Aminoaciduria in Experimental Lead Poisoning[1] (35140)

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Excessive aminoaciduria occurs in humans with lead poisoning (1–3) and experimentally in rats (4) and in rabbits (5). The aminoaciduria has been characterized as "generalized," probably secondary to impaired renal tubular reabsorption and usually accompanied by glycosuria and hyperphosphaturia (3). A renal tubular etiology of the aminoaciduria is supported by morphological studies. Electron microscopy of the kidney in humans (6) and experimental animals (7–9) has shown that the proximal tubular lining cells are the principal site of injury in acute lead poisoning.

Previously reported studies of urinary amino acid excretion in lead poisoning are limited to measurement of total alpha amino nitrogen excretion or paper chromatographic quantitation of individual amino acids. The present study is a report of the urinary excretion and renal clearances of individual amino acids by ion-exchange column chromatography in lead-poisoned rats. The purpose of this study, in addition to determining renal clearances of amino acids, is to characterize any specific abnormalities in the metabolism of individual amino acids.

Methods. Experimental design. Aminoaciduria was measured in two different groups of rats fed a diet containing 1% lead as lead acetate mixed with pulverized laboratory rat chow. All animals were male Sprague–Dawley rats weighing 150–200 g at the start of the experiment.

Group I. For this experiment 13 rats were fed the lead-containing diet and 9 rats fed the same diet without lead acetate served as controls. A single lead-fed and a control rat were sacrificied after 3, 4, 5, 8, 10, 12, and 20 weeks. Three lead-fed and one control rat were sacrificed after 6 and 16 weeks. The rats were placed in metabolism cages 24 hr prior to sacrifice and urine was collected in the presence of thymol crystals as a preservative.

Group II. For the second experiment three rats similar to those used in Group I were fed the same lead-containing diet for 10 weeks. Three other rats served as controls. For the last 7 days of the study, the experimental and control rats were pair-fed in metabolism cages. Urine was collected for the final 24-hr period. The rats were then anesthetized with pentothal (5 mg/100g body weight) and exsanguinated by cardiac puncture. Blood was collected in heparinized syringes and the plasma separated.

Amino acid analysis. Measurement of plasma and urinary amino acids was performed with a Technicon amino acid analyzer using a 21-hr gradient-elution technique. Urine samples were centrifuged and 1/20 of the 24-hr volume was adjusted to pH 2 and applied to the column. Distortion of the elution pattern of the amino acids by the presence of lead in the urine was corrected by adding ethylenediaminetetraacetic acid (EDTA) to the washing solution. Plasma samples were deproteinized with sulfosalicylic acid (10).

Results. The excretion of individual amino acids from 13 lead-intoxicated rats and 9 control rats is summarized in Fig. 1. The period of time each rat was fed the lead-containing diet varied from 2–20 weeks. The total alpha amino nitrogen excretion was determined by summing the amino acid excretion from all 13 rats. The urinary alpha amino nitrogen excretion during the experiment has been correlated with ultrastructural studies of renal morphology and published elsewhere (9). Alpha amino nitrogen excretion for 12 of the 13 rats exceeded the 99%

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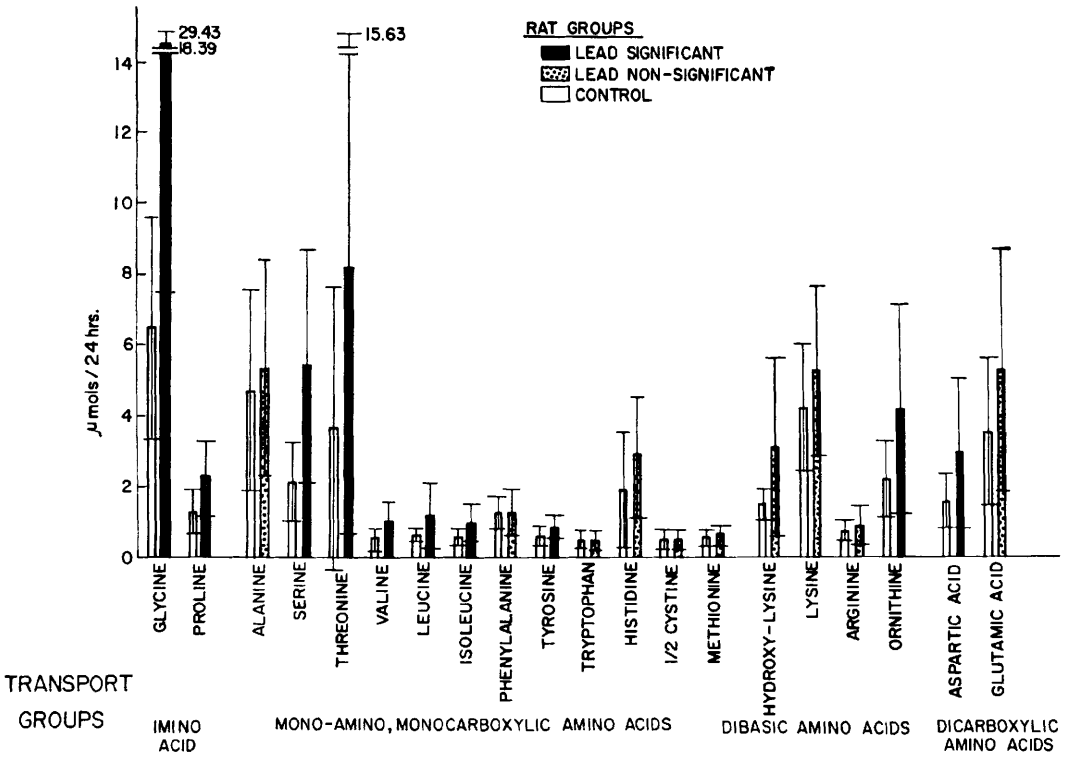

FIG. 1. Twenty-four hour urinary excretion of individual amino acids from 9 rats fed a 1% lead-containing diet and 13 control rats. Values are means ± 2 SD. Those amino acids differing from control values with a probability of less than 0.05 are identified as "lead significant."

confidence levels of excretion for the control rats. The degree of excessive aminoaciduria was approximately two or three times that of control rats.

The results of measurement of the 24-hr excretion of individual amino acids from Group I rats are summarized in Fig. 1. These are arranged in transport groups dependent on similarities in structure as suggested by Milne (11). Nearly all amino acids are excreted in greater amounts in the lead-poisoned rats than in control animals although the urinary excretion of only 10 of the 20 amino acids was increased to the 95% level of significance. Most of these are small neutral monamino, monocarboxylic acids. The increases in ornithine and aspartic acid excretion are also significant, whereas increases in excretion of companion amino acids, lysine and glutamic acid, are only slightly less. The increase in methionine and arginine excretion is slight. No apparent change occurred in the excretion of tryptophan or cystine. Also, there is no apparent relationship between severity of aminoaciduria and time over the 2- to 20-week periods. More recent studies of aminoaciduria after ingestion of the lead-containing diet for 40 and 80 weeks do show a progressive increase in urinary content of aminoacids (unpublished data).

The aminoaciduria from Group II rats is similar to Group I and the result of comparing renal clearances of amino acids in pair-fed lead-poisoned and control rats (Group II) is shown in Fig. 2. Renal clearances for most amino acids in the rat range from 0.0005–0.02 ml/min. Lead poisoning increases the clearance of most amino acids approximately 1–3 times. Exceptions include a decreased clearance of tyrosine, little change in the clearances of glycine and valine, and a very large increase in the clearance of histidine.

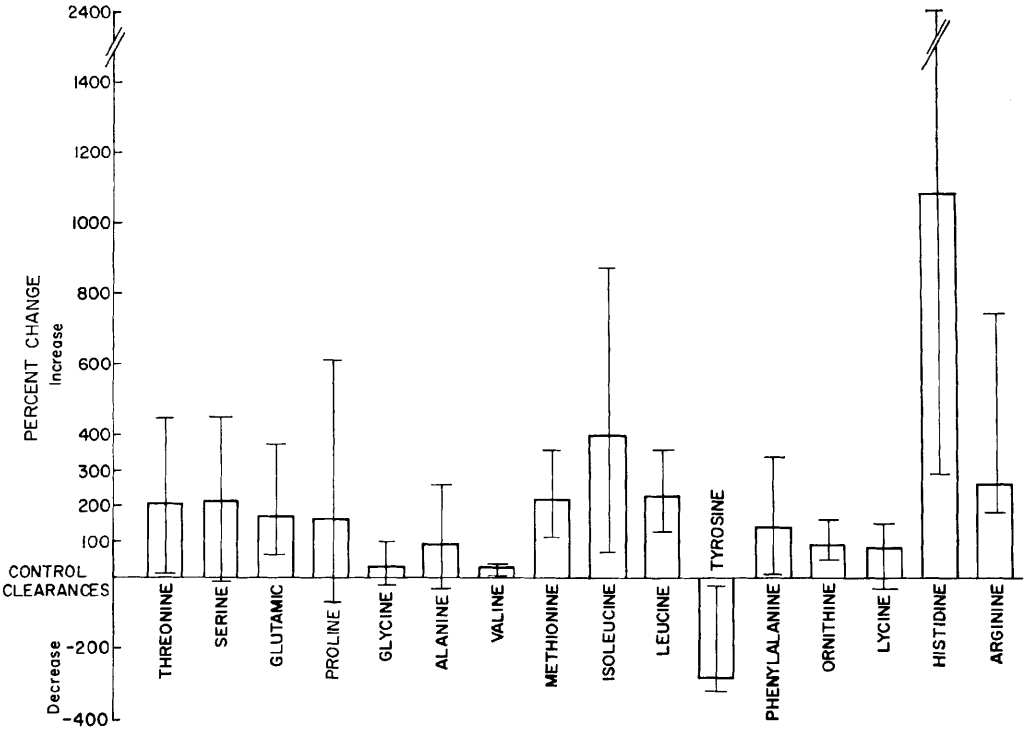

FIG. 2. Change in renal clearance of individual urinary amino acids in rats ingesting 1% lead in drinking water for 10 weeks. Figure indicates mean and range of percentage for three lead-fed rats and pair-fed controls.

The renal clearance of glycine is considerably larger than for other amino acids, (mean = 0.103 ml/min, range = 0.073 - 0.141), but does not increase appreciably in the lead-fed rats. The excessive glycinuria is, therefore, largely overflow aminoaciduria.

Plasma levels of nearly all amino acids from lead-fed animals are either the same or less than plasma levels of control rats (Table I). Exceptions are increases in threonine, glycine, and lysine.

Discussion. These studies demonstrate that the excessive aminoaciduria occurring in lead-poisoned rats is largely secondary to impaired renal tubular function. Amino acid transport in renal tubules of rats does appear to be comparable to man (12). Reabsorption occurs in the proximal tubule and there is absence of reabsorption in distal tubules. There is also competitive interaction between amino acids of related structures but more specific comparison of group interactions has not been performed. Treatment of rats with

TABLE I. Plasma Amino Acids from Rats Ingesting 1% Lead in Drinking Water for 10 Weeks and Pair-Fed Control Rats.[a]

Amino acid	Control animals	Lead animals
Threonine	26 (23–32)	35 (24–47)
Serine	30 (26–34)	23 (17–26)
Glutamic	16 (11–21)	12 (8–16)
Proline	13 (9–16)	14 (8–21)
Glycine	22 (16–28)	46 (36–57)
Alanine	42 (37–50)	44 (36–50)
Valine	13 (13–14)	13 (12–14)
Methionine	5 (4– 6)	5 (4– 5)
Isoleucine	7 (6– 7)	6 (5– 6)
Leucine	16 (14–17)	13 (12–14)
Tyrosine	6 (5– 7)	7 (5– 9)
Phenylalanine	7 (7– 9)	7 (5– 7)
Ornithine	12 (9–16)	10 (7–13)
Lysine	39 (36–42)	51 (47–53)
Histidine	11 (10–11)	7 (6– 8)
Arginine	14 (11–16)	13 (12–17)

[a] Values are mean and range of three animals in μmoles/100 ml.

the drug cycloleucine, known to be competitive with the dibasic amino acids in man, is also competitive with this same group of amino acids in the rat (13).

Lead-induced aminoaciduria in the rat is only moderately severe and is manifested by a 2- or 3-fold increase in urinary excretion of most amino acids. This degree of dysfunction is consistent with the ultrastructural changes previously observed in proximal tubular lining cells by electron microscopy in that cells show evidence of injury but little necrosis is present (9).

Morphological basis for the impaired transport of aminoacids has been investigated. Abnormal mitochondrial ultrastructure occurs in renal tubular cells of humans (6) and rats (9). Mitochondria isolated from kidneys of lead-poisoned rats do have impaired phosphorylative and oxidative abilities (14, 15). It is tempting, therefore, to suggest that the aminoaciduria in lead poisoning is the result of a defect in energy metabolism. A similar degree of aminoaciduria occurring in magnesium deficiency has been related to abnormal mitochondrial morphology and a disturbance of energy metabolism in renal tubular cells (16). Such a basic etiology to proximal tubular dysfunction might be expected to result in glycosuria and hyperphosphaturia, as well as aminoaciduria. These are the components of the triad of proximal tubular defects characteristic of the Fanconi syndrome in humans with toxic or genetic abnormalities of proximal tubules. Children and adults with lead poisoning usually have glycosuria and hyperphosphaturia as well as aminoaciduria (3). The severity of renal tubular dysfunction appears to parallel the degree of intoxication as manifested by other clinical parameters, particularly encephalopathy. Glycosuria has not been found in lead-poisoned rats but this may be a reflection of a high renal threshold for glucose in the rat. Hyperphosphaturia has not been tested for but hypophosphatemia does not occur.

Treatment of lead poisoning in humans with chelating agents (EDTA) reverses the aminoaciduria (3, 17). Such treatment has not been attempted in the rat but *in vitro* treatment of mitochondria isolated from lead-poisoned rats with EDTA does result in improvement of phosphorylative and respiratory ability (14).

The present experiment also suggests abnormalities in the metabolism and urinary excretion of specific amino acids. The increased renal clearance of histidine is much greater than that of other amino acids, whereas, glycine and valine clearances are less than most others, and tyrosine clearance is decreased. Glycine is the only amino acid greatly increased in the plasma. Lesser increases in plasma levels of threonine and lysine may be within experimental limits of normal. The increase in plasma levels of these aminoacids, particularly glycine, suggests a prerenal effect of lead on aminoacid metabolism. The defect in heme synthesis at the level of *d*-aminolevulinic acid hydrose probably accounts for the increased amounts of *d*-aminolevulinic acid (*d*-ala) in the urine in lead-intoxicated people (18) and experimental animals (5, 19). Since *d*-ala is formed by the condensation of glycine and succincyl-CoA (20) the increase in plasma glycine (Table I) and large urinary excretion of glycine (Fig. 1) may be a reflection of the defect in heme synthesis. In the early stages of acute lead poisoning in rabbits, there is simultaneously increased excretions of glycine and *d*-ala suggesting a relationship to the defect in heme synthesis (5). However, glycine has many metabolic alternatives and conversion to *d*-ala is probably not quantitatively a major use of this amino acid.

The disproportionate increase in histidine excretion and marked decrease in tyrosine clearance are not presently explainable. The exceptionally large increase in urinary histidine may be a consistent feature of lead poisoning. Apart from the defect in glycine and *d*-ala, histidine was the only amino acid increased in the urine in the first week of lead poisoning in rabbits (5). Histidine is also one of the most prominent urinary amino acids in human lead poisoning and continues to be excreted in large amounts after correction of generalized aminoaciduria by treatment of EDTA (3).

Another etiology of excessive excretion or altered metabolisms of particular amino acids

in lead poisoning may be the complexing of lead with individual amino acids. Lead content of urine from control rats is approximately 0.1–0.8 μg/ml and increases to a range of 3–9 μg/ml. Lead in urine is thought to be in two forms, inorganic and organic or ligand-bound. The increased amounts of lead appearing in urine in lead toxicity are thought to be largely ligand-bound (21). Identification of specific ligands has not been accomplished but free urinary amino acids are likely candidates for forming metal–ligand complexes (22). Possibilities include the formation of a lead–monoamino–monocarboxylic acid, such as a lead–glycinate. It is of interest that the two amino acids which combine most strongly with metals are histidine and cysteine. The histidine–lead complex may involve a five-membered ring involving carboxylate and alpha amino groups similar to that formed by any alpha amino acid. However, particularly strong binding of lead with histidine might be suspected on the basis of forming a six- or seven-membered ring involving alpha amino or carboxylate groups and imidazole groups. Cysteine may act as a tridentate ligand for lead (23). Whether lead–amino complexes actually occur *in vivo* in lead intoxication has not yet been demonstrated but such phenomena could play a role in the observed excessive aminoaciduria.

Summary. A "generalized" aminoaciduria occurs in lead-intoxicated rats characterized by a 1- to 3-fold elevation in renal clearances of most amino acids. The increase in histidine clearance is exceptionally large, whereas, changes in glycine and valine clearances are slight. Tyrosine clearance is actually decreased.

Plasma glycine is greatly increased; lesser increases in plasma threonine and lysine were found. Plasma levels of other amino acids were the same or less than those of control rats.

The increase in renal clearances of amino acids is thought to result from impaired renal tubular reabsorption secondary to an effect of lead on the metabolism of proximal renal tubular lining cells. Hyperglycinemia may be prerenal reflecting the defect in prophyrin synthesis known to occur in lead poisoning. It is also suggested that the formation of metal–amino acid complexes may contribute to the excessive aminoaciduria.

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