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Title: The Bioavailability of Vitamin B_6 from Selected Foods as Measured by Urinary 4-Pyridoxic Acid in Men Saturated With Pyridoxine

Abstract approved: _____
Dr. Lorraine Miller

The bioavailability of vitamin B_6 in four selected foods (bananas, filberts, soybeans and beef) was determined in five men, aged 22 to 25 years, who were saturated with pyridoxine. The study consisted of a five-day adjustment period followed by a 28-day experimental period. All subjects consumed a constant diet containing 1.34 mg of vitamin B_6 from Monday to Friday of each week, and their self-chosen diets on the weekends. During the experimental period the subjects received 5 mg of pyridoxine each day, including weekends, except on the days when loading doses of crystalline pyridoxine and the selected foods were administered. Doses of 2 mg of crystalline pyridoxine or doses of food containing approximately 2 mg of vitamin B_6 were given. The subjects collected daily 24-hr

urine specimens. Vitamin B_6 bioavailability was determined by comparing the yield of 4-pyridoxic acid in response to the test food doses to the yield of 4-pyridoxic acid in response to the 2mg of crystalline pyridoxine. Compared to the 100 percent bioavailability of 2mg crystalline pyridoxine, the mean percent bioavailability of vitamin B_6 from banana was 131.4 ± 68.2; from filberts, 88.1 ± 13.9; from soybeans, 58.3 ± 24.3; and from beef, 81.5 ± 28.6. Factors affecting bioavailability of vitamin B_6 from these foods are discussed.

The Bioavailability of Vitamin B_6 from Selected Foods as Measured by Urinary 4-pyridoxic Acid in Men Saturated with Pyridoxine

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Typed by Donna Lee Norvell-Race for Patricia Lozano de Gonzalez

♦ *Dedicated, with love, to Carlos and Karla, who made so many sacrifices that I might complete my graduate studies ...*

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INTRODUCTION

The vitamin B_6 content of foods as determined in the laboratory does not indicate the bioavailability of this vitamin. Bioavailability of vitamin B_6 refers to the net utilization of this vitamin in a food, or the fraction of the total vitamin B_6 which has undergone intestinal absorption and functions qualitatively as vitamin B_6. Bioavailability reflects the digestion, absorption and metabolism of an ingested nutrient, and is reflected in the level of this nutrient or its metabolites in body tissues, fluids, and excretory products.

In the past, investigations on vitamin B_6 bioavailability were 50-60 days in length and the test food provided the bulk of the subjects' intake of vitamin B_6 (Leklem, Shultz and Miller, 1980; Lindberg, 1979). If it were possible to measure vitamin B_6 bioavailability from foods by giving loading doses as Wozenski, Leklem and Miller (1980) had done with crystalline vitamin B_6, then these investigations on bioavailability could be less time-consuming and expensive, and more subjects could be tested. There are, however, two problems in this "loading dose" method. The food test dose would need to be large in order to obtain a reliable

measurable change in body fluids or excretory products. In addition, variable results are obtained in normal free-living subjects because of the differences in their vitamin B_6 nutritional status or saturation.

Recently, Tamura and Stokstad (1973) assessed the bioavailability of food folate in subjects saturated with that vitamin by measuring changes in urinary folate excreted in response to test doses of food. Tamura and Stokstad saturated their subjects with pteroylmonoglutamate (PteGlu) in order to obtain increased urinary excretion of folate. Folate excretion following 0.3-0.5 mg of PteGlu was increased about five times by preloading folate saturated subjects with 2 mg of PteGlu. They applied their procedure to measuring the bioavailability of folate in food given as loading doses.

The purpose of the research reported in this thesis was to apply the procedure developed by Tamura and Stokstad for determining the bioavailability of food folate to vitamin B_6: to measure the bioavailability of vitamin B_6 from test food doses administered to men saturated with pyridoxine. Vitamin B_6 bioavailability in the research reported in this thesis was determined by measuring the urinary excretion of 4-pyridoxic acid, a major metabolite of vitamin B_6 which is excreted in urine.

In addition, in support of the use of 4-pyridoxic acid as a measure of vitamin B_6 bioavailability in vitamin B_6-saturated subjects, Lumeng, Lui and Li (1980) recently observed that the

pyridoxal phosphate that was newly formed from pyridoxine added to isolated hepatocytes did not mix freely with endogenous pyridoxal phosphate, but was preferentially converted to and released as pyridoxal and 4-pyridoxic acid. This suggests that the 4-pyridoxic acid excreted by subjects saturated with vitamin B_6 in response to the test doses of food represents the bioavailability of vitamin B_6 in that food: that vitamin B_6 in the food dose had been digested, absorbed and metabolized.

REVIEW OF LITERATURE

History

In 1934, vitamin B_6 was identified by György as essential for preventing a dermatitis, called acrodynia, in the rat (György, 1971). In 1938, the isolation of the pure crystalline vitamin B_6 was announced by five different research groups: those of Lepkovsky, Keresztesy and Stevens, György, Kuhn and Wendt, and Itiba and Miti. Subsequently, the vitamin was synthesized independently by Kuhn and his coworkers in Germany, and by Keresztesky and his associated in the United States (Brin, 1978). When its pyridine ring structure was elucidated in 1939, vitamin B_6 was given the more descriptive name of pyridoxine (PN) (György, 1971). Snell, Guirard and Williams (1942) first recognized the existence of pyridoxal (PL) and pyridoxamine (PM). Since PN, PL and PM are equally effective in animal nutrition, they are referred to collectively as vitamin B_6[1] (Sauberlich and Canham, 1980). The structures of these three free forms of the vitamin and their respective phosphorylated forms are presented in Figure 1. The requirement for vitamin B_6 in the human infant was demonstrated by Synderman et al. (1952).

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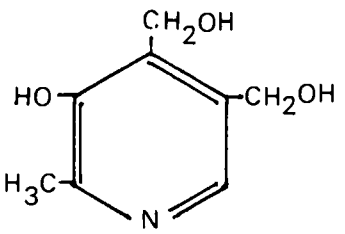
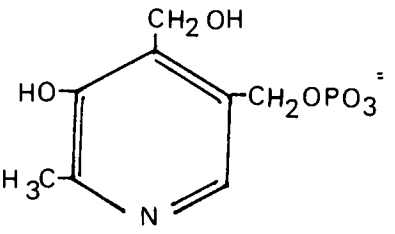
[1]The term vitamin B_6 is used as the generic descriptor for all 3-hydroxy-2-methyl-pyridine derivatives exhibiting qualitatively the biological activity of PN (IUPAC-IUB CMN, 1974).



Pyridoxine (PN)



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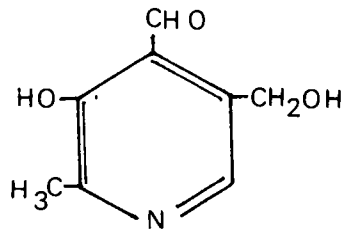
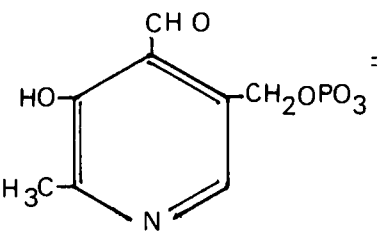
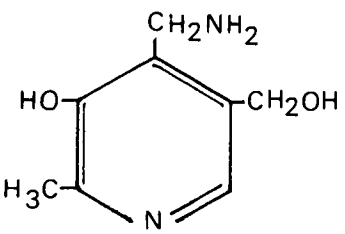


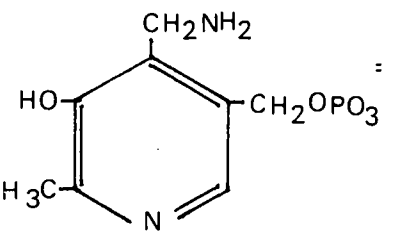
Pyridoxal (PL)



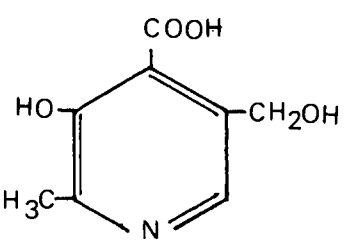
Pyridoxal Phosphate (PLP)



Pyridoxamine (PM)



Pyridoxamine Phosphate (PMP)



4-Pyridoxic Acid (4PA)

Figure 1. Structures of free and phosphorylated forms of vitamin B_6 and the metabolite 4-pyridoxic acid

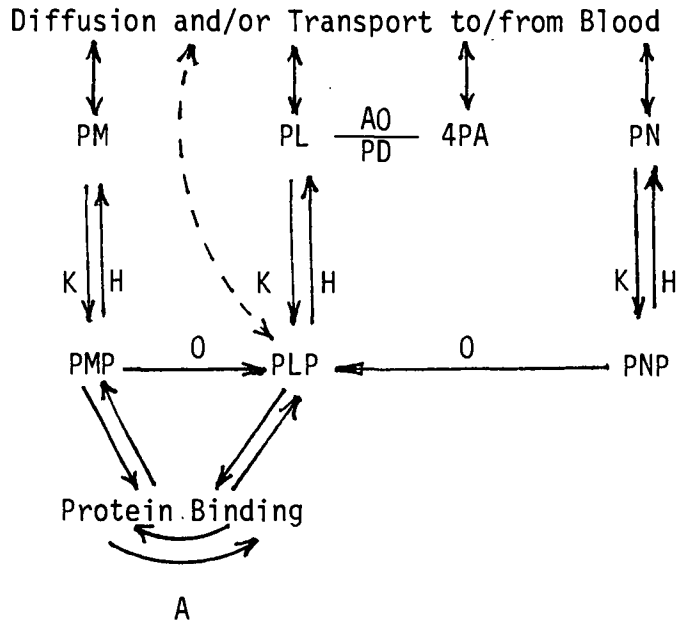
Vitamin B_6 Interconversion

Vitamin B_6 occurs widely in foods. PL and PM occur mainly in animal products, whereas PN is found largely in products of vegetable origin. The three forms are approximately equally effective in supporting animal growth (Sauberlich and Canham, 1980).

The free forms of vitamin B_6 are converted to their respective phosphorylated forms in mammals by the action of the enzymes PL kinase and PNP oxidase (Snell and Haskel, 1971). Adenosine triphosphate (ATP) is the preferred phosphorylating agent. Kidney, liver and brain are rich sources of kinase, whereas skeletal muscle is a poor source (McCormick, Gregory and Snell, 1961). Both kinase (McCormick et al., 1961) and oxidase (Wada and Snell, 1961) activity is high in liver. Reversal of the phosphorylated forms to the free vitamin forms is effected by alkaline phosphatase (Lumeng and Li, 1980).

PL can be oxidized to 4-pyridoxic acid (4PA) (Fig. 1). Conversion of PL to 4PA is catalyzed by either aldehyde oxidase (Schwartz and Kjeldgaard, 1951) or NAD+-dependent pyridoxal dehydrogenase (Stalunovic et al., 1976). Figure 2 shows the interconversion of the various forms of vitamin B_6 and the formation of 4PA.

Lumeng, Lui and Li (1980) incubated isolated hepatocytes with ^{14}C-PN, and measured the formation of various vitamin B_6 compounds. From the results of this investigation they concluded that (1) PLP



AO : Aldehyde Oxidase

H : Alkaline Phosphatase

K : Pyridoxal Kinase

O : Pyridoxamine-P Oxidase

PD : NAD^+-dependent Pyridoxal Dehydrogenase

A : Apo-Pyridoxal-P-Dependent Enzymes

Figure 2. Interconversions of the B_6 vitamers and formation of 4PA (adapted from McCormick and Merril, 1980)

and PMP are the major B_6 vitamers in liver cells, whose intracellular content is tightly regulated so that the excess amounts of these two forms do not accumulate in the presence of the free forms of vitamin B_6; (2) newly formed PLP and PMP do not exchange freely with endogenous intracellular pools of PLP and PMP, and largely constitute rapidly mobilizable pool destined for secretion into the plasma or degradation; and (3) PL, PLP and 4PA are the chief forms of vitamin B_6 released by the liver. These findings give a scientific basis for attempting to measure the bioavailability of vitamin B_6 in test foods by measuring the excretion of urinary 4PA in subjects saturated with vitamin B_6, as presented in this thesis. In PN-saturated subjects, 4PA excreted in response to a test dose should represent that which had been formed from the test dose.

Absorption of Vitamin B_6

The three free forms of vitamin B_6 are quite stable in the intestinal tract and are readily absorbed. Absorption, which occurs primarily in the upper small intestine, can also occur in the jejunum and the ileum, regardless of the size of the dose given. Booth and Brain (1962) gave rats 0.05 to 5 mg of tritium-labeled PN hydrochloride (^{3}H PN HCl) directly into parts of the intestinal tract along with 10 mg of unlabeled PN. The percentage of the radioactive dose excreted when the labeled PN was placed in the jejunum, ileum and colon was 71, 50 and 18 percent, respectively. Booth and Brain suggested that vitamin B_6 was absorbed by passive

diffusion. They observed no tendency for PN uptake to plateau, even after an oral dose of 5 mg. In addition, they did not find evidence that large doses of PN altered the site of absorption from the gastrointestinal tract.

In humans, the jejunum also appears to be the absorption site of vitamin B_6. Oral doses of ^3H PN increasing from 1 to 20 mg resulted in urinary excretion of ^3H PN that increased proportionally to the dose given. Resection of the distal small intestine appeared to have no effect on the ability to absorb PN. A patient having only four feet of proximal jejunum excreted amounts similar to those excreted by normal controls (Brain and Booth, 1964).

Tsuji, Yamada and Nose (1973) used everted ring segments of small intestine from rats to study the uptake of ^3H PN. The rate of uptake of radioactive compounds by intestinal tissue at first was linear and then declined. When the initial concentration in the incubation medium ranged from 0.1 to 4μM of ^3H PN, the ratio of intracellular to extracellular concentration of total ^3H compounds was greater at the beginning than after an hour of incubation. Addition of 4-deoxypyridoxine, an inhibitor of pyridoxal kinase, caused inhibition during long periods of incubation, but little during the five-minute uptake. The distribution of radioactive vitamin B_6 compounds was examined after incubation of the tissue with ^3H PN under various conditions. PN and the three phosphorylated forms were the major vitamin B_6 compounds found, whereas PL, PM and 4PA were minimal. The intracellular PN concentration was

lower than the extracellular one under all conditions examined, indicating that no transport of PN occurred against a concentration gradient. Conversion of ^{3}H PN to the phosphorylated forms was inhibited by the addition of 4-deoxypyridoxine. Tsuji et al. concluded that PN enters the intracellular fluid space by simple diffusion.

This mechanism was confirmed by Middleton (1977), who incubated everted jejunal sacs from rats with solutions of PN ranging from 0.01 to 10 μM. PN uptake was linear with respect to PN concentration. Saturation at still lower concentrations, therefore, is unlikely. Middleton also showed that the presence of metabolic inhibitors like iodoacetamide, 4-deoxypyridoxine HCl, anoxia, and oubain did not produce a significant decrease on the uptake. In a similar study, Middleton (1982) found a linear relationship between PLP concentration and disappearance from perfused segments of rat jejunum with no evidence of saturation at concentrations from 100 to 300 μM of PLP. At concentrations of 1 mM and 3 mM, there was a significant decrease in the disappearance of PLP, indicating saturation of the disappearance mechanisms. The disappearance of 100μM of PLP was reduced by both 1 mM of PM and 1 mM of PMP, the last one significantly.

Urinary Excretion of Vitamin B_6 and 4-Pyridoxic Acid

Vitamin B_6 is excreted in urine as PL, PM and PN, as well as

4PA and some unidentified metabolites (Sauberlich et al., 1972). The major urinary metabolite of vitamin B_6 is 4PA (Rabinowitz and Snell, 1949).

In normal subjects the excretion of 4PA reflects the dietary intake of vitamin B_6. Subjects receiving 1.66 mg of vitamin B_6 daily excreted approximately 50 percent of it as 4PA. They excreted PL in the largest amount, followed by PM and only negligible amounts of PN. After five days of vitamin B_6 deprivation, the subjects' urinary excretion of 4PA and vitamin B_6 was 40 and 20 percent, respectively, of the level they had excreted while receiving 1.66 mg of vitamin B_6 a day (Kelsay, Baysal and Linkswiler, 1968).

In eight men who were receiving 0.16 mg of vitamin B_6 a day, the urinary concentration of 4PA dropped within 5 days. None was detected after 25 days of vitamin B_6 depletion. A daily PN-HCl supplement of 0.6 or 0.9 mg increased the excretion of 4PA, but below the predepletion values (Baysal, Johnson and Linkswiler, 1966). In two infants given a vitamin B_6 deficient diet, the excretion of urinary 4PA fell to zero, and that of urinary vitamin B_6 fell to a range of 0.2 to 2.0 μg daily (Synderman et al, 1952).

Children from two to nine years of age, who were consuming normal diets containing approximately 1.1 mg of vitamin B_6, excreted an average of 48 percent of the vitamin as 4PA. Excretions of 0.16 to 0.30 mg of 4PA per 24 hr appeared to indicate a low intake of the vitamin (Lewis and Nunn, 1977).

Recommended Dietary Allowances for Vitamin B_6

The requirement of vitamin B_6 is increased when high-protein diets are consumed. Baker et al. (1964) administered a liquid formula diet providing 30 or 100 g of protein a day to young adult men. In response to a loading dose of 10 g of DL-tryptophan, the subjects receiving the 100 g of protein daily excreted almost 350 mg of xanthurenic acid per 24 hr after three weeks of vitamin B_6 depletion. Subjects receiving the 30-g protein diet, on the other hand, excreted less than 300 mg of xanthurenic acid after six weeks of depletion. Men fed the 100-g protein diet required at least 1.5 mg of PN-HCl daily; and those fed the 30-g protein diet, 1.25 mg.

Harding, Plough and Friedmann (1959) fed packaged military rations providing 165 g of protein and 1.93 or 2.76 mg of vitamin B_6 to men for 24 days. The subjects consuming the lower level of the vitamin showed a significant rise in xanthurenic acid excretion following a tryptophan load test, whereas the ones receiving the ration containing the higher level did not. Harding et al. concluded that the vitamin B_6 requirement for men was greater than 2 mg per day when 165 g of protein were fed. The recommended dietary allowances (RDA) for adult males was changed from 2.0 mg (NRC, 1974) to 2.2 mg a day (NRC, 1980). The 1980 RDA is based on the range of requirements observed in several studies and the uncertainty of the availability of dietary vitamin B_6. A ratio of 0.02 mg of vitamin B_6 per g of protein eaten was used as the

basis for calculating the RDA for vitamin B_6 (NRC, 1980).

Bioavailability of Vitamin B_6

The vitamin B_6 content of American diets, according to Mangay Chung et al. (1961), is 1.1 to 3.6 mg per day for typical diets and 0.7 to 1.4 mg per day for poor diets. Driskell, Geders and Urban (1976) reported that adult males consumed diets containing 2.14 ± 1.33; and women, diets supplying 1.20 ± 0.52 mg of vitamin B_6 daily.

Although the quantities of vitamin B_6 present in foods have been measured, these data do not represent the amount available to humans. Practical approaches for determining vitamin B_6 bioavailability in a test food include measuring plasma and urinary vitamin B_6 compounds in subjects who are receiving a constant diet in which the test food contributes a large portion of the vitamin, or by measuring vitamin B_6 components in timed blood and urine specimens collected from subjects who have received a loading dose of a test food or crystalline B_6.

Wozenski, Leklem and Miller (1980) administered five levels of PN HCl (0.5, 1, 2, 4 and 10 mg) and equimolar doses (19.44 μmoles) of PN, PL, and PM to five young men. Following a 0.5 mg PN loading dose, the subjects exhibited a rise in plasma total vitamin B_6 and PLP, but not in the urinary excretion of 4PA or vitamin B_6. Although the mean excretion of 4PA increased from 5.6 to 19 μmoles per 24 hr in response to the 0.5 to 10 mg doses, the percent of the

dose excreted as 4PA decreased from 63 to 53, while the dose recovered as urinary vitamin B_6 decreased from 9.3 to 6.9. After the ingestion of PL, the percent rise in plasma total vitamin B_6 and urinary 4PA were significantly greater than after the ingestion of equimolar doses of PN and PM, suggesting that the three B_6 vitamers may be absorbed and metabolized differently or at different rates.

The effect of wheat bran on the bioavailability of vitamin B_6 was determined in 10 young men by Lindberg (1979). When 15 g of bran were added to the diet, the mean urinary 4PA decreased from 42.6 ± 7.5 to 36.6 ± 7.5 percent of vitamin B_6 intake, and the mean fecal vitamin B_6 excreted increased from 34.4 ± 8.7 to 37.2 ± 11 percent.

Vitamin B_6 is less bioavailable from whole wheat (WHW) bread than from white bread enriched with vitamin B_6 (WB6). Leklem et al. (1980) fed young men a constant diet containing 0.38 mg of vitamin B_6, plus WHW or WB6 bread, which supplied 1.2 mg of vitamin B_6 (total vitamin B_6 intake was 1.58 mg). Excretion of 4PA was lower when the subjects were fed WHW than when they had WB6. When the subjects received WHW bread, 4-pyridoxic acid accounted for 37.3 ± 10.2 percent of the dietary vitamin B_6 intake; 6 percent more of the dietary vitamin B_6 was excreted as 4PA when WB6 bread was fed. Plasma PLP and vitamin B_6 were lower with WHW than with WB6 bread. The excretion of urinary total vitamin B_6 accounted for 8 percent of the ingested vitamin B_6, and

a lower level of PL was excreted when WHW was fed than when WB6 was fed.

Tarr, Tamura and Stokstad (1981) used plasma PLP and urinary vitamin B_6 to estimate bioavailability of vitamin B_6 in an average American diet providing 2.3 mg a day of the vitamin. During the study, only 3.2 to 5.7 percent of the vitamin was excreted in urine as vitamin B_6. The bioavailability of vitamin B_6 in the average American diet, as determined by plasma PLP levels, was 71 percent. Tarr et al. did not measure urinary 4PA to determine vitamin B_6 bioavailability.

Nelson, Lane and Cerda (1976) used a triple lumen perfusion tube to assess vitamin B_6 absorption in 15 subjects. Frozen orange juice and three synthetic solutions containing vitamin B_6 in concentrations identical to that in the natural orange juice were perfused into the small intestine by means of an infusion apparatus. Aspiration of the proximal and distal parts was maintained continuously by individual pumps. The mean vitamin B_6 absorption was significantly greater from the synthetic solution (65 percent uptake) than from the orange juice (30 percent uptake). The addition of glucose to the synthetic solution enhanced this difference.

Nguyen, Gregory and Damron (1981) examined the effects of cellulose, pectin and bran on the bioavailability of PN using rat and chick bioassay methods. They did not find any relationship between fiber and the reduction of bioavailability of vitamin

B_6. In the same year, Nguyen et al. (1981) examined the ability of eight purified polysacharides, lignin and wheat bran to bind B_6 vitamers in in vitro systems simulating the jejunal environment. No in vitro binding by pectin was detected. No significant binding of PN by the tested polysacharides, lignin or wheat bran was detected.

The processing and storage of foods has various effects on the bioavailability of vitamin B_6. Losses of vitamin B_6 during food processing and storage may result from interactions of vitamin B_6 with proteins, amino acids or reducing sugars to form complexes of limited bioavailability (Gregory and Kirk, 1981).

As reviewed by Gregory and Kirk (1981), losses of vitamin B_6 in meats during cooking, as determined by rat and microbiological assay procedures, had little or no effect on the bioavailability of B_6 vitamers.

During heat processing of foods, a significant fraction of vitamin B_6 can be converted into the less biologically active pyridoxylamino compounds, particularly ε-pyridoxylysine (Gregory and Kirk, 1978). When food composition and/or processing conditions are such that the concentration of B_6 vitamers is very low and ε-pyridoxyllysine is less than 50 percent of the molar equivalent for PN, the anti-vitamin activity of ε-pyridoxyllysine yields a low apparent bioavailability (Gregory and Kirk, 1981).

MATERIALS AND METHODS

Subject Selection

Subjects were recruited by advertisements placed in campus buildings. Five healthy men from 22 to 25 years of age served as subjects; their physical data are presented in Table 1. They were free from any known metabolic disease, as determined by interview. Each subject exhibited a normal absorption of a 5 g dose of D-xylose, as determined by xylose excretion in urine collected for 5 hr after ingestion of this carbohydrate (Buttery et al., 1975).

Experimental Protocol

This study, which lasted five weeks, was approved by the Human Subjects Committee at Oregon State University. Each subject signed an informed consent form (Appendix 1) approved by this committee before participating in this investigation.

The plan of this five-week study is presented in Table 2. During this investigation the subjects consumed a constant diet (Table 3) containing 1.34 mg of vitamin B_6, as determined[2] with Saccharomyces uvarum as the assay organism by AOAC (1980) procedure except that the chromatography step was omitted. The subjects received this constant diet Monday through Friday of each week, and were not allowed any other food or beverage other than that provided by this

[2]Vitamin B_6 in the constant diet and the test foods was determined by Hussein Kabir.

TABLE 1. Physical Description of the Subjects

Subject No.	Age (years)	Height (m)	Weight	
			Initial (kg)	Final (kg)
1	22	1.89	86.4	85.3
2	25	1.86	84.1	83.2
3	22	1.80	94.4	92.1
4	23	1.81	82.7	81.5
5	25	1.76	69.8	68.6

TABLE 2. Plan of Study on Bioavailability of Vitamin B_6 from Selected Foods in Subjects Saturated With Vitamin B_6

	Week				
	1	2	3	4	5
Constant diet[1]	Daily except Sa[2] and Su[3]				
5 mg PN[4]	Starting on Sa of week 1, daily except on Tu and Th of the remaining four weeks.				
Loading doses					
0 mg PN		Tu,Th			
2 mg PN[4]			Tu,Th		
banana[5]				Tu	
filberts[6]				Th	
cooked soybeans[7]					Tu
beef bologna[8]					Th
Urine collection					
ending at 24 hr		Daily except when indicated otherwise			
ending at 10 and 24 hr:	Th,F	Tu,Th	Tu,Th	Tu,Th	Tu,Th
Blood drawn	M,F	M,W,F	W,F	W,F	W,F
Exercise days[9]	F				F

[1]Containing 1.34 mg vitamin B_6.

[2]Only breakfast was provided

[3]Subjects consumed self chosen diets.

[4]Administered with breakfast.

[5]633 g providing 1.5 mg vitamin B_6.

[6]333 g providing 2.12 mg vitamin B_6.

[7]330 g (weight before cooking) providing 1.53 mg vitamin B_6.

[8]666 g providing 1.57 mg vitamin B_6.

[9]Procedure reported by A-G Wang (1982).

TABLE 3. 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 S. uvarum as the assay organism (AOAC, 1980).

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

[3]In quantities to maintain weight.

diet. Only breakfast was provided on Saturday mornings. During the remainder of each weekend the subjects chose their own diets, which they recorded. Their alcohol intake on weekends was limited to 12 oz of beer.

Starting on day 6 (Saturday of week 1) the subjects received orally 5 mg of PN[3] each day, including weekends, except on Tuesday and Thursday of each week. This 5 mg PN supplement was administered at breakfast; on Sundays the subjects took this supplement at home.

On Tuesday and Thursday of week 2 the subjects received no PN supplement. During week 3 they received 2 mg of PN orally on both Tuesday and Thursday at breakfast. During weeks 4 and 5, approximately 2 mg of vitamin B_6 were supplied by test foods: week 4, the subjects received 633 g of bananas (1.57 mg vitamin B_6) on Tuesday and 333 g of ground filberts (2.12 mg vitamin B_6) on Thursday; and week 5, they received 330 g of soybeans[4] (1.53 mg vitamin B_6) on Tuesday and 666 g of beef bologna (1.57 mg vitamin B_6) on Thursday. The bananas were consumed with breakfast and during the morning; filberts, throughout the day (completed at 5 pm); beef and soybeans, one-third of total quantity at a meal.

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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]Weight before cooking.

An exercise experiment was conducted on the Fridays of weeks 1 and 5. The procedure was described by Ann-Gau Wang (1982). Results will be published elsewhere.

The subjects made complete urine collections throughout this investigation. On Thursday and Friday of week 1, and on test dose days (Tuesdays and Thursdays of weeks 2 to 5)(Table 2), the subjects collected urine ending at 10 and at 24 hr. On the remaining days of study, they collected 24-hr specimens. All urine specimens were collected under toluene. They were measured daily and portions were frozen at -20°C until laboratory determinations were done.

Blood was drawn from the antecubital vein into evacuated heparinized tubes by a registered medical technologist before breakfast of the days indicated on Table 2. Results on plasma vitamin B_6 and erythrocyte transaminase activity will be reported elsewhere.

Urinary 4PA[5] was fluorometrically quantitated after the separation of 4PA from interfering compounds by ion-exchange chromatography (Reddy, Reynolds and Price, 1958).

Completeness of urine collection was checked by measuring creatinine (Technicon Autoanalyzer, Technicon Corp., Tarrytown, N.Y.) by an automated procedure using alkaline picrate (Pino, Benotti and Gardyna, 1965).

Urinary vitamin B_6 was determined by microbiological assay

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[5]Assisted by Linda Barstow.

using S. uvarum as the test organism (Miller and Edwards, 1980).

Preparation of Test Foods

Bananas (4.54 kg) were purchased and refrigerated for six days. The bananas were peeled, the dark mushy parts were removed, and they were cut into approximately 2.5-cm slices. Fifteen milligrams of ascorbic acid were added to prevent the bananas from browning.

Filberts were ground in a blender and in a conventional hand grinder and mixed. To 333-g portions of ground filberts were added 150 ml of corn syrup and 150 ml of water. These ingredients were mixed and kept at room temperature until consumption the following day.

One hundred ten grams of raw soybeans, one vegetable cube[6] and 125 ml of water were mixed together in a casserole and then heated for 10-15 minutes at 176.7°C. The soybeans were autoclaved uncovered for 20 minutes at 102 kPa and stored at 4°C until they were consumed the following day. Each subject received one bowl of cooked soybeans at each meal, a total of 330 g (weight before cooking).

Beef bologna was prepared by mixing 11.34 kg of lean ground beef and 227 g of a commercial bologna mix. The commercial mix contained: salt, dextrose, red and white pepper, sage, monosodium

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[6]Vegex Company, Division of Presco Food Products, Inc., Flemington, New Jersey 08822.

glutamate, oil of sage, capsicum, and calcium phosphate. The bologna was prepared by the Meat Science Laboratory, Department of Animal Science, OSU, and stored frozen. Six hundred and sixty-six grams providing 1.57 mg of vitamin B_6 were consumed by each subject.

Calculation of Percent Bioavailability

Since the subjects were unable to consume the total quantity of any test food at one time, the bioavailability of vitamin B_6 was calculated from the level of 4PA excreted in 24 hr rather than that in 10 and 14-hr urine collections on the days of the test food doses.

The bioavailability of vitamin B_6 in a test food was estimated by comparing the yield of a subject's urinary 4PA on the day of the test food to that on the day the 2 mg PN loading dose was given. Yield of 4PA from a test food was calculated by subtracting the subject's mean 24-hr excretion of 4PA on the two days 0 mg of PN was administered (Tuesday and Thursday of week 2) from the subject's 24-hr excretion of 4PA on the test food day. Yield of 4PA from 2 mg of PN was measured by subtracting the subject's mean 24-hr excretion of 4PA when 0 mg of PN was administered from the subject's mean 24-hr 4PA of the days 2 mg of PN were administered (Tuesday and Thursday of week 3). Since the test food doses did not contain exactly 2 mg of vitamin B_6, the yield of a subject's 24-hr urinary 4PA for the test food was adjusted proportionally so that it would be equivalent to 2 mg of vitamin B_6. It was assumed that the excretion of 4PA by

a subject was proportional to vitamin B_6 intake in the range of 1.5 to 2.2 mg vitamin B_6 intake.

Statistical Analyses

The data were analyzed using the Student's t test on means.

RESULTS AND DISCUSSION

Bioavailability of vitamin B_6 refers to the net utilization of this vitamin from food, or the fraction of the dietary vitamin B_6 which has undergone intestinal absorption and functions qualitatively as vitamin B_6 in the body. The vitamin B_6 compounds in blood and excretory products used to measure vitamin B_6 bioavailability in humans reflect the digestion, absorption and metabolism of the vitamin. A high excretion of vitamin B_6 compounds in urine as well as high levels in plasma suggest high vitamin B_6 bioavailability.

The form of vitamin B_6 in food and the interaction of this vitamin with other dietary constituents within the intestinal tract may influence the digestion and absorption of vitamin B_6. The vitamin B_6 status of the person may also affect the levels of vitamin B_6 parameters used to measure bioavailability.

Previous studies conducted in our laboratory on the bioavailability of vitamin B_6 in various foods involved diets in which test foods provided the bulk of the vitamin. These required long periods of time, and were expensive and time-consuming. The bioavailability from such foods was estimated by measuring urinary, fecal and plasma vitamin B_6 compounds.

The use of loading doses of test foods permits a faster assessment of vitamin B_6 bioavailability, and more subjects can be tested with less expense. Wozenski et al. (1980) suggested that an oral

dose of at least 1 mg of vitamin B_6 is necessary to obtain measurable changes in urinary and plasma vitamin B_6 compounds.

In general, normal human subjects vary widely in the saturation of their tissues with vitamins. Relatively uniform excretion patterns of a water-soluble vitamin can be achieved by saturating the subjects with the vitamin prior to administering it in its crystalline form or as a food test dose and measuring appropriate urinary excretion products (Bauernfeind and Miller, 1978). In this investigation, uniform vitamin B_6 status was achieved by saturating the subjects with supplementary PN until the excretion of 4PA was constant. Under these conditions it was expected that the excretion of 4PA would be proportional to the bioavailability of vitamin B_6 in the test food. Recently, Lumeng et al. (1980) observed that 4PA is a metabolite of recently administered vitamin B_6. They found that in hepatocytes incubated with PN, the newly formed vitamin B_6 compounds, including 4PA, did not mix with the tissue pools of this vitamin.

In view of this observation by Lumeng et al., and of the facility of collecting and handling urine, as well as the sensitivity of the 4PA in assessing vitamin B_6 bioavailability (Leklem et al., 1980), urinary 4PA was used in this study to assess vitamin B_6 bioavailability in four test foods which were administered to subjects saturated with vitamin B_6. It was hypothesized that the excretion of 4PA in response to these test doses represents the bioavailability of vitamin B_6 under these conditions.

Daily 24-hr 4PA Excretion

The levels of urinary 4PA excreted daily during this investigation are presented in Table 4 and Figure 3. Except for subject 5, urinary 4PA in each subject dropped from day 1 to day 5 of the first week when the constant diet was administered without the 5 mg PN supplement. This may indicate that the subjects' diets before participating in this investigation were higher in vitamin B_6 than that of the constant diet, 1.34 mg of vitamin B_6. It is likely that the subjects' previous diets were higher than 1.34 mg, which is only 60 percent of the RDA (NRC, 1980) for adult males (2.2 mg). Driskell et al. (1976) found that young men consumed diets containing 2.14 ± 1.33 mg of vitamin B_6. Leklem et al. (1980) also observed a decrease in urinary 4PA at the end of a preliminary six-day period in a study in which the subjects received 1.58 mg of vitamin B_6 daily. The subjects in the present study were excreting higher mean concentrations of urinary 4PA (6.17 ± 1.47 μmoles/24 hr on day 1 to 5.35 ± 1.47 on day 5) than the subjects studied by Leklem et al. (5.23 ± 1.69 μmoles/24 hr dropping to 4.41 ± 1.58 after 5 days).

It is difficult to determine whether or not the exercise experiment on day 5 affected the excretion of 4PA. We assumed that the modest drop in urinary 4PA on day 5 was not due to exercise, but was a continuation of the gradual decrease in urinary 4PA observed between days 1 to 4.

TABLE 4. Effect of vitamin B_6 supplementation on urinary excretion of 4PA (μmoles/24 hr)

Day	Vitamin B_6 Supplement (μ moles)	Total Intake (μ moles)	Subjects 1	2	3	4	5	Mean ± St Dev.
M 1	0[1]	7.92[2]	7.48	7.37	6.69	5.16	4.13	6.17 ± 1.47
W 3	0	7.92	7.78	6.23	6.23	3.91	5.07	5.84 ± 1.45
Th 4	0	7.92	7.43	5.84	6.35	4.30	4.79	5.74 ± 1.25
F 5[3]	0	7.92	7.17	5.85	6.00	3.51	4.20	5.35 ± 1.47
Sa 6	29.55	-	18.65	20.31	21.04	13.78	15.68	17.89 ± 3.09
Su 7	29.55	-	-	-	-	-	-	- -
M 8	29.55	37.47	25.14	18.12	25.43	21.76	15.07	21.10 ± 4.49
Tu 9	0	7.92	9.55	10.57	10.21	7.23	10.89	9.69 ± 1.46
W 10	29.55	37.47	23.37	16.91	22.66	19.64	22.30	20.98 ± 2.68
Th 11	0	7.92	-	9.87	9.71	6.41	9.08	8.77 ± 1.61
F 12	29.55	37.47	28.42	23.98	23.92	17.22	13.26	21.36 ± 6.04
Sa 13	29.55	-	25.27	21.58	25.58	-	-	24.14 ± 2.23
Su 14	29.55	-	-	-	-	-	-	- -
M 15	29.55	37.47	27.49	26.94	25.53	20.30	22.97	24.65 ± 3.00
Tu 16	11.82[4]	19.74	19.13	19.17	16.32	14.33	13.51	16.49 ± 2.63
W 17	29.55	37.47	21.07	-	25.93	19.92	24.20	22.78 ± 2.77
Th 18	11.82	19.74	15.17	16.08	18.52	13.34	15.96	15.81 ± 1.87
F 19	29.55	37.47	27.74	23.51	27.38	17.63	24.04	24.06 ± 4.07
Sa 20	29.55	-	-	-	-	-	-	- -
Su 21	29.55	-	-	-	-	-	-	- -
M 22	29.55	37.47	28.61	25.01	25.19	21.84	24.82	25.09 ± 2.40
T 23	8.93[5]	16.85	16.72	17.47	13.63	11.74	18.69	15.65 ± 2.87
W 24	29.55	37.47	24.62	25.06	22.66	22.76	22.26	23.47 ± 1.27
Th 25	12.53[6]	20.45	16.97	15.80	16.08	14.70	14.71	15.65 ± 0.97
F 26	29.55	37.47	28.47	25.17	25.58	23.15	24.57	25.39 ± 1.95
Sa 27	29.55	-	21.70	25.38	9.33	21.82	25.33	20.71 ± 6.61
Su 28	29.55	-	-	-	-	-	-	- -
M 29	29.55	37.47	27.45	28.90	21.65	20.06	23.99	24.41 ± 3.75
Tu 30	9.04[7]	16.96	-	14.89	11.44	10.55	12.00	12.22 ± 1.88
W 31	29.55	37.47	28.23	24.52	22.24	22.67	25.60	24.65 ± 2.42
Th 32	9.28[8]	17.20	14.83	13.60	12.68	12.27	14.29	13.53 ± 1.07
F 33[3]	0	7.92	12.37	9.81	10.02	8.52	10.43	10.23 ± 1.39

[1] From day 1 to 5: Adjustment period, no vitamin B_6 supplement

[2] Constant diet containing 7.92 μ moles of vitamin B_6

[3] Exercise days

[4] 2 mg crystalline PN supplement

[5] 633 g banana containing 8.93 μ moles of vitamin B_6

[6] 333 g filberts containing 12-53 μ moles of vitamin B_6

[7] 330 g soybeans containing 9.04 μ moles of vitamin B_6

[8] 666 g of beef sausage containing 9.28 μ moles of vitamin B_6

Vitamin B_6 in constant diet and test foods was assayed with S. uvarum.

Figure 3. Comparison of daily excretion of urinary 4PA and vitamin B_6 in subjects receiving supplementary PN and loading doses of crystalline PN and of test foods.

[1] 0 μmoles vitamin B_6 supplement

[2] 11.82 μmoles vitamin B_6 supplement

[3] 8.93 μmoles vitamin B_6 from banana

[4] 12.53 μmoles vitamin B_6 from filberts

[5] 9.04 μmoles vitamin B_6 from soybeans

[6] 9.28 μmoles vitamin B_6 from beef sausage

From day 1 to 5, the subjects received a constant diet containing 7.92 μmoles of vitamin B_6. Except for Tuesdays and Thursdays, the subjects received daily 5 mg of PN in addition to the constant diet.

The broken line indicates mean value for day 27 excluding data from subject 3.

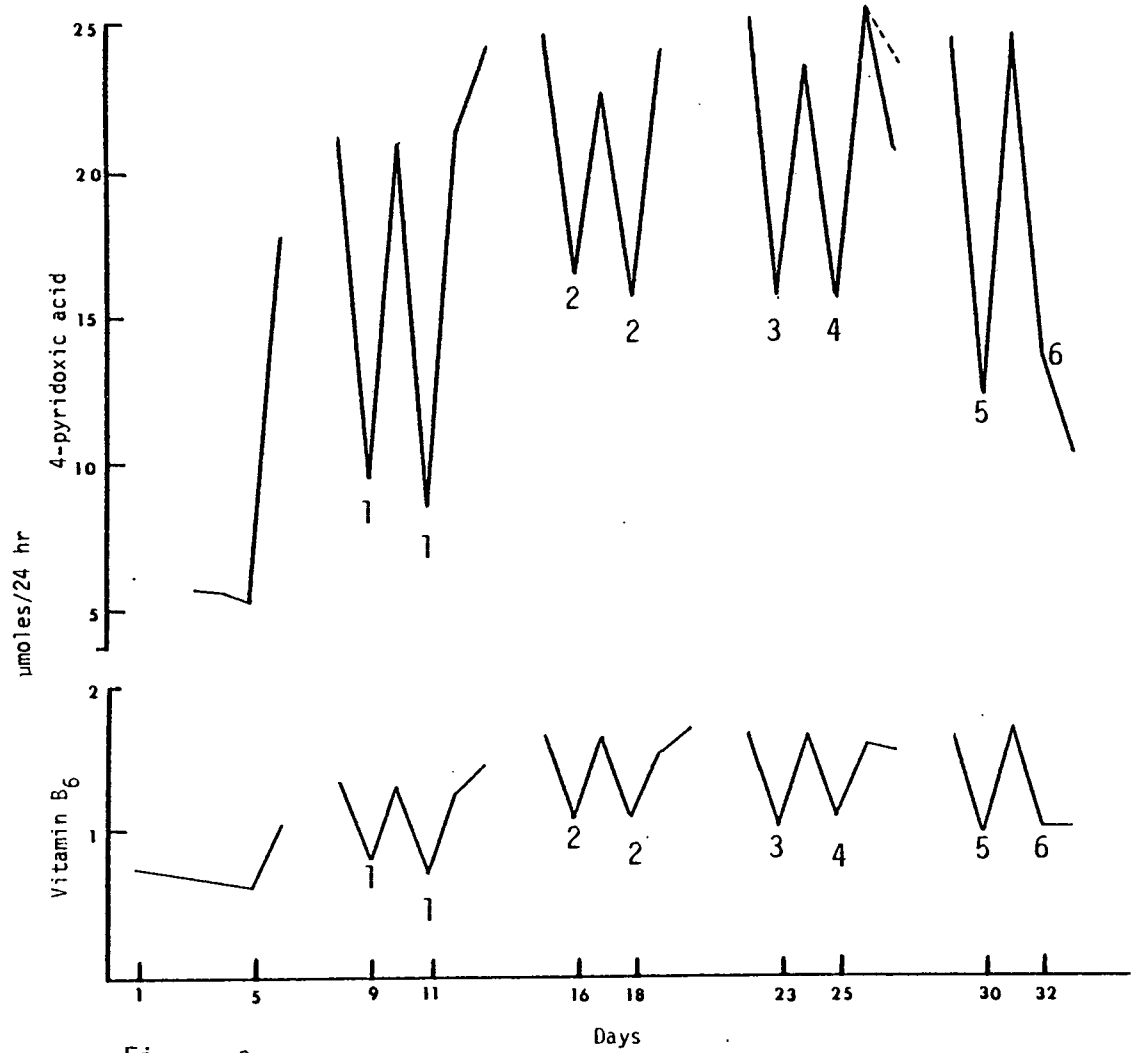
µmoles/24 hr
4-pyridoxic acid
Vitamin B_6
25
20
15
10
5
2
1
1 1 2 2 3 4 5 6
1 5 9 11 16 18 23 25 30 32
Days

Figure 3

On the first day of 5 mg PN supplementation, day 6, the subjects' mean urinary excretion of 4PA increased approximately three-fold (Table 4, Fig. 3). By day 13, after one week of 5 mg PN supplementation, the mean 4PA excretion values were four-fold greater than those during days 1 to 5 when no supplement was given. On days 15, 17, and 19, the days on which the subjects received the 5 mg PN supplement, 4PA excretion reached a plateau and remained relatively constant for the days that the 5 mg PN supplement was given. This suggests that the subjects' tissues were saturated with vitamin B_6, which was the state we desired to study bioavailability in the different test foods.

Unexpectedly, because of the 5 mg supplement on the previous day, on day 27 (end of week 4), there is a decrease in mean urinary 4PA excretion. On that day, subject 3 excreted only 9.33 μmoles of 4PA, while the other four subjects were excreting 4PA in the range of 21 to 25 μmoles/day. It is possible that subject 3 did not take his 5 mg PN supplement on that day. However, even if only the other four subjects are considered, there is still a slight decrease in urinary 4PA on day 27 (Saturday) which is similar to the excretion obtained on day 24 (Wednesday after banana dose).

When no PN supplement was given on days 9 and 11, Tuesday and Thursday of week 2, the subjects' urinary excretion of 4PA dropped, but not to the levels they had excreted during the first days of this study when no PN supplement was administered. This "carry-over" effect of 4PA excretion was not as great on Thursday as on

Tuesday, probably due to the fact that no supplement was administered on the preceding Tuesday.

When the 2 mg PN supplement was given on days 16 and 18, Tuesday and Thursday of week 3, the subjects' pattern of 4PA excretion was similar to that of the preceding week, i.e., slightly less 4PA was excreted on Thursday than on Tuesday. The subjects excretion of 4PA was higher in response to 2 mg of PN than in response to 0 mg PN. Discussion on the excretion of 4PA in response to the food test doses containing approximately 2 mg of vitamin B_6 follows.

Urinary Vitamin B_6 and 4PA

In general, the daily excretion of urinary vitamin B_6 and 4PA followed the same pattern (Fig. 3). Changes in urinary 4PA in response to 0 and 2 mg of PN and the food doses were more marked than those of urinary vitamin B_6, showing that urinary 4PA is more sensitive to changes in vitamin B_6 intake than urinary vitamin B_6. On the days 5 mg of PN were given, both urinary vitamin B_6 and 4PA reached a plateau at the same time, by day 19. The decrease noticed in the excretion of 4PA on day 27 was also observed in urinary vitamin B_6 excretion.

Since urinary 4PA and vitamin B_6 excretion had reached a plateau for the days 5 mg PN was administered, it is likely that the subjects were saturated with vitamin B_6 at that point. Black,

Guirard and Snell (1977) found that both muscle phosphorylase and total muscle vitamin B_6 increased in rats who were fed 10 times the recommended level of vitamin B_6.

Table 5 shows the percent of vitamin B_6 intake excreted as urinary vitamin B_6 and 4PA on selected days at the beginning and end of this study. Together urinary vitamin B_6 and 4PA accounted for approximately 75 percent of the vitamin B_6 ingested on day 5 (67.5 ± 18.6 percent as 4PA and 7.7 ± 1.8 percent as vitamin B_6), and for approximately 72 percent of the vitamin ingested on day 26 of week 4 (67.8 ± 5.2 percent as 4PA and 4.3 ± 1.0 percent as vitamin B_6). The 4PA excretion of days 5 and 26 was approximately 8 and 15 times higher, respectively, than urinary vitamin B_6. During days 1 to 5, fecal vitamin B_6 would have accounted for a large percentage of the remaining dietary vitamin B_6. Unidentified urinary metabolites not measured with the microbiological assay method would account for some additional vitamin B_6. Probably greater quantities of unidentified metabolites were excreted during the 5 mg PN supplementation period.

According to Leklem et al. (1980), 4PA accounted for approximately 35-40 percent of the total dietary vitamin B_6 and urinary vitamin B_6 for approximately eight percent. Wozenski et al. (1980) reported a decrease from 63 to 35 percent of the vitamin B_6 intake excreted as 4PA following doses from 0.5 through 10 mg of PN, even though the mean excretion of 4PA had increased from 5.6 to 19 μmoles/24 hr. In the present study, the higher percentage of 4PA excretion during

TABLE 5. Effect of PN Supplementation on the Percent of Vitamin B_6 Intake Excreted as Urinary Vitamin B_6 and 4PA.

Day	Total intake Vit. B_6				Total percent excretion
	7.92 μ moles		37.47 μ moles		
	4PA	Vit. B_6	4PA	Vit. B_6	
Day 5[1]	67.5± 18.8	7.7± 1.8			75.2
Day 26[2]			67.8± 5.2	4.3± 1.0	72.1

[1]Subjects received no PN supplement on days 1 to 5

[2]Subjects received 5 mg PN for 3 weeks in addition to the constant diet

days 1 to 5 may reflect the subjects' previous vitamin B_6 intake. The higher percentage of 4PA excretion by the subjects after a period of 5 mg supplementation may reflect the effect of vitamin B_6 saturation on the excretion of 4PA. In view of the great capacity of the small intestine to absorb PN (Booth and Brain, 1962), we expect that PN saturated subjects in this study excreted a lower percentage of the total vitamin B_6 intake in feces than that stated by Leklem et al. (1980), around 30 percent.

Excretion of 4PA after 2 mg Crystalline PN Doses

Table 7 presents urinary 4PA excretion during the 10 and subsequent 14 hr after the 0 and 2 mg PN supplements. These values represent means for each subject for the two days they received the 0 and 2 mg PN doses. The yield was obtained by subtracting each subject's mean excretion during each time period after 0 mg PN from the mean excretions during these two periods after the 2 mg supplement. Except for subject 2, the subjects excreted more 4PA during the first 10 hr after the 2 mg dose than during the subsequent 14 hr. These results agree with those reported by Wozenski et al. (1980) who found that in response to doses from 0.5 to 10 mg of PN subjects excreted most of the 4PA between 3 and 8 hr after ingesting the doses.

When 2 mg of crystalline PN were administered on Tuesday and Thursday of week 3, the total yield of 4PA in subjects 1 to 4 was

TABLE 6. Yield of 4-pyridoxic Acid Excretion (μ moles/24 hr)[1]

	Subjects						
	1	2	3	4	5	Mean ±	St Dev.
Crystalline PN[2]	7.60	7.40	7.46	7.01	4.75	6.84 ±	1.19
Banana[3,4]	9.50	9.60	4.86	6.52	11.59	8.41 ±	2.69
Filberts	7.00	5.26	5.77	7.43	4.46	5.98 ±	1.23
Soybeans	-	6.10	1.93	4.88	2.63	3.88 ±	1.94
Beef sausage	6.73	4.31	3.46	6.94	5.48	5.38 ±	1.51

[1]Urine values of 24 hr were used because the test loading doses were consumed throughout the day

[2]Yield was calculated by substracting the 24 hr excretion values from the mean value of the days when the subjects received the 2 mg dose of crystalline PN minus the mean excretion value of the days they received the 0 mg dose of the vitamin

[3]The yield was obtained by substracting the 24 hr excretion of 4 PA values when the subjects received the test loading doses minus the mean excretion value of the days they received the 0 mg dose of the vitamin.

[4]The yield of 4 PA in response to test foods was adjusted from 1.51, 2.12, 1.53 and 1.57 of vitamin B_6 of bananas, filberts, soybeans, and beef sausage, respectively to 2 mg of vitamin B_6 in order to compare with the yield values from crystalline PN.

TABLE 7. Urinary Excretion[1] of 4 PA in μ moles in Vitamin B_6 Supplemented Subjects Who Received 0 and 2 Mg PN Doses

Urine	0 mg. Supplementation[2]					2 mg. Supplement[3]				
	Subject									
	1	2	3	4	5	1	2	3	4	5
10 hr	4.44	4.27	4.32	2.97	4.48	10.23	6.87	9.09	6.91	8.08
14 hr	6.42	5.95	5.63	3.85	5.50	7.76	10.75	8.33	6.92	6.65

Yield	Subject				
	1	2	3	4	5
10 hr	5.79	2.60	4.77	3.94	3.60
14 hr	1.34	4.80	2.70	3.07	1.15

[1]Urine collected during 10-hr period immediately followed the administration of the 0 and 2 mg. doses, and during the subsequent 14 hr. period.

[2]Mean values of urine collected on Tuesday and Thursday of week 2, the first week the subjects received 5 mg PN on M, W and F. The subjects received a constant diet containing 7.92 μ moles of vitamin B_6.

[3]Mean values of urine collected on Tuesday and Thursday of week 3, after two weeks of 5 mg supplementation.

very similar, in the range of 7.01 to 7.60 μmoles/24 hr (refer to Table 6) whereas subject 5 excreted only 4.75 μmoles/24 hr. This subject had excreted the least 4PA at the beginning of the study (4.13 μmoles/24 hr) whereas the others excreted from 5.16 to 7.48 μmoles/24 hr (Table 4).

The bioavailability of crystalline PN was assumed to be 100 percent. Thus the yield of 4PA of 2 mg PN dose in saturated subjects was assumed to reflect the 100 percent bioavailability.

Bioavailability of vitamin B_6 from Test Foods

The bioavailability of vitamin B_6 from the test foods used in this study was measured in 24-hr urine samples rather than in 10- and 14-hr ones. This was done because the food loading doses were consumed throughout the day. The 2 mg crystalline PN doses during week 3 were taken all at one time before breakfast. The rate of digestion and passage through the gastrointestinal tract was shorter for crystalline PN than for vitamin B_6 from foods. In response to the test foods, more variation in urinary 4PA was noticed than after the 2 mg crystalline PN doses, due to differences in composition of the foods selected, rate of digestion, etc. These differences may also have affected the metabolism of vitamin B_6, and subsequently the excretion of 4PA.

The bioavailability of vitamin B_6 from test foods is presented in Table 8 and Figure 4. When banana was consumed, there was more

TABLE 8. Percentage of 4-Pyridoxic Acid Excretion From Test Foods[1]

	Subjects					
	1	2	3	4	5	Mean ± St Dev.
Crystalline PN[2]	100	100	100	100	100	100
Banana	125.0	129.7	65.1	93.0	244.0	131.4 ± 68.18
Filberts	92.1	71.1	77.3	106.0	93.9	88.1 ± 13.9
Soybeans	-	82.4	25.9	69.6	55.4	58.3 ± 24.3[3]
Beef Sausage	88.5	58.2	46.4	99.0	115.4	81.5 ± 28.6

[1]Values for 4 PA excreted after ingestion of test doses of foods were adjusted to 2 mg of vitamin B_6.

[2]The excretion of 4 PA in response to 11.822 μ moles (2 mg) of PN is assumed to be 100%.

[3]t test showed significant difference between urinary 4PA excretion from soybean ($p < 0.05$) as compared to 4PA excretion from crystalline.

Figure 4. Percent bioavailability of vitamin B_6 from test food loading doses as compared to 100 percent bioavailability of crystalline PN in subjects saturated with vitamin B_6. Numbers on bar represent subject number. Last bar for each food represents the mean ± standard deviation.

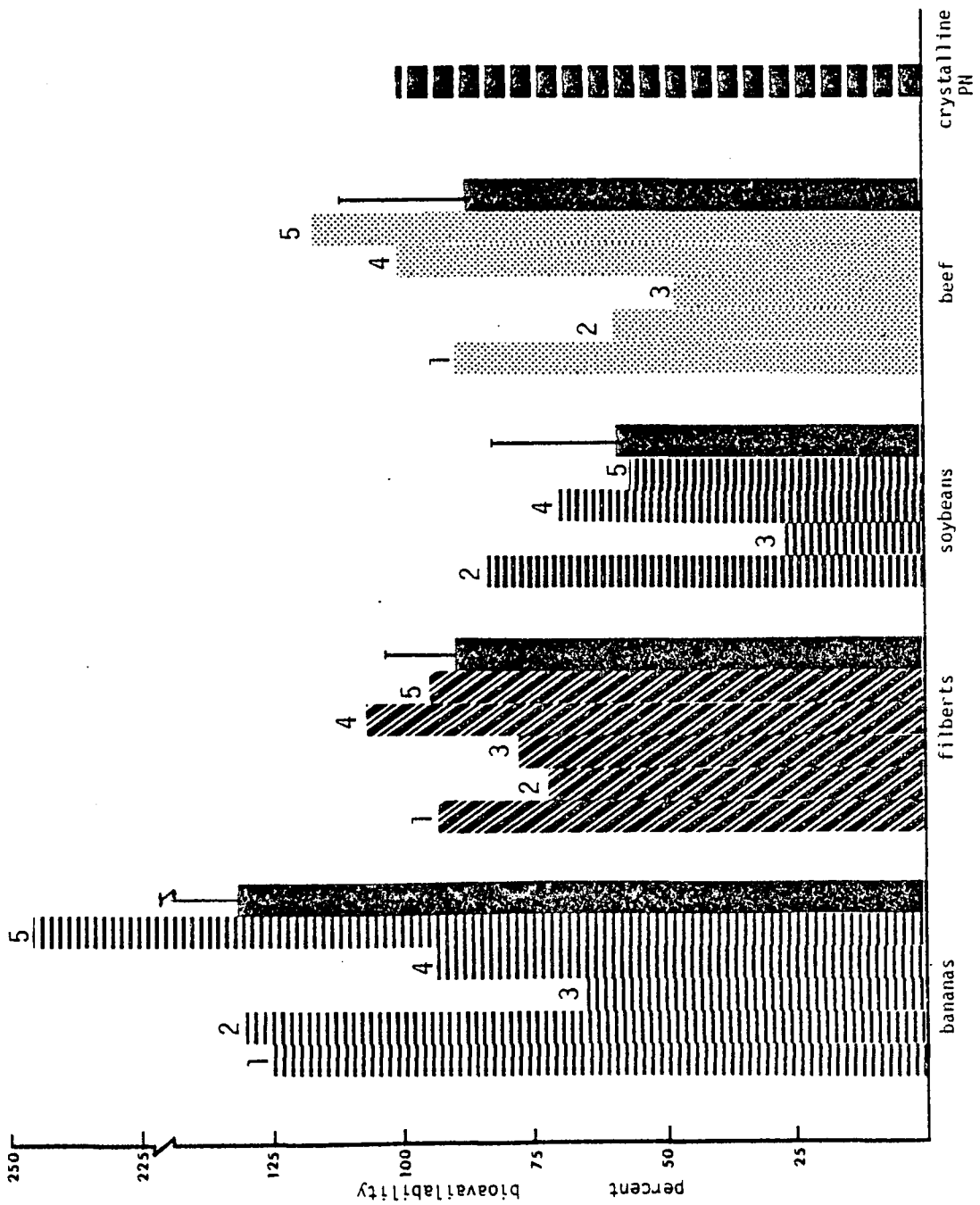
bioavailability
percent
250
225
125
100
75
50
25
bananas
filberts
soybeans
beef
crystalline PH
1
2
3
4
5

Figure 4

than a three-fold variation among subjects in the excretion of 4PA. The mean 4PA excretion on the day banana was administered is extremely high. Compared to the 100 percent bioavailability of vitamin B_6 from 2 mg crystalline PN, the bioavailability of vitamin B_6 from the banana loading dose was higher than 100 percent for subjects 1, 2 and 5, the last subject with a value of 244 percent bioavailability. Subjects 3 and 4, respectively, excreted 65.1 and 93 percent of the vitamin B_6 from bananas.

The bioavailability of vitamin B_6 from filberts appeared to be relatively high, with values ranging from 71.1 to 106.0 percent and a mean of 88.1 ± 13.9 percent. The bioavailability of vitamin B_6 from filberts in subject 4 was slightly higher than 100 percent as compared to the excretion from crystalline PN, and subject 2 excreted 71.1 percent as 4PA. (This lowest value for filberts is higher than the lowest values for other foods tested.) Excretion of 4PA following the test dose filberts was the least variable of that from the other test foods.

Among the four foods tested, soybeans had the lowest bioavailability of vitamin B_6. Urinary 4PA was significantly lower after soybeans than after the 2mg crystalline PN supplement ($p < 0.05$, t test). Among subjects 2 to 5, there was a three-fold difference in percent bioavailability of vitamin B_6 from soybeans. (Subject 1 is not included due to an incomplete urine collection on that day.) Subject 3 excreted only 25.9 percent of vitamin B_6 as 4PA, which is the lowest excretion value for any of the test foods.

When beef was consumed, 4PA excretion by subjects 3, 4 and 5 was higher than after the soybeans. With beef, about a two-fold difference in vitamin B_6 bioavailability was observed. The mean percent of vitamin B_6 bioavailability of beef for the five subjects was 81.5 ± 28.6, and when soybeans was consumed the mean for the four subjects was 58.3 ± 24.3 percent. Subject 2 had a lower 4PA excretion from beef than from soybeans.

Factors Affecting the Bioavailability of Vitamin B_6

Various factors can affect the bioavailability of vitamin B_6. In general, the amount of protein in the diet can affect the levels of various vitamin B_6 parameters used to measure vitamin B_6 bioavailability. Diets high in protein have been reported to decrease 4PA excretion as well as vitamin B_6 (Miller and Leklem, 1978). According to the values in Handbook No. 8 (Agricultural Research Service, 1975), the quantity of protein from the cooked soybeans was approximately three times higher than that in the filberts. Since there was more protein in the soybean dose than in the filberts dose, the low bioavailability of vitamin B_6 from soybeans may be inaccurate due to the effect of dietary protein on 4PA excretions.

Dietary fiber may decrease the bioavailability of vitamin B_6. Leklem et al. (1980) examined the effects of three different types of bread on the bioavailability of vitamin B_6 in adult men. They

found a slightly lower bioavailability of vitamin B_6 from whole wheat bread than from white bread, either enriched with vitamin B_6 or supplemented with PN,and suggested that the fiber in whole wheat bread lowered the bioavailability of vitamin B_6. Lindberg (1979) explored the effect of wheat bran on the bioavailability of vitamin B_6 in another group of men. She also found that wheat bran significantly reduced the bioavailability of vitamin B_6. Nguyen and his colaborators, however, found no relationship between fiber and the reduction of bioavailability of vitamin B_6 *in vitro* (Nguyen et al., 1981) and *in vivo* studies in chicks and rats (Nguyen, Gregory and Damron, 1981).

If fiber influences vitamin B_6 bioavailability, this explains in part the better utilization of vitamin B_6 from beef than from soybeans. Beef contains no fiber while soybeans do. On the other hand, filberts also contain fiber. If high fiber content is a factor in vitamin B_6 bioavailability, we would also expect a lower bioavailability of vitamin B_6 from filbert than from beef. However, only subject 5 had better bioavailability from beef than from filberts, whereas the other four subjects showed a better vitamin B_6 bioavailability from filberts than from beef.

If fiber makes little or no difference, reasons for the better bioavailability of vitamin B_6 from filberts may be due to other factors such as increased transit time due to their fat content of fat, better digestibility, biological individualities, etc.

Digestibility in conjunction with the other components in

foods may be responsible for the relatively low or high vitamin B_6 bioavailability. The coefficient of digestibility of nuts and legumes is 78 percent, and that of fruits and meat, 85 and 95 percent, respectively (Agriculture Handbook No. 8, 1975). The coefficient of digestibility is higher for meat than for soybeans and this may be a contributor for the better utilization of vitamin B_6 from beef. Ganong (1979) observed a higher nitrogen excretion with a diet high in soybeans than with one high in beef. The results from the present experiment agree with those obtained by Leklem, Shultz and Miller (1980) who also reported lower vitamin B_6 bioavailability from soybeans than from beef in a long-term study with adult men.

The fat content of beef versus filberts must also be considered. Although filberts contain 62.4 percent fat (Agricultural Research Service, 1975) and beef bologna approximately 30 percent (Meat Science Laboratory, O.S.U.), the total fat content in these two food doses was comparable. Since fat delays stomach emptying, absorption of vitamin B_6 may be more efficient in the proximal small intestine.

On the other hand, the coefficient of protein digestibility for banana is lower than that of beef, and the bioavailability of vitamin B_6 from bananas was higher than that from beef. The sugar content of the bananas may contribute to the better utilization of the vitamin B_6. Nelson et al. (1976) reported enhanced vitamin B_6 absorption

from a synthetic solution with added glucose than from natural orange juice. Other substances in the bananas also may have increased absorption of the vitamin B_6. Additional explanations for the apparently higher bioavailability of vitamin B_6 from bananas include possibly interfering fluorescent substances in bananas which were not adsorbed on the ion-exchange resins during column chromatography, and an increased vitamin B_6 content of bananas between the time of analysis and their consumption as a test dose. The predominant form of vitamin B_6 in bananas is PN (Orr, 1969).

CONCLUSIONS AND EVALUATION

Determining the bioavailability of vitamin B_6 by measuring the excretion of 4PA in response to test foods by subjects saturated with vitamin B_6 appears to be a promising method and should be explored further. The relative values for bioavailability of vitamin B_6 from beef versus soybeans was similar to that obtained in a longer term study in subjects receiving a normal level of vitamin B_6 intake (Leklem, Shultz and Miller, 1980).

The advantage of this procedure for measuring vitamin B_6 bioavailability are: it appears to be reliable for assessing bioavailability of the vitamin; it is less time-consuming and cheaper; urine is easy to obtain and handle; 4PA is stable and a sensitive indicator of vitamin B_6 intake and bioavailability.

The disadvantages are that the quantities of test foods used in this investigation were larger than those that people would usually consume, and that the gastrointestinal tract has not had time to adjust to the quantity and the composition of some of the test foods.

Criticisms of the research presented in this thesis were that the subjects had not attained complete saturation of their tissues with vitamin B_6 when the 0 mg dose was administered, and that the 2 mg crystalline should not be taken at one time, but in divided doses similar to those of the test foods.

For further experiments we recommend that more subjects be

tested if possible, and that several doses of crystalline PN loading doses, i.e., 0.5 and 1 mg of PN be administered in addition to the 0 and 2 mg doses. For interpretation of the data, composition of the food test doses should be determined in the laboratory, including forms of vitamin B_6, fat, carbohydrates, nitrogen and fiber content.

BIBLIOGRAPHY

Agricultural Research Service. (1975) Agriculture Handbook No. 8, Composition of foods. USDA, Washington, D.C.

A.O.A.C. (1980) Official methods of analysis, 13th ed., pp. 468-469. Association of Official Analytical Chemists, Washington, D.C.

Baker, E. M.; Canham, J. E.; Nunes, W. T.; Sauberlich, H. E. and McDowell. (1964). Vitamin B_6 requirement for adult men. Am. J. Clin. Nutr. 15, 59-66.

Bauernfeind, J. C. and Miller, O. N. (1978) Vitamin B_6: Nutritional and Pharmaceutical Usage, Stability, Bioavailability, Antagonists, and Safety. In: Human Vitamin B_6 requirements (Committee on Dietary Allowance), pp. 78-110. National Academy of Sciences, Washington, D.C.

Baysal, A., Johnson, B. A. and Linkswiler, H. (1966) Vitamin B_6 depletion in man: blood vitamin B_6, plasma pyridoxal phosphate, serum cholesterol, serum transaminases and urinary vitamin B_6 and 4-pyridoxic acid. J. Nutr. 89, 19-23.

Black, A. L.; Guirard, B. M. and Snell, E. E. (1977) Increased muscle phosphorylase in rats fed high levels of vitamin B_6. J. Nutr. 107, 1962-1968.

Booth, C. C. and Brain, M. C. (1962) The absorption of tritium-labeled pyridoxine hydrochloride in the rat. J. Physiol. 164, 282-294.

Brain, M. C. and Booth, C. C. (1964) The absorption of tritium-labelled pyridoxine hydrochloride in control subjects and in patients with intestinal malabsorption. Gut. 5, 241-247.

Brin, M. (1978) Vitamin B_6: Chemistry, Absorption, Metabolism Catabolism and Toxicity. In: Human vitamin B_6 requirements. (Committee on Dietary Allowances), pp. 1-20. National Academy of Sciences. Washington, D.C.

Buttery, J. E.; Kua, S. L. and DeWitt, G. F. (1975) The orthotoluidine method for blood and urine xylose. Clin. Chem. Acta. 64, 325-328.

Driskell, J. A.; Geders, J. M. and Urban, M. C. (1976) Vitamin B_6 status of young men, women and women using oral contraceptives. J. Lab. Clin. Med. 87, 813-821.

Ganong, C. A. (1979) The effect of soy and beef diets on nitrogen and zinc balance in men. Master's thesis. Oregon State University.

Gregory, J. F. and Kirk, J. R. (1978) Vitamin B_6 in foods: assessment of stability and bioavailability, pp. 72-77. In: Human vitamin B_6 requirements. National Academy of Sciences. Washington, D.C.

Gregory, J. F. and Kirk, J. R. (1981) The bioavailability of vitamin B_6 in foods. Nutr. Rev. 39, 1-8.

György, P. (1971) Developments leading to the metabolic role of vitamin B_6. Am. J. Clin. Nutr. 24, 1250-1256.

Harding, R. S.; Plough, I. C. and Friedmann, T. E. (1959) The effect of storage on the vitamin B_6 content of packaged army ration, with a note on the human requirement for the vitamin. J. Nutr. 68, 323-331.

IUPAC-IUB Commission on Biochemical Nomenclature. (1974) Nomenclature for vitamin B_6 on related compounds. Recommendation (1973). Biochem. J. 137, 417-421.

Kabir, Hossein. (1982) Personal communication.

Kelsay, J.; Baysal, A. and Linkswiler, H. (1968) Effect of vitamin B_6 depletion on the pyridoxal, pyridoxamine and pyridoxine content of the blood and urine of men. J. Nutr. 94, 490-494.

Leklem, J. E.; Miller, L. T.; Perera, A. D. and Peffers, D. E. (1980) Bioavailability of vitamin B_6 from wheat bread in humans. J. Nutr. 110, 1819-1828.

Leklem, J. E.; Shultz, T. D. and Miller, L. T. (1980) Comparative bioavailability of vitamin B_6 from soybeans and beef. Fed. Proc. 39, 1558.

Lewis, J. S. and Nunn, K. P. (1977) Vitamin B_6 intakes and 24-hr 4-pyridoxic acid excretion of children. Am. J. Clin. Nutr. 30, 2023-2027.

Lindberg, A. S. (1979) The effect of wheat bran on the bioavailability of vitamin B_6 in humans. M.S. thesis, Oregon State University.

Lumeng, L. and Li, T. K. (1980) Mammalian vitamin B_6 metabolism: Regulatory role of protein-binding and the hydrolysis of pyridoxall phosphate in storage and transport. In: Vitamin B_6 metabolism and role in growth (George Tryfiates, ed.), pp. 27-51. Foods and Nutrition Press.

Lumeng, L.; Lui, A. and Li, T. K. (1980) Plasma content B_6 vitamers and its relationship to hepatic vitamin B_6 metabolism. J. Clin. Invest. 66, 688-695.

Mangay Chung, A. S.; Pearson, W. N.; Darby, W. J.; Miller, O. N. and Goldsmith, G. A. (1961) Folic acid, vitamin B_6, pantothenic acid and vitamin B_{12} in human dietaries. Am. J. Clin. Nutr. 9, 573-582.

McCormick, D. B., Gregory, M. E. and Snell, E. E. (1961) Pyridoxal phospho-kinase assay, distribution, purification, and properties. J. Biol. Chem. 236, 2076-2084.

McCormick, D. B. and Merril, A. H. (1980) Overview of metabolism of vitamin B_6 compounds. In: Vitamin B_6 metabolism and role in growth (George Tryfiates, ed.), p. 3.

Meat Sciences Laboratory, O.S.U. (1979) Personal communication.

Middleton, H. M. (1977) Uptake of pyridoxine hydrochloride by the rat jejunal mucosa in vitro. J. Nutr. 107, 126-131.

Middleton, H. M. (1982) Characterization of pyridoxal 5 phosphate disappearance from in vivo perfused segments of rat jejunum. J. Nutr. 112, 209-275.

Miller, L. T. and Edwards, M. (1981) Microbiological assay of vitamin B_6 in blood and urine. In: Methods in vitamin B_6 nutrition. Analysis and Status Assessment (J. Leblem and R. Reynolds, eds.), pp. 45-55. Plenum Press, New York and London.

Miller, L. T. and Leklem, J. E. (1978) Effect of dietary protein on metabolism of vitamin B_6. Fed. Proc. 37, 449.

National Academy of Sciences, National Research Council (1974). Recommended Dietary Allowances, 8th ed., Washington, D.C.

National Academy of Sciences, National Research Council (1980). Recommended Dietary Allowances, 9th ed., Washington, D.C.

Nelson, E. W.; Lane, H. and Cerda, J. J. (1976) Comparative human intestinal bioavailability of vitamin B_6 from synthetic and natural source. J. Nutr. 106, 1433-1437.

Nguyen, L. B.; Gregory, J. F.; Burgin, C. W. and Cerda, J. J. (1981) In vitro binding of vitamin B_6 by selected polysaccharides, lignin and wheat bran. J. Food Sci. 1860-1862.

Nguyen, L. B.; Gergory, J. F. and Damron, B. L. (1981) Effects of selected polysaccharides on the bioavailability of pyridoxine in rats and chicks. J. Nutr. 111, 1403-1410.

Orr, M. L. (1969) Pantothenic acid, vitamin B_6 and vitamin B_{12} in foods. Home Economics Research Report No. 36. Agricultural Research Service. U.S. Department of Agriculture. Washington, D.C.

Pino, S., Benotti, J. and Gardyna, H. (1965) An automated method for urine creatinine which does not require a dialyzer module. Clin. Chem. 11, 664-666.

Rabinowitz, J. C. and Snell, E. E. (1949) Vitamin B_6 group. XV. Urinary excretion of pyridoxal, pyridoxamine, pyridoxine and 4-pyridoxic acid in human subjects. Proc. Soc. Exp. Biol. Med. 70, 235-240.

Reddy, S. K.; Reynolds, M. S. and Price, J. M. (1958) The determination of 4-pyridoxic acid in human urine. J. Biol. Chem. 233, 691-696.

Sauberlichn, H. E.; Canham, J. E.; Baker, E. M.; Raica, N. and Herman, Y. F. (1972) Biochemical assessment of the nutritional status of vitamin B_6 in the human. Am. J. Clin. Nutr. 25, 629-642.

Sauberlichn, H. E. and Canham, J. E. (1980) Vitamin B_6. In: Modern Nutrition in Health and Disease (Goodhart, R. S. and Shils, M. E., eds.), 6th ed., pp. 216-229. Lea & Febiger, Philadelphia.

Schwartz, R. and Kjeldgaard, N. O. (1951) The enxymatic oxidation of pyridoxal by liver aldehyde oxidase. Biochem. J. 48, 333-337.

Snell, E. E.; Guirard, B. M. and Williams, R. S. (1942) Occurrence in natural products of a physiologically active metabolite of pyridoxine. J. Biol. Chem. 143, 519-5 .

Snell, E. E. and Haskell, B. E. (1971) Metabolism of water soluble vitamins. Compr. Biochem. 21, 47-71.

Stalunovic, M.; Jeremic, V.; Leskovac, V. and Chaykin, S. (1976) New pathway of conversion of pyridoxal to 4-pyridoxic acid. Enzyme. 21, 357-369.

Synderman, S. E.; Holt, L. E.; Carretero, R. and Jacobs, K. (1952) Pyridoxine deficiency in the human infant. Am. J. Clin. Nutr. 1, 200-207.

Tamura, T. and Stokstad, E. L. R. (1973) The availability of food folate in man. Brit. J. Haematol., pp. 513-532.

Tarr, J. B.; Tamura, T. and Stokstad, E. L. R. (1981) Bioavailability of vitamin B_6 and pantothenate in an average American diet in man. Am. J. Clin. Nutr. 34, 1328-1337.

Tsuji, T.; Yamada, R. and Nose, Y. (1973) Intestinal absorption of vitamin B_6. I. Pyridoxal uptake by rat intestinal tissue. J. Nutr. Sci. Vitaminol. 19, 401-417.

Wada, H. and Snell, E. E. (1961) The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. J. Biol. Chem. 236, 2989-2095.

Wang, A. N. G. (1982) The effect of pyridoxine supplementation on erythrocyte aminotransferase activity in man. M.S. thesis, Oregon State University.

Wang, K. (1982) Personal communication.

Wozenski, J. R.; Leklem, J. E. and Miller, L. T. (1980) The metabolism of small doses of vitamin B_6 in men. J. Nutr. 110, 275-285.

APPENDIX

Appendix I

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-hour urine 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 provided 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_____