Bioavailability of pyridoxine in rats fed various levels of casein

Paula R. Trumbo

Department of Foods and Nutrition, Purdue University, West Lafayette, IN, USA

[^{14}C] pyridoxine (PN) was orally administered to male rats and lactating dams fed an 8%, 20%, or 50% casein diet. There was no effect of the level of dietary protein on plasma pyridoxal 5'-phosphate (PLP) concentration. Analysis of fecal radioactivity indicated no dietary effect on the intestinal absorption of [^{14}C]PN. There was greater retention of ^{14}C in the liver when male and lactating rats were fed increased amounts of protein. Increased dietary protein resulted in reduced levels of ^{14}C in the brain of the male rats. There was no dietary effect on the urinary excretion of total ^{14}C or [^{14}C]4-pyridoxic acid. Increasing the level of dietary protein resulted in increased retention of ^{14}C in the liver of the lactating dams, however, the concentration of ^{14}C and the ^{14}C:protein ratio in milk was not affected. Although not significant, the level of ^{14}C in the liver of the suckling pups was two to three times greater when dams were fed 8% protein than when dams were fed 20% or 50% protein. These results demonstrate that altered tissue distribution of vitamin B-6 occurs with varied protein intake without influencing the metabolic utilization and urinary excretion of vitamin B-6 or plasma PLP concentration, the primary assessment parameter for vitamin B-6 status. Furthermore, increased intake of protein by the lactating rat does not influence the concentration vitamin B-6 or the vitamin B-6:protein ratio in milk.

Keywords: vitamin B-6; pyridoxine; bioavailability; lactation; rat

Introduction

Amino acid metabolism requires pyridoxal 5'-phosphate (PLP)-dependent enzymatic reactions, occurring predominantly in the liver. A high protein diet has been shown to aggravate a vitamin B-6 deficiency based on growth and xanthurenic acid excretion following tryptophan administration.[1,2] Itoh and Okada[3] reported increased liver vitamin B-6 levels and increased excretion of 4-pyridoxic acid (4-PA) in the urine when rats were fed a 70% casein diet compared with rats fed a 10% casein diet. The vitamin B-6 status of the rat, as indicated by plasma PLP concentration, was shown to decrease with increased dietary protein when these diets contained inadequate levels of vitamin B-6.[4] Increasing dietary protein (0.5–2.0 g/kg body weight) has been shown to reduce plasma PLP levels and reduce the percentage of oral vitamin B-6 excreted as 4-PA in men.[5] Based on such studies as those mentioned above, there is general agreement that increased dietary protein increases the retention of vitamin B-6 in the liver and possibly affects vitamin B-6 status. Because increased dietary protein appears to increase the requirement for vitamin B-6, the Recommended Dietary Allowance of a vitamin B-6:protein ratio of 0.016 mg/g has been established.[6]

There have been no studies conducted to investigate the effect of liver retention of vitamin B-6 on its availability to other vitamin B-6–requiring organs, as well as to directly assess the metabolic utilization of dietary vitamin B-6 in response to increased dietary protein. Furthermore, there have been no studies conducted to assess the effect of protein intake during pregnancy and lactation on vitamin B-6 hepatic retention and secretion in milk. In this study, the bioavailability and tissue distribution of orally administered [^{14}C]pyridoxine (PN) HCl was assessed in male rats fed varying levels of casein. In addition, lactating dams fed varying levels of dietary casein during gestation and 10 days postpartum were gavaged [^{14}C]PN HCl for assessment of vitamin B-6 content in milk and availability to the suckling pup.

Supported in part by Showalter Research Award. Purdue University Agricultural Experiment Station Journal Series No. 13171.
Address reprint requests to Dr. Paula R. Trumbo, at the Foods and Nutrition Department, Purdue University, West Lafayette, IN 47907 USA.
Received November 8, 1991; accepted February 4, 1992.

Materials and methods

Reagents

Pyridoxal 5-phosphate, 1-octanesulfonic acid, and oxytocin were purchased from Sigma Chemical Co. (St. Louis, MO USA). Water was purified for chromatographic use with a Nanopure II system (Barnstead Co., Newton, MA, USA). [4,5-^{14}C]PN-hydrochloride (91 μCi/mg) was a gift of Hoffman-LaRoche (Nutley, NJ, USA). 2-Propanol (HPLC grade) and phosphoric acid were purchased from Fisher Scientific Co. (Pittsburgh, PA USA). Animal diets were prepared by and purchased from ICN Biochemicals (Costa Mesa, CA USA). Scintillation fluid (Budget-Solve) was purchased from Research Products International Corp. (Mt. Prospect, IL USA).

Animals and diets

For experiment 1, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN USA), weighing 40–60 g, were housed in stainless-steel metabolism cages and fed, ad libitum, a semipurified diet containing 8%, 20%, or 50% casein and 1.5 mg pyridoxine HCl/kg diet *(Table 1)* for 3 weeks. After this period, all rats were fasted for 8 hours and gavaged [^{14}C]PN HCl (2.6 μCi in 150 μL H_2O). A 48-hour fecal collection was conducted following gavage of the isotope. In addition, 48-hours after gavage, blood was collected into heparinized tubes and tissues collected following decapitation of some of the rats. For the remaining rats, 24-hour urine samples were collected for 5 days after gavage of the isotope.

For experiment 2, one-day pregnant, Sprague-Dawley rats (Harlan Sprague-Dawley), weighing 190–230 g, were housed in stainless-steel maternity cages and fed an 8%, 20%, or 50% casein diet, as described for experiment 1 *(Table 1)*, during gestation and 10 days postpartum. Five days postpartum, litters were adjusted to four pups. Ten days postpartum, all rats were gavaged [^{14}C]PN HCl (5.1 μCi in 300 μL H_2O). Twenty-four hours after gavage, all lactating rats were injected (i.p.) with oxytocin (5.0 IU/rat) followed by collection of milk by manual expression. Blood from dams and pups was collected into heparinized tubes and liver was collected following decapitation. Approval for the use of male and pregnant rats in this study was given by the Purdue Animal Care and Use Committee.

Table 1 Nutrient composition of experimental diets

Nutrient	8% Protein	20% Protein	50% Protein
	% weight		
Casein[a]	9.1	22.0	57.4
Cornstarch	15.0	15.0	15.0
Sucrose	60.1	48.1	18.1
Corn oil	4.9	4.8	4.4
Cellulose[b]	6.7	5.0	1.0
Mineral mix[c]	4.0	4.0	4.0
Vitamin mix[d]	1.0	1.0	1.0

[a]Purified high nitrogen casein.
[b]Alphacel.
[c]AIN-76 mineral mix.
[d]Vitamin diet fortification mixture.

Sample preparation

Plasma was obtained after centrifugation of blood. An equal volume of 15% trichloroacetic acid (TCA) was added to plasma. Immediately following centrifugation and precipitation of protein, the supernatant was extracted with an equal volume of ethyl ether for removal of TCA.

Twenty-four hour urine volumes were recorded for each day. For HPLC preparation, urine was deproteinated by ultrafiltration with micropartition tubes and YMT membrane filters (Amicon, Danvers, MA USA). Fecal and tissue samples were homogenized in water. Total homogenate volumes were recorded.

HPLC equipment and method

Chromatographic analyses were conducted with a Rainin HP/HPX Drive Module (Rainin Instrument Co., Woburn, MA, USA), sample injection valve (Rheodyne, model 7125), a fluorescence detector (Model LS 40, Perkin Elmer, Norwalk, CT USA), a Dynamax HPLC Method Manager Integration Software Program (Rainin Instrument Co.) and MacIntosh SE computer (Apple Computer, Inc., Cupertino, CA USA).

Plasma PLP was measured by an isocratic HPLC method with modification.[7] The mobile phase, composed of 0.033 mol/L potassium phosphate, pH 2.2, was employed with a Partisil 10 octadecyl-3 column (Whatman, Clifton, NJ USA). The wavelengths used for fluorometric detection were 295 nm for excitation and 405 nm for emission.

Urine 4-PA was isolated and collected by HPLC using a mobile phase composed of 0.033 mol/L phosphoric acid, pH 2.2 and a Partisil 10 octadecyl-3 column (Whatman).[8] The wavelengths for fluorometric detection were 365 nm for excitation and 405 nm for emission.

Measurement of radioactivity and milk protein

Fecal and tissue homogenates and 4-PA HPLC fractions were measured for radioactivity in scintillation fluid using a liquid scintillation spectrophotometer (Beckman LS 1800, Beckman Instruments, Palo Alto, CA USA). Samples were adjusted for quenching by use of a ^{14}C quench curve. Radioactivity of samples was expressed as percentage of oral dose, nmol/L milk, or pmol/g protein based on the specific radioactivity of the isotope. Protein concentration in milk was determined by the method of Douglass et al.[9] using casein as a standard.

Statistical analysis

Mean values were analyzed by one-way analysis of variance with significant ($P < 0.05$) differences between group means determined by Student-Newman-Keuls multiple pairwise comparisons.

Results

Food intake, weight gain, and plasma PLP concentrations

The level of dietary protein did not affect food intake for either the male or female rats *(Table 2)*. Food consumption by the dams during pregnancy and lactation was approximately two times greater than for the male rats. Increasing the level of protein in the diet increased the growth rate for the male rats. Growth rate was not affected by level of protein in the diet for the pregnant rats. Plasma PLP was measured for as-

Table 2 Food intake, weight gain, and plasma pyridoxal phosphate levels for rats, fed the 8, 20, and 50% protein diets*

	Dietary protein		
	8%	20%	50%
Food intake (g/d)			
Male (exp. 1)	9.4 ± 0.5	9.3 ± 0.1	9.2 ± 0.1
Dam (exp. 2)	20.5 ± 1.5	20.0 ± 1.1	20.0 ± 1.9
Weight gain (g/d)			
Male (exp. 1)	1.6 ± 0.2[a]	3.6 ± 0.1[b]	4.3 ± 0.04[c]
Dam (exp. 2)	6.2 ± 0.4	7.5 ± 0.5	6.5 ± 0.4
Plasma PLP (nmol/L)			
Male (exp. 1)	127 ± 20.7	99 ± 10.1	100 ± 6.5
Dam (exp. 2)	444 ± 26.4	318 ± 37.4	489 ± 55.4
Pup (exp. 2)	238 ± 65.1	249 ± 51.4	186 ± 55.8

*Values represent means ± SEM of 4–6 rats.
Values within each row followed by a different superscript were significantly different ($P < 0.05$).

Table 3 Isotopic distribution in tissues and feces from male rats fed 8, 20, and 50% protein diets*,†

	Dietary protein		
	8%	20%	50%
	Percent oral dose		
Feces	2.29 ± 0.22[a]	1.35 ± 0.21[b]	1.07 ± 0.19[b]
Liver	8.37 ± 0.40[a]	12.10 ± 0.48[b]	17.80 ± 0.54[c]
Muscle	7.57 ± 0.46[a]	9.50 ± 0.60[ab]	9.68 ± 0.57[b]
Kidney	0.98 ± 0.09[a]	1.38 ± 0.14[ab]	1.54 ± 0.13[b]
Brain	0.73 ± 0.08[a]	0.55 ± 0.02[b]	0.48 ± 0.01[b]
Heart	0.35 ± 0.13[a]	0.36 ± 0.02[a]	0.39 ± 0.03[a]
Lung	0.17 ± 0.01[a]	0.19 ± 0.01[a]	0.17 ± 0.01[a]
Spleen	0.24 ± 0.03[a]	0.27 ± 0.04[a]	0.21 ± 0.02[a]
Testes	0.46 ± 0.03[a]	0.46 ± 0.01[a]	0.43 ± 0.01[a]

*Values represent means ± SEM of 4–6 rats.
†Values within each row followed by the same superscript were not significantly different ($P > 0.05$).

sessment of vitamin B-6 status. Plasma PLP values were greater for the dams compared with the male rats. The level of dietary protein fed to the male rats or dams did not influence the plasma PLP levels of the male rats, dams, or pups.

Distribution of radioactivity in feces and tissues

Approximately 97% or greater of the oral dose was absorbed as indicated by one to three percent of the radioactivity measured in the 48-hour post-gavage fecal samples *(Table 3)*. Increased dietary protein slightly, but significantly reduced the fecal excretion of the oral dose.

Because previous studies have demonstrated increased liver vitamin B-6 levels with increased dietary protein, it was of interest to study the effect of the level of dietary protein on the distribution and retention of orally administered [^{14}C]PN HCl among various tissues *(Table 3)*. The liver, being the major organ responsible for the metabolism of vitamin B-6, contained the largest percentage of radioactivity that was orally administered. The percentage of oral dose significantly increased in the liver from 8–18% as the level of dietary protein was increased from 8–50%, respectively. A similar trend was found for the muscle and kidney, however, the dietary effect was not as pronounced. The only organ that demonstrated a reduction in the percentage of oral dose with increasing dietary protein was the brain. The percentage of oral dose measured in the brain was reduced by 34% when dietary protein was increased from 8–50%. The oral dose measured in the heart, lung, spleen, and testes was not influenced by the level of dietary protein.

Excretion of radioactivity in urine

As seen in *Figure 1*, 35–42% of the oral dose was excreted in the urine within 1 day after gavage of the isotope. By day 5, 50–55% of the oral dose was excreted. Greater, however nonsignificant, excretion of radioactivity during this time period was observed for

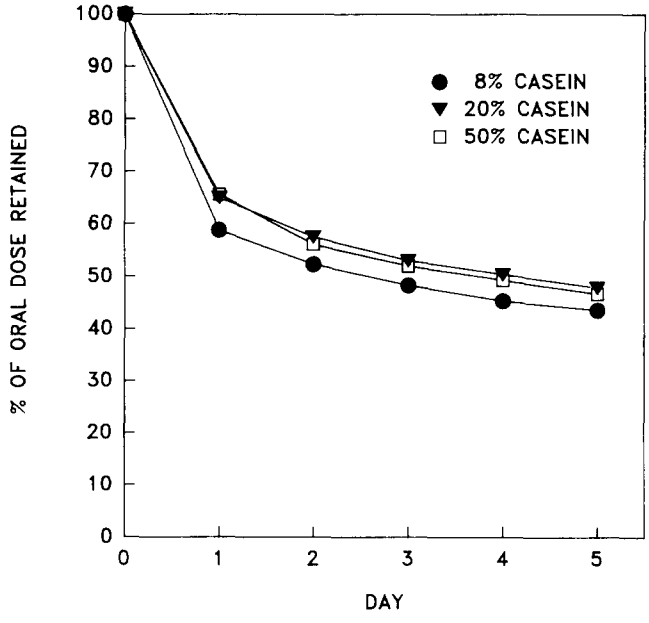

Figure 1 Percentage of oral dose excreted in urine of male rats. Each value is an average of three to seven rats.

rats fed the 8% protein diet compared with rats fed the 20% or 50% protein diet. There was no dietary effect on the excretion of radioactivity for days 2–5. Furthermore, there was no significant difference in the percentage of total radioactivity in the urine that was excreted as 4-pyridoxic acid (10.6 ± 3.0, 6.6 ± 0.9, 11.9 ± 1.2) when rats were fed 8%, 20%, or 50% casein, respectively.

Radioactivity in milk and in dams' and pups' livers

There was no dietary effect on the concentration of radioactivity that was present in the milk samples *(Table 4)*. Furthermore, the level of dietary protein consumed by the dams did not influence the [^{14}C]vitamin

Table 4 Radioactivity in milk and liver when dams were fed 8, 20, and 50% protein diets*,†

	Dietary protein		
	8%	20%	50%
Milk			
pmol/mL	0.77 ± 0.13[a]	0.72 ± 0.06[a]	0.63 ± 0.08[a]
pmol/g protein	8.54 ± 0.71[a]	8.52 ± 0.57[a]	9.25 ± 1.32[a]
Liver, % oral dose			
Dam	7.95 ± 0.78[a]	8.64 ± 0.32[a]	10.60 ± 0.74[b]
Pup	0.95 ± 0.45[a]	0.32 ± 0.05[a]	0.50 ± 0.15[a]

*Values represent means ± SEM of 4–6 rats.
†Values within each row followed by the same superscript were not significantly different ($P > 0.05$).

B-6:protein ratio in milk. There was an increase in the percentage of oral dose present in the liver of the dams when fed increased dietary protein, which was similar to that observed for the male rats, although not as marked. Although not significant, the level of radioactivity in the pup's liver was 2–3 times greater when dams were fed 8% protein compared with pups of dams fed the 20% and 50% protein diets.

Discussion

Vitamin B-6 status, as assessed by plasma PLP concentration, has been reported to be reduced with increased dietary protein when rats were fed diets containing inadequate levels of PN.[4] In this study, rats were fed varied protein diets, marginally adequate in vitamin B-6 (1.5 mg pyridoxine/kg diet), to further investigate the effect of dietary protein on vitamin B-6 status and metabolism. During pregnancy, an increased dietary intake of 40% has been observed.[10] Sloger and Reynolds,[11] however, reported similar food consumption levels for pregnant and non-pregnant rats and a significant increase in food consumption during lactation. In the present study, food consumption was greater during pregnancy and lactation and may explain, in part, the prevention of reduced growth rate for the dams fed the low protein diet, as well as the increased plasma PLP concentration, when compared with the male rats *(Table 2)*. Regardless of the level of protein intake, the plasma PLP concentration was adequate for the male rats and dams.

It was not surprising that the oral dose of [^{14}C]PN HCl was well absorbed *(Table 3)* because Henderson et al.[12] demonstrated that the absorption of PN is greater than 95% and that PN is absorbed by a nonsaturable process. Furthermore, the level of dietary protein would not be expected to affect the intestinal absorption of PN because PN does not have an aldehyde moiety to react with proteins or amino acids.

Increasing the level of dietary protein markedly increased the percent of oral dose retained in the liver *(Table 3)*, which is in agreement with Itoh and Okada.[3] An increase in the activity of PLP-dependent enzymes during increased protein intake has been suggested as a result of changes in amino acid metabolism in the liver.[13] Elevated PLP-dependent transamination was shown to be significantly stimulated as the level of dietary protein was increased to 60%.[14] An increase in radioactivity in muscle, with increased dietary protein (reduced dietary sucrose), was unexpected because it has been suggested that increased glucose consumption increases muscle vitamin B-6 concentration for carbohydrate metabolism.[15] Itoh and Okada[1] observed a two-fold increase in muscle vitamin B-6 when rats were fed a 10% casein diet compared with a 70% casein diet.

The only tissue measured that showed a reduction (34%) in radioactivity with increased dietary protein was the brain. Shane[16] reported that the radioactivity in brain of rats, intraperitoneally injected with [^{14}C]PN, increased with time up to 48 hours after injection and decreased thereafter suggesting that the reduced radioactivity found in the brain of rats fed increased dietary protein *(Table 3)* might be a result of reduced uptake of vitamin B-6. PLP has great biochemical and physiological importance in the brain because many of the neurotransmitters in the brain, including serotonin, norepinephrine, dopamine, γ-aminobutyric acid, and taurine require PLP for their biosynthesis.[17] Although the consequence of long-term ingestion of a high protein diet on brain neurotransmitter levels has not been conducted, no difference was observed in brain norepinephrine, dopamine, or serotonin concentration when rats were fed diets containing 5–75% casein for 11 days.[18]

There was a nonsignificant decrease in excretion of radioactivity in the urine when rats were fed the 20% and 50% casein diets compared with rats fed the 8% casein diet *(Figure 1)*. Reduced urinary excretion in response to increased dietary protein, however, did not compensate for the increased retention of radioactivity in the liver. 4-PA is the major metabolic endproduct of vitamin B-6 metabolism, formed irreversibly in the liver, and therefore is an indicator of vitamin B-6 metabolic utilization. Although there was increased retention of vitamin B-6 in the liver with increased dietary protein, the proportion of vitamin B-6 in the urine that was excreted as 4-PA was not affected. Itoh and Okada[3] reported increased 4-PA excretion in urine when rats were fed diets containing increased levels of protein. In contrast, Miller et al.[5] reported decreased excretion of vitamin B-6, and specifically 4-PA, in the urine when humans were fed increased levels of protein and suggested that PLP was bound to enzymes for amino acid catabolism and therefore less PLP was available for conversion to 4-PA.

The availabililty of vitamin B-6 to the fetus and infant is required for DNA synthesis and the formation of cerebrosides, necessary for myelination of the central nervous system. Abnormal behavior in the young of vitamin B-6–deficient rats has been reported,[19] as well as biochemical and morphological changes in the central nervous system. A vitamin B-6 deficiency in the infant is characterized by epileptiform seizures and irritability.[20] Pups of dams fed a diet marginally deficient in vitamin B-6 throughout gestation and lactation

have been reported to exhibit deficiency symptoms at 10–12 days of age.[21] Because increased retention of vitamin B-6 in the liver was observed when the male rats were fed increased dietary protein, it was of interest to investigate the effect of dietary protein on the secretion of vitamin B-6 in milk, and therefore availability to the suckling pup. Increasing the level of protein in the diet increased the level of radioactivity present in the liver of the lactating rats *(Table 3)*, however, the increase was not as significant as that found for the male rats. Increasing the level of protein in the diet from 11–21% during lactation was shown to significantly increase mammary and liver protein synthesis.[22] Such a finding would suggest reduced amino acid catabolism during pregnancy and would explain, in part, the lack of difference in hepatic radioactivity between dams fed the 8% and 20% protein diet in this study. A significant increase in the level of hepatic radioactivity in dams fed the 50% protein diet would suggest increased amino acid catabolism in the liver.

The concentration of [^{14}C]vitamin B-6 in milk was not affected by increased retention of the isotope in the liver of dams fed 50% protein. Furthermore, the [^{14}C]vitamin B-6:protein ratio in milk was not altered by the dam's protein intake. The level of radioactivity present in the pup's liver was not significantly different among the three dietary groups, however, the level of hepatic radioactivity was two to three times greater for pups when dams were fed the 8% protein diet than when they were fed the 20% or 50% protein diets. Variability in the level of hepatic radioactivity for pups from the 8% protein group contributed to the lack of statistical difference. This variability may be a result of differences in milk consumption because the level of radioactivity in milk was not different among the three groups. In general, most observations have demonstrated that the level of dietary protein in the maternal diet can affect milk production and milk yield without influencing the protein content of milk.[23]

In conclusion, results of this study would suggest that increased protein intake by the male rat results in altered tissue distribution of vitamin B-6 without influencing vitamin B-6 metabolic utilization, urinary excretion, and status. In addition, the level of maternal protein intake does not influence vitamin B-6 status of the lactating dam or the vitamin B-6 concentration and vitamin B-6:protein ratio in milk.

Unlike the rat, human plasma PLP values have been shown to decrease with increased levels of dietary protein.[5] In this human study,[5] pyridoxine was the major source of vitamin B-6, and no difference was observed in the excretion of fecal vitamin B-6 in response to dietary protein. Therefore, reduced plasma PLP concentration in response to increased dietary protein in humans is not solely a result of the consumption of less bioavailable forms of vitamin B-6. Pyridoxal and PLP are the major forms of vitamin B-6 in milk and red meat, respectively. These aldehyde forms of vitamin B-6 have been reported to react with proteins during food processing,[24] resulting in reduced molar activity of the vitamin.[25] Such an occurrence may partially influence the increased requirement for vitamin B-6 that occurs with increased dietary protein in humans.

References

1 Miller, E.C. and Bauman, C.A. (1945). Relative effects of casein and tryptophane on the health and xanthurenic acid excretion of pyridoxine deficient mice. *J. Biol. Chem.* **157,** 551–562

2 Okada, M. and Ochi, A. (1971). The effect of dietary protein level of transaminase activities and fat deposition in vitamin B-6 depleted rat liver. *J. Biochem.* **70,** 581–585

3 Itoh, R. and Okada, M. (1973). Effect of dietary protein level on pyridoxal content in tissues and excretion of pyridoxic acid into urine in normal or pyridoxine-deficient rat. *J. Nutr. Sci. Vitaminol.* **19,** 523–528

4 Nguyen, L.B. and Gregory, J.F. (1983). Effects of food composition on the bioavailability of vitamin B-6 in the rat. *J. Nutr.* **113,** 1550–1560

5 Miller, L.T., Leklem, J.E., and Schultz, T.D. (1985). The effect of dietary protein on the metabolism of vitamin B-6 in humans. *J. Nutr.* **115,** 1663–1672

6 National Research Council (1989). Water Soluble Vitamins. In *Recommended Dietary Allowances*, p. 142–150, National Academy Press, Washington, DC USA

7 Trumbo, P.R. and Gregory, J.F. (1988). Metabolic utilization of pyridoxine-β-glucoside: Influence of vitamin B-6 status and route of administration. *J. Nutr.* **118,** 1336–1342

8 Trumbo, P.R., Gregory, J.F., and Sartain, D.B. (1988). Incomplete utilization of pyridoxine-β-glucoside as vitamin B-6 in the rat. *J. Nutr.* **118,** 170–175

9 Douglass, F.W., Greenberg, R., Farrell, H.M., and Edmondson, L.F. (1981). Effect of ultra-high-temperature pasteurization on milk proteins. *J. Agr. Food Chem.* **29,** 11–15

10 Van Den Berg, H. and Bogaards, J.J.P. (1987). Vitamin B-6 metabolism in the pregnant rat: Effect of progesterone on the (re)distribution in maternal vitamin B-6 stores. *J. Nutr.* **117,** 1866–1874

11 Sloger, M.S. and Reynolds, R.D. (1980). Effects of pregnancy and lactation on pyridoxal-5-phosphate in plasma, blood and liver of rats fed three levels of vitamin B-6. *J. Nutr.* **110,** 1517–1524

12 Henderson, L.M. (1985). Intestinal absorption of B-6 vitamers. In *Vitamin B-6: Its Role in Health and Disease*, (R.D. Reynolds and J.E. Leklem, eds.), p. 25–33, Alan R. Liss, Inc. New York, NY USA

13 Bukin, Y.U. (1976). Activity of pyridoxal kinase and content of pyridoxal phosphate in mammalian tissues under regional and experimental conditions. *Chem. Abst.* **84,** 29

14 Everett, G.B., Mitchell, A.D., and Benevenga, N.J. (1979). Methionine transamination and catabolism in vitamin B-6 deficient rats. *J. Nutr.* **109,** 597–605

15 Leklem, J.E. and Hollenbeck, C.B. (1990). Acute ingestion of glucose decreases plasma pyridoxal 5′-phosphate and total vitamin B-6 concentration. *Am. J. Clin. Nutr.* **51,** 832–836

16 Shane, B. (1982). Vitamin B-6 metabolism and turnover in the ethanol-fed rat. *J. Nutr.* **112,** 610–618

17 Dakshinamurti, K. and Paulose, C.S. (1988). Central nervous system origin of hypertension in the vitamin B-6 deficient adult. In *Clinical and Physiological Applications of Vitamin B-6*, (R.D. Reynold and J.E. Leklem, eds.), p. 159–175, Alan R. Liss, Inc., New York, NY USA

18 Harper, A.E. and Peters, J.C. (1989). Protein intake, brain amino acid and serotonin concentrations and protein self-selection. *J. Nutr.* **119,** 677–689

19 Aycock, J.E. and Kirksey, A. (1976). Influence of different levels of dietary pyridoxine on certain parameters of developing and mature brains in rats. *J. Nutr.* **104,** 111

20 Coursin, D.B. (1964). Vitamin B-6 metabolism in infants and children. *Vitamins Hormones* **22,** 755

21 Kirksey, A., Pang, R.L., and Lin, W. (1975). Effects of different levels of pyridoxine fed during pregnancy superimposed upon growth in the rat. *J. Nutr.* **105,** 607–615

22 Sampson, D.A., Hunsaker, H.A., and Jansen, G.R. (1986). Dietary protein quality, protein quantity and food intake: Effects on lactation and on protein synthesis and tissue composition in mammary tissue and liver in rats. *J. Nutr.* **116,** 365–375

23 Casey, C.E. and Hambidge, K.M. (1983). Nutritional aspects of human lactation. In *Lactation*, (M.C. Neville and M.R. Neifert, eds.), p. 199–266, Plenum Press, New York, NY USA

24 Gregory, J.F. and Kirk, J.R. (1977). Interaction of pyridoxal and pyridoxal phosphate with peptides in a model food system during thermal processing. *J. Food Sci.* **42,** 1554–1558

25 Gregory, J.F. (1980). Effects of pyridoxyllysine bound to dietary protein on the vitamin B-6 status of rats. *J. Nutr.* **110,** 995–1005