AN ABSTRACT TO THE THESIS OF

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Title: THE EFFECT OF VITAMIN B-6 SUPPLEMENTATION ON PLANT PROTEIN UTILIZATION IN ADULTS

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We investigated the effect of pyridoxine supplementation on the utilization of protein in a low-protein, plant-based diet in four subjects (2 men and 2 women), aged 21 to 38 years. Following two days of a negligible protein diet, this 34 day study was divided into three dietary periods: the subjects received a low-protein, plant-based diet during period I for 10 days (no pyridoxine supplement), the same diet but with the addition of 50 mg pyridoxine HCl during period II for 7 days, and their self-chosen diets during period III for 15 days (no pyridoxine supplement). Data for period III will be reported elsewhere. The greatest portion of protein in the experimental diet was furnished by pinto beans (1.02 g

nitrogen) and peanut butter (0.86 g nitrogen); nitrogen intake was kept constant at 4.56 g/d for the men and 4.15 g/d for the women during periods I and II. These diets administered during periods I and II provided 0.907 mg of vitamin B-6 for the men and 0.758 mg of vitamin B-6 for the women and was adequate in other nutrients except for protein.

Overall, the effect of 50 mg pyridoxine HCl supplementation on the utilization of protein in a low-protein plant-based diet was not statistically significant ($p > 0.05$) on the basis of a paired t-test for the parameters measured: nitrogen balance, apparent protein digestibility, as well as plasma and urinary urea nitrogen. Furthermore, we obtained conflicting results, when the subjects received pyridoxine, their plasma urea nitrogen increased slightly (suggesting increased protein degradation), while the percent of total urinary nitrogen excretion as urea nitrogen decreased (suggesting decreased protein degradation). These changes were not statistically significant, but limitations in the nitrogen balance technique and the analytical procedures we used may have contributed to these conflicting results. We suggest that a longer study with more subjects may show a greater improvement of plant protein utilization than we had observed.

THE EFFECT OF VITAMIN B-6 SUPPLEMENTATION ON PLANT PROTEIN UTILIZATION IN ADULTS

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

THE EFFECT OF VITAMIN B-6 SUPPLEMENTATION ON PLANT PROTEIN UTILIZATION IN ADULTS

INTRODUCTION

The effect of quantity of dietary protein on the requirement for vitamin B-6 in animals and in humans has been demonstrated (Cerecedo & Foy, 1944; Cerecedo, Foy & DeRenzo, 1948; Cerecedo & DeRenzo, 1950; Miller, Leklem & Shultz, 1985). More recently, it has been reported that vitamin B-6 requirements in humans and other animals are dependent on dietary protein quality (Daghir & Shah, 1973; Fisher, Willis & Haskell, 1984). Fisher et al. (1984) tested the effect of protein quality on vitamin B-6 in the rat. They found that diets containing an amino acid mixture equivalent to low quality protein had an adverse effect on the vitamin B-6 status of rats fed either suboptimal or ample vitamin B-6 and diets containing an amino acid mixture equivalent to poor quality protein (i.e. maize). Diets containing amino acid mixture equivalent to good quality protein produced maximal growth.

In developing countries where protein energy malnutrition is prevalent, the underlying condition is most often both insufficient dietary protein and energy (Koppert, 1977; McGuire, 1988). Often plants are the protein sources. The consumption of low-protein containing plant foodstuffs

appears to be an important factor contributing to the problem of malnutrition prevalent in these parts of the world which are mostly developing countries (Kakade, 1974). Plant proteins in general are deficient in one or more amino acids. Specifically, cereals are mainly deficient in lysine, while legumes and leaf protein are deficient in methionine.

Vitamin B-6 was reported to improve protein synthesis and food utilization (Sure and Easterling, 1949). Sauberlich (1961) indicated that when a diet was limiting in both pyridoxine and an essential amino acid (tryptophan,valine or methionine), growth of rats was improved by supplementation with either vitamin B-6 or these amino acids (tryptophan, methionine, and valine).

There is a need for additional information concerning the effect of vitamin B-6 supplementation in low protein, plant-based diets. In this research, pinto beans and peanut butter were the major contributors of protein for a study involving 2 men and 2 women. This diet was below the recommended intakes for protein and low in some essential amino acids. (National Research Council (NRC),1980; FAO/WHO/UNU,1985; Orr & Watt,1957; Pike & Brown, 1984).

REVIEW OF THE LITERATURE

Vitamin B-6 was first recognized by György in 1934 as an essential dietary factor for rats (György, 1971). The essentiality of this nutrient for humans was not identified until the 1950's when certain neurological symptoms in infants were attributed to a deficiency of this vitamin (Coursin, 1954; Snyderman et al., 1953).

Vitamin B-6 is a generic descriptor for all 3-hydroxy-5-hydroxymethyl-2-methylpyridine derivatives that "exhibit qualitatively the biological activity of pyridoxine in rats" (American Institute of Nutrition, 1987). Vitamin B-6 includes the three free forms, pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM), as well as their respective phosphorylated forms pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP). These six forms are interconvertible, as shown in Figure 1. PL and PM are the principal forms of vitamin B-6 in animal-derived foods and PN is the principal form in plants (Orr,1969). Some of the PN in plants is in the glycosylated form, 5'-O-(b-D-glucopyranosyl) pyridoxine, which is of limited bioavailability (Kabir,Leklem & Miller, 1983). In animal tissues, PLP and PMP are bound to proteins (Lumeng et al., 1984) The chief metabolite of vitamin B-6 excreted in urine is 4-pyridoxic acid (4-PA) (Wozenski, Leklem & Miller, 1980), also shown in Figure 1.

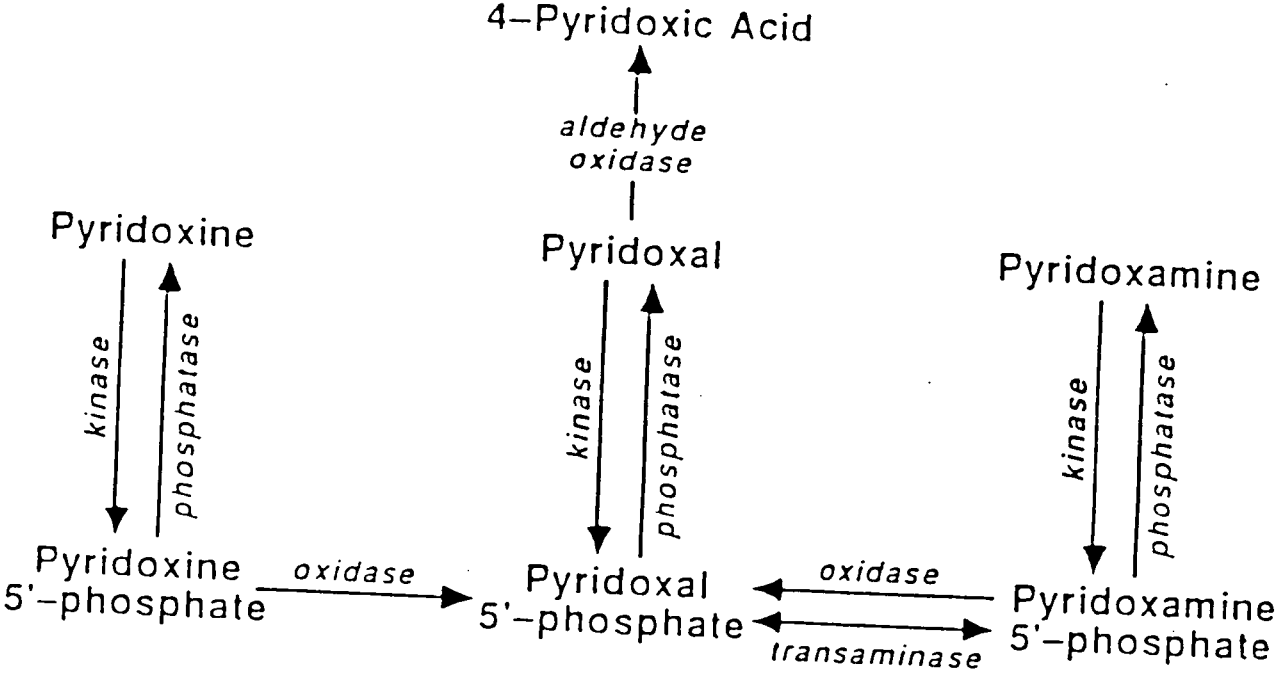

Figure 1. Interconversion of B-6 vitamers in human liver (from Leklem, 1988).

Digestion and absorption of vitamin B-6

Although dietary amounts of PLP and PMP are extensively hydrolyzed by intestinal phosphatase, small amounts of these phosphorylated forms may be absorbed intact. According to Henderson (1984), the mucosal membrane influx and basolateral membrane efflux of non-phosphorylated vitamin B-6 occurs by a nonsaturable, energy-independent process which appears to be passive diffusion.

Metabolism of vitamin B-6

The interconvertibility of the B-6 vitamers in the various tissues of the body depends on the presence of PL kinase and PMP(PNP) oxidase. PL kinase is distributed widely is tissues, while PMP(PNP) oxidase is found in only a few. In the human, PMP(PNP) oxidase appears to be limited to the liver and the red cells; muscle is devoid of PMP(PNP) oxidase activity (Lumeng et al., 1984). PLP in muscle is attached to glycogen phosphorylase, which serves as a reservoir for vitamin B-6 (Black, Guirard & Snell, 1977).

Following absorption, the free forms of vitamin B-6 are transported to the liver where they are converted to their respective phosphorylated forms by PL kinase. PMP and PNP are subsequently converted to PLP by PMP(PNP) oxidase, an FMN-dependent enzyme. PMP can also be reversibly transaminated to PLP. The two coenzyme forms of vitamin B-6, PLP and PMP, are bound to vitamin B-6-dependent

apoenzymes. PLP circulating in plasma is derived from the liver (Lumeng et al., 1984). In the liver PLP is also dephosphorylated to PL, which is subsequently released into circulation attached to albumin, or oxidized by aldehyde oxidase or aldehyde dehydrogenase to 4-PA. PL appears to be the form used by most tissues. Four-pyridoxic acid, a dead-end catabolite, is the chief form of vitamin B-6 excreted in urine (Wozenski et al. 1980).

In the red blood cells, as shown by Anderson et al. (1974), plasma PN is rapidly converted to PL, which is subsequently phosphorylated to PLP. Since PLP and PL are both bound specifically to hemoglobin, the red cells are a circulating reservoir of vitamin B-6. PLP must first be dephosphorylated by an appropriate phosphatase to PL before release into the plasma.

Plasma PLP, extensively bound to albumin, must first be hydrolyzed to PL in order to be transported across plasma membranes. Anderson et al., (1971) suggested that serum alkaline phosphatase may play a role in plasma PLP degradation. In vitro experiments by Lumeng et al. (1984), however, demonstrated that plasma alkaline phosphatase and cellular elements in whole blood contribute very little to the hydrolysis of plasma PLP.

Metabolic function of vitamin B-6

Vitamin B-6 has many functions, most of which are related to protein and amino acid metabolism. PLP is the coenzyme for a large number of enzymes participating in amino acid metabolism. These reactions include: transamination; racemization; alpha, beta or alpha, gamma - elimination; decarboxylation; oxido-reduction; dehydration and desulfhydration. Aminotransferases, providing an interface between amino acid metabolism and ketogenic and glycogenic reactions, catalyze the reversible formation of an alpha-keto acid and pyridoxamine 5'-phosphate. Amino acid decarboxylases irreversibly form amines, including the neurotransmitters serotonin, epinephrine, norepinephrine, dopamine and gamma-amino butyric acid. The synthesis of heme depends upon the formation of delta-aminolevulinic acid from the PLP-catalyzed condensation of succinyl-CoA and glycine followed by decarboxylation. Some PLP-dependent enzymes effect the loss and transfer of amino acid side chains, e.g. cysteine desulfhydrase in the formation of pyruvate, and serine transmethyltransferase in the formation of $N_{5,10,}$ methylenetetrahydrofolic acid. PLP is necessary for the formation of niacin from tryptophan, and carnitine from lysine.

Vitamin B-6 plays an important role in the maintenance and function of the immune system through its influence on nucleic acid and protein synthesis. PLP is an

integral part of glycogen phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate. In lipid metabolism, PLP catalyzes the condensation of L-serine with palmitoyl-CoA, forming 3-dehydrosphingosine, a precursor of sphingolipid. More recently, the role of PLP in steroid hormone function has been identified. Figure 2, adapted from Leklem (1988), summarizes the function of PLP as a coenzyme.

Measurement of vitamin B-6 status

Various biochemical procedures have been developed for evaluating vitamin B-6 status in humans (Sauberlich, Dowdy & Skala, 1974; Leklem & Reynolds, 1981; Gregory, 1988). These procedures can be divided into direct and indirect methods. For measuring vitamin B-6 status it is desirable to measure two or more indices of status (Leklem & Reynolds, 1981) .

Vitamin B-6 compounds in blood and plasma

Vitamin B-6 compounds in plasma fall rapidly during vitamin B-6 depletion and rise following repletion or supplementation with the vitamin (Baysal, Johnson & Linkswiller, 1966). PLP, the principal form of vitamin B-6 in plasma, has been considered to be a sensitive, reliable indicator of vitamin B-6 status. Besides PLP, another

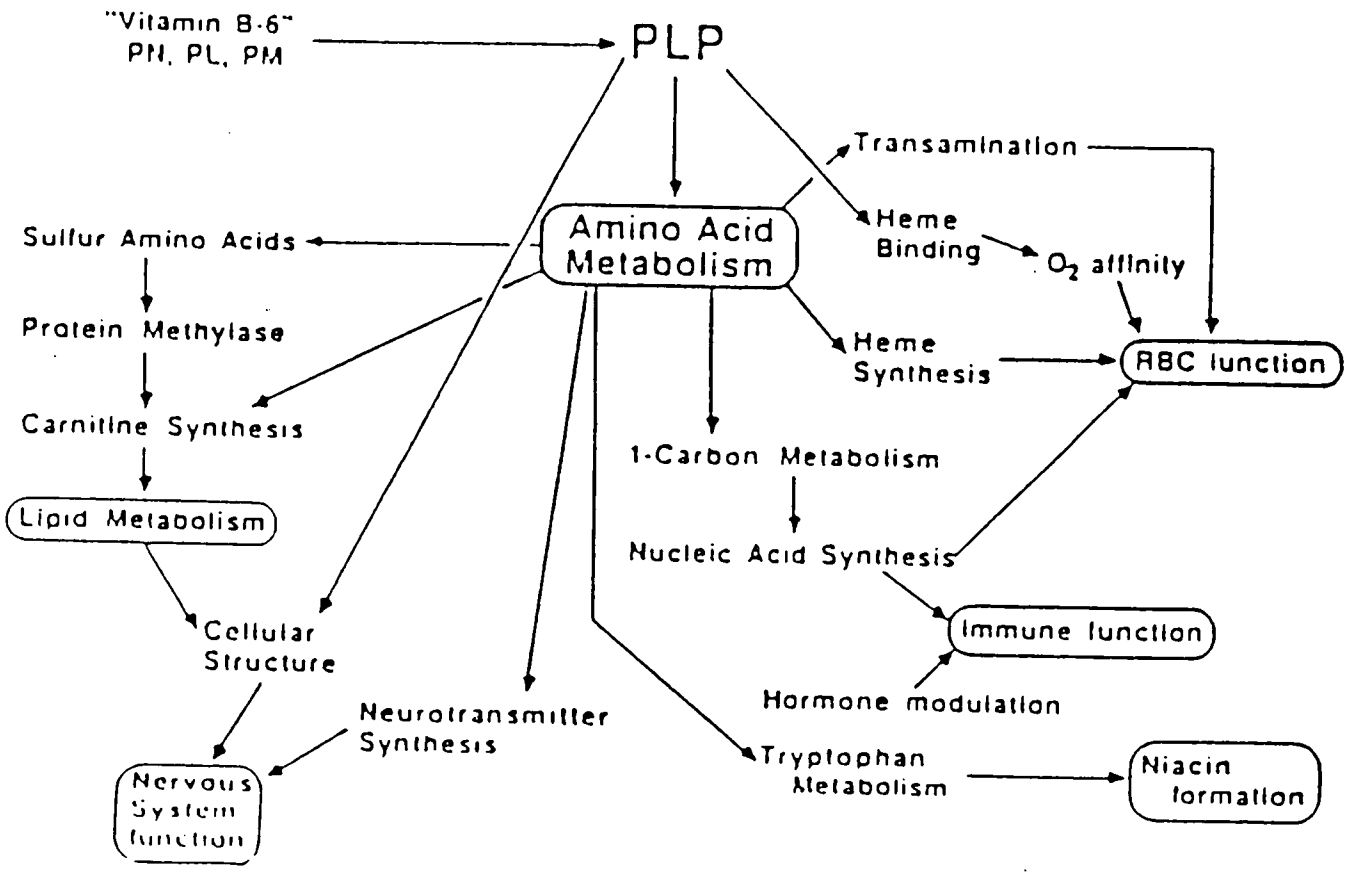

Figure 2. Functions in which pyridoxal 5'phosphate (PLP) acts as a coenzyme or binds with proteins and modifies the action of the protein (from Leklem, 1988).

circulating form of vitamin B-6 that is available to tissues is PL. Recent research indicates that PL, which operates independently of PLP in circulation, may more closely reflect the vitamin B-6 available to the tissues than PLP. (Lumeng et al., 1984)

Tentative guidelines for evaluating vitamin B-6 nutritional status in adults using plasma pyridoxal 5'-phosphate have been proposed by Shultz & Leklem (1981). To the knowledge of the author, no normal values for plasma PL are available in the literature. Vitamin B-6 status can also be determined by measuring plasma total vitamin B-6 . It is measured in hydrolyzed blood or plasma by microbiological assay with Saccharomyces uvarum, which responds to the PL, PM, and PN (Miller & Edwards, 1981). This procedure, however, gives no information on the individual B-6 vitamers in circulation.

Urinary excretion of 4-PA and vitamin B-6

The major urinary metabolite of vitamin B-6 is 4-PA. Vitamin B-6 appears in the urine mainly as pyridoxal and to a lesser amount as pyridoxamine; pyridoxine and phosphorylated forms may also be excreted (Kelsay, Baysal & Linkswiller, 1968). Urinary excretion of 4-PA and total vitamin B-6 reflect dietary intake of vitamin B-6: they rise with increased intake of vitamin B-6 and decrease during vitamin B-6 deprivation (Baysal et al., 1966). According to

Wozenski et al. (1980), young men who were given oral loading doses from 2.4 umoles to 48.6 umoles of pyridoxine excreted about half of the dose as 4-PA and from 5-10% as vitamin B-6. Shultz and Leklem (1981) have proposed tentative guidelines for using urinary concentrations of 4-PA and total vitamin B-6 to assess vitamin B-6 status in humans.

Tryptophan load test

Lepkovsky, Roboz & Gaagen-Samit, (1943) were the first to recognize that vitamin B-6 deficient rats spontaneously excreted large amounts of xanthurenic acid, a metabolite of tryptophan, in urine. This finding eventually led to the development of the tryptophan load test in humans.

Several enzymes in the tryptophan-kynurenine pathway require PLP. Vitamin B-6 deficiency may affect this pathway at one or more points. In response to a tryptophan load test, persons deficient in vitamin B-6 excrete elevated amounts of kynurenine, hydroxykynurenine, kynurenic acid and xanthurenic acid in urine (Brown, 1981). According to Brown, the tryptophan load test clearly indicates the functional adequacy of coenzyme (PLP) levels, whereas the measurement of plasma PLP and urinary 4-PA reflect intake of vitamin B-6 and, possibly, tissue levels of the vitamin.

Erythrocyte alanine (EALT) and aspartate (EAST) amino-transferase activity.

The activities of these PLP- dependent enzymes, indicate long term vitamin B-6 status. The stimulated and unstimulated activities, respectively, of both enzymes are measured with and without the in vitro addition of the coenzyme, PLP. Vitamin B-6 nutriture is assessed by the activity coefficients of EALT and EAST: activity measured with PLP added in vitro divided by activity measured without added PLP. In general, the higher the activity coefficient, the greater the deficiency of vitamin B-6.

Recommended dietary allowance for vitamin B-6

The ratio, 0.02 mg of vitamin B-6 per gram of protein eaten, was used to calculate the recommended dietary allowances for vitamin B-6 for adults and children (National Research Council (NRC), 1980). The RDA for men, assuming that they receive 110 g of protein daily, is 2.2 mg of vitamin B-6 per day. The RDA for adult women, 2.0 mg per day, is based on an assumed intake of 100 g of protein per day. To cover the demands for pregnancy and lactation, respectively, an additional 0.6 mg and 0.5 mg of vitamin B-6 are recommended for pregnant and lactating women. Use of steroid hormones as oral contraceptive agents appears not to increase the requirement for vitamin B-6.

For the young infant, 0.3 mg of vitamin B-6 is considered adequate. For 7- to 12- month old infants who are receiving a mixed diet, an allowance of 0.6 mg is recommended. For children older than one year, the RDA is based on the same vitamin B-6 to protein ratio as for adults, 0.02 mg of vitamin B-6 per g of protein eaten.

Metabolism of supplementary vitamin B-6

The metabolism of vitamin B-6 can also be investigated by analyzing blood and urine collected from individuals who are given either single doses of vitamin B-6 or supplementary vitamin B-6 over a period of time. Wozenski et al. (1980) examined the changes in concentrations of vitamin B-6 compounds in blood and urine collected after the administration of single small oral doses of vitamin B-6 to 5 men, aged 24 to 32 years. Wozenski et al. observed that 1/2 to 1 hr following ingestion of 24 to 48.6 umoles of pyridoxine, plasma total vitamin B-6 concentrations increased several-fold more than plasma PLP concentrations. As mentioned previously, the subjects excreted in urine about half of the ingested PN dose as 4-PA and from 5-10% as vitamin B-6.

Speitling, Hesker & Kubler, (1988) assessed the pharmacologic effects of chronic high vitamin B-6 dosage in ten males, aged 20 to 30 years, who received 40 mg (194.5 umoles of PN) of pyridoxine HCl (PN HCl) for 30 days. On the first day of supplementation, the subjects' mean plasma

PLP concentration increased from 78 nmol/L to 298 nmol/L. By the 4th day of PN HCl supplementation, the subjects' mean plasma PLP concentrations reached 500 nmol/L and remained constant at this level during the remaining 26 days of supplementation. During the first 8 days of PN HCl supplementation, mean daily 4-PA excretion increased from 69 to 135 umol/day. It remained constant at the latter value during the remaining 22 days the subjects received the PN HCl supplement. Approximately two-thirds of the ingested PN HCl was excreted as 4-PA.

When PN HCl supplementation was discontinued, urinary 4-PA decreased in a biphasic manner. Four-pyridoxic acid excretion in urine decreased after supplementation was discontinued. Presupplement plasma PLP levels were reached by 16 days, and urinary 4-PA by 7 days (Speitling et al., 1988).

Speitling et al. evaluated PLP concentrations in tissues by measuring EALT and EAST. The presumed elevated concentrations of PLP in erythrocytes were associated with increased EAST and EALT activities after 30 days of PN HCl supplementation. When the supplement was discontinued, EALT activity measured with and without added PLP decreased within two days and remained constant during the subsequent 38 days. EAST activity, on the other hand, measured with PLP added in vitro remained elevated, but EAST activity without added PLP decreased.

Pyridoxine toxicity

Schaumburg & Berger (1988) reviewed studies on PN toxicity in animals and humans. Sensory neuropathy produced by high dose of PN depended on the dosage . Moderately high levels of PN intoxication, 200 mg/kg/day in rats, produced an unsteady gait after about six weeks. Animals dosed with an extremely high level, 600 or 1200 mg/kg/day in rats or 1 g/kg/day in dogs, developed a severe locomotor ataxia of gait within a few days.

In human studies it is claimed that the ingestion of pyridoxine 200 mg/day for 33 days produces a dependency syndrome with withdrawal symptoms following the cessation of the drug. Humans who have ingested moderately high dose of PN (200 mg-10 g) have developed sensory neuropathies which are largely reversible.

Extraordinarily high levels of vitamin B-6 were observed in individuals receiving 180 g of PN HCl intravenously over a two-day period administered to the patient for mushroom poisoning. The patients developed a striking sensory neuropathy syndrome which included sensory ataxia of all limbs, inability to swallow, autonomic disturbances, loss of sensation of entire body, absence of tendon reflexes and slight weakness. Recovery was poor. Thus, it appears that massive doses of pyridoxine hydrochloride in man produces a syndrome mimicking that produced

in animals, reflecting irreversible destruction of dorsal root ganglion cell.

Proteins and their major functions

Proteins, the indispensable constituents of living protoplasm, participate in all vital processes. They contain nitrogen-containing amino acids which differ in structure.

Proteins are essential for growth, a function in which they cannot be replaced by fat and carbohydrate. Proteins provide the amino acids, the building stones for tissue synthesis. The body constantly undergoes wear and tear, the repair of which requires amino acids. Also, proteins supply new materials for the synthesis of digestive juices, hormones, plasma proteins, hemoglobin, certain vitamins, and enzymes.

Furthermore, protein can be used as an energy source, but it is wasteful to use it for this purpose since carbohydrates and fats also provide calories. Physiologically, each gram of protein supplies about 4 Kcal (16.7 Kj) of energy. In addition, proteins function as buffers, helping to maintain the reactions of various media, such as plasma, cerebrospinal fluid, and intestinal secretions (Passmore, et al., 1974).

Classifications of proteins

Proteins are classified nutritionally as either animal proteins or vegetable proteins, or as biologically complete or incomplete. Animal proteins contain all of the essential amino acids in adequate amounts, whereas vegetable proteins may be deficient in one or more essential amino acids. Thus, animal proteins generally have a higher nutritive value than plant proteins. Essential amino acids are those which cannot be synthesized by the body and must therefore be supplied by diet. Non-essential amino acids can be synthesized in the body. A biologically complete protein contains all of the essential amino acids in adequate amounts to meet human requirements. A biologically incomplete protein is deficient in one or more essential amino acids. Most of the vegetable proteins are classed as biologically incomplete, although mixtures of vegetable protein (rice and beans,for example) may present all of the amino acids in adequate quantities. Therefore, amino acids in mixtures of various proteins may complement one another as long as the proteins are not all lacking the same essential amino acid(s). The ultimate fate of the amino acids not used for synthesis is the removal of nitrogen for the formation of urea and their direct and indirect release as energy from the carbon skeleton (Passmore et al., 1974).

Protein requirements

The protein requirement of a healthy adult is defined as the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in persons who are in energy balance at a modest level of physical activity (FAO/WHO/UNU, 1985). For children and pregnant or lactating women an additional amount of protein required to support tissue growth or milk formation is incorporated into this estimate of protein requirements consistent with good health.

An individual's protein requirement is affected by his/her age, sex and weight. Requirements are often given by age groups, and changes in the variables that characterize these groups involve changes in the average nutritional requirement. The FAO/WHO/UNU (1985) has defined six main age ranges(in years) : 0-3, 3-10, 10-18, 18-30, 30-60, 60+. Passmore et al. (1974) stated that the protein requirement can be experimentally determined from obligatory nitrogen losses when the subjects are fed a nitrogen-free diet. The FAO/WHO/UNU Expert Consultation (1985) reported that the sum of obligatory losses in urine and feces of young adult males is approximately 49 mg of nitrogen per kg of body weight. An amount of 5-8 mg of N/kgBw was added to this figure to allow for miscellaneous, unmeasured nitrogen losses (sweat, etc.).

The FAO/WHO/UNU Expert Consultation (1985) stated an estimated mean protein requirement of 0.60 g/kg per day of highly digestible, good quality protein, such as that from meat, milk, egg and fish. This mean value is slightly higher than the "safe level": 0.58 and 0.52 g/kg body weight per day, recommended earlier for adult men and women, respectively (Passmore et al., 1974). These requirements are valid only when the energy requirement is fully met. If total energy intake is inadequate, some dietary protein is used for energy and is therefore not available to satisfy protein needs (Passmore et al., 1974). Further study by Garza, Scrimshaw & Young et al. (1976) indicated that a high energy intake is required to maintain nitrogen balance at the level of 0.57 g/kg of dietary protein. These authors cited a Japanese study which showed that the estimated mean requirement for egg protein in Japanese men who were maintaining their weight was 0.56 g/Kg and could be reduced to 0.42 g/Kg by providing a higher energy intake (57 Kcal/Kg). Based on FAO/WHO/UNU (1985), which reported an average protein requirement of 0.60 g/kg/day, the addition of 25% (2 SD) above the average physiological requirement was estimated to meet the needs of all but 2.5% of individuals within the population. Therefore, 0.75 g/kg of good quality protein was thought to correspond to the lower end of the safe range of protein intake for adult men and women. Similarly, the National Research Council in the US

considered a 75% efficiency of protein utilization of a mixed protein diet compared with the reference protein and recommended an allowance of 0.8 grams of mixed proteins per kilogram of body weight per day.

Special consideration should be given to older adults, whose energy intake and needs tend to fall progressively with age, whereas the amount of protein per kg of body weight needed for N equilibrium is not reduced and may even increase. Since many age-related body changes appear to occur continuously throughout adult life, protein allowances for adults should ideally be those that best preserve bodily functions from early adulthood to old age (FAO/WHO/UNU, 1985).

According to the NRC it is prudent to ensure that the elderly receive 12% or more of their energy intake in the form of protein, especially since the elderly often have recurring episodes of chronic diseases, requiring the repletion of body protein in convalescence. It was found that more whole egg protein had to be added to the diet of the elderly than to the diet of young adults in order to achieve N equilibrium. Indeed, 0.8 g of whole egg protein per kg of body weight was needed to ensure nitrogen equilibrium in the elderly, a level twice that predicted by the factorial method and also greater than the recommended level of 0.57 g/kg body weight (NRC, 1980).

For pregnant women, the protein allowance must cover needs for both maternal and physiological adjustments as well as growth and development of the conceptus. According to NRC, an additional protein intake of 30 g/day above the non-pregnant allowance is recommended during pregnancy for adult women. This estimate is based on the retention of 16 mg of N/kg body weight daily and on 50% utilization of dietary protein. A pregnant adolescent should receive the protein allowance based on her non-pregnant body weight (0.9 g/kg for those aged 15-18; 1.0 g/kg for those aged 11/14) plus an additional 30 g/day for the pregnancy. For the nursing mother, an addition of 20 g/day to that for non-pregnant and non-lactating women is recommended (NRC,1980).

For infants the allowances are based on the amount of protein provided by the quantity of milk required to ensure a satisfactory rate of growth. This is estimated by NRC to be 2-2.4 g/kg/day by the sixth month. For infants older than six months, the allowance for protein, 1.5 g of milk protein per kg per day has been adjusted upward to allow for 75% efficiency of utilization of proteins from a mixed diet.

The allowances for children and young people are calculated from information of growth rates and body composition assuming that the efficiency of protein utilization for growth is comparable to that observed for maintenance in adults. The allowances decrease gradually

from 2.0 g/kg at 0.5-1 year to 0.8 g/kg at 18 years of age (NRC, 1980).

Health implications of inadequate protein-energy intake

With consideration of the protein requirement and its relationships with energy intake (protein-energy ratio requirements or intakes), several studies have considered the prevalence of inadequate calorie and protein intakes as a major health problem in populations characterized by insufficient food supply and feedings.

Siegel & Hoover (1982) reported that food production in many less developed countries (LDC) actually fell behind population growth, resulting in an inadequate daily food supply and thus a deficient caloric consumption in relation to nutrition requirements. These same authors reported that from 1972-1974 regions with insufficient energy supplies included Africa and Asia, with deficiencies of 9% and 8%, respectively, and an Asian group of centrally planned economies, with a deficiency of 3%. Regional protein supplies per capita were similar, but the differences were greater in protein supplies than in energy available and more closely associated with regional differences in per capita income. According to Seigel and Hoover, the degree of dietary inadequacy in many parts of the world is rather severe, and the severity of this problem is expected to increase in the future. If this prediction is realized,

there may be increased mortality in susceptible age groups, especially the elderly and the very young.

There is a growing concern by national and international authorities about the extent and increase of malnutrition in developing countries (Koppert, 1977; McGuire, 1988). McGuire (1988) reported that the prevalence of preschool child malnutrition in the 1980's was estimated as 15.3% in Latin America, 29.5% in Africa, 36.6% in Asia and the Near East (excluding Pakistan, Bangladesh, and India), 71.7% in Bangladesh, 64% in Pakistan, and 29% world-wide.

It is recognized that complex factors are involved in the malnutrition syndrome including: food shortages, inadequate food distribution (Koppert, 1977; Siegel & Hoover, 1982 and McGuire, 1988), short breastfeeding and early weaning due to pregnancy (Koppert, 1977; Aykroyd, 1970; McLaren, 1966; Stetler et al., 1980; Lemaire & DeMaegd, 1986), family income, education and cultural habits (Koppert, 1977, Bairagi, 1980), diseases (Koppert, 1977, McGuire, 1980, Siegel & Hoover, 1982, Victora et al., 1986), inadequate water supply and national food policy (Koppert, 1977). Koppert considered that malnutrition could be seen, therefore, as an environmental problem varying from country to country, but also between rural and urban districts within a country, and between fertile and poor land or high or low rainfall in rural areas.

Drought, deforestation, erosion, and misuse of land resources (overgrazing), urbanization and environmental refugees due to economic or political factors are environmental factors involved in the risk of malnutrition. A recent nutritional survey conducted in drought affected regions of Somalia concluded that the prevalence of malnutrition (undernutrition) in the surveyed population was higher than the estimates reported during the non-drought periods in the same country and comparable to other drought-prone sub-Saharan countries (Centers for Disease Control, 1988). Furthermore, 20 to 26% of the children who were between 80 and 84% of median weight-for-height were at risk of malnutrition following further weight loss due to an insufficient food supply until any potential harvest season. Illness accentuated problems of malnutrition. Diarrheal episodes and deficiencies in vitamins A and C were also reported (Centers for Disease Control, 1988). Recommendations resulting from the survey emphasized the early provision of food, including calories and protein, to prevent further nutritional deterioration.

Although Glantz (1987) considered that droughts do not necessarily result in famines or severe food shortages in nations which can cope with drought by appropriate management (i.e., irrigation), he concluded that drought affects farming (crops and livestock) adversely, and therefore, inadequate agricultural production results.

Glantz discussed drought as an effect of various factors, mainly human activities leading to the modification of land surfaces, deforestation, overgrazing, desertification, and woodcutting for fuel and construction. Such activities increase the surface albedo of the earth, and consequently, limit cloud formation and precipitation. In particular, Roche (1989) reported that massive deforestation is associated with rural impoverishment in Africa, not only because of the resulting ecological effects, but also the decline in food production due to soil degradation or low rainfall and even in the reduction of plant species usually used as direct food sources (i.e. fruit and leaves). In developing countries, where most people are engaged in agriculture and where the nutritional economy depends on agricultural production, the impact of drought is devastating and results in sharp price increases, rising import of food, changes in malnutritional status of the population at risk, and surges in migration from the countryside to urban centers. In the part of sub-Saharan Africa affected by prolonged drought and famines, migrants often move to refugee camps in a weakened condition, totally dependent on food relief (Glantz, 1987). In Somalia (Centers for Disease Control, 1988), It was found that in such conditions, the risk of malnutrition and vitamin deficiencies rises in a population increasingly dependent on food aid consisting largely of grains low in vitamin A

and C. This problem is also aggravated in nations plagued by internal conflicts. Cases of famine in Mozambique, Angola, Sudan, and Ethiopia were reported associated with drought and social conflicts and wars (Glantz, 1987). Parenti et al. (1987) evaluated the health status of 239 Ethiopian refugees in the United States and observed that these subjects had illnesses similar to those found in refugees from both developed and developing countries. Their illnesses included intestinal parasites, 36.7% (mostly Giardia lamblia, Trichuris trichiura, and Schistosoma mansoni) and anemia, 14.9%. Tuberculine positivity was reported for 71.9% with 1% prevalence of active tuberculosis. These refugees had good nutritional status in contrast to the malnutrition noted in Ethiopian refugees in Somalian refugee camps. Health studies in 194 Indochinese refugees in the USA also identified infectious diseases such as tuberculosis, intestinal parasites, skin infections, and malaria (Erikson and Hoang, 1980). An impressive number of cases of anemia, hematological abnormalities (37%), and psychiatric disorders were also reported.

Urbanization leads to cultural changes which are often adverse. Aykroyd (1970) emphasized the effect of urbanization on the nutrition of infants and young children. The most important effect is early weaning, usually due to the mother being employed, to social attitudes which discourage breastfeeding and to the economic aspects that

sometimes make artificial feeding unsatisfactory and unsanitary. Early weaning in these circumstances promotes protein calorie malnutrition particularly in its marasmic form. Koppert (1977) stated that the groups most vulnerable to protein-energy malnutrition (PEM) are pregnant and lactating women and young children under the age of 5 years.

McLaren (1988) reviewed an adult form of PEM due to starvation and protein depression in hospital patients in the Third World and Western countries during injury and sepsis. Adult PEM resulting from starvation coincides closely with the findings in infantile marasmus (McLaren, 1988). In fact, McLaren (1966) and Roussouw (1989) presented marasmus as a total inanition in the child with severe and continued restriction of both calories and protein as well as of all other nutrients, often due to failure to breastfeed, inadequate breast milk supply, or the use of very diluted breast milk substitute. Another form of PEM is often referred to as kwashiorkor, which is fundamentally of dietary origin and primarily affects children who are weaned on traditional family starchy or sugary foods (McLaren, 1966).

Figure 3, adapted from McLaren (1988), shows the pathogenesis of kwashiorkor which results from protein lack with sufficient calories. A similar result is produced by the metabolic changes resulting from an acute infection such as measles. However, Roussouw (1989) in his study of

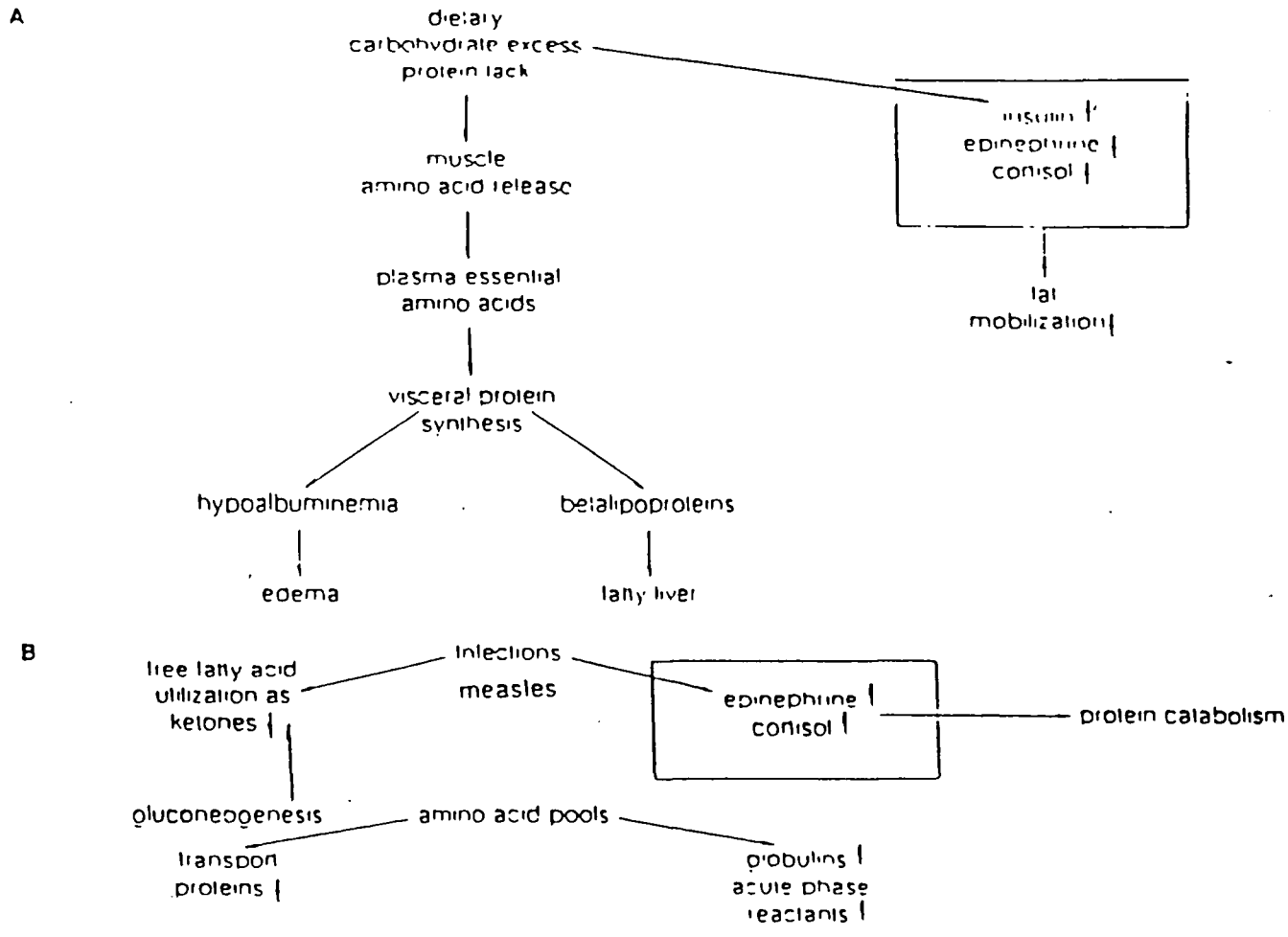

Figure 3. Pathogenesis of Kwashiorkor (A) and similar effects of an acute infection (B) (adapted from McLaren, 1988).

kwashiorkor in 2-18 month-old North American infants found that protein deficiency is an essential prerequisite for the development of kwashiorkor and marasmic kwashiorkor. His findings supported the evidence that a low ratio of protein to energy disrupts the usually hypometabolic response to dietary deficiency, leading to hypoalbuminia and consequent edema.

In parts of the world where marasmus dominates the picture (i.e., in most of the Near and Far East, in North Africa, and much of Latin America), rapid succession of pregnancies and early weaning of children seem to be highly relevant (Scrimshaw, 1964 and McLaren, 1966). Scrimshaw (1964) also indicated that frequent pregnancies act as a major host factor in malnutrition in women of childbearing age. When the demands of pregnancy and lactation are not met by the diet, the mother's own tissues become depleted. If the postpartum diet is not sufficient to allow repletion, the mother becomes progressively more poorly nourished. These conditions affect also the health of successive offspring. In Jordon, 78% of questioned mothers of malnourished children weaned their children because they were pregnant again (McLaren, 1966). A similar situation was reported by Lemaire & DeMaegd (1986) where 17% of early weaned Burundian infants (less than 2 years) presented PEM. Clinical examination showed that 2% and 1.7% of 0-5 year-old children had symptoms of marasmus and pre-kwashiorkor,

respectively. Stetler et al. (1980), who examined the nutrition of 6-23 month old Togolese preschool children, found that prevalence of acute undernutrition occurred during the weaning period, reflecting inadequate food intake patterns during the transition from breastfeeding to the household diet. Referring to previous studies in Togo, these authors indicated that 23.2% of rural households had energy intakes that were less than 80% of the recommended amount, and 35.2% had similarly deficient protein intakes.

In the etiology of PEM, McLaren (1966) emphasized the effect of environmental variables on nutritional status. He stated that stress such as infectious diseases and maternal deprivation play an important but incidental role. The synergistic effect of disease and malnutrition is exacerbated in preschool children and women of childbearing age. Victora et al. (1986) reported that infections contribute to malnutrition not only through their adverse metabolic effects (including nitrogen loss) but also by reducing appetite, decreasing nutrient absorption, and accelerating the intestinal transit of food. Episodes of diarrhea are particularly responsible for poor growth in children. Indeed, early reports (McLaren, 1966) showed that the two main measures which prevent protein-calorie malnutrition are (1) diet, by providing supplementary food and (2) control of infection, especially through immunization at an early age. However, considering the complexity

of interacting factors involved in the etiology of protein-calorie malnutrition, the conquest of that health problem depends on action in a number of inter-related, socio-cultural, educational, sanitary and economic aspects. There is no doubt, for example, that efficient programs to prevent protein-energy malnutrition include supplying appropriate foods, educating mothers (nutrition education) in longer breast-feeding, improving child feeding practices and hygienic measures which reduce infectious diseases (McLaren, 1966, Aykroyd, 1970, Caliendo, 1979 and McGuire, 1988).

To eliminate protein-energy malnutrition in a population, individuals must receive enough of the right food (locally produced or imported), especially food fulfilling protein requirements. The great difficulty of producing an abundance of foods such as milk, meat and eggs in most developing countries is well recognized (Aykroyd, 1970). However, they have other foods such as fish, fruit, legumes, cereals and vegetables as sources of major nutrients, minerals and vitamins, which offer more scope for increased production and consumption.

In relation with dietary protein, requirements for vitamin B-6 have been studied by several authors.

Requirements of vitamin B-6 as influenced by protein intake

The requirement for vitamin B-6 by animals (Cerecedo & Foy, 1944; Daghir & Shah, 1973) and humans (Baker et al., 1964; Miller & Linkswiller, 1967; Miller, Leklem & Shultz, 1985) is related to dietary protein . The RDA for vitamin B-6, 2.0 mg/day for adult women and 2.2 mg/day for adult men, is based on the ratio of 0.02 mg of vitamin B-6 per g of protein (NRC, 1980).

Okada & Ochi (1971) showed in rats that a high-protein diet produced an increase in the activity of liver amino-transferases, presumably in response to the necessity for increased catabolism of amino acids. In vitamin B-6 deficient animals, the activity of those liver amino-transferases was strongly reduced by a high-protein diet. Later, Itoh & Okada (1973) fed rats different levels of protein with or without pyridoxine. They found that the growth of rats receiving the 70% protein diet without pyridoxine was severely retarded. The concentration of pyridoxal was the highest in the livers of rats receiving the 70% casein diet with pyridoxine and the lowest level in those receiving the same amount of protein without pyridoxine. In kidney and muscle, pyridoxal level were higher in the 10% casein group than in the 70% casein group. The vitamin B-6 deficient animals excreted more 4-PA than vitamin B-6 intake; in the groups receiving pyridoxine, the

animals fed a 70% protein diet excreted twice as much 4-PA as the animal fed the 10% protein diet.

Cerecedo, Foy & DeRenzo (1948) investigated the effect of a low-protein diet supplemented with sulfur-containing amino acids on the development of vitamin B-6 deficiency in rats. These researchers found that animals receiving an unsupplemented low-protein diet were resistant to vitamin B-6 deficiency. Addition of cystine or methionine to the low-protein diet aggravated the development of vitamin B-6 deficiency in rats. Of the two amino acids, methionine seemed to have a more pronounced effect. Cerecedo & DeRenzo (1950) later reported the effect of supplementing a low-protein, vitamin B-6 deficient diet with tryptophan and other sulfur-free amino acids. The rats receiving threonine, lysine, or serine did not develop symptoms of vitamin B-6 deficiency sooner than unsupplemented controls. The tryptophan-supplemented rats survived as long as the controls, while methionine-fed rats developed lesions of vitamin B-6 deficiency more quickly, and succumbed much sooner than both the control and the tryptophan supplemented groups. This is taken to mean that high-protein diets do not seem to be harmful to vitamin B-6 deficient rats because of the increased amount of tryptophan ingested. It is more probable that this effect is brought about by the intake of larger amounts of methionine or other sulfur-containing amino acids.

In humans, a high-protein diet produces an effect on vitamin B-6 metabolism similar to that produced in rats. In humans depleted of vitamin B-6, a diet high in protein produced abnormal tryptophan metabolism sooner than did a diet low in protein (Miller & Linkswiller, 1967; Baker, et al., 1964). Miller and Linkswiller observed that young men fed a 54 g protein diet developed abnormal tryptophan metabolism after 40 days of vitamin B-6 depletion. Those receiving 154 g of protein, in contrast, developed abnormal tryptophan metabolism after 14 days of vitamin B-6 depletion. An intake of 1.66 mg of vitamin B-6 daily was sufficient to maintain normal tryptophan metabolism whether the protein intake was 54 or 150 g.

In a similar investigation, Baker et al. observed that young men receiving a 100-g protein diet exhibited abnormal tryptophan metabolism within two weeks of vitamin B-6 deprivation. The subjects fed a 30-g protein diet excreted elevated amounts of xanthurenic acid in response to tryptophan loading after 6 weeks of vitamin B-6 deprivation. Baker et al. concluded that under the conditions of their study the optimal intake of vitamin B-6 (as PN HCl) for subjects receiving a 100-g protein diet was 1.75 to 2.0 mg per day, while subjects receiving a 30-g protein diet need 1.25 to 1.75 mg per day.

Miller et al. (1985) examined the effect of dietary protein on vitamin B-6 metabolism in humans receiving an

adequate but not excessive amount of vitamin B-6. In their experiment, 8 men were fed a semi-purified diet containing 0.5, 1, and 2 g of protein per kg of body weight and vitamin B-6 intake was kept constant at 1.5 mg per day. Urinary 4-PA, plasma total vitamin B-6, and plasma PLP were inversely related with protein intake, i.e., as protein intake increased, the concentrations of these vitamin B-6 compounds decreased. Their results suggest that with increased intake of dietary protein, more vitamin B-6 is retained in the body for increased catabolism of amino acids. It was also concluded that when assessing vitamin B-6 metabolism in humans protein intake must be considered.

Requirement of vitamin B-6 as affected by protein quality

Fisher et al. (1984) tested the effect of protein quality on vitamin B-6 status in rats. The rats were fed purified diets containing amino acid mixtures supplying the equivalent of low-quality (LQ) or good quality (GQ) proteins. The effect of protein quality was tested at two levels of vitamin B-6, 0.2 and 0.7 mg/kg diets. After six weeks, vitamin B-6 was evaluated by determining urinary 4-PA, plasma PLP, and total vitamin B-6 in the liver. They found that at both levels of vitamin B-6 intake, mean values of all three indices were lower in the rats fed LQ protein than in rats fed GQ protein. Additionally, LQ protein appeared to have an adverse effect on the vitamin B-6 status

of rats fed either suboptimal or ample vitamin B-6. The results suggest a minor but consistent deleterious effect of LQ protein on vitamin B-6 status in rats, regardless of vitamin B-6 intake.

Hudson et al. (1989) investigated protein utilization by young women who consumed animal or plant protein diets at three levels of vitamin B-6 intake. After adjustment period the subject received 1.55 g protein/kg BW of either animal protein (mostly dairy and poultry products) or plant protein (mainly various types of beans) and 0.5, 1.0, and 2.0 mg of vitamin B-6 successive per day for 14-21 day period. Measured apparent protein digestibility for animal protein was 94.6%, significantly higher than that of plant protein, 88.4%. Nitrogen balance was not influenced by vitamin B-6 intake. The results of their research showed that short term low vitamin B-6 intake in subject receiving adequate amount of protein did not affect protein utilization in humans as determined by protein digestibility and N balance.

Vitamin B-6 and protein utilization

Vitamin B-6 is generally required for enzymatic reactions involving the non oxidative degradation and interconversion of amino acids. Sure & Easterling (1949) noted that pyridoxine improved utilization of proteins and their synthesis in rats. Other investigators reviewed by Sauberlich (1961) pointed to an increased oxidation of amino

acids in rats deprived of vitamin B-6 and fed a diet containing 40% casein. These investigators observed that vitamin B-6 deficient rats have an increased production of urea, a high blood urea concentration, and an increased urinary excretion of nitrogen. Hawkins, Leonard & Coles (1959) reported that rats fed a vitamin B-6 deficient diet excreted elevated amounts of urinary nitrogen. This is consistent with impaired utilization of amino acids.

Sampson, Young & Kretsch (1988) pointed out that because of the role of vitamin B-6 in amino acid and nucleic acid metabolism, the inadequacy of dietary vitamin B-6 may depress protein synthesis. They found that, in rats, marginal vitamin B-6 intake depressed liver protein absolute synthesis rates, liver wet weight, and liver protein content. Also, marginal vitamin B-6 intake tended to depress the rate of fractional muscle protein synthesis, but increased protein synthesis in the kidneys. Their data demonstrated that marginal vitamin B-6 intake alters protein synthesis in liver and muscles of adult male rats.

Swan, Wentworth & Linkswiller (1964) fed six young men a partially purified diet which supplied 0.16 mg of vitamin B-6 daily and supplemented with 0.9 mg of pyridoxine when biochemical changes became apparent. Nitrogen balance, however, was not affected by vitamin B-6 depletion or repletion.

MATERIALS AND METHODS

Subject selection

Subjects were recruited by announcements in nutrition classes and by advertisements on bulletin boards and in the campus newspaper. Subjects were selected according to the following criteria: age 20 to 40 years; no illness or medical condition requiring constant supervision of a physician; no history of intestinal, renal or metabolic disorder which would affect absorption, metabolism or excretion of vitamin B-6 (e. g., bowel surgery, colitis, diabetes, asthma); no use of vitamin or other nutritional supplements for at least four weeks before the study; no food allergies; no alcohol consumption greater than 2 oz pure alcohol per week; no smoking for at least 6 months before the study; no use of drugs which influence vitamin B-6 metabolism (e.g., isoniazid, penicillamine) or determination (e.g., antibiotics); good dietary habits; and appropriate weight for height, as compared to 1983 Metropolitan Life Insurance Co. tables on desirable weights for men and women, as cited by Public Health Services (1988). On the basis of these criteria, two men and two women, aged 21 to 38 years, were selected to participate in this 34-day study. The subjects' data are presented in Table I. Before participating in this investigation each subject was advised of the purpose and design of the

TABLE I: Descriptive Data of the Subjects

Subject	Sex	Age	Nationality	Height cm	Weight kg	Relative Weight %
M-2	M	21	American	190.5	75.3	97
M-6	M	25	American	182.8	106.8	151
F-4	F	38	Burundian	178.0	67.8	102
F-8	F	37	Indonesian	149.0	57.0	122

Relative weight = usual weight / desirable weight x 100.
Data on desirable weights are from 1983 Metropolitan Life Insurance Co. tables as given in: US Department of Health and Human Services Public Health Services (1988). The Surgeon General's report on Nutrition and Health. DHHS (PHS) Publication no 88-50210. Washington DC. 1988; 282-3

experiment. When their questions were answered and they agreed to participate in this investigation, the subjects signed an informed consent form which was approved by the Oregon State Univserity Human Subjects Committee.

Experimental design

In a two-day preliminary period (d 1-2) of this 34-day study the subjects were fed a diet (Table II) which contained 1.17 g (men) and 1.13 g (women) nitrogen and 0.907 mg (men) and 0.758 mg (women) of vitamin B-6. The purpose of this negligible protein diet was to deplete the subjects' protein stores which shortens the subjects' adaptation to the low protein diet (Pellet & Young 1980), which was administered during the subsequent period.

During the 17-day experimental period (days 3 to 19), a low-protein, plant-based diet containing 4.56 (men) and 4.15 (women) of nitrogen and 0.907 mg (men) and 0.758 mg (women of vitamin B-6 was administered to the subjects. The diet was low in nitrogen so that the determination of protein ulitization would be more sensitive (Pellet & Young, 1980). To assess the effect of vitamin B-6 on the utlization of protein, 50 mg of pyridoxine hydrochloride (McKesson Laboratories, Fairfield, CT) were administered daily to the subjects on days 13 to 19.

TABLE II: Negligible Protein Diet Fed to the Subjects During First Two Days of the Study.

Menu	Amount	
	Men	Women
Breakfast :		
Orange juice, frozen reconstituted, g	240	240
Bread, low-protein enriched, g	45	45
Margarine, g	as needed	
Jelly or jam, g	if desired	
Cherry pie filling, canned, g	175	175
Non-dairy creamer, g	60	60
Coffee or tea	if desired	
Calcium carbonate, tablets[1]	2	2
Lunch:		
Soup:		
Potatoes, canned, g	35	35
Carrots, canned, g	35	35
Vegetable broth powder, tsp	1	1
Noodles, low-protein, g (before cooking)	43	43
Bread, low-protein, g	85	43
Pears, canned, drained, g	150	150
syrup, g	30	30
Coffee or tea	if desired	
Dinner:		
Apple juice, g	240	240
Spaghetti		
Spaghetti noodles, low-protein, g (before cooking)	170	170
Spaghetti sauce, g	240	120
corn oil, g	10	10
Green beans, canned, g	100	100
Juice, g	15	15
Peach halves, canned, g	200	200
syrup, g	30	30
Graham crackers, g	14	14

[1] Fred Meyer certified calcium antiacid tablets. Each tablet contains 500 mg of calcium carbonate, providing 200 mg of calcium. Distributed by Fred Meyer, Portland, OR 97242.

TABLE III: Experimental Diet Fed to the Subjects During the Seventeen Days of the Study.

Menu	Amount	
	Men	Women
Breakfast :		
Orange juice, frozen reconstituted, g	240	240
Shredded wheat, g	25	25
Bread, low-protein enriched, g	45	45
Margarine, g	as needed	
Jelly or jam, g	if desired	
Non-dairy creamer, g	60	60
Coffee or tea	if desired	
Calcium carbonate[1], tablets	2	2
Feosol[2], tablets	1	1
Pyridoxine chloride[3], tablets	1	1
Lunch:		
Peanut butter, g	20	20
Soup:		
Potatoes canned, g	35	35
Carrots canned, g	35	35
Vegetable broth powder, tsp	1	1
Salt, tsp	1/4	1/4
Pepper, dash	1	1
Noodles, low-protein, g (before cooking)	43	43
Bread, low-protein,g	85	85
Pears, canned, g	150	150
syrup, g	30	30
Calcium carbonate, tablets	2	2
Dinner:		
Apple juice, frozen reconstituted, g	240	240
Pinto beans, canned, g	95	95
Spaghetti noodles, low-protein, dry, g (before cooking)	90	45
Spaghetti sauce, canned, g	200	100
Bread, low-protein enriched, g	45	45
Green beans, canned, g	100	100
Peaches, canned, g	200	200
syrup, g	30	30
Graham crackers, g	14	14
Calcium carbonate, tablets	1	1

[1] Fred Meyer Certified Calcium Antacid Tablets. Each tablet contained 500 mg of Ca carbonate, providing 200 mg of Ca. Distributed by Fred Meyer, Portland, OR 97242.

[2] Smith Kline Corp., Philadelphia Pa. 19101. Each tablet contains 200 mg of dried ferrous sulfate USP 165 or 65 mg of elemental iron.

[3] McKesson Laboratories, McKesson & Robbins Drug Company, Fairfield, CN 06430. One tablet supplies 50 mg of pyridoxine HCl; one tablet was given with breakfast on days 13 to 19.

The experimental diet administered to the subjects on days 3 to 19 is given in Table III. Creamy peanut butter (20 g; Hoody Peanut Butter, Hoody, Inc., Beaverton, Oregon) supplied 0.86 g of nitrogen and 0.065 mg of vitamin B-6 and canned pinto beans (95 g, drained; Teasdale Pinto Beans, distributed by CMB Foods Inc.) supplied 1.02 g of nitrogen, and 0.50 mg of total vitamin B-6 daily. Low-protein bread mix (Kingsmill Foods Company Limited, Scarborough, Ontario, Canada), and imitation spaghetti and noodles (Gentil Aglutella, distributed by Ener-G Foods, Inc., Seattle, WA 84487) were used so that most of the nitrogen in the low-protein, plant-based, experimental diet would be supplied the peanut butter and pinto beans. These low-protein products were obtained from Ned-Diet Laboratories, Inc., Minnetonka, MN 55343. The margarine (Nucoa, Best Foods, CPC International Inc., Englewood, NJ 07632) that used in this investigation was not clarified since it contained no milk or any other source of nitrogen, except lecithin. All foods were purchases at one time and were from the same lot.

The calculated nutrient (Food Processor II, 1988) content of this diet (not including margarine and other protein and vitamin B-6 free foods, which were fed in variable amounts to maintain the subjects' weight) was 1429 Kcal, 30.2 g protein, 1555 RE vitamin A, 0.77 mg thiamin, 0.72 mg riboflavin, 12 mg niacin, 1.13 mg vitamin B-6,

380 mcg folate, 134 mg vitamin C, 294 mg calcium and 10.3 mg iron. This diet was supplement daily with 1000 mg of calcium and 65 mg of elemental iron in the form of tablets; the low-protein bread was prepared with the addition of thiamin, riboflavin, niacin and folate so that the subjects' recommended dietary allowances for these nutrients would be met. Instructions for the preparation of the enriched low-protein bread and other menu items on the experimental diet are given in the appendix.

During the preliminary and experimental periods (days 1-19), all meals were prepared by the investigator and served in the metabolic unit of Department of Nutrition and Food Management at Oregon State University. While participating in the study, the subjects were allowed to consume only the foods and beverages prepared for them or permitted. In addition to the experimental diet, subjects consumed margarine, sugar, jelly, jam, hard candies and sugar to maintain their initial body weight. Margarine consumed by each subject was weighed daily. Consumption of the remaining vitamin B-6- and nitrogen-free sources of fat and carbohydrate as well as tea and coffee were recorded daily by each subject on their daily activity sheet (given in appendix 1-3). During this 19-day period, the subjects weighed themselves each morning before breakfast and recorded their weight. They also kept a record of their physical activity and any unusual events (e.g., gave

seminar, had a headache, etc.). Starting on day 5, 45 g of additional non-protein bread and 5 tablespoons of vegetable oil were added to the experimental diet administered to M-2 and M-6 to prevent weight loss. Subject M-2 received an additional 10 teaspoons of vegetable oil. All subjects, starting by the third day of the study received 45 g of extra bread at dinner.

During the last 15 days of the study (d 20 to 34), the subjects ate their self-chosen diet. The purpose of this period was to determine the rate at which the body disposed of the supplementary vitamin B-6 which was given on days 13-19 during the preceding period. The subjects recorded their daily food intake and collected urine and gave (20 mL) blood at regular intervals. Results of this 15-day "wash-out" period will be reported elsewhere.

Food composites

Blended food composites of the preliminary and experimental diets were analyzed for nitrogen and total vitamin B-6 content. Some foods were analyzed separately: peanut butter and pinto beans, because they were the major sources of dietary nitrogen and vitamin B-6; and low-protein bread, spaghetti and spaghetti sauce, because consumption of these foods varied among the subjects.

Urine collections

Daily during the first 19 days of the study and at regular intervals during the 15-day "wash-out" period, the subjects collected their 24-hour urine specimens. Urine was collected under a layer of toluene in polyethylene bottles and was stored at 4° C. On the day following each 24-hour collection, the volume of each subject's specimen was measured, mixed and an aliquot was frozen at -22° C pending analysis for urea nitrogen, creatinine, nitrogen, 4-pyridoxic acid and total vitamin B-6.

Blood collections

Blood (20mL) was collected from fasting subjects before breakfast by a registered medical technologist on day 1 (preliminary period), d 10 and 12 (low-protein diet), d 17 and 19 (low-protein diet + pyridoxine) and d 22, 24, 29, and 34 ("wash-out" period). Blood was drawn from the antecubital vein into 10 mL evacuated tubes which contained heparin as an anticoagulant. After removing blood for the determination of hemoglobin and hematocrit, the blood was centrifuged at top speed for 20 minutes at 4°C. Plasma was removed from the cellular elements of the blood and aliquots were stored frozen at -22°C. for determination later of urea nitrogen, total vitamin B-6, and PLP.

Fecal collections

Subjects collected their feces in air-tight plastic containers which the subjects identified with their initials, date and time of defecation. A fecal marker of FDC Blue No 1 (50 mg of FDC Blue No 1 mixed with 200 mg of methylcellulose given in a gelatin capsule) was administered to the subjects with breakfast on days 3, 8, 13, and 19. Feces were stored frozen at -22° C. The feces were transferred from the plastic containers to weighed paint pails with the addition of the water that had been used to rinse the plastic collection containers. Specimens showing the marker up to the appearance of the next marker were pooled. Water, half the weight of the feces plus rinsings, was added to each composite. After closing the pails tightly, the feces were mixed thoroughly on a paint mixer for 15 minutes. A portion of each composite was analyzed for nitrogen and another one was stored frozen at -22° C.

Laboratory analyses

Total nitrogen

Total nitrogen of the experimental diets, urine and feces were analyzed by a boric acid modification of the Kjeldahl method (Scales & Harrison, 1929). The subjects' daily urine was analyzed for nitrogen. Daily fecal nitrogen excretion was estimated by dividing total nitrogen in the

composite by number of days over which feces had been collected. Nitrogen balance was calculated as the difference between daily total nitrogen excreted in urine and feces and total nitrogen intake. No corrections were made for endogenous, menstrual and dermal losses of nitrogen. Apparent protein digestibility was estimated by dividing the difference between nitrogen intake and fecal nitrogen excretion by nitrogen intake, and multiplying the dividend by 100.

Urea nitrogen

Plasma and urinary urea nitrogen were determined by using an automated procedure with a Technicon Autoanalyzer (Technicon, Inc., Ardsley, NY) (Henry, Cannon & Winkleman, 1974).

Urinary creatinine

Urinary creatinine was measured on a Technicon Auto Analyzer by an automated procedure utilizing the Jaffe reaction (Pino, Benotti & Gordyna, 1965).

Vitamin B-6

Total vitamin B-6 in plasma and urine was determined using Saccharomyces uvarum (ATCC 9080) as the assay organism by the method of Miller and Edwards (1981). Vitamin B-6 in

the food composites was determined by the AOAC method (1980).

Hemoglobin and hematocrit

Hemoglobin was determined after conversion to cyanomethemoglobin; and hematocrit, by a micro procedure. (Henry, 1968; Richterich, 1968).

Calculated nutrient analyses

Nutrient content of the preliminary and low-protein experimental diets as well as the subjects' self-chosen diets during the wash-out period was estimated by computer using the Food Processor II nutrient database and from information obtained from manufacturers.

Statistical analyses

For variables measured, means and standard deviation (SD) were computed. The paired t-test was used to test for any sinificant difference between variable means of the two experimental dietary periods (d8-12 and 15-19) ($p \leq 0.05$) (Steel & Torrie, 1960).

RESULTS

In general, the low-protein, plant-based diet was well accepted by the subjects. It did not cause them any major discomfort, except weight loss in subject M-2 and constipation in subject F-4. Except for subject F-4, the subjects' hemoglobin and hematocrit value were in the normal range for men and women. Subject F-4's values improved during the study.

Body weight and energy intake

The subjects' mean body weight and energy intake (calculated) during the 19-day study are presented in Table IV. Except for subject M-2, the subjects maintained their weight after the first week of the experiment. Subject M-2 found it difficult to tolerate the additional fat and carbohydrate required to maintain his initial weight.

Urinary urea nitrogen

The effect of pyridoxine supplementation on the mean excretion of urinary urea nitrogen (UUN) in subjects who received a low-protein, plant-based diet is presented in Table V. The day-to-day variability in the subjects' excretion of UUN is shown in Figure 4. Both Table V and

TABLE IV: Mean Body Weight[a] & Energy Intake of Subjects who were Receiving a Low-Protein Diet and PN as Supplement.

	Subjects							
	M-2		M-6		F-4		F-8	
	Weight	Energy	Weight	Energy	Weight	Energy	Weight	Energy
	Kg	Kcal	Kg	Kcal	Kg	Kcal	Kg	Kcal
Dietary period negligible protein (d 1-2)	76.3	1549	107.7	2210	68.1	1549	57.2	1634
low protein (d 3-7)	75.5±0.25	2201±117	107.7±0.47	2609±285	67.5±0.70	1812±117	56.8±0.21	1802±86
(d 8-12)	74.8±0.41	2606±188	106.2±0.18	3078±279	67.6±0.59	1888±68	56.9±0.26	2030±91
low protein + 50 mg PN HCl (d 13-19)	73.7±0.41	2744±253	106.0±0.22	3074±96	67.3±0.11	1860±63	56.7±0.28	2084±159

[a]: Mean ± SD

TABLE V: Effect of Pyridoxine (PN) Supplementation on Mean Urinary Urea Nitrogen Excretion (UUN) in mmol/d[a] in Subjects Fed a Low Protein Plant Based Diet.

	Subjects			
Dietary Period	M-2	M-6	F-4	F-8
Preliminary (Negligible protein) (d1-2)	205	224	215	186
Low protein				
d3-7	135 ± 44[a]	125 ± 27	119 ± 23	80 ± 13
d8-12	100 ± 16	76 ± 34	86 ± 18.5	78.2 ± 21
Low protein + 50 mg PN HCl				
d13-14	95	66	93	55
d15-19	98 ± 25	84 ± 60	86 ± 26	87 ± 26

[a] mean UUN ± SD

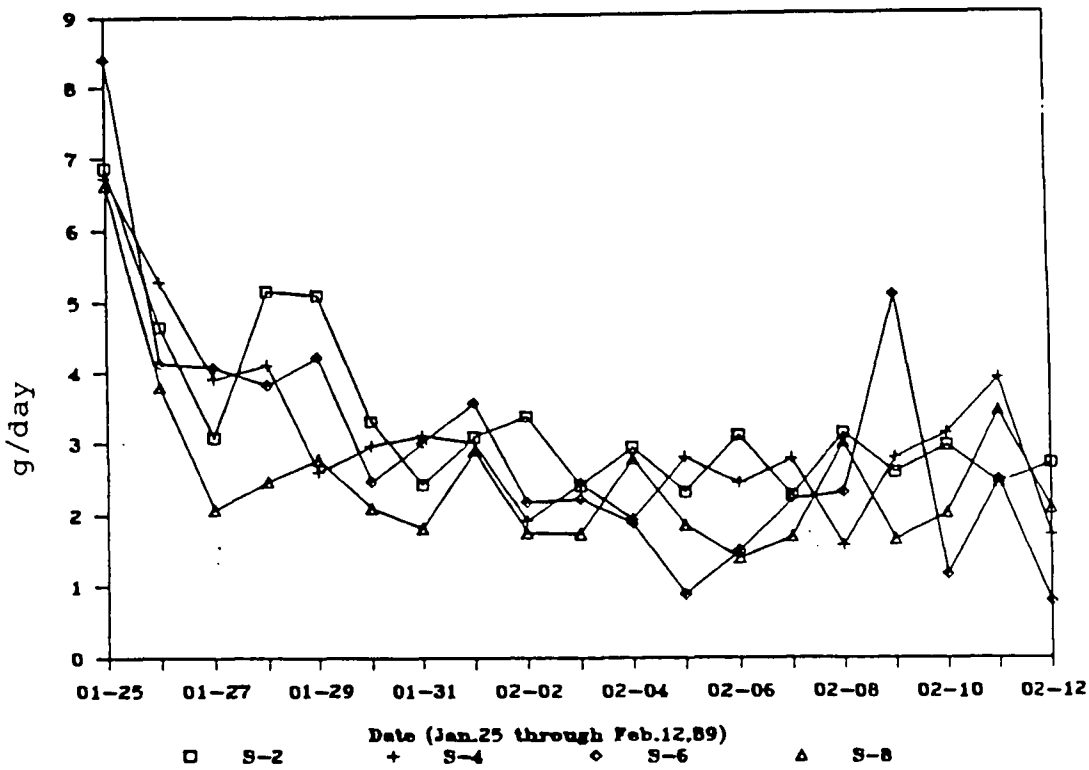

Figure 4. Daily urinary urea nitrogen (UUN) in g/d, excretion during the experimental dietary periods.

On 1/25 & 1/26 the subjects received a negligible protein diet. From 1/27 to 2/12 they received a low-protein, plant-based diet. This diet was supplemented with 50 mg PN HCl from 2/6 to 2/12.

Figure 4 show the drop in UUN excretion between days 1 and 7 as the subjects adapted to the low-protein experimental diet. Excretion of UUN leveled off thereafter and was not affected by the pyridoxine supplement administered daily on days 13 to 19. On the basis of the paired t test, comparing each subject's mean UUN excretion on d 8-12 and d 15-19, there was no statistically significant difference in UUN excretion. The elevated excretion of UUN by M-6 on d 16 (Fig. 4) was due to an irregularity in urine collection.

Plasma urea nitrogen

Table VI presents the results of plasma urea nitrogen (PUN) in blood that had been drawn on d 1 (preliminary period), d 10 and 13 (low-protein diet), and d 17 and 20 (low-protein diet + pyridoxine). Since UUN had stabilized by day 7 (Figure 4, Table V), and little change occurred during d 13 and 19, the mean of the data for each subject during the low-protein diet and during low-protein diet + pyridoxine periods were compared to determine the effect of pyridoxine supplement on PUN. Although the effect of pyridoxine was not statistically significant, it should be noted that PUN concentration in three of the four subjects increased when they received the pyridoxine supplement.

TABLE VI: Effect of PN Supplementation on Plasma Urea Nitrogen (PUN) (mmol/l) in Subjects Receiving a Low Protein Plant Based Diet.

	Subjects			
Dietary Period	M-2	M-6	F-4	F-8
Preliminary (d1-2) (negligible protein)				
d1	3.8	5.5	4.8	5.3
Low protein (d3-12)				
d10	1.6	1.8	1.6	3.6
d13	2.0	1.8	2.0	1.3
Low protein + PN HCl (d13-20)				
d17	2.4	2.0	2.2	1.9
d20	2.4	2.4	2.2	1.4

Nitrogen balance and apparent protein digestibility

The effect of pyridoxine supplementation on nitrogen balance and apparent protein digestibility is shown in Table VII. The nitrogen balance was calculated on the basis of daily nitrogen intake, urinary nitrogen excretion and fecal nitrogen of the last 5 days of the low-protein diet and last 4 days of the low protein diet supplemented with pyridoxine. Nitrogen balance was not reported for d 3-8, because the subjects' UUN excretion was not yet stable (Fig.4), indicating that the subjects had not yet adapted to the low protein diet. It was calculated for d 15-19 of the pyridoxine supplementation period to allow subjects two days to adapt to pyridoxine. All subjects were in negative nitrogen balance on the low protein diet, and the pyridoxine supplement had no effect on the nitrogen balance.

In general, apparent protein digestibility, as shown by the subjects' fecal excretion of nitrogen, increased during the period of low-protein diet + pyridoxine. Overall, protein digestibility was lower than expected. There were no statistically significant differences ($p>0.05$) between apparent protein digestibility on the low protein diet and the low-protein diet supplemented with pyridoxine, even though protein digestibility increased in each of the 4 subjects during the pyridoxine supplementation period.

TABLE VII: Effect of PN Supplementation on Nitrogen Balance (g/d) and Percent Protein Digestibility in Subjects Receving a Low Protein Plant Based Diet.

	Subjects							
	M-2		M-6		F-4	F-8		
Dietary Period	Low protein	Low protein +50mg PN HCl	Low protein	Low protein +50mg PN HCl	Low protein	Low protein +50mg PN HCl	Low protein	Low protein +50mg PN HCl
Vitamin B-6 intake (mg/d)	<1[a]	51[b]	<1	51	<1	51	<1	51
Nitrogen (g/d) Intake	4.56	4.60	4.56	4.56	4.15	4.15	4.15	4.15
Urinary N	3.93 ± 0.71[c]	4.04 ± 0.41[d]	3.77 ± 1.23	4.96 ± 1.33	3.59 ± 0.70	3.57 ± 0.70	2.79 ± 0.16	2.81 ± 0.54
Fecal N	(FN)1.69	1.61	2.29	2.02	1.14	0.73	1.85	1.64
Nitrogen balance[e]	-1.07	-1.06	-1.50	-2.42	-0.28	-0.17	-0.50	-0.30
Protein digesti-bility[f]	63	64	50	56	73	83	55	60

[a] Subjects receiving constant diet supplying 4.56 g N and 0.91 mg vitamin B-6 (males), and 4.15 g N and 0.76 mg vitamin B-6 (females), during days 3 to 12 of 19 days experimental period on low protein diet. Data collected from days 8 to 12.

[b] Subjects receiving same constant diet supplying 4.56 g N (males) and 4.15 g N (females) and 0.758 mg of vitamin B-6 supplemented by 50 mg PN on days 13 to 19. Data collected from days 15 to 19.

[c] Mean ± SD for days 8 to 12.

[d] Mean ± SD for days 15 to 19.

[e] N balance = N intake - (urine N + fecal N).

[f] Protein digestibility (%) = 100 x (N intake - Fecal N)/N intake

Percent of total urinary nitrogen excretion as urea nitrogen

The effect of a low-protein diet supplemented with pyridoxine on these two parameters is compared in Table VIII. In general, there was a decrease in excretion of the percentage of total N as urea N (UUN/UN x 100) in three of the four subjects when the pyridoxine supplement was administered. This difference was, however, not statistically significant.

Urinary creatinine excretion

The subjects' mean daily urinary creatinine excretion is shown in Table IX. Urinary excretion of creatinine decreased in subjects M-2 and F-2 during the 19-day experiment. Except for subject F-4, however, there was no decrease in mean urinary creatinine excretion in the other subjects during the last 5 days of the low protein diet period and the low protein diet supplemented by vitamin B-6. However, the difference between urinary creatinine excretion on the low protein diet and on the low protein diet supplemented with vitamin B-6 was not statistically significant ($p>0.05$).

TABLE VIII: Effect of PN Supplementation on Mean Total Urinary N (UN) Excretion (g/d); Mean Urinary Urea N (UUN) Excretion (g/d) and Percent UUN of UN Excretion in Subjects' Receiving Low Protein, Plant-Based Diet.

Subjects	Dietary period	UN	UUN	$\frac{UUN}{UN}$ x 100
M-2				
d8-12	Low protein[a]	3.93 ± 0.71[b]	2.81 ± 0.44	71
d15-19	Low protein + 50 mg PN HCl	4.04 ± 0.41	2.75 ± 0.28	68
M-6				
d8-12	Low protein	3.77 ± 1.23	2.13 ± 0.95	57
d15-19	Low protein + 50 mg PN HCl	4.96 ± 1.23	2.35 ± 1.67	47
F-4				
d8-12	Low protein	3.29 ± 0.62	2.41 ± 0.49	73
d15-19	Low protein + 50 mg PN HCl	3.57 ± 0.70	2.41 ± 0.72	67
F-8				
d8-12	Low protein diet	2.79 ± 0.16	2.19 ± 0.58	79
d15-19	Low protein + 50 mg PN HCl	2.81 ± 0.54	2.43 ± 0.74	87

[a] Subjects received constant diet supplying 4.56 g N (males) and 4.15 g N (females) and 0.907 mg vitamin B-6 (males) and 0.758 mg vitamin B-6 (females) during day 8 to 12 of experimental period.

[b] Mean ± SD.

[c] subject receiving constant diet supplying 4.56 g N (males) and 4.15 g N (females) and 0.907 mg vitamin B-6 (males) and 0.758 mg vitamin B-6 (females) supplemented by 50 mg PN on day 13 to 19 of experimental period

TABLE IX: Effect of PN Supplementation on Mean Daily Urinary Creatinine Excretion (mmol/d) IN Subjects Receiving Low-Protein Plant Based Diet.

	Subjects			
Dietary period	M-2	M-6	F-4	F-8
Preliminary (negligible protein) d 1-2	18.3	17.1	13.5	10.4
Low protein d 3-7	17.8±2.1[a]	19.3±2.2	12.6±1.2	10.3±1.3
d 8-12	17.1±2.1	17.6±3.0	11.3±2.0	9.6±1.7
Low protein d 10-14	17.6	17.7	11.2	11.0
Low protein + 50 mg PN HCl d 15-19	16.8±1.5	19.6±7.4	9.5±3.3	10.8±0.9

[a]Mean ± SD

DISCUSSION

The aim of this research was to investigate the effect of pyridoxine supplementation on the utilization of a low-protein plant-based diet by healthy young adults. The main source of dietary protein was pinto beans and peanut butter in a diet that supplied 4.56 g N/day for males and 4.15 g N/day for females. The diet was low in protein, so that the improvement of nitrogen balance (i.e., more N retained) could be seen easily (Pellet and Young, 1980) if pyridoxine improved protein utilization. The subjects' daily protein intakes in this investigation were below the Recommended Daily Allowance (NRC, 1980 and FAO/WHO/UNU, 1985) for adults. Also, this diet was low (Orr and Watt, 1957) in some essential amino acids. (Amino acid content of diet was compared to the requirements for amino acids for men and women as given in Pike and Brown, 1984).

Our research to ascertain whether or not the pyridoxine supplementation (50 mg) would improve protein utilization in humans was suggested by a number of animal studies. Sure and Easterling (1949) noted that pyridoxine improved food utilization and protein synthesis in rats. Sauberlich (1961) found that growth and efficiency of weight gain in rats were improved by increased levels of either pyridoxine (0.5 to 6 mg/kg diet) or the amino acids under the study, which was lacking in the diet. Adequate dietary pyridoxine,

through association with various enzymatic activities, will permit a greater portion of limiting amino acid to be incorporated into protein for growth and protect against catabolic losses.

In this present investigation in humans, pyridoxine supplementation may possibly have had a slight effect on some of the parameters we used to measure protein utilization. However, due to the small number of subjects and variability among subjects, these affects were not statistically significant. Additionally, the results of this investigation may have been affected by some cumulative errors inherent in nitrogen balance study technique (Kies, 1977; Pellet and Young, 1980).

Plasma and urinary urea nitrogen

Plasma and urinary urea nitrogen has been suggested as a means to evaluate protein utilization (Bodwell, 1977). After the addition of 50 mg pyridoxine HCL to the diet, the subjects' mean plasma urea nitrogen levels were slightly higher than before vitamin B-6 supplementation (Table VI). Indeed, three of the four subjects had an increased plasma urea nitrogen level after supplementation. Elevated plasma urea nitrogen levels may indicate that there is increased degradation of protein (Taylor, Scrimshaw and Young, 1974), suggesting that more protein was degraded when the subjects received the pyridoxine supplement than without it. Urinary

urea nitrogen excretion (Table V), on the other hand, appeared not to be affected by pyridoxine supplementation.

Additionally, except for subject M-6, the subjects' urinary urea nitrogen excretion was not related to their plasma urea nitrogen levels.

Relationship between body weight and energy intake with nitrogen balance

Because of the importance of adequate energy intake in nitrogen balance determination and protein requirements, a goal for this study was to maintain the subjects' body weight. With an increase of caloric intake after the experimental diet was initiated, each subject except M-2 maintained his or her weight (Table IV). Subject M-2 could not tolerate the additional dietary fat and carbohydrate which were needed to maintain his weight throughout the study. Furthermore, subject M-2 was more physically active than the other subjects under study, as shown in his daily activity sheets. Increase in physical activity requires adjustment of energy requirements (FAO/WHO/UNU, 1985). Inadequate energy intake in these conditions results in weight loss and negative nitrogen balance. Protein allowances suggested by FAO/WHO (1973) and the US National Research Council (NRC) (1974) are based on amounts of dietary protein needed to maintain nitrogen (N) balance in healthy adults in laboratory tests. Both allowances are valid only if the need for dietary energy is met. The NRC's

recommended dietary allowances (RDA) for energy is 2,700 Kcal/day or 38.6 Kcal/kg for the average American who engages in no heavy activity but does walk purposefully for 1 hour daily. The FAO/WHO report suggests an average need of 46 Kcal/kg for a moderately active man, and the allowance for the lightest activity level is 42 Kcal/kg. These figures are 10 to 20% higher than the RDA.

During the experimental periods (d 3-19), the daily kcal intake for subject M-2 varied from 2210 to 2744 kcal/day or 29.1 to 37.2 Kcal/kg (Table IV). His caloric intake was close to that of the RDA (1974) for energy for the average American (2,700 Kcal/day or 38.6 Kcal/kg), but his caloric intake was lower than that of the FAO/WHO (1973) for the average need for a moderately active man (46 Kcal/kg) for the lightest activity level (42 Kcal/Kg). In fact, M-2 had a high activity level (from his activity sheets)--more than one hour daily of activities such as walking, skiing, and skating, especially in the supplementary period. In addition, he was not only a student, but had a job at the same time. Thus, his energy intake was low compared to his daily activity. That explains his weight loss and probably the non-improvement for the energy balance during the supplementary period. The daily caloric intakes of M-6 (24.4 Kcal/Kg) and F-4 (26.7 to 27.6 Kcal/Kg) were lower than that of the RDA (1974) and FAO/WHO (1973), but these subjects did not lose weight: their daily activity

was low, and they probably adjusted to their caloric intake. Subject F-8 had a daily caloric intake of 1802 to 31.72 to 36.75 Kcal/Kg, and showed a small increase in her weight and a small improvement in N balance after supplementation.

Nitrogen balance and apparent protein digestibility

Pyridoxine had no effect on nitrogen balance (Table VII). Except for M-6, our data show a trend towards improvement of nitrogen balance with pyridoxine supplementation and/or further adaptation to the experimental diet. An explanation of subject M-6's higher nitrogen losses with PN supplementation than without is not readily available. His calorie intake during PN supplementation was adequate, and his weight was stable.

The subjects' high fecal nitrogen, in general, contributed to their negative nitrogen balance. We do not know exactly what increases fecal nitrogen loss, but we do know from the findings of Kies and Fox (1977) that increased fecal loss can contribute to negative nitrogen balance. We believe that the high fecal nitrogen loss is due in part to the fact that most of the subjects were unaccustomed to a plant protein diet (pinto beans and peanut butter). Subject F-4, for example, who had a relatively low fecal and urine excretion, was accustomed to a plant diet. A longer experimental period might have allowed the subjects to adjust better to the dietary protein source.

Apparent protein digestibility improved slightly in each subject during the period of pyridoxine supplementation (Table VII). The subjects' apparent protein digestibility ranged from 50 to 73% on the low-protein diet and 56 to 83% in the supplementary pyridoxine diet period (Table VII). Improvement in the apparent protein digestibility may be due also to adjustment to the diet. Hudson et al. (1989) found that protein digestibility and nitrogen balance were not affected by vitamin B-6 supplementation of less than 2 mg/day. The reported coefficient of digestibility of legumes and nuts (i.e. dried beans, peanut butter) is 78% (Watt & Merrill, 1963), and the apparent protein digestibility of our own subjects was lower than the 85% figure suggested by FAO/WHO (1975). Calloway and Kretsch (1978), who fed their subjects a Guatemalan diet high in plant protein, found that the apparent protein digestibility was also low, only 69%.

According to Kies (personal communication), apparent protein digestibility may not be a good estimate of protein digestibility when a low-protein diet is used. She suggested subtracting endogenous fecal nitrogen from total fecal nitrogen may result in digestibility values that are closer to values in the literature. Using 12 mg N/kg body weight to calculate endogenous fecal N loss (FAO/WHO/UNU, 1985), values for apparent protein digestibility are

obtained that are closer to values in the literature, from about 70 to 90%.

Percent of total urinary nitrogen excretion as urea nitrogen (UUN/UN x 100)

The data show a lower percentage of UNN/UN in three of the four subjects during the pyridoxine supplementation period (Table VIII), which suggests an improvement of protein utilization in those subjects. Calloway and Kretsch (1978), in their experiment with subjects on a Guatemalan diet, found that the subjects' percent of total urinary nitrogen as urea nitrogen was also around 75%. In conjunction with a low level of protein intake relative to need, a low percentage of urea nitrogen in relation to total nitrogen excretion is associated with improved efficiency of protein utilization.

Comments on analytical procedures to measure nitrogen

These results (Table VIII) which suggest improvement in protein utilization as a result of pyridoxine supplementation are in conflict with our results with plasma urea nitrogen (Table VI), which suggest increased protein catabolism during pyridoxine supplementation. We have no explanation for these inconsistent results. A problem in determining urinary nitrogen, urinary urea nitrogen, and plasma urea nitrogen was that we were measuring these analytes at very low concentrations, which could produce

bigger experimental errors. The concentration of plasma and urinary urea nitrogen in the subjects' specimens were often lower than the least concentrated standard. Low concentrations of nitrogen in urine and food by the Kjeldahl method were also difficult to measure. Some of the inconsistent results obtained in this research may be explained by these analytical problems.

SUMMARY AND CONCLUSIONS

The effect of pyridoxine supplementation on protein utilization of a low-protein, plant-based diet was investigated in four subjects (2 men and 2 women), aged 21 to 38 years. Following a two day adjustment period of a negligible protein diet, this 32 day study was divided into three dietary periods: subjects received a low-protein diet during period I (10 days), this same plant-based, low-protein diet + 50 mg pyridoxine HCl supplement during period II (7 days), and consumed their self-chosen diets during period III (15 days). Data obtained for period III will be reported later. The greatest portion of protein was furnished by pinto beans (1.02 g nitrogen) and peanut butter (0.86 g nitrogen); total nitrogen was kept constant at 4.56 g/day for the male subjects and 4.15 g/day for the female subjects during periods I and II. These diets of periods I and II provided 0.907 mg of Vitamin B-6 for males and 0.758 mg of Vitamin B-6 for females and was adequate in other nutrients.

Overall, the effect of 50 mg pyridoxine supplementation on a low-protein plant-based diet was not statistically significant ($p > 0.05$) on the basis of a paired t-test for all of the parameters that we measured. Furthermore, conflicting results were obtained. When the subjects received pyridoxine, their plasma urea nitrogen

concentrations increased slightly (suggesting increased protein degradation), while the percent of total urinary nitrogen excretion as urea nitrogen decreased (suggesting decreased protein degradation). Limitations in the nitrogen balance technique and the analytical procedures we used may have contributed to these discrepancies.

In future studies in which low-protein diets are fed to human subjects, sensitivity of methods used to measure nitrogen excretion and urea nitrogen in specimens should be adjusted for the low concentrations of these analytes. This can be achieved by making larger sample sizes or by using a lower dilution of sample for analysis.

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APPENDICES

APPENDIX 1-1

Recipe for low-protein enriched bread

'UNIMIX' LOAF
1 pouch (375g) 'UNIMIX'[1]
1 tsp. granulated sugar containing added vitamins[2]
1 3/4 cups warm water (105 - 110 F)
1 1/2 tsp. fast-acting dry yeast
1/2 tsp. salt

In a 3 quart bowl, dissolve sugar in warm water. Add yeast and stir well. Let stand 10 minutes, then add UNIMIX and salt and stir until 'UNIMIX' is just moistened. Beat mixture with: table top mixer--2 minutes at low speed or electric portable mixer--3 minutes at medium speed or large spoon--4 minutes. Dough should be light and sticky. Lightly grease a 9x5x3 inch bread pan. A smaller or larger loaf pan will result in an inferior loaf. Pour batter into prepared pan. Place pan in a cold oven and let rise to the edge of the pan--about 40-50 minutes. Turn oven temperature to 375 F with bread in the oven and bake for 45 minutes or until golden brown and it sounds hollow when the top is tapped with the knuckles. Remove bread from oven. Cool 5 minutes. Remove from pan immediately or the loaf will become soggy. Cool on wire rack. Serve plain or toasted.

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[1] Unimix baking mix, Kingsmill Food Company Ltd., Scarborough, Ontario Canada, obtained from Med Diet, Inc., Minnetonka, MN 55343.

[2] 1 tsp. sugar contained 2.4 mg of thiamin, 3.3 mg of riboflavin, 33 mg of niacin and 0.6 mg of folic acid. Each subject received approximately one-third loaf daily.

APPENDIX 1-2

To cook spaghetti or noodles

Before cooking, weigh _____ g of uncooked spaghetti or noodles, using the double beam balance, not the top-loading electronic one.

Total weight of spaghetti or noodles to cook = _____g
x _____ (number of subjects).

Heat to boiling, _____ cups of water. Add to water 1 tablespoon of vegetable oil and 1 tablespoon of salt.

Add weighed spaghetti to boiling water. When water again starts to boil, start counting time. Cook spaghetti or noodles for ____ minutes.

To weigh individual portions of spaghetti or noodles: use double beam balance and no electronic one for this.

weight of colander or pan empty _____ g
weight of colander or pan plus drained spaghetti or noodles _____ g
weight of spaghetti or noodles _____ g

weight of spaghetti or noodles/number of subjects = one portion of spaghetti.

APPENDIX 1-3

Daily activity record

Name_____ Date_____

Extra calories consumed:

Soft drinks, oz._____

Sugar, tsp._____

Hard candies, _____

Gum drops, _____

_____

Activity record: (Please estimate number of hours spent)

Sleeping_____

Sitting_____

Walking_____

Eating_____

Other--please describe_____

_____

How do you feel today?_____

Drugs or medication taken_____

Any unusual event_____

Turn in activity sheet each morning at breakfast. Thank you.