AN ABSTRACT OF THE THESIS OF

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Title: THE METABOLISM OF VITAMIN B6 IN HUMANS AND GUINEA PIGS

Abstract approved by: Lorraine T. Miller

The purposes of the research presented in this thesis were: (1) to determine the precision with which it is possible to measure changes in vitamin B6 compounds in the blood and urine following oral doses of levels of vitamin B6 (as pyridoxine) which are in the range of the normal daily intake of this vitamin; (2) to compare the effect of three free forms of vitamin B6 (PL, PM, PN) at these same levels using the same assays; and (3) to compare the response of guinea pigs to that of humans when the animals are given three free forms of vitamin B6 at physiological levels.

The effect of small incremental doses of pyridoxine (PN) (0.5 - 10 mg) and of equimolar doses of PN, pyridoxamine (PM) and pyridoxal (PL) were studied in five healthy young men. On the day before and during the day of the dose, the subjects were on a controlled diet that supplied 1.6 mg of vitamin B6 each day. During

other days of the week, the subjects were on self-selected diets.

Timed blood and urine samples were obtained on the day each dose was administered. The parameters measured were: plasma vitamin B6 (PB6), plasma pyridoxal phosphate (PLP), urinary vitamin B6 (UB6) and urinary 4-pyridoxic acid (4PA). Variables reflecting the response to each dose for each of these parameters were calculated in two ways; (1) the percent increase of the maximal post-response value over the pre-response value; and (2) the area under the curve bounded by the values obtained and the times of the samples.

For all eight of the variables so calculated, the relationship to the PN doses given were linear in the 0.5 to 10 mg range. Maximal levels of plasma PLP and PB6 were reached at 1/2 hr after the dose for the 0.5, 1, 2, and 4 mg levels of PN. At the 10 mg level, plasma B6 peaked at 1/2 hr for 3 subjects and at 1 hr for 2 subjects. Plasma PLP peaked at 1 hr following the 10 mg PN dose. PB6 was much more responsive to the loading doses than was PLP. The PB6:PLP ratio was maximal at 1/2 hr following the doses. Maximal values of urinary 4PA and UB6 were found in the first 3 hr after the dose. The ratio 4PA:UB6 decreased with increasing PN dose levels and varied for each collection period following the dose.

The same variables were calculated for the study of a comparison of 19.44 μmole doses of PN, PM and PL. The PB6 peaks

occurred at 1/2 hr for PL and PN, and at 1 hr for PM. The PLP peaks occurred at the following times: PN, 1/2 hr; PM, 3 hr; and PL, 1 hr. Maximal levels of UB6 and 4PA were reached in the first three hr after the dose for all three forms. The percent increase and area variables were able to distinguish between nearly all the responses to the three forms of vitamin B6 administered at the 19.44 μmole level. The PB6 response was largest following the PL dose, but the PLP levels were lower after PL than after either PM or PN. The 4PA values were highest following the PL dose, indicating that the PL dose was metabolized to 4PA rather than converted to plasma PLP.

Some of the nutrient contents of the self-selected diets were found to be significantly correlated with some response variables. There was no relationship found between either body weight and 4PA excretion on the day before the dose, or between ascorbic acid intake on the self-selected diets and 4PA excretion on the day before the dose.

Strenuous exercise was found to significantly affect plasma PLP levels in subjects who had received the loading doses of PN.

In another study, three groups of guinea pigs were each given their Recommended Dietary Allowance of vitamin B6 as PM, PL or PN. The same parameters were measured as for the humans. There were no significant differences between the groups of animals in body

weight, organ weights (spleen, liver, kidney, brain), plasma B6 or PLP, or in formed elements of the blood (hemoglobin, hematocrit, white blood cells, red blood cells). Urinary 4PA and UB6 were significantly higher in animals receiving PN.

The Metabolism of Vitamin B6 in Humans and Guinea Pigs

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ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
P_i	Inorganic phosphorous
PN	Pyridoxine
PNP	Pyridoxine-5'-phosphate
PM	Pyridoxamine
PMP	Pyridoxamine-5'-phosphate
PL	Pyridoxal
PLP	Pyridoxal-5'-phosphate
%PB6	For plasma vitamin B6, the percent increase of the highest level for the day over the lowest level for the day
%PLP	For plasma pyridoxal phosphate, the percent increase of the highest level for the day over the lowest level for the day
%UB6	For urinary vitamin B6, the percent increase of the highest level for the day over the mean level for the day before
%4PA	For urinary 4-pyridoxic acid, the percent increase of the highest level for the day over the mean level for the day before
PB6AREA	For plasma vitamin B6, the area bounded by the times, response variables obtained, and the lowest level of the response value for that day

UB6AREA	For urinary vitamin B6, the area bounded by the times, response variables obtained, and the lowest level of the response value for that day
4PAAREA	For urinary 4-pyridoxic acid, the area bounded by the times, response variables obtained, and the lowest level of the response value for that day
PLPAREA	For plasma pyridoxal phosphate, the area bounded by the times, response variables obtained, and the lowest level of the response value for that day
UB6TH	For urinary vitamin B6, total 24 hr excretion on the dose day expressed as an hourly average
4PATH	For urinary 4PA, total 24 hr excretion on the dose day expressed as an hourly average
TUB64PA	Total urinary excretion of UB6 and 4PA on the dose day = 24 (UB6TH + 4PATH)
4PA:UB6	4PATH/UB6TH
PB6:PLP	%PB6/%PLP
4PA	4-pyridoxic acid
4PAP	4-pyridoxic acid-5'-phosphate

THE METABOLISM OF VITAMIN B6 IN HUMANS AND GUINEA PIGS

I. INTRODUCTION

The U. S. Western Regional Experiment Stations have a cooperative research project under way, which will investigate nutrient bioavailability (Project W-143). The Nutrition Research laboratory at Oregon State University is responsible for (1) developing methodology on vitamin B6 bioavailability, and (2) comparing the bioavailability of vitamin B6 from different food items.

The study reported here is preliminary to testing the effect of foods. This study focuses on the methodology and the ability to measure the appearance of vitamin B6 compounds in the blood and urine of subjects who had received an oral dose of the crystalline form of each B6 vitamer.

Previous studies on the urinary excretion of vitamin B6 compounds had employed 70-100 mg doses of the vitamin (Rabinowitz and Snell, 1949), or had used smaller doses in subjects previously saturated with vitamin B6 (Brain and Booth, 1964). The methodology available for the measurement of 4-pyridoxic acid (Reddy, Reynolds and Price, 1958) and of plasma pyridoxal phosphate (Peffers, 1977; Appendix C) are more specific and precise than the methods used in these earlier papers. The absorption of small amounts of vitamin

B6 has been measured directly in the jejunum (Nelson, Lane and Cerda, 1976), but blood and urinary levels of vitamin B6 compounds were not determined.

Since the long range goal of the regional project is to measure changes in the availability of normal levels of vitamin B6 with changes in protein and fiber in the diet, it is desirable to be able to measure the effect of doses of vitamin B6 which are comparable to the usual dietary intake.

The purpose of the research presented in this thesis is three-fold:

(1) To determine the precision with which it is possible to measure changes in vitamin B6 compounds in the blood and urine following oral doses of levels of vitamin B6 as pyridoxine which are in the range of the normal daily intake of this vitamin;

(2) To compare the effect of three free forms of vitamin B6 (PN, PM, PL) at these same levels using the same assays; and

(3) To compare the response of guinea pigs to that of humans when the animals are given three free forms of vitamin B6 (PN, PM, PL) at physiological levels.

II. REVIEW OF LITERATURE

Vitamin B6

Importance

Biochemical

Since the definition of the essentiality of vitamin B6 in the rat in 1936 (Harris, 1968), a myriad of functions has been identified for this nutrient. Nearly all of the enzymes for which vitamin B6 is a coenzyme deal with the metabolism of amino acids (Sauberlich, 1968). The α-amino groups of many amino acids are removed by transaminases. Another group of vitamin B6-dependent enzymes are decarboxylases which will form amines from tyrosine, histidine, dihydroxyphenylalanine and tryptophan. Vitamin B6 is also required for the conversion of cysteine to pyruvic acid, and in the synthesis of δ-amino levulinic acid, a porphyrin precursor (Sauberlich and Canham, 1973). The vitamin has been identified as an integral part of phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate. Other vitamin B6-dependent enzymes include oxidoreductases, bacterial isomerases, desulfhydrases and deaminases (Sauberlich, 1968). The vitamin also functions in the bacterial and mold synthesis of tryptophan from indole and serine, in the cleavage of cystathionine to homoserine, and in the formation of aldehydes both through amine oxidation and through aldol formation (Sauberlich,

1968). In all, some 60 enzymes have been found to require vitamin B6. The vitamin has been related to lipid metabolism (Sauberlich and Canham, 1973), although this relationship is complicated by meal feeding in rats (Angel and Song, 1973).

Physiological

The availability and metabolism of vitamin B6 can be affected by many seemingly unrelated factors and physiological conditions. The symptom resulting from vitamin B6 deficiency is a function of the origin of the abnormality (Brown, 1972) and of such factors as age and sex (György, 1964). Paul György has pointed out that vitamin B6 deficiency in young animals and infants appears as convulsions, whereas older subjects develop microcytic sideroblastic anemia. Vitamin B6-dependent convulsions are more common in female infants and vitamin B6-dependent anemia is more common in male adults.

Abnormal vitamin B6 nutriture can result from conditions such as inadequate intake, defective delivery (absorption and transport) alterations in metabolism, excessive loss (excretion or formation of complexes with drugs), increased need (pregnancy, fever, high protein intake), or through apoenzyme defects (Brown, 1972).

Intake. The Recommended Dietary Allowance (RDA) for vitamin B6 is 2.0 mg for adults (National Academy of Sciences, 1974).

Foods which are good sources of vitamin B6 include organ meats, whole grains and cereals, nuts and some fruits and vegetables (banana, pepper, avocado) (U. S. Department of Agriculture, 1969). The occurrence of vitamin B6 deficiency symptoms as a result of poor intake is limited. No world population has been identified as being at great risk due to inadequate intake of vitamin B6 (National Academy of Sciences, 1968).

More recently, Driskell, Geders and Urban (1976) found that 18- to 25-year-old students were consuming more protein and less vitamin B6 than the 1974 Recommended Dietary Allowances. The vitamin B6 intake using a 2-day food record, was 1.81 mg for males and 1.29 mg for females.

Inadequate intake can result from destruction of the vitamin B6 content of the food during processing. This has been shown in milk by Tomarelli, Spence and Bernhart (1955). Convulsive seizures and abnormal electroencephalograms were observed in infants fed an autoclaved commercial milk formula low in vitamin B6. These symptoms were responsive to vitamin B6 therapy (Coursin, 1964).

Delivery. Defective delivery of vitamin B6 can result from abnormal absorption or transport. Malabsorption syndromes will affect the uptake of the vitamin. Most of vitamin B6 is transported in the blood bound to albumin (Lumeng, Brashear and Li, 1974). Pathological conditions which decrease albumin (protein malnutrition,

burns, trauma, steroid treatment, cirrhosis of the liver) could result in a lower amount of circulating vitamin B6. The conversion of PN to PL, two of the B6 vitamers, has been shown to occur in red blood cells (Anderson et al., 1971). A decrease in the number of circulating red cells, therefore, might have a relationship to the distribution of the vitamin.

Excessive Loss. Excessive loss of vitamin B6 can occur within the body in such cases as the use of antagonists or the ingestion of interfering agents. Isoniazid, used in the treatment of tuberculosis, binds to the vitamin (forming an isoniazid-pyridoxal hydrazone) and causes its inactivation. Penicillamine semicarbazide and cycloserine act in a similar manner (Sauberlich and Canham, 1973) forming coenzymatically inactive addition compounds with PL or PLP (Unna and Honig, 1968). Alcohol ingestion has been linked to accelerated hydrolysis of pyridoxal phosphate (Lumeng and Li, 1974). Since liver cirrhosis is also a condition which would decrease the metabolism of vitamin B6, alcoholics may be prone to a deficiency of the vitamin.

Excessive loss of the vitamin may occur through enhanced renal clearance. In this study the diuretic action of caffeine has been examined with respect to its possible effect on the results obtained here.

Relative Deficiencies. A relative deficiency of vitamin B6 could occur when the metabolic demands for the vitamin exceed the intake.

Examples of such conditions of excess demand are pregnancy, fever (Brown, 1972) and high protein intake (Miller and Linkswiler, 1967; Itoh and Okado, 1973). Hunter and Harper (1977) induced several vitamin B6-dependent enzymes with high-protein diets. They believed that the high apoenzyme concentrations would tend to inhibit the depletion of the vitamin.

High intakes of vitamin B6 can also alter metabolism. Cohen et al. (1973) found that high doses of pyridoxine (50 mg/kg diet) resulted in an increase in body and liver weight in rats and in a greater incorporation of cystine into reduced glutathione than into oxidized glutathione. They postulated that the reduced glutathione would play a role in the control of the transsulfuration that occurs in the formation of cystathionine, and that this may be the mechanism by which megavitamin B6 therapy functions in the treatment of homocystinuria. Pyridoxine has also been shown to increase hepatic tyrosine transaminase activity in rats (Green and Gordon, 1963) and cystathionase activity in humans (Cohen et al., 1973).

The use of oral contraceptives may alter vitamin B6 metabolism (Lumeng, Cleary and Li, 1974). The need for vitamin B6 has been linked to several other physiological conditions, among these decreased immune response (Axelrod, 1971) and decreased incidence of dental caries (Hillman, 1964).

Apoenzyme Defects. Another way in which vitamin B6 deficiency might appear, is through an alteration of the affinity of the apoenzyme for the vitamin. This is believed to be the reason for an increased need for vitamin B6 in some inborn metabolic errors (Brown, 1972). Examples of clinical conditions which fall in this category include B6-responsive convulsive seizures in infants (Sauberlich and Canham, 1973), pyridoxine-responsive anemia (Harris, 1964) and in some cases of cystathioninuria (Brown, 1972). Vitamin B6 metabolism is also altered in patients with Down's syndrome (Young, 1973; Brown, 1972).

Metabolism

Definition and Chemistry

Vitamin B6 exists naturally in six different forms: pyridoxine (PN); pyridoxamine (PM); pyridoxal (PL); and the phosphorylated forms of each (PNP, PMP, PLP). The chemical structures of the forms and their interrelationships are depicted in Figure 1. There is little information on the relative biological activity of each of the forms in man (National Academy of Sciences, 1974). In the rat, it was found that the relative activities of PM, PN and PL vary depending on the mode of administration. When mixed in the ration, PM and PL were less active in growth promotion than PN. When each form

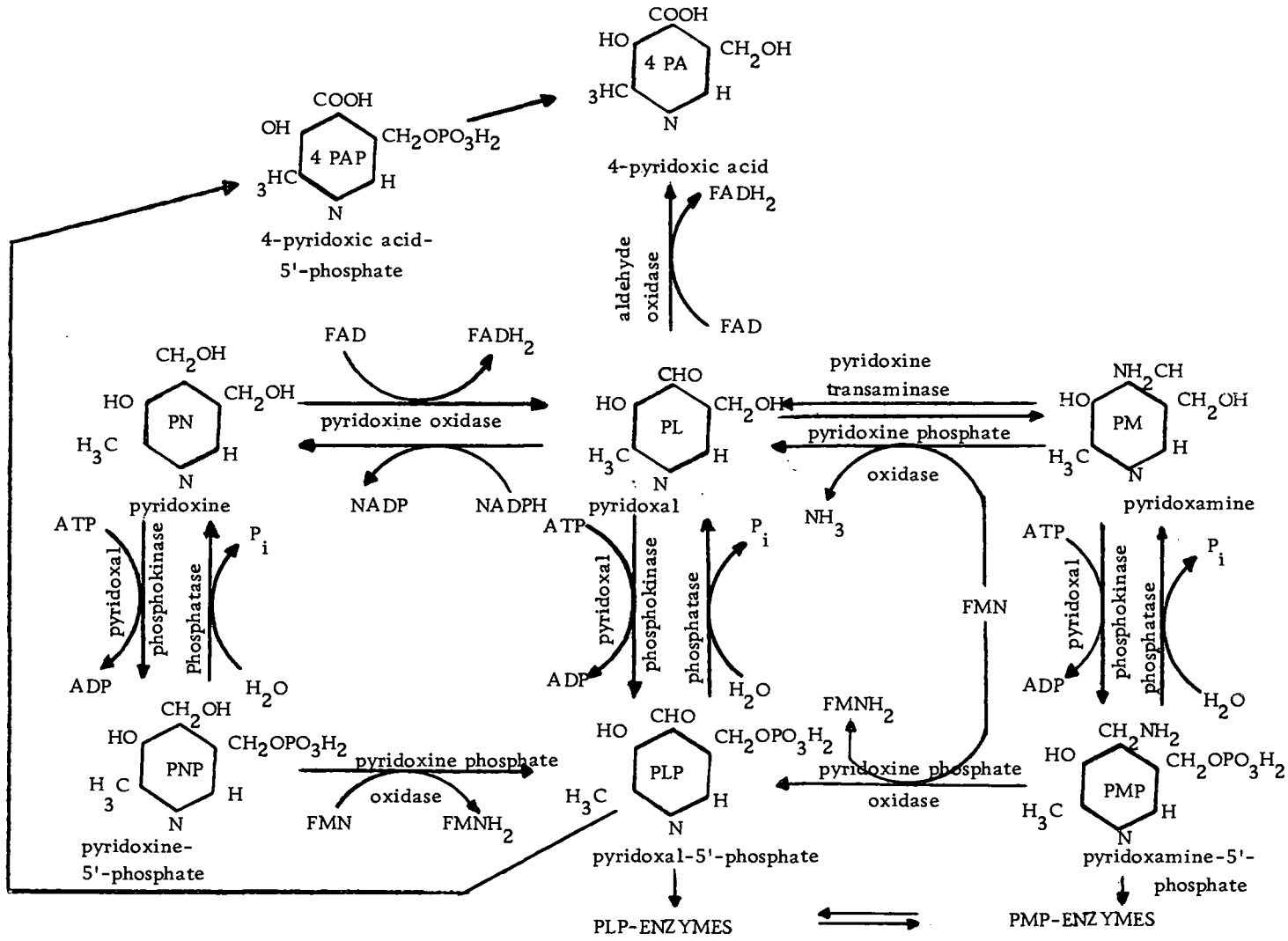

Figure 1. Metabolic interconversion of vitamin B6 and the formation of 4-pyridoxic acid. (From Sauberlich, 1968, Snell, 1964, and Contractor and Shane, 1970).

was given orally or intraperitoneally, however, they were all equally active (Sarma, Snell and Elvehjem, 1946).

Sources

PL and PM are the more prevalent forms in animal products and PN predominates in foods of vegetable origin (U. S. Department of Agriculture, 1969).

Absorption

Absorption of PN has been shown to occur mainly in the jejunum of the rat (Booth and Brain, 1962). Small amounts of the PN given orally were absorbed in the ileum and colon. The method of absorption was thought to be by passive diffusion. This has been corroborated in vitro by Middleton (1977), using an everted sac technique.

Previous studies of the absorption of vitamin B6 in humans have used doses of crystalline forms of the vitamin that were large compared to the National Research Council Recommended Dietary Allowances (National Academy of Sciences, 1974). Rabinowitz and Snell (1949) measured the urinary excretion of PL, PM, PN and 4PA in humans following 72- or 80-mg oral doses of each of PN, PM and PL. Booth and Brain (1964) saturated both human subjects and patients with idiopathic steatorrhea with 200 mg of PN before administering a labeled dose of 1 mg PN. Urinary excretion of the vitamin was used to

determine the extent of absorption. They found that saturation with a 200-mg parenteral injection of PN resulted in larger amounts of ^{3}H-PN excreted in the urine in the first 24 hr following an oral ^{3}H-PN dose, than without saturation. From the rate of urinary excretion, the majority of the oral dose was absorbed within the first 4 hr after the injection. The ^{3}H-radioactivity excreted in urine by normal subjects saturated with 200 mg of PN given intravenously was linear following oral doses of ^{3}H-PN between 1 and 100 mg. Some patients with idiopathic steatorrhea excreted less pyridoxine under the same conditions than the normal subjects described above. The extent of impairment of absorption in these patients, however, could not be correlated with other intestinal function tests. The levels of vitamin B6 in the blood were not measured.

Levels of vitamin B6 in the blood as well as in the urine were measured in two women by Contractor and Shane (1968). They found no significant variation in the levels of vitamin B6 compounds in blood and urine of normal female subjects during their menstrual cycles. Oral doses of 100 mg of PN caused increases in vitamin B6 compounds in blood and urine. In one case, plasma PLP was still elevated 4 days after the dose. Again the 100 mg dose of PN given these women was 50 times the present Recommended Dietary Allowance for vitamin B6 (National Academy of Sciences, 1974).

Lumeng et al. (1974) used 25 and 50 mg daily doses and

measured plasma PLP for a period of 20 days. The plasma PLP levels rose in the first days of the supplementation, then plateaued by the third or fourth day at either dose level. The plasma PLP levels returned to the initial levels within 5 days following termination of the 25 mg supplementation. Five days after the termination of the 50 mg supplement, plasma PLP values had not returned to their initial levels.

Anderson et al. (1971) measured changes in whole blood, plasma and red cells after oral ingestion of 50 mg of PN. The Lactobacillus casei-active vitamin B6 compounds in the red cells increased more than the vitamin B6 in the plasma within 1 hr after the dose. Since L. casei measures only PL (+ PLP after hydrolysis), the appearance of L. casei-active vitamin B6-compounds after a PN dose meant that PN was converted to PL, and that this conversion probably took place in the red cell. Both PL and PLP were found in red cells, but only PL was found in plasma.

More recent studies of absorption of vitamin B6 have employed techniques which make possible direct measurement of absorption by quantitating the amount of an infused solution that disappears from a given section of the intestinal tract (Cooper et al., 1966). This method has been used to compare the bioavailability of both a PN solution and of a mixture of crystalline PLP, PM and PN with that of the vitamin B6 in orange juice (Nelson, Lane and Cerda, 1976). Mean

vitamin B6 absorption was greater from the synthetic solution than from orange juice. The addition of glucose to a synthetic mixture of vitamin B6 to promote water transport in the intestine did not increase the absorption of vitamin B6 over that of a vitamin B6-saline solution. No blood or urine samples were taken, and no dietary control was imposed prior to the test infusions.

Interconversions Between the Different Forms of Vitamin B6

The known metabolic interconversions between the six B6 vitamers are shown in Figure 1. Lumeng et al. (1974) have shown that in dogs the liver is the principal, if not the sole, organ responsible for the production of plasma PLP from either PL or PN, when these vitamers were given intravenously. Anderson et al. (1971) have shown that the red blood cell is capable of converting PN to PL and had suggested that PL may be the main transport form of the vitamin. McCoy and Columbini (1972) have discussed the interconversions of the forms of vitamin B6 in mice. The amount of an intravenous dose of ^{14}-C-PN retained is characteristic for each tissue. The maximum percent retention in the brain was 0.4; in liver, 12.2; and in carcass, 86.7. In the brain, in contrast to liver and carcass, the maximum percent retention occurred at 5 min rather than 1 min. In the liver, phosphorylation proceeded more rapidly than oxidation, whereas both were comparable in the brain. In the whole carcass, oxidation was the

predominant initial pathway of PN metabolism. Four to 7 days after the initial changes, the liver seemed to maintain a constant rate of interconversion between PLP and PMP. In studies with vitamin B6-deficient mice, with convulsogenic mice, and with the effects of amphetamine in mice, it was demonstrated that the percent retention of PMP is increased relative to the other B6 vitamers. McCoy and Colombini concluded that this effect may be due to an increased vitamin B6 metabolism.

Long-term feeding of massive doses of PN caused a shift in the concentrations of the B6 vitamers in rat liver (Cohen et al., 1973). The concentrations of PLP and PMP were decreased and the concentrations of PL and PM increased. These changes were not observed in blood and brain. A decrease in PMP and PLP was shown to occur in mouse brain after high doses of PL or PN were given intraperitoneally (Bain and Williams, 1960). In contrast, mice receiving vitamin B6-depletion diets lost a disproportionate amount of PLP in the brain and liver (Lyon, Bain and Williams, 1962; Bain and Williams, 1960).

Storage

Organ Levels. Total vitamin B6 content of blood and organs for various species have been summarized by Storvick and Peters (1964). Bain and Williams (1960) have given more information on distribution

of B6 vitamers. Johansson et al. in experiments on the excretion of labeled pyridoxine in rat (1966a) and man (1966b) have estimated total body reserves to be 25-80 μg/day and 40-150 mg/day, respectively. The urinary excretion of the labeled isotope lead these authors to the proposal that the overall metabolism of vitamin B6 in humans involves both a small body compartment with a rapid turnover rate, and a larger storage compartment with a slower turnover rate. Krebs and Fischer (1964) have suggested that the large amount of PLP in muscle phosphorylase (possibly half of all the vitamin B6 in the body) may be a reservoir for the vitamin. Black, Guirard and Snell (1977) explored the accessibility of the vitamin from muscle phosphorylase as a vitamin B6 reservoir. They found that starvation depletes muscle phosphorylase, releasing vitamin B6 to be available for liver alanine and aspartate transaminase, both of which increase during starvation.

Blood. The levels of vitamin B6 circulating in the blood are much more immediately reflective of dietary intake than are organ levels. Lumeng et al. (1974) have shown that PLP in human plasma is bound predominantly to albumin. Some unbound PLP also exists. Anderson et al. (1971) postulated that the main transport form of vitamin B6 in the plasma is PL. More recently, however, Anderson et al. (1974) concluded that PLP is the main transport form of vitamin B6. Lumeng et al. (1974) have shown that PLP bound to albumin does

not readily enter erythrocytes. If PLP is the main transport form of the vitamin, the target cells must have a means by which they can take in the PLP. Alkaline phosphatase has been proposed as the enzyme which may function in this respect (Lumeng et al., 1974).

Excretion

Normal daily excretion of vitamin B6 and its metabolites has been studied. The daily excretion of vitamin B6 compounds includes 4PA (about 50% of the vitamin B6 ingested) (Reddy, Reynolds and Price, 1958), 4PAP (Contractor and Shane, 1970), vitamin B6 itself (mainly as PL and to a lesser extent PM), and a number of unidentified metabolites (Sauberlich, 1974). Using the same methods as reported here, Perera (1977) reported that in young men, 37-44% of a 1.58 mg daily intake of vitamin B6 from natural foods was recovered as urinary 4PA. On a 1.58 mg vitamin B6 intake from a diet of natural food the average urinary excretion of vitamin B6 was found to be 153 μg/day (Perera, 1977).

The distribution of the forms of vitamin B6 recovered in the urine is dependent on the forms ingested (Rabinowitz and Snell, 1949). Ingestion of 100 mg of PL resulted in much larger amounts of 4PA in human urine than did 100 mg of PM or PN. Following the PL or PN doses, the major forms of the compounds excreted were the same as those that were fed. Following the PM dose, however, both PM and

PL were excreted in equal amounts. The percent of the dose recovered was 70 for PL, 45 for PN, and 31 for PM. The greatest portion of the doses was excreted as vitamin B6 or 4PA within 5 hr following ingestion of the dose. There have been no studies in humans on the relationship of small graded oral intakes of vitamin B6 to the amount of vitamin B6 and 4PA excreted in the urine. Booth and Brain (1962) gave graded oral doses of 0.5 to 5 mg of ^{3}H-PN to rats with simultaneous parenteral injections of unlabeled PN. Within the dose range used, the ^{3}H-PN excreted was linearly related to the ^{3}H-PN fed. There was only one 12-hr urine collection made following the oral dose.

The phosphorylated form of 4PA has been shown to account for as much as 20% of the vitamin B6 metabolites in rat and man (Contractor and Shane, 1970).

Perhaps the most widely used procedure for evaluating vitamin B6 status has been the tryptophan load test. Following a 2-5 g tryptophan load, individuals depleted of vitamin B6 will excrete amounts of tryptophan metabolites (xanthurenic acid, 3-hydroxy kynurenine, kynurenine and kynurenic acid) that are greater than the levels excreted by normal subjects. Some question has been raised about the validity of using this method for the estimation of normal vitamin B6 nutriture. It has been suggested that the tryptophan load may present

the body with a stress that may alter the utilization of the ingested vitamin (Brown, 1972).

The amount of vitamin B6 and 4PA recovered in the urine are dependent on dietary components other than vitamin B6. Although the vitamin B6 in human urine is not much affected by the level of protein (Sauberlich, 1974), the amount of 4PA has been shown to be increased in rats when dextrin was present in the diet (Sarma et al., 1946). Young (1973) found an inverse relationship in humans between weight and 4PA excretion, while Perera (1977) found the relationship to be direct. A direct relationship between dietary ascorbic acid and 4PA excretion has been reported (Kraut and Imhoff, 1968).

Effect of Exercise

Exercise causes a rise in plasma free fatty acids, an increased utilization of exogenous carbohydrate, and an accumulation of lactic acid during the period of oxygen debt (Bell, Davidson and Scarborough, 1969). Levels of enzymes which increase following exercise include lipoprotein lipase (Nikkilä, Torsti and Penttilä, 1963), malic dehydrogenase, glutamic-oxaloacetic transaminase (GOT) and lactic dehydrogenase (Sangster and Beaton, 1966). Glutamic-pyruvic transaminase had been reported to increase with exercise, but this has not been supported by subsequent studies (Sangster and Beaton, 1966; Chen and Marlatt, 1975). The ability of exercise to induce serum enzyme

levels is markedly reduced with training (Sangster and Beaton, 1966) and is also dependent on the duration of the exercise (Critz, 1966). GOT is a vitamin B6-dependent enzyme. Another vitamin B6-dependent enzyme which plays a role in exercise physiology is glycogen phosphorylase.

Guinea Pig Requirements

Vitamin B6 was identified as essential for the guinea pig by Reid (1954). Deficiency symptoms included anorexia, retarded growth, roughness and thinning of haircoat and, in some cases, in convulsions, and enlarged kidneys and adrenals. The Recommended Dietary Allowance for guinea pigs on a 30% protein diet has been set at 2.0-3.0 mg/kg dry diet (Reid, 1964), or 1.3 mg/kg body weight (National Academy of Sciences, 1972). This is a very high allowance compared to the 2.0 mg allowance set for human adults (National Academy of Sciences, 1974). There are no data on the changes in blood and urinary levels of vitamin B6 compounds following administration of the Recommended Dietary Allowance of the three non-phosphorylated forms of vitamin B6 to guinea pigs.

III. METHODS

Human Diet Study

Subject Selection

Five apparently healthy males served as subjects in this study. Prior to beginning the study, the subjects were determined to be metabolically normal on the basis of the following criteria: (1) freedom from disease (as determined by interview); (2) normal liver function and blood chemistry as determined by automated analysis; (3) normal xylose absorption (Henry, Cannon and Winkelman, 1974) as a measure of intestinal absorption; (4) adequate vitamin B6 status as determined by plasma and urinary vitamin B6; (5) avoidance of drugs, including alcohol (on weekdays) and vitamin supplements for the duration of the study; (6) normal weight and physical activity (not engaged in competitive sports); and (7) adequate dietary intake as determined by dietary history.

The subjects' vital statistics are given in Table 1. Results of tests made prior to the study are included in Table 2. Subject 4 was the only person outside the normal range for xylose absorption, with 0.79 g of xylose excreted following a 5 g xylose load. Subject 4 also had a high bilirubin level (2.9 mg/dl), but since his SGOT and alkaline phosphatase values were normal, it was decided to allow him to remain in the study.

Table 1. Vital statistics of the human subjects

Subject	Age (yr)	Height (m)	Weight (kg)	
			Initial	Final
SB	32	1.75	77.7	77.7
WG	26	1.75	69.1	70.5
DM	28	1.80	70.5	69.1
AM	26	1.73	69.5	66.8
SS	24	1.83	88.2	88.2
Mean	27.2	1.77	75.0	74.5
sd	± 3.0	±0.04	± 8.2	± 8.7

Table 2. Biochemical evaluation of human subjects prior to study

Parameter	Subject Mean ± Std. Deviation	Range	Normal Range[a] and Unit
Blood urea nitrogen	19.2 ± 4.3	13.6 - 24.7	5.0 - 22.0 mg/dl
Glucose	16.0 ± 5.0	89.4 - 101.8	70.0 - 115.0 mg/dl
Chloride	102.8 ± 1.4	101.6 - 105.0	96.0 - 107.0 meq/l
Sodium	141.9 ± 1.6	139.1 - 143.0	135.0 - 148.0 meq/l
Potassium	4.5 ± 0.5	4.0 - 5.3	3.5 - 5.3 meq/l
Xylose absorption	1.6 ± 0.5	0.79 - 2.24	1.2 - 2.4 g excreted[b]
Plasma vitamin B6	13.7 ± 4.8	8.0 - 19.0	12.8 ± 4.5 ng/ml[c]
Urinary vitamin B6 (hydrolyzed)	122. ± 43.2	61 - 166	168 ± 43 ug/24 hr[c]
Globulin	2.0 ± 0.4	1.5 - 2.6	
A/G	2.1 ± 1.1	1.5 - 3.6	
Initial hematocrit	48 ± 3	45 - 52	46.5 ± 7.7 %[d]
Triglycerides	96.3 ± 9.2	87.5 - 111.7	50.0 - 155.0 mg/dl
Lactic dehydrogenase	130.9 ± 29.7	79.5 - 154.2	56.0 - 150.0 U/L
SGOT	29.8 ± 8.2	15.3 - 34.4	11.0 - 38.9 U/l
SGPT (only 2 values)	53.6 , 18.8		13.0 - 41.0 U/l
Phosphorous	3.9 ± 0.5	3.5 - 4.7	2.5 - 4.5 mg/dl
Calcium	9.5 ± 0.3	9.2 - 9.8	8.7 - 10.7 mg/dl
Alkaline phosphatase	20.5 ± 5.1	13.8 - 26.9	9.0 - 35.0 U/l
Bilirubin	1.1 ± 1.0	0.5 - 2.9	0 - 1.5 mg/dL
Uric acid	5.0 ± 0.9	4.1 - 6.6	2.0 - 7.0 mg/dl
Total protein	6.7 ± 0.1	6.6 - 6.9	5.5 - 8.0 g/dl
Albumin	4.7 ± 0.5	4.0 - 5.4	3.8 - 5.2 g/dl
Cholesterol	170.3 ± 18.4	153.6 - 201.8	150.0 - 300.0 mg/dl
Initial hemoglobin	15.6 ± 1.1	14.6 - 17.4	13 - 18 g/dl[b]

[a]Normal values for fasting plasma analyzed on Sequential Multiple Analyzer, Good Samaritan Hospital, Corvallis, Oregon

[b]Henry, et al., 1974

[c]Nutrition Laboratory, Department of Foods and Nutrition, Oregon State University

[d]Wohl and Goodhardt, 1968, p. 1208

Dietary histories were done to determine whether the subjects were consuming adequate diets and whether foods containing substantial amounts of vitamin B6 were commonplace in their diets. Subject histories also included questions on food allergies, daily physical activity and smoking. The study was approved by the Human Subjects Committee of Oregon State University on December 5, 1975. Consent forms approved by the Human Subjects Committee of Oregon State University were signed by the subjects.

Experimental Protocol

A summary of the dietary regimen, and of blood and urine sampling periods is given in Table 3.

Diets and Doses

The subjects were asked to record their food intake daily throughout the experimental period and to restrict alcohol consumption to the weekends. The subjects were on self-selected diets on Friday through Tuesday of each week, and during these periods they were asked to limit their intake of foods allowed in order to maintain their daily intake of vitamin B6 at approximately 1.5 mg. The three meals preceding and the three meals following the dose were controlled and provided in the metabolic unit in the Department of Foods and Nutrition at Oregon State University. Breakfast, lunch and dinner

Table 3. Experimental protocol, human study

Diet	Length of Experimental Samplings	Doses (Amounts and Forms)	Parameters Measured
Friday - Tuesday self-selected approx. 1.5 mg B6 Wednesday controlled 1.5 mg B6 Thursday controlled no breakfast low B6 lunch regular B6 dinner	Total experiment 8 weeks Friday - Tuesday -24 hr urine samples -diet records Wednesday -24 hr urine samples -diet records Thursday -Urine 4 x 4 hr[a] 1 x 8 hr -Blood 6 x after dose at 0, 0.5, 1, 3, 5, 8 hr	Each week different Week / Form / Level mg 1 / PN / 0 2 / PN / 0.5 3 / PN / 1 4 / PN / 2 5 / PN / 4 6 / PN / 10 7 / PM / (4)[b] 8 / PL / (4)	Urine -4-pyridoxic acid -vitamin B6 -creatinine Plasma -vitamin B6 -pyridoxal phosphate Diet records nutrient intake

[a]For the week of 0 and 0.5 mg PN, the first two sampling periods were both 4 hr, instead of the 3 and 5 hr periods used for the other weeks

[b]Equimolar doses (19.44 μmoles) of PN, PM and PL were given that were equivalent to 4 mg of PN

were provided on Wednesdays, the days prior to the dose days. The subjects received no breakfast during the morning of the dose day (Thursday), then lunch and dinner were given. Breakfast was given on the day following the dose (Friday). The menu for the meals provided and the vitamin B6 content as determined from food composites are given in Table 4. The carrots, which contributed a substantial amount of vitamin B6 to the diet, were given for lunch on the day prior to the B6 dose (Wednesday), but not on the day of the dose (Thursday). The calculated nutrient composition of the food provided is given in Table 5. Foods known to contain negligible amounts of vitamin B6 were allowed to the subjects as "free foods" (Table 4). Records were kept of the amounts of the "free foods" eaten and the calories in these foods were averaged and presented in Table 5.

Doses of PN were given to measure the effect of level of vitamin B6 (Table 3). One dose was given each week except for levels 0 and 0.5 mg PN, which were given in the same week. The levels of pyridoxine hydrochloride (given as pyridoxine) (Calbiochem, San Diego, California) administered were 0.5, 1, 2, 4, and 10 mg. Pyridoxamine dihydrochloride (PM) (Calbiochem, San Diego, California) was given in an amount which was the molar equivalent (19.44 μmoles = 5.04 mg) of the 4 mg dose of pyridoxine. Similarly, pyridoxal hydrochloride (PL) (Sigma, St. Louis, Missouri) was

Table 4. Controlled diet provided in metabolic unit[a]

Menu (g)	Vitamin B6 Content (mg)[b, c]
Breakfast	
125 half and half	
54 hard cooked egg	
25 white bread	
30 cornflakes	
125 orange juice	
30 fresh banana	0.29
Lunch	
250 whole milk	
50 white bread	
30 bologna	
39 oatmeal raisin cookies	
40 raisins	
34 chocolate bar	0.31
100 carrots	0.22
Lunch with carrots	0.53
Dinner	
250 whole milk casserole	
75 ground beef	
60 raw macaroni	
25 green pepper	
10 dehydrated onion	
40 tomato paste, canned	
50 white bread	
90 peaches, canned	
30 peach syrup, heavy	
75 vanilla ice cream	0.77
	1.59

[a]On Wednesday, all three meals were provided. The vitamin B6 doses were given instead of breakfast on Thursday. Lunch was the same as Wednesday except that the carrots were omitted. Dinner was the same as on Wednesday. Only breakfast was given on Friday. Other meals on Friday through Tuesday were self-selected. A 1.5 mg vitamin B6 intake on the self-selected days was desired.

[b]Assayed by Margaret Edwards, using Saccharomyces uvarum (ACAC, 1975).

[c]"Free foods" allowed and recorded every day included: carbonated beverage, margarine, butter, sugar, spices, coffee, tea, jam, salt, hard candy, non-dairy creamers.

Table 5. Nutrient composition of controlled diet provided in metabolic unit[a]

Nutrient	Amount	%RDA[b]
Protein	77.8 g	139
Fat	94.5 g	-
Carbohydrate	299.8 g	-
Fiber	4.02 g	-
Calcium	1195 mg	149
Phosphorous	1280 mg	160
Iron	14.0 mg	140
Sodium	2056 mg	-
Potassium	3428 mg	-
Vitamin A value	15,730 IU[c]	315
Thiamin	2.65 mg	189
Riboflavin	2.31 mg	144
Niacin	13.5 mg[d]	75
Ascorbic acid	119 mg	264
Pantothenic acid	4.50 mg	-
Vitamin B6	1.59 mg[e]	73
Vitamin B12	3.54 µg	118
Calories		
Meals	2317	86
"Free foods"	605[f]	22
Total	2922	108

[a]Calculated from Handbook 8 (U. S. Department of Agriculture, 1968)

[b]Recommended Dietary Allowances, National Academy of Sciences, 1974

[c]Does not include vitamin A from "free foods" listed in Table 4

[d]Not including tryptophan

[e]Assayed by S. uvarum (AOAC, 1975)

[f]Allowed to maintain weight and satisfy appetites

administered in an amount equimolar to the 4 mg PN dose (19.44 μmoles = 3.96 mg).

All doses were given dissolved in a total volume of 200 ml of water. The 4 mg PN, PM and PL, and the 10 mg PN doses were given in 100 ml of water, which was followed by a 100 ml portion of water used to rinse the glass containing the dose. Denker, Jagerstad and Westesson (1976) found an increase in folate levels in portal plasma when the test dose of folate was given in 200 ml of water rather than in 4 ml. It was, therefore, decided to use 200 ml portions of water as carriers of the vitamin B6 doses given in this study.

An experiment was conducted to determine the effect of exercise on response to a 1 mg dose of PN. Meals were self-selected on the day preceding the running experiment. One mg of PN was given to the fasting subjects, who were asked to run for 15 min, or to capacity, between 1 1/2 and 1 3/4 hr following the dose of PN. Blood was drawn at 0, 1/2, 1, 2, and 3 hr after the dose.

Blood Sample Collection

Blood was sampled six times on the day of the vitamin B6 doses. Each sample was 10 ml, drawn by a registered medical technician from the antecubital vein of the forearm into heparinized vacutainer tubes. The sampling periods, timed with respect to the

administration of the B6 dose, were 0, 0.5, 1, 3, 5 and 8 hr. The subjects were fasting until immediately following the 5 hr sample, at which time a lunch containing 0.31 mg of vitamin B6 was served (Table 4). The 8-hr sample, therefore, occurred 3 hr after lunch. The blood sampling times during the exercise experiment were 0, 0.5, 1, 2 and 3 hr after the dose. Since the exercise period was between 1.5 and 1.75 hr after the dose, blood samples were taken approximately 30 min before and 15 min after the exercise period.

Urine Collections

Total 24 hr urine collections under toluene were made daily, except for the day on which the vitamin B6 dose was given. Once received, samples were refrigerated or frozen until analysis. On the dose day, there were 5 separate urine collection periods. The collection periods ended at the following times after each dose: 3, 8, 12, 16 and 24 hr. (The first two dose levels, 0 and 0.5 mg PN, had urine collection periods ending at 4, 8, 12, 16 and 24 hr after the dose.)

Guinea Pig Study

Animals

The guinea pigs used were a short haired, bi- or tri-colored mixed strain. Weanling males, weight 162 to 232 g were used. The

animals were obtained from Dr. Mary Meekle, Kresge Speech and Hearing Clinic, University of Oregon Medical School, Portland, Oregon. They were housed in pairs in stainless steel, wire mesh-bottomed cages at the Small Animal Laboratory, Oregon State University.

Diets and Feeding

A summary of the dietary and sampling regimen is given in Table 6. The composition of the experimental diet is shown in Table 7. The guinea pigs had difficulty adjusting to the semi-synthetic diet.

Table 6. Experimental protocol, guinea pig study

Diet	Length of Experiment and Samplings	Doses (Amounts and Forms)	Parameters Measured
Same every day B6 deficient semi-synthetic	8 weeks Blood 24 hr urine Weight gain Final organ weights	Daily oral dose = RDA (1.3 mg/kg body weight) Group 1 PN (6)[a] Group 2 PM (6) Group 3 PL (5)	Weights Urine creatinine 4-pyridoxic acid vitamin B6 Blood WBC, RBC, Hct, Hb Plasma PLP Plasma vitamin B6

[a](Number of animals in each group.)

Table 7. Composition of semi-synthetic guinea pig diet[a]

Basic Diet g/kg		Salt Mix (g/75 g)	
Casein (vitamin free)[b]	300	$CaHPO_4$	29.1
Cornstarch	200	K acetate	20.78
Cellulose[c]	150	KCl	6.45
Sucrose	100	$CaCO_3$	4.95
Salt mixture	75	NaCl	4.8
Glucose	87	MgO	4.13
Corn oil	60	$MgSO_4$	3.825
Water soluble vitamin mixture[d]	10	Fe citrate	0.533
		$MnSO_4$	0.308
Fat soluble vitamin mixture in corn oil[e]	10	KIO_3	0.0125
Arginine	2	$CuSO_4$	0.004
Ascorbic acid	2	$ZnCO_3$	0.108
Choline	2	Na_2MoO_4	6.25 mg
Inositol	2	Na_2SeO_3	0.27 mg

[a]The diet is essentially that of Liu et al. (1967) with 7.5% salt mixture and no vitamin B6. The Na_2MoO_4 and Na_2SeO_3 were added to the diet as done by Fox and Briggs (1960).

[b]Casein purchased from ICN, Cleveland, Ohio

[c]Alphacel purchased from ICN, Cleveland, Ohio

[d]The following amounts were present per kg of diet: (in mg) Thiamin, 16; vitamin B6, 0; riboflavin, 16; pantothenate, 40; niacin, 200; biotin, 0.5; folic acid, 10; and vitamin B12, 0.05.

[e]The following levels of fat-soluble vitamins were present per kg of diet: (in mg) menadione, 2; vitamin A acetate, 6; tocopherol, 20; and vitamin D_3, 0.04.

Animals which failed to thrive were not used in the experiment. The average growth curves for this mixed strain on a pelleted commercial diet and on the semi-synthetic diet are shown in Figure 2. The pelleted diet growth curve which is for the same mixed strain of animals as purchased, was obtained from Dr. Meekle's laboratory in Portland, Oregon.

The vitamin B6 doses were given orally between 9 and 10 AM each day in a 75% sucrose solution. The amount of vitamin B6 given was equal to the Recommended Dietary Allowance for guinea pig (1.3 mg/kg body weight, National Academy of Sciences, 1972). The casein in the semi-synthetic powdered diet was analyzed for vitamin B6 in our laboratory, using Sacharomyces uvarum (carlsbergensis) (AOAC, 1975).[1] The casein contained 0.36 mg vitamin B6/g. The average daily feed consumption was 27 g, which would amount to a daily intake of 2.89 μg of vitamin B6 from the casein (0.22% of the RDA).

Bleeding Method

The guinea pigs were anaesthetized with a sequential combination of CO_2 and ether. The animals were placed in a closed container on a screen over dry ice as described by Hoar (1969). The surgically anaesthetized state was maintained with the periodic administration of a nose cone containing ether. The animals were bled by cardiac

[1] Casein was analyzed for vitamin B6 by Eva Benson.

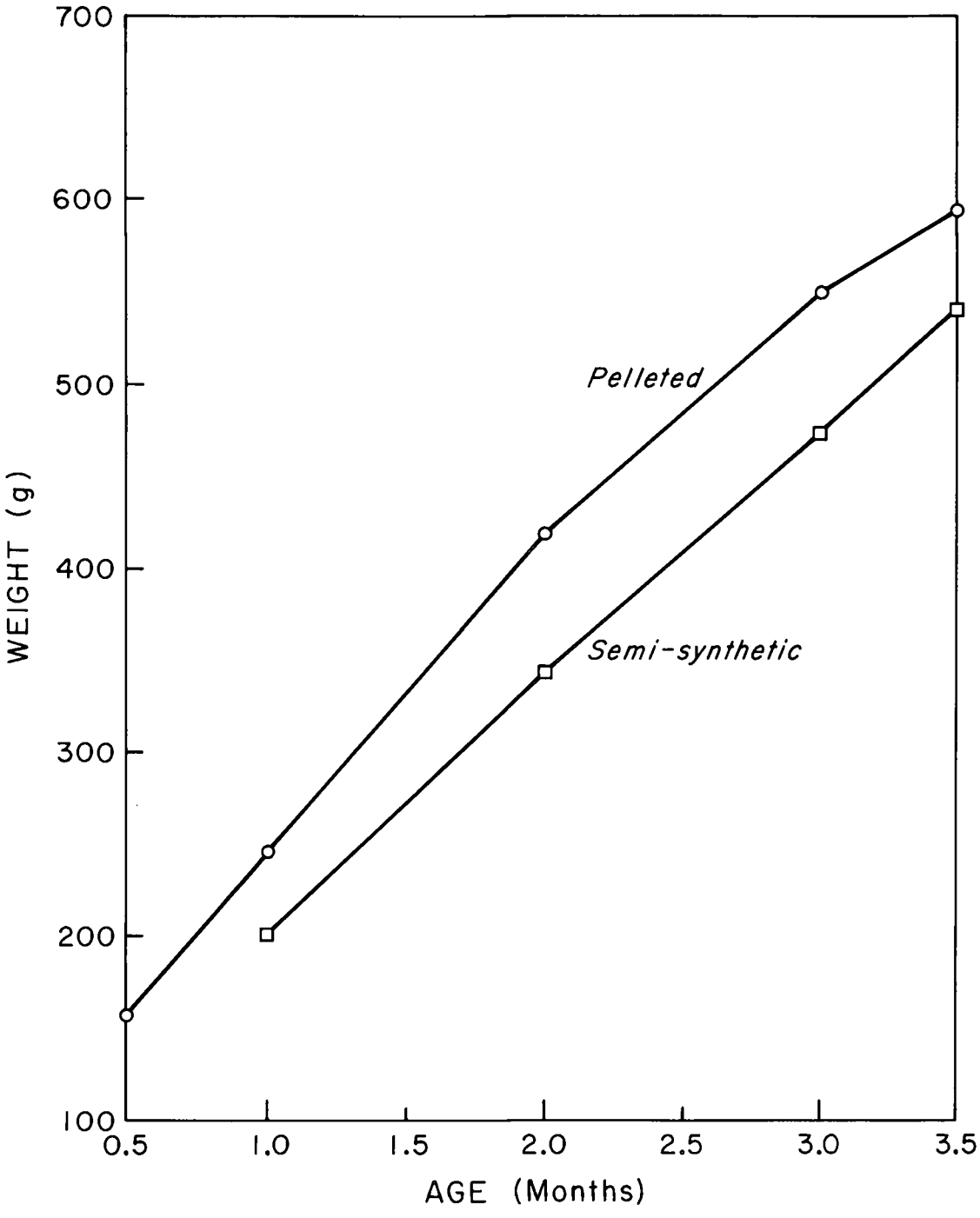

Figure 2. Guinea pig normal growth curves on pelleted and on semi-synthetic rations. Pelleted diet curve from Dr. Mary Meekle, Kresge Speech and Hearing Clinic, University of Oregon Medical School, Portland, Oregon. Semi-synthetic curve obtained with the same strain of guinea pigs, using the diet given in Table 7.

puncture. Less than 10% of their total blood volume was drawn at any one time.

Urine Collections

The urine collections were carried out in metabolic cages. The samples were collected for 24 hr under toluene in brown bottles surrounded by ice. The animals were given their daily doses of vitamin B6 at the start of the 24-hr period. The following day's dose was not given until the 24-hr urine collection was completed.

Laboratory Analyses

Creatinine

Creatinine in the urine was determined on a Technicon Autoanalyzer (Technicon Corporation, Tarrytown, New York) by an automated modification of the Jaffe reaction (Pino et al., 1965).[2]

Xylose Absorption

Xylose absorption was measured following a 5 g dose of xylose with the orthotoluidine colorimetric method of Harris (1969) and Buttery (1975). The xylose absorption test results are shown in Table 2.

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[2]Creatinine in urine was determined by Gilda Coblentz.

Formed Elements of Blood

Hematocrit and hemoglobin were determined by standard methods.[3] Hemoglobin and hematocrit were measured once weekly for each subject to insure they were not becoming anemic from the frequency of blood samplings.

Red and white cell counts were obtained with the use of a hemacytometer.[4]

Chemical Blood Profile

An automated method for chemical screening was done on plasma samples from each fasting subject by the Sequential Multiple Analyzer-12 (SMA-12) at the Good Samaritan Hospital, Corvallis, Oregon. The results showed all subjects to be normal for the parameters of interest. The parameters measured, values obtained and normal values are given in Table 2.

Pyridoxal Phosphate

The method of Curry and Hewitt (1974) as modified by Peffers (1977) was used for assaying pyridoxal phosphate in human plasma.[5]

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[3]Hemoglobin and hematocrit were determined by Gilda Coblentz.

[4]Margaret Edwards helped with the white cell counts.

[5]Diane Peffers conducted the PLP assays on the human plasma.

A similar method developed by Woodring was used for the analysis of the guinea pig plasma (Appendix C). For both methods, L-tyrosine-1-^{14}C is decarboxylated by pyridoxal phosphate-dependent tyrosine decarboxylase. The labeled carbon is released as $^{14}CO_2$, which is trapped and quantitated by liquid scintillation counting.

The measurements of PLP in the plasma by the apotyrosine decarboxylase methods used here (Appendix C; Peffers, 1977) is specific for that vitamer. Either perchloric acid or TCA is used to precipitate the plasma proteins and are thought thereby to release the protein-bound PLP.

Vitamin B6

Plasma and urine were assayed with Saccharomyces uvarum (carlsbergensis) ATCC 9080 for vitamin B6.[6] The urine samples were hydrolyzed and the method of Kokkeler (1976) was followed. Trichloroacetic acid extracts of the plasma were used for analysis by the same S. uvarum method (Kokkeler, 1976).

The organisms used for microbiological assays for plasma vitamin B6 are differentially responsive to the various non-phosphorylated forms (Storvick et al., 1964; Storvick and Peters, 1964). S. uvarum has gained wide acceptance as the organism of choice

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[6] Plasma and urinary vitamin B6 were determined, respectively, by Eva Benson and Margaret Edwards.

(Storvick and Peters, 1964). This organism responds nearly equally to all three non-phosphorylated forms of vitamin B6. The method used here precipitates plasma proteins with TCA, which should release the protein-bound PLP. A 30-min autoclaving period hydrolyzes the phosphate from the vitamers. Thus, the values represent the total concentration of the three non-phosphorylated forms of the vitamin.

4-Pyridoxic Acid

The 4-pyridoxic acid method used was that of Reddy et al. (1958).[7] The 4-pyridoxic acid is quantitated fluorometrically, following separation of the 4PA from interfering compounds by ion exchange chromatography.

Food Composites

Composites were made of each meal, as well as of the carrots served at lunch. Aliquots from these composites were hydrolyzed, and analyzed for vitamin B6 by the S. uvarum method (AOAC, 1975).[8] The vitamin B6 content of the food composites of the meals provided are given in Table 4.

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[7] Gilda Coblentz helped with 4PA determinations.

[8] Food composites were analyzed by Margaret Edwards.

Dietary Records

Dietary records were kept by each subject for every day of the study. In an effort to increase the accuracy of the records, the subjects were asked to be as complete and descriptive as possible, and were instructed in methods of quantity estimation. The foods described were coded for computer nutrient analysis.[9] The nutrient data bank used was developed by Ohio State University Hospitals and School of Allied Medical Professions (Schaum, Mason and Sharp, 1973) and modified for use by Oregon State University, Department of Foods and Nutrition.

Data Analysis

Some analyses of variance and regressions were done with the SPSS package through the Cyber system of the Oregon State University Computer Center.[10] Pooled error estimates using the whole human study were calculated for each variable of interest. These error estimates were used in one way analysis of variance on PN levels and the different B6 vitamers. Analyses of variance on guinea pig data were done manually. The effect of dietary intake on responses was examined

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[9] Mbuki Mwamufiya helped with the coding.

[10] Josh Burke of the Department of Statistics at Oregon State University was the programmer and statistical consultant.

by calculating correlation coefficients. Areas under time response plots were computed by a summation of trapezoidal areas on a BASIC program run in the OS-3 system of the Oregon State University Computer Center.[11]

[11] The BASIC program was written by Mark Borgenson.

IV. RESULTS AND DISCUSSION

Loading Doses of B6 Vitamers in Man

Effect of Level of PN

General

Data representing the subjects' responses to a test dose were calculated in two ways: (1) the percent increase of the highest level for the day over the lowest level for the day (usually the fasting level for plasma, and for urine, the 24-hr mean value of the day before the dose); and, (2) the area under the curve bounded by the times and response variables obtained. The lowest level of the response values for that day was used as the baseline.

Blood Responses

Plasma B6. The subjects' mean responses of plasma B6 to the different levels of PN are shown in Figure 3. The individual plasma B6 values for all subjects for each time period are shown in Appendix A, Table A.1. The values for Subject 4 at the 0.5 mg PN level 2 and 3 hr samples were not included in the mean. This is discussed in the exercise section below. The values for the initial (0 hr) samples were close to the range of plasma vitamin B6 values usually obtained from fasting subjects in this laboratory (12.8 ± 4.5 ng/ml plasma). Except for the 10-mg dose, vitamin B6 in plasma peaked at 0.5 hr following

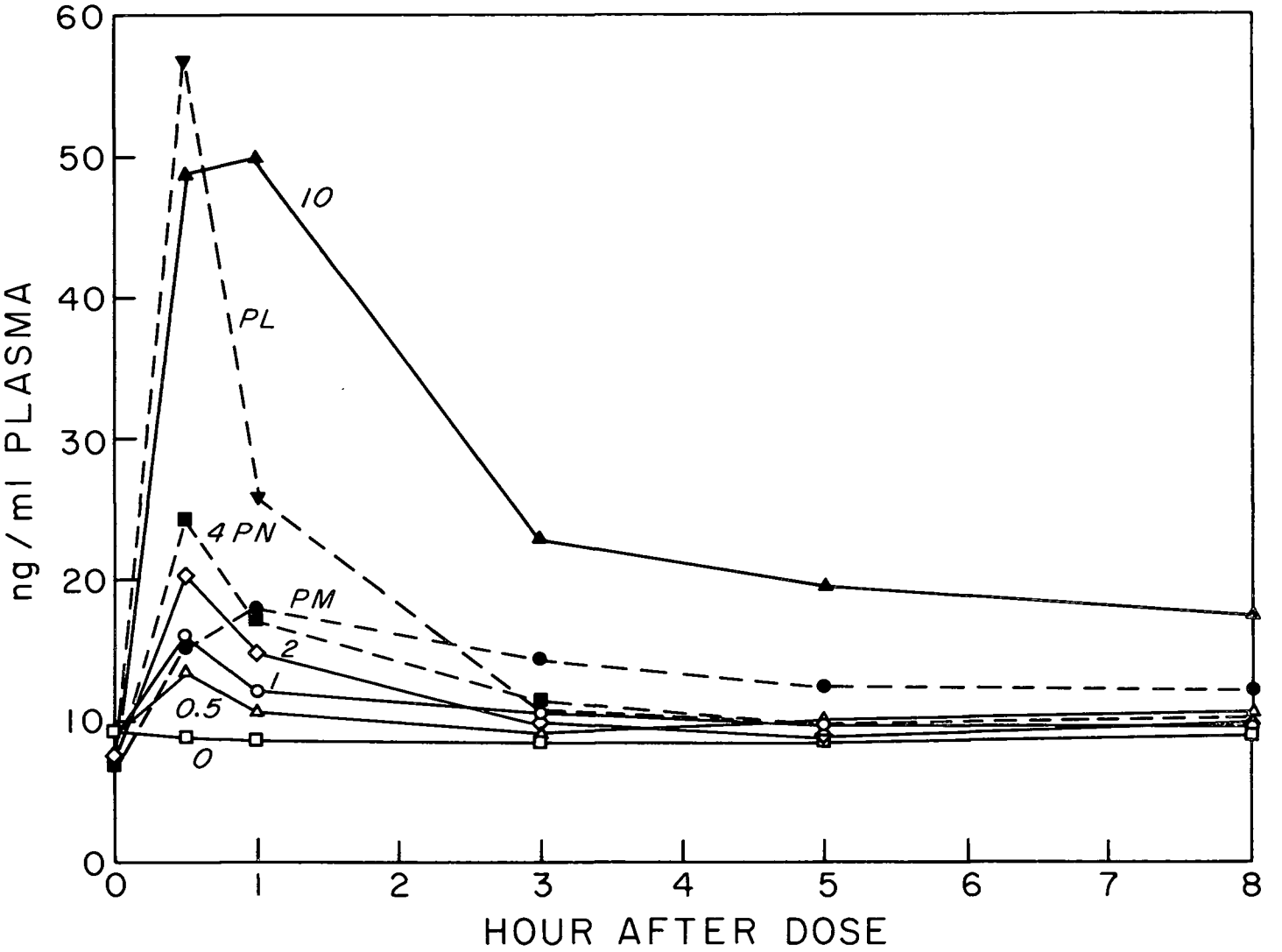

Figure 3. Mean plasma vitamin B6 for all PN levels and for equimolar doses of pyridoxine (PN), pyridoxamine (PM) and pyridoxal (PL). The 0.5, 1, 2, 4, and 10 (mg) are all PN doses. PM, PN and PL are equimolar doses (19.4 μmoles of each B6 vitamer) (- - -).

the PN dose. At the 10-mg level, there was more variation among subjects. Plasma vitamin B6 peaked at either 0.5 or at 1 hr. This is why the mean curve for all subjects at the 10-mg level appears truncated at the peak (Figure 3). Using higher levels of PN, other studies showed peak levels of vitamin B6 compounds occurring longer after the dose than reported here. Contractor and Shane (1968) found peak values to occur at 2 hr following 100 mg of oral PN. Anderson et al.[1] (1971) administered 50 mg of PN and measured peak blood responses in whole blood, plasma and in red blood cells at 1 hr. For the 0 to 4 mg PN doses, plasma vitamin B6 returned to predose levels within 5 hr following the dose (Figure 3). Even at 24 hr, for the 10 mg PN dose, the blood levels of PB6 did not return to the predose fasting levels (Figure 3 and Appendix A, Table A.1).

The results of analysis of variance for the effect of level is shown in Appendix B, Table B.1. The overall effect of level, as measured by %PB6 and PB6AREA was significant ($p < 0.01$, Appendix B, Table B.1). The mean PB6AREA and %PB6 for all subjects and the levels between which significant differences existed are presented in Appendix A, Table A.1. The smallest differences between levels which were significant are given the same superscripts in Appendix A, Table A.1. For example, the response of vitamin B6 in the plasma, expressed as area under the curve, was sufficiently precise to be able to distinguish between a loading dose of 0 mg (5211 ng-min/ml x 10)

and 2 mg (16115 ng-min/ml x 10), but not between one of 0 and 1 mg. The %PB6 variable was more sensitive than PB6AREA (Appendix A, Table A. 1). Significant differences existed between the 0 and the 1 mg PN level ($p < 0.05$) of the %PB6 variable (111 and 188%, respectively).

Experiments to follow this one plan to determine the effect of certain dietary components on the bioavailability of vitamin B6. From these results, it appears that the magnitude of the effect of the dietary component in question on the bioavailability of PN would have to be equal to a change equivalent to a dose of 2 mg of PN, before a significantly different ($p < 0.05$) change would occur in the levels of plasma vitamin B6, calculated as PB6AREA. Thus, since the difference between the 0 mg and the 2 mg level is the smallest increment distinguishable by calculating plasma B6 area, if a constant diet containing 2 mg of vitamin B6 were given, a change in average availability to the body of 2 mg would be necessary before a discernable change could be noticed in the PB6AREA. It is possible that differences between small increments of PN doses may be perceptible if the subjects were saturated (Tamura and Stokstad, 1973; Brain and Booth, 1964). The saturated condition, however, does not duplicate conditions normally occurring in humans.

The relationships between PN level and %PB6 and between PN

level and PB6AREA were significantly linear with $p < 0.01$ (Appendix B, Table B.4 and Figure 4).

Plasma Pyridoxal Phosphate. The response of PLP to the different levels of PN are shown in Figure 5. The individual and mean PLP values for all subjects for each time period are shown in Appendix A, Table A.2. The PLP values for the initial (0 hr) samples were similar to those obtained by Peffers (1977) for fasting male subjects. She found a mean value of 8.7 ± 2.8 ng/ml plasma for 9 men receiving a diet supplying 1.85 mg of vitamin B6. The mean PLP response to the various levels of PN were not as great as were the responses of PB6 for the same dose levels. (Compare Figures 3 and 5.) Differences in response of plasma vitamin B6 and PLP largely reflect the fact that these two methods are measuring different compounds. The S. uvarum is responsive to all forms of the vitamin in the plasma, whereas the PLP assay is specific for that form only. The appearance of PLP in the plasma following a dose of PN is subject to the metabolic regulation involved in the formation of PLP from PN, namely oxidation and phosphorylation. For this reason the PLP peaks might be expected to occur later than the PB6 peaks (Figures 3 and 5). Since PLP is the predominant active form of the vitamin in plasma, it might also be expected that the plasma levels would remain relatively constant as a rapidly available reservoir for PLP enzymes. This idea is supported by the fact that in this study the PB6 values

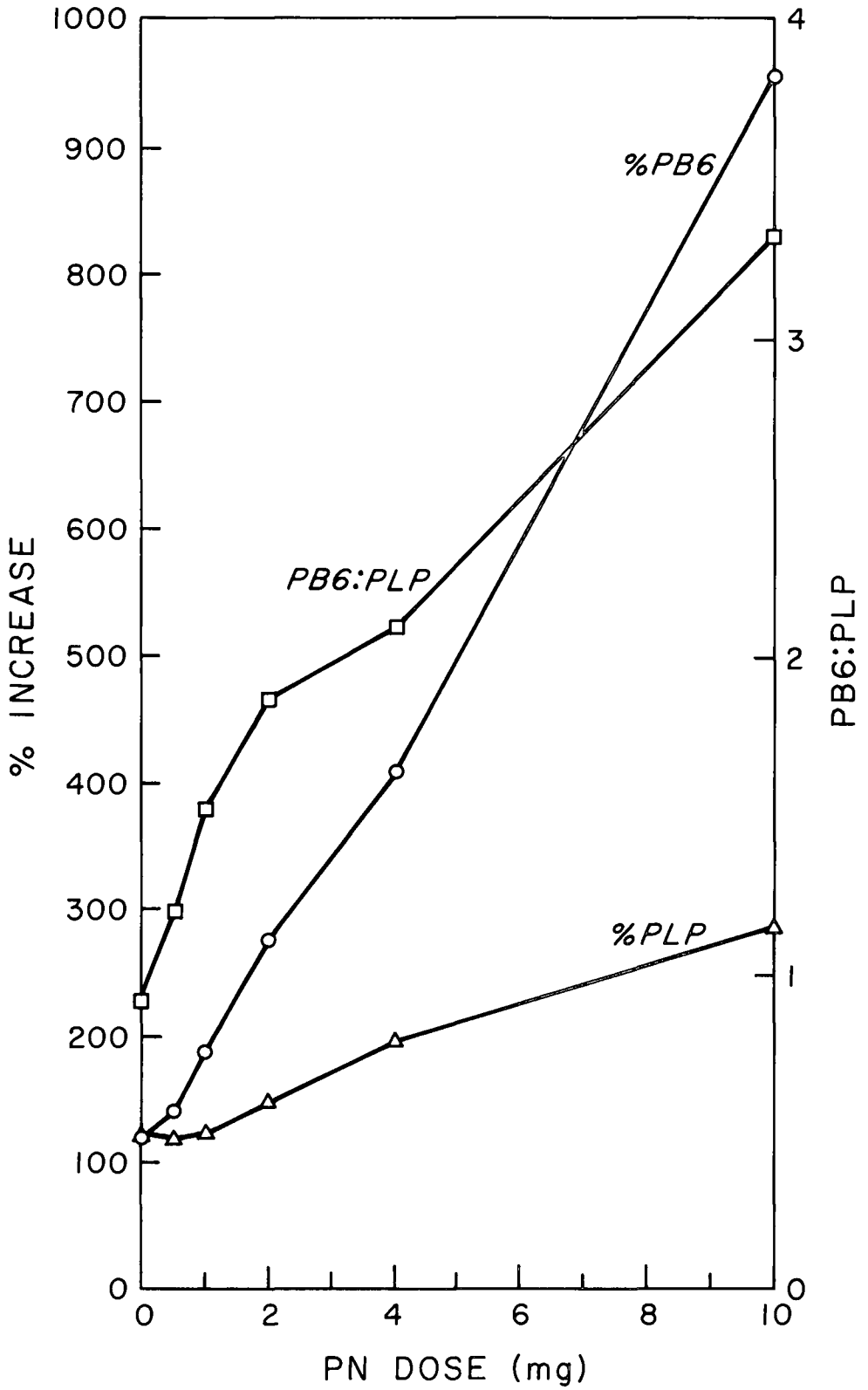

Figure 4. Mean %PB6, %PLP and ratio %PB6;PLP as a function of pyridoxine (PN) dose level.

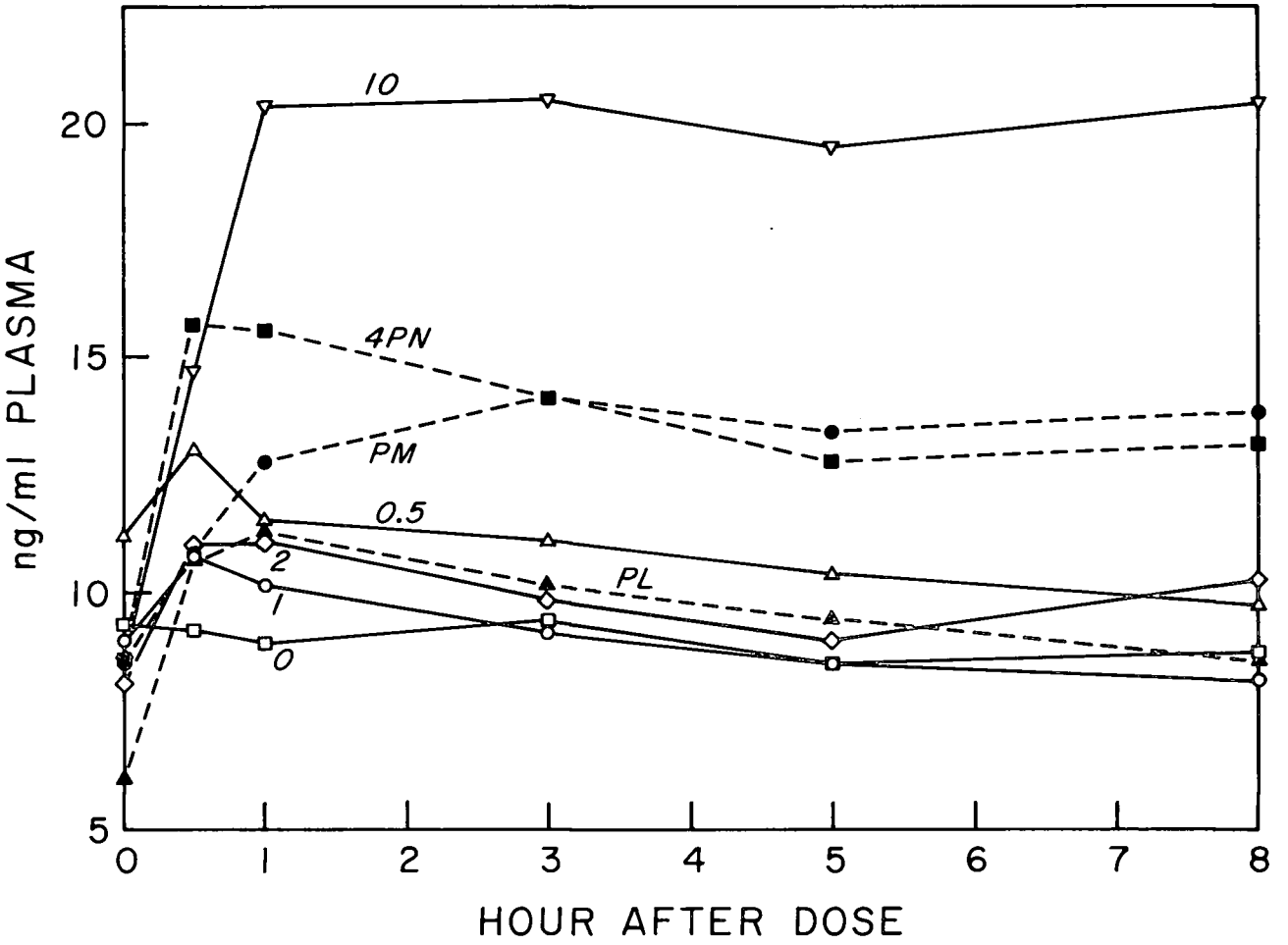

Figure 5. Mean plasma pyridoxal phosphate (PLP) for all pyridoxine (PN) levels and for equimolar doses of pyridoxine (PN), pyridoxamine (PM) and pyridoxal (PL). The 0.5, 1, 2, 4 and 10 (mg) are all PN doses. PM, PN and PL are equimolar doses (19.4 μmoles) of each B6 vitamer (- - -).

following the PN dose were less modulated than the PLP values (Compare Figures 3 and 5). The 8-hr plasma values for vitamin B6 and for PLP (Figures 3 and 5) are nearly the same. This lends weight to the idea that PLP is the principal form of the vitamin in the plasma.

Up to and including the 4 mg level, the peaks occurred at 0.5 hr following the dose. At the 10 mg level, the maximum values were reached at one hour, and the values remained constantly high for the remainder of the usual 8-hr sampling period. The fasting PLP levels at 27 hr following the 10 mg dose were still nearly double the predose fasting levels (Appendix A, Table A.2). The PLP areas for the varying levels and the %PLP are presented in Appendix A, Table A.2. The area parameters calculated in this study could be used as an indication of the relative predominance of PLP or of S. uvarum responsive B6 forms during the 8-hr period encompassed by the calculated areas. If the PB6 areas (Appendix A, Table A.1) are compared to the PLPAREAs (Appendix A, Table A.2), it is apparent that PLPAREAs account for a large portion of the total PB6 area, indicating that PLP is indeed the predominant form in the plasma. This would support the theories of Lumeng et al. (1974) and Anderson et al. (1974). Both %PLP (Figure 5) and PLPAREA increased with increasing PN dose, and both increased linearly (Appendix B, Table B.4). The overall effect of PN level on %PLP and on PLPAREA was significant ($p < 0.01$, Appendix B, Table B.2). The lowest levels which were significantly different from each other are designated in Appendix A, Table

A.2. PLPAREA could distinguish between the 2 mg and the 4 mg PN levels (8605 and 25,681 ng-min/ml x 10, respectively), whereas %PLP was significantly different between the 1 and 2 mg PN doses (1233 and 1475%, respectively). The %PLP variable was a more precise method of evaluating the dose responses than was PLPAREA. The %PLP was, however, less sensitive than %PB6 (Appendix A, Tables A.1 and A.2). Timed responses of plasma PLP following small doses of PN have not been measured in other investigations.

The Ratio of PB6 to PLP. The ratio of PB6 to PLP was calculated for each time period. Individual values and subject means are given in Appendix A, Table A.3 and plotted in Figure 6. The ratio increases at the 0.5 hr sample for all PN levels. Therefore, there is relatively more PB6 than PLP available for assay at 0.5 hr than at the other sampling times following the dose. From Figure 6, it appears that, except for the 4 mg PN level, the ratio increases with increasing dose. The anomaly of the 4 mg dose is markedly reduced when the data is reported as the ratio of the percent basal values for PB6 and PLP (PB6:PLP = %PB6:%PLP). Both individual and mean percent basal values and significantly different levels are given in Appendix A, Table A.3. The PB6:PLP variable proved to be more precise for distinguishing between the doses than either %PB6 or %PLP alone (Appendix A, Tables A.1-A.3). Thus, the ratio could distinguish between 0.5 and 1 mg, while %PB6 was unable to show

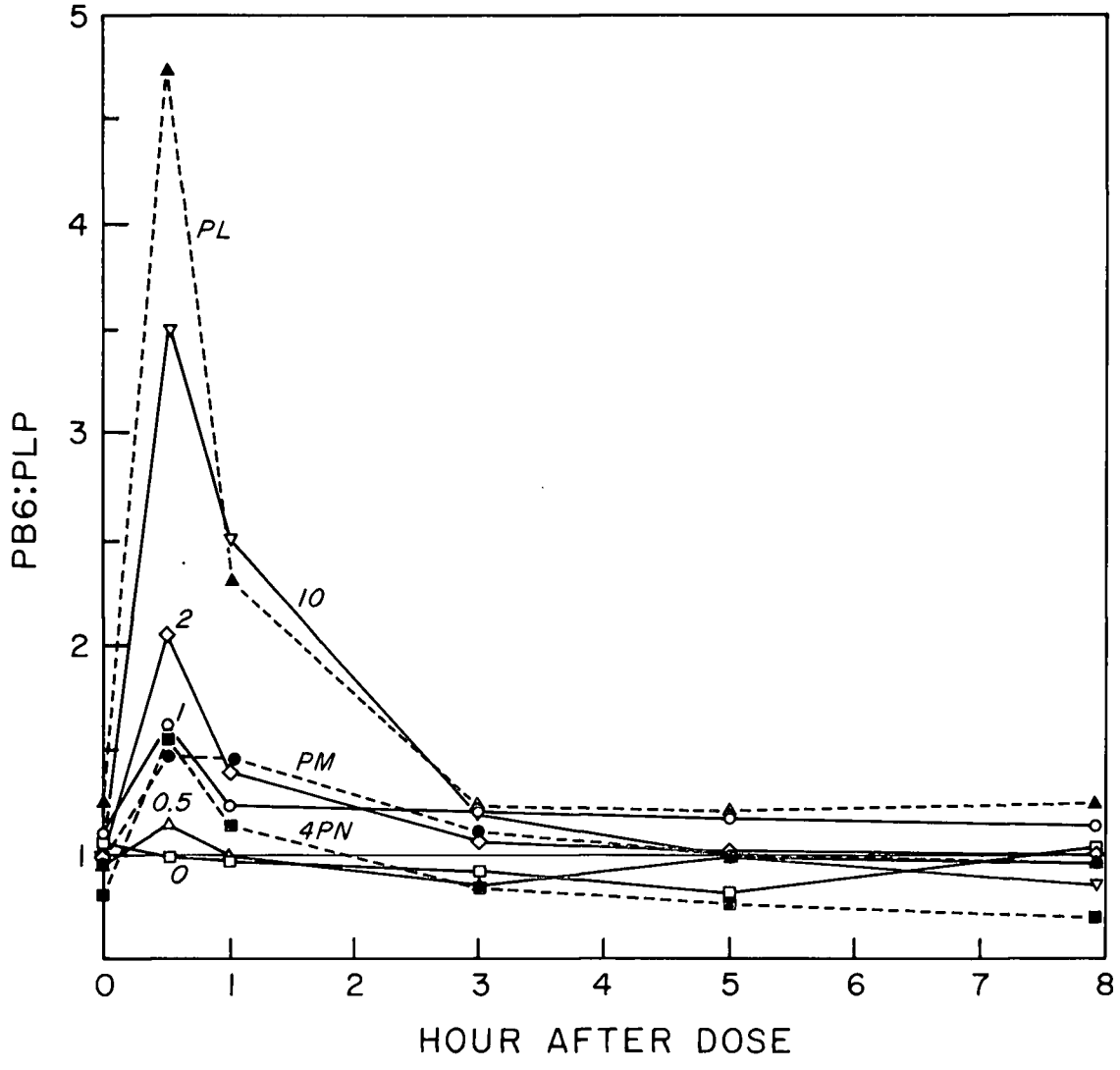

Figure 6. Mean ratio PB6:PLP for all pyridoxine (PN) levels and for equimolar doses of PN, pyridoxamine (PM) and pyridoxal (PL). The 0.5, 1, 2, 4 and 10 (mg) are all PN doses. PM, PN and PL are equimolar doses (19.4 μmoles of each B6 vitamer) (- - -).

significant differences for less than a 1 mg dose change. That is, a significant difference was seen for PB6 between 0 and 1 mg, but not between 0.5 and 1 mg (Appendix A, Table A.1). For %PLP, the smallest increment which was significant was that between 1 and 2 mg (Appendix A, Table A.2).

The percent basal values following the doses for PB6, PLP and PB6:PLP are plotted against dose level in Figure 4. There was a significant linear effect of PN dose on PB6:PLP (Appendix B, Table B.4). Although the linear effect was significant, inspection of the PB6:PLP line in Figure 4 indicates that there is a change in slope between the 2 and the 4 mg levels of PN, indicating that it is possible that the response of %PB6 and %PLP to different levels of PN is not linear over the range of 0 to 10 mg. The PLP values contribute more variability to the PB6:PLP ratio than do the PB6 values. This is supported by the fact that the F for linearity was larger for %PB6 than for %PLP (Appendix B, Table B.4). Clarification of the effect of PN level on PB6:PLP may be obtained with the use of smaller and more regular increments of the doses.

Urine Values

General. Urine volumes were the same or higher on the days during which timed collections were made, than on the days when one urine collection was made over an entire 24-hr period. The subjects

were encouraged to drink normal amounts of the allowed beverages to insure that urine samples could be given during each of the timed periods, but some samples, particularly the first following the dose, were still very low in volume (30 ml in one case).

4PA. The individual and mean 4PA values for all subjects for each collection period are given in Appendix A, Table A.4 and the means are plotted in Figure 7. The value of 4PA obtained for each sample was divided by the number of hours the sample was collected. The plotted points represent the hourly average excretion of 4PA for the time period immediately preceding that point. Thus, the 0 hr point is the average excretion of 4PA for the 24 hr preceding the dose, and the 3 hr point is the hourly average for the first 3 hr following the dose. Figure 7 indicates that for PN the maximum rate of excretion of 4PA occurred in the first 4 hr following the dose. Following a 100 mg PN load, Rabinowitz and Snell (1949) found peak values of 4PA to occur in the 2-5 hr sampling period. This seems to be somewhat later than the peak observed here, but this difference may be due either to the difference in the size of the doses used (100 mg versus 0.5 - 10 mg) or to the difference in the timing of the urine collection period (2 and 3 hr versus 3 and 5 hr). Differences between the results reported here and those reported by Rabinowitz and Snell (1949) may also be due to differences in the 4PA methods used. The method of Reddy et al. (1958) used in this study is less hampered by interfering

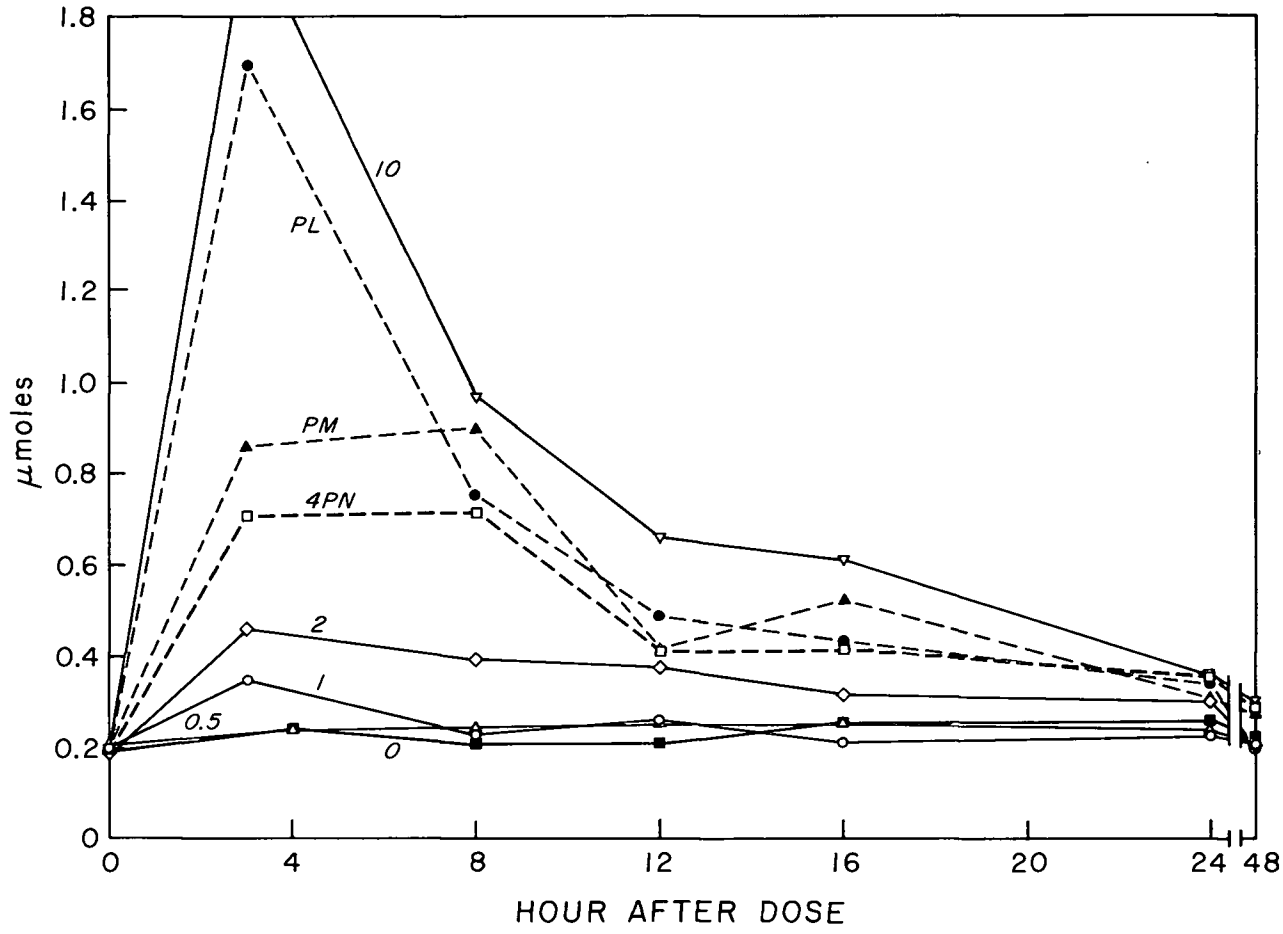

Figure 7. Mean urinary 4PA for all pyridoxine (PN) levels and for equimolar doses of PN, pyridoxamine (PM) and pyridoxal (PL). The 0.5, 1, 2, 4 and 10 (mg) are all PN doses. PM, PN and PL are equimolar doses (19.4 µmoles) of each B6 vitamer (- - -). Each point represents the hourly average excretion for the time period immediately preceding that point. Maximum 4PA excretion for the 10 mg PN level was 2.05 µmoles.

substances than is the method used by Rabinowitz and Snell (1949).

The mean 4PA content of the 4 mg level samples was constant for the first two periods following the dose. Some individuals peaked during the first collection period and others during the second (Appendix A, Table A.4). The mean percent of the PN dose excreted as 4PA decreased from 61.7 for the 0.5 mg PN level to 34.5 for the 10 mg level (Appendix A, Table A.5). The values for 4PA on the days before and after the dose day are given in Appendix A, Table A.6. The only appreciable difference between the 24 hr 4PA values on the day before and after the PN dose occurred at the 10 mg PN level. The mean 24 hr 4PA values on the day before the dose were 4.43 ± 0.97 μmoles and on the day after the dose 7.42 ± 1.77 μmoles.

The overall effects of level on percent basal and 4PAAREA were significant ($p < 0.01$, Appendix B, Table B.1). The individual and mean values for 4PAAREA and %4PA are given in Appendix A, Table A.4, which also indicates which levels were significantly different from other levels. Maximum precision of the response of 4PA to various levels was obtained with the 4PAAREA variable, which was able to distinguish between the 0 and the 2 mg PN doses (Appendix A, Table A.4), which had respective values of 1534 and 4203 nmoles-hr/hr. The smallest difference between PN dose levels which was significant for the %4PA variable was the 3 mg difference between the 1 mg (213%) and the 4 mg (521%) levels (Appendix A, Table A.4). The

4PAAREAs and %4PA (Figure 8) were both linearly related to dose ($p < 0.01$, Appendix B, Table B.4). When calculated as μmole/hr, the 4PA was also linearly related to PN dose (4PATH, Appendix B, Table B.4, $p < 0.01$).

Urinary Vitamin B6. Urinary vitamin B6 values were measured on hydrolyzed samples. The individual and mean vitamin B6 values for all subjects for each collection period are given in Appendix A, Table A.7, and the means are plotted in Figure 9. Urinary vitamin B6 values are presented as μmoles per hour in the same manner as were the urinary 4PA values. Also, as for the 4PA values, the maximum rate of excretion of UB6 occurred in the first 4-hr period following the PN dose. This is in agreement with Rabinowitz and Snell (1949), who found that the maximum rate of urinary excretion of vitamin B6 occurred within 2 hr following a 100 mg PN dose. Brain and Booth (1964) found that 80% of the total urinary excretion of a 1 mg ^{3}H-PN dose occurred within 4 hr after oral administration. This subject had previously been saturated with 200 mg PN given intravenously.

The amount of vitamin B6 excreted during this first period increased with increasing doses of PN (Appendix A, Table A.7). The relationships of UB6AREA, %UB6 (Figure 8) and UB6TH were all linear with respect to dose level (Appendix A, Table A.7 and Appendix B, Table B.4). Within a 1-100 mg range, Brain and Booth (1964) found a linear relationship between oral doses of ^{3}H-PN and the

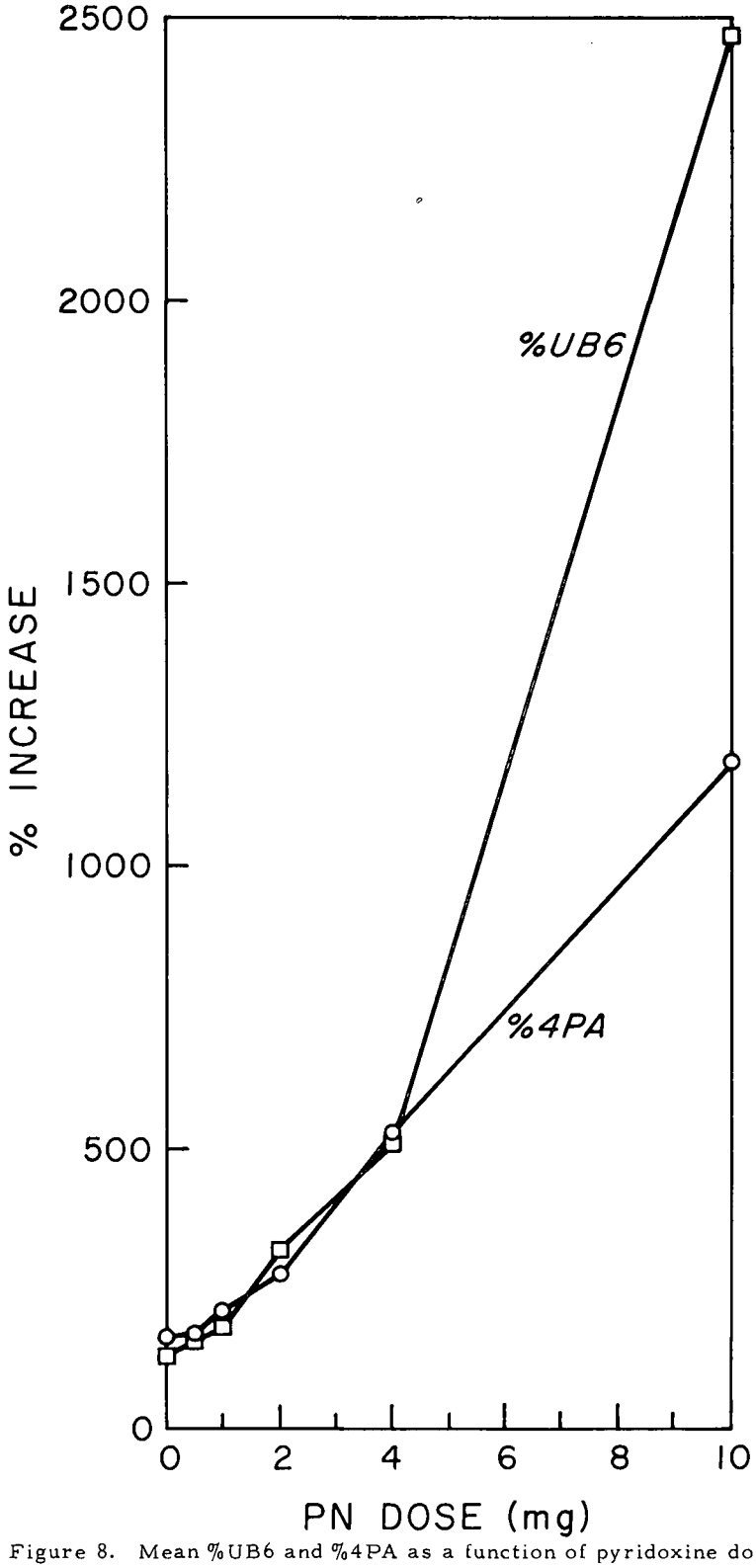

Figure 8. Mean %UB6 and %4PA as a function of pyridoxine dose level.

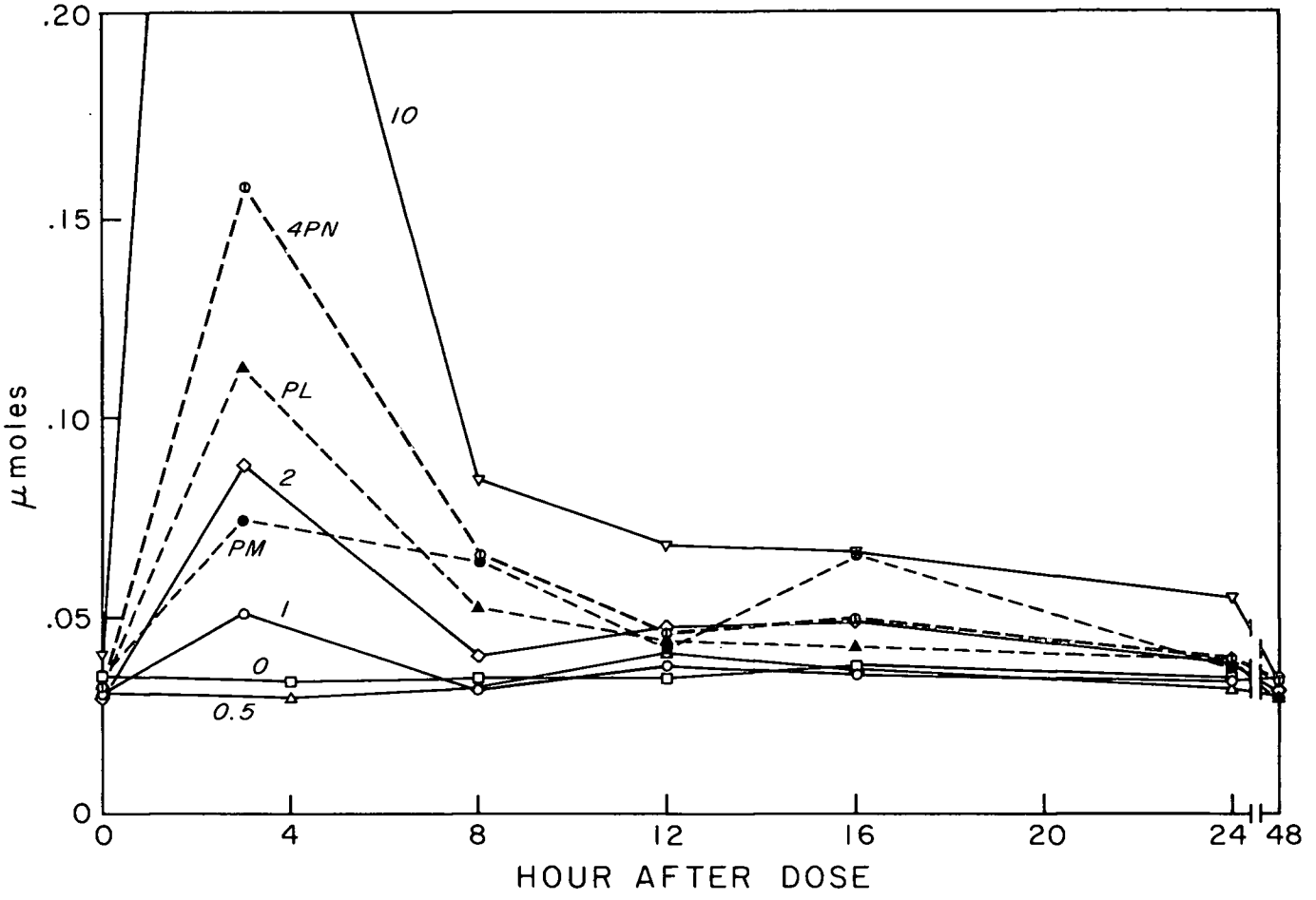

Figure 9. Mean urinary vitamin B6 for all pyridoxine (PN) levels and for equimolar doses of PN, pyridoxamine (PM) and pyridoxal (PL). The 0.5, 1, 2, 4 and 10 are all PN doses (mg). PM, PN and PL are equimolar doses (19.4 umoles) of each B6 vitamer (- - -). Each point represents the hourly average excretion for the time period immediately preceding that point. Maximum UB6 excretion at 10 mg PN level was 0.82 umoles.

amount of radioactivity excreted. The subjects had been given 200 mg PN intravenously. Similarly, in the rat, Brain and Booth (1962) found the ^{3}H-PN fed and that excreted in the urine to be linearly related between 0.05 and 5.0 mg. Their rats were also given simultaneous parenteral injections of PN. The overall effects of dose level on %UB6 and UB6AREA were significant ($p < 0.01$, Appendix B, Table B.1). The individual and mean values for UB6AREA and %UB6 and the levels which were significantly different from each other are shown in Appendix A, Table A.7. The smallest significant difference between PN levels for UB6AREA was between the 1 and 2 mg doses (3279 ± 1045 and 5733 ± 1414 nmole-hr x 10/hr, respectively). The %UB6 variable could discern a significant difference between the 0 (131%) and the 2 mg dose (320%). Urinary vitamin B6 was better able to distinguish between the lower levels of PN than was 4PA. (Compare Appendix A, Tables A.4 and A.7.) There was a significant difference between 1 mg and 2 mg for UB6AREA (Appendix A, Table A.7), but the minimum difference that could be measured by 4PA was 2 mg (4PAAREA, Appendix A, Table A.4). The mean % of the PN doses excreted as urinary vitamin B6 decreased from 8.7 in the 24 hr following the 0.5 mg dose to 6.9 following the 10 mg dose (Appendix A, Table A.5). There was not any appreciable change in the 24 hr UB6 values between the days before and after each of the doses (Appendix A, Table A.6).

The example of UB6AREA (Appendix A, Table A.7) illustrates a phenomenon that is also seen for other parameters measured. That is, that often no significant difference will be observed between 0 and a certain level, but there will be a significant difference for the same incremental increase between the two next higher levels. Here, for instance, there is no significant difference between 0 and 1 mg, but there is a significant difference between 1 and 2 mg, even though the difference between both pairs is 1 mg. The precision of the method is therefore, dependent on both the magnitude of the difference between two levels, and on the absolute level of the dose administered. It is apparent from the data here that a given amount above 0 must be reached before significant differences are perceived.

UB6 and 4PA. Total 24 hr excretion of UB6 and 4PA (TUB64PA) were added and regressed against dose (Appendix A, Table A.5). This sum was found to be linearly related to dose (Appendix B, Table B.4). The TUB64PA in μmoles per 24 hr is given in Appendix A, Table A.5. The percent of the dose recovered as TUB64PA decreased with increasing PN dose (Appendix A, Table A.5). The percent of the dose recovered was high compared to the 45-52% recovered in urinary vitamin B6 and 4PA by Perera (1977), with intakes averaging 8.97 μmoles per day. The crystalline forms of the vitamin used in this study may have been absorbed more completely than the vitamin given in the food by Perera. A higher absorption of the dose would result

in a larger fraction of the dose recovered in the urine, than in the feces. Rabinowitz and Snell (1949) recovered 41.4% of the 82 mg PN dose given, after analyses of urine for PL, PM, PN and 4PA. This is the same as the 41.4% recovery that is reported in Appendix A, Table A.5 for the 10 mg dose. TUB64PA decreased from 97.9% of the 0 dose to 41.4% of the 10 mg PN dose (Appendix A, Table A.5).

The fact that the TUB64PA decreased with increasing dose could be explained in either of two ways. One explanation for this decrease is that there could be a decreased amount of PN absorbed at the higher doses. Since fecal collections were not made in this experiment, total recovery of the administered dose could not be made. Another explanation might be that other vitamin B6 metabolites (such as 4PAP) could be formed in larger proportions at the higher dose levels. If this were happening, measurement of UB6 and 4PA only would not account for all the PN absorbed from the intestine (Sauberlich, 1974; Contractor and Shane, 1974).

The cumulative percent of intake of TUB64PA calculated for each period and averaged for all subjects (Table 8) indicates that for all PN levels, 13.3-43.3% of the dose was excreted in the first collection period. This period coincides with the times of the maximum increases of PLP and vitamin B6 in the plasma. The larger the fasting dose, the longer it took to be metabolized (Tables 8 and 9). The ratio 4PA:UB6 (= 4PATH:UB6TH) (Appendix A, Table A.5) was

Table 8. Mean TUB64PA for each collection period for all levels of PN and for PM and PL doses as cumulative % of intake[a]

Level (mg)	Form	Hour after Dose					
		0-3	4-8	9-12	13-16	17-24	0-24
0		-	110.4	46.8	64.6	98.0	98.0
0.5	PN	43.3	50.0	37.6	48.8	71.9	71.9
1	PN	24.3	36.6	32.4	42.2	62.9	62.9
2	PN	16.9	32.6	33.9	42.9	59.5	59.5
4	PN	13.4	30.6	32.3	39.4	51.5	51.6
10	PN	17.7	27.4	30.4	35.3	41.4	41.4
(4)	PM	14.1	35.3	36.1	43.7	54.2	54.2
(4)	PL	27.4	43.9	44.3	51.7	51.9	51.9

[a]For calculation of the % cumulative intake, the vitamin B6 intakes of the 0-3 hr period included the dose only. The 4-8 hr period included the dose plus the vitamin B6 content of lunch. The vitamin B6 intake of the other periods included the vitamin B6 in the dose, that of lunch and of dinner.

Table 9. Mean ratio 4PA:UB6 for each collection period for all levels of PN, and for equimolar dose of PM and PL

Level (mg)	Form	Hour after Dose					
		0-3[a]	4-8[a]	9-12	13-16	17-24	0-24
0		7.19	6.04	6.04	6.52	6.76	6.58
0.5	PN	7.95	7.66	6.31	5.67	7.21	7.11
1	PN	6.83	7.33	7.03	6.96	7.75	7.28
2	PN	5.22	9.79	7.97	6.34	7.77	7.31
4	PN	4.48	10.97	9.03	8.53	9.11	8.14
10	PN	2.49	11.55	11.59	9.24	6.64	5.11
(4)	PM	13.75	13.83	10.15	8.07	8.30	10.42
(4)	PL	21.87	11.52	10.90	10.47	9.16	12.08

[a]For the week of 0 and 0.5 mg PN, the first two sampling periods were both 4 hr, instead of the 3 and 5 hr periods used for the other weeks

regressed against PN dose level but was not found to be linear (Figure 8 and Appendix B, Table B.4). At the 10 mg level, the ratio fell off considerably, indicating that at this level proportionately much more vitamin B6 was spilled into the urine than at the other levels (Appendix A, Table A.5). At the 10 mg level, therefore, it appears that the capacity of the subjects to metabolize the PN doses had been reduced since the 4PA:UB6 was decreased. Table 9 gives 4PA:UB6 for each dose level and each collection period. During the initial collection period, the ratio of 4PA:UB6 decreases with increasing PN dose levels. Since it is shown in Appendix A, Tables A.4 and A.7 that the amounts of both UB6 and 4PA increase with increasing level of PN during the first urine collection period, the decrease in 4PA:UB6 for this same period is due not to a decrease in either component, but to a relatively greater increase in the amount of vitamin B6 excreted in the urine during that first period. Therefore, most of the portion of the dose that will not be metabolized is excreted in the first period. During all the other timed urine collection periods (Periods up to 24 hr, Table 9) 4PA:UB6 increased with increasing levels of PN. (The only exception to this was the fifth period at the 10 mg PN dose level.) This indicates that there was relatively more vitamin B6 metabolized to 4PA during these periods than during the initial 3 hr periods, and that during these periods the relative amount of vitamin B6 metabolized to 4PA increased with increasing dose.

Therefore, although the 24 hr total 4PA:UB6 decreased at the 10 mg level, it can be seen from Table 9 that the ratio changes between periods as well as between dose levels.

Effect of Form of Vitamin B6

General

Three forms of vitamin B6 were given in amounts equimolar to 4 mg PN. The subjects were sampled for blood and urine at timed intervals following the dose in the same manner as was done for the studies on the effect of level of PN.

Blood Responses

PB6. The mean response curves for plasma vitamin B6 for the three forms of vitamin B6 administered are shown in Figure 3. Both individual values and subject means are given in Appendix A, Table A.8. The plasma vitamin B6 following equimolar doses (19.44 μmoles) of PL and PN peaked at 1/2 hr. With PM the mean peak was more diffuse and occurred at 1 hr; the subjects did not all peak at the same time. Three reached maximum levels at 1 hr, one at 1/2 hr, and one at the 3 hr sample. The %PB6 was highest for the PL dose. Since the S. uvarum assay is nearly equally responsive to all non-phosphorylated forms of vitamin B6, the differences in %PB6 between the forms is most likely due to differences in absorption rather than to

differences in rates of interconversion of the vitamers. A logical extension of this study would be to analyze the urine samples collected after each dose for the presence of each form of vitamin B6.

The plasma vitamin B6 values following the PM dose took much longer to return to fasting levels than did the plasma vitamin B6 values following the PN or PL doses. The overall effect of form on the %PB6 values was significant ($p < 0.01$, Appendix B, Table B.1). The different forms did not have a significant effect on PB6AREA (Appendix B, Table B.1). Values for %PB6 and PB6AREA are given in Appendix A, Table A.8. The forms which were statistically different from each other are designated in Appendix A, Table A.8. The %PB6 variable was able to distinguish ($p < 0.01$) between all three forms at the 4 mg level.

Plasma PLP. As in the experiment of the effect of PN level, the plasma PLP responses to the three forms of vitamin B6 were not as great in magnitude as the PB6 responses to the same doses (Appendix A, Tables A.8 and A.9; Figures 4 and 6). The maximum values of plasma PLP occurred later than the maximum PB6 values. This result might be expected since the tyrosine decarboxylase assay is specific for PLP and the formation of PLP is dependent on the conversion reactions depicted in Figure 1. The mean maximum plasma PLP values were reached at 1/2 hr for PN, at 1 hr for PL, and at 3 hr for PM. There was, however, considerable variation

among the subjects with respect to the time required after the dose to reach maximal plasma PLP values (see individual PLP values, Appendix A, Table A.9).

The analysis of variance for the effect of form on %PLP and PLPAREA indicated that only for PLPAREA was there a significant difference due to form ($p < 0.01$, Appendix B, Table B.1). The forms which were significantly different from each other are designated in Appendix A, Table A.9. The overall effect of form on %PLP was not significant. At the 4 mg level, PLPAREA could distinguish between PN (25,681 ng-min/ml x 10) and PL (14,448), and between PM (22,006) and PL, but not between PN and PM.

Lumeng et al. (1974) have shown that PLP is formed in the liver. McCoy and Columbini (1972) have shown in the livers of mice that following an intravenous dose of ^{14}C-PN, phosphorylation proceeded more rapidly than oxidation. It would be expected from these studies that the plasma PLP would be highest following the PL dose. Nearly the opposite was found in this study. There was no significant difference between the %PLP values for the three forms. The PLPAREAs were smallest following the PL dose. This may reflect a homeostatic control that regulates the level of plasma PLP. These results are not inconsistent with those of Colombini and McCoy. The liver could be rapidly phosphorylating the doses administered, but there may be

other factors controlling the release of PLP into the peripheral blood.

PB6:PLP. Consistent with the study of PN levels, the PB6:PLP ratio (Figure 6, Appendix A, Table A.10) reflects the fact that PB6 is a parameter much more responsive to the presence of each of the three forms in the plasma than is the tyrosine decarboxylase assay for pyridoxal phosphate. The most reasonable explanation for the difference between the plasma vitamin B6 and the plasma PLP responses to these three B6 vitamers is that the formation of PLP is a function of more metabolic regulation than the B6 vitamers measured by the S. uvarum assay.

Urine Values

4PA. The individual and mean 4PA values for each form and period are given in Appendix A, Table A.11 and the means are graphed in Figure 7. The values are calculated and plotted in the same manner as were the 4PA values from the experiment of the effect of level of PN. The subject means following the PL dose reached maxima in the first collection period. The PM and PN curves plateaued for the first two periods before falling off. The PM curve reached its maximum value in the second time period (4-8 hr after the dose). The times of occurrence of the peaks of urinary 4PA were similar to those obtained with 100 mg doses used by

Rabinowitz and Snell (1949). In their experiments, the 4PA after the PL dose peaked earlier than did the urinary 4PA after either the PN or PM doses. The curves reported by Rabinowitz and Snell (1949) were spiked for PL and flattened in appearance for PN and PM. The relative size of the peaks for the different forms were slightly different for the 100 mg doses used by them. The decreasing order of magnitude of the peaks they found was PL > PN > PM. Figure 7 indicates that the order of magnitude of the peaks obtained in this experiment was PL > PM > PN. It seems reasonable that PL would result in the highest concentration of 4PA, since it requires only oxidation to form 4PA (Figure 1). The difference between the results given here and those reported by Rabinowitz and Snell (1949) for the urinary excretion of 4PA following the PM and PN doses may not be significant. In this experiment the calculated 4PAAREA and %4PA variables were not significantly different between forms. Another explanation for the smaller PN peak may be that less of this dose was absorbed. This argument for a difference in amount of the dose absorbed is supported by the differences in the PB6 values for the different forms (p. 63).

The 24 hr values for 4PA on the days before and after each of the doses are given in Appendix A, Table A.12. Only for the PM dose did there appear to be any difference in the before and after values. This may not be a consistent trend for all subjects since data for two

subjects were missing. Either not all of the PM dose was completely metabolized by the following 24-hr period, or the self-selected diet on the day following the dose had higher levels of vitamin B6 than on other Fridays.

The rise in the PM curve for three subjects in the fourth period (12 hr, Figure 7) may be related to the timing of the evening meal (served at 10 1/2 hr following the dose), or to the timing of the urine collections.

The means for %4PA and 4PAAREA are given in Appendix A, Table A.11. The overall effect of form on these parameters was significant (Appendix B, Table B.1). The forms which differed significantly from each other are designated in Appendix A, Table A.11. The 4PAAREA could distinguish between PN (7,219 nmole-hr/hr) and PL (12,329) and between PM (9,571) and PL, but not between PN and PM. The %4PA variable was also able to measure significant differences between PL (1007%) and PN (521%) and between PL and PM (631%), but not between PN and PM. The 4PAAREA and %4PA variables differed between forms in the same way as did the absolute peaks discussed above. The amounts of each form converted to 4PA were in decreasing order PL > PM > PN.

UB6. The individual and mean UB6 values for each period are reported in Appendix A, Table A.12 and the means are plotted in Figure 9. All calculations were done as for the PN level experiment.

The maximum values for the different forms were all reached in the first period, and fell thereafter, but did not return to the initial levels until the following day (Appendix A, Tables A.6 and A.13; Figure 9).

The timing of the peaks were similar to those seen by Rabinowitz and Snell (1949) who used 100 mg doses of the three forms. They found the greatest rate of excretion of each of the forms in the urine within the first 5 hours after the dose. Since they measured each of the vitamers in the urine rather than total vitamin B6, a comparison of the relative magnitude of the peaks for the vitamers with those found in this study would not be valid.

The PM curve also rose at the 16-hr time period. Again, as for the 4PA, this may have been due to either the evening meal or to the timing of the urine collections.

The effect of form on %UB6 and UB6AREA was not significant (Appendix B, Table B.1). The individual and mean values for the %UB6 and UB6AREA parameters are given in Appendix A, Table A.13. The 24-hr excretions of the total of UB6 and 4PA (TUB64PA = 24(4PATH + UB6TH)) are reported in Appendix A, Table A.14. The TUB64PA excreted as percent of the dose were virtually identical for all forms (51-53%). The ratios 4PA:UB6 (%4PA:%UB6) for each period and for the total 24 hr periods are given in Table 9. Both PM and PL doses resulted in lower 24-hr excretions of UB6 than did PN (Appendix A, Table A.13). Concomitantly, the amount of 4PA

excreted for PM and PL was more than the 4PA excreted following the PN dose. This indicates that more of the PM and PL doses were metabolized to 4PA than was the PN dose (Table 9). When this is expressed as 4PA:UB6 the decreasing order of conversion to 4PA is PL > PM > PN (Table 9).

The percentages of the doses recovered were comparable to those found by Rabinowitz and Snell (1949) who used 72-80 mg doses of the vitamers. The total percent recoveries for all urinary vitamin B6 forms measured by them were 30.4 for PM, 41.4 for PN and 65.6 for PL. The recoveries obtained here were 53.3% for PM, 51.6 for PN and 50.9 for PL (Appendix A, Table A.14). There was less variation among forms at the 4 mg level than at the higher levels used by Rabinowitz and Snell (1949).

Effect of Exercise on Plasma Responses

The original reason for conducting this experiment was to explain the great rise in PB6 values for subject 4 two hours after the 0.5 mg PN dose (Figure 10). Subject 4 said that he had run two miles between the 1 and 2 hr samples, so it seemed that strenuous exercise might have caused his aberrant PB6 results. For comparative purposes, the data for the plasma area variables after the 1 mg PN dose without exercise were recalculated and abbreviated to include the same time periods as those used during the exercise experiment.

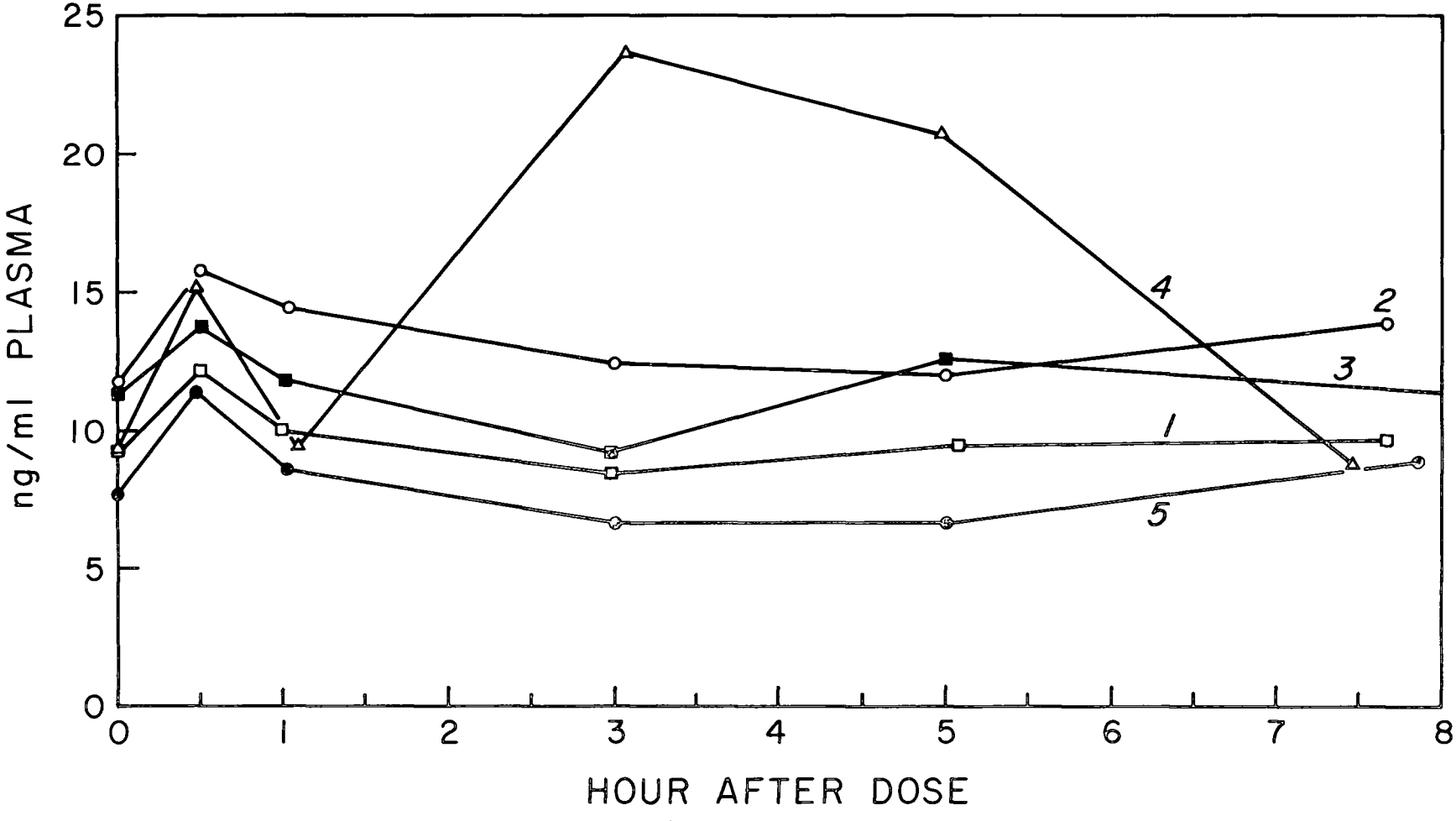

Figure 10. Individual plasma vitamin B6 following an oral 0.5 mg pyridoxine dose. Subject 4 ran 2 miles between the 1 hr and 3 hr samples.

The PB6AREA and PLPAREA for individuals during the exercise experiment are shown in Table A.15, Appendix A, and are plotted in Figure 11. To see the effect of exercise on these parameters for each individual, Figure 11b should be compared with Figure 12 for PB6, and Figure 11a with Figure 13 for PLP. The subject means without exercise are shown in the 1 mg levels of Figure 4 (PLP) and 3 (PB6).

The areas bounded by the PB6 curves were not significantly different between the 1 mg doses with or without exercise (Figures 11b and 12; Appendix B, Table B.3). Although for two subjects, the PB6 values rose slightly following the exercise, the differences were not enough to affect the area response to the dose. For the PLP areas, however, four out of five of the subjects showed a definite change in PLP levels after exercise (Figure 11a, Appendix A, Table A.15). These changes were large enough to significantly affect the response to the dose, as measured by PLPAREA ($p < 0.05$, Appendix B, Table B.3). For two individuals (3,2), the level of PLP in the blood was higher following the exercise than it was after the dose.

Muscle phosphorylase is utilized during exercise for energy production from glycogen. The rise of PLP in the plasma following exercise may be a response by the liver or other organs to the need for energy. The results of this experiment do not show whether the magnitude of the PLP rise following exercise is independent of dose.

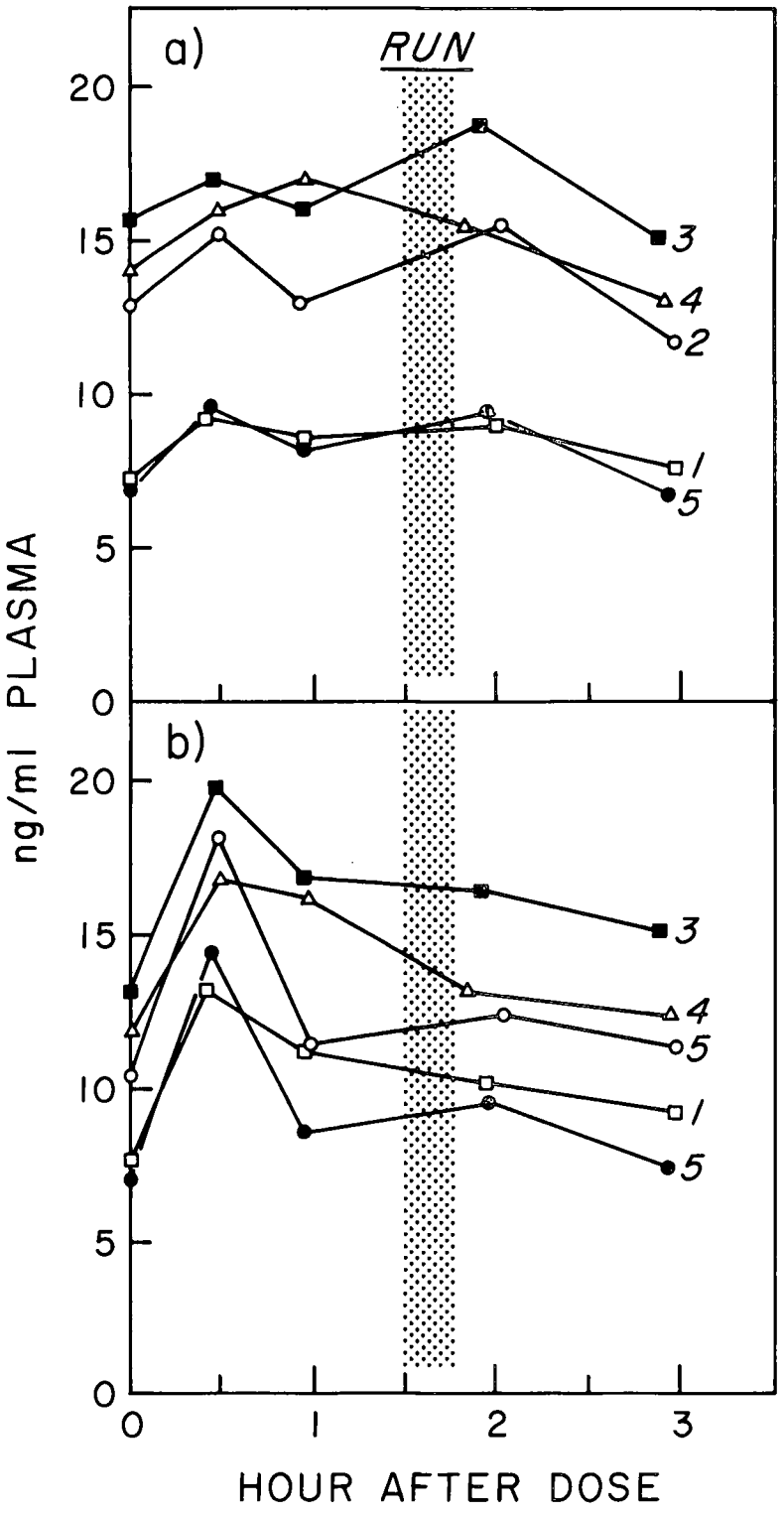

Figure 11a. Individual plasma pyridoxal phosphate with exercise and following an oral 1 mg pyridoxine dose.

b. Individual plasma vitamin B6 with exercise and following an oral 1 mg pyridoxine dose.

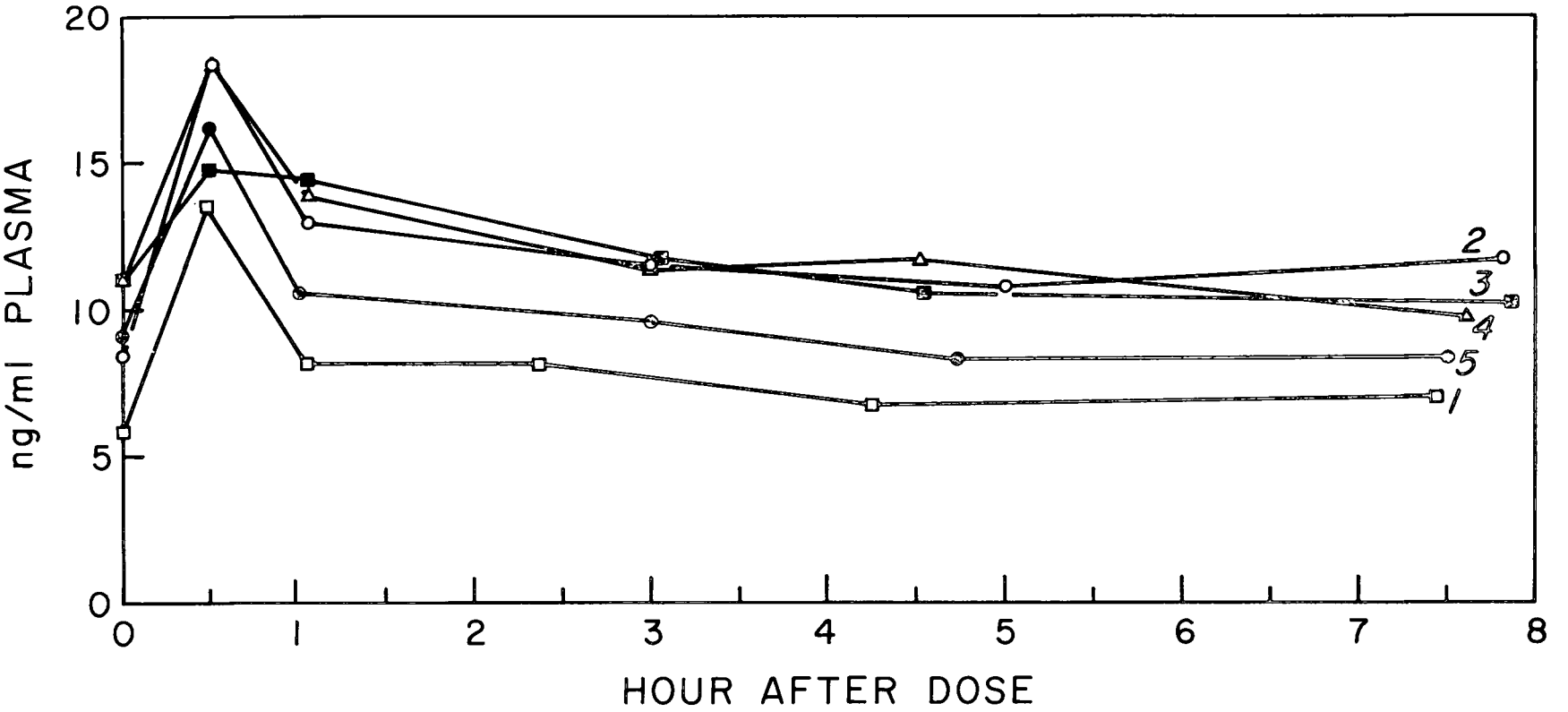

Figure 12. Individual plasma vitamin B6 following an oral 1 mg pyridoxine dose.

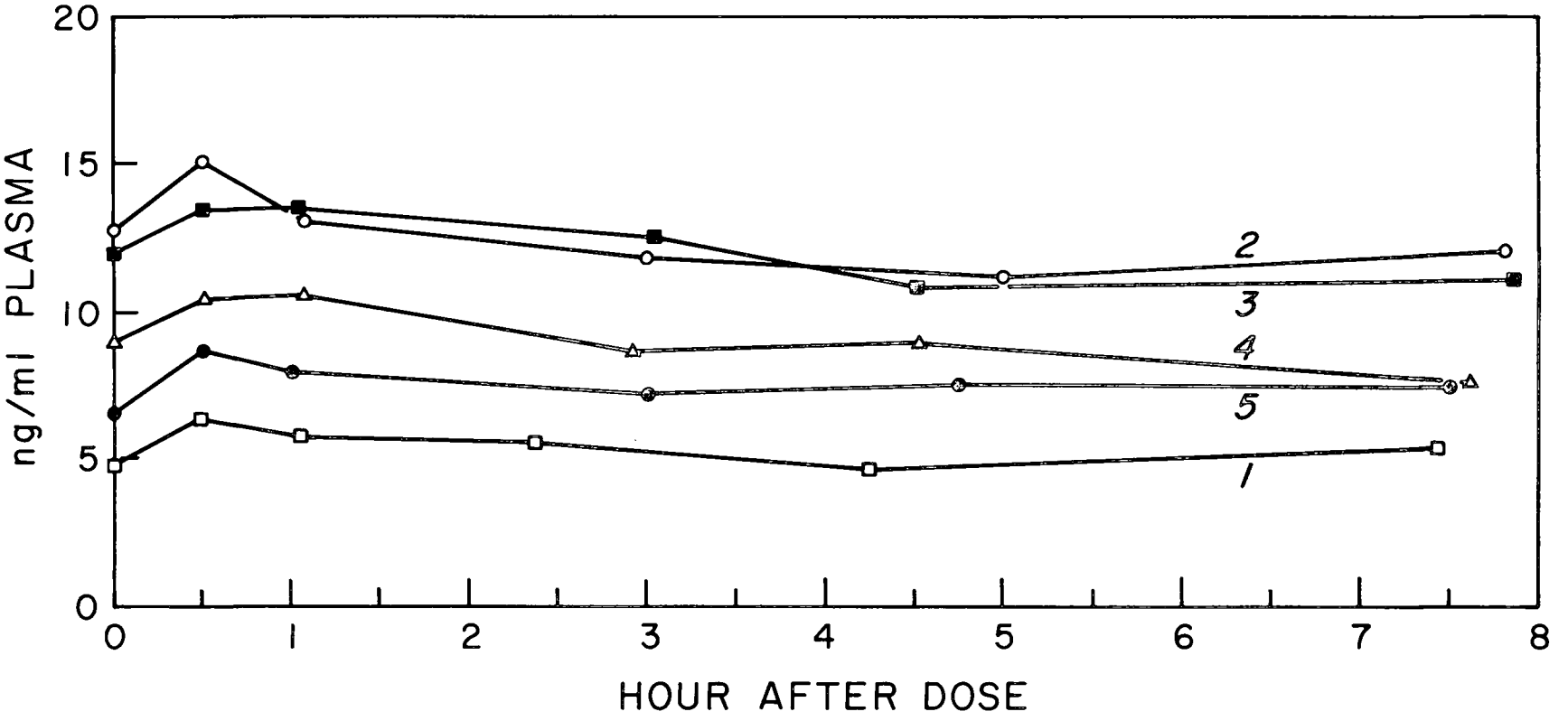

Figure 13. Individual plasma PLP following an oral 1 mg pyridoxine dose.

Since the subjects had received nothing but the 1 mg PN dose, this experiment may have simulated the starvation study of Black et al. (1977) in which muscle phosphorylase was shown to release PLP. The transaminases which would be increasing during this period (liver alanine and aspartate transaminase during starvation (Black et al., 1977) and glutamic-oxaloacetic transaminase from exercise (Critz, 1966), would be able to use the PLP being liberated in the plasma. It is possible that the maximum levels of PLP were not obtained because of the timing of the samples. Samples drawn during and immediately after the exercise would have given a clearer picture of the plasma PLP changes. It is not clear why the plasma vitamin B6 levels did not also rise following exercise. Mobilization of the enzyme as measured by changes in plasma PLP can occur within a half hour. It is possible that plasma samples drawn during or immediately after exercise may have shown higher PLP values than those drawn 15 min after the completion of the exercise. Critz (1966) has shown that the duration of the exercise has an effect on enzyme levels. Glutamic-oxaloacetic transaminase increased during the first 0.5 hr of an exercise period, and then decreased for the remainder of the exercise.

No definite relationship could be drawn here between the training of the subject and the rise in PLP following exercise.

The original 3 and 5 hr PB6 values of Subject 4 with the 0.5

mg PN dose were probably erroneous. This experiment seems to eliminate exercise as a cause for his outlying PB6 values. Of all the subjects partaking in the exercise experiment, Subject 4 was the only one showing consistently no response in plasma values of either vitamin B6 or PLP (Figure 11a and 11b).

These results indicate that physical activity should be restricted during the period of timed blood drawings for the measurement of plasma pyridoxal phosphate.

Relationship of Self-Selected Diet to Dose Responses

Nutrient contents of the self-selected diets (Friday through Tuesday) are presented in Table 10. Intake of nutrients not reported in Table 10 were adequate. The 1.51 mg daily average of vitamin B6 was what we desired. The mean for calories was sufficient to maintain weight during the study. None of the subjects had an appreciable weight change during the study (Table 1). The mean protein intake of 90 g was comparable to the 80 g level fed in the controlled meals in the metabolic unit. There was not, therefore, an appreciable change in the level of protein ingested by the subjects on the controlled diet and the dose days compared to their normal diet. This eliminated a period of adjustment to a different protein level, which might have affected the utilization of vitamin B6 (Miller and Linkswiler, 1967). The ascorbic acid intake varied considerably among individuals.

Table 10. Nutrient content of self-selected diets[a, b]

Week (before level)	Vitamin B6 (mg)	Calories (kcal)	Protein (g)	Fiber (g)	Ascorbic Acid (mg)	Alcohol (g)	Caffeine (mg)
1, 2[c]	1.28[d]	2077	84.6	4.1	82	4.6	85
(0, 0.5 mg PN)	±0.45	±416	±20.1	±2.9	±35	±7.8	±92
	64%[e]	77%	151%	-	183%	-	-
3	1.43	2626	94.2	4.0	125	12.4	112
(1 mg PN)	±0.24	±467	±14.8	±1.8	±126	±1.3	±85
	72%	97%	168%	-	277%	-	-
4	1.58	2461	87.0	4.7	93	12.9	128
(2 mg PN)	±0.54	±511	±26.2	±1.8	+39	±15.2	±167
	79%	91%	155%	-	206%	-	-
5	1.54	2455	89.8	3.7	94	11.4	180
(4 mg PN)	±0.42	±802	±27.0	±2.3	±69	±17.3	±160
	77%	91%	160%	-	208%	-	-
6	1.52	2444	81.2	5.1	98	22.6	149
(10 mg PN)	±0.37	±599	±19.9	±1.9	±82	±26.6	±100
	76%	91%	145%	-	218%	-	-
7	1.69	2552	95.3	6.6	108	2.8	108
(4 mg PM)	±0.51	±254	±15.6	±3.3	±100	±3.8	±150
	84%	95%	170%	-	240%	-	-
8	1.56	2702	107.7	4.7	123	4.4	132
(4 mg PL)	±0.42	±499	±14.6	±2.0	±81	±3.8	±152
	78%	100%	192%	-	273%	-	-
Overall mean	1.51	2465	90.8	4.6	98	10.2	128
	±0.41	±521	±20.2	±2.3	±75	±6.9	±31
	75%	91%	162%	-	222%	-	-

[a] Friday through Tuesday, inclusive

[b] Intake of other nutrients was adequate (2/3 RDA, National Academy of Sciences, 1974)

[c] Two dose levels were done in one week

[d] Subject means for each week ± standard deviation

[e] 1974 Recommended Dietary Allowances (RDA) for adults (National Academy of Sciences, 1974)

Ascorbic acid is related to 4PA excretion in a subsequent section. Correlations between some nutrients in the self-selected diets and responses following each dose are discussed below.

One way of estimating the effect of the self-selected diets on the experiment is to examine the initial values for the parameters measured. Since the initial values did not vary outside normal ranges and did not vary widely among subjects, it may be concluded from these initial values that the one day of controlled diet was sufficient to bring all subjects to within a small range of blood and urine values. Compare initial values on Figures 3, 5, 7 and 9 and standard deviations for initial mean values Appendix A, Tables A.1, A.2, A.4, A.7, A.8, A.9, A.11 and A.12).

Statistical correlations were done for each dose between selected nutrients calculated from the weekly diet records of the self-selected diets (Friday through Tuesday) and response variables following the Thursday doses of the same week. The significant correlations are indicated in Table 11. The correlations that were significant show no definite pattern. The number of significant correlations were fairly evenly distributed throughout the weeks. The fact that these correlations are significant may be meaningless considering the accuracy of the dietary records and their nutrient calculation. However, the appearance of these significant correlations with so few subjects would argue for extending the period of controlled diet prior

Table 11. Significant correlation coefficients between certain compounds in self-selected diets and response variables for each week[a]

Dietary Compound	Dose Response Variable															
	UB6AREA		4PAAREA		PB6AREA		PLPAREA		%PLP		%PB6		%UB6		%4PA	
	r	wk	r	wk	r	wk	r	wk	r	wk	r	wk	r	wk	r	wk
Ascorbic Acid	0.86	5	0.82	4			0.87	5	-0.82	6	0.95	7	-0.83	2	0.84	4
									-0.99**	7			-0.99**	8		
Protein							0.86	6	-0.99**	8	-0.90	6			-0.90	6
											-0.93	8			0.98*	7
Calories	0.94	7			0.94	2					-0.98**	6				
Vitamin B6					0.91	2									0.99**	6
Alcohol							-0.92*	5	0.87	3	0.89*	2			0.82	3
									0.98*	8	0.97**	4			-0.82	4
											0.91*	6				
Caffeine			0.89*	1	-0.81	6	-0.83	3	0.83	2	0.81	4			0.87	2
			0.99**	2	-0.95*	7	-0.92	8								
					-0.93	8										

Significance: No star, $p < 0.10$; *, $p < 0.05$; **, $p < 0.01$

[a]

Week	1	2	3	4	5	6	7	8	
Level	0	0.5	1	2	4	10	(4)	(4)	mg
Form		PN	PN	PN	PN	PN	PM	PL	

to the administration of the fasting vitamin B6 dose, and perhaps for stricter controls of alcohol and caffeine, in particular.

Relationship of Weight to 4PA

Both positive (Perera, 1977) and negative (Young, 1973) relationships between body weight and 4PA excretion have been reported. Simple correlations were calculated for each week between body weight and total 24 hr 4PA excretion on the day before each dose. There was no significant relationship found in this study between weight and 4PA excretion.

Relationship of Ascorbic Acid Intake to 4PA

Ascorbic acid intake has been directly related to 4PA excretion (Kraut and Imhoff, 1968). Correlations were done between ascorbic acid intake of the subjects on their self-selected diets and the 4PA excretion on the day before the dose. No significant correlations were found.

Loading Doses of B6 Vitamers in the Guinea Pig

Blood Responses

PB6

The mean guinea pig PB6 values following two months of vitamin B6 doses are reported in Table 12. The samples were taken 24 hr

Table 12. Guinea pig body and organ weights, blood elements, and vitamin B6 compounds in blood and urine for equimolar doses of PN, PM and PL

Parameter	Unit	B6 Vitamer PN	PM	PL
Organ weights	g			
Brain		3.50 ± 0.33	3.56 ± 0.35	3.55 ± 0.29
Spleen		0.70 ± 0.11	0.61 ± 0.14	0.62 + 0.17
Kidney		4.48 ± 0.49	4.36 ± 0.68	4.06 ± 0.53
Liver		20.79 ± 4.33	21.20 ± 1.76	21.85 ± 3.47
Final body weight	g	764 ± 71	700 ± 93	695 + 117
Weight gain	g	142 ± 137	118 ± 174	107 ± 106
Red blood cells	$10^6/mm^3$	5.88 ± 0.56	5.85 ± 0.53	5.52 ± 0.42
White blood cells	no/mm^3	8936 ± 4256	7984 ± 3973	8740 ± 6624
Hematocrit	%	45.8 ± 3.7	44.4 ± 4.7	40.4 ± 2.2
Hemoglobin	gm/100 ml	14.7 ± 2.1	14.2 ± 1.2	13.5 ± 0.6
Plasma PLP	ng/ml	7.5 ± 2.3	7.4 ± 1.3	7.1 ± 1.3
Plasma vitamin B6	ng/ml	17.4 ± 2.5	18.2 ± 3.0	11.7 ± 0.4
PB6/PLP		2.32	2.46	1.64
Urine volume	ml	69 ± 49	123 ± 100	54 ± 21
Urinary creatinine	mg/ml	0.55 ± 0.41	0.27 ± 0.12	0.66 ± 0.25
Urinary 4PA	um/μg creat.	71.6 ± 18.3^{a}	37.6 ± 13.7^{a}	57.3 ± 25.1
Urinary vitamin B6	um/μg creat.	34.6 ± 15.4^{B,C}	9.7 ± 5.1^{C}	5.02 ± 1.5^{C}
R4PA/UB6		2.07	3.90	11.4

^{a,B,C} Significant: Capital superscript, $p < 0.01$; small superscript, $p < 0.05$

after the last vitamin B6 dose. The significant differences between groups are shown in Appendix B, Table B.2. From these values, it seems that equimolar doses of PN, PM and PL (1.3 mg/kg body weight) did not significantly affect the levels of plasma vitamin B6 in these guinea pigs. The values for fasting plasma vitamin B6 in the guinea pigs were higher than the fasting vitamin B6 values in humans. The normal fasting values for humans obtained with this method are 12.8 ± 4.5 ng/ml of plasma.

Plasma PLP

The mean plasma PLP values for each group of guinea pigs are reported in Table 12. As for plasma vitamin B6, the plasma PLP values for guinea pigs were within the usual range of fasting values obtained in this laboratory with these methods for humans. This is in spite of the fact that the Recommended Dietary Allowance for guinea pigs is many times that for humans when calculated on a body weight basis. The overall effect of form on PLP values in guinea pigs was not significant (Appendix B, Table B.2).

Urine Responses

4PA

The volumes of guinea pig urine collected were very difficult to measure. The high concentration of salts obliterated the distinction

between the urine and the protective toluene layer used during sample collection. Since the urine volumes were uncertain, values for 4PA and UB6 are given per g creatinine in the urine.

The mean 4PA values for each group are given in Table 12. Analysis of variance (Appendix B, Table B.2) indicated that the overall effect of form was significant ($p < 0.05$). The 4PA values were highest for the PN group and lowest for PM. These last two groups were significantly different from each other, as shown in Table 12.

It is not clear whether the differences between the forms in the guinea pig urinary values are due to variations in rates of absorption or in rates of metabolism. Scudi et al. (1940) have shown in dogs and humans that the urinary excretion of vitamin B6 was the same for 50 and 100 mg doses of PN, whether given orally or intravenously. Thus, PN is absorbed nearly completely. Differences in the 4PA values in this report may mean that PM and PL are less well absorbed than PN (Table 12), but variations in metabolism among the forms cannot be ruled out from the data presented here.

UB6

The guinea pig UB6 values are presented in Table 12 and are expressed as μmoles per g urinary creatinine. Table 12 also gives the results of the statistical treatment of differences between groups. The overall effect of form was significant ($p < 0.01$, Appendix B,

Table B.2). The urinary vitamin B6 values following the PN dose were different from those after either the PM or PL doses. PM and PL were not different from each other. The ratios of 4PA:UB6 for each form are given in Table 12. The ratios are not significantly different from each other. The ratio for the PL dose was the most different statistic, indicating that for this form relatively less UB6 and relatively more 4PA was produced by the guinea pig than for the other forms (Table 12).

Guinea Pig Organ Weights and Growth Records

Data on weight gains, organ weights and the results of statistical analysis for the differences between the different forms of vitamin B6 are shown in Table 12 and Appendix B, Table B.2. Guinea pig weight gains after two months were: PN, 141.5 g; PM, 118.2 g; and PL, 107.4 g. This fact seems to corroborate the evidence from the urinary vitamin B6 and 4PA values, that the guinea pig absorbed PN more efficiently than the other two forms. There was more UB6 and 4PA found in the urine of the PN fed guinea pigs than there was found in the other two groups, yet the average weight gain was not significantly greater with PN than with the other forms. The weight gains were within the normal weight gains for the mixed strain of animals used. The normal weight gains for this strain on a pelleted

commercial diet and on the semi-synthetic powdered diet are shown in Figure 2.

There was no significant difference (Table 12) between the groups in selected organ weights (liver, kidney, spleen, and brain) whether expressed on a weight basis, or as a percent of the final body weight.

Organ enzyme levels (pyridoxine oxidases, transaminases, or pyridoxal phosphokinases) may have shown differences between the groups. The animals were considered to be in general good health. The white blood cell counts indicated that there were some animals with infections. Rapid weight loss, followed by death had eliminated several animals from the study. Although the white blood cell counts of some of the animals remaining were high, they were included in the final data since the animals had not started to lose weight.

Comparison of Species

Blood Levels

The three forms had no significant effect on guinea pig blood values. A comparison of species, therefore, is limited to urinary values.

Urine Data

There was less of a difference in the 4PA:UB6 ratios between the forms for the human than there was for the guinea pigs. The

ratios for humans are given in Table 9 and for guinea pigs in Table 12. The relative differences between the vitamin B6 forms in terms of UB6 were the same for both species. Vitamin B6 was excreted in the greatest amounts following the PN dose for both species. The 4PA values also showed that more 4PA was produced with PL than with PM for both species. The 4PA values for the PL dose in the guinea pig, however, were intermediate between those following the PN and PM doses. In the human, the PM and PL 4PA values were nearly the same and the PN values were slightly lower. This species difference may mean that more of the PL was metabolized by the guinea pig than by the human, or that less of the PN dose was metabolized to 4PA relative to the other forms than in the guinea pig. A summary of the species differences is presented below.

Variable		Order of Magnitude	
		Human	Guinea Pig
PLP	% AREA	PN, PM > PL	PN, PM, PL
PB6	% AREA	PL > PN > PM	PN, PM, PL
UB6	% AREA	PN > PM, PL	PN > PM, PL
4PA	% AREA	PL > PM, > PN	PN > PL > PM

> appreciably greater than

, approximately equal to

V. SUMMARY AND CONCLUSIONS

The purposes of the research presented in this thesis were: (1) to determine the precision with which it is possible to measure changes in vitamin B6 compounds in the blood and urine following oral doses of levels of vitamin B6 (as pyridoxine) which are in the range of the normal daily intake of this vitamin; (2) to compare the effect of three free forms of vitamin B6 (PL, PM, PN) at these same levels using the same assays; and (3) to compare the response of guinea pigs to that of humans when the animals are given three free forms of vitamin B6 at physiological levels.

The effect of small incremental doses of pyridoxine, PN (0.5 - 10 mg), and of equimolar doses of PN, PM and PL were studied in five healthy young men. On the day before and during the day of the dose, the subjects were on a controlled diet that supplied 1.6 mg of vitamin B6 each day. During other days of the week, the subjects were on self-selected diets.

Timed blood and urine samples were obtained on the day each dose was administered. The parameters measured were: plasma vitamin B6 (PB6) plasma pyridoxal phosphate (PLP), urinary vitamin B6 (UB6) and urinary 4-pyridoxic acid (4PA). Variables reflecting the response to each dose for each of these parameters were calculated in two ways: (1) the percent increase of the maximal post-

response value; and (2) the area under the curve bounded by the values obtained and the times of the samples.

From the results obtained here, of the parameters used, those which were best able to distinguish between the smallest differences in fasting dose levels were %PB6 (Appendix A, Table A.1), UB6AREA (Appendix A, Table A.7) and PB6:PLP (Appendix A, Table A.3). The smallest level of PN between which each variable could distinguish were: UB6AREA, 1 and 2 mg; 4PAAREA, 0 and 2 mg; PB6AREA, 0 and 2 mg; PLPAREA, 2 and 4 mg; %PLP, 1 and 2 mg; %PB6, 0 and 1 mg; and %UB6, 0 and 2 mg.

The same variables were calculated for the study of a comparison of 19.44 μmole doses of PN, PM and PL. The PB6 peaks occurred at 1/2 hr for PL and PN., and at 1 hr for PM. The PLP peaks occurred at the following times: PN, 1/2 hr, PM 3 hr and PL, 1 hr. Maximal levels of UB6 and 4PA were reached in the first three hours after the dose for all three forms. The percent increase and area variables were able to distinguish between nearly all the responses to the three forms of vitamin B6 administered at the 19.44 μmole level. Those response parameters best able to distinguish between the PN, PM and PL forms at the 4 mg level were: %PB6 (Appendix A, Table A.8), 4PAAREA (Appendix A, Table A.11) and %4PA (Appendix A, Table A.11). The level above zero which must be reached to show differences varies with the parameter measured.

The correlations between some nutrients in the self-selected diets before the dose and some responses indicated that it may be beneficial to extend the period of controlled diet before the dose is given, and in particular, to restrict caffeine and alcohol.

The precision with which the parameters used were able to measure differences in dose levels (Appendix A, Table A.7) makes it advisable to start with a dietary level of vitamin B6 somewhat higher than 1.5 mg, if the expected effect of the other nutrient to be tested is that of lowering the vitamin B6 available to the body. The precision of the measurements made here was dependent on reaching a certain minimum level of vitamin B6 intake, below which changes in availability of the nutrient would be masked by subject variation. Thus, for example, UB6AREA was sufficiently precise to measure between the 1 and 2 mg PN doses, but not between the 0 and 1 mg levels, although there is a difference of 1 mg between both pairs.

The results of the experiment that measured the effect of exercise on plasma responses indicate that physical activity should be restricted during periods of blood sampling for the measurement of plasma pyridoxal phosphate.

Three groups of guinea pigs were each given their Recommended Dietary Allowances of vitamin B6 as PM, PN, or PL. The same parameters were measured as for the humans. There were no significant differences between the groups of animals in body weight,

organ weights (spleen, liver, kidney, brain), plasma B6 or PLP, or in formed elements of the blood (hemoglobin, hematocrit, white blood cells, red blood cells). Urinary 4PA and UB6 were significantly higher in animals receiving PN.

A comparison between the two species studied revealed that guinea pigs metabolize relatively more PN to 4PA than do humans at the dose levels used.

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APPENDICES

Table A.1. Plasma vitamin B6 for all PN levels (ng/ml)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PB6**	PB6AREA** (ng-min/ml x 10)
0	PN	1	10.6	9.0	10.6	7.0	10.4	10.2		100	11,882
		2	13.0	12.2	11.2	10.6	10.0	12.2		94	4,688
		3	10.0	10.2	8.6	8.4	8.2	8.2		102	1,387
		4	8.6	8.4	8.0	9.0	8.8	9.0		105	3,515
		5	4.8	5.2	5.0	7.4	4.6	5.4		154	4,583
		Mean	9.4	9.0	8.7	8.5	8.4	9.0		111[a]	5,211[b]
		sd	±3.0	±2.6	±2.5	±1.4	±2.3	±2.5		±24	±3,958
0.5	PN	1	9.2	12.2	10.0	8.4	9.4	9.6		133	5,007
		2	11.8	15.8	14.4	12.4	12.0	13.8		134	6,028
		3	11.2	13.8	11.8	9.2	12.6	11.2		123	10,534
		4	9.4	15.2	9.4	23.8[c]	20.8[c]	8.8		162	-
		5	7.5	11.4	8.6	6.6	6.6	8.8		152	5,145
		Mean	9.8	13.7	10.8	9.2	10.2	10.4		141	6,679
		sd	±1.7	±1.9	±2.3	±2.4	±2.7	±2.1		±16	±2,610
1	PN	1	5.8	13.6	8.2	8.2	6.8	7.2		235	9,382
		2	8.4	18.4	13.0	11.6	10.8	11.8		219	17,086
		3	11.0	14.8	14.4	11.8	10.6	10.2		135	7,948
		4	10.8	18.4	13.8	11.4	11.6	9.8		170	10,183
		5	9.0	16.2	10.6	9.6	8.4	8.4		180	5,424
		Mean	9.0	16.3	12.0	10.5	9.6	9.5		188[a]	9,825
		sd	±2.1	±2.1	±2.6	±1.6	±2.0	±1.8		±40	±4,476

Table A.1 (continued)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PB6**	PB6AREA** (mg-min/ml x 10)
2	PN	1	5.8	23.4	11.6	8.6	6.4	6.8		403	14,779
		2	11.0	26.6	19.0	12.6	12.8	16.0		242	20,119
		3	8.4	17.6	14.0	11.0	10.0	11.0		210	15,030
		4	6.8	19.4	17.2	9.8	8.0	9.0		285	18,814
		5	6.4	15.4	13.0	8.4	7.0	7.2		241	11,833
		Mean	7.7	20.5	15.0	10.1	8.8	10.0		276	16,115[b]
		sd	±2.1	±4.5	±3.1	±1.8	±2.6	±3.7		±76	± 3,340
4	PN	1	6.4	25.4	14.2	9.8	9.4	9.0		397	22,692
		2	6.8	28.6	19.0	13.6	11.6	12.6		421	36,594
		3	7.6	31.2	20.6	12.2	9.0	8.8		411	24,958
		4	7.0	11.6	19.0	13.2	10.6	8.8		271	24,604
		5	4.6	25.0	13.6	9.2	8.4	8.0		544	27,414
		Mean	6.5	24.4	17.3	11.6	9.8	9.4		409	27,252
		sd	±1.1	±7.6	±3.2	±2.0	±1.3	±1.9		±97	± 5,486
10	PN	1	4.8	59.5	40.2	15.9	14.4	13.8	10.5	1240	79,348
		2	8.7	78.0	52.8	23.4	23.7	22.2	14.7	897	106,712
		3	7.8	45.0	56.4	31.8	21.3	18.9	14.4	723	101,879
		4	7.2	25.5	71.4	30.6	22.8	17.4	12.3	992	107,090
		5	4.5	37.5	29.7	13.8	15.6	14.1	8.4	933	67,218
		Mean	6.6	50.0	50.1	23.1	19.6	17.3	12.1	957	92,449
		sd	±1.9	±19.8	±15.9	±8.2	±4.3	±3.5	±2.7	±187	±18,131

** Overall effect of level on variable significant, $p < 0.01$
a, b Smallest levels between which significant differences exist, $p < 0.05$
c Not included in mean

Table A.2. Plasma PLP for all PN levels (ng/ml)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PLP**	PLPAREA** (ng-min/ml x 10)
0	PN	1	9.1	8.4	9.6	8.4	9.6	4.7		106	-
		2	15.3	13.6	12.7	12.5	12.4	14.8		97	3,289
		3	8.3	9.4	7.9	7.3	7.1	7.4		113	1,971
		4	10.3	9.0	8.7	11.1	9.0	8.5		87	4,401
		5	3.7	5.4	5.9	7.6	4.3	5.4		205	9,272
		Mean	9.3	9.2	9.0	9.4	8.5	8.2		122	4,733
		sd	±4.2	±2.9	±2.5	±2.3	±3.0	±4.0		±48	±3,185
0.5	PN	1	13.0	15.8	14.1	13.0	14.0	12.7		122	4,342
		2	16.6	19.0	16.5	17.7	15.1	21.2		115	11,082
		3	11.1	12.6	11.3	9.1	9.5	11.6		114	5,841
		4	9.4	10.4	9.1	9.6	7.6	8.3		111	5,308
		5	5.7	7.6	6.7	6.1	5.7	6.5		133	2,548
		Mean	11.2	13.1	11.5	11.1	10.4	9.8		119[a]	5,824
		sd	±4.1	±4.5	±3.9	±4.4	±4.1	±2.9		± 9	±3,195
1	PN	1	4.8	6.3	5.7	5.5	4.6	5.3		131	2,838
		2	12.7	15.1	13.1	11.8	11.2	12.1		119	4,416
		3	11.9	13.4	13.5	12.5	10.8	11.1		113	5,138
		4	8.9	10.4	10.6	8.7	8.9	7.6		119	6,393
		5	6.5	8.7	7.9	7.2	7.5	7.4		134	4,876
		Mean	9.0	10.8	10.2	9.1	8.6	8.7		123[b]	4,732
		sd	±3.4	±3.5	±3.4	±3.0	±2.7	±2.8		± 9	±1,288

Table A.2 (continued)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PLP**	PLPAREA** (ng-min/ml x 10)
2	PN	1	4.5	6.6	6.9	6.3	5.5	5.6		153	7,014
		2	12.3	17.1	15.2	14.0	12.4	18.0		139	11,199
		3	9.9	12.9	12.6	11.5	10.3	11.8		130	7,199
		4	6.4	8.6	11.0	8.9	7.8	7.8		172	10,371
		5	7.0	10.0	9.5	8.5	8.1	8.1		143	7,240
		Mean	8.0	11.0	11.0	9.8	8.8	10.3		147[a,b]	8,605[c]
		sd	±3.1	±4.1	±3.1	±3.0	±2.6	±4.9		±16	±2,014
4	PN	1	6.1	12.1	11.3	10.1	9.5	9.9		198	19,050
		2	12.0	21.0	20.5	17.4	16.8	18.8		175	29,014
		3	10.1	20.2	18.9	16.4	16.2	15.6		200	31,320
		4	8.1	9.7	13.3	14.3	12.9	12.5		177	22,844
		5	6.9	15.6	13.9	12.2	11.5	12.5		226	26,176
		Mean	8.6	15.7	15.6	14.1	13.4	13.9		195	25,681[c]
		sd	±2.4	±4.9	±3.9	±3.0	±3.1	±3.4		±21	±4,878
10	PN	1	6.4	13.1	22.9	11.4	14.6	16.5	13.4	358	42,669
		2	13.4	23.4	25.3	27.1	25.4	25.8	18.5	202	57,860
		3	10.0	14.3	20.7	26.0	20.3	23.6	16.1	260	56,123
		4	8.6	10.3	19.5	23.5	22.3	20.9	14.4	273	58,304
		5	4.5	10.3	13.2	14.6	15.3	15.4	12.0	342	46,526
		Mean	8.6	14.3	20.3	20.5	19.6	20.4	14.9	287	52,296
		sd	±3.4	±5.4	±4.6	±7.1	±4.6	±4.5	±2.5	±64	±7,205

** Overall effect of level on variable significant $p < 0.01$

a, b, c Smallest levels between which significant differences exist, $p < 0.05$

Table A.3. Plasma ratio PB6:PLP for all PN levels

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PB6:%PLP**
0		1	1.16	1.07	1.10	0.83	1.08	2.17		.09
		2	0.85	0.90	0.88	0.85	0.81	0.82		.10
		3	1.20	1.09	1.09	1.15	1.15	1.11		.09
		4	0.83	0.93	0.92	0.81	0.98	1.06		.12
		5	1.29	0.96	0.85	0.97	1.07	1.00		.07
		Mean	1.07	0.99	0.97	0.92	1.02	1.00		.09
		sd	±0.21	±0.09	±0.12	±0.14	±0.13	±0.13		±.02
0.5	PN	1	0.71	0.81	0.71	0.65	0.67	0.76		.11
		2	0.71	0.83	0.87	0.70	0.79	0.65		.12
		3	1.01	1.10	1.04	1.01	1.33	0.97		.11
		4	1.00	1.46	1.03	2.46[a]	2.74[a]	1.06		.15
		5	1.32	1.50	1.28	1.08	1.16	1.35		.11
		Mean	0.95	1.14	0.99	0.86	0.99	0.96		.12[b]
		sd	±0.25	±0.33	±0.21	±0.22	±0.31	±0.27		±.02
1	PN	1	1.21	2.16	1.44	1.49	1.48	1.36		.18
		2	0.66	1.22	0.99	0.98	0.96	0.98		.18
		3	0.92	1.10	1.07	0.94	0.98	0.92		.12
		4	1.21	1.77	1.30	1.31	1.30	1.28		.14
		5	1.38	1.86	1.34	1.33	1.12	1.14		.13
		Mean	1.08	1.62	1.23	1.21	1.17	1.14		.15[b, c]
		sd	±0.29	±0.45	±0.19	±0.24	±0.22	±0.19		±.03

Table A.3 (continued)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	24	%PB6:%PLP**
2	PN	1	1.29	3.55	1.68	1.37	1.16	1.21		.26
		2	0.89	1.56	1.25	0.90	1.03	0.89		.17
		3	0.85	1.36	1.11	0.96	0.97	0.93		.16
		4	1.06	2.26	1.56	1.10	1.03	1.15		.17
		5	0.91	1.54	1.37	0.99	0.86	0.89		.17
		Mean	1.00	2.06	1.39	1.06	1.01	1.01		.19[c]
		sd	±0.18	±0.90	±0.23	±0.19	±0.11	±0.15		±.04
4	PN	1	1.05	2.10	1.26	0.97	0.99	0.91		.20
		2	0.57	1.36	0.93	0.78	0.69	0.67		.24
		3	0.75	1.54	1.09	0.74	0.56	0.56		.21
		4	0.86	1.20	1.43	0.92	0.82	0.70		.15
		5	0.67	1.60	0.99	0.75	0.73	0.64		.24
		Mean	0.78	1.56	1.14	0.83	0.76	0.70		.21
		sd	±0.18	±0.24	±0.20	±0.11	±0.16	±0.13		±.04
10	PN	1	0.75	4.54	1.76	1.39	0.99	0.84	0.78	.35
		2	0.65	3.33	2.09	0.86	0.93	0.86	0.79	.44
		3	0.78	3.15	2.72	1.22	1.05	0.80	0.89	.28
		4	0.84	2.48	3.66	1.30	1.02	0.83	0.85	.36
		5	1.00	4.00	2.25	0.95	1.02	0.92	0.70	.27
		Mean	0.80	3.50	2.50	1.14	1.00	0.85	0.80	.34
		sd	±0.13	±0.79	±0.74	±0.23	±0.05	±0.04	±0.07	±.01

** Overall effect of level on ratio significant, $p < 0.01$

[a] Not included in mean

[b, c] Levels between which significant differences exist, $p < 0.05$

Table A.4. Urinary 4PA for all PN levels (nmoles/hr)

Level (mg)	Form	Subject	Hour After Dose					4PATH	%4PA**	4PAAREA**
			0-3[a]	4-8[a]	9-12	13-16	17-24	0-24		(nmole-hr/hr)
0		1	242	242	161	290	258	242	178	1,814
		2	243	210	225	258	185	218	117	1,000
		3	305	203	215	315	243	254	114	1,436
		4	225	178	225	145	234	207	160	1,130
		5	168	193	193	215	240	208	242	2,288
		Mean	236	205	204	245	232	226	162	1,534[b]
		sd	±50	±20	±30	±70	±30	±20	±42	± 526
0.5	PN	1	242	209	403	129	226	239	260	2,308
		2	290	260	305	213	236	257	109	1,092
		3	360	193	225	248	225	246	167	1,298
		4	183	275	243	178[c]		205	136	970
		5	95	263	95	240	204	183	180	2,080
		Mean	234	240	254	208	223	226	170	1,550
		sd	±100	±40	±110	±50	±10	±30	±57	± 605
1	PN	1	215	155	387	274	266	258	248	2,328
		2	317	274	175	398	206	261	179	2,546
		3	390	226	360	260	333	310	144	1,890
		4	380	216	175	128	346	225	197	2,162
		5	413	248	193	143	240	239	295	2,208
		Mean	343	224	258	241	258	259	213[d]	2,227
		sd	±80	±40	±110	±110	±50	±30	±59	± 240

Table A.4.(continued)

Level (mg)	Form	Subject	Hour After Dose 0-3[a]	4-8[a]	9-12	13-16	17-24	4PATH 0-24	%4PA**	4PAAREA** (nmole-hr/hr)
2	PN	1	343	206	419	403	266	311	254	3,745
		2	640	372	423	380	303	392	281	4,288
		3	553	562	415	400	280	415	304	5,759
		4	413	440	408	240	310	354	270	4,277
		5	343	356	208	135	319	280	272	2,945
		Mean	458	387	375	312	296	350	276	4,203[b]
		sd	±130	±130	±90	±120	±20	±60	±18	±1,028
4	PN	1	773	889	322	419	395	537	390	7,345
		2	957	236	368	528	271	408	433	5,641
		3	810	812	553	530	259	537	367	8,350
		4	627	890	538	255	470	553	394	6,990
		5	380	754	268	335	360	425	830	7,770
		Mean	709	716	410	413	351	492	482[d]	7,219
		sd	±220	±270	±130	±120	±90	±70	±196	±1,017
10	PN	1	2,812	850	403	773	539	216	1803	19,969
		2	2,370	960	565	600	421	288	1097	16,484
		3	1,400[c]		1,128	735	230	395	614	16,612
		4	1,807	1,404	743	123	458	372	941	16,424
		5	1,197	640	445	821	169	275	907	12,080
		Mean	2,047	964	657	610	363	309	1072	16,313
		sd	±700	±320	±300	±280	±160	±70	±444	±2,802

** Overall effect of level significant $p < 0.01$

a For the week of 0 and 0.5 mg PN, the first two sampling periods were both 4 hr, instead of the 3 and 5 hr periods used for other weeks

b,d Smallest levels between which significant differences exist, $p < 0.05$

c Combined urine sample from two collection periods

Table A.5. Urinary vitamin B6, 4-pyridoxic acid, T4PAUB6 and recovery of dose for all PN levels

Level (mg)	Form	Subject	UB6	4PA	TUB64PA	UB6	4PA	TUB64PA	4PA:UB6
				(μmoles)			% dose		
0		1	0.98	5.81	6.78				5.94
		2	0.74	5.23	5.96				7.04
		3	0.88	6.05	6.97				6.90
		4	0.73	4.97	5.69				6.76
		5	0.81	4.99	5.80				6.19
		Mean	0.83	5.41	6.24	13.0	84.9	97.9	6.57
		sd	±0.10	±0.49	±0.53				±0.48
0.5	PN	1	0.94	5.74	6.68				6.11
		2	0.74	6.17	6.90				8.34
		3	0.82	5.90	6.72				7.19
		4	0.65	4.92	5.58				7.64
		5	0.70	4.39	5.10				6.32
		Mean	0.77	5.42	6.20	8.7	61.7	70.4	7.12
		sd	±0.12	±0.74	±0.80				±0.93
1	PN	1	0.87	6.19	7.06				7.11
		2	0.87	6.26	7.13				7.21
		3	0.83	7.44	8.27				8.94
		4	0.78	5.40	6.17				6.98
		5	0.93	5.74	6.67				6.17
		Mean	0.86	6.21	7.06	7.6	55.3	62.9	7.28
		sd	±0.06	±0.77	±0.78				±1.01
2	PN	1	1.20	7.46	8.68				6.20
		2	1.30	9.41	10.71				7.23
		3	1.23	9.96	11.20				8.09
		4	1.06	8.50	9.57				8.04
		5	0.95	6.72	7.68				7.09
		Mean	1.15	8.41	9.57	7.1	52.3	59.4	7.33
		sd	±0.14	±1.34	±1.44				±0.78
4	PN	1	1.58	12.89	14.46				8.17
		2	1.80	9.97	11.59				5.46
		3	1.40	12.89	14.29				9.22
		4	1.26	13.27	14.52				10.51
		5	1.39	10.20	11.59				7.32
		Mean	1.49	11.81	13.29	5.8	45.8	51.6	8.14
		sd	±0.21	±1.67	±1.55				±1.91
10	PN	1	3.68	21.70	25.38				5.90
		2	4.78	19.94	24.72				4.17
		3	3.34	20.50	23.83				6.12
		4	3.45	19.56	23.83				5.66
		5	3.60	13.20	16.80				3.68
		Mean	3.77	18.89	22.91	6.9	34.5	41.4	5.11
		sd	±0.48	±3.33	±3.48				±1.10

Table A.6. 4PA and UB6 for the days before, during and after all PN doses (umoles/24 hr)

Level (mg)	Form	Subject	4PA			UB6		
			Wednesday	Thursday	Friday	Wednesday	Thursday	Friday
0		1	3.90	5.80	3.71	1.05	0.98	0.68
		2	5.28	5.22	6.73	0.80	0.74	0.80
		3	6.64	6.09	5.17	1.16	0.88	0.79
		4	3.51	4.96	4.88	0.56	0.73	0.73
		5	2.37	4.99	3.51	0.58	0.81	0.74
		Mean	4.34	5.41	4.74	0.83	0.83	0.75
		sd	±1.66	±0.51	±1.15	±0.27	±0.10	±0.05
0.5	PN	1	3.71	5.74	3.90	0.67	0.94	0.69
		2	6.73	6.16	6.01	0.80	0.74	-
		3	5.17	5.90	6.09	0.79	0.82	0.91
		4	4.88	4.93	5.74	0.73	0.65	0.68
		5	3.51	4.40	2.77	0.74	0.70	0.58
		Mean	4.80	5.43	4.90	0.75	0.77	0.72
		sd	±1.30	±0.73	±1.49	±0.05	±0.12	±0.14
1	PN	1	4.19	6.19	3.71	0.68	0.87	0.80
		2	5.33	6.26	6.01	0.71	0.87	-
		3	6.47	7.44	7.75	0.78	0.83	1.11
		4	4.63	5.40	5.86	0.73	0.77	0.69
		5	3.35	5.74	2.95	0.65	0.93	0.60
		Mean	4.79	6.21	5.25	0.71	0.86	0.80
		sd	±1.18	±0.77	±1.93	±0.05	±0.06	±0.22
2	PN	1	3.95	7.47	4.50	0.78	1.21	0.74
		2	5.46	9.41	5.82	0.80	1.30	0.88
		3	4.43	9.97	3.51	0.72	1.23	0.58
		4	3.90	8.51	5.27	0.69	1.06	0.80
		5	3.14	6.73	4.61	0.57	0.95	0.64
		Mean	4.18	8.42	4.74	0.71	1.15	0.73
		sd	±0.85	±1.34	±0.87	±0.10	±0.14	±0.12
4	PN	1	5.46	12.88	2.28	0.89	1.58	0.45
		2	5.30	9.90	6.64	0.87	1.79	0.90
		3	5.30	12.89	4.26	0.83	1.40	0.64
		4	5.43	13.26	8.19	0.76	1.26	0.92
		5	2.18	10.20	3.45	0.50	1.39	0.53
		Mean	4.74	11.81	4.96	0.77	1.49	0.69
		sd	±1.43	±1.66	±2.41	±0.16	±0.21	±0.21
10	PN	1	3.74	21.71	5.18	0.80	3.68	0.73
		2	5.18	19.94	6.90	0.87	4.78	0.89
		3	5.46	20.49	9.49	0.92	3.34	-
		4	4.60	19.56	8.92	0.69	3.45	0.91
		5	3.16	13.21	6.61	0.72	3.59	0.77
		Mean	4.43	18.98	7.42	0.80	3.77	0.83
		sd	±0.97	±3.33	±1.77	±0.10	±0.58	±0.09

Table A.7. Urinary vitamin B6 for all PN levels (nmoles/hr)

Level (mg)	Form	Subject	Hour After Dose						%UB6**	UB6AREA** (nmole-hr/hr x 10)
			0-3[a]	4-8[a]	9-12	13-16	17-24	0-24		
0		1	38	43	31	52	41	41	118	2,628
		2	33	24	36	40	26	31	120	1,952
		3	41	40	33	37	35	37	84	1,188
		4	31	27	38	19	34	30	164	2,272
		5	22	35	31	40	36	34	166	2,534
		Mean	33	34	34	38	34	34	131[b]	2,115
		sd	±7	±8	±3	±12	±5	±4	±35	±581
0.5	PN	1	34	25	66	30	40	39	236	3,002
		2	35	24	35	37	27	31	111	1,876
		3	42	30	32	43	29	34	132	1,672
		4	17	35	49	-	-	27	160	2,552
		5	19	43	19	36	28	29	139	2,590
		Mean	29	31	40	37	31	32	156	2,338[c]
		sd	±11	±8	±18	±5	±6	±5	±48	± 549
1	PN	1	37	18	50	47	35	36	177	4,560
		2	52	35	33	51	25	36	177	3,401
		3	50	34	34	35	29	35	155	1,644
		4	48	27	33	16	38	32	157	3,480
		5	64	39	34	24	39	39	236	3,308
		Mean	50	31	37	35	32	36	180	3,279[d]
		sd	±10	±8	±7	±15	±6	±2	±33	±1,045

Table A.7 (continued)

Level (mg)	Form	Subject	Hour After Dose 0-3[a]	4-8[a]	9-12	13-16	17-24	0-24	%UB6**	UB6AREA** (nmole-hr/hr x 10)
2	PN	1	54	23	61	95	38	50	291	7,767
		2	153	34	45	49	38	54	457	6,305
		3	81	55	48	56	37	51	270	5,773
		4	79	44	50	25	38	44	272	4,516
		5	73	42	32	20	39	40	308	4,302
		Mean	88	40	47	49	38	48	320[b]	5,733[C,d]
		sd	±34	±12	±11	±30	±1	±6	±78	±1,414
4	PN	1	118	88	43	67	43	66	319	7,837
		2	254	54	47	68	38	75	745	12,379
		3	167	58	52	46	27	58	480	9,262
		4	131	54	48	19	41	53	414	8,221
		5	122	72	38	42	43	58	585	9,197
		Mean	158	65	45	48	39	62	509	9,379
		sd	±58	±15	±5	±20	±7	±9	±164	±1,786
10	PN	1	742	78	40	105	60	153	2,225	35,991
		2	1,126	75	65	79	56	199	3,093	49,804
		3	697	-	112	88	57	140	1,815	31,008
		4	722	111	66	28	44	144	2,498	33,645
		5	817	70	55	31	56	150	2,717	35,352
		Mean	821	83	68	66	55	150	2,469	37,160
		sd	±176	±19	±27	±35	±6	±24	±485	±7,326

** Overall effect of level significant, $p < 0.01$

a For the week of 0 and 0.5 mg PN, the first two sampling periods were both 4 hr, instead of the 3 and 5 hr periods used for the other weeks.

b, C, d Smallest levels between which significant differences exist: Capital superscript $p < 0.01$; small superscript, $p < 0.05$.

Table A.8. Plasma vitamin B6 for equimolar doses of PN, PM and PL (ng/ml)

Level	Form	Subject	Hour After Dose						%PB6**	PB6AREA (ng-min/ml x 10)
			0	0.5	1	3	5	8		
4 mg =	PN	1	6.4	25.4	14.2	9.8	9.4	9.0	397	22,692
19.44		2	6.8	28.6	19.0	13.6	11.6	12.6	421	36,594
μmoles		3	7.6	31.2	20.6	12.2	9.0	8.8	411	24,958
		4	7.0	11.6	19.0	13.2	10.6	8.8	271	24,604
		5	4.6	25.0	13.6	9.2	8.4	8.0	544	27,414
		Mean	6.5	24.4	17.3	11.6	9.8	9.4	409[A,B]	27,252
		sd	±1.1	±7.6	±3.2	±2.0	±1.3	±1.8	±97	±5,486
19.44	PM	1	6.0	16.2	15.6	16.0	12.4	11.1	270	36,214
μmoles		2	9.4	20.4	25.8	14.8	13.0	14.4	275	32,608
		3	8.2	16.8	19.2	14.6	13.2	13.2	234	30,535
		4	8.0	10.4	15.6	18.2	13.8	13.0	228	31,966
		5	7.0	12.4	14.4	13.0	10.2	9.8	206	20,965
		Mean	7.7	15.2	18.1	14.9	12.5	12.3	243[B,C]	30,458
		sd	±1.3	±3.9	±4.7	±2.2	±1.4	±1.8	±29	±5,704
19.44	PL	1	6.6	58.5	25.4	11.2	9.0	9.2	886	39,612
μmoles		2	8.6	53.6	24.2	12.2	12.2	12.4	623	38,241
		3	11.8	69.9	28.8	14.0	13.0	-	592	33,334
		4	8.0	69.7	29.3	12.0	11.4	11.2	871	46,373
		5	6.8	32.9	20.4	8.6	8.8	9.4	484	25,320
		Mean	8.4	56.9	25.6	11.6	10.9	10.6	691[A,C]	36,576
		sd	±2.4	±15.2	±3.6	±2.0	±1.9	±1.5	±179	±7,829

** Overall effect of form, significant $p < 0.01$

A, B, C Forms between which significant differences exist, $p < 0.01$

Table A.9. Plasma pyridoxal phosphate for equimolar doses of PN, PM and PL (ng/ml)

Level	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	%PLP	PLPAREA** (ng-min/ml x 10)
4 mg =	PN	1	6.1	12.1	11.3	10.1	9.5	9.9	198.4	19,050
19.44		2	12.0	21.0	20.5	17.4	16.8	18.8	175.0	29,014
μmoles		3	10.1	20.2	18.9	16.4	16.2	15.6	200.0	31,320
		4	8.1	9.7	13.3	14.3	12.9	12.5	176.5	22,844
		5	6.9	15.6	13.9	12.2	11.5	12.5	226.1	26,176
		Mean	8.6	15.7	15.6	14.1	13.4	13.9	195.2	25,681[A]
		sd	±2.4	±4.9	±3.9	±3.0	±3.1	±3.4	±20.9	±4,878
19.44	PM	1	5.5	6.8	8.1	10.7	10.6	9.0	194.5	19,589
μmoles		2	12.6	15.7	18.7	16.9	16.2	17.1	148.4	20,265
		3	8.3	11.7	13.0	14.1	12.4	13.9	169.9	22,760
		4	8.5	10.9	12.8	15.8	12.9	14.6	185.9	24,963
		5	6.9	9.8	11.2	12.6	11.7	11.1	182.6	22,452
		Mean	8.4	11.0	12.8	14.0	12.8	13.1	176.3	22,006[B]
		sd	±2.7	±3.2	±3.9	±2.5	±2.1	±0.1	± 17.9	±2,144
19.44	PL	1	4.4	9.8	9.7	8.2	7.5	7.4	220.5	17,364
μmoles		2	7.9	12.0	11.7	11.6	11.1	11.8	151.9	16,781
		3	10.0	12.7	14.1	12.6	11.9	-	141.0	11,437
		4	6.2	10.6	12.7	10.9	-	8.7	204.8	19,187
		5	5.7	8.4	8.3	7.3	7.1	6.6	147.4	7,473
		Mean	6.0	10.7	11.3	10.1	9.4	8.6	173.1	14,448[A, B]
		sd	±3.6	±1.7	±2.3	±2.3	±2.5	±2.3	± 36.5	±4,851

** Overall effect of form, significant $p < 0.01$

A, B Forms between which significant differences exist, $p < 0.01$

Table A.10. Plasma ratio of vitamin B6 to pyridoxal phosphate for equimolar doses of PN, PM and PL

Level	Form	Subject	Hour After Dose 0	0.5	1	3	5	8	%PB6: %PLP
4 mg = 19.44 μmoles	PN	1	1.05	2.10	1.26	0.97	0.99	0.91	0.20
		2	0.57	1.36	0.93	0.78	0.69	0.67	0.24
		3	0.75	1.54	1.09	0.74	0.56	0.56	0.21
		4	0.86	1.20	1.43	0.92	0.82	0.70	0.15
		5	0.67	1.60	0.99	0.75	0.73	0.64	0.24
		Mean	0.78	1.56	1.14	0.83	0.76	0.70	0.21
		sd	±0.18	±0.34	±0.20	±0.11	±0.16	±0.13	±0.04
19.44 μmoles	PM	1	1.09	2.38	1.93	1.50	1.17	1.23	0.14
		2	0.75	1.30	1.38	0.88	0.80	0.84	0.19
		3	0.99	1.44	1.48	1.04	1.06	0.95	0.14
		4	0.94	0.95	1.22	1.15	1.07	0.89	0.12
		5	1.01	1.27	1.29	1.03	0.87	0.88	0.11
		Mean	0.96	1.47	1.46	1.12	0.99	0.96	0.14
		sd	±0.13	±0.54	±0.28	±0.23	±0.15	±0.16	±0.03
19.44 μmoles	PL	1	1.50	5.97	2.62	1.37	1.20	1.24	0.40
		2	1.09	4.47	2.07	1.05	1.10	1.05	0.41
		3	1.18	2.59	2.04	1.11	1.09	-	0.42
		4	1.29	6.58	2.31	1.10	-	1.29	0.43
		5	1.19	3.92	2.46	1.18	1.24	1.42	0.33
		Mean	1.25	4.71	2.30	1.16	1.16	1.25	0.40
		sd	±0.16	±1.60	±0.25	±0.13	±0.07	±0.15	±0.04

Table A.11. Urinary 4-pyridoxic acid for equimolar doses of PN, PM and PL (nmole/hr)

Level	Form	Subject	Hour After Dose 0-3	4-8	9-12	13-16	17-24	4PATH 0-24	%4PA**	4PAAREA* (nmole-hr/hr)
4 mg = 19.44 μmoles	PN	1	773	889	332	419	395	537	390	7,345
		2	957	236	368	528	271	408	433	5,641
		3	810	812	553	530	259	537	367	8,350
		4	627	890	538	255	470	553	394	6,990
		5	380	754	265	335	360	425	1,019	7,770
		Mean	709	716	410	413	351	492	521^{A}	7,219^{B}
		sd	±220	±270	±130	±120	±90	±70	±280	±1,017
19.44 μmoles	PM	1	1,696	541	322	349^{c}		553	1,162	10,598
		2	817	718	380	455	295	489	358	6,799
		3	553	1,446	448	513	280	624	673	9,872
		4	350	857	570	336^{c}		485	459	7,930
		5	-	889	387	612	330	463	502	12,658
		Mean	854	890	420	527	302	523	631^{d}	9,571^{e}
		sd	±590	±340	±90	±80	±30	±70	±318	±2,294
19.44 μmoles	PL	1	1,803	412	177^{c}		370	494	1,387	11,511
		2	2,817	1,848	1,113	155	409	1,085	1,374	23,190
		3	1,157	568	328	283	418	504	519	6,849
		4	1,560	124	110	948	310	500	1,157	11,729
		5	1,150	806	353	353	184	490	599	8,365
		Mean	1,697	752	476	435	338	615	1,007^{A,d}	12,329^{B,e}
		sd	±680	±660	±440	±350	±100	±260	±420	±6,417

Overall effect of form on variable, significance: ** = $p < 0.01$; * = $p < 0.05$

A, B, d, e Forms between which significant differences exist: Capital superscripts, $p < 0.01$; small superscripts, $p < 0.05$

c Combined urine sample from two collection periods

Table A.12. 4PA and UB6 for the days before, during and after equimolar doses of PN, PM and PL (μmoles/24 hr)

Level	Form	Subject	4PA			UB6		
			Wednesday	Thursday	Friday	Wednesday	Thursday	Friday
4 mg = 19.4 μmoles	PN	1	5.46	12.88	2.28	0.89	1.58	0.45
		2	5.30	9.80	6.64	0.87	1.79	0.90
		3	5.30	12.89	4.26	0.83	1.40	0.64
		4	5.43	13.26	8.19	0.76	1.26	0.92
		5	2.18	10.20	3.45	0.50	1.39	0.53
		Mean	4.73	11.81	4.96	0.77	1.49	0.69
		sd	±1.43	±1.66	±2.41	±0.16	±0.21	±0.21
19.4 μmoles	PM	1	3.51	13.27	-	0.82	1.26	-
		2	5.46	11.74	5.10	0.85	0.98	0.81
		3	5.17	14.97	-	0.85	1.52	-
		4	4.50	11.65	6.83	0.86	1.07	0.64
		5	4.24	11.12	7.01	0.80	1.25	0.86
		Mean	4.57	12.55	6.31	0.84	1.22	0.77
		sd	±0.77	±1.57	±1.06	±0.03	±0.21	±0.12
19.4 μmoles	PL	1	3.12	11.85	2.73	0.59	1.22	.20
		2	4.91	26.03	5.28	0.76	1.41	0.96
		3	5.35	12.09	-	1.17	1.30	-
		4	3.24	12.01	4.57	0.69	1.07	0.72
		5	4.61	11.77	5.90	0.67	1.07	0.76
		Mean	4.25	14.75	4.62	0.78	1.21	0.33
		sd	±1.01	±6.31	±1.37	±0.23	±0.15	±0.33

Table A.13. Urinary vitamin B6 for equimolar doses of PN, PM and PL (nmoles/hr)

Level	Form	Subject	Hour After Dose 0-3	4-8	9-12	13-16	17-24	UB6TH 0-24	%UB6	UB6AREA (nmole-hr/hr x 10)
4 mg =	PN	1	118	88	43	67	43	66	319	7,837
19.44		2	254	54	47	68	38	75	745	12,379
μmoles		3	167	58	52	46	27	58	480	9,262
		4	131	54	48	19	41	53	414	8,221
		5	122	72	38	42	43	58	585	9,197
		Mean	158	65	45	48	39	62	509	9,379
		sd	±58	±15	±5	±20	±7	±9	±164	±1,786
19.44	PM	1	104	44	33	50[a]		53	305	4,922
μmoles		2	71	35	31	49	34	41	201	3,138
		3	78	106	45	72	32	64	299	8,178
		4	41	56	60	36[a]		45	169	2,136
		5	16	81	37	75	43	52	241	8,710
		Mean	62	62	41	65	36	51	243	5,417
		sd	±34	±31	±12	±14	±6	±9	±59	±2,944
19.44	PL	1	99	48	30[a]		55	51	403	5,811
μmoles		2	20	43	39	48	32	59	153	4,172
		3	109	61	41	49	38	54	224	4,839
		4	87	40	49	23	40	45	301	5,081
		5	68	68	45	46	19	44	245	6,880
		Mean	77	52	44	42	37	51	265	5,357
		sd	±35	±12	±5	±12	±13	±6	±93	±1,034

[a] Combined urine sample from two collection periods

Table A. 14. 24 hr urinary vitamin B6, 4-pyridoxic acid, T4PAUB6, and recovery of dose for equimolar doses of PN, PM and PL

Level	Form	Subject	UB6	4PA	TUB64PA	UB6	4PA	TUB64PA	4PA:UB6
				(μmoles)			% dose		
4 mg =	PN	1	1.58	12.89	14.46				8.17
19.44		2	1.80	9.79	11.59				5.46
μmoles		3	1.40	12.89	14.29				9.22
		4	1.26	13.27	14.52				10.51
		5	1.39	10.20	11.59				7.32
		Mean	1.49	11.81	13.29	5.8	45.8	51.6	8.14
		sd	±0.21	±1.66	±1.55				±1.91
19.44	PM	1	1.26	13.27	14.53				10.53
μmoles		2	0.98	11.74	12.72				11.94
		3	1.52	14.98	16.49				9.85
		4	1.08	11.64	12.72				10.85
		5	1.25	11.11	12.37				8.92
		Mean	1.22	12.55	13.77	4.7	48.6	53.3	10.42
		sd	±0.21	±1.58	±1.74				±1.13
19.44	PL	1	1.22	11.86	13.07				9.71
μmoles		2	1.41	23.57	24.98				18.41
		3	1.30	12.10	13.39				9.34
		4	1.08	12.00	13.08				11.18
		5	1.07	11.76	12.84				11.77
		Mean	1.21	14.26	15.47	4.7	46.2	50.9	12.08
		sd	±0.15	±5.21	±5.32				±3.68

Table A.15. Plasma vitamin B6 and pyridoxal phosphate with and without exercise following a 1 mg PN dose (ng/ml)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	2[a]	3	%	AREA (ng-min/ml x 10)
Vitamin B6 with exercise									
1	PN	1	7.6	13.2	11.2	10.2	9.2	174	6,020
		2	10.4	18.2	11.4	12.4	11.6	175	5,181
		3	13.2	19.8	16.8	16.4	15.2	150	7,269
		4	11.8	16.8	16.2	13.2	12.4	142	4,903
		5	7.0	14.4	8.6	9.6	7.4	206	6,550
		Mean	10.0	16.5	12.8	12.4	11.2	169	5,985
		sd	±2.7	±2.7	±3.5	±2.7	±3.0	±25	± 973
	without exercise								
1	PN	1	5.8	13.6	8.2	8.2	8.2	235	6,682
		2	8.4	18.4	13.0	12.3	11.6	219	11,852
		3	11.0	14.8	14.4	13.2	11.8	135	5,565
		4	10.8	18.4	13.8	12.7	11.4	170	5,648
		5	9.0	16.2	10.6	10.2	9.6	180	5,484
		Mean	9.0	16.3	12.0	11.3	10.5	188	7,046
		sd	±2.1	±2.1	±2.6	±2.1	±1.6	±40	±2,730

Table A.15 (continued)

Level (mg)	Form	Subject	Hour After Dose 0	0.5	1	2[a]	3	%	AREA (ng-min/ml x 10)
Pyridoxal phosphate with exercise									
1	PN	1	7.2	9.2	8.6	9.0	7.6	128	3,680
		2	12.8	15.2	12.9	15.4	11.7	120	5,389
		3	15.7	16.9	16.0	18.7	15.1	108	7,304
		4	14.1	16.0	17.0	15.4	13.1	121	3,994
		5	6.8	9.6	8.2	9.4	6.7	141	3,284
		Mean	11.3	13.4	12.5	13.6	10.8	124	4,730[b]
		sd	±4.1	±3.7	±4.1	±4.2	±3.6	±12	±1,643
	without exercise								
1	PN	1	4.8	6.3	5.7	5.6	5.5	131	2,068
		2	12.7	15.1	13.1	12.5	11.8	119	3,622
		3	11.9	13.4	13.5	13.0	12.5	113	4,811
		4	8.9	10.4	10.6	9.6	8.7	119	2,020
		5	6.5	8.7	7.9	7.6	7.2	134	3,044
		Mean	9.0	10.8	10.2	9.7	9.1	123	3,113[b]
		sd	±3.4	±3.5	±3.4	±3.2	±3.0	± 9	±1,166

[a] 2 hr values for without exercise 1 mg PN dose computed from curves

[b] Significant difference, $p < 0.05$

Table B. 1. Analysis of variance of the overall effect of PN level and of different B6 vitamers in humans

Parameter	df	MS	EMS	F
PN level				
UB6AREA	5, 28	922, 315, 404	8, 761, 000	105. 28**
4PAAREA	5, 28	163, 628, 124	7, 713, 645	21. 21**
PB6AREA	5, 27	5, 468, 965, 173	49, 915, 081	109. 57**
PLPAREA	5, 27	1, 803, 098, 796	13, 868, 332	130. 02**
%PLP	5, 28	21, 889	827	26. 46**
%PB6	5, 28	504, 490	9, 129	55. 26**
%UB6	5, 28	4, 171, 968	34, 108	122. 32**
%4PA	5, 27	635, 248	59, 151	10. 74**
UB6TH	5, 28	1.16×10^{-2}	9.18×10^{-5}	126. 36**
4PATH	5, 28	0. 24	0. 01	22. 18**
TUB64PA	5, 28	206	6	31. 15**
4PA:UB6	5, 28	5. 20	2. 63	1. 98
PB6:PLP	5, 28	3. 83	0. 12	31. 88**
FORM				
UB6AREA	2, 28	26, 571, 290	8, 761, 000	3. 03
4PAAREA	2, 28	32, 703, 426	7, 713, 645	4. 24*
PB6AREA	2, 27	112, 198, 035	49, 915, 081	2. 25
PLPAREA	2, 27	163, 988, 941	13, 868, 332	11. 83**
%PLP	2, 27	713	827	0. 86
%PB6	2, 27	257, 178	9, 129	28. 17**
%UB6	2, 27	108, 566	34, 108	3. 19
%4PA	2, 27	325, 212	59, 151	5. 45**

Significance * p 0. 05; ** p 0. 01

Table B. 2. Analysis of variance for the overall effect of different B6 vitamers in the guinea pig

Parameter	df	MS	EMS	F
Weight gain	2, 13	1, 622. 21	19, 492. 73	0. 08
UB6	2, 11	1, 286. 4	64. 78	19. 86**
4PA	2, 11	1, 309. 8	321. 83	4. 07*
PB6	2, 4	27. 5	6. 13	4. 49
4PA:UB6	2, 11	5. 98	3. 53	1. 70
PLP	2, 10	2. 66	5. 05	0. 53
Spleen weight	2, 13	6.99×10^{-3}	1.91×10^{-2}	0. 37
Kidney weight	2, 13	3.73×10^{-1}	3.45×10^{-1}	1. 08
Liver weight	2, 13	5.99×10^{-1}	10. 30	0. 06
Brain weight	2, 10	5.20×10^{-3}	1.06×10^{-1}	0. 05

Significance * $p < 0.05$; ** $p < 0.01$

Table B. 3. Analysis of variance for the effect of exercise

Parameter	df	MS	EMS	F
%PB6	1, 8	839	9, 129	0. 09
PB6AREA	1, 4	2, 817, 486	5, 487, 563	0. 51
%PLP	1, 8	0. 10	827	1.21×10^{-4}
PLPAREA	1, 4	6, 538, 340	351, 685	18. 59*

* Significant $p < 0.05$

Table B.4. Analysis of variance for the linearity of the PN levels

Parameter	df	MS	EMS	F
UB6AREA	1,28	4,451,725,842	8,761,000	508.13**
4PAAREA	1,28	815,944,639	7,713,645	105.78**
PB6AREA	1,27	26,755,430,000	49,915,081	536.02**
PLPAREA	1,27	8,848,262,328	13,868,332	638.02**
%PLP	1,28	108,091	827	130.65**
%PB6	1,28	2,516,559	9,129	275.65**
%UB6	1,28	19,740,200	34,108	578.75**
%4PA	1,27	3,151,861	59,151	53.29**
UB6TH	1,28	5.6×10^{-2}	9.18×10^{-5}	610.02**
4PATH	1,28	1.205	0.011	109.55**
TUB64PA	1,28	1,026	6.63	154.89**
4PA:UB6	1,28	10.30	2.63	3.92
PB6:PLP	1,28	18.48	0.12	154.03**

** Significant, $p < 0.01$

APPENDIX C

Tyrosine Decarboxylase Assay for Plasma PLP

Deproteinization of Plasma

To 9 volumes of plasma were added 1 volume of 50% TCA. This was well mixed and allowed to stand for 30 min, at 20° C, after which it was centrifuged for 30 min at 20° C, 1900 x g. The supernatant was removed for analysis.

Enzyme Preparation

Twenty mg tyrosine decarboxylase (Sigma, St. Louis, MO) was ground mechanically and then suspended in 4.0 ml sodium acetate buffer (0.1 M, pH 5.7). The suspension was allowed to stand for 5 min and then centrifuged for 30 min at 0° C, 2100 x g. The supernatant was used for analysis.

Assay

To Kontes incubation flasks (Kontes Glass Co., Vineland, NJ) were added: buffer solution, 0.5 ml sodium acetate buffer (0.1 M, pH 5.7); 0.1 ml tyrosine decarboxylase (0.5 mg/ml); 0.025 ml PLP standard solution, deproteinized plasma, or buffer. (Standard PLP solutions were used in the range of 0.1 to 1.0 ngm/0.025 ml).

The flasks were incubated for 30 min at 20° C, swirling the mixture occasionally. The flasks were then capped with serum stoppers. Protosol (0.1 ml) (New England Nuclear, Boston, MS) was added to

liquid scintillation vials which were set in an ice bath for 10 min. Substrate (0.5 ml) was injected through the serum stoppers. (The working substrate was 2 μmole/ml unlabeled L-tyrosine and 10 nCi/ml L-tyrosine-1-^{14}C.) The flasks were evacuated by means of a hypodermic needle attached to a vacuum for 10 sec, and then incubated for 15 min at 37° C in a shaking water bath. The reaction was stopped by injecting 0.4 ml 6 n HCl. The collection of CO_2 was continued in the shaking water bath for 30 min at 37° C, after which the vials were removed, and triton fluor was added and the vials were counted. (Triton fluor contained one part triton X-100 and 2 parts toluene scintillator solution composed of 8.25 g PPO and 0.25 g POPOP per liter of toluene.)