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EFFECT OF LOW LEAD EXPOSURE ON THE LEVEL OF DELTA-AMINOLEVULINIC ACID DEHYDRATASE ACTIVITY

S. Milić, M. Stanković, Vera Delić

Institute of Occupational and Radiological Health, Belgrade

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The examination of the activity of delta-aminolevulinic acid dehydratase in blood has been carried out in workers in a printing office with a low occupational exposure to inorganic lead.

A reduction of the enzyme activity was found in a group of 99 workers. In this group, the mean values of urinary levels of lead, delta-aminolevulinic acid and corproporphyrin were found to be within normal limits. The mean enzyme activity amounted to 77 per cent of that of the control group. The control group was composed of 33 nonexposed healthy subjects.

In the group slightly exposed to lead in about 60 per cent of workers ALA-dehydratase activity values were below normal level.

A statistically significant difference ($t = 3.77$; $P < 0.001$) in the mean values of ALA-dehydratase activity was obtained between the exposed and the control subjects.

The estimation of ALA-dehydratase activity provides a very sensitive test of increased lead intake, even at lead concentrations in air below the MAC value. The test therefore seems to be too sensitive to be of use in industrial medicine, especially in the control of workers with evidently increased lead exposure.

In the last decades industrial medicine has shifted away from a pure applied and descriptive discipline towards basic scientific research. As a result of this modern concept great advances have been made in the knowledge of how toxic agents, foreign to the body, actually act. However, a great deal of uncertainty still remains in the interpretation of many findings and in the assessment of the relationship between clinical symptoms and biochemical observations.

From the viewpoint of possible metabolic activities, lead has been shown to be involved in many processes. The application of the concept

of biochemical lesions has directed the attention of toxicologists to the development of screening methods, suitable for the early diagnosis of lead poisoning. Chemical tests, based on this approach, may supplement classic methods of physical and hematological examination.

More recently, the attention has been called to the value of urinary delta-aminolevulinic acid (ALA) determination for the early detection of increased lead absorption (1–4). Increased ALA excretion has been found in lead workers with or without symptoms as well as in animal experiments. Since *Mauzerall* and *Granick* (5) developed an excellent method for the determination of ALA concentrations, this biochemical parameter has generally come in use in the control of occupationally exposed workers. By this method is determined the total amount of urinary aminoketones of which ALA constitutes a part, the remainder chiefly consisting of aminoacetone. ALA determination has proved suitable to define the quantity of metabolically active lead in the organism and the values obtained were highly correlative with blood lead, urinary lead and coproporphyrin levels.

It has been recognized that lead exerts an inhibitory action along the porphyrinic chain and interferes in the biosynthetic pathway leading to heme synthesis. The enzymes, located in the erythrocytes, are inhibited also by some other heavy metals and SH-group inactivating substances (6, 7).

In this study attention has been paid to the dehydratase of delta-aminolevulinic acid (ALA-D), enzyme which accomplishes the conversion of delta-aminolevulinic acid (ALA) into porphobilinogen (PBG) and is localized in the mature red cells of the peripheral blood. Suppression of the activity of ALA-D has been demonstrated both in the lead affected subjects (8–11) and in the rabbits intoxicated by lead salts (12, 13). Experiments performed on animals demonstrated that the enzyme inhibition occurs at extremely low lead concentration (i. v. or s. c. injections). Similarly, in experiments with rabbits the inhibition of ALA-D preceded the appearance of coproporphyrinuria. Hence, it is concluded that the blockage of ALA-D may be considered the earliest and most sensitive sign of increased lead absorption.

The purpose of our study was to examine ALA-D activity in workers very slightly exposed to lead (in concentrations below MAC value) when other urinary parameters such as lead and coproporphyrin remained unchanged and ALA was in some cases slightly increased. The problem of medical control and prevention suggested an investigation of the validity of ALA-D activity determination as exposure test in workers with low exposure to lead.

MATERIAL AND METHODS

Urinary lead, coproporphyrin (CP) and ALA levels were measured in a group of 99 workers in a printing office, slightly exposed to lead. The concentrations of lead in air were from 0.020 to 0.145 mg/m³ – Parallel blood ALA-D activity measurements were taken. In order to avoid the problem of 24-hour urine sampling, urine was collected between 6–7 a. m. A physical examination of workers showed no sign of lead effect.

A control group consisted of 33 nonexposed healthy subjects living in a village far from urban environmental pollution.

ALA-D activity was measured in fresh heparinized whole blood by direct colorimetric estimation of the amount of PBG produced from added ALA after incubation for 60 minutes according to a modification of the original method of *Gibson* et al. (6) developed by *Bonsignore* et al. (10). The results were corrected for haematocrit value, and enzyme activity was calculated from the formula:

$$\frac{1{,}000 \times 12.5\ (A_{60}-A_0)}{\text{per cent haematocrit}}$$

where A_{60} and A_0 stand for the absorbencies at 60 and 0 minutes respectively and 12.5 is the dilution correction of blood.

Urinary lead was estimated by the polarographic method (14). Coproporphyrin was determined spectrophotometrically (15). This method involves quantitative extraction of coproporphyrin from urine with ether, followed by purification of the ether layers and extraction of coproporphyrin into dilute HCl. ALA in the urine was determined by the method of *Grabecki* et al. (16).

RESULTS AND DISCUSSION

The results of our investigations are presented in Table 1 and statistical analysis of the results in Table 2. They show a significant difference ($t = 3.77$; $P < 0.001$) in ALA-D activity between 99 exposed workers and 33 control subjects. The normal level of activity determined in our laboratory in a group of nonexposed subjects agrees with those found by *Bonsignore* et al. (10).

The mean value of enzyme activity in the exposed group was found to be 77 per cent of that of the control group. In spite of the marked reduction of enzyme activity, the mean values of urinary levels of lead, ALA and CP were not increased in lead workers – typographers. Practically, no value reached the upper normal limit, except some values of ALA in the urine.

Table 1

The values of ALA-D activity, urinary CP, ALA and Pb

Tests	Control group		Exposed group	
	Range	Mean	Range	Mean
ALA-D, units/ml Er	76–122	92.33	7–125	71.32
CP, mg/lit	0.008–0.078	0.034	0.017–0.173	0.044
ALA, mg/lit	1.35–8.50	5.07	1.0–22.0	7.25
Pb, mg/lit	0.008–0.053	0.020	0.010–0.092	0.037

In the group slightly exposed to lead, about 60 percent of workers had ALA-D activity below normal (below 80 units per ml of RBC). The level of ALA in urine was increased in 17 percent and the level of coproporphyrin only in 2 percent of workers. The values of lead in urine were below 0.092 mg/l.

Table 2

The significance of the difference of the mean values of ALA-D activity between the groups

Groups	Number of cases	Mean value (X)	Stand. deviat. (s)	Difference in mean values	t – test P
Exposed	99	71.32	29.74	21.01	$t = 3.77$
Control	33	92.33	14.42		$P < 0.001$

According to *Bonsignore* et al. (12, 17) the activity of ALA-D is strongly inhibited in experimental lead intoxication both »in vivo« and »in vitro«. These authors (13) recorded a decrease in enzyme activity up to 20 per cent in rabbits after intravenous treatment with lead acetate (20 gama Pb/kg) for 10 days. *De Bruin* (11) reported a decrease in ALA-D activity up to 20 percent in a group of lead processing workers, most of them with raised urinary levels of CP and ALA. In another paper *De Bruin* (18) reported a lower reduction of activity of about 33 percent in a group of 30 typographers. In our group of 99 printing office workers exposed to low lead concentrations the reduction was lower – about 23 percent compared to the control group. ALA-D activity diminished gradually and at a slow rate under the influence of a small intake of lead salts (18). *De Bruin* also observed that the lowest dose (intravenous) capable to cause a detectable change in enzyme activity is 5–10 gamma Pb/kg body weight.

ALA-D phenomenon is associated with the apparent capability of lead to exert definite biochemical effects at concentration levels approximating the MAC value (0.150 mg Pb per m^3), or even below this limit as in our study. From our results it is obvious that ALA-D activity determination is the most sensitive test for lead exposure. It seems, however, from our and previous reports, that this test is perhaps too sensitive to be of use in industrial medicine, especially in the control of workers with evident lead exposure. Owing to lowered activity observed in the case of moderate exposure, difficulties are encountered in discriminating actual lead poisoning from the stages due to low exposure.

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Sažetak

UTICAJ NEZNATNE EKSPOZICIJE OLOVU NA NIVO AKTIVNOSTI DELTA-AMINO-LEVULINSKE DEHIDRATAZE

Ispitivana je aktivnost dehidrataze delta-amino-levulinske kiseline u krvi radnika jedne štamparije koji su bili izloženi niskom stepenu ekspozicije neorganskog olova.

U grupi od 99 radnika nađena je smanjena aktivnost enzima. U ovoj grupi srednje vrednosti nivoa olova, delta-amino-levulinske kiseline i koproporfirina bile su u normalnim granicama. Srednja vrednost aktivnosti enzima iznosila je 77% u odnosu na kontrolnu grupu. Kontrolnu grupu sačinjavale su 33 neeksponovane zdrave osobe.

U grupi neznatno eksponovanoj olovu, kod oko 60% radnika je vrednost za aktivnost ALK-dehidrataze bila ispod normalnog nivoa.

Dobijena je statistička značajna razlika ($t = 3,77$; $P < 0,001$) u srednjim vrednostima aktivnosti ALK-dehidrataze eksponiranih radnika i kontrolne grupe.

Ispitivanje aktivnosti ALK-dehidrataze predstavlja neobično osetljiv test povećane apsorpcije olova, čak i pri koncentracijama olova u vazduhu ispod MDK vrednosti. Stoga je izgleda, ovaj test suviše osetljiv za primenu u industrijskoj medicini, naročito za kontrolu radnika kod kojih je povećana ekspozicija olovu potpuno očigledna.

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Institut za medicinu rada
i radiološku zaštitu, Beograd