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Comparative Toxicity to Rats of Lead Acetate From Food or Water

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Lead is an ubiquitous contaminant of food and water supplies. Under some circumstances, this contamination can be quite severe, as described in case reports of clinical lead poisoning in which food (BERITIC & STAHEDLJAK 1961) or water (BACON et al. 1967, COVELL 1975, WILSON 1968) was the source of lead. It is not clear at present if lead is more toxic when added to food or to water because literature relating to this specific problem is limited (CASTLES et al. 1976, RABINOWITZ et al. 1975). Because of the general effects of food intake on reduction of lead absorption in humans (RABINOWITZ et al. 1975), it is possible that lead-contaminated water is more toxic than lead-contaminated food. The purpose of this study was to determine if equal concentrations of lead acetate added to food or to water were equally bioavailable to rats.

MATERIALS AND METHODS

Animals and experimental design: Forty-five male albino Sprague-Dawley rats (Blue Spruce Farms, Altamont, NY) were divided into three groups and fed a nutritionally adequate, purified diet (AIN-76, AMERICAN INSTITUTE OF NUTRITION 1977) prepared by Ziegler Brothers, Gardners, PA, for 6 weeks. Groups I and II received the standard diet and Group III received the diet supplemented with 200 µg lead as lead acetate/g. Rats in Group II received a drinking solution containing 200 µg lead acetate/ml. Rats in Groups I and III received deionized water. Analysis of the diets by atomic absorption spectrophotometry showed lead concentrations of 209.7±26.2 µg/g (mean ± standard deviation) and <2 µg/g for the lead-supplemented and control diets, respectively. Iron content of the diets was 50 µg/g and calcium content was 0.54%.

The rats were housed individually in stainless steel cages. Food and water were supplied ad libitum; consumption of both was measured throughout the experimental period. Twenty-four-hr urine collections for measurement of δ-aminolevulinic acid (δ-ALA) were made during the fifth week of the study. Urine was collected in foil-covered 50 ml Erlenmeyer flasks containing 0.25 ml glacial acetic acid. Samples were stored in the dark at 5°C for 1 week before assay.

At the time of sacrifice, rats were anesthetized with Nembutal (50 mg/kg body weight) and blood was collected by cardiac puncture. Vacutainers from lots previously established as not being

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contaminated with lead above 0.1 μg were used for collection of blood samples for lead analysis. Kidneys and femurs were obtained by dissection, freed of adhering tissues, and fresh weights were obtained.

Analytical methods: Urinary δ-ALA acid was determined by the method of DAVIS & ANDELMAN (1976). Free erythrocyte protoporphyrins (FEP) were measured by the method of PIOMELLI (1977). Complete blood counts and differential white cell determinations were performed. Determination of lead in blood, kidney, and femur samples was carried out by anodic stripping voltammetry using a Trace Metals Analyzer (Environmental Sciences Associates, Bedford, MA).

Histological methods: Three-four mm cross-sectional slices of whole kidneys were fixed in buffered formalin. Sections were prepared, stained with hematoxylin and eosin, and evaluated for the presence of intranuclear inclusion bodies in the renal proximal tubule lining cell.

Statistical methods: The Student *t*-test was used to determine statistical significance of differences between mean values. A test for homogeneity of regression lines (OSTLE & MENSING 1975) was performed to determine if relationships between blood lead and urinary δ-ALA and between blood lead and FEP were equivalent for data obtained from rats receiving lead in food or in drinking water.

RESULTS

Food consumption, water consumption, and weight gain: Lead-related differences in food and water consumption were observed in both groups of lead-exposed rats (Table 1). Food consumption was reduced significantly in both groups of lead-treated rats compared with controls. Lead ingestion at this concentration did not impair food utilization; the ratio of food consumed per unit of weight gain was not significantly different among the three groups. Rats exposed to lead in drinking water consumed significantly less water than rats exposed to lead in food. Rats fed the lead-supplemented diet consumed slightly less lead than rats whose drinking water was supplemented with lead.

Hematological effects: Hemoglobin values in rats exposed to lead-supplemented water were significantly lower ($p<0.05$) than those of rats consuming a lead-containing diet (Table 1). Hematocrit values were significantly reduced in both groups of lead-treated rats when compared with control values. Erthrocyte counts (RBC X 10^6) were 7.40 ± 0.45, 7.28 ± 0.49, and 7.58 ± 0.49 for Groups I, II, and III, respectively. Corresponding values for total white cell counts (WBC X 10^3) were 4.5 ± 2.1, 5.5 ± 2.3, and 5.7 ± 2.3 for Groups I, II, and III, respectively. RBC and WBC counts for the lead-exposed animals (Groups II and III) were not significantly different from those of the control group (I).

Effects on lead levels in blood, femur, and kidney: Blood lead levels were markedly elevated after 6 weeks of exposure to food or

TABLE 1

Weight gain, food and water consumption, hematological values, and tissue lead concentration in rats exposed to lead in food or in drinking water[a]

Parameter	Group I Control	Group II 200 µg Pb/ml water	p	Group III 200 µg Pb/g food	p	p II vs III
Initial body wt, g	90 ± 7	90 ± 9	NS[b]	90 ± 8	NS	NS
Final body wt, g	322 ± 25	304 ± 26	<0.05	288 ± 24	<0.0005	<0.05
Femur wt, g	0.87 ± 0.09	0.83 ± 0.06	NS	0.76 ± 0.07	<0.005	<0.05
Kidney wt, g	1.31 ± 0.12	1.49 ± 0.23	<0.01	1.34 ± 0.16	NS	<0.05
Food intake, g	809 ± 56	735 ± 90	<0.01	714 ± 90	<0.0025	NS
Water intake, ml	993 ± 115	836 ± 95	<0.0005	979 ± 259	NS	<0.05
Calcd lead intake						
from food, mg	1.6 ± 0.1	1.5 ± 0.2	NS	150 ± 19	<0.0005	
from water, mg	<0.0001	167 ± 19	<0.0005	0.0001	NS	<0.0125
Hemoglobin, g/dl	14.8 ± 0.6	13.5 ± 1.0	<0.0005	14.2 ± 0.9	NS	<0.05
Hematocrit, %	41.8 ± 1.5	36.1 ± 3.3	<0.0005	37.0 ± 2.6	<0.0005	NS
FEP, µg/dl	14 ± 1	21 ± 7	<0.0025	23 ± 6	<0.0025	NS
δ-ALA µg/24 hr	64 ± 15	362 ± 207	<0.0005	369 ± 200	<0.0005	NS
Blood lead, µg/dl	<3	68 ± 40		72 ± 22		NS
Femur lead, µg/g	0.28 ± 0.18	157 ± 56	<0.0005	206 ± 50	<0.0005	<0.01
Kidney lead, µg/g	0.14 ± 0.09	13 ± 2	<0.0005	13 ± 1	<0.0005	NS

[a]Values represent mean ± standard deviation; N=15 animals per group. p values estimated by t-test.
[b]NS=not significant at 0.05 probability level.

water containing equal concentrations of lead. There was no significant difference between blood lead concentrations resulting from lead ingested from food or from water (Table 1).

Marked increases in renal size (as percent of body weight) were apparent in the rats in Groups II and III. Kidney weight was 0.40±0.02% of body weight in control animals. Corresponding values for rats exposed to lead in water or food were 0.49±0.05% (significantly different from control value, $p<0.0005$) and 0.47±0.07% ($p<0.0005$), respectively.

Lead accumulation in kidney tissue was comparable in both lead-treated groups (Table 1) and represented an approximate 100-fold increase over renal lead values for control animals. Lead accumulation in the femur was significantly greater in rats exposed to lead in food than in rats ingesting lead in drinking water.

δ-ALA and FEP: δ-ALA and FEP were significantly elevated in both groups of lead-treated rats; no significant differences were observed between Groups II and III (Table 1).

Histological observations: No animals in Group I exhibited intranuclear inclusion body formation or any significant degenerative changes of any kind. Group II rats exhibited rare lead inclusion body formation in 6 of the 15 animals. These animals also exhibited occasional focal accumulations of chronic inflammatory cells and occasional cloudy swelling of tubular cells. Five of the 15 rats receiving lead in the water (Group III) exhibited rare intranuclear inclusion body formation. Inflammatory cell changes and cloudy swelling were observed in this group similar to those seen in the group receiving lead in food (Group II).

Relationships between FEP, urinary δ-ALA, and blood lead: Relationships between FEP and blood lead and between urinary δ-ALA and blood lead are shown in Figures 1A and B, respectively. Individual FEP values are expressed as FEP/hemoglobin ratios as suggested by PIOMELLI (1977). Statistical analysis indicated that a single regression line could be fitted to each set of values. The r values for FEP/Hb vs blood lead and for urinary δ-ALA vs blood lead are 0.66 and 0.78, respectively.

DISCUSSION

Overall, the results of this study demonstrate no marked differences in the bioavailability to the rat of lead added as lead acetate to food or water. For some individual parameters, such as femur lead concentration and hemoglobin, differences between animals exposed to lead via water or food could be observed. However, these changes did not indicate consistently greater availability of lead from food or from water. Changes which are more directly indicative of lead toxicity did not differ between the two routes of lead exposure, for example, renal pathology, renal and blood lead concentrations, and most hematologic indices.

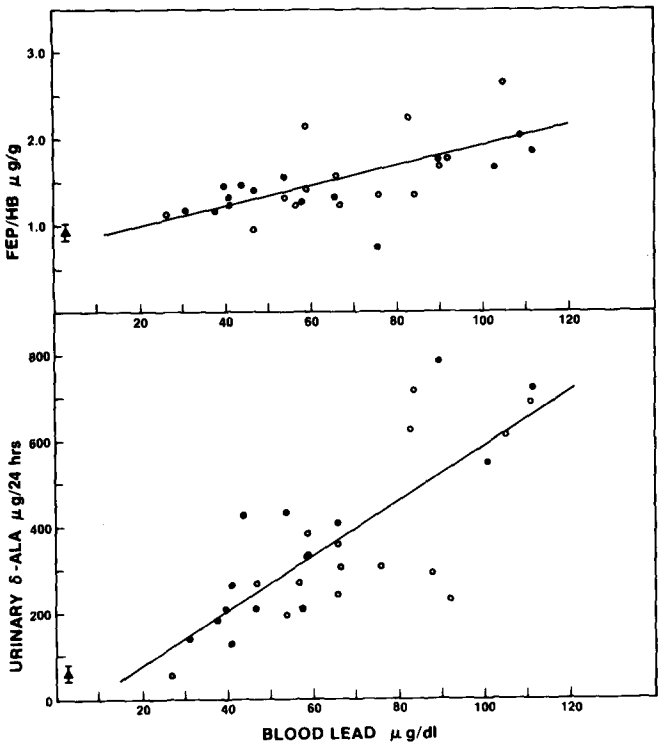

Figure 1. Relationship between FEP/Hb and blood lead (A) and urinary δ-ALA and blood lead (B) in rats receiving lead acetate in food or in drinking water for 6 weeks. ●, Group II: 200 µg Pb/ml drinking water; O, Group III: 200 µg Pb/g food. Values for control group (▲): blood lead <3 µg/dl, FEP/Hb 0.97± 0.08 µg/g, urinary δ-ALA 64±15 µg/24 hr.

Urinary δ-ALA was the most sensitive indicator of lead effect examined, and displayed a strong correlation (r=0.78) with the blood lead level. Significant elevations in FEP were also observed, but the magnitude of the changes was considerably less than that observed with changes in urinary δ-ALA. In addition, the correlation between FEP/Hb and blood lead was poorer (r=0.66) than the correlation between urinary δ-ALA and blood lead.

Although marked increases in FEP are known to occur following chronic exposure to lead (PETER & REYNOLDS 1978, SASSA 1978, EISINGER 1978, EISINGER et al. 1978), less information is available on elevation of FEP caused by more acute lead exposure. Protoporphyrins incorporated into reticulocytes in the course of hematopoieses are retained in the erythrocytes for the duration of their lifespan. As such, increases in FEP content of erythrocytes reflect the level of lead present during erythropoiesis averaged over the lifespan of the red cell (EISINGER 1978, SASSA 1978). In

rats, the lifespan of the red cell is 45-68 days. The 42-day duration of the exposure of rats to lead in the present study was probably insufficient to cause the pronounced elevations in FEP observed in chronically lead-intoxicated monkeys (KNEIP et al. 1976), although species differences cannot be discounted. Increases in FEP are a function of lead dosage as well as duration of exposure. SASSA et al. (1973) and ALVARES et al. (1972) observed increased FEP levels in rats fed lead acetate in their food for 5 weeks; however, these animals received considerably more lead (20 mg Pb/g diet) than did the rats used in the present study (0.20 mg Pb/g diet).

In this study the bioavailability of lead organically incorporated into food was not evaluated. Literature on this topic is sparse, but CASTLES et al. (1976) reported that lead incorporated *in vivo* into oysters was as bioavailable to rats as lead acetate added to the animal's diet at equal concentrations. Whether lead in other plants or animals differs in bioavailability is not currently known.

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