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The bioavailability of vitamin B-6 from four selected foods was investigated in five men, aged 22 to 25 years, who were receiving a pyridoxine supplement. The subjects received a constant diet containing 1.34 mg of vitamin B-6 throughout this five-week study, except on Saturdays and Sundays when they ate their self-chosen diets. Starting on day 6 of week 1, following a five-day adjustment period, the subjects received orally 5-mg crystalline pyridoxine supplement daily, except on Tuesday and Thursday of each week. On these two days, the subjects were given orally 0 mg or 2 mg of crystalline pyridoxine, or test doses of bananas, filberts, soybeans and beef which contained

around 2 mg of vitamin B-6. Vitamin B-6 was determined by microbiological assay with Saccharomyces uvarum. Vitamin B-6 bioavailability in the test food was determined by comparing 24-hour urinary total vitamin B-6 in response to the test food doses to that excreted following a 2-mg crystalline pyridoxine dose in each subject. Compared to the 100 percent bioavailability of the 2-mg crystalline PN dose, the average vitamin B-6 bioavailability in bananas was 115 $\pm$ 32% and that in filberts, soybeans and beef was 93 $\pm$ 8%, 73 $\pm$ 20% and 87 $\pm$ 7%, respectively. The metabolism of vitamin B-6 in pyridoxine-supplemented subjects was also investigated by measuring changes in plasma total vitamin B-6 which increased and was stablized after three weeks of pyridoxine supplementation. It was concluded that urinary total vitamin B-6 in pyridoxine-supplemented subjects can be used as a measure of vitamin B-6 bioavailability from test food doses.

Bioavailability of Vitamin B-6 from Test Foods and Metabolism of Vitamin B-6 in Men Receiving Supplementary Pyridoxine

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDIX TABLES

Bioavailability of Vitamin B-6 from Test Foods and Metabolism of Vitamin B-6 in Men Receiving Supplementary Pyridoxine

INTRODUCTION

The accuracy of dietary quality assessments depends on information concerning both the content and bioavailability of nutrients present. The determination of nutrients in foods is of little value unless these data can be interpreted with respect to the bioavailability of those nutrients. The term bioavailability, as used here, refers to the fraction of a nutrient in food which is suitable for intestinal absorption and metabolic utilization.

Little quantitative information is available on the bioavailability of vitamin B-6 from foods in humans. The bioavailability of vitamin B-6 as affected by wheat bran (Miller et al., 1979), citrus pectin (Miller, Shultz and Leklem, 1980), protein (Miller and Leklem, 1978) and beef vs. soybeans (Leklem, Shultz and Miller, 1980) has been estimated in subjects who were given a constant diet over a relatively long period of time (50 to 55 days for each investigation). Recently, Wozenski, Leklem and Miller (1980) reported on the metabolism of small doses of

crystalline vitamin B-6 in young man. If bioavailability of vitamin B-6 in foods could be determined similarly, by measuring changes in concentration of urinary vitamin B-6 in response to a test food dose, then the determination of vitamin B-6 bioavailability would be less time-consuming and expensive, and more subjects could be tested.

Tamura and Stokstad (1973) investigated the bioavailability of food folate in male adults by giving a test dose of food. Each subject was maintained in a "folate-saturated" condition, so that the increase in urinary folate following small doses of crystalline folate or food folate was proportional to the bioavailability of this vitamin.

The purpose of the research reported in this thesis was to estimate the bioavailability of vitamin B-6 from bananas, filberts, soybeans and beef in young adult men by an adaptation of the procedure developed by Tamura and Stokstad. Test doses containing 1.5 to 2 mg of vitamin B-6 were administered to young men who were supplemented with crystalline pyridoxine. Vitamin B-6 bioavailability was measured by comparing urinary total vitamin B-6 excretion after a test food dose to that excreted in response to a 2-mg crystalline pyridoxine dose.

The wide extent of vitamin usage was reviewed by English and Carl (1981). Little information is available on the effect of excessive vitamin B-6 intake on the metabolism of this vitamin. In the present study, the metabolism of vitamin B-6 in the pyridoxine-supplemented subjects was also assessed by measuring plasma total vitamin B-6. Plasma total vitamin B-6 levels were compared to the activities of the erythrocyte aminotransferases which were reported by Wang (1982).

REVIEW OF LITERATURE

Background

The isolation of crystalline vitamin B-6 was reported by György, Lepkovsky and three other groups in 1938. Within a year, vitamin B-6 was identified as a pyridine and was named pyridoxine (PN) (Brin, 1978). Studying the growth requirement of various microorganisms, Snell, Guirard and Williams (1942) recognized the existence of two other forms of vitamin B-6 in addition to PN: pyridoxal (PL) and pyridoxamine (PM). Pyridoxal 5-phosphate (PLP), the coenzyme form of vitamin B-6, is concerned with a vast number and variety of enzyme systems. So far, over 60 PLP-dependent enzymes have been reported (Sauberlich and Canham, 1980). The reactions catalyzed by these PLP-dependent enzymes are mainly involved in various aspects of amino acid metabolism, including transamination, racemization, decarboxylation, desulfhydration and dehydration.

PLP is also a component of glycogen phosphorylase. This enzyme catalyzes the breakdown of glycogen to form glucose-1-phosphate (White et al., 1978). PLP is bound to ϵ-amino group of a lysine residue of the enzyme. Since the 5-phosphate group of PLP is needed for the activation of phosphorylase (Fischer, Rocker and Saari, 1970), it appears that PLP plays a structural or conforma-

tional role in this enzyme (White et al., 1978). Since phosphorylase constitutes about 5% of muscle protein, and muscle accounts for around 40% of the body weight, Krebs and Fischer (1964) proposed that the enzyme might serve as a physiological repository for vitamin B-6. By experimenting with weanling rats, Black, Guirard and Snell (1977) have shown that the content of the phosphorylase reservoir rises and falls with changes in vitamin B-6 supply. Whether or not vitamin B-6 storage will affect the activity of this enzyme is still unclear (Anonymous, 1978).

Interconversion of Vitamin B-6 Forms

The interconversions of the various forms of vitamin B-6 have been studied intensively. McCormick, Gregory and Snell (1961) observed in mammalian tissue that PL phosphokinase not only phosphorylated PL, but PN and PM as well. PN may be either oxidized to PL or phosphorylated to pyridoxine phosphate (PNP), which, in turn, is oxidized to PLP. Subsequent work by others showed that two distinct enzymes catalyze these two oxidation reactions: PN oxidase oxidizes PN only (Morino et al., 1960), and PNP oxidase catalyzes the oxidation of both PNP and pyridoxamine phosphate (PMP) to PLP (Wada and Snell, 1961).

In vivo studies have supplemented the suggested

possible metabolic pathways deduced from the *in vitro* studies on individual enzymes. Johansson, Lindstedt and Tiselius (1968) demonstrated a rapid conversion of PN to PNP in mouse liver and carcass. Similar results were obtained by Colombini and McCoy (1970) with the use of ^{14}C-labelled PN. Johansson et al. (1974) subsequently attempted to formulate a metabolic model for the interconversion of the different forms of vitamin B-6 in liver. The results that they obtained strongly suggested that phosphorylation is also the first step in the conversion of PM to PLP. Fig. 1 shows the interconversion of the various forms of vitamin B-6 and the formation of 4-pyridoxic acid (4-PA).

Absorption of Vitamin B-6

The initial studies on absorption of vitamin B-6 were conducted in 1940 by Scudi, Unna and Antopol. They investigated the absorption of PN by measuring urinary PN. Later, Brain and Booth (1964) estimated the urinary excretion of radioactive substances following a dose of ^{3}H-labelled PN-HCl administered orally to mice which had been saturated with unlabelled PN. Excretion of a 1-mg dose was maximal at 60 to 90 minutes, suggesting that PN is absorbed from the upper small intestine. Experiments in rats by Booth and Brain (1962) led to similar conclusions.

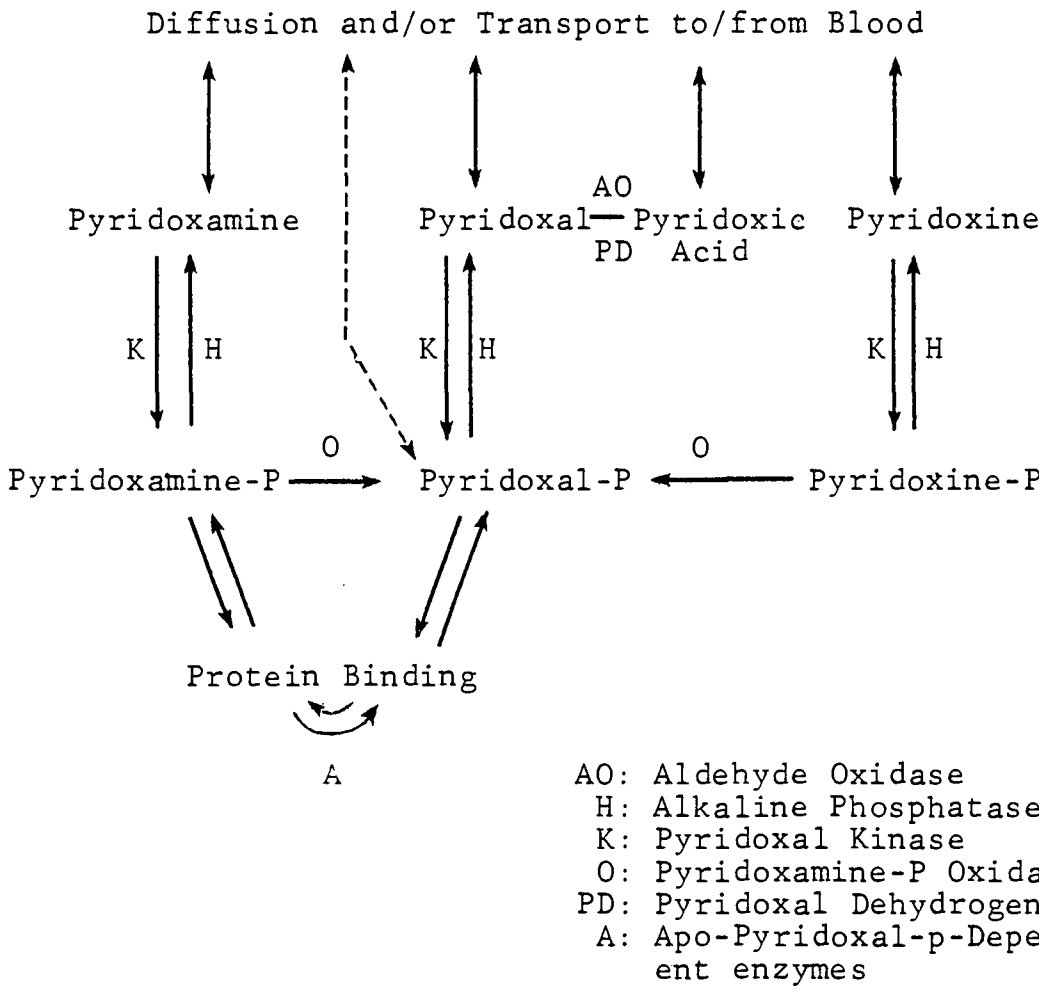

Fig. 1. Interconversions of the B-6 vitamins and formation of 4-PA (from McCormick and Merrill, 1980.)

Booth and Brain also suggested that PN is absorbed from the ileum and, to a small extent, from the colon. The linear relationship between oral dose and urinary excretion suggested that the absorption of PN is by simple passive diffusion (Brain and Booth, 1964; Booth and Brain, 1962). Utilizing everted intestinal sacs, Serebro et al. (1966) were unable to demonstrate tha accumulation of PN against a concentration gradient in tissue or serosal fluid. This further supported the suggestion that PN is absorbed by passive diffusion.

Recently, the kinetics of mucosal membrane transport of unphosphorylated vitamin B-6 were determined with the use of everted rings or isolated, perfused segments of rat jejunum (Middleton, 1977; Meinen, Aeppli and Rehner, 1977; Mehansho, Hamm and Henderson, 1979). The data presented in these studies are also compatible with the suggestion that vitamin B-6 is absorbed by passive diffusion in rats. Middleton (1977) observed a substantial portion of radioactivity appeared in phosphorylated forms of vitamin B-6 in tissue. Phosphorylation is considered to occur intracellularly, since PL kinase has been reported to be present in the intestine (McCormick et al., 1961). However, Middleton also found radioactivity in unphosphorylated forms of vitamin B-6. This suggested the possibility of not only the phosphorylation of PN, but also the subse-

quent dephosphorylation of vitamin B-6 in the mucosal cell. Similar observations were also shown by Mehansho et al. (1979).

Studies clearly indicate that the mechanisms involved in the absorption of phosphorylated vitamin B-6 are more complicated than passive diffusion seen for the free forms of vitamin B-6. Middleton (1979) investigated the intestinal absorption of PLP in vivo in rat jejunum by using a perfused segment model. The disappearance of PLP was linear with respect to time. Middleton proposed that the disappearance of PLP was mediated both by alkaline phosphatase hydrolysis, which occurred either at the brush-border or in the lumen of the intestine, and by a second mechanism which may represent absorption of the intact PLP. Mehansho et al. (1979) also made a similar suggestion. The latest report by Middleton (1982) substantiated his earlier proposal on the site of alkaline phosphatase hydrolysis.

Evidently vitamin B-6 can be synthesized in the intestinal tract and absorbed by animals deficient in the vitamin. Sarma, Snell and Elvehjem (1946) investigated the effect of various dietary carbohydrates on the development of vitamin B-6 deficiency in rats. They observed that when dextrin was substituted for sucrose or glucose in the diet, considerable growth occurred in rats fed a vitamin

B-6 deficient diet. In addition, Okuda, Hsu and Chow (1960) reported that supplementing a vitamin B-6 deficient diet with D-sorbitol increased the concentration of vitamin B-6 in urine and in the livers of young adult rats. Okuda et al. also explained their results on the basis of increased synthesis of vitamin B-6 by intestinal micro-organisms.

Urinary Excretion of Vitamin B-6

Kelsay, Baysal and Linkswiler (1968) investigated the effect of vitamin B-6 depletion on the urinary excretion of vitamin B-6 in adult males. A marked decrease in urinary vitamin B-6 occurred in the subjects when they received only 0.16 mg of vitamin B-6 daily. A decreased excretion of vitamin B-6 by vitamin B-6 depleted human subjects was also observed by Baysal, Johnson and Linkswiler (1966) and Donald et al. (1971).

In a similar controlled study with adult males by Baker et al. (1964), the urinary excretion of free vitamin B-6 correlated well with changes in dietary PN. The subjects' urinary free vitamin B-6 decreased progressively with time when the subjects received a vitamin B-6 deficient diet; this low urinary excretion of free vitamin B-6 paralleled the increase in abnormal tryptophan metabolites

following tryptophan loading.

Although the vitamin B-6 requirement is related to protein intake (NAS, 1980), Baker et al. (1964) observed that the level of dietary protein had little effect on the rate at which urinary vitamin B-6 decreased in men fed diets deficient in this vitamin. Similar results were obtained by Kelsay et al. (1968) and Canham et al. (1969).

Supplementary PN increased the urinary excretion of vitamin B-6 by vitamin B-6-depleted subjects (Baker et al., 1964; Baysal et al., 1966; Donald et al., 1971). Donald et al. observed that the percentage of dietary vitamin B-6 excreted as urinary vitamin B-6 decreased from the end of the depletion phase to the repletion phase as vitamin B-6 deficient subjects were being repleted with increasing amounts of PN. This suggested to Donald et al. that vitamin B-6 was being retained by the tissues.

Vitamin B-6 occurred in urine mainly as PL and, to a lesser amount, as PM when subjects received 1.5 mg of crystalline PN in addition to a controlled diet containing 0.16 mg of vitamin B-6 (Kelsay et al., 1968); during vitamin B-6 deficiency, the ratio of urinary PL to PM was reversed. Although PN was the main form of vitamin B-6 in a diet high in wheat bread fed to human subjects by Leklem et al. (1980), PL was the major form of vitamin B-6 in urine. Leklem et al. suggested that urinary vitamin

B-6 is not only a reflection of unmetabolized vitamin B-6, but also of vitamin B-6 which has been metabolized.

Since urinary vitamin B-6 drops immediately in response to a vitamin B-6 deficient diet, it may be of limited value as an indicator of vitamin B-6 deficiency in humans. It is more useful as a reflection of the subjects' recent dietary intake of vitamin B-6 (Sauberlich et al., 1972).

Wozenski et al. (1980) studied the metabolism of small oral doses of PN and of equimolar doses of PN, PL and PM in young men by measuring vitamin B-6 compounds in timed urine and blood samples collected after each dose. Their data showed that the rate of urinary vitamin B-6 excretion increased linearly with increasing PN doses. The response to the PM dose was slower than for PN or PL, which suggests that PM is absorbed more slowly or metabolized differently, or both, than PL or PN.

By measuring urinary vitamin B-6, Tarr, Tamura and Stokstad (1981) investigated the availability of vitamin B-6 in an average American diet in healthy individuals. The excretion of vitamin B-6 in urine increased with increasing intake of vitamin B-6 (1.1 mg, 2.3 mg and 2.7 mg/day) and represented approximately 5% of the total vitamin B-6 intake. When combining their data with those from earlier experiments, Tarr et al. observed that the relationship between vitamin B-6 intake and urinary

excretion appeared to follow a sigmoidal curve in the range of vitamin B-6 intakes studied (0.16 mg to 2.7 mg/day).

Biodegradation of Vitamin B-6

Four-pyridoxic acid (4-PA) is formed from the oxidation of PL or PLP (fig. 1) and is the predominant metabolite of the three forms of vitamin B-6 (Wozenski et al., 1980). This compound has no vitamin B-6 activity (Snell and Rannefeld, 1945) and cannot be degraded further. The routine use of urinary 4-PA measurement in metabolic studies and nutritional assessment has been limited by the cumbersome laboratory procedure for determining 4-PA.

Approximately 30 to 60% of the dietary intake of vitamin B-6 in normal, adequately nourished men (Johansson et al., 1966; Kelsay et al., 1968), women (Donald et al., 1971), and children (Lewis and Nunn, 1977) was excreted as 4-PA in urine. Lewis and Nunn (1977) advised against using a percentage of vitamin B-6 intake excreted as 4-PA as the sole criterion of vitamin B-6 adequacy. If vitamin B-6 intake has been low and is low at the time of the urine collection, the percentage of dietary vitamin B-6 excreted as 4-PA would appear adequate. In this situation, vitamin B-6 is being retained in the body and less

4-PA is excreted. Sauberlich et al. (1972) stated that if one accepts tentatively a conversion of 40% of daily ingested vitamin B-6 to 4-PA, guidelines of acceptable levels of 4-PA excretion can be calculated from the recommended daily allowance of vitamin B-6.

Under normal conditions, urinary 4-PA reflects the intake of vitamin B-6. This relationship has been observed in human metabolic studies in which the intake of vitamin B-6 was controlled (Baysal et al., 1966; Linkswiler, 1967; Kelsay et al., 1968; Donald et al., 1971). Yess et al. (1964) found that the decrease in 4-PA excretion in man depleted of vitamin B-6 paralleled the increase in excretion of tryptophan metabolites following a 2 g loading dose of L-tryptophan. Others (Snyderman, 1953; Baysal et al., 1966; Linkswiler, 1967; Kelsay et al., 1968; Donald et al., 1971) also have reported decreased excretion of 4-PA during vitamin B-6 deficiency. In some studies, little or no 4-PA was detected after a period of vitamin B-6 deprivation.

Blood Vitamin B-6

Total Vitamin B-6 in Blood.

Giving a controlled diet to healthy human subjects, Baysal et al. (1966) demonstrated that the concentration

of vitamin B-6 in blood fell rapidly during vitamin B-6 depletion and rose following supplementation with PN. Similar results have also been observed by Kelsay et al. (1968) and Donald et al. (1971). The decrease in blood vitamin B-6 in subjects fed a vitamin B-6 deficient diet suggests that this measurement can be used as an indicator of vitamin B-6 status.

Baker et al. (1967) conducted the only nutrition survey on 642 New York City school children in which a significant number of serum samples were analyzed for vitamin B-6 levels. Miller et al. (in process) who compared vitamin B-6 status in children taking no vitamin supplements with those taking vitamin supplements including vitamin B-6, reported that the vitamin takers had significant higher mean plasma total vitamin B-6 than non-vitamin takers. Plasma total vitamin B-6 was measured for determining vitamin B-6 absorption and metabolism in subjects who had received orally small doses of crystalline vitamin B-6 (Wozenski et al., 1980).

Pyridoxal Phosphate in Blood.

PLP in blood has been measured by various analytical methods including enzymatic (Chabner and Livingston, 1970; Lumeng and Li, 1974), microbiological (Haskell and Snell,

1972) and fluorometric (Contractor and Shane, 1968) procedures. Lumeng, Brashear and Li (1974) demonstrated the effect of supplementary PN on plasma PLP in normal subjects who received 25 mg of PN daily by mouth. Plasma PLP increased and reached a maximal plateau within 4 days and remained elevated until the PN supplement was discontinued.

Due to the considerable fluctuation of the levels of the vitamin B-6 in blood, Contractor and Shane (1968) suggested that blood PLP is a better index of vitamin B-6 status than blood vitamin B-6. The main advantage, however, in measuring PLP levels in blood is that this is the functional form of the vitamin. Under normal conditions, PLP is distributed approximately equally between plasma and erythrocytes (Bhagavan, Colemen and Coursin, 1975).

Liver is the primary source of PLP in plasma. Plasma PLP is synthesized from either PN or PL (Lumeng et al., 1974). In human plasma, PLP is bound to albumin (Lumeng et al., 1974; Anderson et al., 1974). Albumin has multiple PLP-binding sites (Dempsey and Christensen, 1962); this enormous binding capacity of albumin accounts for the rise in plasma PLP values as PN intake is increased (Lumeng, Ryan and Li, 1978). Lumeng and coworkers (1974) suggest that albumin-bound PLP, being virtually nontransportable

into the cells, may help in maintaining an equilibrium between the plasma and tissue concentrations of the vitamin.

Aminotransferase Activity in Blood.

Since PLP and PMP are the coenzymes for aspartate aminotransferase and pyruvate aminotransferase, a deficiency of vitamin B-6 gives rise to a decreased saturation of these holoenzymes with their coenzyme, resulting in decreased enzyme activity. This has been observed in vitamin B-6 deficient human subjects (Cavill and Jacobs, 1967; Hamfelt, 1967) and rats (Cheney, Curry and Beaton, 1965; Cheney, Sabry and Beaton, 1967).

The effect of vitamin B-6 depletion on serum aminotransferase activities was studied in men by Baysal et al. (1966). The consumption of a partially purified diet containing 0.16 mg vitamin B-6 resulted in a significant decrease in serum aminotransferase activity; pyruvate aminotransferase appeared to be affected more than aspartate aminotransferase. These results are in agreement with those of Brin et al. (1960) and Cheney et al. (1965) in rats. Since serum pyruvate aminotransferase was affected more by vitamin B-6 depletion than aspartate aminotransferase, Baysal et al. suggested that the apoenzyme of aspartate aminotransferase may have a greater affinity for PLP than does the one of pyruvate aminotransferase.

Measurement of plasma or serum aminotransferases are of limited use for assessing vitamin B-6 status because of the wide range of activity observed among normal individuals.

From the results of a controlled vitamin B-6 depletion-repletion study, Raica and Sauberlich (1964) concluded that the stimulation of erythrocyte aminotransferases by PLP added *in vitro* was a more reliable index of vitamin B-6 status than the measurement of aminotransferase activity alone (basal activity) in erythrocytes. During vitamin B-6 depletion in adult males, *in vitro* stimulation of aminotransferase by added PLP increased; after oral administration of vitamin B-6, aminotransferase activity rose and *in vitro* stimulation by added PLP decreased concomitantly. Other investigators (Hamfelt, 1966; Cavill and Jacobs, 1967; Cinnamon and Beaton, 1970; Woodring and Storvick, 1970; Krishnaswamy, 1971) have expressed the view that the erythrocyte aminotransferase activity is a good indicator of vitamin B-6 status.

Vitamin B-6 Requirement for Man

Vitamin B-6 status in man is estimated on the basis of biochemical parameters (Sauberlich, 1974). The requirement of vitamin B-6 is increased when high-protein diets are consumed (Baker et al., 1964; Miller and Linkswiler,

1967; Canham et al., 1969). According to Linkswiler (1978), the data from these studies indicate that the vitamin B-6 requirement for men consuming diets containing 100 to 150 g of protein is between 1.5 to 2.0 mg/day, and the requirement is 1.0 to 1.5 mg when the protein intake is 100 g or less.

A ratio of 0.02 mg of vitamin B-6 per gram of protein was suggested as a basis for calculating vitamin B-6 allowances. Consequently, the Food and Nutrition Board of the National Research Council (NAS, 1980) recommended 2.2 mg/day of vitamin B-6 as the dietary allowance for adult males. This was based on the customary protein intake in the United States (110 g/day), which usually exceeds the RDA for protein, and the uncertainty of the availability of dietary vitamin B-6.

Bioavailability of Vitamin B-6 in Foods

Food Sources of Vitamin B-6.

Vitamin B-6 occurs widely in foods. Meats (especially liver), some fruits and vegetables, wheat germ, wheat bran and whole grain cereals are good sources of this vitamin (Orr, 1969). PN, PL and PM in foods have been determined. Generally, fruits and vegetables contain a higher proportion of the total vitamin B-6 as PN than either of

the other two forms of vitamin B-6, while PL and PM are the predominant vitamin B-6 form in animal products (Rabinowitz and Snell, 1948; Polansky and Murphy, 1966).

Effect of Food Processing.

Most studies concerning the stability of vitamin B-6 during food processing have dealt with the effect of heat treatment. Richardson, Wilkes and Ritchey (1961) compared the retention of vitamin B-6 in various canned foods preserved by conventional heating, freezing, and irradiation. Both heat processing and irradiation cause high losses of vitamin B-6. Processing of beans (Raab, Luh and Schweigert, 1973) at 120°C for 30 to 40 minutes has been reported to have a maximum destruction of 20% of the total vitamin B-6. Yen, Jonsen and Baker (1976) found that the roasting of shelled corn in the production of animal feed resulted in losses of available vitamin B-6.

Recently, a dehydrated food model system fortified with various forms of vitamin B-6 was subjected to a roasting process to determine the effect of roasting on vitamin B-6. The relative degradation of PN, PM and PLP was 50 to 70% by both microbiological and semiautomated fluorometric assay methods. The vitamin B-6 remaining after roasting at 180°C for 25 minutes was fully bioavailable as determined by rat bioassay (Gregory and Kirk,

1978a). By using a model food system, they also investigated the nature and activity of vitamin B-6 bound in heat-processed foods. A significant fraction of vitamin B-6, especially PL and PLP, can be converted during heat treatment of foods into the less biologically active pyridoxal-amino compounds, particularly ε-pyridoxyllysine (Gregory and Kirk, 1977, 1978a, 1978b). These interactions have been reviewed (Anonymous, 1978; Gregory and Kirk, 1981).

Effect of Food Composition.

Leklem et al. (1980) reported the results of a bioavailability study in which the naturally occurring vitamin B-6 in whole wheat bread was utilized less well than the vitamin B-6 in white bread fortified with or without PN-HCl. Leklem et al. (1980) also observed that the vitamin B-6 is less available from soybeans than from beef. Dietary fiber was suggested to exert a weak inhibitory effect on the utilization of the vitamin in both studies. A similar effect has been noticed by other workers (Miller et al., 1979; Gregory, 1980). Investigating the effect of pectin supplementation in human beings on vitamin B-6 bioavailability, Miller et al. (1980) found that pectin had no effect on the utilization of dietary vitamin B-6. Although pectin may have increased the synthesis of vitamin B-6 by the intestinal microflora, as indicated by increased

fecal vitamin B-6, this vitamin was not absorbed as determined by urinary vitamin B-6 or 4-PA. The effect of cellulose, pectin and bran on the bioavailability of PN were examined using rat and chick bioassay methods by Nguyen, Gregory and Damron (1981). The dietary fibers tested had no important deleterious effects on the bioavailability of PN. Using a triple-lumen perfusion technique, Nelson, Lane and Cerda (1976) compared intestinal absorption of vitamin B-6 from a synthetic solution and orange juice. Since vitamin B-6 absorption was significantly greater from a synthetic source than from orange juice, Nelson et al. postulated that the bound forms of this vitamin in natural products may lower absorption.

The bioavailability of vitamin B-6 in foods has been reviewed thoroughly by Gregory and Kirk (1978b, 1981).

MATERIALS AND METHODS

Subjects in the Study

Five young male volunteers served as subjects. Their ages, body weights and heights are presented in table 1. All subjects were in good health and were without a history of any known metabolic disease, as determined by a questionnaire. All subjects exhibited normal absorption of D-xylose, as determined by the excretion of xylose in urine[1] collected during the 5-hr. period immediately following the ingestion of 5 g D-xylose (Harris, 1969).

The protocol of this study was approved by the Human Subjects Committee at Oregon State University. After having received a detailed explanation of the study, the subject signed an informed consent form (Appendix table 1) approved by this committee.

Diet

In this five-week study (April 21 to May 24, 1980), the subjects received a constant diet containing 1.34 mg of vitamin B-6[2] per day from Monday to Friday of each

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[1]Performed by Lynda Barstow, Foods and Nutrition Dept., OSU.

[2]Vitamin B-6 in the constant diet was determined by using Saccharomyces uvarum as the assay organism (AOAC, 1980 chromatography step omitted). The assay was performed by H. Kabirmedianshah.

Table 1. Ages, weights and heights of the subjects.

Subject	Age (yr.)	Height (cm)	Initial Weight (kg)	Final Weight (kg)
1	22	189	86.6	85.5
2	25	172	84.3	83.4
3	22	180	94.5	92.3
4	23	181	83.0	81.7
5	25	176	70.0	68.8
Mean±S.D.	23±2	180±6	83.7±8.9	82.3±8.6

week. Only breakfast was served on Saturdays. The detailed diet is shown in table 2. In addition to the foods listed in table 2, margarine, jelly, hard candy, sugar and carbonated beverages were made available to the subjects for them to maintain a constant body weight. A record of their daily consumption of these items was maintained. No other foods or drinks were allowed on the days when the three meals were provided. Self-chosen diets were consumed at the remaining meals on Saturday and all day Sunday; on the weekends, the consumption of alcoholic beverages was limited to 16 oz of beer or 8 oz of wine. On the weekends, the subjects were instructed to avoid foods high in vitamin B-6 and to keep an exact record of the types and amounts of foods and beverages they consumed.

Procedure

The subjects maintained their normal activities during the study. No strenuous exercise, however, was allowed. Each subject recorded his physical activity daily.

During the first 5 days of week 1 (adjustment period), the subjects received the constant diet (table 2) without any PN supplement. Starting on day 6 of week 1 and during the remaining four weeks of this investiga-

Table 2. Constant diet

Breakfast	g
Orange juice, frozen reconstituted	230
Bread, white enriched	50
Applesauce, canned	75
Rice cereal, enriched, oven popped	30
Milk, nonfat, reconstituted	240
Margarine	variable
Honey	variable
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	1/4 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

tion, the subjects received an oral supplement of 5 mg of PN[3] each day except on Tuesdays and Thursdays. The 5 mg supplement was given with breakfast. On Sundays, the subjects came into the laboratory to take their PN supplements. There was no PN supplement on the last two days of the study (May 23 and 24).

The following loading doses were administered on Tuesdays and Thursdays of weeks 2 to 5: 0 mg of PN on Tuesday and Thursday of the second week; 2 mg of PN on Tuesday and Thursday of the third week; bananas , 633 g (1.51 mg vitamin B-6) on Tuesday and filberts, 333 g (2.12 mg vitamin B-6) on Thursday of the fourth week; soybeans, 330 g[4] (1.53 mg vitamin B-6) on Tuesday and beef bologna, 666 g (1.58 mg vitamin B-6) on Thursday of the fifth week. The preparation of these selected foods is given in table 3. The 2 mg crystalline PN dose was administered at breakfast. Since the test food doses were large in volume, the subjects consumed part of the "food dose" at breakfast and finished it as soon as they could during the rest of the day. They finished eating the bananas by lunch, and filberts by 5 o'clock in the afternoon; one-third of the

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[3]Prepared from pyridoxine-HCl and dissolved in 0.5 percent acetic acid (17 mg/ml). Five and two milliliter portions were frozen. They were thawed just before administering.

[4]The weight before cooking.

Table 3. Preparation of test foods

Food	Procedure
Bananas	1. Peel and remove the dark/mushy areas. 2. Cut the remainder into 2.5 cm slices. 3. Weigh 633 g of the slices into a container for each subject. 4. Add 10 ml of ascorbic acid solution (0.00134%) to the fruits in each container.
Filberts	1. Grind filberts. 2. Weigh out portions of 333 g filberts. 3. Add 150 ml of corn syrup and 150 ml of H_2O to each portion, then mix.
Soybeans	1. Place in each casserole 110 g (raw weight) of soybeans and 125 ml of H_2O. 2. Autoclave uncovered for 20 minutes at 102 kPa. 3. Store in refrigerator at 4°C. 4. Before serving, stir in 240 ml of H_2O and 1 Veg-ex[1] cube. 5. Heat at 176.5°C for 15 minutes, covered. 6. Serve one casserole at each meal.
Beef Bologna[2]	1. For every 11.4 kg of lean ground beef, mix in 8 oz of commercial sausage mix. The commercial mix contains: salt — monosodium glutamate dextrose — oil of sage red & white pepper — capsicuim sage — calcium phosphate 2. Serve 222 g of bologna at each meal.

[1]Distributed by Vegex Co., Division of Presco Food Products, Inc., Flemington, NJ 08822.

[2]The beef bologna was prepared by the Meat Science Lab., Dept. of Animal Science, OSU.

quantity of soybeans and beef bologna was consumed at each meal. The vitamin B-6 content of these foods[5] was determined by using Saccharomyces uvarum as the assay organism by A.O.A.C. method without the chromatography step (1980).

Urine and Blood Sample Collections

Complete 24-hr. urine collections were made throughout the study. On Thursday and Friday of week 1 and on Tuesdays and Thursdays of weeks 2 to 5, timed urine collections were made: one during the first 10 hr. (7:00 a.m. to 5:00 p.m.), the second for the remaining 14 hr. (5:00 p.m. to 7:00 a.m.). Urine was collected in bottles containing toluene and was refrigerated. After each 10-, 14- and 24-hr. urine collection, the urine was mixed and measured; portions were stored at -20°C until assayed.

Blood was collected on days 1 and 5 of the week 1 (adjustment period); on Wednesdays and Fridays of the remaining four weeks; and on the last day of the study. Blood (20 ml) was drawn from fasting subjects from the antecubital vein by a registered medical technologist[6] into evacuated tubes containing heparin. Plasma was

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[5]These assays were carried out by H. Kabirmeidanshah.

[6]Lynda Barstow, Foods and Nutrition Dept., OSU.

separated immediately from the cells and stored frozen (-40°C) until analyzed for total vitamin B-6.

Laboratory Analyses

The concentration of vitamin B-6 in urine and plasma was determined by microbiological assay with Saccharomyces uvarum (Miller and Edwards, 1981). Total vitamin B-6 was determined in hydrolyzed urine (5 ml of urine hydrolyzed with 25 ml of 0.1N HCl at 102 kPa for 30 minutes). Free vitamin B-6 was measured in unhydrolyzed urine (5 ml urine + 25 ml H_2O). Each sample was adjusted to pH 4.5 and diluted to 50 ml before assay. PN was used to prepare the standard curve. The mean percent recovery of 0.5 ug PN added to the urine was 95 $\pm$ 8%, ranging from 81 to 110% (n = 20). Plasma proteins were precipitated before assaying for plasma total vitamin B-6: 15 ml of 10% trichloroacetic acid were added to 3 ml of plasma to precipitate the protein. After centrifugation, the supernatant was autoclaved at 102 kPa for 30 minutes. Under these conditions PLP, the major form of vitamin B-6 in plasma, is hydrolyzed to PL (Sauberlich et al., 1972). PL was used to prepare the standard curve for the plasma total vitamin B-6 assay. Due to the limited volume of plasma sample available, no recovery of PL added to plasma was determined. All vitamin B-6 assays were conducted in

subdued light to minimize losses by photolysis.

Completeness of 24-hr. urine collections was determined by measuring urinary creatinine[7] by an automated modification of the Jaffe reaction (Pino et al., 1965). Bioavailability of vitamin B-6 from "food doses" was also assessed by the urinary 4-PA excretion[8] using the method of Reddy, Renolds and Price (1958). Erythrocyte aminotransferases[9] (EAspAT and EAlaAT) were assayed by using the method of Woodring and Storvick (1970). The data on urinary 4-PA (Gonzalez, 1983) and erythrocyte aminotransferases (Wang, 1982) are presented elsewhere.

Exercise Experiment

An exercise experiment was conducted on Friday of week 1 and Friday of week 5. Details and results will be presented elsewhere.

Calculation of Bioavailability

The vitamin B-6 bioavailability was measured by com-

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[7]Creatinine in urine was determined by Lynda Barstow on a Technicon Autoanalyzer (Technicon Corp., Tarrytown, NY)

[8]Performed by Patricia Gonzalez and Lynda Barstow.

[9]Performed by Ann-Gau Wang.

paring the urinary total vitamin B-6 levels after giving the test foods to the 24-hr. urinary excretions measured on the days of the 2-mg crystalline PN doses were in each subject. Since the test foods did not contain exactly 2 mg of vitamin B-6, the urinary excretion of this vitamin from each food was adjusted to be equivalent to 2 mg of vitamin B-6. The assumption was made that in this range of vitamin B-6 intake, the excretion of urinary vitamin B-6 is proportional to intake.

Calculations were based on the assumption that 2-mg crystalline PN administered on Tuesday and Thursday of week 3 is 100% bioavailable. The bioavailability of vitamin B-6 in a food fed as determined by urinary vitamin B-6 was calculated as follows:

$$\frac{(A - C) \times \dfrac{\text{2 mg of vitamin B-6}}{\text{vitamin B-6 in food}}}{B - C} \times 100\%$$

where A= the 24-hr. urinary excretion of vitamin B-6 on test food day.
B= the average 24-hr. urinary excretion of vitamin B-6 on the days of 2-mg PN dose (Tuesday and Thursday of week 3).
C= the average 24-hr. urinary excretion of vitamin B-6 on the days of 0-mg PN dose (Tuesday and Thursday of week 2).
B - C= yield from 2-mg PN dose in each individual.
A - C= yield of vitamin B-6 from a test food.

Statistical Analyses

The significance of weekly changes of urinary and plasma total vitamin B-6 levels was tested by the paired t-test. Total vitamin B-6 excretion during week 2 to week 5 in response to the crystalline PN doses and supplements (o mg, 2 mg and 5 mg) was analyzed by linear regression.

RESULTS

Urinary Excretion of Vitamin B-6

Fig. 2 presents the mean daily urinary excretion of total vitamin B-6 throughout this study. This figure also includes urinary 4-PA for comparison. Individual data of urinary vitamin B-6 are given in appendix table 2.

Urinary total vitamin B-6 decreased in all subjects during the adjustment period (week 1) when no PN supplement was given. On the first day of the adjustment period (4/21), the mean excretion of urinary total vitamin B-6 was 0.77 $\pm$ 0.12 μmol/24 hr., ranging from 0.67 to 0.93 μmol/24 hr. At the end of this period (4/25), the mean excretion of urinary total vitamin B-6 was 0.61 $\pm$ 0.13 μmol/24 hr., ranging from 0.48 to 0.79 μmol/24 hr. Comparing the mean urinary vitamin B-6 from week to week (fig. 3) on the days the five subjects received the 5-mg PN supplement, we observed that urinary total vitamin B-6 increased and reached a plateau at the beginning of the third week (5/5, after two weeks of PN supplement) and remained relatively constant thereafter. Urinary total vitamin B-6 increased significantly ($p<0.01$) from week to week until week 3.

Table 4 shows the average urinary total vitamin B-6

Fig. 2 Comparison of the mean daily excretion patterns of urinary total vitamin B-6 and 4-PA in subjects receiving oral supplement of PN as well as the loading doses of crystalline PN and test foods.

The subjects received a constant diet containing 1.34 mg of vitamin B-6 and no PN supplement during the adjustment period (April 21 to April 25, day 1 to day 5 of week 1).

Starting on day 6 of week 1 (April 26) until day 5 of week 5 (May 23), the subjects received daily, except on Tuesdays and Thursdays, 5 mg of PN at breakfast in addition to the constant diet containing 1.34 mg of vitamin B-6.

[1]0 mg PN dose
[2]2 mg PN dose
[3]1.51 mg vitamin B-6 dose from bananas
[4]2.12 mg vitamin B-6 dose from filberts
[5]1.53 mg vitamin B-6 dose from soybeans
[6]1.58 mg vitamin B-6 dose from beef bologna

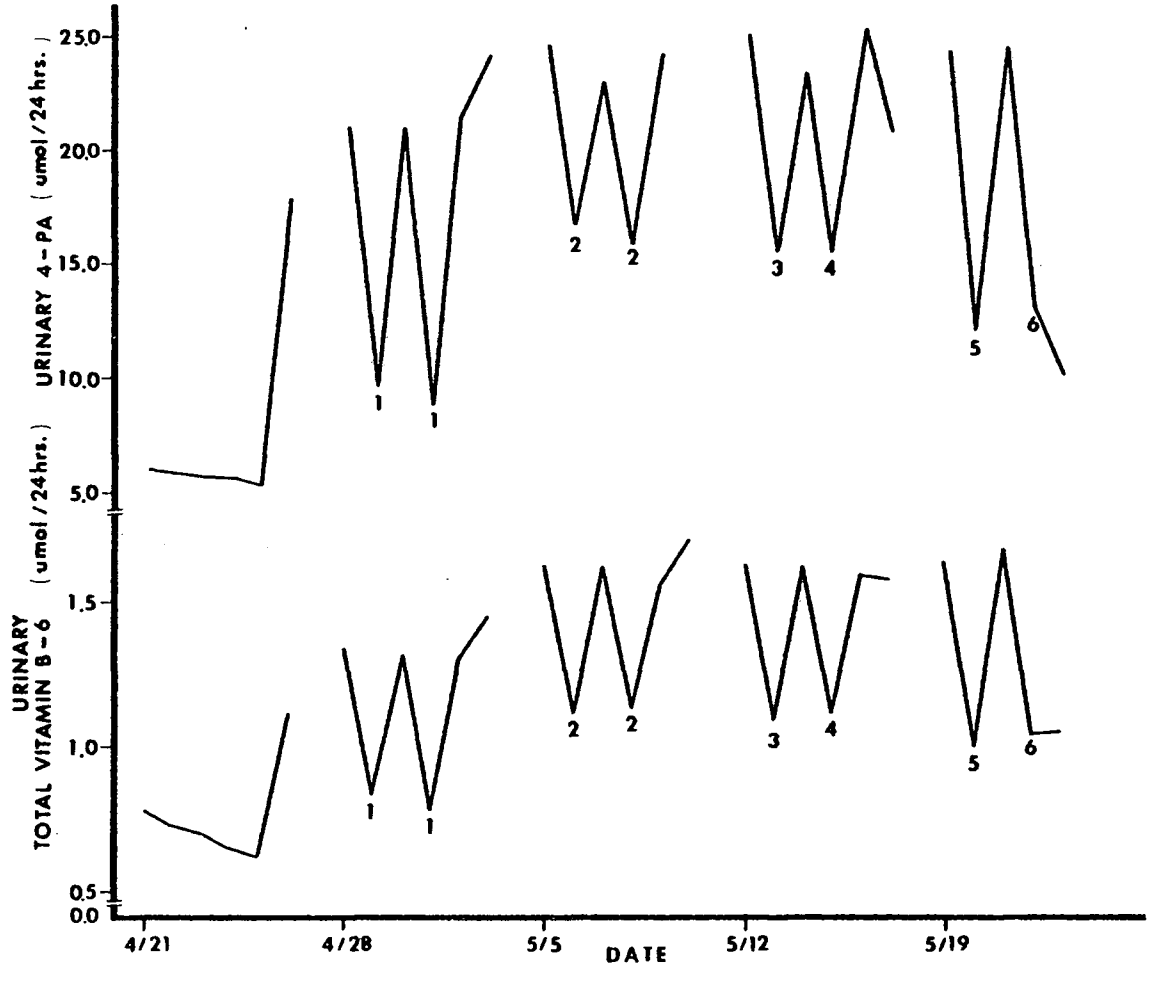
URINARY 4-PA (umol/24 hrs.)
25.0
20.0
15.0
10.0
5.0
URINARY TOTAL VITAMIN B-6 (umol/24hrs.)
1.5
1.0
0.5
0.0
1
1
2
2
3
4
5
6
4/21
4/28
5/5
5/12
5/19
DATE

Fig. 2

Fig. 3 Effect of 5 mg PN supplementation on the mean weekly changes in urinary total vitamin B-6, urinary 4-PA and plasma total vitamin B-6 (means $\pm$ S.D.)

The mean values for week 1 were taken from the day 5 of week 1 (the end of adjustment period) for urinary vitamin B-6, 4-PA and plasma vitamin B-6. Starting on day 6 of week 1 until day 5 of week 5, the subjects received daily, except on Tuesdays and Thursdays, 5 mg of PN at breakfast.

The mean values for urinary total vitamin B-6 and 4-PA for weeks 2 to 4 were taken from the average of Monday, Wednesday and Friday of the five subjects; the means for week 5 were from the average of Monday and Wednesday of the five subjects.

The mean value of plasma total vitamin B-6 was from the average of Wednesday and Friday of the five subjects for weeks 2 to 5.

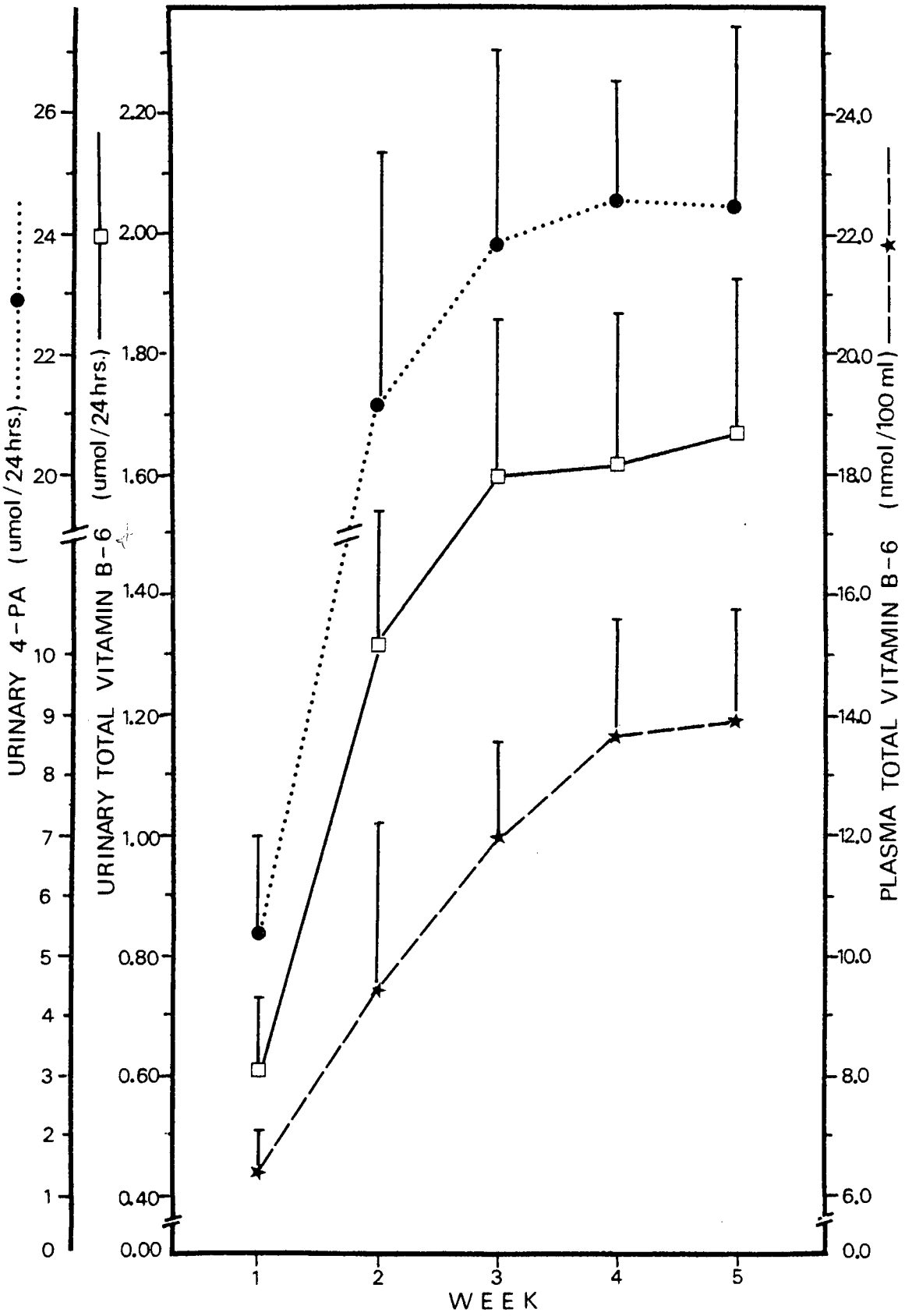
URINARY 4-PA (umol/24 hrs.)
URINARY TOTAL VITAMIN B-6 (umol/24 hrs.)
PLASMA TOTAL VITAMIN B-6 (nmol/100 ml)
WEEK
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
20
22
24
26
0.00
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
0.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0

Fig. 3

Table 4. Average urinary total vitamin B-6 and 4-PA and percent recoveries of total vitamin B-6 intake in response to the PN dose and supplement in the five subjects[1].

PN Dose (mg)	Total vitamin B-6 intake[2] (μmol/day)	Urinary total vitamin B-6 (μmol/day)	Recovery as B-6 (%)	Urinary 4-PA (μmol/day)	Recovery as 4-PA (%)	Urinary B-6 and 4-PA (μmol/day)	Recovery as B-6 and 4-PA (%)
0	7.9	0.7 ± 0.1[3]	8.9 ± 1.6	5.8 ± 1.3	73.4 ± 16.5	6.4 ± 1.4	81.0 ± 17.7
0	7.9	0.8 ± 0.1[4]	10.1 ± 1.3	9.3 ± 1.5	117.7 ± 19.0	10.1 ± 1.5	127.8 ± 19.0
2	19.7	1.1 ± 0.1[5]	5.6 ± 0.5	16.2 ± 2.2	82.2 ± 11.2	17.3 ± 2.1	87.8 ± 10.7
5	37.5	1.6 ± 0.3[6]	4.3 ± 0.8	24.4 ± 2.7	65.1 ± 7.2	26.0 ± 2.5	69.3 ± 6.7

[1]Mean ± S.D. for all the values.
[2]The amount of vitamin B-6 from PN dose or supplement, and constant diet, which supplied 7.9 μmol/day.
[3]Average of 24-hr. urinary excretion of week 1 when 0 mg PN dose was given.
[4]Average of 24-hr. urinary excretion on Tuesday and Thursday of week 2 when 0 mg PN dose was given. Daily 5 mg PN supplement was started on day 6 of week 1.
[5]Average of 24-hr. urinary excretion on Tuesday and Thursday of week 3 when 2 mg PN dose was given.
[6]Average of 24-hr. urinary excretion on Mondays, Wednesdays and Fridays of week 3, 4 and 5 when 5 mg PN was given (except Friday of week 5).

and 4-PA excretions and percent recoveries of vitamin B-6 intake. The mean total vitamin B-6 excretion of the five subjects was 0.8 ± 0.1 μmol/24 hr. after 0-mg PN dose (on Tuesday and Thursday of week 2, after having 5 mg PN supplement for 3 days); 1.1 ± 0.1 μmol/24 hr. following 2-mg PN doses (on Tuesday and Thursday of week 3), and 1.6 ± 0.3 μmol/24 hr. in response to 5 mg of PN supplement on Mondays, Wednesdays and Fridays of the last three weeks. These changes were statistically significant ($p<0.01$). The percent of total vitamin B-6 recovered as urinary total vitamin B-6 decreased from 5.6 (intake of 3.34 mg or 19.7 μmol vitamin B-6) to 4.3 (total intake of 6.34 mg or 37.5 μmol vitamin B-6). The regression line calculated from the urinary total vitamin B-6 excretion of each subject as the result of crystalline PN dose and supplement feeding is shown in fig. 4. The correlation coefficient of this line is 0.8394.

Fig. 5 shows that the urinary free vitamin B-6 comprises two-thirds or slightly more of the urinary total vitamin B-6 during the adjustment period (mean, 67 ± 7%) and the PN supplement period (means, 72 ± 7 to 75 ± 7%). There were no significant differences in the percentage of free/total vitamin B-6 excretion among the days of 0-mg dose (with or without PN supplement), 2-mg PN dose and 5-mg PN supplement. Individual data for 24-hr. free

Fig. 4 Urinary total vitamin B-6 excretion in response to the crystalline PN doses and supplements.

Five-milligram PN was given in addition to the constant diet containing 1.34 mg of vitamin B-6. Data points for 0-mg PN were taken from Tuesday and Thursday of week 2 (total vitamin B-6 intake was 1.34 mg, subject had received 5-mg PN daily for 3 days); for 2-mg PN were taken from Tuesday and Thursday of week 3 (total vitamin B-6 intake was 3.34 mg); for 5-mg PN were taken from Mondays, Wednesdays and Fridays of weeks 3 to 5 (Friday of week 5 was not included).

The regression line was calculated by the least-squares method. Each dot represents a value from a subject at each level of PN.

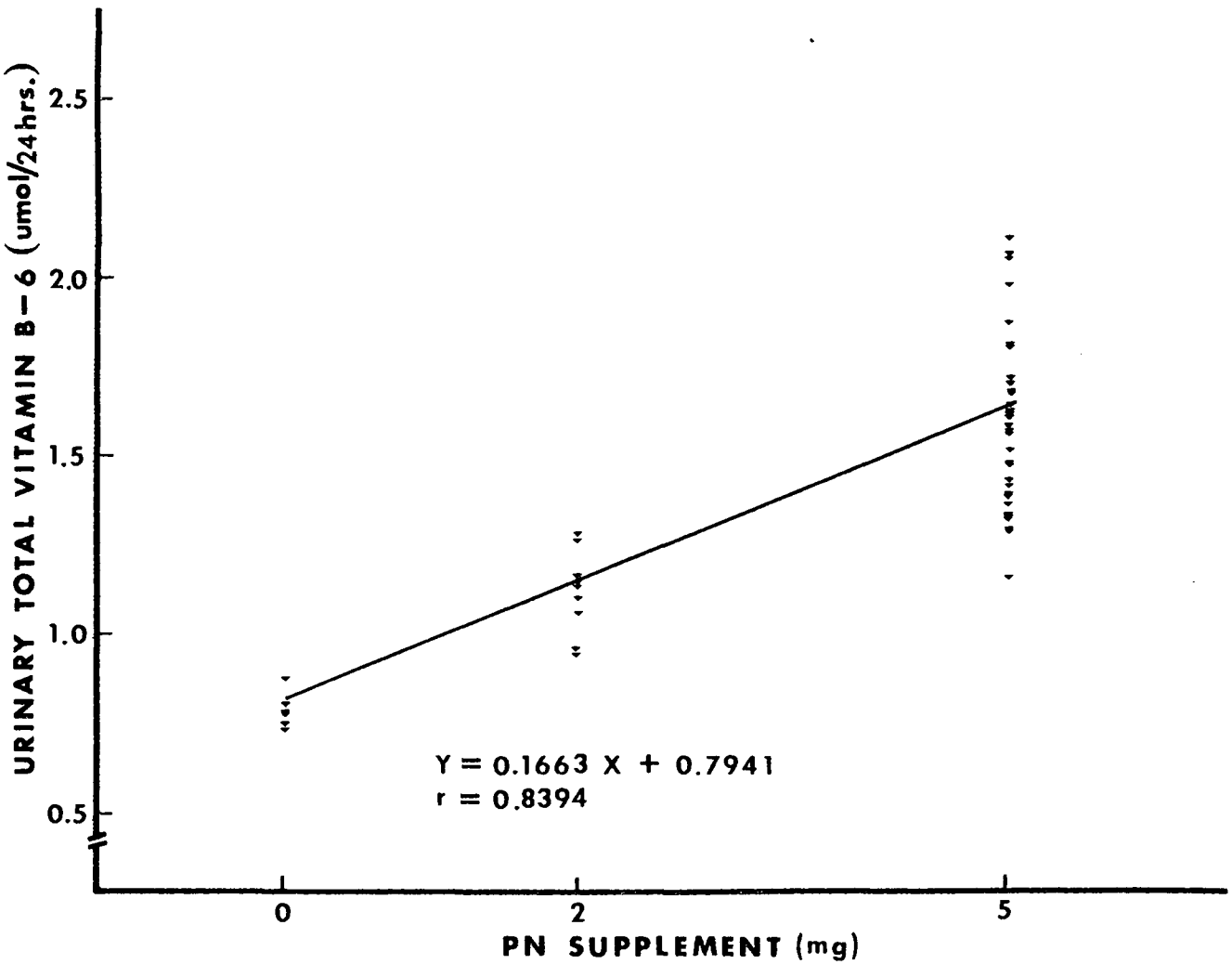
URINARY TOTAL VITAMIN B-6 (umol/24hrs.)
2.5
2.0
1.5
1.0
0.5
Y = 0.1663 X + 0.7941
r = 0.8394
0
2
5
PN SUPPLEMENT (mg)

Fig. 4

Fig. 5 Ratio of the 24-hr. urinary free vitamin B-6 to total vitamin B-6. Subjects receiving different levels of PN in addition to the constant diet. Values for mean ± S.D. for total vitamin B-6 excretion, free vitamin B-6 excretion. Percentages of free/total vitamin B-6 excretion are also given.

Bound vitamin B-6 was released by acid hydrolysis and was calculated by substracting the free vitamin B-6 from total vitamin B-6.

Constant diet containing 1.34 mg of vitamin B-6 was served Monday through Friday of each week.

[1]Average of the urinary vitamin B-6 excretion from the five subjects during the adjustment period (day 1 to day 5; constant diet only).
[2]Average of urinary vitamin B-6 excretion from the five subjects on Tuesday and Thursday of week 2 when 0-mg PN dose was given and subject had received 5 mg PN, starting day 6 of week 1.

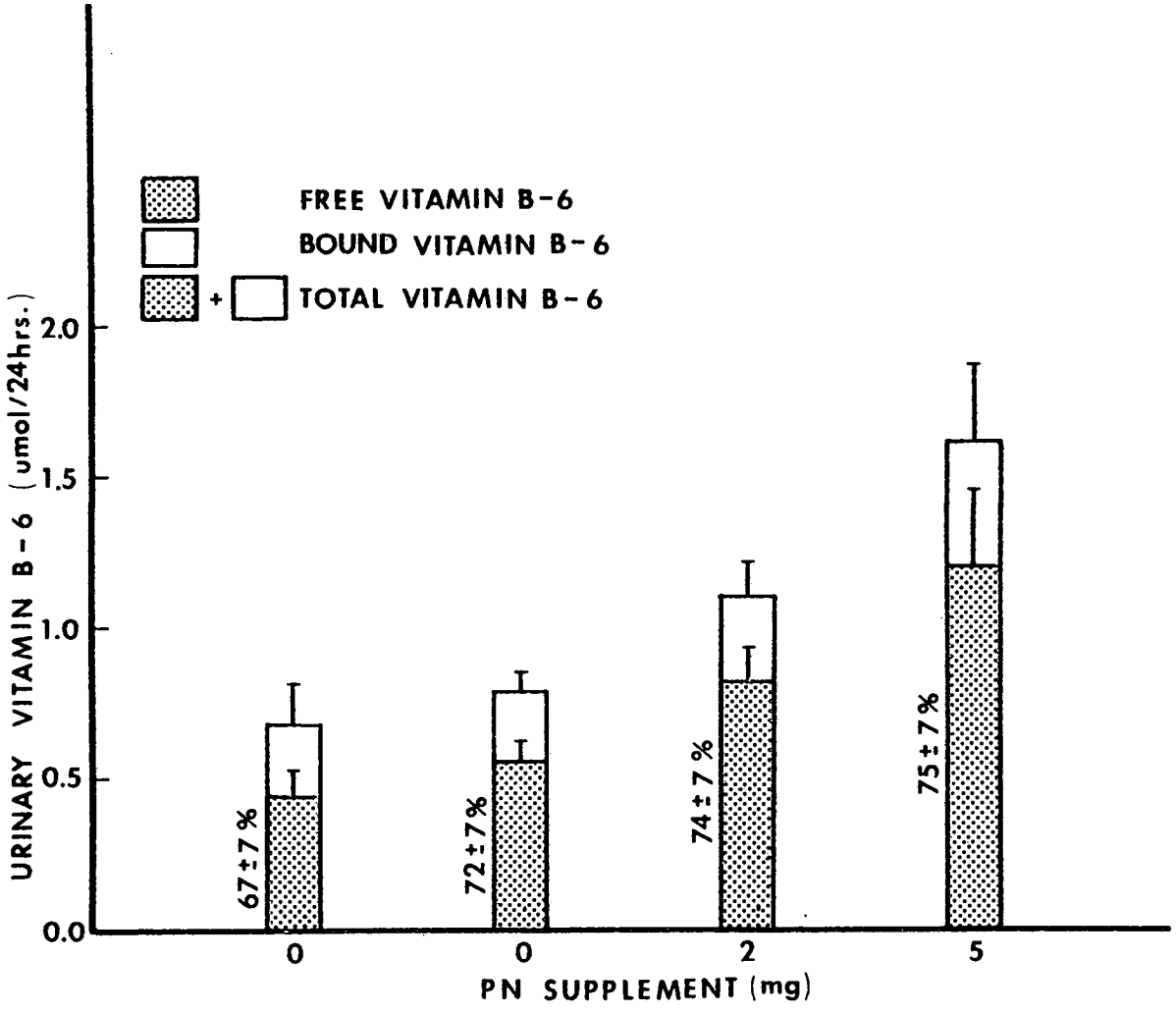
FREE VITAMIN B-6
BOUND VITAMIN B-6
+ TOTAL VITAMIN B-6
URINARY VITAMIN B-6 (umol/24hrs.)
2.0
1.5
1.0
0.5
0.0
67±7%
72±7%
74±7%
75±7%
0
0
2
5
PN SUPPLEMENT (mg)

Fig. 5

vitamin B-6 excretion and the ratio of 24-hr. free vitamin B-6 to total vitamin B-6 excretion are present in appendix tables 3 and 4, respectively.

Total Vitamin B-6 in Timed Urine Collection

The total vitamin B-6 excretion in 10-hr. and 14-hr. timed urine samples in response to the loading doses is shown in table 5. When the subjects received no PN dose, they excreted more total vitamin B-6 in 14-hr. urine than in the 10-hr. one. In response to 2 mg of crystalline PN dose, the mean total vitamin B-6 in the 14-hr. urine collection was higher than in the preceding 10-hr. one due to subject 2 on May 8 when he excreted more vitamin B-6 in the 10-hr. urine collection than the 14-hr. one. When the values from subject 2 are not included, the mean vitamin B-6 excretion in 10-hr. urine (0.58 ± 0.08 μmol/24 hr.) was comparable to that in the 14-hr. urine (0.55 ± 0.06 μmol/24 hr.). However, the 10-hr. yield of urinary vitamin B-6 was greater than the 14-hr. one in four of the five subjects (except subject 2) (fig. 6 and table 6).

On the food test dose days, the mean excretion of total vitamin B-6 in 14-hr. urine was greater than those in 10-hr. urine. Since the subjects were not able to consume the large amount of foods at one meal, the bio-

Table 5 Urinary total vitamin B-6 in 10-hr. and 14-hr. timed urine samples in response to the loading doses

			Food Doses[3]			
	0-mg PN[1]	2-mg PN[2]	Bananas	Filberts	Soybeans	Beef Bologna
	← (umol) →					
10-hr.	0.35±0.03[4]	0.55±0.08 (0.58±0.08)[5]	0.48±0.13	0.46±0.13	0.42±0.05	0.44±0.06
14-hr.	0.46±0.03	0.58±0.08 (0.55±0.06)	0.61±0.11	0.67±0.05	0.57±0.08	0.60±0.07

[1]Average of the means of total vitamin B-6 excretion for each subject on Tuesday and Thursday of the second week.

[2]Average of the means of total vitamin B-6 excretion for each subject on Tuesday and Thursday of the third week.

[3]Bananas contain 1.51 mg vitamin B-6; filberts, 2.12 mg vitamin B-6; soybeans, 1.53 mg vitamin B-6; beef bologna, 1.58 mg vitamin B-6.

[4]Mean±Standard Deviation.

[5]Average of the means of total vitamin B-6 excretion from 4 subjects on Tuesday and Thursday of the third week, subject 2 was not included.

Fig. 6 Yields of urinary total vitamin B-6 from 10-hr. and 14-hr. urine in response to 2-mg crystalline PN supplement.

The yield was determined by substracting the subject's mean 10-hr. and 14-hr. excretion of total vitamin B-6 when 0 mg of PN was administered (Tuesday and Thursday of week 2) from the subject's mean 10-hr. and 14-hr. vitamin B-6, respectively, of the days 2 mg of PN were administered (Tuesday and Thursday of week 3).

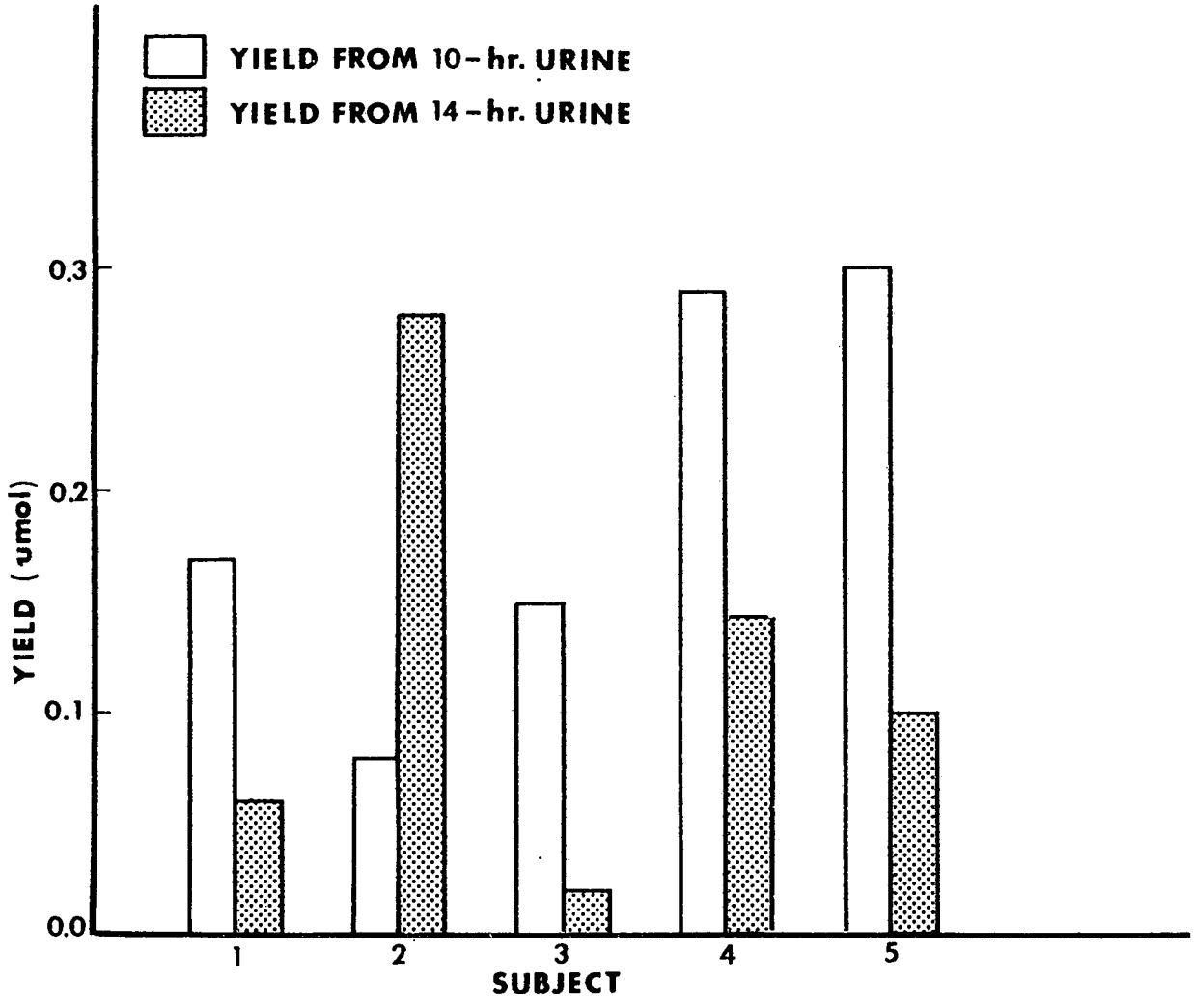
YIELD FROM 10-hr. URINE
YIELD FROM 14-hr. URINE
0.3
0.2
0.1
0.0
YIELD (umol)
1
2
3
4
5
SUBJECT

Fig. 6

Table 6 Yields of urinary total vitamin B-6 from 2 mg PN dose.

Urine	Yield (μmol)[1] subject 1	2	3	4	5	Mean+S.D.
10-hr	0.17	0.08 (0.18)[2]	0.15	0.29	0.30	0.20±0.09
14-hr	0.06	0.28 (0.18)	0.02	0.14	0.10	0.12±0.10
24-hr	0.23	0.36 (0.36)	0.17	0.43	0.40	0.32±0.11 (0.32±0.11)

[1]Means of 2 values for each subject; the yield of total vitamin B-6 from 2 mg of PN was measured by substracting the subject's mean 10-hr., 14-hr. and 24-hr. excretions of vitamin B-6 when 0 mg PN was given from the subject's mean 10-hr., 14-hr. and 24-hr. vitamin B-6, respectively, of the days 2 mg of PN were administered (Tuesday and Thursday of week 3).

[2]The datum from 5/8 (Thursday of week 3) was not included.

availability of vitamin B-6 in foods was calculated based on the yield of 2 mg PN from 24-hr. urine sample. As shown in table 6, the average yield from 2 mg of PN dose was 0.32 ± 0.11 umol/24 hr., ranging from 0.17 to 0.43 umol/24 hr.

Bioavailability Determined by Urinary Total Vitamin B-6

The bioavailability of vitamin B-6 from the four foods tested is given in table 7. The average bioavailability for bananas was 115 ± 32% and that of filberts, soybeans and beef bologna was 93 ± 8%, 73 ± 20% and 87 ± 7%, respectively. The large variation among the subjects in their ability to utilize vitamin B-6 in these foods is suggested from the data.

Plasma Total Vitamin B-6

Plasma total vitamin B-6 was measured on Wednesdays and Fridays of each week during the study; the weekly means of plasma vitamin B-6 are shown in fig. 3. The initial mean of plasma total vitamin B-6 status from five subjects was 6.40 ± 0.70 nmol/100 ml (appendix table 5). Plasma total vitamin B-6 increased significantly ($p<0.05$) from week to week until week 3 when it stablized.

Table 7 Bioavailability of vitamin B-6 from four test foods.

Food	Amount of food dose	Food vitamin B-6 content	Bioavailability[1] Subject 1	2	3	4	5	Mean±S.D.
	(g)	(mg)	← (%) →					(%)
Bananas	633	1.51	126	72	100	120	159	115±32
Filberts	333	2.12	98	84	94	103	85	93± 8
Soybeans	330[2]	1.53	-[3]	78	46	95	72	73±20
Beef Bologna	666	1.58	81	86	82	98	91	87± 7

[1]Calculation was based on the assumption that the 2-mg crystalline PN dose was 100% available for each subject. The bioavailability of vitamin B-6 from each food was adjusted to be equivalent to 2 mg of vitamin B-6.

[2]Weight before cooking.

[3]The bioavailability of vitamin B-6 from soybeans in subject 1 was not available due to an incomplete urine collection on Tuesday of week 5 (May 20).

DISCUSSION

Vitamin B-6 in the Urine

According to Sauberlich et al. (1972), urinary vitamin reflects a subject's recent dietary intake of the vitamin. The results of this study show that there was a carryover effect of the subjects' preceding diet on vitamin B-6 excretion. The gradual decrease in urinary total vitamin B-6 observed during the adjustment period (week 1) (fig. 2), suggests that the level of vitamin B-6 in the constant diet was below that of the self-chosen diets ingested by the subjects before participating in the study. The constant diet contained 1.34 mg of vitamin B-6, which is below the recommended dietary allowances (NAS, 1980) for adult males (2.2 mg/day). The vitamin B-6 content of the average American diet, as calculated by Tarr et al. (1981), is 2.3 mg/day.

The carryover effect of the subjects' preceding vitamin B-6 intake on urinary vitamin B-6 was more dramatic on Tuesday and Thursday of week 2. On these two days, the subjects' mean urinary vitamin B-6 was higher (0.8 umol/24 hr.) than during the preceding week (0.7 umol/24 hr.). This result was expected because of the 5-mg PN supplement administered daily during the preceding weekend and the following Monday and Wednesday. A higher

urinary total vitamin B-6 would have been expected on the days when 0 mg PN dose was given (week 2) if the subjects' urinary vitamin B-6 had been stablized already at the beginning of the second week (fig. 3). The subjects did not excrete a constant elevated amount of urinary vitamin B-6 on the days they received the 5-mg PN supplement until the beginning of week 3 (fig. 3).

As expected, the PN-supplemented subjects excreted more urinary vitamin B-6 after the 2-mg PN dose than after the 0-mg one. Wozenski et al. (1980) observed that urinary vitamin B-6 increased in response to increasing PN doses in subjects receiving a diet containing 1.5 mg of vitamin B-6. During the 5-mg PN supplementation period, the subjects' urinary excretion in response to 0-mg, 2-mg PN doses and 5-mg PN supplement was linear (fig. 4).

On day 6, the first day 5 mg of PN were given with the constant diet, the amount of urinary total vitamin B-6 expressed as a percentage of intake changed from 7.6% on day 5 (the end of adjustment period) to 2.9%. After the subjects had received the 5-mg PN supplement for a longer period of time, the percent of the vitamin B-6 intake excreted as total vitamin B-6 increased to 4.3% (table 4). Additionally, a lower percentage of the vitamin B-6 intake was excreted as urinary vitamin B-6

during the 5-mg PN supplement than during the first week of the study. This suggests that as PN supplementation continued, more of the dose was converted to 4-PA (Gonzalez, 1983), retained in the body, or converted to other unidentified products not measured by the microbiological assay method (Sauberlich et al., 1972).

Woodring and Storvick (1970) observed that the urinary excretion of free vitamin B-6 increased and correlated closely with vitamin B-6 intake when a 50-mg PN supplement was given to their subjects. The 5-mg PN supplement in the present study seemed to have no effect on the free/total vitamin B-6 excretion ratio (fig. 5). The ratio increased only slightly, from 67% of the adjustment period to 72% of the 0-mg PN dose in the second week after the 5-mg PN supplement during the first weekend as well as the following Monday and Wednesday.

Correlation Between Urinary Total Vitamin B-6 and 4-PA

Similar urinary excretion patterns of 4-PA and total vitamin B-6 were observed in this study: the amounts excreted decreased gradually during the adjustment period and paralleled the doses of crystalline PN and test foods in PN-supplemented subjects (fig. 2). In other studies, urinary 4-PA and vitamin B-6 were significantly correlated

to vitamin B-6 intake (Baysal et al., 1966; Kelsay et al., 1968; Lewis and Nunn, 1977). The urinary excretion of 4-PA, in some cases after a loading dose of PN, has been used to assess vitamin B-6 status (Shane and Contractor, 1980). The excretion of total vitamin B-6 and 4-PA in response to the 5-mg PN supplement was stable by week 3, after two weeks of PN supplementation (fig. 3).

Urinary 4-PA accounted for a greater proportion of the PN doses (table 4), an observation also made by Wozenski et al. (1980). The relationship between the percentage recovery of 4-PA and the size of PN doses showed the same trend as urinary total vitamin B-6 (table 4). As vitamin B-6 intake increased from 7.9 umol to 37.5 umol, the percent of this dietary intake recovered as 4-PA and vitamin B-6 combined decreased from 81 to 69.

The mean 4-PA excretion and the percentage recovery of 4-PA following the PN doses were higher in this study than those in other reports (Johansson et al., 1966; Donald et al., 1971; Lewis et al., 1977; Wozenski et al., 1980). Johansson et al. (1966),who conducted a study on the metabolism of labelled PN in man, reported that only 15 to 20% of the administered isotope were excreted within the first day. From their data, Johansson et al. suggested a two-compartment model for estimating the overall metabolism of PN in human subject. The urinary 4-PA

originating from the reservoir compartment was constantly diluted with 4-PA originating from dietary PN. Lumeng, Liu and Li (1980) observed that 4-PA was a metabolite of recently administered vitamin B-6 and this newly formed 4-PA did not freely exchange with endogenous B-6 vitamers. Brain and Booth (1964) found that within 24 hours, the percentage excretion of a 1-mg oral dose of ^{3}H-PN increased from 17% to 50% if given simultaneously with 100 mg of unlabelled PN dose. The higher urinary excretion of 4-PA in the present study is most likely due to the PN supplement which the subjects received almost daily and the resultant carryover effect from the supplement.

Excretion of Total Vitamin B-6 In Response To 2-mg PN Dose

In response to both the crystalline PN and food doses, more total vitamin B-6 was excreted during the 14-hr. urine collection than the 10-hr. one (table 5). This was due to the higher vitamin B-6 content (0.78 mg) of dinner in contrast to 0.56 mg of vitamin B-6 total from breakfast and lunch. On the food test dose days, no expected excretion pattern was noticed because it was impossible for the subjects to consume the entire food test dose at breakfast. Due to the individual variability in absorption and meta-

bolism of vitamin B-6, each subject served as his own control in this study. The yield of 2 mg PN dose from 10-hr. urine was higher than that from 14-hr. urine in four out of the five subjects (fig. 5 and table 6). Wozenski et al. (1980) reported that the rate of urinary vitamin excretion was maximal during the first three hours after the PN doses.

Bioavailability of Vitamin B-6 from Selected Foods

For the water-soluble vitamins (except vitamin B-12), the concentration of the vitamins or their metabolites in urine are commonly used in assessing bioavailability (Bauernfeind and Miller, 1978). In the present study, levels of urinary total vitamin B-6 in PN-supplemented subject were used to assess the bioavailability of vitamin B-6 in selected foods. Tarr and coworkers (1981) felt that urinary vitamin B-6 was a reliable indicator of vitamin B-6 bioavailability in humans.

According to Melnick et al. (1945), the technique for determining bioavailability of water-soluble vitamins in humans involves the comparison between the urinary excretion of the vitamin or its metabolite in response to a known amount of the vitamin in the test material and the crystalline vitamin. Since PN is absorbed readily

from the intestinal tract by humans (Bauernfeind and Miller, 1978), we considered the bioavailability of pure PN to be 100%. In order to have the relatively uniform excretion pattern from the subjects, we supplemented them with the vitamin prior to the bioavailability experiments. Tamura and Stokstad (1973) used a similar technique to study the bioavailability of food folate in man. In the present study, the assumption was made that in PN-supplemented subjects urinary vitamin B-6 excreted in response to a food dose would reflect the bioavailability of this vitamin.

The wide range of bioavailability from each food observed in the present study (table 7) suggests individual variability in the digestion, absorption and metabolism of vitamin B-6 from each food dose. For example, there was a two-fold difference in the bioavailability of vitamin B-6 from bananas and soybeans. Even among the subjects, the bioavailability of vitamin B-6 from the different foods did not show the same trend. Subject 5, for example, showed the highest bioavailability of vitamin B-6 from bananas and the lowest (with subject 2) from filberts. Among the four foods tested, the bioavailability of vitamin B-6 was the highest from bananas and, in descending order, from filberts, beef bologna and soybeans.

The bioavailability of vitamin B-6 in bananas was

greater than or equal to 100% for four of the five subjects (table 7). One possible reason for this high bioavailability of vitamin B-6 is that the carbohydrate in bananas may affect the absorption of vitamin B-6. The estimated amount of carbohydrate in the banana dose was 140 g (Watt and Merrill, 1963). Nelson et al. (1976) observed that the absorption of vitamin B-6 from a synthetic glucose-saline mixture exceeded that from a synthetic saline solution. The amount of vitamin C added to the banana dose to prevent oxidative browning was small (15 mg) in the present study, it had no effect on vitamin B-6 bioavailability. Shultz and Leklem (1982) reported that the percentage of total vitamin B-6 excretion remained unchanged when the subjects were given 0.5 g or 1 g of vitamin C orally with 2 mg of PN-HCl. Possibly other substances in bananas may have increased the bioavailability of vitamin B-6. Another possible explanation is that the subjects finished eating 633 g of bananas by lunch on the experiment day. To have such a large quantity of food in this short period of time may have resulted in a lower vitamin B-6 retention than from food test doses that were divided among the three meals. Possibly, if the banana dose had been divided among the three meals, less vitamin B-6 would have been excreted, and consequently vitamin B-6 bioavailability as measured by this

technique, might have been lower. An additional explanation for the apparently higher bioavailability of vitamin B-6 from bananas includes their high digestibility.

The mean bioavailability (93 ± 8%) of vitamin B-6 in filberts was also high. The subjects consumed the 333 g of filberts in small amounts throughout the day. This may have contributed to the high bioavailability of vitamin B-6 from filberts if by comparison the difference in vitamin retention level did affect the food vitamin B-6 bioavailability. Since the fat content of filbert dose was high (208 g; Watt and Merrill, 1963), the time needed for food to pass through the digestive tract may have been prolonged, which may have enhanced the amount of vitamin B-6 absorbed by the intestinal tract. In addition, the added syrup and carbohydrate content of the filbert dose (56 g, total; Watt and Merrill, 1963) may partly account for the high bioavailability (Nelson et al., 1976). The high fiber content of the filberts (10 g crude fiber; Watt and Merrill, 1963) apparently did not affect the bioavailability of vitamin B-6.

The average vitamin B-6 bioavailability for soybeans was 73 ± 20%, ranging from 46 to 95% (table 7). In the present study, soybeans were autoclaved for 20 minutes at 102 kPa and reheated in an oven at 176.5°C for 15 minutes (table 3). Thermal processing may favor the formation of

the less biologically active pyridoxylamino compounds (Gregory and Kirk, 1977). The distribution of PL, PN and PM in soybeans is 44%, 44% and 12%, respectively (Orr, 1969). On the other hand, cooking increases the digestibility and protein utilization of soybeans (Wolf, 1978) and hence possible vitamin B-6 bioavailability.

Vitamin B-6 bioavailability in beef bologna was higher than that in the soybeans (table 7), which is in agreement with a previous report by Leklem et al. (1980). Part of this difference in bioavailability may be accounted for by the differences in digestibility and fat content between these two foods. Animal protein is more digestible than plant protein. This could partly explain the differences in bioavailability of vitamin B-6 from soybeans and beef, but not from beef and filberts. Compared to the soybeans, beef bologna contained about 10 times more fat (200g vs. 19 g; Watt and Merrill, 1963), which could have slowed the passage of beef through the digestive tract. The fiber, which had been reported to exert a weak inhibitory effect on bioavailability of foods (Leklem et al., 1980; Leklem, Shultz and Miller, 1980) was not present in the beef. The soluble carbohydrate in soybeans probably did not contribute to increased vitamin B-6 bioavailability, since the principle oligo-

saccharides in soybeans in addition to sucrose, raffinose and stachyose, are not digested in the small intestine (Smith and Circle, 1978). In addition, the ability to digest such a large volume of soybeans could be low and varied under these physiological conditions.

From the results of this study, the filberts showed higher vitamin B-6 bioavailability than beef. The total fat content in filberts and beef bologna was comparable (208 g vs. 200 g; Watt and Merrill, 1963). Filberts contain fiber which beef does not. Fiber in this situation may not have exerted an inhibitory effect on vitamin B-6 bioavailability. Nguyen et al. (1981) observed that there was no relationship between fiber and the reduction of bioavailability of vitamin B-6 in vivo study in chicks and rats.

In the present study, the bioavailability of vitamin B-6 from the test foods as determined by urinary 4-PA showed the similar trend among foods as determined by the urinary vitamin B-6 (Gonzalez, 1983), but the differences in bioavailability among those foods were less variable when assessed by urinary vitamin B-6 due to the lower excretion of urinary vitamin B-6 in relation to 4-PA (fig. 2). In some of the vitamin B-6 bioavailability studies, there was no change in urinary vitamin B-6 when the subjects received daily a constant diet

containing 1.5 mg vitamin B-6 and no PN supplement, and the difference in bioavailability from foods was determined by urinary 4-PA and fecal vitamin B-6 (Leklem et al., 1980).

Relationships Among Urinary and Plasma Total Vitamin B-6 and Erythrocyte Aminotransferase Activities

Plasma total vitamin B-6, which includes both free vitamin B-6 and PLP, is a more sensitive indicator of the absorption of oral doses of crystalline vitamin B-6 than plasma PLP (Wozenski et al., 1980). It measures not only the vitamin B-6 which was metabolized, but also the portion of test dose which had been absorbed and not yet metabolized or excreted.

In our experiment, the mean level of plasma total vitamin B-6 decreased from 7.35 $\pm$ 1.18 nmol/100 ml on day 1 to 6.40 $\pm$ 0.70 nmol/100 ml on day 5 of the adjustment period (appendix table 5). These values closely parallel those of the urinary total vitamin B-6 (fig. 3) as well as 4-PA (Gonzalez, 1983) during this period.

PLP constitutes more than 50% of the vitamin B-6 in human blood plasma (Li and Lumeng, 1981; Miller and Edwards, 1981). Contractor and Shane (1968) noted the elevated plasma vitamin B-6 compounds in subjects who had taken 100 mg of PN-HCl daily for 4 days. In a recent study (Lumeng et al., 1980), the vitamin B-6 compounds in the

plasma of 6 healthy human subjects were measured before and after the oral administration of 100 mg PN-HCl for 1 to 3 weeks. After PN supplementation, the plasma concentrations of PLP and PL increased five-fold or more, whereas little or no change occurred in the concentrations of PM, PN and PMP. Lumeng et al. (1974) found that plasma PLP reached the plateau within 4 days after oral supplementation with 25 mg of PN daily. In the present study, the plasma total vitamin B-6 had doubled after 3 weeks of 5 mg PN supplementation (fig. 3). This relatively slow and modest increase is most likely due to smaller PN supplement.

Comparing the levels of urinary and plasma total vitamin B-6 in response to the 5-mg PN supplement, we noticed that the urinary total vitamin B-6 and 4-PA plateaued one week earlier than plasma total vitamin B-6. This suggested that the storage of vitamin B-6 in red blood cells and muscle phosphorylase might still be continuing while the mechanism governing urinary excretion of total vitamin B-6 and 4-PA had stablized. No comparison between urinary and plasma vitamin B-6 had been made in other supplement studies.

Both basal and PLP-stimulated erythrocyte aminotransferase activities increased and percent stimulation by added PLP decreased after only three days of PN supple-

mentation and continued throughout the 4 weeks of 5-mg PN supplementation (Wang, 1982). In spite of the approximately equal distribution of PLP between plasma and erythrocytes in normal subjects studied by Bhagavan et al. (1975), erythrocyte PLP levels increased faster than plasma levels after PN loading. In view of the increasing erythrocyte aminotransferase activities, Wang (1982) suggested that the level of erythrocyte PLP was increasing throughout the 5-mg PN supplementation period. From the increased basal and PLP-stimulated activities of erythrocyte aminotransferase, Wang also speculated that erythrocyte aminotransferase may be another PLP-binding protein in addition to hemoglobin in erythrocytes. The binding of PLP to erythrocyte apoaminotransferases may serve as another reservoir for vitamin B-6 in the body. Although plasma vitamin B-6 had reached a plateau by week 4, aspartate aminotransferase and pyruvate aminotransferase activities were still rising (fig. 7). Wang suggested that after plasma vitamin B-6 had reached a plateau, red blood cells were still continuing to pick up PN and converting it to PLP,which in turn enhanced the stability of these enzymes. In a recent investigation, Fonda and Harker (1982) who incubated ^{3}H-PN with isolated erythrocytes observed that 80% of the radioactivity was attached to hemoglobin. In subjects taking a PN supplement, how-

Fig. 7. The effect of PN supplementation on the mean basal and PLP-stimulated activities of pyruvate aminotransferase (represented by ——— and ----, respectively), of asparate aminotransferase (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 subjects received 5 mg PN daily, except on Tuesday and Thursday 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 regression equations.
(revised from Wang, 1982)

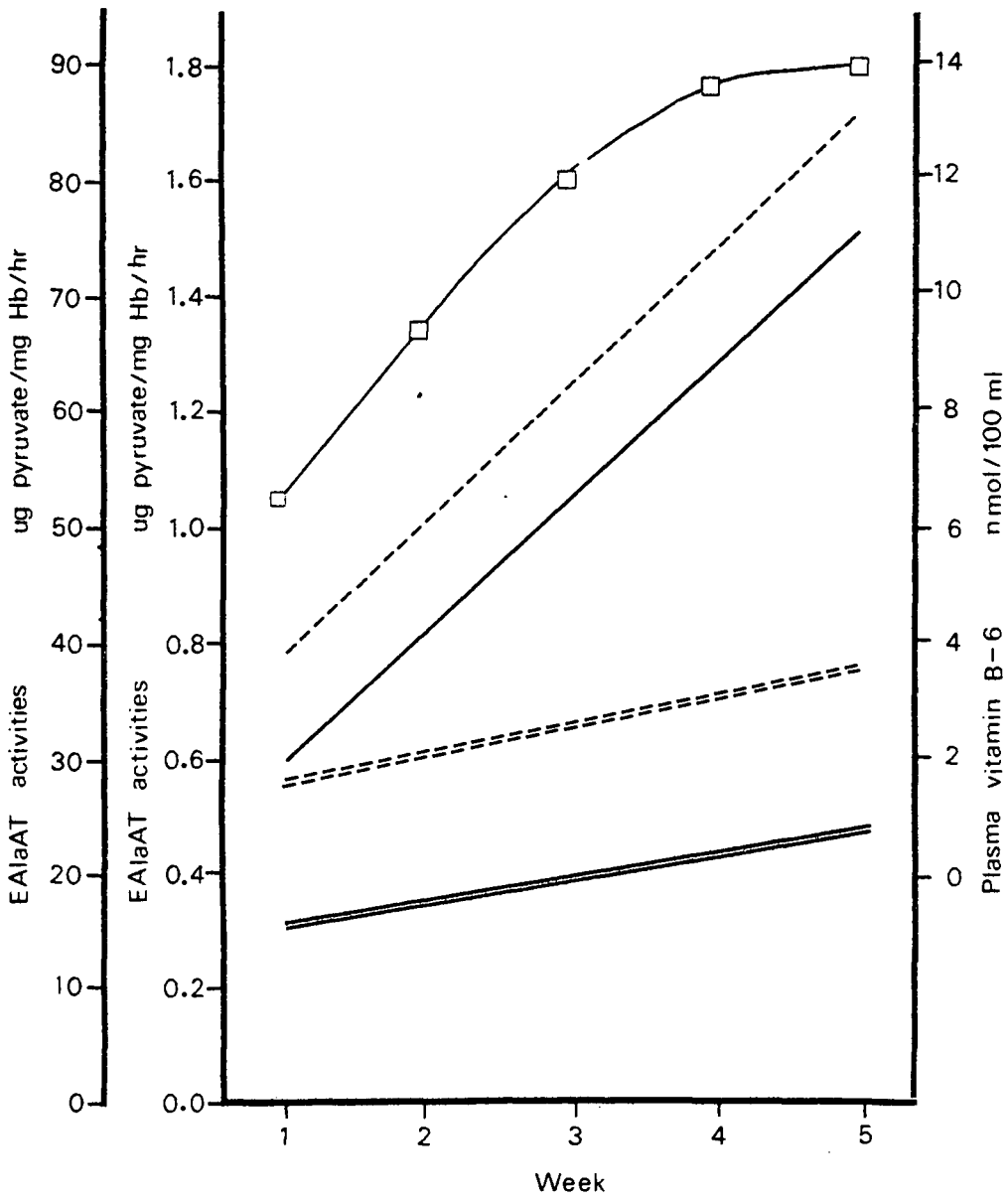
EAlaAT activities
ug pyruvate/mg Hb/hr
90
80
70
60
50
40
30
20
10
0
EAlaAT activities
ug pyruvate/mg Hb/hr
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Plasma vitamin B-6
n mol/100 ml
14
12
10
8
6
4
2
0
1
2
3
4
5
Week

Fig. 7

ever, PLP may be bound to erythrocyte aminotransferases as well as to hemoglobin.

CONCLUSIONS

The results of this investigation suggest that the bioavailability of vitamin B-6 can be estimated by giving test food doses to subjects who had been receiving 5-mg crystalline PN supplements. Since the food doses are large in volume and since the metabolism may be affected by the amount of vitamin B-6 in the dose given at one time, we recommend that both the crystalline PN dose and the food doses be divided evenly among the three meals.
In future studies, explanations for differences in the bioavailability of vitamin B-6 from various foods should be sought: whether or not vitamin B-6 bioavailability is affected by the form of vitamin B-6 in the food and whether or not bound forms of vitamin B-6 are biologically available. Digestibility of the food doses should be measured. In addition, graded amounts of crystalline PN dose (i.e., 0.5 mg, 1.0 mg, 1.5 mg and 2.0 mg) should be administered in order to establish linearity within the range of food doses, so that bioavailability could be determined from a regression line for each subject. In order to study the metabolism of supplementary vitamin B-6, the uptake of PLP by erythrocyte aminotransferases could be investigated by feeding ^{3}H-PN supplements to rats.

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APPENDICES

Appendix
Table 1

CONSENT FORM

Spring, 1980

I have read the description of this study and have had any questions answered concerning this study. I give my consent to participate in this study. Consent is given to have blood drawn as indicated in the description of the study. I further give my consent to collect 24 hoururine samples and understand that a complete urine collection is required as a part of this study. I give my consent to participate in the exercise phase of this study as described and understand the risks associated with bicycling involved. I understand that I must keep a record of all food eaten on those days when meals are not provided.

I understand that I will consume no other food on those days when meals are provided. I understand that no alcohol is to be consumed except on weekends in the amounts indicated. I understand that I am not to be involved in any strenuous exercise other than that described in the information sheet.

I understand that I will be paid $2.50 per day for each day I am in the study with the understanding that complete urine collections are peovided and adequate diet records provided.

I understand that I am free to withdraw from the study at any time and further that the investigators in this study can withdraw me from the study at any time. I understand that all data pertaining to me will be kept strictly confidential.

Name_____ Date_____

Witness_____ Date_____

Appendix
Table 2. 24-hr. urinary total vitamin B-6 excretion (umol/24 hr.)

Date \ Subject	1	2	3	4	5	Mean ± S.D.
4/21	0.79	-	0.93	0.67	0.70	0.77 ± 0.12
4/22	0.70	0.64	0.92	0.69	0.67	0.72 ± 0.11
4/23	0.70	0.54	0.93	0.69	0.61	0.69 ± 0.12
4/24	0.66	0.53	0.85	0.65	0.55	0.64 ± 0.12
4/25	0.72	0.57	0.79	0.53	0.48	0.61 ± 0.13
4/26	1.38	1.16	0.95	1.25	0.80	1.11 ± 0.23
4/28	1.61	1.27	1.10	1.52	1.22	1.34 ± 0.21
4/29	0.88	0.80	0.80	0.88	0.75	0.82 ± 0.06
4/30	1.26	1.24	1.18	1.72	1.21	1.32 ± 0.22
5/ 1	-	0.77	0.78	0.78	0.73	0.77 ± 0.02
5/ 2	1.42	1.33	1.06	1.63	1.01	1.29 ± 0.26
5/ 3	1.32	1.40	1.19	1.86	1.54	1.46 ± 0.26
5/ 5	1.42	1.61	1.34	1.98	1.81	1.63 ± 0.27
5/ 6	1.14	1.15	0.95	1.28	1.10	1.12 ± 0.12
5/ 7	1.67	-	1.30	1.87	1.63	1.62 ± 0.24
5/ 8	1.06	1.15	0.97	1.26	1.17	1.12 ± 0.11
5/ 9	1.33	1.65	1.16	1.98	1.70	1.56 ± 0.32
5/10	1.59	1.58	1.10	2.43	1.94	1.72 ± 0.49
5/12	1.57	1.37	1.47	2.11	1.64	1.63 ± 0.29
5/13	1.09	0.99	0.92	1.23	1.22	1.09 ± 0.14
5/14	1.44	1.48	1.68	1.87	1.70	1.63 ± 0.18
5/15	1.11	1.12	0.96	1.31	1.10	1.12 ± 0.13
5/16	1.32	1.52	1.29	2.05	1.80	1.60 ± 0.33
5/17	1.21	1.66	0.77	2.32	1.98	1.59 ± 0.61

Appendix
Table 2. (continued)

5/19	1.40	1.62	1.39	1.72	2.07	1.64 ± 0.28
5/20	-	1.01	0.85	1.15	0.96	1.00 ± 0.11
5/21	1.56	1.40	1.59	1.80	2.11	1.69 ± 0.27
5/22	1.02	1.04	0.90	1.17	1.05	1.04 ± 0.09
5/23	1.03	0.91	0.99	1.05	1.25	1.05 ± 0.13

Appendix
Table 3 24-hr. urinary free vitamin B-6 excretion (umol/24 hr.)

Date \ Subject	1	2	3	4	5	Mean ± S.D.
4/21	0.44	-	0.68	0.45	0.46	0.51 ± 0.12
4/22	0.39	0.41	0.63	0.50	0.46	0.48 ± 0.10
4/23	0.38	0.38	-	0.52	0.41	0.42 ± 0.07
4/24	0.35	0.35	0.59	0.47	0.40	0.43 ± 0.10
4/25	0.43	0.39	0.54	0.38	0.37	0.42 ± 0.07
4/26	0.79	0.59	0.69	0.82	0.57	0.69 ± 0.11
4/28	1.03	0.84	0.85	1.17	0.79	0.94 ± 0.16
4/29	0.53	0.56	0.53	0.69	0.49	0.56 ± 0.08
4/30	0.77	0.84	0.89	1.24	0.86	0.92 ± 0.18
5/ 1	-	0.60	0.61	0.60	0.54	0.59 ± 0.03
5/ 2	0.85	0.94	0.81	1.19	0.73	0.90 ± 0.18
5/ 3	0.87	1.11	0.82	1.43	1.03	1.05 ± 0.24
5/ 5	0.97	1.18	1.13	1.49	1.39	1.23 ± 0.21
5/ 6	0.71	0.80	0.80	0.98	0.85	0.83 ± 0.10
5/ 7	1.05	-	1.13	1.50	1.27	1.23 ± 0.20
5/ 8	0.67	0.87	0.72	1.03	0.97	0.85 ± 0.15
5/ 9	0.76	1.19	0.78	1.56	1.26	1.11 ± 0.34
5/10	1.10	1.23	0.81	1.75	1.69	1.32 ± 0.40
5/12	1.05	1.04	1.13	1.65	1.34	1.24 ± 0.26
5/13	0.70	0.76	0.69	0.99	0.91	0.81 ± 0.13
5/14	0.94	1.15	1.11	1.57	1.41	1.24 ± 0.25
5/15	0.75	0.94	0.77	1.06	0.90	0.88 ± 0.13
5/16	0.90	1.12	0.98	1.62	1.35	1.19 ± 0.29
5/17	0.83	1.16	0.65	1.93	1.58	1.23 ± 0.53

Appendix

Table 3. (continued)

5/19	0.92	1.15	1.20	1.34	1.69	1.26 ± 0.28
5/20	-	0.78	0.65	0.91	0.76	0.75 ± 0.11
5/21	0.98	1.09	1.29	1.28	1.62	1.25 ± 0.24
5/22	0.71	0.90	0.72	0.92	0.84	0.82 ± 0.10
5/23	0.71	0.79	0.86	0.85	1.06	0.85 ± 0.13

Appendix

Table 4. Percentage of 24-hr. urinary free/total vitamin B-6 excretion (%)

Date \ Subject	1	2	3	4	5	Mean ± S.D.
4/21	56	-	73	67	66	66 ± 7
4/22	56	64	68	72	69	66 ± 8
4/23	54	70	69	75	67	66 ± 9
4/24	53	66	69	72	77	67 ± 8
4/25	60	68	68	72	77	69 ± 6
4/26	57	51	73	66	71	64 ± 9
4/28	64	66	77	77	65	70 ± 7
4/29	60	70	66	78	65	68 ± 7
4/30	61	68	75	72	71	69 ± 5
5/ 1	-	78	78	77	74	77 ± 2
5/ 2	60	71	76	73	73	71 ± 6
5/ 3	66	79	69	77	67	72 ± 6
5/ 5	68	73	84	75	77	75 ± 6
5/ 6	62	70	84	77	77	74 ± 8
5/ 7	63	-	87	80	78	77 ±10
5/ 8	63	76	74	82	77	74 ± 7
5/ 9	57	72	67	79	74	70 ± 8
5/10	69	78	74	72	87	76 ± 7
5/12	67	76	77	78	82	76 ± 6
5/13	64	77	75	80	75	74 ± 6
5/14	65	78	66	84	83	75 ± 9
5/15	68	84	80	81	82	79 ± 6
5/16	68	74	76	79	75	74 ± 4
5/17	69	70	84	83	80	77 ± 7

Appendix
Table 4. (continued)

5/19	66	71	86	78	82	77 ± 8
5/20	-	77	76	79	79	78 ± 2
5/21	63	78	81	71	77	74 ± 7
5/22	70	87	80	79	80	79 ± 6
5/23	69	87	87	81	85	82 ± 8

Appendix

Table 5. Weekly mean of plasma total vitamin B-6 (nmol/100 ml) in the five subjects.

Week	Date	subject 1	2	3	4	5	Mean ± S.D.
1	4/21[1]	8.20	8.14	7.78	7.30	5.33	6.40 ± 0.70[2]
	4/25	7.24	5.87	7.06	6.10	5.75	
2	4/30	15.86	8.80	7.36	11.31	6.10	9.38 ± 2.81
	5/ 2	18.30	9.34	7.12	9.28	7.72	
3	5/ 7	13.47	10.71	9.58	11.61	13.05	11.98 ± 1.36
	5/ 9	14.12	11.37	11.25	12.51	12.15	
4	5/14	15.62	11.79	11.61	14.90	14.00	13.72 ± 1.86
	5/16	16.94	11.61	12.21	14.00	14.48	
5	5/21	15.92	12.09	11.61	15.74	14.30	13.93 ± 2.01[3]
	5/24	14.84	10.65	11.25	13.05	12.45	

[1]Mean of the plasma total vitamin B-6 from the 5 subjects on 4/21 (the first day of the study) is 7.35 ± 1.18 nmol/100 ml.

[2]Average of the five subjects on 4/25.

[3]Average of the five subjects on 5/21.