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Interactions of Lead and Glutathione with Delta-Aminolevulinic Acid Dehydratase * **

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Abstract. Because there were tendencies for normalization of lead-sensitive parameters in animals with low lead burdens, these phenomena were examined *in vivo* and *in vitro*. Lead inhibited the enzyme delta-aminolevulinic acid dehydratase (ALA-D) dose-dependently and not competitively or irreversibly noncompetitively *in vitro*. The lead-blocked portion of ALA-D could be reactivated at least partially by glutathione. This part was higher in lead-contaminated animals than in the control group, though there were no significant differences in the free part of the enzyme. The free part of the enzyme was normalized by a resynthesis of the molecule. Therefore the determination of the lead-blocked part and the part reactivated by glutathione, seemed more suitable for determining the chronic lead burden than the measurement of a possible adapted total activity of ALA-D.

Key words: ALA-D — Lead — Glutathione — Free and Blocked Part of ALA-D.

Zusammenfassung. Weil sich in Tierversuchen bei niedriger Bleibelastung Tendenzen zur Normalisierung der bleiempfindlichen Parameter zeigten, wurde dieser als Adaptation interpretierte Vorgang *in vivo* und *in vitro* geprüft. Blei hemmte *in vitro* die Delta-Aminolävulinsäure-Dehydratase (ALA-D) dosisabhängig und nicht kompetitiv oder irreversibel kompetitiv. Der durch Blei blockierte Anteil der ALA-D war bei vermehrt bleikontaminierten Versuchstieren höher als bei Kontrolltieren. Dagegen bestanden im freien Anteil keine signifikanten Unterschiede zwischen den beiden Gruppen. Die Normalisierung des freien Anteils erfolgte über eine Neusynthese des Enzyms. Somit erscheint die Messung des durch Blei inaktivierten, durch Glutathion reaktivierten Anteils dieses Fermentes eher geeignet, eine chronische Bleieinwirkung festzustellen, als die Messung der möglicherweise wieder normalisierten ALA-D-Aktivität.

Schlüsselwörter: ALA-D — Blei — Glutathion — Freier und blockierter Teil von ALA-D.

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The results of our own experiments in sheep treated with different doses of lead via drinking water show that the lead induced changes of some parameters have tend to normalize after some weeks of lead exposure. In these experiments the excretion of delta-aminolevulinic acid in urine decreased after an initial increase and reached the values of control animals after some weeks (Prigge, 1971; Prigge and Hapke, 1971; Hapke, 1972). Bonsignore (1961) made similar observations in rabbits, in which the excretion of delta-aminolevulinic acid remained normal though the lead application continued. Lehnert (1971) interpreted this as correction of a disturbed equilibrium.

In the same way the activity of the delta-aminolevulinic acid dehydratase (ALA-D) in the blood of the sheep increased after a short period of inhibition in the beginning of the lead application (Hapke, 1972; Fassbender, 1973). The inhibition of the ALA-D explains the augmented excretion of the delta-aminolevulinic acid in the urine. Only in a late state of the symptomless intoxication (i.e. more than 3 months) the activity of ALA-D remained low.

As these phenomena can be interpreted as an adaptation to small amounts of lead, the enzyme ALA-D was investigated in relation to its inhibition by lead and to its reactivation by thiols like glutathione. The part of ALA-D activity blocked by lead, should be measured by the reactivation experiments.

Methods

The activity of ALA-D and of the reactivated part of ALA-D was measured by the method of Haas *et al.* (1972) with a few variations. The measurements were carried out 1. for determination of normal values in different species, 2. for the determination of the enzyme after oral and intravenous application of lead nitrate in rats and rabbits, and 3. for in vitro experiments after addition of lead or glutathione to the blood.

1. The normal values were determined in 7 healthy men, 9 horses, 25 dogs of different breeds, 20 beagles, 15 cows, 16 pigs, 13 rats and 4 rabbits, using fresh blood with heparin.

2. 96 female Sprague-Dawley rats were used for the determination of the enzyme activity after oral application of lead. The animals with an average weight of [illegible] g were divided into three groups. The first group served as control group and received water. The second group received water with 0.02[illegible] % lead nitrate ($Pb(NO_3)_2$), the third group received water with 0.04[illegible] % lead nitrate. They were fed with "Ssniff Fertigfutter". The water and feed consumption was measured daily and, based on the body weights, the daily dose of lead was calculated. The rats received 14.7 and 27.5 mg lead per kg and day. Out of each group 6 rats were killed by decapitation in deep chloroform anesthesia after 1, 2 and 4 weeks and after 5 months (13, 14 and 15 animals). The activity of the ALA-D was measured in the blood, the lead concentration was measured in the blood, liver, kidney, in [illegible] and in the hair of the animals[1]. The determination of the lead was performed

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photometrically using the ... metric method. In four male and female rabbits with ... body weight of 2.5 kg (Feed: Hoeing Fertigfutter) 1 mg lead/kg body weight was injected intravenously. Before treatment and for 17 days thereafter the activity of ALA-D and of the reactivated part of the ALA-D were measured.

3. The activity of ALA-D was measured in fresh blood of 10 dogs, 8 pigs and 21 cows *in vitro* by adding increasing doses of lead (0.1; 0.4; 0.7 and 1.0 ppm). In two specimens of cow blood 1.5 ml of a 0.1 M glutathione solution was added to the blood incubated with lead.

To investigate the mechanism of inhibition of the ALA-D by lead, the method of Lineweaver and Burk was used in 4 blood specimens of cows in the following manner: To 4 of 8 samples with 0.2 ml blood was added 0.55 ml water, to 4 other samples 0.29 ml water and 0.16 ml lead solution of 1 ppm, that gives a lead concentration of 0.4 ppm in blood. After an incubation time of 15 min increasing amounts of substrate: 0.025; 0.1; 0.25 and 0.5 ml of a 0.02 M delta-aminolevulinic acid solution, pH 6.8, were added to the samples. The substrate was filled up to 1.0 ml. To interrupt the next step, the transformation of porphobilinogen to coproporphyrinogen, all specimens were incubated in a nitrogen atmosphere for 1 h (Lichtmann and Feldmann, 1963). The further procedures were the same as described above.

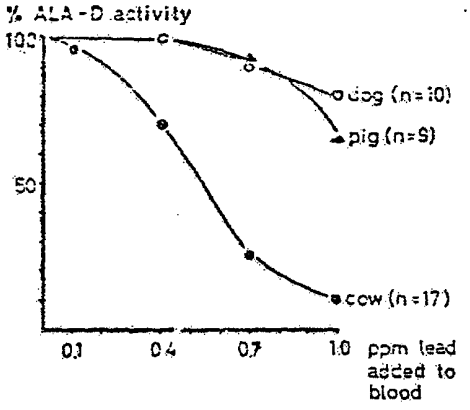
Results

The activity of ALA-D was inhibited in a dose-related manner in vitro by lead. There were some species-differencies, as indicated in Fig. 1. An amount of lead which inhibits only 10 or 20% of the activity in dog and pig blood blocks nearly 90% of the activity in cattle blood.

The inhibition of the ALA-D activity by lead can be demonstrated by the graphic method of Lineweaver and Burk. This inhibition of the ALA-D activity by lead was not competitively or irreversibly competitively. As indicated in the Fig. 2, the maximal velocity of the porphobilinogen production and the velocity of the reaction in the presence of lower concentrations of the substrate are reduced by lead. The space between the two curves is that amount of the enzyme, which is blocked by lead.

The ALA-D inhibited by lead could be reactivated *in vitro* by glutathione (Fig. 3). The reactivation of the blocked part of the enzyme was not complete by glutathione. The more the enzyme was inhibited by lead, the less the activity could be increased by glutathione. The space between the two curves in Fig. 3 represents that part of the enzyme, which is reactivated by glutathione (or which was blocked by lead previously).

The determination of the two parts of ALA-D, the free and the blocked or reactivated part, in different animal species and in men with normal lead exposure show, that only in men and cattle, the two lead-sensitive species, could the ALA-D activity be reactivated by glutathione. Experiments with penicillamine (2.5 to 40 mM in blood of cattle) and dimercaprol (0.25 to 25 mM) did not produce uniform results. The



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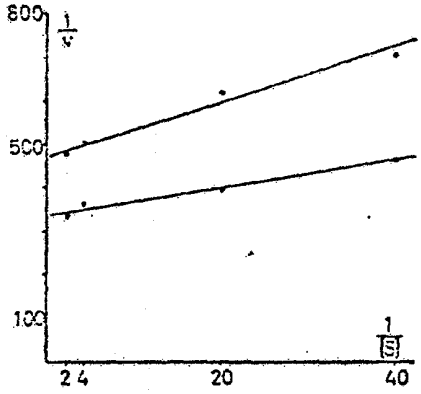
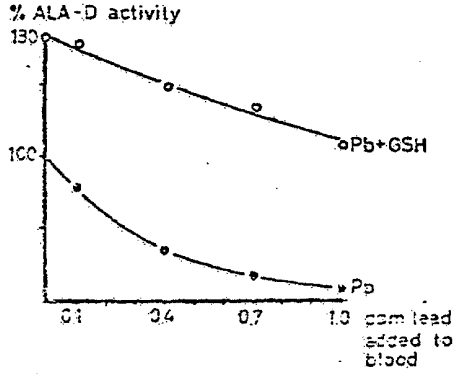


Fig. 2

Fig. 1. Dose-depending inhibition of ALA-D by different concentrations of lead *in vitro* in dogs, pigs, and cows

Fig. 2. Relations between the reciproke velocities of the enzyme-ALA-reaction and the reciproke substrate concentration in blood of cattle, demonstrated in the way of Lineweaver and Burk



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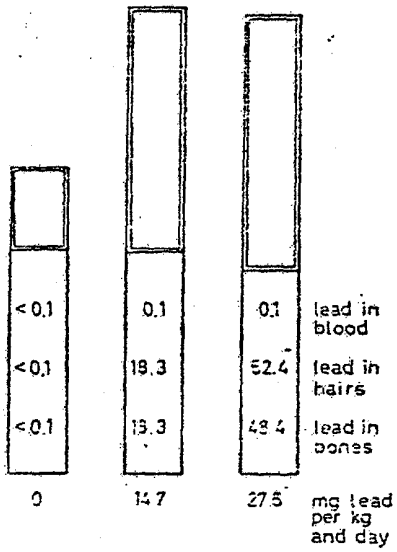

Fig. 4

Fig. 3. Dose relations between ALA-D activity and lead concentration (added to blood specimen of cows) or lead plus glutathione (0.1 M) concentration

Fig. 4. Free (lower part of the columns) and by glutathione reactivated (i.e. by lead blocked) form (double-lined rectangles) of ALA-D in the blood of rats, fed with different doses of lead. The numbers in the columns indicate the lead concentration in blood, hairs and bones of the animals (ppm)

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Table 1. ALA-D and re
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	ALA-D	
	$\bar{x}$	s
man	924	284
cattle	240	128
dog 1	405	262
dog 2[a]	255	130
pig	1040	388
horse	0.0—0.1	
rat 1	159	4(
rat 2[b]	170	5(
rabbit	2152	

[a] A group of 20 be
dog 1 = bastards of diff
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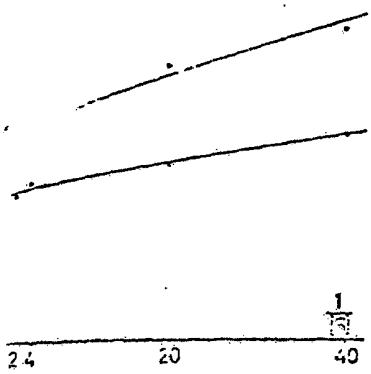

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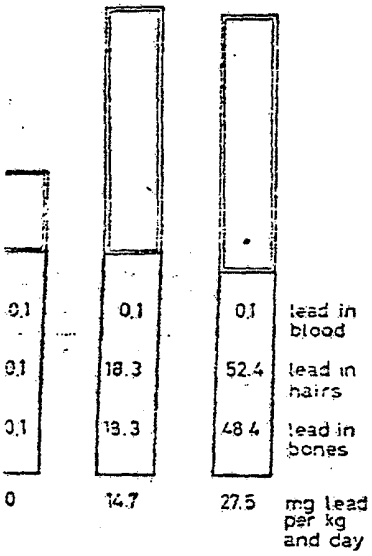

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Table 1. ALA-D and reactivated ALA-D in different species not exposed to lead (µM porphobilinogen formed per hour and l cells and hour

	ALA-D			reactivated ALA-D			ALA-D react. / ALA-D
	$\bar{x}$	s	n	$\bar{x}$	s	n	
man	924	284	7	1814	547	7	1.96
cattle	240	125	18	423	155	9	1.78
dog 1	405	202	25	351	202	19	—
dog 2[a]	255	130	20	274	128	20	—
pig	1040	385	16	713		3	—
horse	0.0–0.1		9	0.0–0.8		9	—
rat 1	160	49	13	213	53	13	1.34
rat 2[b]	170	50	6	159	15	6	—
rabbit	2152		4	1302		4	—

[a] A group of 20 beagle dogs in the same age and the same body weight; dog 1 = bastards of different ages and weights.

[b] young rats in the age of 8 weeks, body weight between 120 and 130 g; rat 1 = adult rats.

reactivation of the partly blocked ALA-D did not depend on the concentration of these two compounds in blood. For details see Table 1. In horses no activity of ALA-D could be determined. The highest activity was measured in rabbits. The reactivated part of the ALA-D was higher in older rats than in younger ones.

It is concluded that in naturally occuring lead contaminations the ALA-D activity of sensitive species as men and cattle is partly blocked by lead. This effect can be explained by the ubiquity of lead in the environment. The rest of ALA-D is brought up to the necessary activity by a new synthesis.

In rats which received lead for 5 months the free and the reactivated parts of ALA-D were measured at the end of the experiment. The activity of ALA-D in the test animals was as high as in controls (Fig. 4). The small differences were not significant. The concentrations of lead in hair and bones were high in the lead-fed animals. In blood no lead was found in spite of the contamination of the animals with high amounts of lead during 5 months. The lead-blocked and glutathione reactivated part of the ALA-D activity increased in the lead-fed animals higher than in control animals. The action of lead in these animals was only demonstrable by determination of the reactivated part of ALA-D. The level and duration of the contamination could be determined by the amount of the lead in organs such as bones and hair (Stierner, 1972). There were no differences between the groups with regard to the body weight and the feed efficiencies. At the beginning of the development of

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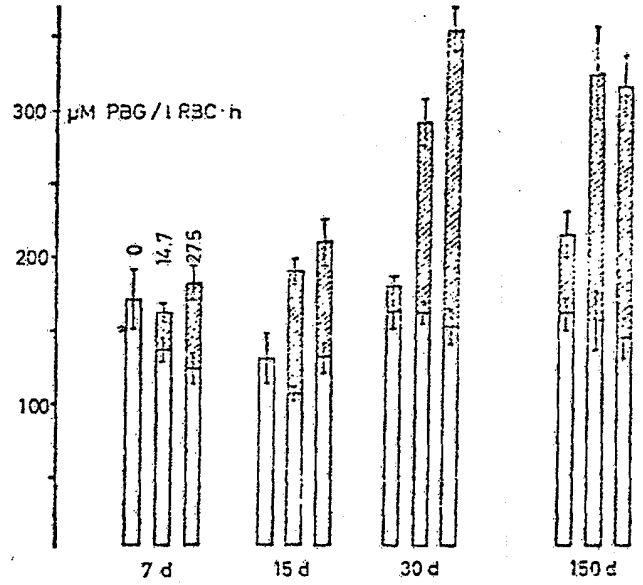
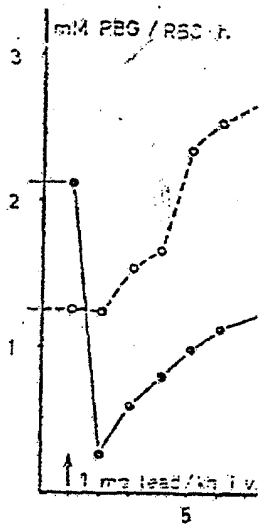


Fig. 5. Activity of ALA-D (white columns) and of reactivated ALA-D (expressed as µMoles porphobilinogene, produced per liter red blood cells and hour) in rats, fed with two different doses of lead for several times. After 7 days the whole ALA-D activity is partly blocked by lead in a dose-related manner. This blocked part increases during the experiment and the free part regenerates to the control values

chronic lead intoxication a short reduction of the free part of ALA-D was seen in rats. The amount of this reduction was that part, which could be reactivated by glutathione (Fig. 5, upper part of the columns) after the lead contamination of 1 week. Then the blocked part increased slowly and the free part showed the trend to normalization (Fig. 5, columns after 14 days). In control animals a part of ALA-D was also blocked as they got older; however, these animals did not receive lead-free food. The lead content of the food was between 0.1 and 0.3 ppm dry substance.

To study *de novo* synthesis of the ALA-D, which was reduced previously by lead, lead was injected intravenously into rabbits. The injection of 1 mg lead/kg body weight decreased the activity of ALA-D immediately (Fig. 6). The whole activity then increased (i.e. the sum of free and reactivated activity) as a result of increased free enzyme. The observation that the whole activity seems to be lower than the free part, is due to the method: The development of the color of porphobilinogen with Ehrlich's reagent is inhibited by thiols *in vitro* (see also



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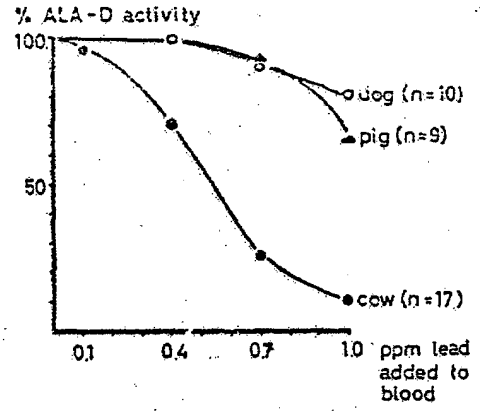

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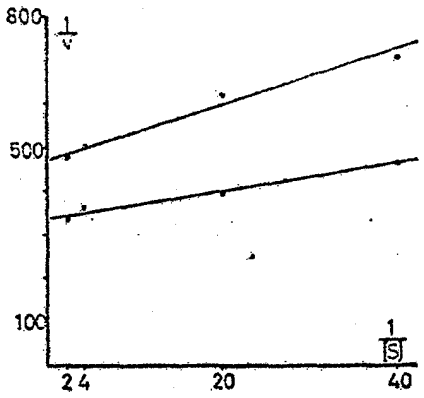

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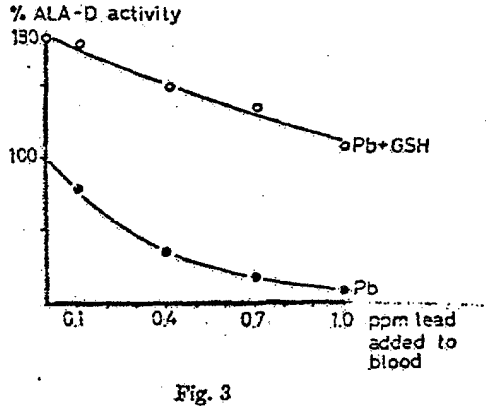

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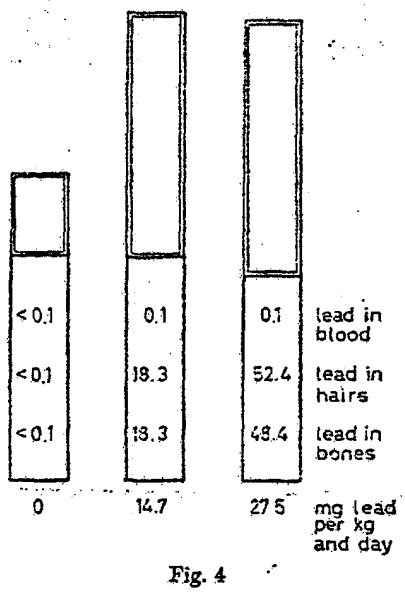

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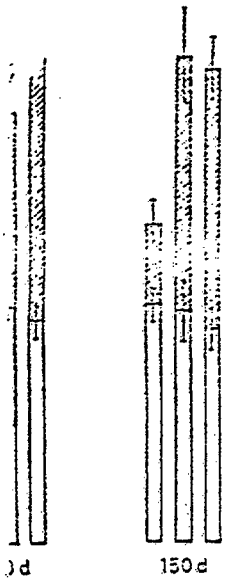

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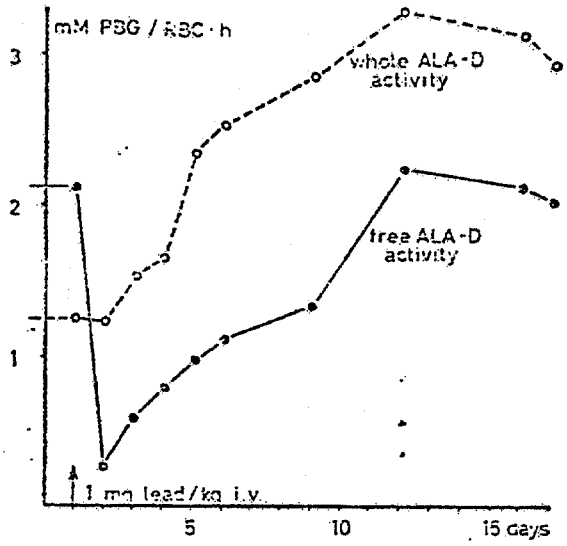

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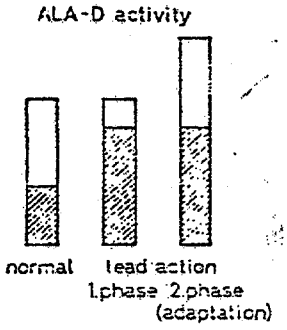

Fig. 7

Fig. 6. Decreases of the ALA-D activity in the blood of 4 rabbits after i.v. injection of 1 mg lead per kg body weight. Free ALA-D (lower line) and whole ALA-D after reactivation with glutathione. The curves show that the normalization is caused by an increase of the free part of ALA-D

Fig. 7. Scheme of the lead action on ALA-D activity in normal and in lead exposed animals. 1 phase = increase of the blocked part of ALA-D; 2 phase = increase of the free part of ALA-D to normal values

Table 1 for other species). The activity of the free part of ALA-D increased relatively quickly, in parallel with the increase of the whole activity. Therefore, we interpret the increase as due to a new synthesis of ALA-D and not to a slow liberation of lead.

Discussion

As indicated by the results, the part of the ALA-D which is blocked by lead can be reactivated by the addition of glutathione. By this method the degree of the lead contamination can be determined. The sensitivity of ALA-D to lead and the reactivation of the blocked enzyme by glutathione differ between species.

After chronic lead contamination, the reactivated part of ALA-D increases and the free part tends to normalize. Haas *et al.* (1972) established too, that the part of ALA-D reactivated by glutathione increases

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with increasing lead concentrations of the blood in men. In sensitive species like men and cattle a part of ALA-D is blocked by the lead in the normal environment. The remaining part of ALA-D is supplemented by a new syntheses of the enzyme.

We conclude that established adaptation to small lead amounts may be explained by the following scheme (*Fig. 7*): In the normal environment a part of ALA-D is blocked even in individuals who are not lead-intoxicated. With increasing lead concentration in the environment, the blocked part of ALA-D increases first. By a regulation mechanism the organism is able to supplement the lost part.

The results indicate that, due to the unavoidable lead contamination of the environment, the enzyme patterns of all organisms are under the influence of lead. Therefore the question of a non-toxic level of lead cannot be answered. The state before the lead contamination begins strongly affects the action of lead at higher doses. Hernberg *et al.* (1970 a, b) considered that a non-toxic level could not be determined for lead.

Short lasting and abrupt increases of lead intake cause an inhibition of the free ALA-D activity. Slow changes of the lead intakes can be partly compensated by the healthy organism. This phenomenon is interpreted as adaptation.

The most sensitive parameter for determining lead action on organisms has been shown by these investigations and of those of Haas *et al.* (1972) to be the measurement of the reactivated part of ALA-D. With this test, the action of lead can be determined, even if the other parameters such as increased excretion of delta-aminolevulinic acid in urine or decrease of the ALA-D activity on blood tend to normalize.

References

Bonsignore, D.: Biochemical approach to the physiopathology of porphyrin metabolism in lead poisoning. Lav. umano 13, 606—617 (1961)

Fassbender, C. P.: Versuche zur Enzymdiagnostik der subklinischen Bleivergiftung bei Schafen. Inaug.-Diss., Hannover 1973

Haas, T., Mache, W., Schaller, K.-H., Mache, K., Klavis, G., Stumpf, R.: Zur Bestimmung der Delta-Aminolävulinsäure-Dehydratase und ihrer diagnostischen Wertigkeit. Int. Arch. Arbeitsmed. 30, 87—104 (1972)

Hapke, H.-J.: Subklinische Bleivergiftung bei Schafen. International Symposium "Health Aspects of Environmental Pollution with Lead". Amsterdam 1972

Hernberg, S., Nikkanen, J.: Enzyme inhibition by lead under normal urban conditions. Lancet 1970 I, 63 (1970a)

Hernberg, S., Nikkanen, J., Tola, S., Vaikanen, S., Nordman, C. H.: Erythrocyte ALA-dehydratase as a test of lead exposure. Int. Conf. Chem. Poll. Hum. Ecol., Prague 1970 b

Lehnert, H.: Biokybernetische Probleme der Bleibelastung. Referat VDI-Kommission Reinhaltung der Luft, 19. April 1971, Düsseldorf (1971)

Lichtmann, H. C., Fe
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Hannover 1971
Prigge, E., Hapke, I
Bleivergiftung be
Sterner, W.: Die Haa
gen geeignete Met
und Tier. Arch. L

DUP040007938

Lichtmann, H. C., Feldmann, F.: In vitro pyrrole and porphyrin synthesis in lead poisoning and iron deficiency. J. clin. Invest. 42, 630 (1963)
Prigge, E.: Versuche zur Frühdiagnose der Bleivergiftung bei Schafen. Inaug.-Diss., Hannover 1971
Prigge, E., Hapke, H.-J.: Die Feststellung einer experimentellen subklinischen Bleivergiftung bei Schafen. Dtsch. tierärztl. Wschr. 79, 475–482 (1971)
Sterner, W.: Die Haaranalyse — eine besonders für epidemiologische Untersuchungen geeignete Methode zur Feststellung von Schwermetallbelastungen bei Mensch und Tier. Arch. Lebensmitt.-Hyg. 10, 209–213 (1972)

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