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Pyridoxal Phosphate Concentrations Determined Postmortem as an Indication of Antemortem Vitamin B-6 Status (42480)

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Abstract. In anticipation of studies relating vitamin B-6 status determined at autopsy to known pathologic causes of death, the postmortem stability of pyridoxal phosphate (PLP) in the plasma, skeletal muscle, and liver of pigs was assessed. Concentrations of plasma K, Mg, Na, and Ca also were examined for postmortem stability using the pig as an experimental model. At 1 hr after death, the mean plasma PLP concentration was essentially unchanged from that observed prior to death. Thereafter, plasma PLP concentration increased with increasing postmortem time interval and was 2.3 times greater than initial by 6 hr postmortem and 7.6 times greater than initial by 12 hr postmortem ($P < 0.01$). Skeletal muscle and liver PLP content were 35% lower than initial by 6 hr postmortem ($P < 0.01$). Plasma K and Mg were significantly higher by 1 hr postmortem ($P < 0.01$) while plasma Na concentration was significantly lower by 1 hr postmortem ($P < 0.01$). Plasma Ca concentration was not significantly different at any measured time point. Knowledge of the postmortem time interval appears to be required in order to evaluate the antemortem vitamin B-6 status using pyridoxal phosphate values derived from autopsy samples.

Vitamin B-6 deficiency has been implicated in the etiology of several diseases (1) including heart disease (2, 3) and cancer (4). In addition, a pharmacologic effect of this vitamin has recently been reported for the partial management of patients with sickle cell anemia and asthma who possess an apparent vitamin B-6 deficiency or an altered metabolism of the vitamin (5, 6). In view of the emerging relationship between various illnesses and vitamin B-6 metabolism and status, it may be useful to determine the vitamin B-6 nutritional status of individuals at autopsy and relate these findings to known illnesses or pathologic causes of death. In anticipation of such investigations with humans, we used the pig as an animal model to study the postmortem stability of pyridoxal phosphate in plasma, muscle, and the liver.

Materials and Methods. Antemortem blood samples were obtained with a heparinized 5-ml syringe and a 16-gauge needle from the superior vena cava of pigs weighing 10–13 kg. The blood was centrifuged at 740*g* for 15 min and the resulting plasma stored at −20°C until analyzed for pyridoxal phosphate (PLP), sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) concentrations.

Preliminary experimentation indicated that the process of postmortem blood sampling caused a more rapid onset of *in situ* postmortem blood clotting than would normally occur in an undisturbed animal. Therefore, Na heparin was injected into the superior vena cava (2800 USP K units/kg body wt) immediately before death to prevent the premature onset of postmortem blood clotting. Although the administration of Na heparin prior to death is a departure from normally occurring conditions at time of death of humans, it is important to note that plasma and serum PLP concentrations determined from antemortem samples are equivalent (unpublished observations). In addition, the onset of postmortem blood clotting may occur either rapidly or many hours after death, depending on the circumstances of death and environmental conditions at which the body is maintained (7, 8). Thus, either plasma or serum samples may be obtained at autopsy. The use of unclotted blood in the present investigation greatly fa-

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cilitated the collection of multiple blood samples several hours apart from the same animal (sampling procedure described below).

Following Na heparin administration, the pigs were asphyxiated with CO_2 and approximately 200 mg each of muscle from the right hind quarter and liver tissue were obtained and are considered antemortem samples. Muscle and liver samples were homogenized with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) in 100 vol (w:v) ice-cold 0.2 *M* sodium phosphate buffer (pH 7.4) and stored at −20°C until analyzed for PLP and protein content.

Following death, the pigs were maintained in a room at 25°C, humidity 45%. Postmortem blood samples were obtained by opening the chest wall and pericardial sac; then blood was removed with a 2-ml syringe and 18-gauge needle at 1, 6, and 12 hr postmortem from the superior vena cava, inferior vena cava, right atrium, and aorta. To prevent leakage of heparinized blood from the above blood sampling sites, the needles were left in place and a new syringe attached to them until the next blood sampling. Between blood samplings, saline-dampened cheesecloth was placed over the exposed abdominal and chest cavity and covered with plastic wrap to prevent dessication of internal tissues. Postmortem muscle and liver tissues were obtained at 6 and 12 hr after death. All postmortem samples were processed and stored as described above for the antemortem samples.

Plasma, muscle, and liver PLP were determined using a modified (9) L-tyrosine apodecarboxylase enzymatic assay of Chabner and Livingston (10) utilizing L-[1-^{14}C]tyrosine and partially purified tyrosine apodecarboxylase isolated from *Streptococcus faecalis* (11). Protein contents of muscle and liver were determined by the method of Lowry *et al.* (12). Plasma Ca and Mg concentrations were determined using flame atomic absorption spectrometry with plasma samples diluted in 0.5% lanthanum. Plasma Na and K concentrations were determined using flame atomic emission spectrometry with the plasma diluted in deionized H_2O. The Student Newman–Kuels multiple-range comparison test (13) was utilized to determine differences between the means.

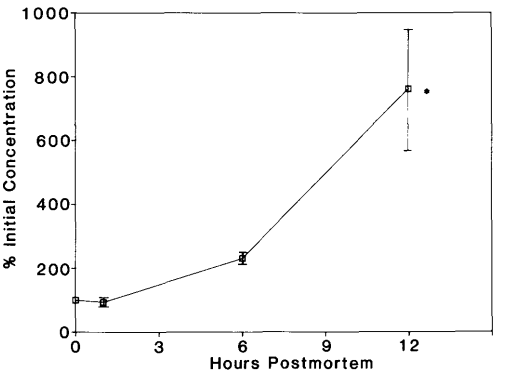

FIG. 1. Postmortem change in plasma pyridoxal phosphate concentration in pigs asphyxiated with CO_2 and maintained at 25°C, humidity 45%. *Significantly different ($P < 0.01$) from antemortem concentrations.

Results. *Postmortem changes of pyridoxal phosphate.* Figure 1 depicts the changes in plasma PLP that occur postmortem. Each point represents the mean of seven to nine plasma samples obtained from either the superior vena cava, the inferior vena cava, the right atrium, and/or the aorta of two to five pigs asphyxiated with CO_2 and maintained at 25°C, humidity 45%. The values are expressed as a percentage of the initial antemortem concentration with each pig serving as its own control. The mean (±SEM) antemortem plasma PLP concentration was 19 ± 1 n*M*. PLP concentrations of plasma samples obtained 1 hr after death were essentially the same as antemortem plasma PLP concentrations and no consistent difference was observed between sampling sites. However, the mean plasma PLP concentration at 6 hr postmortem was 2.3 times greater than antemortem and 7.6 times greater than antemortem by 12 hr postmortem ($P < 0.01$).

Similar postmortem increases in plasma PLP were observed in one pig killed by a lethal dose of Na pentabarbital administered peritoneally (data not shown). This implies that postmortem changes observed for pigs killed by asphyxiation with CO_2 are probably not related to increased blood acidosis which is produced by breathing CO_2 (14). Interestingly, when heparinized whole blood obtained from live pigs was incubated *in vitro* in the dark at room temperature, the plasma PLP concen-

tration gradually decreased to approximately 65% of initial concentration by 12 hr (data not shown). This indicates that the postmortem changes in plasma PLP concentration which occur *in situ* are not related exclusively to processes associated with plasma and erthrocytes. Rather, the postmortem changes in plasma PLP concentration may be due to interaction of the blood vessel and other tissues with plasma and erythrocyte constituents.

Figures 2 and 3 represent changes in muscle and liver PLP contents that occur after death. The mean (±SEM) initial muscle and liver PLP contents were 58 ± 6 and 31 ± 4 pmol PLP/mg protein, respectively. In contrast to the increase of PLP observed in plasma, the PLP content of muscle and liver decreased to 64% of antemortem levels by 6 hr postmortem ($P < 0.01$). At 12 hr postmortem, muscle and liver PLP content had decreased to 58 and 54% of their respective antemortem values.

Postmortem changes of plasma minerals. The Na heparin solution that was injected immediately prior to death contained 11,190 μg Na/ml compared to the mean antemortem plasma Na concentration of 2970 μg/ml. Approximately 2 ml of this Na heparin solution was slowly injected into the superior vena cava and allowed to circulate for 2–3 min before death. Using the mean hematocrit of the pigs utilized (30.0) and assuming that blood comprises 8.8% of total body weight (14), the contribution to plasma Na concentration from the administered Na heparin is approximately 30 μg/ml or 1% of the antemortem concentration.

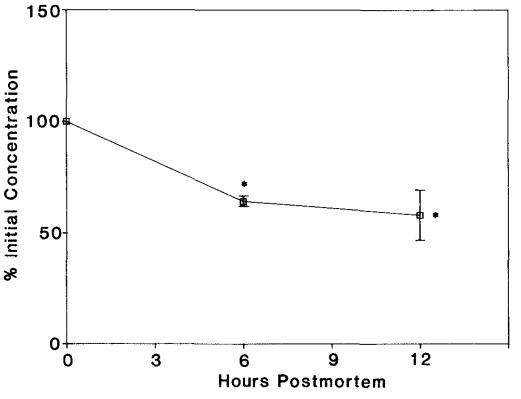

FIG. 2. Postmortem change in skeletal muscle pyridoxal phosphate content in pigs asphyxiated with CO_2 and maintained at 25°C, humidity 45%. *Significantly different ($P < 0.01$) from antemortem content.

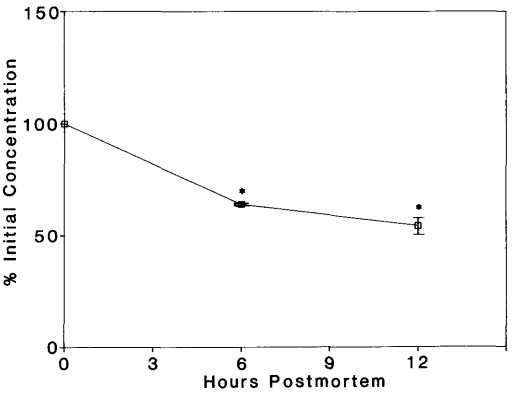

FIG. 3. Postmortem change in liver pyridoxal phosphate content in pigs asphyxiated with CO_2 and maintained at 25°C, humidity 45%. *Significantly different ($P < 0.01$) from antemortem content.

The mean postmortem changes of plasma Na, K, Mg, and Ca concentrations are shown in Fig. 4. Na concentration is significantly lower by 1 hr postmortem ($P < 0.01$) and gradually decreases to 81% of antemortem concentration by 12 hr. Plasma potassium concentration increased rapidly after death and was significantly elevated at 1 hr postmortem compared to the mean antemortem concentration ($P < 0.01$). Plasma K concentration continued to increase such that by 12 hr postmortem, the mean plasma K concentration was 5.1 times greater than the antemortem K concentration. Plasma Mg concentration increased to a mean value 2.1 times greater than the antemortem concentration by 1 hr postmortem ($P < 0.01$). Plasma Mg concentration continued to increase with increasing postmortem time interval and was 3.4 times greater than antemortem concentration by 12 hr. In contrast, plasma Ca concentration was not significantly different from antemortem concentrations at any meaured time point.

Discussion. The present investigation was undertaken to determine the *in situ* postmortem stability of PLP. The determination of plasma PLP is of interest because currently it is considered the single best method for vitamin B-6 nutritional status assessment (15, 16).

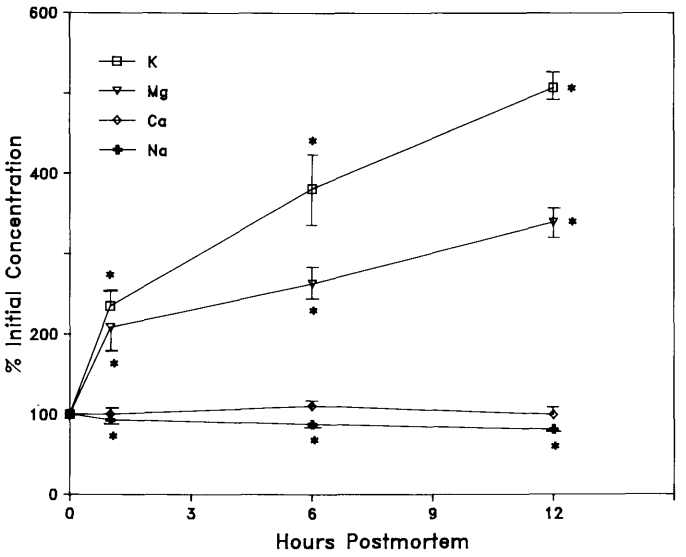

FIG. 4. Postmortem change in plasma K, Na, Ca, and Mg concentration in pigs asphyxiated with CO_2 and maintained at 25°C, humidity 45%. *Significantly different ($P < 0.01$) from antemortem concentrations.

The study was conducted in anticipation of investigations designed to relate vitamin B-6 status determined from autopsy samples to known pathologic causes of death. Because the pig is biochemically similar to humans with respect to metabolism in general and also in their plasma concentration of PLP (17), we chose the pig as an appropriate animal model for studying the stability of PLP in samples obtained at autopsy. It should be emphasized that similar studies of the postmortem stability of PLP in human samples must be performed before valid interpretation of PLP values determined from autopsy samples can be made. Data from the present investigation with pigs indicate that the determination of PLP in autopsy samples could be of use in estimating vitamin B-6 status immediately prior to death. Because plasma PLP concentrations increase after death rather than decrease, the postmortem determination of plasma PLP concentrations might be used to detect severe antemortem vitamin B-6 deficiency. An inadequate vitamin B-6 status would be indicated by postmortem plasma PLP concentrations less than those reported to indicate adequate antemortem vitamin B-6 status (18). However, postmortem plasma PLP concentrations equal to or greater than the concentrations suggested as indicating adequate vitamin B-6 nutriture must be interpreted with caution. Because of the postmortem increase in plasma PLP concentration, marginal deficiencies will be difficult to detect unless the postmortem samples are obtained very quickly after death and the postmortem time interval is known.

For ethical reasons, the determination of skeletal muscle and liver PLP content currently are not employed for human vitamin B-6 status assessment. However, skeletal muscle may be useful for estimating vitamin B-6 status at autopsy. Lumeng *et al.* reported that skeletal muscle PLP content was approximately threefold lower in vitamin B-6-deficient rats compared with that of sufficient controls. In addition, skeletal muscle PLP is correlated strongly with plasma PLP concentrations during both vitamin B-6 deficiency and sufficiency (19). Considering the degree of change observed at autopsy for skeletal muscle PLP in pigs (35% lower by 6 hr postmortem) and the magnitude of difference in skeletal muscle PLP content between deficient and sufficient rats (threefold lower), the postmortem determination of skeletal muscle PLP might be of use in estimating antemortem vitamin B-6 status. However, normal values for human skeletal muscle PLP content are currently unavailable and would need to be established before muscle PLP could be utilized

for this purpose. Alternatively, skeletal muscle autopsy control samples (such as from accident victims) could be used in investigations concerning vitamin B-6 status and known pathologic causes of death. Liver PLP content, compared with plasma and muscle, is much more refractory to changes in dietary vitamin B-6 intake (19) and therefore is of limited usefulness for assessing vitamin B-6 status.

The postmortem increase in plasma PLP and decrease in muscle and liver PLP can be partially accounted for by the hemoconcentration which occurs after death due to failure of the Na-K-ATP pump. Cessation of the Na-K-ATP pump allows for a redistribution of Na^+, Cl^-, and K^+ between intra- and extracellular space (20), leading to a net movement of H_2O from extra- to intracellular space. Because PLP does not readily cross cell membranes (21–23) plasma PLP concentrations should increase as hemoconcentration occurs. Conversely, as H_2O passes into intracellular space, the PLP content of muscle and liver would be diluted. Postmortem redistribution of H_2O from extra- to intracellular space has been demonstrated by measuring inulin space of rats sacrificed by ether asphyxiation or tracheal occulation (24). Hodgkinson and Hambleton reported a twofold decrease in extracellular fluid volume by 22 hours postmortem in rats stored at 4°C (24). Thus, the postmortem increase in plasma PLP concentration partially can be explained on the basis of H_2O redistribution after death. Serum and plasma alkaline phosphatase activities have been reported to increase after death (25–28) and would be expected to cause a decrease in the concentration of PLP via hydrolysis to yield pyridoxal and inorganic phosphate. However, serum and plasma phosphorus concentrations also increase after death (8, 29) and may inhibit the alkaline phosphatase-catalyzed degradation of plasma PLP. Although the effect of temperature was not assessed in the present study, several investigations have lead to the conclusion that postmortem changes are delayed by refrigeration of the body after death (24, 26, 29, 30). Therefore, postmortem changes in plasma PLP concentration also might be slowed by refrigeration of the body.

With respect to postmortem plasma mineral concentrations, postmortem changes previously have been reported for dogs, humans, and rats and are similar to those reported here for pigs. Schoning and Strafuss used samples obtained from dogs sacrificed with an overdose of Na pentabarbital and observed a rapid postmortem rise in K, a gradual decrease in Na, and no change in serum Ca concentrations (29). Jetter (8) and Naumann (31) reported a rapid postmortem elevation of human serum or plasma K concentrations and no change in plasma or serum Ca concentrations. Hodgkinson and Hambelton reported a postmortem increase in serum K and Mg and a decrease in Na concentrations in rats (24). Thus, our animal model utilizing pigs yields results for postmortem changes in plasma mineral concentrations which are similar to those obtained with other animal models. This observation suggests that postmortem changes in PLP concentrations also may be comparable across species.

The present investigation provides information concerning vitamin B-6 status assessment at autopsy. We observed a postmortem increase in plasma PLP and a postmortem decrease in skeletal muscle and liver PLP in pigs. Thus, knowledge of the postmortem time interval appears to be requisite for valid interpretation of PLP determinations in samples obtained at autopsy. Additional investigations utilizing human autopsy samples should be conducted to provide more definitive information as to the use of postmortem tissues for antemortem vitamin B-6 status assessment, as it may relate to disease state and cause of death.

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1. Reynolds RD, Leklem JE. Vitamin B-6: Its Role in Health and Disease. New York, Liss, 1985.
2. Willett WC. Does low vitamin B-6 intake increase the risk of coronary heart disease? In: Reynolds RD, Leklem JE, Eds. Vitamin B-6: Its Role in Health and Disease. New York, Liss, pp337–346, 1985.
3. Serfontein W, Ubbink J, DeVilliers L, Rapley C, Becker P. Plasma pyridoxal-5-phosphate level as risk index for coronary artery disease. Atherosclerosis **55**: 357–361, 1985.
4. Reynolds RD. Vitamin B-6 deficiency and carcinogenesis. In: Poirier LA, Newberne P, Pariza M, Eds. Role of Essential Nutrients in Carcinogenesis. New York, Plenum, in press, 1987.

5. Natta CL, Reynolds RD. Apparent vitamin B-6 deficiency in sickle cell anemia. Amer J Clin Nutr **40:** 235–239, 1984.
6. Reynolds RD, Natta CL. Depressed plasma pyridoxal phosphate concentrations in adult asthmatics. Amer J Clin Nutr **41**:684–688, 1985.
7. Spriggs AO. The art and science of embalming. Champion Co., Springfield, OH, 1954.
8. Jetter WW. Postmortem biochemical changes. J Forensic Sci **4**:330–341, 1959.
9. Reynolds RD. Vitamin B-6. In: Kaplan LA, Pesce AJ, Eds. Clinical Chemistry—Methods and Techniques. Mosby, St. Louis, in press, 1987.
10. Chabner B, Livingston D. A simple enzyme assay for pyridoxal phosphate. Anal Biochem **34**:413–423, 1970.
11. Lumeng L, Lui A, Li T-K. Microassay of pyridoxal phosphate using tyrosine apodecarboxylase. In: Leklem JE, Reynolds Rd, Eds. Methods in vitamin B-6 nutrition: Analysis and status assessment. New York, Plenum, pp57–67, 1981.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem **193**:265–275, 1951.
13. Multiple Comparison Test. Wang Laboratories, Inc., Lowell, MA, 1978.
14. Vander AJ, Sherman JH, Luciano DS. Human physiology: The mechanisms of body functions. New York, McGraw-Hill, pp327–365, 1980.
15. Li T-K, Lumeng L. Plasma PLP as an indicator of nutritional status: Relationship to tissue vitamin B-6 content and hepatic metabolism. In: Leklem JE, Reynolds RD, Eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York, Plenum, pp289–296, 1981.
16. Leklem JE, Reynolds RD. Recommendation for status assessment of vitamin B-6. In: Leklem JE, Reynolds RD, Eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York, Plenum, pp389–392, 1981.
17. Coburn SP, Mahuren JD, Guilarte TR. Vitamin B-6 content of plasma of domestic animals determined by HPLC, enzymatic and radiometric microbiological methods. J Nutr **114**:2269–2273, 1984.
18. Shultz TD, Leklem JE. Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake in adults. In: Leklem JE, Reynolds RD, Eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York, Plenum, pp297–320, 1981.
19. Lumeng L, Ryan MP, Li T-K. Validation of the diagnostic value of plasma pyridoxal 5′-phosphate measurements in vitamin B-6 nutrition of the rat. J Nutr **109**:545–553, 1978.
20. Ganong WF. Review of medical physiology. Los Altos, CA, Lange Medical Publications, pp1–20, 1979.
21. Anderson BB, Fulford-Jones CE, Child JA, Beard MEJ, Bateman CJT. Conversion of vitamin B-6 compounds to active forms in the red blood cell. J Clin Invest **50**:1901–1909, 1971.
22. Mehansho H, Hamm MW, Henderson LM. Transport and metabolism of pyridoxal and pyridoxal phosphate in the small intestine of the rat. *J Nutr* **109**:1542–1551, 1979.
23. Fonda ML, Eggers DK. Vitamin B-6 metabolism in the blood of young adult and senescent mice. Exp. Gerontol **15**:465–472, 1980.
24. Hodgkinson A, Hambleton J. Elevation of serum calcium concentration and changes in other blood parameters after death. J Surg Res **9**:567–574, 1969.
25. Coe JI. Postmortem chemistries on blood with particular reference to urea nitrogen, electrolytes, and bilirubin. J Forensic Sci **19**:33–42, 1974.
26. Schoning P, Strafuss AC. Postmortem sera and cerebrospinal fluid enzymes. J Forensic Sci **25**:344–348, 1980.
27. Entickap JB. Biochemical changes in cadaver sera in fatal heart attacks. J Forensic Sci **7**:135–146, 1960.
28. Naumann HN. Postmortem liver function tests. Amer J Clin Pathol **26**:495–505, 1956.
29. Schoning P, Strafuss AC. Postmortem biochemical changes in canine blood. J Forensic Sci **25**:336–343, 1980.
30. Schoning P, Strafuss AC. Postmortem biochemical changes in canine cerebrospinal fluid. J Forensic Sci **25**:60–66, 1980.
31. Naumann HN. Body chemistry after death. J Amer Med Assoc **171**:2278, 1959.

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