Effects of Lead on Heme-Synthesizing Enzymes and Urinary δ-Aminolevulinic Acid in the Rat[1] (37292)

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Although the anemia caused by lead poisoning is seldom severe, a disturbance in heme synthesis is one of the important effects of lead toxicity. This disturbance is characterized by decreased activity of aminolevulinic acid dehydrase (ALAD) in tissues and an increased excretion of aminolevulinic acid (ALA) in the urine to such an extent that analyses for ALA and for ALAD are useful indicators of the incidence of lead toxicity in humans (1, 2). The present investigation was undertaken to provide information on the dose and time relationships existent between lead intake, ALA, ALAD and another enzyme, aminolevulinic acid synthetase (ALAS), said to be the normally rate-limiting enzyme in heme synthesis. We have also attempted a biochemical interpretation of the changes found in these parameters of heme synthesis in the early stages of lead toxicity.

Materials and Methods. One hundred male Sprague–Dawley albino rats weighing 100–110 g were used in each of two sections of this experiment. After being habituated to the basal diet (Table I) for at least 3 days each rat was given 0, 500, 1500 or 3000 μg of lead as lead acetate in 2 g of diet following a 24-hr fast in Sect. 1, and 0, 100, 250 or 500 μg of lead in Sect. 2. All rats ate the 2 g of diet within 2 hr and basal diet was given *ad libitum* again at this time. Urine samples were collected throughout the study and urinary ALA and creatinine were measured (3, 4). At scheduled times after lead treatment five animals from each treatment group were etherized and blood, liver and kidneys were obtained from each animal. The tissues were put in ice-cold test tubes and ice-cold water was added acording to the volume or weight of the tissues. After homogenization the ALAS and ALAD activities were determined according to the methods of Marver *et al.* (5) and Bonsignore, Calissano and Cartasegna (6) and are expressed as percentage of control values.

Results. Since the results of the 0 and 500 μg lead dosages replicated in the two sections of the experiment were similar, the data of these sections are treated as a unit in the following discussion. The results of urinary ALA analyses are presented in Table II. The two lower levels of lead exposure did not affect urinary ALA excretion, while the two highest dosages produced almost a 4-fold increase within 12 hr. The response to the 500 μg lead treatment was evident within 24 hr. At the three highest dose levels the effect of the single exposure persisted through 5 days, attaining maximum values at Days 3–4.

The effect of lead on ALAD activity of blood, liver and kidney is summarized in Table III. The maximum decrease in ALAD activity was evident at 6 hr after the three highest lead dosages and the degree of decrease was dose-related. In contrast to the ALA data, the ALAD activity was affected by the two lowest lead treatments, although the effects were of smaller magnitude and developed later than those produced by higher lead treatments. Although there was a tendency for recovery of ALAD activity within the 6-day period following exposure, recovery was not complete at the higher dose levels. The liver data deviated from blood and kidney data in indicating a strong

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TABLE I. Composition of Basal Diet for Rats.

	%
Casein[a]	20
Corn oil	8
Cellulose[b]	3
Vitamin mixture[c]	5
Mineral mixture[d]	4
Glucose[e]	60

[a] Vitamin-free test casein, Whitson Products, Bordon Co., New York.

[b] Solka Floc, Brown Co., Boston.

[c] Contained in mg/kg of mixture: thiamine HCl, 40; riboflavin, 100; pyridoxine HCl, 30; Ca pantothenate, 320; biotin, 2; niacin, 360; folic acid, 4; vitamin B_{12}-mannitol (B_{12} present at 0.1%), 160; menadione, 4; choline chloride, 30,000; glucose to make 1000 g vitamin A palmitate (250,000 USP units/g) added to give 1000 USP units vitamin A/100 g diet; calciferol (850,000 USP units/g) added to give 125 USP units vitamin D/100 g diet.

[d] Mineral mixture 4164, General Biochemicals, Chagrin Falls, OH.

[e] Cerelose, Corn Products Refining Co., New York.

stimulation of ALAD activity following the early decrease.

ALAS activity in liver and kidney was not affected by treatment and averaged 16.9 ± 0.8 and 10.2 ± 1.6 μg of ALA formed/g of fresh tissue. Values obtained for blood ALAS activity are presented in Table IV only for the higher levels of lead, since the lower dosages had not significantly altered the values obtained for ALA excretion. The initial significant effect of lead on blood ALAS activity was a stimulation at 12 hr with the two highest lead dosages and at 2 days with the 500 μg treatment. This was followed by a decrease by the second day in animals given the two highest lead treatments. ALAS analyses were not made on tissues of animals beyond the second day after exposure to lead.

Comment. These data show that the initial increase of ALA excretion in response to a single dose of lead is preceded in time by the decrease of ALAD activity and coincides with an increase of ALAS activity in the blood. The data for ALAS and ALAD indicate that normally blood, liver and kidney can utilize 1.6-, 8.6-, and 8.0-fold more ALA than they produce. Thus, increased ALA excretion as a result of decreased ALAD activity alone might be expected to occur only if ALAD of normal activity was lowered to less than 60, 12 and 12%, respectively, in blood, liver and kidney. Since depressions of those magnitudes occurred only in blood, it may be assumed that blood was the source of the increased ALA excretion in the lead-toxic animals. Further support of this conclusion is seen in the data indicating that in blood there was an early, though brief, increase in ALAS activity. The persistence of ALA excretion through the 5 posttreatment days is probably related to the slow recovery of blood ALAD activity as the single dose of lead was transferred from blood to bone.

The changes in activity of blood ALAS

TABLE II. Effects of Lead on Urinary ALA.[a]

After treatment (days)	Pb consumed (μg)					
	0	100	250	500	1500	3000
0.25	10.9 ± 1.5	12.4 ± 2.0	9.8 ± 2.6	12.0 ± 1.4	11.5 ± 2.0	8.3 ± 2.0
0.5	14.7 ± 5.4	9.9 ± 5.4	11.3 ± 5.4	20.6 ± 3.8	39.2 ± 5.4[c]	33.6 ± 5.4[b]
1	14.2 ± 3.3	14.3 ± 4.2	13.4 ± 4.2	24.9 ± 3.0[b]	39.8 ± 4.2[c]	34.5 ± 4.2[c]
2	14.9 ± 5.9	15.1 ± 7.3	12.3 ± 7.3	31.0 ± 5.1[b]	52.6 ± 7.3[c]	50.1 ± 7.3[c]
3	19.8 ± 7.7	21.0 ± 7.7	20.9 ± 7.7	48.2 ± 5.4[c]	79.9 ± 7.7[c]	64.4 ± 7.7[c]
4	11.5 ± 9.9	16.6 ± 9.9	5.6 ± 9.9	48.0 ± 7.1[c]	76.8 ± 9.9[c]	81.4 ± 9.9[c]
5	13.7 ± 9.4	20.0 ± 9.4	4.5 ± 9.4	41.8 ± 6.7[b]	66.3 ± 9.4[c]	61.9 ± 9.4[c]
6	15.9 ± 4.6	14.1 ± 4.6	11.9 ± 4.6	18.1 ± 3.2	12.5 ± 4.6	12.0 ± 4.6

[a] Urinary ALA (μg/mg urinary creatinine ± SEM).

[b] Significant difference from control at: 5% level; [c] 1% level.

TABLE III. Activity of ALAD in Tissues as Percentage of Control.[a]

Tissue	After treatment (days)	Pb consumed (μg) 100	250	500	1500	3000
Blood	0.25	82	86	33[b]	8[b]	7[b]
	0.5	77	43[b]	44[b]	32[b]	20[b]
	1	72[b]	34[b]	35[b]	34[b]	25[b]
	2	72[c]	51[b]	47[b]	56[b]	40[b]
	6	83[b]	72[b]	82[b]	69[b]	65[b]
Liver	0.25	92	83	75[b]	52[b]	41[b]
	0.5	94	81[b]	71[b]	86[b]	83[b]
	1	86	93	87[b]	97	67[b]
	2	100	85	104	245[b]	255[b]
	6	102	117[c]	98	197[b]	190[b]
Kidney	0.25	82[c]	88	48[b]	23[b]	18[b]
	0.5	85	68[b]	62[b]	52[b]	38[b]
	1	86[b]	65[b]	62[b]	48[b]	34[b]
	2	81[c]	70[b]	66[b]	71[b]	69[b]
	6	101	100	93	86[b]	86[b]

[a] Control values ± SEM: Blood: 16.7 ± 0.8 μg ALA used/ml blood; liver: 146.0 ± 7.5 μg ALA used/g liver; kidney: 81.6 ± 4.3 μg ALA used/g kidney.
[b] Significant difference from control at: 1% level; [c] 5% level.

and ALAD which we observed as results of a single dose of lead may be explained on the basis of their sites of action. ALAD, the first enzyme to be affected by lead, functions in the cell cytoplasm, whereas ALAS acts within mitochondria (7). Since lead must enter the cytoplasm before encountering the mitochondria, it would seem reasonable for the cytoplasmic enzyme to be affected first, other things being equal. Although ALAD is known to be inhibited by lead (8), the mechanism of its inhibition is not known (9). The apparent "overshoot" of ALAD activity in liver during the late stages of this experiment is an instance of a phenomenon often seen in enzyme activity following release of inhibition. In this instance it might be a result of release of inhibition as lead concentration decreased after dosage, or it might be an adaptive response to presence of elevated substrate (ALA) levels. An increase of ALAD activity in presence of increased ALA has been reported (10, 11).

TABLE IV. Percentage Activity of ALAS in Blood vs Control.[a]

After treatment (days)	Pb consumed (μg) 500	1500	3000
0.25	82	78	99
0.5	53	234[b]	305[b]
1	92	76	89
2	163[b]	56[c]	54[b]

[a] Control value = 10.2 ± 0.5 (SEM) μg ALA formed/ml blood.
[b] Significant difference from control at: 1% level; [c] 5% level.

The initial increase of ALAS activity in the blood early in the exposure to lead may represent an increase of the enzyme in response to increased substrate, succinyl-CoA. This principle has been established as a mode of action of porphyria-inducing drugs (10–13). Inhibition of succinate oxidation by low levels of lead has been observed in mitochondria (14, 15), and this can lead to increased succinyl-CoA levels and hence an increase of ALAS activity (16). While this sequence of events has not been verified in animal tissues subjected to lead *in vivo*, it is presented here as an hypothesis worthy of further investigation.

Summary. In a study of early effects of lead on delta aminolevulinic acid (ALA) metabolism in rats a 4-fold increase in con-

centration of this metabolite was found in the urine within 12 hr after a single oral dose of lead acetate. This corresponded in time with a 3-fold increase in blood ALA synthetase (ALAS) and was preceded by at least 6 hr by a 90% decrease in blood ALA dehydrase (ALAD). Urinary ALA reached a peak at about 3–4 days and had returned to control levels at 6 days. Tissue ALAD increased towards normal throughout the 6-day period and was about twice the control values in liver after the second day. Blood ALAS decreased to half-normal by the second day.

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