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Renal Concentrating Ability in Pyridoxine Deficiency.* (30552)

ROBERT P. DAVIS† AND ROBERT F. SLOOP, JR.‡ (Introduced by Harry Eagle)
Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, and Department of Medicine and Unit for Research in Aging, Albert Einstein College of Medicine, Yeshiva University, New York City

Renal concentrating ability depends upon the integrity of a variety of anatomic, physiologic and biochemical functions. Apparently specific disorders of the renal concentrating mechanism have been described in potassium depletion(1), during calcium infusion(2-4), and in protein starvation(5-10). While the mechanism of loss of renal concentrating power is not clear in any of these disorders, the least is known about the effect of protein deprivation on renal concentrating ability. Some studies(6-8) suggest that a defect in urea production is responsible, while studies by Hendrikx and Epstein(9) and by Manitius, Pigeon and Epstein(10) indicate that this is an insufficient explanation for the entire mechanism of the loss of renal concentrating ability.

A function specifically related to metabolism of amino acids and proteins could conceivably be the basis of this lesion. Pyridoxine is a major coenzyme of amino acid metabolism. It has several functions and is particularly involved in transamination reactions required for protein synthesis. It is conceivable that the defect in renal concentrating ability of protein starvation is related to a defect in one of the reactions for which pyridoxine is a coenzyme. Pyridoxine deficiency has already been shown to be associated with microcytic anemia(11,12), hyperoxaluria with stone formation(13), and a number of biochemical abnormalities resulting from defective transamination processes(14). Because of our interest in other aspects of pyridoxine metabolism,§ we were able to examine renal concentrating ability in the pyridoxine deficient rat.

Materials and methods. These studies were conducted on male rats of the Sprague-Dawley strain. The animals weighed 107-147 g at start of the experiment, which was carried out over 5 months. The animals were pair-fed throughout the study. All received the same basic vitamin-free diet (vitamin B-Complex Test Diet, free of vit A, D and B complex, from Nutritional Biochemicals Corp.). Control animals received this diet supplemented by a standard "complete" vitamin mixture (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp.). The experimental group received this standard vitamin-free diet supplemented with this vitamin mixture free of pyridoxine and its derivatives. Water intake was unlimited.

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Pyridoxine deficiency was evaluated by measurement of xanthurenic acid excretion following administration of a standard dose of l-tryptophane(15). The rats were given 100 mg of l-tryptophane (Nutritional Biochemicals Corp.) in a volume of 10 ml by stomach tube. They were placed in metabolic cages and the following 24-hour volume of urine was collected in the dark with toluene as the preservative. The cages were designed to permit collection of urine free of contact with feces. Xanthurenic acid was measured in an aliquot of urine by a modification(16) of the method of Lepkovsky *et al*(17).

Further physiologic evidence of pyridoxine deficiency was obtained by measurement of the rise in systolic blood pressure in depleted and control rats by means of the tail sphygmomanometer.‖

The maximum renal concentrating ability of each rat was tested by a slight modification of the method of Hollander *et al*(1). The rats were deprived of food and water beginning at 10:15 AM on the day of test. Each rat was given 50 milliunits of vasopressin-in-oil (Parke Davis and Co., Pitressin-in-oil, lot U 231A) subcutaneously at 10:15 AM and again at 3:15 PM. At 6:45 PM, the rats were stimulated to empty their bladders by brief electric shock. The completeness of bladder emptying is unknown but probably comparable in control and deficient rats. Overnight urine was collected as described by Hollander *et al*(1). It was usually possible to collect a separate final urine specimen at the end of the experiment by a repeat electrical shock. This final voiding was at least as highly concentrated as the overnight collection in every case. The osmolality of the urine was determined cryoscopically in a Fiske osmometer calibrated with standard solutions of sodium chloride.

To test the effect of a solute load on ability to concentrate urine, the test was repeated one week later with the modification that during the overnight collection the rats were allowed access to water bottles containing 2% urea. Although precise determination of water ingestion was not carried out, both control and experimental rats drank comparable volumes of urea solution as determined by calibration of water bottles.

Statistical analysis of the results was performed with application of Student's "t" test. Results with a value of P 0.05 are considered not to be significant. Kidneys from both control and pyridoxine deficient animals were fixed in buffered formalin and embedded in paraffin; sections were prepared by conventional histological techniques. The sections were stained with hematoxylin and eosin and examined by light microscopy.

Results. Weights. On the program described above, at first both control and experimental animals slowly gained weight. The experimental group gained at a slightly slower rate. After 2 months the control rats weighed an average of 220 g; the experimental group average was 190 g. The control group on pair-fed intake continued thereafter to maintain a constant weight, while each rat of the experimental group lost a mean of 10 g in weight over the remainder of the study period. Typical acrodynia was seen in the experimental group.

Degree of pyridoxine deficiency. Degree of pyridoxine deficiency was measured by the tryptophane load test. Excretion of xanthurenic acid following a 100 mg tryptophane load was 3.01 ± 0.49 mg/24 hr (mean ± standard error) in the control group and 19.25 ± 2.85 mg/24 hr in the pyridoxine deficient group. The results are highly significant (P .001).

The systolic blood pressure of a group of control animals was 109 ± 4 mm Hg, (mean ± standard error) while that of the pyridoxine deficient animals was 129 ± 9 mm Hg. These results are probably statistically significant (P .05, one-tailed test) and are compatible with previous findings(18).

Ability to form a concentrated urine. Maximum urinary osmolality following deprivation of water and administration of vasopressin was no different (P .40) in pyridoxine deficient rats than in their pair-fed control rats (Table I). Both groups of rats, however, had significantly reduced renal concentrating ability when compared with re-

‖ The authors would like to thank Dr. James Woods and Miss Billie Sioux Bush for assistance in these measurements.

TABLE I. Effect of Pyridoxine Deficiency on Renal Concentrating Mechanism.

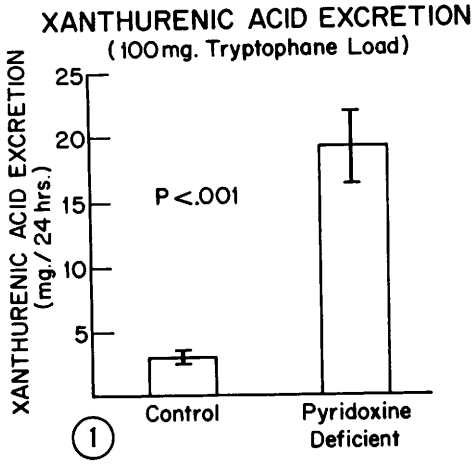
	Maximum urinary osmolality*		
	Before urea	After urea	Difference (urea)
	Control group		
KA	1748	2986	1238
KB	1088	3564	2476
KG	1712	2556	844
KD	1352	2716	1364
Mean	1475	2956	1480
Standard error	157	219	350
Significance (P)			<.02†
	Pyridoxine deficient group		
XA	2195	2290	95
XB	2450	1570	−880
XC	1124	2016	892
XD	1465	2668	1203
XE	1460	2542	1082
Mean	1739	2217	478
Standard error	249	197	1235
Significance (P)	>.40‡	<.05‡	>.50†

* Measured following dehydration and vasopressin administration (see text).
† Comparison of maximum osmolality for each group of rats before *vs* after urea administration.
‡ Comparison of control *vs* pyridoxine deficient rats.

ported values determined by the same procedure in normal rats of the same strain (1).

Because the diets of both groups were nutritionally insufficient, presumably to the same degree as a result of pair-feeding, a deficiency of solute was considered to be a possible basis for this concentrating defect. Water intake was approximately equal in the 2 groups. Accordingly the animals were retested for renal concentrating ability following administration to each rat of an amount of urea calculated to provide the equivalent of solute arising from 3-5 g of dietary protein. Under these circumstances, maximum urinary concentrating ability of the control rats rose significantly ($P<.02$) to normal (Table I). In the pyridoxine deficient rats the rise in maximum urinary osmolality following urea administration was not significant ($P>.50$). Before urea the maximum concentrating ability of the control group was not significantly greater than that of the pyridoxine deficient group. After urea, the control group rose sufficiently more than the deficient group so as to create a significant difference between them ($P<.05$). Histologic examination of the kidneys from pyridoxine deficient and pair-fed control rats demonstrated¶ no anatomic lesion in the renal medulla which might account for a concentrating defect. The tubules were normal in caliber and configuration and there was no evident abnormality of the tubular epithelium, the loops of Henle, vasa recta or interstitium.

Discussion. Pyridoxine deficiency was readily established in the rat as judged by the abnormality in tryptophane metabolism.



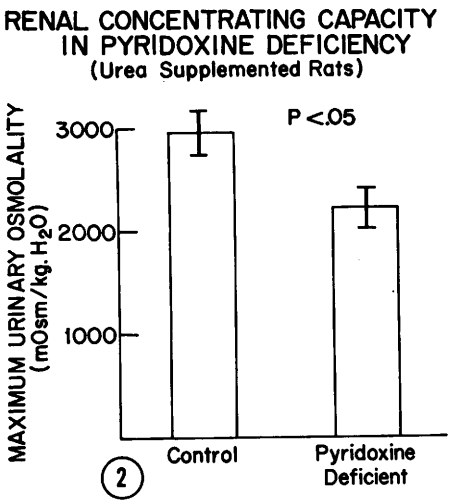


FIG. 1 and 2

¶ We would like to thank Dr. Richard Dobsen for assistance in preparation of tissue sections and Dr. Rosamond Janis for reviewing the histological sections.

The excretion of xanthurenic acid in the control rats was of the same order of magnitude as described by Brown and Price(15). The pyridoxine deficient animals showed a 6-fold increase in excretion of xanthurenic acid suggesting a marked abnormality in tryptophane metabolism. The elevation of blood pressure, while modest in the small group of rats in which it was measured, is probably statistically significant and is consistent with a previous report(18) in pyridoxine deficient rats.

The initial study of renal concentrating capacity showed no difference between control and pyridoxine deficient rats. Both groups, however, had diminished concentrating power when compared to reported values for normal rats. Both groups, furthermore, were nutritionally insufficient in that the control animals were pair-fed to the reduced intake of the pyridoxine deficient rats. A limited solute intake by itself imposes a limitation on renal concentrating capacity, apart from intrinsic defects in the concentrating mechanism(6,8). This deficiency, in fact, accounts for the major portion of the limited renal concentrating capacity of protein deficiency(5,6). In order to correct for this enough urea was administered to provide that amount of solute equivalent to the estimated deficit caused by the 30-50% decrease in the daily protein intake of both groups. Urea administration fully corrected the concentrating defect of the control animals, but there remained a significant small reduction in renal concentrating capacity of pyridoxine deficient rats.

It is possible that concomitant potassium depletion accounts for this defect in concentrating ability, despite adequate dietary potassium intake by the pyridoxine deficient rats. Diamant and Guggenheim(19) have noted that the muscle of pyridoxine deficient rats contains less potassium and more sodium than pair-fed and *ad libitum* fed control rats. The 10% decline in muscle potassium described by these authors is smaller than that usually associated with the renal concentrating defect of experimental potassium depletion(1,20). However, the defect described by us is not significantly different from that seen in minimal degrees of potassium deficiency(1). Yet serum potassium concentration is normal in the pyridoxine deficient rats(19) in contrast to the usual finding in potassium deficient rats(21). Furthermore, Diamant and Guggenheim suggest that the electrolyte changes were secondary to alterations of adrenal cortical function in pyridoxine deficiency in that the distorted electrolyte content of muscle and impaired excretion of a sodium chloride load were restored to normal by administration of cortisone. A previously described impairment in the handling of a water load was similarly corrected by cortisone(22). These features are not described in potassium depletion and the renal concentrating defect of pyridoxine deficiency probably has another basis.

While the defect in renal concentrating power in pyridoxine deficiency is small and would not appear to be of major physiologic significance, it is strikingly comparable to that reported to persist in protein deficiency following urea administration(9,10). The defect in concentrating mechanism in protein starvation is not fully corrected by urea, which itself improves the ability of the kidney of the rat to concentrate non-urea solutes. Pyridoxine has many roles in intermediary metabolism, but its major function appears to be that of a coenzyme in transamination reactions of protein metabolism. It is possible that the renal concentrating defect of protein insufficiency is due to a concomitant defect in pyridoxine dependent metabolism, although not necessarily a deficiency of pyridoxine itself. This proposed defect may well be an insufficiency of a substrate for pyridoxine-requiring reactions. Alternatively it is possible that pyridoxine deficiency produces a deficiency of a specific amino acid metabolite necessary for the integrity of the renal concentrating mechanism. A role for lysine in the concentrating function of the kidney has been demonstrated by Radford(23). Specific studies of pyridoxine metabolism in protein depletion would be of interest, as would be studies of the effect of specific intermediates of amino acid metabolism on renal concentrating power in both pyridoxine deficiency and protein starvation.

Summary. Pyridoxine deficiency was induced in rats by feeding a diet deficient in pyridoxine. Control rats were pair-fed to the intake of the experimental group. Pyridoxine deficiency was demonstrated in the experimental group by increased xanthurenic acid excretion following a tryptophane load and by development of hypertension. Renal concentrating ability, measured as maximum urinary osmolality following dehydration and vasopressin administration, was impaired in both pyridoxine deficient and control rats. Since both groups were protein depleted as a result of the pair-feeding, urea was given to both groups and renal concentrating ability remeasured. The control group now had normal concentrating ability, whereas the maximum urinary osmolality of the pyridoxine deficient rats was significantly reduced. These results suggest that pyridoxine or some aspect of its metabolism is essential for normal renal concentrating ability.

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Effects of Temperature on Electroencephalogram of the Caiman.* (30553)

L. C. PARSONS† AND S. E. HUGGINS (Introduced by R. A. Huggins)
Department of Biology, University of Houston, Houston, Texas

In a previous study made in this laboratory(1) the normal electroencephalographic pattern of the South American caiman, *Caiman sclerops,* for the cerebral hemispheres, optic lobes, and cerebellum was described. The cerebellar hemispheres showed a dominant "beta" rhythm of 18-24 cycles per second with an amplitude of 10-20 μv; superimposed there was an intermittent alpha-like rhythm of 7-12 cycles per second with an amplitude of 20-40 μv. In the optic lobe region the dominant rhythm was 6-8 cycles per second with an amplitude of 20-25 μv and superimposed was a faster 14-18 rhythm

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† Present Address: Dept. of Physiol., Univ. of Texas Med. Br., Galveston, Texas.