.62
		PLP-stimulated activity	0.82	0.76	0.78	0.78	0.82
		percent stimulation	31.70	30.80	29.30	31.10	31.40
	5	basal activity	0.38	0.36	0.38	0.34	0.36
		PLP-stimulated activity	0.48	0.44	0.46	0.42	0.44
		percent stimulation	25.00	24.00	24.10	23.70	24.10
5	1	basal activity	1.88	1.76	1.94	1.82	1.86
		PLP-stimulated activity	2.00	1.88	2.06	1.94	1.98
		percent stimulation	7.40	6.80	6.20	6.60	6.50
	3	basal activity	1.48	1.44	1.52	1.30	1.32
		PLP-stimulated activity	1.64	1.58	1.66	1.44	1.46
		percent stimulation	10.80	9.70	9.20	10.80	10.60
	5	basal activity	0.68	0.64	0.78	0.64	0.66
		PLP-stimulated activity	0.76	0.70	0.84	0.70	0.72
		percent stimulation	11.80	9.40	7.70	9.40	9.10

[1]Week of study. Week 1, no PN supplement was given; week 5, subjects had received four weeks of PN supplement.

[2]Fasting blood collected before breakfast.

[3]Four hours after breakfast, the subjects had pre-exercise blood drawn, then started exercise experiment. The exercise consists of pedalling a bicycle ergometer for 21 minutes. The work required to pedal the bicycle was increased every 7 minutes. Before lunch, collect blood at the end of exercise (post) and after 30 minutes (+30). Two hours after exercise, another blood sample was collected (+2 hr.).

[4]Measured in unit of µg pyruvate/mg Hb/hr.

[5]No PLP added to assay medium.

[6]PLP added to assay medium.

[7]Calculated by: $\dfrac{\text{EAlaAT(or EAspAT) with PLP} - \text{EAlaAT(or EAspAT) without PLP}}{\text{EAlaAT(or EAspAT) without PLP}} \times 100$.