Increased ALA dehydratase activity and spleen weight in lead-intoxicated rats. A consequence of increased blood cell destruction[1]

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Summary. Lead was given in the diet (1%) to rats from birth and at different times the animals were studied for delta amino levulinic acid dehydratase (ALAD) activity, spleen weight, ^{59}Fe incorporation in erythrocytes and ^{51}Cr-labeled erythrocytes survival. The increased ALAD and spleen weight found after lead treatment is explained as a consequence of a shortened survival, which results in a younger age of circulating erythrocytes with higher ALAD activity.

It has almost become a dogma that lead intoxication depresses delta amino levulinic acid dehydratase activity (ALAD) in blood[2,3]. We were the more astonished to find that rats, which received 1% of lead from birth on, showed increased levels of ALAD from an age of 1 month. At the same time, it was noted that the spleen of these lead-intoxicated animals had greatly increased in size. It seemed reasonable to assume that both effects reflect lead-induced changes in erythrocyte survival, and we have therefore followed incorporation and loss of ^{59}Fe in erythrocytes, and loss of ^{51}Cr-labeled erythrocytes at different times in rats receiving lead from birth.

Methods. Immediately after delivery, the mothers and their litter were given a diet containing 1% of lead as lead acetate. At different times thereafter, blood from 1 animal of a litter was removed and labeled in vitro with ^{51}Cr. The labeled washed erythrocytes together with 1 μCi of ^{59}Fe citrate were injected i.v. to the litter mates.

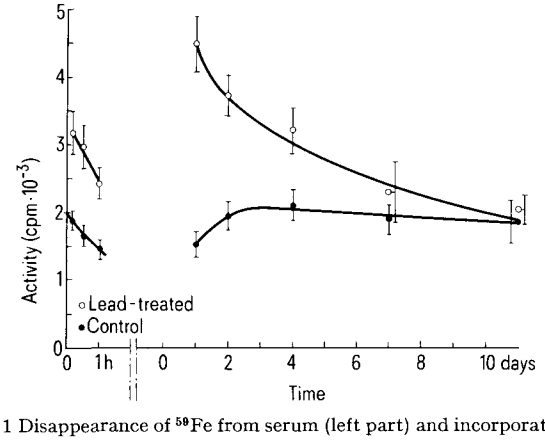

Fig. 1 Disappearance of ^{59}Fe from serum (left part) and incorporation into erythrocytes of control and lead-treated rats. All rats of age 1–4 months were combined in the calculation of the data.

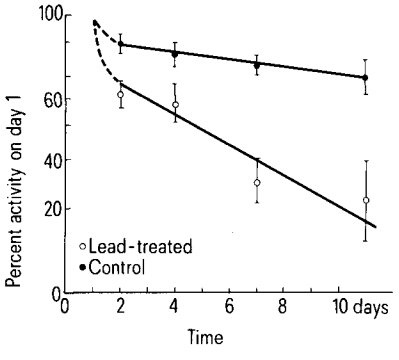

Fig. 2 Disappearance of ^{51}Cr labeled erythrocytes injected into control and lead-treated rats.

Blood samples were taken 10, 30, 60 min and 1, 2, 4, 7 and 11 days later and counted for ^{59}Fe and ^{51}Cr-gamma-activity using a Packard Multichannel scintillation spectrometer with sodium iodide crystal. The ^{51}Cr-activity was corrected for interference by ^{59}Fe. In some samples serum and erythrocytes were counted separately to ascertain that during the 1st h all activity is in the serum, and from day 1 on in erythrocyte hemoglobin. After 7 or 11 days, the animals were sacrificed, and bone marrow, spleen, kidney and liver were counted. ALAD was determined by the European Standard Method[4]. The data shown on organ weights and ALAD contain also those from animals assayed for brain biochemistry (Gerber et al., unpublished results).

Results and discussion. Activity of ALAD in the blood and spleen weight increase significantly from the age of 1 month when rats receive lead from birth (table). In addition, hematocrit is diminished and body growth is permanently retarded. Kidney weight is slightly reduced compared to animals of the same age up to 4 months, and is markedly increased from 1 year. If kidney weight is related to body weight, an increase is discernible from 1 month on.

In figure 1 all data on iron incorporation of animals of 1, 2, 3 and 4 months have been combined as no significant differences exist among the different age groups. The animals of 1-month age appeared, however, to attain maximal incorporation earlier (day 1) than older ones. Fe^{59} activity in serum falls rapidly after injection and with an equal slope in control and lead-treated animals, but the intercept with the ordinate is significantly higher in the later ones. This observation might reflect a lower distribution volume of iron after lead treatment. Incorporation in erythrocytes in greater and occurs at an earlier time in lead-treated than in control animals. Fe^{59} activity in erythrocytes does not decrease significantly in controls during the 11-day observation period, whereas it declines markedly in the lead-treated rats indicating a shorter survival of the erythrocytes. This postulate is confirmed by the behaviour of the ^{51}Cr-labeled erythrocytes, which also show a more rapid fall after lead treatment (figure 2). Significantly more ^{59}Fe is found in spleen (102 ± 15 vs 7.06 ± 1.36 cpm/mg) and liver (27.6 ± 2.9 vs 5.84 ± 1.18 cpm/mg) of lead treated than in control rats, and a similar although smaller difference is also observed for ^{51}Cr activity, whereas no effect of lead-treatment on activity is seen in the other organs studied. The results demonstrate that lead-treatment shortens markedly survival of the erythrocytes and that destruction occurs mainly in

1 Supported by Contract No. 140-76-12 ENV.B. of the E. C. Environmental Research Program.

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Age (months)	Body weight (g) Control	Lead-treated	Spleen weight (mg) Control	Lead-treated	Kidney weight Control	Lead-treated	ALAD units/ml erythrocytes Control	Lead-treated	Hematocrit Control	Lead-treated
0.5	36.9±1.0	23.2±3.7	102±13	78± 8	255±77	176±17	17.3 ±0.8	12.5±3.2	39.2±2.1	37.5±1.2
1	60.6±2.2	40.6±1.6	182±6	318±40	344±14	325±13	7.66±0.44	13.7±1.20	40.2±1.0	26.5±2.5
2	153±9	45.5±5.3	337±33	706±126	746±25	608±29	3.75±0.31	11.9±2.30	44.8±1.3	27.5±2.2
3	224±14	125±7	345±25	1190±203	894±52	727±35	3.40±0.46	16.4±7.31	45.2±1.8	26.4±2.6
4	210±12	120±11	340±16	806±135	946±21	802±25	2.98±0.18	19.3±5.4	45.9±2.3	34.7±2.4
7	284±13	125±1.5	354±23	918±60			2.96±0.15	23.3±2.5	46.2±1.9	28.9±2.9
12	296±39	169±12	311±46	1112±155	1126±87	1457±170	3.43±0.67	30.2±0.4	45.3±2.1	22.4±2.7
14 (0.5% Pb)			295±25	952±60	1145±68	2194±280			44.9±1.8	33.2±3.5

Values are means ± SE. All data are from animals given 1% of lead except the incomplete data after 14 months (0.5% lead).

spleen leading to an increase in organ size. More young erythrocytes are therefore present in the blood circulation of lead-treated than in control rats. It may be postulated that ALAD activity, as that of many other erythrocyte enzymes, is only a remnant from the synthesizing stage and diminishes as the erythrocytes age. The greater number of young erythrocytes would thus imply an increased ALAD activity as has indeed been found.

It has long been known that lead shortens erythrocyte survival possibly by damaging their membrane[5,6]. In recent years this aspect has somewhat receded in the general interest, as the depression of blood ALAD and the excess excretion of delta amino levulinic acid focused attention on hem synthesis. Clearly. ALAD in blood cannot be a limiting factor, otherwise anemia would follow the marked depression observed in persons only mildly intoxicated with lead. Moreover ALAD inhibition cannot be complete, otherwise excess urinary excretion and accumulation in erythrocytes of porphyrins could not occur. Indeed, ALAD activity in organs appears much less depressed than it is in blood and under certain conditions may increase (Gerber, unpublished results; Lauwerys, personal communication). The present investigation confirms that, when lead intoxication is severe, survival of erythrocytes becomes the critical factor for changes in blood while ALAD may actually increase in circulating erythrocytes.

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Inhibition of noradrenaline release from sympathetic nerves by pentobarbital[1]

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Summary. Pentobarbital concentrations of 10–100 μM selectively inhibited the noradrenaline release evoked by activation of the nicotinic receptors on the terminals sympathetic nerves of the rabbit heart. Higher concentrations also decreased the noradrenaline release induced by KCl or by electrical stimulation of the nerve axons.

The site and mechanism of action of barbiturates on the nerve cell is not yet known. Previously, in-vitro studies provided evidence that barbiturates are capable of inhibiting Na^+ conductance[3,4] and Ca^{2+} permeability[5,6] of the cell membrane; however, the concentrations which are necessary for this inhibition would cause severe poisoning in vivo. In the present study, the terminal sympathetic nerves of the rabbit heart were used as a model for the investigation of the membrane actions of the drugs.

Methods. The experiments were made on isolated hearts of rabbits (either sex) weighing 1.6–2.8 kg. All details of the methods used have been described previously[7]. Briefly, the hearts (some of them with an intact postganglionic sympathetic nerve supply[8]) were perfused with Tyrode solution (33 °C) at a constant flow rate of 25 ml/min. The composition of the solution was as follows (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.6; ascorbic acid 0.06 (aeration with 95% oxygen and 5% carbon dioxide). The noradrenaline concentration in the perfusate was measured spectrofluorimetrically by a modification of the trihydroxyindole method. In addition, heart rate and tension developed by the hearts were determined.

Results and discussion. Pentobarbital at concentrations up to 1 mM neither significantly altered the spontaneous noradrenaline output, nor the ability of the heart to remove exogenous noradrenaline (table). Since most of the exogenous noradrenaline removed during the passage through the coronary vessels of the rabbit heart is taken up into the noradrenergic neurons[9,10], we conclude that pentobarbital does not inhibit the noradrenaline uptake into the cardiac sympathetic nerves. Hence, changes of noradrenaline output from the hearts caused by this drug are due to alterations of noradrenaline release from the nerves.

Figure 1 shows that pentobarbital at concentrations up to 100 μM selectively inhibited the noradrenaline release evoked by activation of the nicotinic receptors with acetylcholine (muscarinic receptors blocked with atropine); there is evidence that such receptor sites exist in the mem-