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Lead Dosage and the Role of the Intranuclear Inclusion Body

An Experimental Study

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Biological parameters known to be affected in lead poisoning were measured in rats following ingestion of graded dosages of lead. Intranuclear inclusion bodies are formed in renal tubular lining cells with smaller doses of lead than produce other changes. Decreased body weight is the next most sensitive abnormality. This is followed by increased delta-aminolevulinic acid (ALA) excretion, reticulocytosis, renal edema, and aminoaciduria. Anemia only occurs at the highest lead dosage. Over a wide range of lead ingestion, urinary lead excretion remains constant, although renal lead content increases. Quantitative lead analyses of cell organelles show that lead is concentrated within the inclusion bodies. Relatively small amounts of lead are present in the cytoplasm and mitochondria. It is suggested that soft-tissue lead accumulates in the intranuclear inclusion body, thereby sparing toxic injury to cytoplasmic organelles.

THE METABOLIC effect of trace quantities of lead derived from environmental exposure is not completely understood. It is not known whether a small amount of lead in body stores, that is, the body burden of lead, is in some way harmful or subclinically toxic.[1,2] There is no doubt that lead is potentially lethal to humans and experimental animals, and the clinical manifestations of overt toxicity are well defined. However, when compared to the effects of other heavy metals such as cadmium and mercury, clinical lead toxicity only occurs upon exposure to relatively large dosage. In addition, there is no clearly recognized biological requirement for lead. If a large amount of lead in body tissues is overtly harmful and none is required, accumulation of even trace amounts in body tissues may be undesirable or detrimental to optimal health. However, such a subclinical effect is yet to be defined. Moreover, any subclinical effect is unlikely to be recognized until a better understanding of the metabolism of lead is obtained, particularly in terms of its effects on cellular organelles and metabolism.

The need to attain this level of understanding is becoming increasingly urgent. Patterson estimates that man, by mining and industrial usage, introduces approximately 100 times more lead into the environment annually than would occur natural-

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Table 1.—*Renal Retention and Urinary Excretion of Lead by Rats Given Varying Doses of Lead in Drinking Water for Ten Weeks*

Animal Groups: Lead Content of Drinking Water (mg/ml)	Lead Ingested (mg/Day/Rat)*	Renal Lead (mg/gm Wet Weight)	Urinary Lead Excretion (μg/Day/Rat)†	% of Ingested Lead Excreted in Urine
Control group	0.006	0.5	7.0	...‡
0.08	2.3	0.8	28.0	1.21
0.20	7.0	2.3	65.0	0.93
0.40	12	8.1	60.0	0.50
1.20	21	30.4	64.0	0.31
4.00	67	36.6	60.0	0.07
10.00	220	276	132.0	0.06

* Volume of drinking water × lead content: average daily intake measured for one week for each group of rats.
† Twenty-four hour urine samples from all rats of each group were pooled before analysis.
‡ See text for explanation (Results).

ly. This is reflected in body tissue levels. Recent surveys indicate that present day blood levels of the North American adult averages 0.25μg of lead per milliliter rather than an inferred natural level of 0.0025μg of lead per milliliter. Blood lead levels of persons with clinically recognizable lead poisoning are only slightly higher, 0.5μg to 0.8μg of lead per milliliter. The question exists, therefore, whether man has some threshold of tolerance to lead and, if so, what is the nature of such a mechanism and how can its limits be recognized.[2]

The present report is an experimental study of the sensitivity of various biological parameters in the rat to exposure to graded doses of lead. Particular interest is given to the kidney where the accumulation of lead is associated with the formation of intranuclear inclusion bodies and renal tubular dysfunction.

Materials and Methods

Experimental Design.—There are two aspects of the study. The first portion deals with the sensitivity of a number of biological parameters known to be abnormal in lead poisoning. Forty-two white, male Sprague-Dawley rats, weighing 150 gm to 175 gm, were divided into seven groups of six rats. All rats were caged in pairs and fed standard laboratory chow.

Six groups were given tap water containing the following quantities of lead in the form of lead acetate: 0.08, 0.20, 0.40, 1.20, 2.00, and 10.0 mg of lead per milliliter. The control group received tap water without added lead. The animals were weighed weekly and 24-hour urine collections were made prior to killing at 10 weeks of age. The animals were anesthetized with pentobarbital (5 mg/100 gm body weight) and exsanguinated by cardiac aspiration into a heparinized syringe. Both kidneys were quickly excised, weighed, and a small portion of cortex was fixed in 2.5% glutaraldehyde with 0.1 M sodium cacodylate. The remainder of the kidneys was frozen at −20 C until analyzed for lead and β-glucuronidase.

The second phase of the experiment concerns measurement of lead content of organs and organelles in young-adult, white, Sprague-Dawley male rats given pulverized 1% lead as lead acetate mixed with powdered laboratory chow for about four months. Similar rats fed the same chow without added lead served as controls. Both groups were given tap water for drinking.

Analytical Methods.—Packed red-cell volumes were determined by centrifugation of capillary tubes of heparinized blood using a microhematocrit centrifuge.

Reticulocytes on blood smears were stained with 0.5% methylene blue. The dye was mixed with heparinized blood and the stain in a capillary tube. Blood cells and stain were allowed to stand for ten minutes before making smears on glass slides. At least 2,000 red blood cells (RBC) were counted on slides from each rat.

Urinary delta-aminolevulinic acid (ALA) was determined by the method of Davis employing prefilled, disposable exchange columns.[3]

Urinary α-amino nitrogen was determined by totaling the individual amino acids which had been separated and measured by ion-exchange column chromatography employing an automatic amino-acid analyzer with 120 × 0.6-cm column containing cation exchange resin. Prior to analysis, proteins were precipitated from 0.025 of a 24-hour urine sample by adding an equal amount of 10% sulfosalicylic acid and centrifuging in the cold (4 C). Distortion of the

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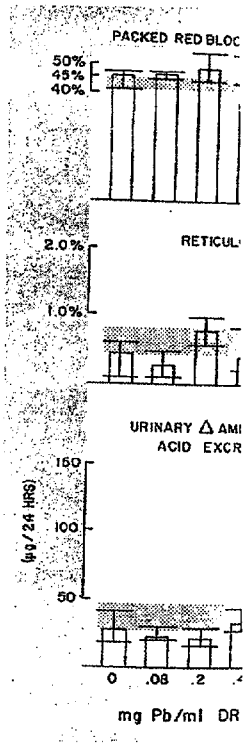


elution pattern of amino acids by the presence of lead remaining in the urine was corrected by adding 0.2% edetic acid (EDTA) to the washing solution.

Lead was measured by wet digestion of tissue and reaction with dithizone according to the method of Bessman and Layne.[4] Fractionation of cells for isolation of organelles and nuclear inclusion bodies was performed by differential centrifugation by methods previously described from this laboratory.[5]

Beta-glucuronidase was assayed by the method of Gianetto and de Duve.[6]

Results

The relationship of increasing ingestion to renal retention and urinary excretion of lead is shown in Table 1. For the control group, urinary excretion of lead slightly exceeds the amount ingested with drinking water. Environmental lead in laboratory chow ingested by this group must account for the relatively large urinary excretion. Balance studies on humans ingesting environmental quantities of lead have shown that only 5% to 10% of ingested lead is absorbed from the gastrointestinal tract.[7]

Animals given increased doses of lead excrete a very much smaller fraction in their urine—the more lead ingested, the smaller the percent appearing in the urine. However, the actual amount of lead appearing in the urine remains constant over a wide range of increased lead ingestion (0.20 mg to 4.00 mg of lead per milliliter of drinking water). This suggests some limit to the amount of lead excreted by the kidney. The amount absorbed, however, must increase with quantity ingested, since retention by the kidney increases with dosage.

The result of increasing doses of lead on hematological parameters is shown in Fig 1. The packed red-cell volume appears to in-

Fig 1.—Hematologic effects of increasing doses of lead in drinking water fed to rats for ten weeks.

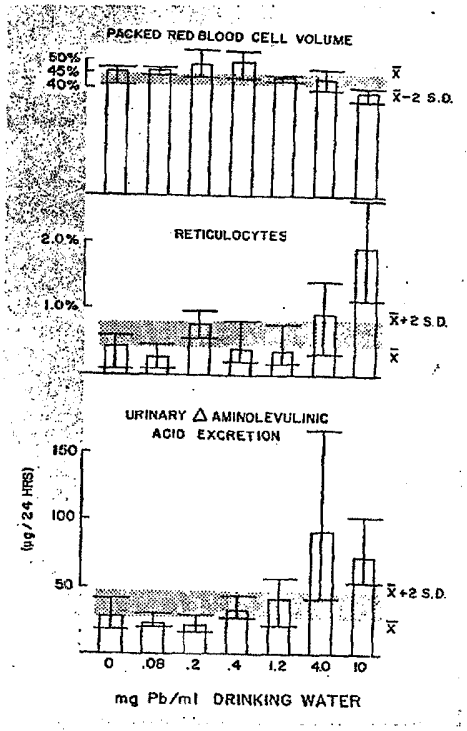
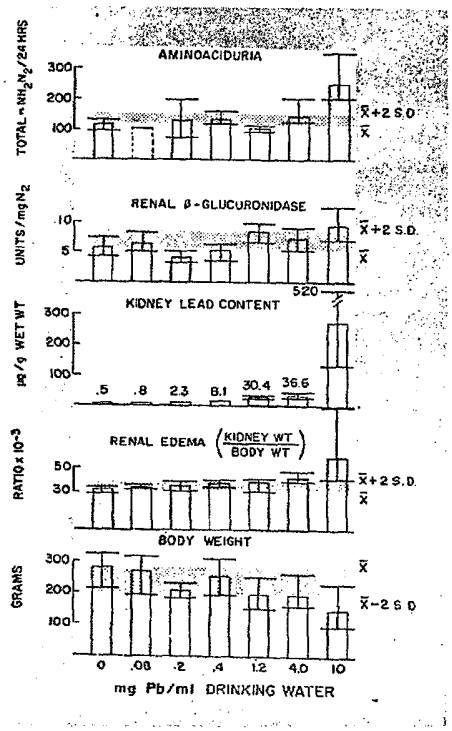


Fig 2.—Renal effects and body weight resulting from increasing doses of lead in drinking water fed to rats for ten weeks.



crease in rats receiving small doses of lead, but declines at higher dosage levels. At 4.0 mg of lead per ml of drinking water, reticulocytes and ALA excretion are increased, whereas significant anemia only occurred at the highest level of lead ingestion.

A number of other parameters which reflect injury to the kidney and effect on body weight are presented in Fig 2. Amino-acid excretion was more variable in the lead-fed rats than in the control animals, but is not constantly elevated except in the rats receiving the highest dosage of lead.

A few rats receiving 1.2 and 4.0 mg of lead per ml of drinking water had elevated renal β-glucuronidase levels, and even the rats in the groups receiving the highest dose showed some overlap with controls. Increments in lead content of kidneys and renal edema expressed as

$$\frac{\text{kidney weight}}{\text{body weight}}$$

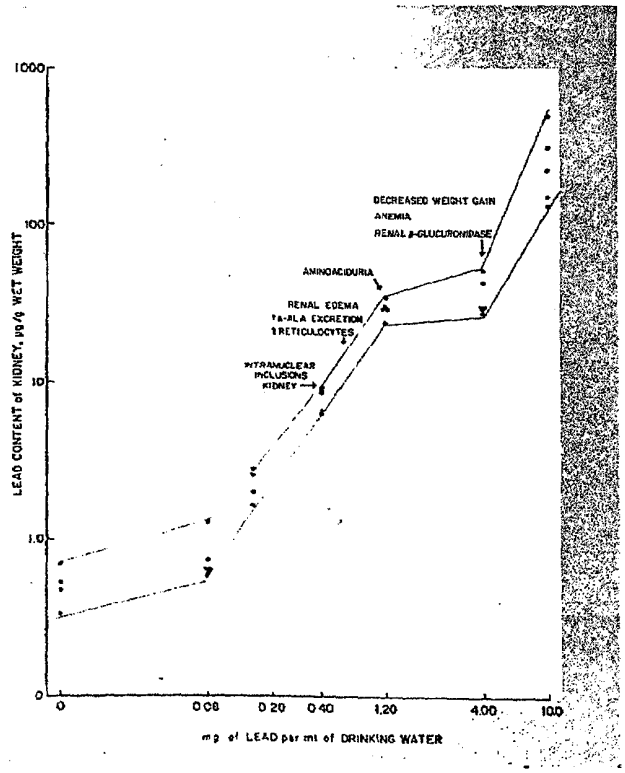
show a gradual progression from control values. Although renal edema commences with low lead dosage, it is not clearly discernible from control levels until lead ingestion reaches 4.0 mg of lead per milliliter of drinking water. Body weight is also quite variable among rats ingesting smaller doses of lead, but rats receiving 1.2 mg lead per milliliter of drinking water have consistently lower than normal body weight.

The expression of lead effects at different dosage levels is related to lead content of kidneys (Fig 3). Apart from irregular increments at doses of 0.08 and 4.0 mg of lead per ml of drinking water, the rise in renal lead content is predictable, although increments at the highest dosage (10 mg of lead per milliliter of drinking water) are quite variable.

The most sensitive parameter of lead poisoning in the rat is the presence of intranuclear inclusions. These bodies, easily recognized on histological examination in the kidneys of rats fed 0.40 mg of lead per milliliter of drinking water, have a characteristic ultrastructural appearance. An electron photomicrograph of an inclusion body is shown in Fig 4. The morphological and histochemical features have been well described in the past, and recent study in this laboratory has shown that most of the lead retained by the kidney in lead poisoning is located within these inclusion bodies.[5]

Other soft tissues in the rat have only a small fraction of the kidney lead concentration (Table 2). Analysis of lead content of organelles obtained by differential centrifugation shows that the concentration of lead in control rats is highest in nuclei in the kidney (Table 3). Increase in organelle lead content, therefore, is not uniform. The mito-

Fig 3.—Correlation of lead content of kidney and different doses of lead fed to rats for ten weeks with expression of lead toxicity.



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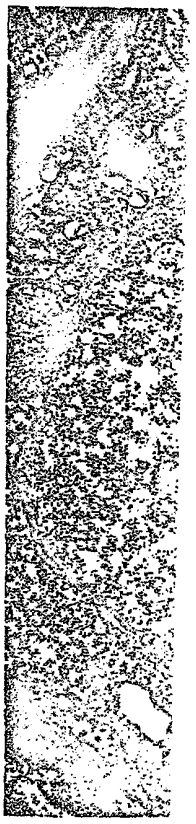

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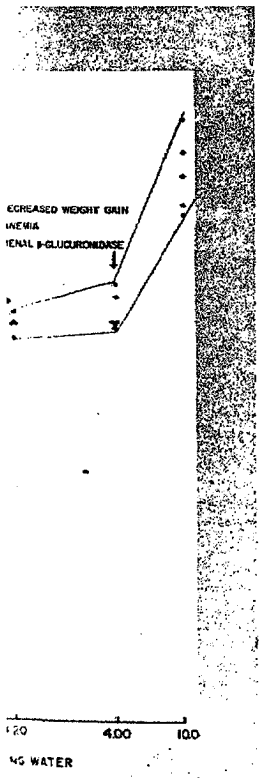

chondrial increment in lead-poisoned rats is only three or four times control values, whereas, the increment in nuclei is about 30 times. Lead per milligram of protein is even more concentrated in the intranuclear inclusion bodies, and suggests that the large increase in nuclear content of lead in kidneys of lead poisoned rats is related to the formation of the intranuclear inclusion bodies.

Comment

The most sensitive index of increased ingestion of lead, apart from actual urinary lead content, is the formation of intranuclear inclusion bodies. The clinical usefulness of this parameter is obviously very limited. It is possible, however, to recognize these inclusions in urine sediment from persons with clinical lead poisoning.[8] Increased urinary ALA excretion and reticulocytosis occur at the same dosage of lead as does renal tubular dysfunction manifested by amino-aciduria and renal edema. Similar levels of sensitivity to lead by the hematopoietic and renal systems may be only coincidental. However, both femur (containing bone marrow) and kidney contain higher concentrations of lead than other organs in the rat and, perhaps even more important, the biochemical manifestations in both organs are related to a common organelle, the mitochondrion. Evidence for involvement of the mitochondrion in the anemia of lead poisoning comes from two sources. Delta-aminolevulinic acid, a precursor of porphyrin,

Fig 4.—Nucleus of proximal renal tubular-lining cell from kidney of rat ingesting water containing 10.0 mg of lead per ml for 10 weeks. Inclusion body (arrow) contains dense staining central core surrounded by fibrillary outer core (× 14,000).

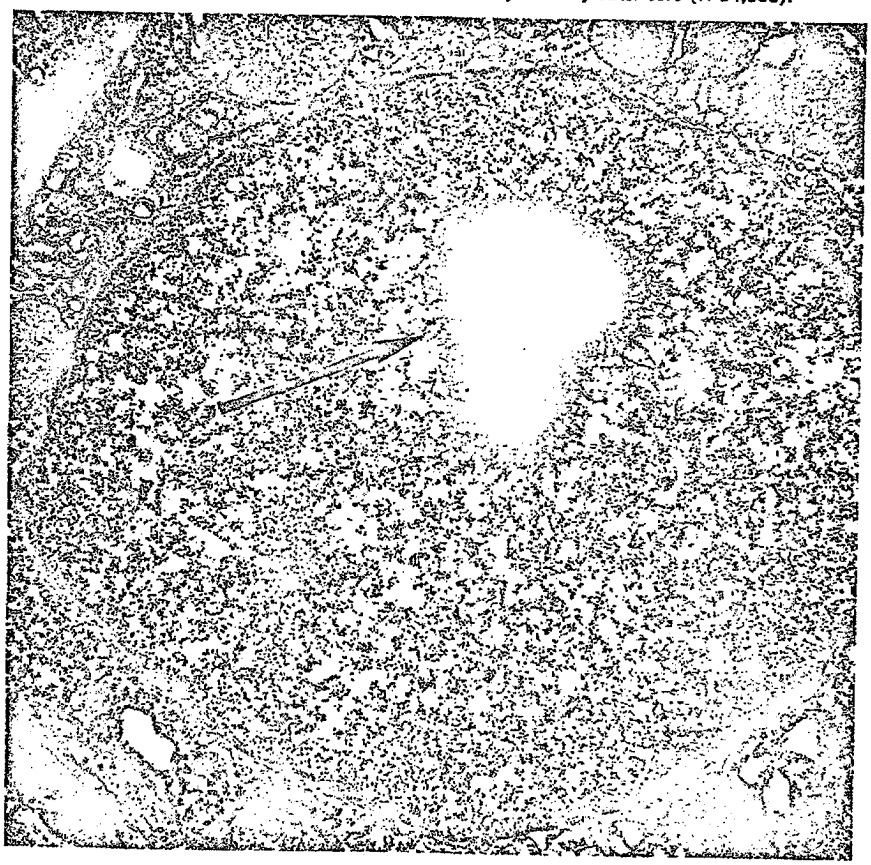

Table 2.—*Lead Content* of Various Tissues in Control and Lead-Poisoned Rats*

Tissue	Control Rats	Rats Fed 1% Lead Acetate 15 to 17 Weeks
Femur	2.7	731
	2.4	666
Kidney	0.8	84
	1.0	54
Liver	0.4	5.5
	0.4	4.8
Heart	1.7	0.7
	1.4	0.9
Brain	0.4	1.8
	0.4	1.9
Blood (μg/ml)	0.1	1.8
	0.2	1.6

* Wet weight; μg of lead per gm of tissue.

Table 3.—*Lead Content* of Cellular Organelles From Rat Kidney*

	Control	Rats Fed 1% Lead Acetate 15 to 17 Weeks
Whole kidney homogenate	0.0016-0.016(8)	0.53-1.10(4)
Mitochondria	0.034-0.056(3)	0.13-0.16(2)
Nuclei	0.3-0.6(2)	11.5-15.7(4)
Isolated inclusion bodies	...	49.2-68.5(4)

* Content, μg of lead per mg of protein.

is synthesized within the mitochondrion of the reticulocyte, and increased ALA excretion is thought to reflect impairment by lead of ALA dehydrase.[9] The function of the enzyme, as well as other steps in heme synthesis, is dependent on intact mitochondrial respiration.[10] Also, respiration of reticulocytes from bone marrow is decreased in lead-poisoned rats.[11] Renal tubular dysfunction in lead poisoning is also believed to be related to impairment of mitochondrial function.[12-14]

Decrease in rate of body-weight gain is another sensitive indicator of the metabolic effects of lead. Possible contributing factors include decreased intestinal absorption, uncoupled oxidative phosphorylation, and excessive loss of urinary amino acids. The latter two defects are clearly shown to be operative in rats receiving larger doses of lead, but changes in these parameters are not descernible at the small doses at which decreased weight gain becomes apparent.

The pathology of lead poisoning in rats and humans is comparable with respect to the hematopoietic and renal organ systems. In humans, however, the central nervous system (CNS) is also very sensitive to the toxic effects of lead, particularly in children in whom an acute encephalopathy and coma are likely to be manifestations of acute intoxication. The severity of the CNS disease in humans is likely to preclude the development of chronic renal disease. However, it has been suggested that acute childhood lead poisoning may influence the development of chronic renal disease later in life.[15] This relationship is not confirmed in another similar study.[16]

Correlations between biochemical and clinical manifestations of lead poisoning have been conducted in humans particularly for the purpose of improving early diagnosis of lead poisoning among lead industry workers. Increased ALA excretion and urinary lead content are both useful indicators of early lead intoxications.[17,18] Also, the occurrence of amino-aciduria in acute lead intoxication in children is well documented,[19] and Clarkson and Kench have demonstrated varying degrees of amino-aciduria in a group of lead factory workers.[20] However, the relationship of renal tubular dysfunction with a parameter of heme metabolism has not to our knowledge been studied in humans.

The urinary excretion of lead remains relatively stable over a wide range of lead dosage. The amount retained by the kidney progressively increases over this dosage range. Also, the increased retention of lead appears to be related to formation of intranuclear inclusion bodies. Recent studies have shown that renal excretion of lead occurs by two mechanisms, glomerular filtration and transtubular excretion.[21] Lead entering tubular-lining cells from adjacent capillaries is excreted into tubular fluid. The stability of the lead content of urine suggests that the amount of lead actually excreted by these renal routes is limited although renal content increases. Measurement of lead content in organelles shows that the concentration within the nucleus increases more rapidly than within whole kidney homogenate or a subcellular mitochondrial fraction. Studies on the composition of the intranuclear inclusion bodies have shown that they are composed of a lead-protein complex, although the nature of the protein is at present

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unknown.[5] Nevertheless, it must function by accumulating lead within the nucleus, thus minimizing the concentration of lead within the cytoplasm.

A corollary of this hypothesis is that mitochondria have a limited tolerance to lead. Mitochondria isolated from rats fed 1% lead acetate for ten weeks or longer show partial impairment of respiratory and phosphorylative abilities.[14] The present study shows that preparations of mitochondria isolated from rats fed a similar diet for a longer period contain 0.13μg to 0.16μg of lead per milligram of protein. This amount of lead associated with mitochondria must be near the upper limits compatible with function. Recent studies in this laboratory have shown that the adenosine diphosphate-(ADP) stimulated respiration of mitochondria isolated from kidneys of normal rats is completely inhibited when incubated in vitro for ten minutes with 0.15μg to 0.20μg of lead per milligram of protein. Intranuclear complexing of lead must therefore limit transport or transtubular flow of diffusible lead. Toxic injury to cytoplasmic organelles is thereby spared. This mechanism may be important in man's ability to tolerate continued exposure to trace or environmental quantities of lead.

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Nonproprietary and Trade Names of Drug

Pentobarbital—*Nembutal.*

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