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

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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 coproporphyrin 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 83 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 ($t = 8.77; P < 0.001$) 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 Mauersrall and Granick (5) developed an excellent
method for the determination of ALA concentrations, this biochemical
parameter has generally come into use in the control of occupationally
exposed workers. By this method is determined the total amount of urin-
ary aminoketones of which ALA constitutes a part, the remainder
chiefly consisting of aminoacetone. ALA determination has proved suit-
able to define the quantity of metabolically active lead in the organism
and the values obtained were highly correlated with blood lead, urinary
lead and coproporphyrin levels.

It has been recognized that lead exerts an inhibitory action along the
porphyric 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 ami-
noleuvinonic 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 af-
acted subjects (8-11) and in the rabbits intoxicated by lead salts (12, 13).
Experiments performed on animals demonstrated that the enzyme inhibi-
tion occurs at extremely low lead concentration (i. v. or s. c. injec-
tions). 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 un-
changed and ALA was in some cases slightly increased. The problem of
medical control and prevention suggested an investigation of the valid-
dity 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/m$^3$ - 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 hematocrit value, and enzyme activity was calculated from the formula:

$$1,000 \times 12.5 \left( A_{60} - A_9 \right)$$

per cent hematocrit

where $A_{60}$ and $A_9$ 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 ($t = 3.77; P \leq 0.001$) 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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Control group</th>
<th>Exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA-D, units/ml Er</td>
<td>76–122</td>
<td>7–125</td>
</tr>
<tr>
<td>CP, mg/lit</td>
<td>0.008–0.078</td>
<td>0.007–0.017</td>
</tr>
<tr>
<td>ALA, mg/lit</td>
<td>1.35–8.50</td>
<td>1.0–22.0</td>
</tr>
<tr>
<td>Pb, mg/lit</td>
<td>0.008–0.053</td>
<td>0.004–0.009</td>
</tr>
</tbody>
</table>

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 0.0092 mg/l.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of cases</th>
<th>Mean value (X)</th>
<th>Stand. deviat. (s)</th>
<th>Difference in mean values</th>
<th>t - test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>99</td>
<td>71.32</td>
<td>29.74</td>
<td>21.01</td>
<td>t = 3.77</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Control</td>
<td>33</td>
<td>92.33</td>
<td>14.42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Bonsignore et al. (12, 17) the activity of ALA-D is strongly inhibited in experimental lead intoxication both \(\text{in vivo}\) and \(\text{in vitro}\). 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.
AI.A-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 m³), 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.

References


Sažetak

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

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

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

U grupi neznatno ekspozovanoj olovu, kod oko 60% radnika je vrednost za aktivnost AI.K-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 osjetljiv test povećane apsorpcije olova, čak i pri koncentracijama olova u vazduhu ispod MDK vrednosti. Stoga je izgleda, ovaj test suvremen i osjetljiv za primenu u industrijskoj medicini, naročito za kontrolu radnika kod kojih je povećana ekspozicija olova potpuno obzirom.


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