The effect of zinc and pH on the behaviour of δ -aminolevulinic acid dehydratase activity in baboons exposed to lead

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ABSTRACT Four adult male baboons (*Papio ursinus*) were exposed to a cloud of lead oxide dust to induce changes in the status of δ -aminolevulinic acid dehydratase (ALAD). Enzyme activity fell rapidly to a steady state as blood lead levels rose above normal. Exogenous zinc was shown to activate the enzyme, and the antagonistic effect of zinc on *in vivo* and *in vitro* lead inhibition was demonstrated for baboons. In baboons not exposed to lead dust, ALAD showed an activity optimum at pH 7.1 which shifted to pH 6.8 with *in vitro* addition of lead. In baboons exposed to lead dust, with raised blood lead, activity optima were observed at pH 6.8 and 6.2, while the optimum at pH 7.1 was absent.

Erythrocyte δ -aminolevulinic acid dehydratase (ALAD) activity is a highly sensitive indicator of lead exposure (Tola, 1972) and has been used mostly for the demonstration of exposure of low intensity (De Bruin and Hoolboom, 1967; Haeger-Aronsen *et al.*, 1971; Hernberg *et al.*, 1969; Hernberg and Nikkanen, 1970). Nikkanen *et al.* (1972) demonstrated a shift in pH optimum in lead-exposed patients which did not occur when lead was added *in vitro*, although in both cases ALAD was inhibited. This is compatible with allosteric inhibition and ALAD has been shown to exhibit many characteristics of an allosteric enzyme (Nandi and Shemin, 1968).

Zinc which, added *in vitro* has an activating effect (Border *et al.*, 1976a) at certain concentrations (Finelli *et al.*, 1974; Abdulla and Haeger-Aronsen 1971), has been shown to override the inhibitory effect of lead on ALAD (Abdulla and Haeger-Aronsen, 1973; Meredith *et al.*, 1974; Finelli *et al.*, 1975) at various pH values (Border *et al.*, 1976b). Zinc also prevented the development of clinical signs of lead poisoning in the young growing horse (Willoughby *et al.*, 1972).

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The baboon appears to be an excellent metabolic model for abnormal exposures to ingested lead (Hopkins, 1970; Cohen *et al.*, 1974; Goldstein *et al.*, 1975) and has been employed to obtain a more systematic approach in the definition of dose and dose-rate relationships to both clinical and laboratory observation of toxic effects (Kneip *et al.*, 1971).

In the present study the baboon has been used to demonstrate the antagonistic effect of zinc at various pH values which was shown in humans (Border *et al.*, 1976b). Using baboons it was possible to extend these experiments to include *in vivo* lead exposure.

Materials and methods

Four adult male baboons of the local species (*Papio ursinus*) were used in the study. They were captured in the wild state shortly before the experiments began and were housed individually under normal animal house conditions.

Lead exposure was achieved *in vivo* by exposing the animals to a lead oxide dust cloud. Dusting continued for 26 days at 1.50 mg/m³ (S.D. 0.43 mg/m³) for 4 hours daily with Pb₃O₄ (Mean Mass Diameter 3.2 micron; Bulk Diameter < 5 micron).

Blood was withdrawn under light anaesthesia (Sernylan), using disposable syringes. Samples were collected in 25 ml plastic tubes (3033 Tissue

Culture Tubes, Falcon) fitted with wax-coated cork stoppers. Heparin (Boots, 500 U/ml) was added to the tubes at 10 μ l per ml of blood where uncoagulated blood was required.

The *in vitro* addition of zinc and lead before assay and the measurement of ALAD activity at various pH values have been described by Border *et al.* (1976b). Blood lead and zinc levels were determined using a Beckman 444 atomic absorption spectrophotometer. In calculating ALAD activity the overall mean packed cell volume (PCV) for each animal was used. Where pH curves are shown, the pH value of the actual incubation mixture is given and not that of the buffer. These values were obtained from Fig. 1, which emphasises that the buffer used in the incubation does not compensate entirely for the addition of the various assay components.

Fig. 1 Standard correction curve showing the relationship between buffer pH values and the effective pH of the ALAD assay.

Results

During the course of the experiment various observations were made for each animal. Figure 2 illustrates the mean values $\pm$ standard deviation obtained for blood lead and ALAD activity.

The initial mean blood lead was 9.9 μ g/100 ml (0.48 μ mol/l) over 6 samplings, with the individual means ranging from 6.0 to 16.7 μ g/100 ml (0.29 to 0.81 μ mol/l). Twenty-four hours after dusting started the mean value had risen to 19.0 μ g/100 ml

Fig. 2 The relationship between blood lead and ALAD activity determined at pH 7.10 in four baboons. * = value for a single animal.

(0.92 μ mol/l) and two days later stood at 41.0 μ g/100 ml (1.98 μ mol/l). This level was maintained during the dusting period and only began to drop 11 days after exposure stopped. After 21 days' recovery, mean blood lead was 31.0 μ g/100 ml (1.50 μ mol/l).

Mean serum zinc levels started at 76.7 μ g/100 ml (1.17 μ mol/l) over 2 samplings ranging from 74.5 to 78.8 μ g/100 ml (1.14 to 1.21 μ mol/l). During the rest of the experiment this level was maintained at a mean value of 78.3 $\pm$ 11.9 μ g/100 ml (1.20 $\pm$ 0.18 μ mol/l) over seven samplings.

PCV values for each animal throughout the experiment did not vary appreciably. The overall mean was 0.48 with individuals ranging from 0.416 $\pm$ 0.024 to 0.565 $\pm$ 0.025. To calculate individual ALAD activities, the respective mean PCV values were used.

Before lead dusting was started the mean ALAD activity for all animals was 729 μ mol porphobilinogen (PBG)/litre red blood cells (RBC)/h at pH 7.1, which is slightly higher than that reported by Goldstein *et al.* (1975) for infant baboons. Dusting started at 0900 on day 15 and continued until 1300. At 1400 no drop in ALAD activity had occurred. After 24 hours, however, the mean activity had dropped to 438 μ g PBG/litre RBC/h without further exposure. This indicates that there was a definite absorption lag with the Pb_3O_4 used. Subsequently enzyme activity dropped further and remained relatively constant for the duration of dusting. In the recovery stage of the experiment there was a slight increase in ALAD activity coupled with the falling blood lead levels, but this was not statistically significant.

Both before and during exposure to lead dust, the behaviour of ALAD activity was examined at pH values ranging from 5.9 to 7.7. The effect of adding lead and zinc ions *in vitro* over this range was also studied. The antagonistic effect of zinc to lead inhibition has been demonstrated over a range of pH values in human blood (Border *et al.*, 1976b) and the same is true for baboons. Figure 3 illustrates

Fig. 3 Typical patterns of ALAD activity in response to the *in vitro* addition of lead and zinc. A: Before *in vivo* dusting with Pb_3O_4 . B: After dusting.

the sort of response obtained in one of the animals and is typical of all four involved in the study. Before dusting (Fig. 3a), the control pH activity curve is relatively symmetrical with an optimum at about pH 7.10. By adding an arbitrary amount of exogenous zinc (0.53 mmol Zn/litre blood) to the assay, activity is increased appreciably. Exogenous lead (0.053 mmol Pb/l blood) reduces overall enzyme activity and the pH optimum is shifted lower (pH 6.8). When zinc is added with lead, the antagonistic

effect of zinc is observed and the pH optimum is again lower than that of the control.

Once the animal has been dusted with lead (Fig. 3b), ALAD activity is much lower, with the pH optimum (6.8) again lower than the undusted control. Addition of exogenous lead reduces activity still further. The picture changes when exogenous zinc is added to the blood from dusted animals. Zinc added to the control blood activates the enzyme appreciably, giving two pH optima, one at pH 6.8 and another at pH 6.2. Zinc added to the assay with exogenous lead again has an antagonistic effect and two optima are observed at 6.8 and 6.2.

Discussion

The results presented in Fig. 2 confirm that the adult male baboon (*Papio ursinus*) can be used as a primate model for the human response to lead exposure (Goldstein *et al.*, 1975). The study on ALAD activity over a range of pH values has emphasised that correction is necessary if absolute pH values are to be obtained. The original assay method of Burch and Siegel (1971) has been modified (Border *et al.*, 1976a) to use a 0.25 M sodium phosphate buffer. Even at this concentration the buffers cannot entirely compensate for the components of the assay mixture (Fig. 1). Addition of haemolysate to the buffer in the proportions of the assay, increases the pH of the mixture. This effect is more marked at lower pH values. Subsequent addition of zinc and/or lead does not alter the pH but the final addition of ALA-HCl to initiate reaction leads to the overall picture shown in Figure 1. If the correction is applied from pH 5 to pH 8, there is a corresponding non-linear reduction of the range of more than 25%, which is significant if absolute pH values are required.

The various pH optima illustrated in Fig. 3 correspond to a certain extent with the data obtained by Nikkanen *et al.* (1972) in human blood. This similarity would probably be closer if actual pH was recorded in their case. In the present study a single optimum (pH 7.1) was obtained with undusted control baboons. This activity was enhanced by exogenous zinc without a shift of the optimum. The *in vitro* addition of lead shifted the optimum to pH 6.8 and the antagonistic effect of zinc on lead inhibition is most marked at this pH. This finding suggests that lead alters the normal enzyme in baboons to produce this shift. This could be explained as an involvement of the allosteric nature of ALAD similar to that suggested by Nikkanen *et al.* (1972) for the human enzyme.

Once the baboons had been exposed to lead *in vivo*, the pattern changed (Figure 3b). Obviously,

ALAD activity was much lower and the control curve was relatively featureless. However, this residual activity could be enhanced by adding zinc, and two definite optima were then obtained. The peak at pH 6.8 matches that in blood from baboons not exposed to lead dust to which lead is added *in vitro*, and probably corresponds to the normal enzyme in the 'lead form'. This intermediate peak was not reported in humans with *in vivo* exposure (Nikkanen *et al.*, 1972). The activity optimum at pH 6.2 is evident in the presence of zinc and was demonstrated after prolonged exposure of the baboons to lead *in vivo*. This optimum would appear to be the result of *de novo* production of an enzyme with ALAD activity. The same pattern is obtained when zinc overrides the effect of added lead. Work is proceeding to determine how early this activity can be demonstrated and whether it is attributable to the induction of an isozyme. The possible contribution of this component under physiological conditions will determine whether it is of any significance in the body.

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