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Lead Increases Urinary Zinc Excretion in Rats

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Abstract

The major purpose of this study was to determine whether acute or chronic Pb exposure would increase urinary excretion of zinc in the rat. Four groups of unanesthetized rats were given 0, 0.03, 0.3, or 3 mg Pb (as acetate) kg intravenously, and urinary excretion of zinc, sodium, and potassium was monitored for 6 h. Only at the highest dose was urinary Zn excretion significantly elevated; there were no significant changes in sodium and potassium excretion at any dose. Two other groups of rats were studied for 9 weeks in metabolism cages before and during administration of either 500 ppm Pb (as acetate) or equimolar Na acetate in the drinking water. Two days after Pb treatment and continuing through day 35, Zn excretion was elevated in the Pb-exposed animals; beyond this day, zinc excretion became similar in the two groups. The difference in Zn excretion was not the result of lower water intake by the Pb-treated animals. At sacrifice (70 days after starting Pb exposure), Pb-exposed animals had lower Zn content of the plasma and testis, but there was no difference in kidney Zn. Plasma renin activity was significantly higher in Pb-exposed animals. We conclude that chronic Pb exposure in rats can result in some degree of decreased tissue zinc, which is, at least in part, secondary to increased urinary losses of zinc.

Index Entries: Zinc excretion, and lead; lead, and urinary Zn excretion; zinc deficiency; lead, effect on zinc balance; zinc, in testis; renin, effect of lead on; lead, effect on renin.

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Introduction

We previously demonstrated that the acute administration of lead to anesthetized dogs produced a rapid marked increase in the urinary excretion of zinc (*1*). The present studies test the hypothesis that chronic administration of lead, in doses that do not produce renal damage, would elevate urinary zinc excretion, thereby possibly contributing to the development of zinc deficiency. To our knowledge there have been only three published studies that deal with the chronic effects of lead on zinc metabolism: In a study of mice, Seth et al. (*2*) found no effect of lead administration on the concentrations of zinc in kidney and liver; El-Gazzar et al. (*3*), in a study with rats, found that the addition of 100 ppm Pb to drinking water caused a decrease in plasma, liver, and bone zinc concentrations; Mahaffey et. al. (*4*), also studying rats, found that 200 ppm Pb decreased kidney, brain, and femur zinc. In none of these studies was urinary excretion of zinc measured.

To test the effect of Pb exposure on zinc excretion, rats were given either lead acetate (500 ppm Pb) or sodium acetate chronically in their drinking water and their urines were collected daily or three times weekly, using metabolism cages. However, before undertaking this long-term experiment, we studied the acute effects of lead in this same species to satisfy ourselves that lead can enhance zinc excretion in species other than dog.

Methods

Acute Lead Exposure

Male rats weighing 275–300 g (Charles River, Wilmington, Mass.) were housed five per cage and placed on Teklad Rat and Mouse Chow (zinc content = 28 ppm). On the day of the experiment, the animals were randomly assigned to one of four experimental groups ($N = 6$ per group) receiving 0, 0.03, 0.3, or 3 mg. Pb (as Pb acetate) /kg. The lead solutions or sodium acetate for the control animals was freshly prepared in 5% dextrose; they contained 1.71 mg acetate/mL (equal to the acetate in the 3 mg/kg dose) and were administered intravenously in a volume of 0.1 mL/100 g body weight. Immediately prior to the injection, each animal received 2 mL demineralized water/100 g body weight via stomach tube (to insure adequate urine flows) and was then lightly anesthetized with ether. The femoral vein was exposed for injection of the treatment dose, after which the incision was closed with wound-clips. Each animal was placed in a stainless steel solder-free metabolism cage for collection of urine during the next 6 h. At the end of this period, the animals were removed from the cages and their bladders were emptied manually by firm pressure on the abdomen; this urine was added to the total collection. Each animal was then anesthetized with sodium pentobarbital (50 mg/kg. ip), and a terminal blood sample was collected by aortic puncture, using EDTA as anticoagulant.

Chronic Lead Exposure

Fifteen male rats (initial weights, 275–300 g) were housed individually in stainless-steel solder-free metabolism cages. The rats were matched by weight and assigned to one of two drinking-water groups, either 500 ppm Pb (as Pb acetate) or equimolar sodium acetate, both dissolved in demineralized water. Prior to beginning these regimens, baseline urinary excretory data were collected for 6 days, during which all animals were given demineralized drinking water *ad libitum* and fed Purina Basal purified chow (Catalog 5755, zinc content 20 ppm) prepared daily as a mush as follows: 500 mL demineralized water was added to 500 g of pellets, and the mixture allowed to stand refrigerated for 24 h. Food was presented daily from 5 PM to the next morning and then removed. This procedure facilitated the clean collection of urine, with minimal food contamination. Urine was collected daily, the volume measured and stored frozen for later analysis. Water intake was also recorded. The cages and collection vessels were washed daily and rinsed with demineralized water before replacing the rats in them.

Following the 6-day baseline period, the drinking water was switched to either Pb acetate or Na acetate, which was continued throughout the remainder of the experiment. Both were administered *ad libitum* from experimental day 7 through day 18. We noted that daily water intake of the Pb-treated animals averaged less than that of controls (14.1 ± 0.9 mL vs 32.0 ± 1.4) as did urine volume (7.6 ± 0.4 mL vs 21.0 ± 1.1), and to evaluate whether this reduced water intake and urine volume contributed to altered urinary zinc excretion, the drinking water was restricted for both groups to 25 mL on days 19–21, then to 10 mL for days 22–29, and again to 25 mL on days 30 to the termination of the experiment on day 70. On day 43, both groups of animals were switched to a low zinc diet (Purina low zinc purified diet, catalog No.5886); this diet contained 8–10 ppm Zn, but was otherwise identical to the Purina Basal Chow. Urine collections (each 24 h long) continued either daily or thrice weekly until day 64. The animals were killed by decapitation on day 70, with blood collected for measurement of blood Pb, plasma sodium, potassium, zinc, creatinine, renin activity (PRA), and hematocrit. The trunk blood was collected for 10–15 s into prechilled tubes containing EDTA as anticoagulant; it was immediately spun at 4°C and the plasma removed and frozen for later analysis. One kidney and testis was taken for later zinc analysis.

Analytical Methods

Urine and plasma zinc concentrations were determined by flame atomic absorption (Varian) as previously described (*1*). Tissue zinc was measured after digesting the kidney or testis in a muffle furnace and redissolving in 6*N* nitric acid. Blood Pb was measured by flameless atomic absorption (Varian). Plasma sodium and potassium concentrations were measured by flame photometry, and endogenous plasma creatinine was measured by the method of Bonsnes and Taussky (*5*). Plasma renin activity was measured by radioimmunoassay according to previously published methods (*6*). All data were expressed at the mean ± standard error of the mean. Statistical comparisons are by Student's *t*-test for unpaired observations.

Results

Table 1 summarizes all data obtained from the four groups of rats acutely exposed to varying doses of lead administered intravenously. Rats receiving either 0.03 or 0.3 mg Pb/kg showed no difference in zinc excretion from controls for the 6-h collection period. Zinc excretion was significantly elevated in the animals that received the highest tested dose (3.0 mg. Pb/kg). There were no significant differences in urine volume, sodium or potassium excretion, plasma zinc concentration, or body weight between any of the groups.

Urinary zinc excretion for the two groups of chronic animals for the first 42 days of the experiment are summarized in Fig. 1. Baseline zinc excretion was quite stable for the two groups of animals for the 6 days prior to beginning lead treatment. Two days after starting lead treatment, zinc excretion by the lead-treated animals began consistently to exceed that of controls, and this pattern persisted at every measured day through day 35. One major reason for the difference between the two groups during days 8–16 was that the control rats manifested a decrease in zinc excretion (relative to that group's baseline) during this period; the reason for this is unknown. By day 17, zinc excretion of the control animals had returned to pretreatment levels, and the difference between the groups from this day on clearly resulted from increased zinc excretion by the Pb-treated rats.

Because of problems inherent in multiple comparisons and the variability of zinc excretion from day to day, we chose not to do statistical comparisons for each day, but rather to compare total zinc excretion by the two groups of animals for two 6-day periods, the baseline before Pb (or sodium acetate) treatment (experimental days 1–6) and days 10–15 after Pb treatment (experimental days 16–21). As shown in Fig. 2, there was no difference in excretion between the two groups for the

TABLE 1
Effects of Acute Intravenous Injections of Pb Acetate or Sodium Acetate (0 dose) on Urinary Excretion and Plasma Zinc Concentration in Rats[a]

	Pb dose, mg/kg			
	0	0.03	0.3	3.0
$U_{Zn}V$ (μg/6 h)	1.98 ± 0.17	1.90 ± 0.11	2.18 ± 0.26	4.08 ± 0.64[b]
Urine Volume (mL/6 h)	8.1 ± 1.0	7.8 ± 0.8	7.6 ± 0.4	6.2 ± 0.5
$U_{Na}V$ (μmol/6 h)	575 ± 154	518 ± 105	491 ± 57	334 ± 73
$U_{K}V$ (μmol/6 h)	830 ± 141	692 ± 70	899 ± 104	765 ± 93
$[Zn]_p$ (μg/dL)	94.5 ± 9.1	91.9 ± 4.9	93.9 ± 5.7	85.4 ± 9.0

[a] $U_{Zn}V$ denotes urinary zinc excretion; similarly for $U_{Na}V$ and $U_{K}V$.
$[Zn]_p$ = plasma zinc concentration.
[b] $p < 0.01$, compared to 0 dose. $N = 6$ in each group.

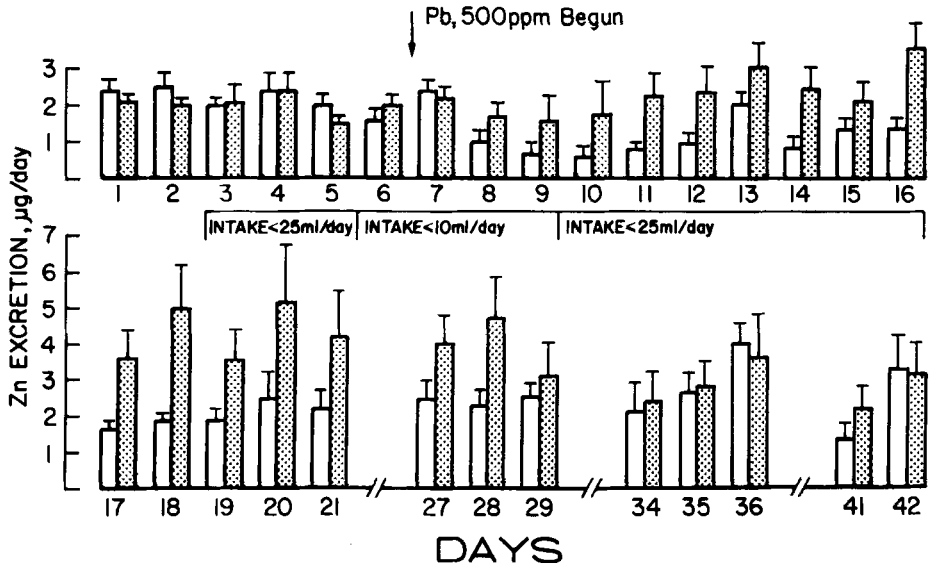

Fig. 1. Mean urinary zinc excretion (± 1 SEM) for control (open bars) and lead-treated animals (hatched bars). Breaks in the *X*-axis indicate periods where no collections were obtained. Changes in the drinking water regimen are shown above the excretion bars: Pb or Na acetate water *ad libitum* was begun at the end of day 6 and continued throughout day 18; water consumption was restricted to 25 mL/day (days 19 > 21 and days 30–42) or 10 mL day (days 22–29). Number of animals = 8 in control group and 7 in experimental group

baseline days, but on days 10–15 after Pb exposure, zinc excretion by Pb-exposed animals was approximately twice as high ($P < 0.05$) as that of the controls.

Figure 3 emphasizes the consistency of the Pb effect; it summarizes the responses of all individual animals by comparing the highest daily rate of excretion exhibited by each animal during the pretreatment (baseline period) and the 30-day treatment periods. Maximal daily zinc excretion in control rats only changed by 0.85 ± 0.33 µg, while in lead-exposed rats the urinary zinc excretion changed by 4.53 ± 0.91 µg. This difference is statistically significant ($P < 0.03$). Figure 3 also demonstrates that some of the control animals exhibited maximal zinc excretion during a baseline day (despite the fact that the baseline period was only 6 days compared to the 36-day treatment period), whereas all lead-exposed animals manifested their maximal urinary zinc excretion during a treatment day.

Because the Pb-exposed animals lowered their water intake, it was possible that the elevated zinc excretion was caused by physiological changes secondary to reduced water intake or urine volume. If this were the case, then restriction of water intake by control animals on days 19–29 should have had the same effect; however, as shown in Fig. 1, urinary zinc excretion was completely unaffected by reduction of water intake to levels even lower (10 mL/day) than the mean value of the Pb-exposed animals (14 mL/day).

Figure 1 demonstrates that the increased zinc excretion of the Pb-exposed rats did not persist beyond the fourth week of treatment. From days 34–36 on, there

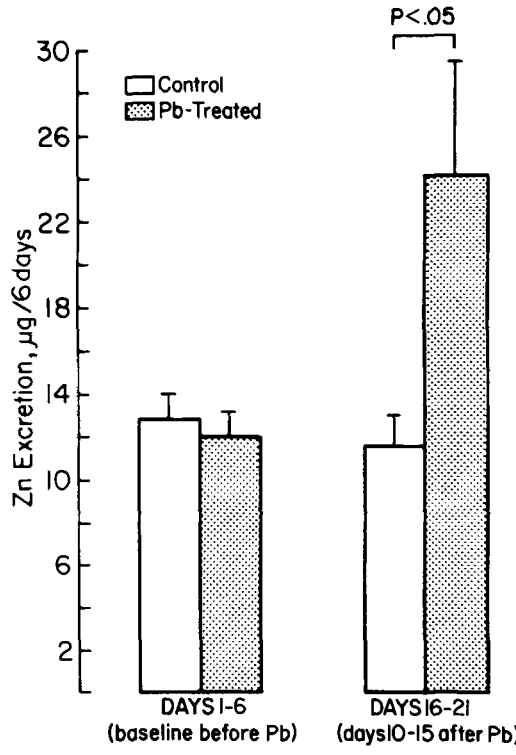

Fig. 2. Total urinary zinc excretion for the 6-day baseline collection period and for the peak-effect 6-day period during Pb exposure. Control animals are shown in open bars, lead-exposed animals in hatched bars.

were no consistent differences between the two groups. A lowered zinc diet was instituted from days 43 to termination to see whether this might magnify any Pb-induced effect on zinc excretion. However, this diet produced no significant change in urinary zinc excretion by either group.

Data obtained at sacrifice (on day 70) are given in Table 2. There were no differences between the two groups in body weight, hemtocrit, plasma concentrations of creatinine, sodium, and potassium, or kidney weight. Plasma zinc concentration was significantly lower in the lead-exposed animals. There was no difference in kidney zinc content; however, testis weight and zinc content were significantly decreased in Pb-exposed animals. Plasma renin activity was approximately doubled in the Pb-exposed animals ($P < 0.05$). Blood lead concentration was 47.6 $\pm$ 1.6 µg/dL in the Pb-exposed animals.

Discussion

The results of the first series of experiments confirmed that rats, like dogs, respond to an acute intravenous dose of 3 mg Pb/kg with a significant increase in urinary Zn excretion. The response occurred within 6 h of the injection and was not associated

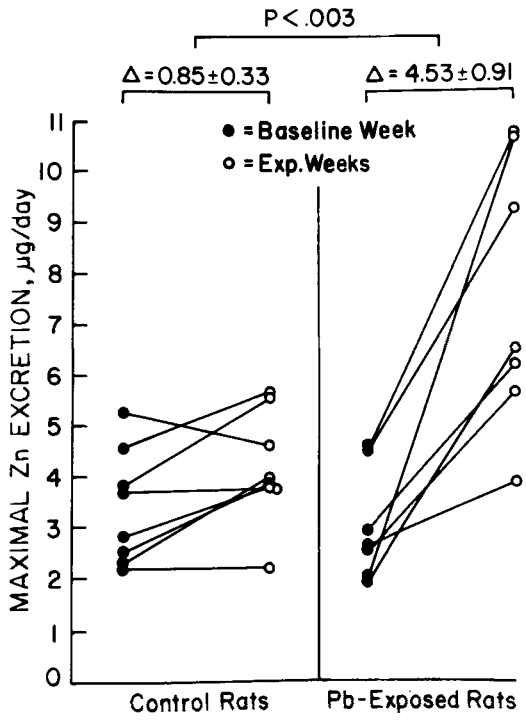

Fig. 3. Individual animals' maximal daily zinc excretion during baseline and experimental days.

with changes in urinary excretion of water, sodium, or potassium. We have previously reported increased excretion of sodium and potassium after Pb injections (*5*), and the reason for failure to observe an increase in the present series is not known. However, one likely possibility is that the use of ether in the present animals altered baseline sodium excretion, since sodium excretion in the present control animals averaged 600 μmol/6 h compared to 250 μmol/6 h in the previous study. The 3 mgPb/kg dose in the previous study increased Na excretion approximately by 400 μmol/6 h, a value quite similar to the present control animals' value. The very high baseline excretion may have precluded any further increase in electrolyte excretion.

The results of the long-term experiments demonstrate that chronic exposure to 500 ppm Pb, a dose that does not produce renal damage (*7, 8*), can also produce a significant increase in urinary zinc excretion. The change in zinc excretion is delayed for several days and is limited in duration (it appears to have run its course by the fourth week of treatment). The mechanism of this hyperzincuria was not studied in the present experiments. Our previous experiments with dogs (*1*), utilizing acute exposure to high doses of Pb, demonstrated two contributory effects: (1) increased plasma concentration of ultrafilterable zinc; (2) alteration of proximal tubular transport of zinc (either increased secretion or decreased reabsorption). The latter effect, perhaps representing interaction of the two metals at a common trans-

TABLE 2
Effects of Chronic Pb-Exposure on Various Blood and Tissue Parameters in Rats[a]

	Control (n = 8)	Pb (500 ppm) (n = 7)
Hematocrit	46 ± 0.8	45 ± 2.4
Blood [Pb], μg/dL	1.7 ± 0.5	47.6 ± 1.6
Plasma [Na], mM	142 ± 0.6	141 ± 1.2
Plasma [K], mM	6.8 ± 0.1	6.5 ± 0.2
Plasma [Zn], μg/dL	114 ± 6.5	92.7 ± 5.4[b]
Plasma [creatinine], μg/mL	5.1 ± 1.1	4.9 ± 0.5
Plasma renin activity, ng/mL/h	9.1 ± 1.3	18.4 ± 4.1[b]
Body weight, g	485 ± 10.3	486 ± 11.2
Kidney weight, g	1.41 ± 0.04	1.47 ± 0.06
Kidney Zn, μg	43.1 ± 1.0	42.8 ± 2.0
Kidney [Zn], μg/g	30.6 ± 0.1	29.2 ± 1.2
Testis weight, g	1.75 ± 0.02	1.63 ± 0.04[b]
Testis Zn, μg	56.1 ± 1.3	48.8 ± 1.77[c]
Testis [Zn], μg/g	32.1 ± 0.8	29.9 ± 0.5[b]

[a]Plasma [K] values are artifactually elevated because of method of sacrifice (decapitation).
[b]$P < 0.05$.
[c]$P < 0.01$.

port pathway, seemed to be the predominant one. However, these acute affects need not be the responsible factors in the chronic experiments.

It is probable that the disappearance of the hyperzincuria after approximately 1 month was the result of triggering the homeostatic responses that lower zinc excretion in response to the zinc deficiency developed during that time. Evidence documenting the existence of some degree of zinc deficiency, at least by the end of the experiment, are the decreases in plasma and testis zinc concentrations.

If we assume that, for a 2-week period, urinary zinc excretion was doubled, the "excess Zn excretion" would be 2 μg/day × 14 days or 28/μg. Although 28 μg is only 0.33% of total body zinc in a rat (about 8400 μg in the adult male 350 g rat [9]), it may reflect a much larger percentage of those body stores that are easily mobilized. For example, this amount exceeds the entire plasma zinc content (plasma volume is approximately 5% of body weight and plasma Zn concentration is about 1 μg/mL). We do not mean to imply that increased urinary zinc excretion is the only means by which lead induced zinc deficiency, since oral lead administration may have also decreased gastrointestinal zinc absorption, as documented by El-Gazzar et al. (*3*).

The chronic experiment also offered an opportunity for gaining information concerning possible interactions of lead and zinc on the renin–angiotensin hormonal system, since the rats were sacrificed while on a relatively low zinc diet. We have previously demonstrated (*10*) that chronic exposure to 500 ppm Pb elevated plasma

renin activity; the degree of elevation was essentially the same in the present study, indicating that the degree of zinc deficiency produced did not, itself, affect plasma renin.

What is the relevance of these studies for human populations? The blood Pb concentration of these rats (drinking 500 ppm Pb) averaged 47.6 μg/dL, a value exhibited by many urban dwellers and workers exposed to lead. Two previous studies documenting decreased tissue zinc in Pb-exposed rats utilized even lower doses of 200 ppm (*4*) and 100 ppm (*3*); mean blood Pb concentration in the 200 ppm study was 45 μg/dL. Clearly, it will be important to test even lower doses, since all studies are consistent with the possibility that some degree of zinc deficiency might be a consequence of relatively low levels of lead exposure in human beings.

Acknowledgments

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