BUTTERWORTH HEINEMANN

Activities of hepatic vitamin B_6 metabolizing enzymes and concentrations of vitamin B_6 vitamers in tissues of chronically azotemic rats

Ien-Lan Wei and Tze-Kong Young*

*Laboratory of Nutrition, Faculty of Nursing and *Institute of Physiology, National Yang-Ming University, Taipei, Taiwan*

The activities of hepatic vitamin B_6 metabolizing enzymes and the concentrations of vitamin B_6 vitamers in plasma and liver were studied in uremic rats. The uremic group of rats was prepared by a two-stage surgical procedure. Food intake of the control and uremic groups was not significantly different. The loss of renal function of the uremic rats was evident by the significantly decreased creatinine clearance value as well as the elevated serum urea levels. Concentrations of each liver B_6 vitamer and plasma total vitamin B_6 were not significantly different between the control and uremic groups. Plasma pyridoxal 5'-phosphate concentrations were 31% ($P < 0.05$) lower; however, pyridoxal levels were 39% ($P < 0.05$) greater in the uremic group than in the control group. Activities of hepatic pyridoxal kinase and pyridoxamine (pyridoxine) 5' -phosphate oxidase were similar for both groups, whereas the hepatic pyridoxal 5' -phosphate phosphatase activities were significantly lower for the uremic rats. Experimental results indicated that uremic animals could synthesize pyridoxal 5' -phosphate normally. The reduction in plasma pyridoxal 5' -phosphate of uremic animals was not caused by decreased synthesis or increased degradation of pyridoxal 5' -phosphate in the liver. Increased hydrolysis of plasma pyridoxal 5' -phosphate was responsible for the significant reduction in plasma pyridoxal 5' -phosphate in the uremic rats. The normal concentration of plasma total vitamin B_6 in the uremic rats resulted from the significantly elevated plasma pyridoxal levels. Based on the results of this study, plasma total vitamin B_6 would apparently not be an appropriate biochemical index for assessing vitamin B_6 status in chronic renal disease. (J. Nutr. Biochem. 6: 494–498, 1995.)

Keywords: uremia; vitamin B_6 metabolizing enzymes; pyridoxal phosphate; pyridoxal; rats

Introduction

Six biologically active forms of vitamin B_6 are pyridoxine (PN), pyridoxamine (PM), pyridoxal (PL), pyridoxine 5'-phosphate (PNP), pyridoxamine 5' -phosphate (PMP), and pyridoxal 5' -phosphate (PLP). PLP acts as a coenzyme, especially in protein metabolism.[1] Studies[2–6] have demonstrated that chronic renal-failure patients had significantly lower plasma PLP concentrations. In contrast to these reports, other investigators found that chronic renal-failure patients had normal concentrations of plasma total vitamin B_6 and were not deficient in vitamin B_6.[7–10] In our previous animals study,[11] chronic renal failure rats had significantly lower plasma PLP; however, these rats had normal levels of plasma total vitamin B_6. The principal forms of vitamin B_6 vitamers in the plasma are PLP and PL.[12,13] No difference in plasma total vitamin B_6 but significantly decreased plasma PLP in uremic animals strongly indicates that the distribution of vitamin B_6 vitamers may be deranged in chronic renal disease.

Liver is the major organ in vitamin B_6 metabolism.[14] Vitamin B_6 in food is absorbed in the forms of PL, PN, or

Address reprint requests to Ien-Lan Wei, Ph.D., R.D. Laboratory of Nutrition, Faculty of Nursing, National Yang-Ming University, Taipei 112, Taiwan.
Received October 27, 1994; revised March 15, 1995; accepted April 18, 1995.

Nutritional Biochemistry 6:494–498, 1995

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(95)00079-F

PM. These nonphosphorylated forms of vitamin B_6 are transported into the liver and phosphorylated by pyridoxal kinase (PL kinase; EC 2.7.1.35) to their respective phosphorylated compounds. The PNP and PMP are oxidized to PLP by pyridoxamine (pyridoxine) 5′ -phosphate oxidase [PMP (PNP) oxidase; EC 1.4.3.5]. When PLP is synthesized beyond the binding capacity of cellular protein, it is dephosphorylated by pyridoxal 5′ -phosphate phosphatase (PLP phosphatase). Lumeng et al.[15] demonstrated that liver is the chief organ that supplies plasma PLP. Accordingly, studies of the activities of hepatic enzymes involved in vitamin B_6 metabolism could provide us with a better understanding of the mechanisms by which plasma PLP was reduced in the chronically uremic condition.

This study was carried out in experimentally induced uremic rats. Activities of hepatic enzymes-PL kinase, PMP (PNP) oxidase, and PLP phosphatase were determined. The concentrations of liver and plasma vitamin B_6 vitamers were also measured.

Materials and Methods

Sprague-Dawley male rats (Experimental Animal Center of National Yang-Ming University, Taipei, Taiwan), weighing 150 to 200 g, were randomly divided into two groups. The uremic group of rats was prepared by a two-stage surgical procedure.[16] Two thirds of the left kidney were removed, following 1 week later by contralateral nephrectomy. The control group of rats was sham-operated by laparotomy. Animals were individually housed in stainless steel cages in a room maintained at a constant temperature (23°C) and humidity (50%), with alternating 12 hr periods of light and dark. Rats of both groups were allowed free access to water and AIN-76A diet (ICN Biochemicals, Cleveland, OH USA).[17] The animal care procedures followed the established guidelines of the Animal Care and Use Committees of the National Science Council, Taiwan. The food intake of the animals was daily recorded. The body weights of the rats were measured once a week.

Nine weeks after the operation, rats were placed in individual metabolic cages to collect 24-hr urine specimens for the determination of creatinine. The animals were deprived of food at 1700 hr on the day before death. Animals were killed by decapitation between 0900 and 1100 hr. Blood was collected in tubes with or without anticoagulant-ethylenediaminetetraacetate. The serum was used for measurements of urea and creatinine. Plasma was stored at −30°C for analyses of vitamin B_6 vitamers. Liver was quickly excised and weighed. For each gram of liver, ice-cold sucrose (0.25 mol/L, 9 mL) was added, and the tissue was homogenized with a tissue homogenizer (polytron PT3000, 9,000 rpm for 30 sec). The homogenates were centrifuged at 4°C for 30 min at 18,000*g*. The supernatant was stored in aliquots at −80°C for later determination of PL kinase and PMP (PNP) oxidase activities. The pellets were resuspended in sucrose solution (0.25 mol/L, 4 mL) and rehomogenized on the polytron. The pellet fractions were divided into aliquots and stored at −80°C for subsequent determination of PLP phosphatase. The activities of PL kinase, PMP (PNP) oxidase, and PLP phosphatase were determined at pH 7.4 according to the procedure of Ubbink and Schnell.[18] Vitamin B_6 vitamers were determined by HPLC. Preparations of the samples for HPLC determinations of B_6 vitamers were based on the previous method of Furth-Walker et al.[19]

The HPLC system consisted of a solvent delivery system (Waters model 501, Millipore, Milford, MA, USA), a universal LC injector (Waters U6K), a data module (Waters 745 B), a scanning fluorescence detector (Waters 470) with a 5-μL flow cell with excitation wavelength set at 325 nm and emission wavelength at 400 nm, and an infusion pump (Harvard Apparatus, Southnatick, MA, USA) for delivery of the postcolumn reagent. A reverse-phase analytic column (Waters 10-μm particle size, C18 μBondapak, 3.9 × 300 mm) was used. The chromatographic conditions followed the method of Edwards et al.[20] except that the flow rate was 1 mL/min. A postcolumn reagent (250 mmol/L Na_2HPO_4, to which 1 mg/mL of sodium metabisulfite was added just before use) was introduced to the column effluent through a mixing tee at a flow rate of 0.09 mL/min. The exact concentrations of the vitamin B_6 stock solutions were determined spectrophotometrically from the molar absorbances of the B_6 compounds.[21] Due to the sensitivity of vitamin B_6 to light, all procedures for the determination of B_6 vitamers were conducted in a room illuminated by yellow fluorescent lights.

Serum urea was measured with a BUN analyzer 2 (Beckman, Brea, CA, USA). Creatinine in serum and urine samples was measured with a creatinine analyzer 2 (Beckman). The protein content of the liver was determined according to the method of Lowry et al.[22]

Data were analyzed by means of the SPSS/PC+ statistics computer program (SPSS/PC+ V.4.0, SPSS Inc., Chicago, IL, USA). Food intake of the animals was analyzed by repeated measurements. All other parameters were evaluated by Student's *t* test, and $P < 0.05$ was considered statistically significant.

Results

Food intake, body weight, and kidney function

Food intake of the uremic rats was not significantly different from that of the control animals (*Figure 1*). However, their weight gains were less during the 9-week study period than those of the controls. The initial body weights of the control and uremic rats were similar. At the end of the study, uremic animals weighed significantly less than control rats (*Table 1*). The kidney function of the rats was evaluated by serum urea concentrations and creatinine clearance values. The uremic group had significantly greater serum urea concentrations than the control group. The creatinine clearance value was less in the uremic group than that in the control group (*Table 1*).

Plasma and liver vitamin B_6 vitamers

The plasma total vitamin B_6 (PLP + PL) concentrations were not significantly different between the control and ure-

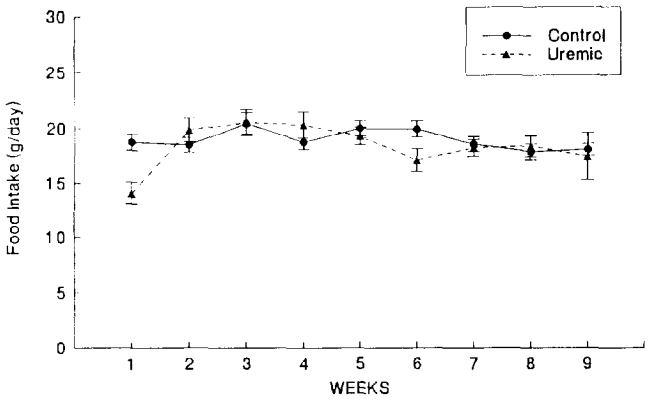

Figure 1 Food intake of control and uremic rats. The average food intake of control and uremic rats was not significantly different. Each point represents the average food intake (mean ± SE) of 12 rats in the control and 9 rats in the uremic groups.

Table 1 Body weight, serum urea, and creatinine clearance of control and uremic rats*†

Parameter	Control	Uremic
Body weight (g)		
initial	171.1 ± 3.0	169.6 ± 4.8
final	423.8 ± 10.0[a]	363.6 ± 16.1[b]
Serum urea (mmol/L)	1.7 ± 0.2[b]	13.7 ± 3.5[a]
Creatinine clearance (μL/sec/100 g of body weight)	8.19 ± 0.45[a]	3.27 ± 0.56[b]

*The results are mean ± SE of 12 rats in control and 9 rats in uremic groups.
†Values in the same row with different superscript differ significantly ($P < 0.05$).

mic rats (*Table 2*). The concentrations of plasma PLP of uremic rats were 31% lower ($P < 0.05$) than those of controls. In contrast to the PLP, the plasma PL concentrations in uremic animals were increased 39% ($P < 0.05$) over the control levels. Concentrations of each liver B_6 vitamer from both groups were similar (*Figure 2*).

Activities of hepatic enzymes of vitamin B_6 metabolism

Hepatic enzyme activities of vitamin B_6 metabolism are presented in *Table 3*. The activities of PL kinase and PMP (PNP) oxidase were not statistically different between the control and uremic groups. The PLP phosphatase activities were found to be approximately 25% lower ($P < 0.05$) in uremic rats.

Discussion

Some of the abnormalities, e.g., central nervous system depression, convulsions, changes in plasma amino acids, increased oxalate production, and depression of immune responses observed in chronic renal failure patients, are similar to those of vitamin B_6 deficiency.[6] Furthermore, the renal failure patients had significantly lower plasma PLP concentrations. Hence, patients with chronic renal failure are believed to be efficient in vitamin B_6.[2–6] When blood levels of total vitamin B_6 have been measured, however, these patients had normal values of plasma total vitamin B_6

Table 2 Plasma pyridoxal phosphate (PLP), pyridoxal (PL), and total vitamin B6 (PLP + PL) concentrations of control and uremic rats*†‡

Vitamer	Control (nmol/L)	Uremic (nmol/L)
PLP	596.1 ± 44.4[a]	412.1 ± 22.6[b]
PL	343.6 ± 24.0[b]	477.8 ± 53.0[a]
PLP + PL	939.7 ± 60.9	889.9 ± 64.6

*The results are mean ± SE of 12 rats in control and 9 rats in uremic groups.
†Values in the same row with different superscript differ significantly ($P < 0.05$).
‡Abbreviation used: PL, pyridoxal; PLP, pyridoxal 5′-phosphate.

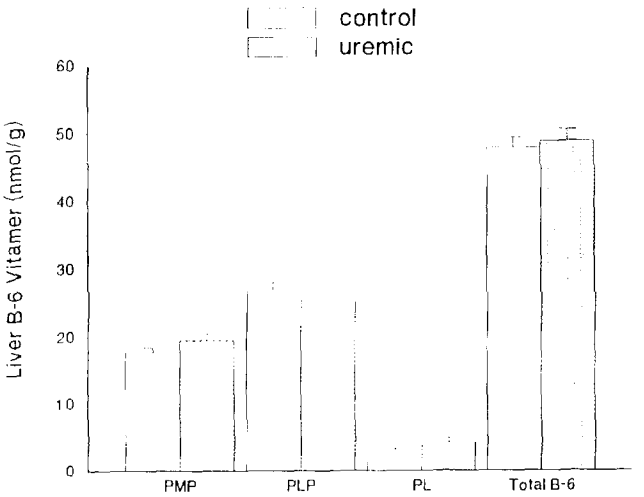

Figure 2 Concentrations of vitamin B_6 vitamers in liver of control and uremic rats. PMP, pyridoxamine phosphate; PLP, pyridoxal 5′-phosphate; PL, pyridoxal. Results are mean ± SE of 12 rats in control and 9 rats in uremic groups. Concentrations of the each B_6 vitamer and total vitamin B_6 in liver of control and uremic groups were not significantly different.

and were reported not to be deficient in vitamin B_6.[7–10] Studies have shown that plasma PLP and total vitamin B_6 concentrations respond to changes in dietary vitamin B_6 intake.[12] These controversial findings regarding vitamin B_6 status in chronic renal failure patients reported by the previous studies mentioned previously could be caused by differences in dietary vitamin B_6 intake or alteration in vitamin B_6 metabolism. The animal model of uremic rats was recently used in our previous study to investigate the metabolism of vitamin B_6 in chronic renal disease, indicating that uremic animals had significantly lower plasma PLP concentrations; however, their plasma total vitamin B_6 concentrations were not different from the controls.[11] The food intake of the uremic rats was similar to that of controls. These findings indicated that vitamin B_6 metabolism was deranged in chronic renal failure. The mechanisms by which plasma PLP was lowered were investigated in this study.

The kidney function of the rats in the uremic group in the present study was impaired as shown by a significantly

Table 3 Activities of hepatic vitamin B6 metabolizing enzymes in control and uremic rats*†‡

Enzyme	Control	Uremic
PL kinase (nmol/hr × mg of protein)	11.39 ± 0.86	12.34 ± 0.90
PMP (PNP) oxidase (nmol/hr × mg of protein)	3.64 ± 0.41	3.55 ± 0.22
PLP phosphatase (nmol/hr × mg of protein)	66.17 ± 3.51[a]	49.91 ± 3.78[b]

*The results are mean ± SE of 12 rats in the control and 9 rats in uremic
†Values in the same row with different superscript differ significantly ($P < 0.05$)
‡Abbreviation used: PL, pyridoxal; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; PNP, pyridoxine 5′-phosphate.

greater serum urea concentration and a 60% reduction in creatinine clearance value. The body weight gains of the uremic animals were significantly less than those of the controls. However, the food intake of the uremic rats was not different from the control rats. This indicated that the reduced body weight gain of the uremic animals was not related to food intake and was possibly caused by the metabolic effects of the loss of renal function as well as the alteration in vitamin B_6 metabolism. The uremic rats had significantly lower plasma PLP concentrations; however, their plasma total vitamin B_6 concentrations were not different from those of the controls. These results are consistent with the findings of our previous investigation.[11] The reduction in plasma PLP in uremic animals was not related to vitamin B_6 intake.

Liver is the primary source of plasma PLP and the major organ of vitamin B_6 metabolism.[15] The activities of hepatic PL kinase and PMP (PNP) oxidase of uremic rats in this study were not significantly different from those of the controls, indicating that chronic renal failure rats synthesized PLP normally. Previous studies[3,4] have indicated that administration of vitamin B_6 to chronic renal-failure patients normalized plasma PLP levels. Our present findings confirmed those previous results of normal activities of PLP synthesizing enzymes in chronic renal disease.

The most intriguing findings of this study was that the PLP phosphatase activity was significantly reduced in the uremic animals. The mechanism responsible for the decreased activity of hepatic PLP phosphatase cannot be determined from the present results. We postulate that the decreased activity of hepatic PLP phosphatase may be a physiological attempt to normalize the plasma PLP levels in the uremic rats. According to Lui et al.[23] the newly synthesized PLP in the liver is more accessible to the PLP degradation enzyme and the transport systems for secretion of PLP to circulation. The hepatic PLP phosphatase, a PLP degradation enzyme, hydrolyzes the phosphorylated forms of vitamin B_6 to their respective nonphosphorylated compounds which are further oxidized to the inactive metabolite 4-pyridoxic acid.[14] Decreasing the activity of hepatic PLP phosphatase in the uremic rats would reduce the amount of PLP being hydrolyzed in the liver. Consequently, the amount of PLP accessible to the transport systems would increase. Generally, if the activities of PLP synthesizing enzymes in the liver are not altered and the activity of hepatic PLP degradation enzyme is lowered, the concentrations of PLP in the liver would likely be elevated. However, the concentrations of PMP and PLP in the liver of uremic rats of this study were comparable to those of the control rats. These results indicated that the release of PLP from liver to circulation was not affected in uremic animals.

The dynamic equilibrium of plasma PLP depends upon hepatic synthesis, tissue extraction, and degradation.[15] The significantly lowered plasma PLP in the uremic rats of the present study was not related to the synthesis, degradation, or release of PLP by the liver. Based on the observed results of this study, increased hydrolysis of plasma PLP in circulation was responsible for the significantly decreased concentration of plasma PLP in the uremic rats. An altered distribution of plasma PLP and PL was observed in uremic rats of this study. The plasma PLP and PL of control rats comprised approximately 63 and 37% of the plasma total vitamin B_6, respectively. However, the plasma PLP of the uremic rats accounted for approximately 47% whereas PL accounted for 53% of the total vitamin B_6 in plasma. The reduction in plasma PLP ($P < 0.05$) was accompanied by an elevation in plasma PL ($P < 0.05$) levels in the uremic rats, indicating that the hydrolysis of plasma PLP to PL was increased in these animals. Increased clearance of plasma PLP has been observed in a human study as well. Spannuth et al.[5] administered PLP intravenously to control subjects and uremic patients and determined the plasma PLP concentrations serially. The plasma clearance of the administered PLP was significantly greater for the uremic patients than for the controls. The increased clearance of plasma PLP in the uremic patients was attributed to the presence of an inhibitory factor by Spannuth et al.[5] These investigators observed that the recovery of PLP added directly to uremic plasma was significantly lower than recovery from the normal plasma. Serum alkaline phosphatase has been suggested to cause increased hydrolysis of plasma PLP in certain conditions.[24,25] However, Spannuth et al.[5] observed no relationship between serum alkaline phosphatase activity and plasma PLP concentrations in chronic renal failure patients. A previous animal study[11] also demonstrated that serum alkaline phosphatase was not involved in lowering plasma PLP of chronic renal failure animals. Toxic chemicals and amines in the circulation of uremic patients were speculated to cause increased hydrolysis of plasma PLP.[6] This possibility has not yet been studied.

Increased hydrolysis of plasma PLP in the uremic animals reduced the supply of the coenzyme to peripheral tissues. Although the capability of synthesizing PLP in the liver was not impaired in the uremic animals, a gradual decrease in vitamin B_6 status would occur if the dietary intake of the vitamin B_6 was not sufficiently high to surpass the hydrolysis rate of plasma PLP. Accordingly, supplementation of vitamin B_6 appears to be essential in chronic renal disease.

In summary, uremic rats had normal activities of hepatic PL kinase and PMP (PNP) oxidase and that their hepatic PLP phosphatase activities were low. The reduction in plasma PLP of uremic animals was not caused by decreased synthesis or increased degradation of PLP in the liver. Increased hydrolysis of plasma PLP was responsible for the reduction in plasma PLP levels. The normal concentration of plasma total vitamin B_6 observed in the uremic animals was caused by the changes in the distribution of plasma PLP and PL. Therefore, the application of the plasma total vitamin B_6 to assess vitamin B_6 nutritional status was inappropriate in the chronic renal disease.

Acknowledgments

This work was supported by the National Science Council of Taiwan under grant number NSC81-0412-B010-33.

References

1 Merrill, A.H., Jr. and Burnham, F.S. (1990). Vitamin B-6 In *Present knowledge in Nutrition* (M.L. Brown, ed.), pp. 155–162,

International Life Sciences Institute, Nutrition Foundation, Washington, DC USA
2 Gressner, A.M. and Sittel, D. (1985). Plasma pyridoxal 5' -phosphate concentrations in relation to apoaminotransferase levels in normal, uremic, and post-myocardial infarct sera. *J. Clin. Chem. Clin. Biochem.* **23,** 631–636
3 Lacour, B., Parry, C., Drueke, T., Touam, M., Kreis, H., Bailly, M., and Durand, D. (1983). Pyridoxal 5'-phosphate deficiency in uremic undialyzed, hemodialyzed, and non-uremic kidney transplant patients. *Clin. Chim. Acta* **127,** 205–215
4 Teehan, B.P., Smith, L.J., Sigler, M.H., Gilgore, G.S., and Schleifer, C.R. (1978). Plasma pyridoxal-5' -phosphate levels and clinical correlations in chronic hemodialysis patients. *Am. J. Clin. Nutr.* **31,** 1932–1936
5 Spannuth, C.L. Jr., Warnock, L.G., Wagner, C., and Stone, W.J. (1977). Increased plasma clearance of pyridoxal 5' -phosphate in vitamin B-6 deficient uremic man. *J. Lab. Clin. Med.* **90,** 632–637
6 Stone, W.J., Warnock, L.G., and Wagner, C. (1975). Vitamin B-6 deficiency in uremia. *Am. J. Clin. Nutr.* **28,** 950–957
7 Marumo, F., Kamata, K., and Okubo, M. (1986). Deranged concentrations of water-soluble vitamins in the blood of undialyzed and dialyzed patients with chronic renal failure. *Intern. J. Artif. Organs* **9,** 17–24
8 Ramirez, G., Chen, M., Worth Boyce, Jr., H., Fuller, S.M., Butcher, D.E., Brueggemeyer, C.D., and Newton, J.L. (1986). The plasma and red cell vitamin B levels of chronic hemodialysis patients: A longitudinal study. *Nephron* **42,** 41–46
9 Debari, V.A., Frank, O., Baker, H., and Needle, M.A. (1984). Water soluble vitamins in granculocytes, erythrocytes, and plasma obtained from chronic hemodialysis patients. *Am. J. Clin. Nutr.* **39,** 410–415
10 Heaf, J.G. (1982). Liver function tests and pyridoxine levels in uremia. *Nephron* **30,** 131–136
11 Wei, I.-L. and Young, T.-K. (1994). Vitamin B-6 metabolism is altered in chronic renal failure rats. Nutr. Res. **14,** 271–278
12 Leklem, J.E. (1990). Vitamin B-6: A status report. *J. Nutr.* **120** 1503–1507
13 Lumeng, L., Lui, A. and Li., T.-K. (1980). Plasma Content of B-6 vitamers and its relationship to hepatic vitamin B-6 metabolism. *Am. J. Clin. Invest.* **66,** 688–695
14 Ink, S.L. and Henderson, L.M. (1984). Vitamin B-6 metabolism *Ann. Rev. Nutr.* **4,** 455–470
15 Lumeng, L., Brashear, R.E., and Li., T.-K. (1974). Pyridoxal 5'-phosphate in plasma: source, protein-binding, and cellular transport. *J. Lab. Clin. Med.* **85,** 334–343
16 Sterner, G. (1979). Experimental chronic renal failure in rats. *Nephron* **24,** 207–208
17 American Institute of Nutrition (1980). Second report of the ad hoc committee on standards for nutritional studies. *J. Nutr.* **110,** 1726
18 Ubbink, J.B. and Schnell, A.M. (1988). High-Performance liquid chromatographic assay of erythrocyte enzyme activity levels involved in vitamin B-6 metabolism. *J. Chromatogr.* **431,** 406–412
19 Furth-Walker, D., Leibman, D., and Smolen, A. (1989). Changes in pyridoxal phosphate and pyridoxamine phosphate in the blood, liver and brain in the pregnant mouse. *J. Nutr.* **119,** 750–756
20 Edwards, P., Liu, P.K.S., and Rose, G.A. (1989). A simple liquid chromatographic method for measuring vitamin B-6 compounds in plasma. *Clin. Chem.* **35,** 241–245
21 Peterson, E.A. and Sober, H.A. (1954). Preparation of crystalline phosphorylated derivatives of vitamin B-6. *J. Am. Chem. Soc.* **76,** 169–175
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
23 Lui, A., Lumeng, L., and Li, T.-K. (1981). Metabolism of vitamin B-6 in rat liver mitochondria. *J. Biol. Chem.* **256,** 6041–6046
24 Anderson, B.B. O'Brien, H., Griffin, G.E., and Mollin, D.L. (1980). Hydrolysis of pyridoxal 5'-phosphate in plasma in conditions with raised alkaline phosphatase. *Gut* **21,** 192–194
25 Barnard, H.C., deKock, J.J., Vermaak, W.J.H., and Potgiete, G.M. (1987). A new perspective in assessment of vitamin B-6 nutritional status during pregnancy in humans. *J. Nutr.* **117,** 1303–1306