Zinc Content in Tissues of Pyridoxine Deficient Rats. (30130)

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Hsu *et al*(1) observed that a deficiency of pyridoxine in rats resulted in an elevation of serum Na and muscle K, accompanied by a reduction of muscle Na level. In spite of the shift of cellular equilibrium of these 2 electrolytes in tissues, there was no alteration in blood and plasma volumes(2). Dietary deprivation of pyridoxine brought about an increase of fecal excretion and of radioactivity in the brain and testicles, but a reduction in the liver and heart of injected Mn^{54}(3). Physiological consequences of pyridoxine deficiency with regard to other minerals are (a) an impairment of iron absorption(4,5), and (b) an increase in the frequency of formation of kidney stones(6,7,8). The influence of pyridoxine deficiency on Zn metabolism has not been investigated although changes in certain Zn metalloenzymes in pyridoxine-deprived rats have been observed (9,10,11). The present communication reports the effect of pyridoxine deficiency on Zn concentrations in plasma and other tissues and on distribution of injected radioactive Zn-65 in various tissues.

Experimental procedure. Four-week-old male and female rats (40-60 g) and 8-week-old male rats (150-170 g) were used. They were either of the Wistar strain obtained from National Institutes of Health or the McCollum strain from our stock colony.

Preparation of deficient animals. In a given experiment, one strain of rats was used randomly divided into 2 groups comparable with respect to age, body weight, and sex. The first group received a high protein diet deficient in pyridoxine(3). The second group (control) received an identical ration with a supplement of pyridoxine HCl (20 mg/kg). In one instance, the antagonist of pyridoxine, desoxypyridoxine HCl (50 mg/kg), was added to pyridoxine-deficient diet and given to five 8-week-old male rats in order to intensify the deficiency of vitamin B_6. A total of 4 sets of pyridoxine-deficient and control rats was prepared. Food consumption of the control animals was restricted to that amount ingested by the rats receiving the pyridoxine deficient diet. To minimize zinc contamination, rats were housed individually in galvanized cages which were coated with an epoxy resin (Fisher Scientific product) and isolated in an air-conditioned room away from general laboratory equipment and personnel. Deionized drinking water was offered *ad libitum* to all animals. This procedure was used to insure comparable dietary Zn intake which may affect its concentrations in tissues. Thiamine and riboflavin deficient rats and their corresponding controls were prepared according to the procedure previously reported(13). They were also housed individually in galvanized cages coated with epoxy resin and received their corresponding diets *ad libitum.*

Determination of Zn content of tissues. Following a period of 4 to 8 weeks of feeding, pyridoxine-deficient rats and their respective controls were starved overnight and then exsanguinated by cardiac puncture under Nembutal anesthesia. The heparinized blood was centrifuged, and plasma was immediately separated. The various tissues were dissected, rinsed with deionized water, blotted dry, weighed, and stored at $-5°$ for no more than a week. The Zn content of the tissues was determined according to the dithizone method of Vallee and Gibson(12).

Retention of injected Zn-65. The pyridoxine-deficient and control animals were injected intramuscularly with 0.5 ml physiological saline containing 1.5 μc of carrier-free Zn-65 as zinc chloride. They were immediately transferred to individual metabolism cages for collection of urine and feces, and killed 24 hours after administration of radiozinc. The various organs and entire G.I. tract were removed, rinsed with a saline solution, and solubilized in 30% KOH solution. The feces were moistened with water and homogenized with concentrated sulfuric

TABLE I. Zn Levels in Tissues of Pyridoxine-Deficient Rats.*

Tissues	Pyridoxine-deficient	Pyridoxine-fed
Plasma	1.37 ± .041†‡	2.49 ± .085
Liver	25.4 ± .12‡	34.6 ± 1.56
Pancreas	19.1 ± .58‡	35.2 ± 2.63
Brain	19.3 ± .49	13.3 ± .45
Kidneys	22.2 ± .33	23.1 ± .58
Spleen	20.7 ± .15	21.5 ± .23
Heart	13.8 ± .63§	16.6 ± .39

* Zn concentrations are expressed as μg/ml for plasma, and μg/g wet weight for other tissues.
† Mean and standard error of mean.
‡ $P < .01$.
§ $P < .05$.
Six male rats were used in each group for an experimental period of 6 weeks.

acid. Zinc-65 radioactivity in the tissues and feces preparations was measured in a well-type scintillation counter.

Results and discussion. Pyridoxine deficiency in our rats was evident from a retarded growth rate even after 2 weeks of feeding. Extension of dietary deprivation to 4 weeks or longer resulted in acrodynia and skin lesions.

The data in Table I indicate that the zinc content in plasma, liver, pancreas, and heart of pyridoxine-depleted rats was significantly lower than that of pyridoxine-treated animals. No substantial differences in Zn concentrations were observed in other tissues examined.

Table II summarizes the effect of thiamine or riboflavin deficiency on hepatic Zn levels in rats as compared to the animals depleted of pyridoxine. Although food was given *ad libitum* to thiamine and riboflavin experimental animals, rats depleted of thiamine or riboflavin had approximately the same amount of hepatic Zn as their respective controls. Again, it was observed (Table II) that hepatic Zn levels were reduced in pyridoxine-deficient rats.

TABLE II. Comparison of Liver Zn Levels in Thiamine, Riboflavin, and Pyridoxine-Deficient Rats.

Diet	Exp period (days)	Body wt (g)	Zn (μg/g wet tissue)
Thiamine-deficient (4)	15	176 ± 3.1*	26.3 ± 2.72
Thiamine-fed (4)		175 ± 1.6	23.5 ± 3.07
Riboflavin-deficient (5)	56	195 ± 3.3	28.6 ± .95
Riboflavin-fed (5)		271 ± 8.8	27.4 ± .56
Pyridoxine-deficient (6)	28	175 ± 10.2	17.8 ± 1.88†
Pyridoxine-fed (6)		219 ± 5.1	29.3 ± 1.30

* Mean and standard error of mean.
† $P < .01$.
Figures in parentheses indicate number of male rats used.

In other studies, we have demonstrated that zinc deficient rats retained more injected radioactive Zn-65 in their tissues than those receiving a zinc supplemented diet(14). Since pyridoxine-deficient rats exhibited a decrease in Zn content in certain tissues, an increased uptake of administered Zn-65 would be expected. Our results (Tables III and IV), indeed, show such an increase in tissue concentration of injected radiozinc. Uptake of Zn-65 by the plasma and liver of pyridoxine-deficient male and female rats was significantly higher than that of their respective controls. Pyridoxine deficiency induced by feeding desoxypyridoxine further increased the retention of injected Zn-65 in kidney, pancreas, and testis and epididymis. The amount of the isotope measured in the G.I. tract and feces of deficient groups was also significantly increased. Since the major route of excretion of oral or injected Zn is by way of the bile into the gut, an increased elimination of injected Zn-65 in the feces and G.I. tract of pyridoxine-deficient animals would suggest that deficiency of this vitamin enhances the rate of excretion of radiozinc from the body. It has been reported that the activities of certain zinc-containing enzymes are changed in pyridoxine-deficient rats. These include a decrease in liver lactic dehydrogenase(9) and an increase in plasma alkaline phosphatase(10). Tulpule and Patwardhan(11) reported that the activity of hepatic glutamic dehydrogenase was reduced when the rats were depleted of fat and pyridoxine alone. Recent studies by Huber *et al* (15) indicated that pyridoxine-deficient rats had less insulin activity than their controls. All of these findings, in conjunction with our present observations, indicate the possibility of Zn depletion and concomitant functional

TABLE III. Effect of Pyridoxine Deficiency on Tissue Distribution of Injected Zn-65 in Rats.*

Tissues	Male		Female	
	Pyridoxine-deficient (6)	Pyridoxine-fed (6)	Pyridoxine-deficient (5)	Pyridoxine-fed (5)
Plasma	.144 ± .008†‡	.121 ± .003	.180 ± .003‡	.142 ± .010
Liver	2.24 ± .03§	1.81 ± .01	2.23 ± .05‡	1.82 ± .02
Kidney	2.78 ± .02	2.51 ± .1	2.66 ± .01	2.41 ± .3
G.I. tract:				
Upper	4.55 ± .16	4.31 ± .09	9.75 ± .18	8.57 ± .07
Middle	6.51 ± .66	5.97 ± .33	8.99 ± .43	7.22 ± .56
Lower, plus feces	15.8 ± 1.43	12.9 ± .51	19.47 ± 1.16‡	14.89 ± .89
Total	26.9 ± 1.1‡	22.9 ± .6	38.2 ± 1.3‡	30.7 ± 1.6

* Expressed as per cent of injected Zn-65 activity per ml of plasma or per g wet weight of tissue or, in the case of G.I. tract and feces, per section (rather than per g) i.e., upper, middle, and lower with feces.

1.5 μc of carrier-free Zn-65 injected I.M. Rats were killed 24 hr later.

† Mean and standard error of mean.

‡ $P < .05$.

§ $P < .01$.

Figures in parentheses indicate number of rats used. Experimental period for males was 4 weeks; for females, 5 weeks.

changes as a result of feeding a pyridoxine-deficient diet. However, it is not clear whether the changes in zinc metalloenzyme and insulin activities observed in pyridoxine-deficient rats are a consequence of zinc deficiency or whether this vitamin has a specific role in relation to these substances.

TABLE IV. Effect of Pyridoxine Deficiency Induced by Feeding Desoxypyridoxine on Tissue Distribution of Injected Zn-65 in Rats.*

Tissues	Desoxypyridoxine (5)	Pyridoxine (6)
Plasma	.142 ± .003†‡	.110 ± .004
Liver	2.27 ± .13‡	1.60 ± .04
Kidney	1.96 ± .04	1.62 ± .03
Pancreas	2.27 ± .31§	.93 ± .07
Brain	1.03 ± .02	.83 ± .03
Testis and epididymis	.402 ± .041‡	.281 ± .020
Seminal vesicles and coagulating glands	.1834 ± .004	.1987 ± .007
Prostate glands	.0583 ± .005	.0481 ± .001
G.I. tract:		
Upper	6.30 ± .34	4.89 ± .31
Middle	5.63 ± .82	4.27 ± .50
Lower, plus feces	20.4 ± 1.95‡	14.9 ± .50
Total	32.34 ± 1.46‡	24.05 ± 1.22

* Expressed as per cent of injected Zn-65 activity per ml of plasma or per g wet weight of tissue or, in the case of G.I. tract and feces, per section (rather than per g) i.e., upper, middle, and lower with feces.

2.5 μc of carrier-free Zn-65 injected I.M. Rats killed 22 hr later.

† Mean and standard error of mean.

‡ $P < .05$.

§ $P < .01$.

Figures in parentheses indicate number of male rats used for an experimental period of 8 weeks.

Summary. Pyridoxine deficiency in rats resulted in a decreased Zn content in the plasma, liver, pancreas, and heart tissues and an increase of Zn-65 uptake in the plasma and liver after an intramuscular injection of radiozinc. A highly significant increase in Zn-65 radioactivity was also observed in the gastrointestinal tract and feces of pyridoxine-deficient rats. This phenomenon appeared to be specific for pyridoxine since deficiency of thiamine or riboflavin did not affect hepatic Zn concentrations.

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Anomalous Hemadsorption by Cell Cultures.* (30131)

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The adhesion of red blood cells to viral infected cell cultures was discovered by Vogel and Shelokov(1) and employed for identification of different types of viruses(1,2). Since then, hemadsorption has been utilized in a variety of ways to assay, identify and study viruses(3-7).

In the present report, a consistent hemadsorption by primary kidney cell cultures and stable lines of monkey kidney cell cultures not known to be infected with viruses is described. Critical requirements for this phenomenon were the presence of calcium ions or treatment of the red blood cells with periodate.

Material and methods. Fresh chicken red blood cells (RBC's) were obtained as a stabilized suspension from the Baltimore Biological Laboratory, Baltimore, Md. RBC's from other animals were collected fresh in citrated blood specimens. All RBC's were centrifuged immediately before use, washed twice in physiological saline (0.85%) and resuspended as a 5% suspension in physiological saline solution. For application to cell cultures, the RBC's were prepared in 0.2% suspension in buffer solutions.

Buffer solutions were prepared at pH 4.0 to 5.8 with 0.15 M acetate and at pH 6.9 to 9.3 with 0.04 M veronal containing 0.1 M NaCl.

Cell cultures were grown from freshly trypsinized tissues (primary cultures), subcultures of these (secondary cultures), or from stable lines of cells. For tests, the cultures were grown in Leighton tubes with a flat bottom area 5 × 1 cm. The medium for routine cell cultures has been described(8). Eagle's minimum medium(9) and Medium 199(10) also were used at times. Media routinely contained 5% unheated calf serum obtained from Baltimore Biological Laboratory. Other sera were from the same source.

BSC-1, a stable line of green monkey *(Cercopithecus aethiops)* kidney cells, was obtained from Microbiological Associates, Inc., Bethesda, Md. RK-13 cells were obtained from Dr. Hope Hopps, Bureau of Biological Standards, National Institute of Health. The LLC-MK 2 Rhesus monkey kidney derived stable line was obtained from the American Type Culture Collection Cell Repository, Rockville, Md.

For general treatment of cultures with RBC's, growth medium was removed; the cells were washed twice with physiological saline and overlaid with 1.5 ml RBC's in test solutions. After 30 minutes at room temperature (25°C), the cultures were agitated, the RBC suspension removed and the cultures washed twice with Earle's balanced salt solution. The hemadsorption was evaluated microscopically. Values were ascribed as (—) for absence of adhering RBC's, (±) for some scattered adherence and (+) to (+++++) for increasing amounts of RBC's up to apparent maximal adsorption. A complete

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