5-aminolevulinate dehydratase activity in blood of rabbits given tin or lead

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ABSTRACT The activity of 5-aminolevulinate dehydratase (ALAD) in rabbit blood is significantly inhibited by tin. Intravenous administration of tin (0.48 or 4.8 $\mu\text{mol/kg}$ body weight) causes a decrease in the activity of the enzyme by 60% or 94% respectively. The effects of tin and lead on ALAD differ: inhibition by tin is not affected by pre-incubation at 50–60°C, whereas the inhibitory effect of lead is increased by the same pretreatment. The optimum pH for rabbit blood ALAD is 6.8 in control rabbits. This optimum shifts to pH 5.8–6.0 in the blood of tin-treated rabbits, with or without pre-incubation at 60°C for 5 min, while a similar shift is prevented by the same pre-incubation after lead treatment. Recovery to normal activity is faster after tin than after lead treatment.

A fall in the activity of 5-aminolevulinate dehydratase (ALAD) [5-aminolevulinate hydro-lyase, EC 4.2.1.24] in blood is the most sensitive index of lead exposure (De Bruin and Hoolboom, 1967; Hernberg *et al.*, 1972). However, we have found that the activity of this blood enzyme is also inhibited by inorganic tin. This fact must be considered when the activity of ALAD is measured during the medical examination of lead-exposed workers. The present report deals with the effect of tin on ALAD activity in rabbit blood and suggests a method for discriminating between the effects of tin and lead on this enzyme.

Materials and methods

ANIMALS

Four groups of adult male rabbits (New Zealand White) of 2.5–3.0 kg body weight were used. Each group consisted of four rabbits.

METAL SOLUTIONS

Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) and lead acetate [$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$] were each dissolved in 5% glucose.

DOSE AND ROUTE OF ADMINISTRATION

Group A (control) received 5% glucose; Group B (Pb) received 4.8 $\mu\text{mol Pb/kg}$; Group C (low Sn)

received 0.48 $\mu\text{mol Sn/kg}$ and Group D (high Sn) received 4.8 $\mu\text{mol Sn/kg}$. Each animal was injected with 0.5 ml/kg body weight via the marginal ear vein once a day for three consecutive days.

BLOOD SAMPLING

Blood drawn from the marginal ear vein was haemolysed by freezing on solid carbon dioxide. Heparin was used as an anticoagulant agent. About 1 ml of blood was collected from the marginal vein on the 1st, 2nd, 3rd, 6th, 9th, 14th, 23rd, 37th, and 55th days after the first injection. On the 1st and 3rd days an additional 2–4 ml of blood was collected for experiments on the effect of heat treatments or on the pH dependency.

ASSAY FOR ALAD ACTIVITY

ALAD activity was assayed according to the method of Chiba *et al.* (1976). ALAD activity was represented by absorbance at 556 nm (A_{556}) or μmol porphobilinogen (PBG) formed. The effect of heat treatment on ALAD activities was estimated from differences in values without and after pre-incubation at 40°, 50°, or 60°C. The method of Nikkanen *et al.* (1972) was used to study pH dependency. All determinations were carried out in duplicate.

Results

EFFECT OF TIN OR LEAD ON ALAD ACTIVITY

ALAD activities determined on the 1st, 2nd, 3rd,

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Fig. 1 ALAD activity before and after heat treatment at 60°C for 5 min in blood from rabbits injected iv with lead or tin. ● Group A (control); ■ Group B (4.8 $\mu\text{mol Pb/kg}$); ▲ Group C (0.48 $\mu\text{mol Sn/kg}$); △ Group D (4.8 $\mu\text{mol Sn/kg}$); ↑ Injection; | $\pm$ SD.

Fig. 2 Effect of temperature and duration of pre-incubation on ALAD activity in rabbit blood. (a) Group A (control); (b) Group B (4.8 $\mu\text{mol Pb/kg}$ iv); (c) Group D (4.8 $\mu\text{mol Sn/kg}$ iv). Blood samples were collected on the first day after single injection. Heat treatment: 60°C (●); 50°C (■); 40°C (▲).

6th, 9th, 14th, 23rd, 37th, and 55th days after the first injection are shown in Fig. 1. It can be seen that ALAD activity decreased significantly after a single injection in Groups B, C, and D ($P < 0.01$). In tin-treated animals (Groups C and D) activities became normal soon after the cessation of treatment, whereas recovery in the lead-treated animals (Group

Fig. 3 pH dependency of ALAD activity in rabbit blood. Blood was from Group A (control) (●), Group B (4.8 $\mu\text{mol/kg}$ of lead) (■), and Group D (4.8 $\mu\text{mol/kg}$ of tin) (▲). Blood was collected on the 3rd day after the first injection. Open symbols represent values after 5 min pre-incubation at 60°C.

B) was slow. Five minutes' pre-incubation at 60°C resulted in an increase in ALAD activity in Group B comparable to that in Group A (control), whereas the activities in Groups C and D stayed as low as that before heat treatment.

EFFECT OF TEMPERATURE AND DURATION OF HEAT TREATMENT ON ALAD ACTIVITY

ALAD activity in blood from intact rabbits fell to 33–25% after a 5-min pre-incubation at 60°C. The same pre-incubation increased the activity of blood ALAD after lead treatment to the level of heat-treated control samples. Heat treatment caused only a slight increase after tin treatment. Results with different pre-incubation schedules are shown in Fig. 2. In Group A (control) the activity decreased with increasing temperature and with time. In Group B (Pb), the activity increased by a factor of about 10 when the sample was heated at 60°C for 2.5 min, and by a factor of about 6 when the sample was heated at 50°C for 20 min; pre-incubation at 40°C did not produce any change in the activity. In Group D, pre-incubation at 60°C for 5 min increased activity by a factor of only 1.4, and pre-incubation at 50°C or 40°C did not influence activity at all.

On the 3rd day after the first injection, the pH-dependency of blood ALAD activity was estimated, with the results shown in Fig. 3. The optimum value for control rabbits was pH 6.8, and for those given tin or lead, pH 5.8–6.0. After heat treatment of blood samples, the optimum pH in blood from tin-injected rabbits stayed at the same pH range as that before heat treatment, whereas that in blood from lead-injected rabbits increased to pH 6.8.

Discussion

In the course of experiments on the effect of different

metals (Pb, Sn, Hg, As and Zn) on the activity of ALAD (Chiba and Kikuchi, 1974), it was noticed that tin, like lead, decreased ALAD activity. The effect of tin on porphyrin synthesis must be taken into account, therefore, when the activity of ALAD is used as a measure of lead exposure.

The effects of tin and lead on ALAD activity differ as follows: (1) recovery of ALAD activity in rabbits injected with tin is more rapid than in those injected with lead (Fig. 1); (2) heat treatment does not affect ALAD activity after tin treatment, but increases ALAD activity after lead treatment to the level of control heat-treated blood samples (Fig. 1); (3) the shift in optimum pH from 6.8 to 5.8–6.0 is the same in tin or lead treatments, but heat treatment prevents this pH shift in the blood of lead-treated rabbits.

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