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δ-ALAD Activity Variations in Red Blood Cells in Response to Lead Accumulation in Rock Doves (*Columba livia*)

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The enzyme δ-aminolevulinic acid dehydratase (δ-ALAD, E.C. 4.2.1.24), catalyses the second step of the haeme biosynthetic pathway and is required to maintain the haemoglobin and cytochrome content in red cells. δ-ALAD is not only found in bone marrow cells, the major site of haeme synthesis, but also in circulating erythrocytes and other tissues. An inverse correlation was found between δ-ALAD activity in red blood cells and lead concentration in the blood. The degree of δ-ALAD inhibition in erythrocytes has been widely accepted as a standard bioassay to detect acute and chronic lead exposure in humans (Fujita et al. 1982) and in avians (Dieter 1979; Eastin et al. 1983). The value of this parameter as an indicator for environmental lead has been often reported in doves (Ohi et al. 1974; Hutton & Goodman 1980; Kendall and Scanlon 1982).

In lead-treated rats, an increase in δ-ALAD activity in bone marrow cells and in blood samples was shown by radioimmunoassay at 5 and 9 days after the treatment (Fujita et al. 1981). Similarly, the amount of δ-ALAD in human erythrocytes was increased in workers with moderate lead exposure (Fujita et al. 1982). Although blood δ-ALAD seems be more sensitive to lead in avian species than in mammals (Hutton & Goodman 1980), the usefulness of blood δ-ALAD activity as an index of lead exposure has already been questioned by Hutton (1983) in the pigeon and by Jaffe et al. (1991) in humans.

The present investigation studied the toxic effects of lead on rock dove red blood cell δ-ALAD activity in two situations: in doves treated with lead acetate in the laboratory and in doves exposed to the environment of Alcalá de Henares. The final lead blood concentrations were lower in the environmental than in the laboratory doves (Tejedor and González 1992). δ-ALAD activity in bone marrow cells and the relationships between lead accumulation and enzyme activity in red cells, are examined.

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MATERIALS AND METHODS

Two groups of twenty adult doves (male and female) were kept in our laboratory located on the outskirts of Alcalá de Henares. Two other groups of twenty male doves were exposed during March through May, 1989, to the environment at two different schools in the city of Alcalá de Henares (Zone I in Santiago Street, the center of the old town; and Zone II in Caballería Española Street, an area with heavy traffic). All were fed water and grain "ad libitum" which were free of lead contamination.

Laboratory doves were weighed and a suitable dose of lead acetate (5 mg/Kg equivalent to 2.77 ppm of Pb in total bodyweight) was administered orally once a week, for one, two, three or four weeks. One week after the last dose, blood samples were extracted from the braquial vein, doves killed and the femur and tibia removed. Pools of bone marrow cells were isolated from long bones as described by Tejedor et al. (1984) and both types of samples were kept at 4°C.

δ-ALAD activity was performed in triplicate, within 3 h of sample collection, according to the Standardised European method (Berlin and Schaller 1974). 200 uL of whole blood or bone marrow cells suspension were incubated at 37°C in a mixture containing (final volume 3.5 mL): ALA (0.01 M), phosphate buffer (0.1M) at pH 6.4 for exactly 60 min. The enzyme reaction was quenched by the addition of 1 mL $HgCl_2$ (5mM)/TCA (612mM). After centrifuging for 10 min at 20,000 g, the supernatant was filtered through acid-resistant filter paper (Whatman N° 54). 1 mL of filtrate was mixed with 1 mL modified Ehrlichs's reagent and the absorbance at room temperature was read after 5 min at 555 nm. The results were expressed as umol porphobilinogen generated per litre RBC per hour at 37°C (PBG umol/L/h). The triplicate values for absorbance of a sample had a standard deviation of less than 2%.

The haematocrit was determined in heparinized capillary tubes, centrifuged for 8 min at 11,000 rpm (Haemofuge, Heraeus). Haemoglobin was measured with Drabkin's reagent by a standard method (Zijlstra and Van Kampen 1981). Protein was estimated according to Lowry et al. (1951), using bovine serum albumin as standard. Student's t test was applied to determine the statistical significance.

RESULTS AND DISCUSSION

The haematological parameters were not highly modified by lead presence. There was no significant lead effect on haemoglobin levels during treatment or exposition (data not shown) and the haematocrit decreased only slightly in both groups (Table 1). The decreases in haematocrit were statistically similar in both groups of environmentally exposed male rock doves (15% in Zone I and 12% in Zone II) and in the treated doves (15% in males and 11% in females). Blood lead levels were substantially lower in the urban

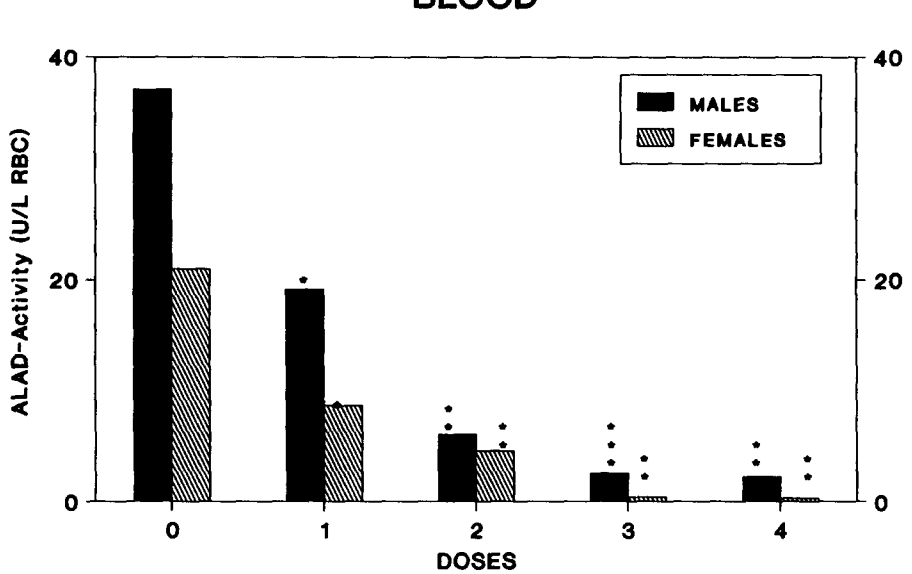

Figure 1. Blood δ-ALAD activity during lead treatment in rock doves. Superscripts denote statistical differences between each dosed groups and control: *** $p<0.001$, ** $p<0.01$, *$p<0.05$.

Table 1. Variations of haematocrit (%) in treated or exposed lead rock doves.

	Treated			Exposed males	
	Males	Females		Zone I	Zone II
Doses			Weeks		
Control	64.8±4.0	61.8±2.2	Control	64.8±4.0	64.8±4.0
1	63.0±1.0°	59.5±0.6*	4	56.1±1.5*	61.0±0.4*
2	62.3±2.0°	59.7±0.5*	6	57.9±1.2*	58.8±1.3*
3	57.3±2.0*	55.0±2.0*	8	57.7±1.6*	57.3±0.9*
4	55.5±0.1*	55.0±1.0*	10	54.8±2.7*	57.1±2.3*

.n=5, values are expresed X±SE. Superscripts within columns denote statistical differences between each treated or exposed group and control:* $p<0.05$, ° $p<0.1$.

doves than in the treated doves (Tejedor and González 1992) and there was no correlation between haematocrit decrease and blood lead increase levels. A similar effect has been found in other avian (Hoffman et al. 1981) and mammalian species (Tomokuni et al. 1989; Falke and Zwennis 1991).

Blood δ-ALAD activity in treated male and female rock doves began to decrease with the first lead doses (Fig. 1). Males show an

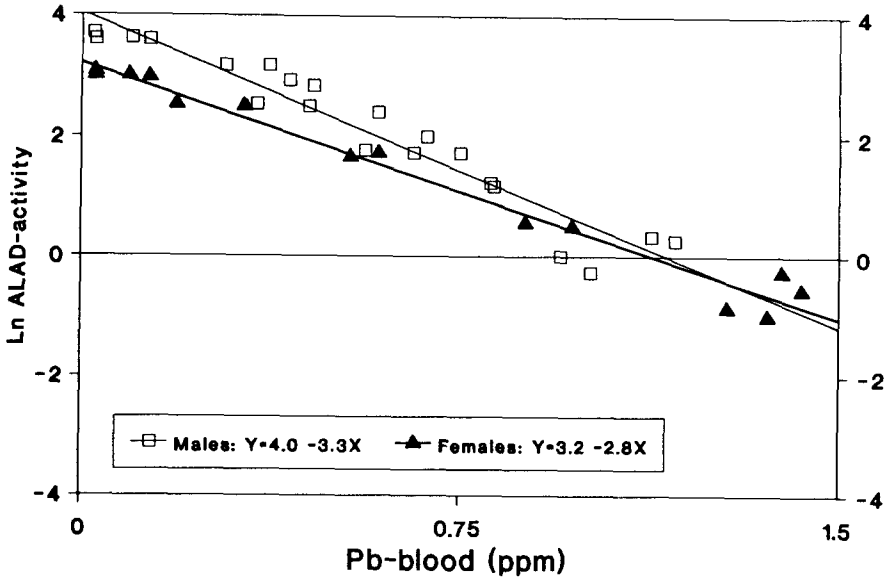

Figure 2. Linear regression of Ln δ-ALAD activity and lead concentrations in blood of treated rock doves (Correlation coefficient is 0.95 for males and 0.98 for females).

inhibition of 50% after the first dose and 93% at the end of treatment. Females present a similar behaviour with a more marked decrease of δ-ALAD activity, 60% of inhibition after the first dose and 98% at the end of treatment. This higher inhibition of blood δ-ALAD activity in female doves is consistent with higher lead blood accumulation (Tejedor and González 1992). An inverse correlation between δ-ALAD activity and blood lead levels can be established for both sexes (Fig. 2). This relationship confirms the reported hypothesis that presents blood δ-ALAD in rock doves as a sensitive indicator for environmental lead, since these birds present a higher inhibitory response than other species (Ohi et al. 1974; Hutton & Goodman 1980). Dieter et al. (1978 and 1979) presented a similar relation in ducks, although they did not note sex differences. Nevertheless, our results in doves indicate that the relative δ-ALAD activity inhibition in females (-2.8) is slightly lower than in males (-3.3)(Fig. 2).

δ-ALAD activity in the bone marrow cells (Fig. 3) of untreated and unexposed male rock doves (0.8 U/g Protein) is higher than that present in their blood (0.1 U/g Protein). δ-ALAD activity in Fig. 1 is expresed as U/L RBC, the equivalence is 0.1 U/g Protein = 38 U/L RBC. Bone marrow cells δ-ALAD is also inhibited after the first lead dose (Fig. 3) and the maximum inhibition, reached after the second dose, is 75%. However, in all cases the inhibition level in bone marrow cells was lower than that in blood, a similar result has been found (Tomokuni et al. 1989). Moreover, δ-ALAD activity began to recuperate from the third dose onwards, in spite of the continued rise in blood and bone lead levels during the

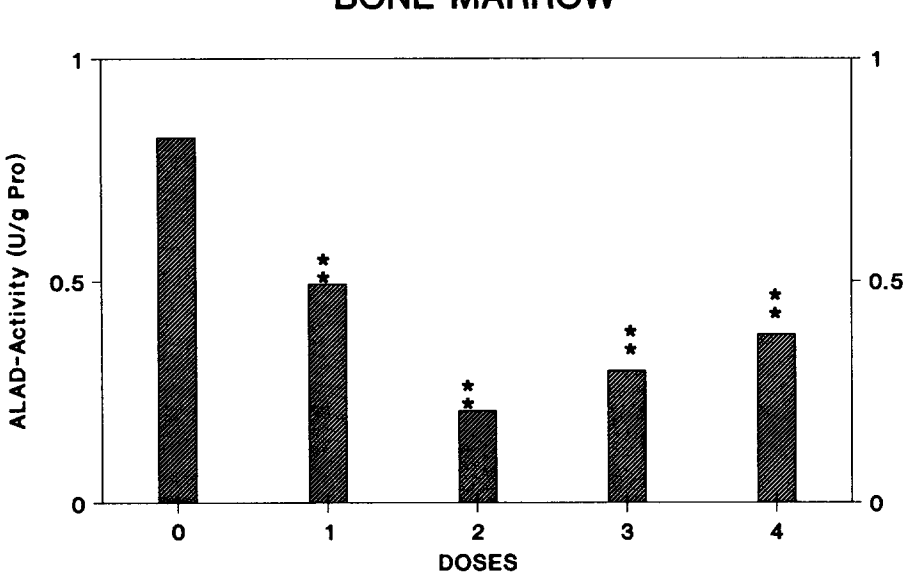

Figure 3. δ-ALAD activity in bone marrow cells during lead treatment in male rock doves. Superscripts denote statistical differences: $^{**}p<0.01$, $^{*}p<0.05$.

same period (Tejedor and González 1991). This data can be explained by an enzymatic induction effect similar to the one proposed by Fujita et al. (1981) and consequently a recuperation in blood δ-ALAD can be expected during the following weeks.

On the other hand, the level of blood δ-ALAD activity in the male rock doves exposed to the environment of Alcalá de Henares City (Fig. 4), decreased to 50% of the control values. In both zones, activity decreased gradually during the first weeks, but was recuperated after the 8th week. The inhibitory effect is more intense in Zone I than in Zone II, although the variations in both exposure groups were only significant ($p<0.05$) when each one was compared with the control population. Recuperation of δ-ALAD activity, which is mentioned earlier in the bone marrow cells of the treated doves (Fig. 3), can be explained as an increase in the enzyme through an enzymatic induction mechanism triggered by the decrease in blood activity levels. Fujita et al. (1982) gave a similar explanation for blood δ-ALAD levels in workers with moderate and continued lead exposure, as did Yagminas et al. (1990) in red blood cells from lead-treated male rats.

Although the differences between the two zones were nearly insignificant, both blood lead level and blood δ-ALAD inhibition were higher in Zone I (Fig. 4), which may explain why δ-ALAD recuperation begins later. The δ-ALAD activity inhibition found in rock doves exposed to the Alcalá de Henares City environment for 10 weeks is lower than that described in other urban centers (Ohi et al. 1974; Hutton 1980). This differences may be due to the use

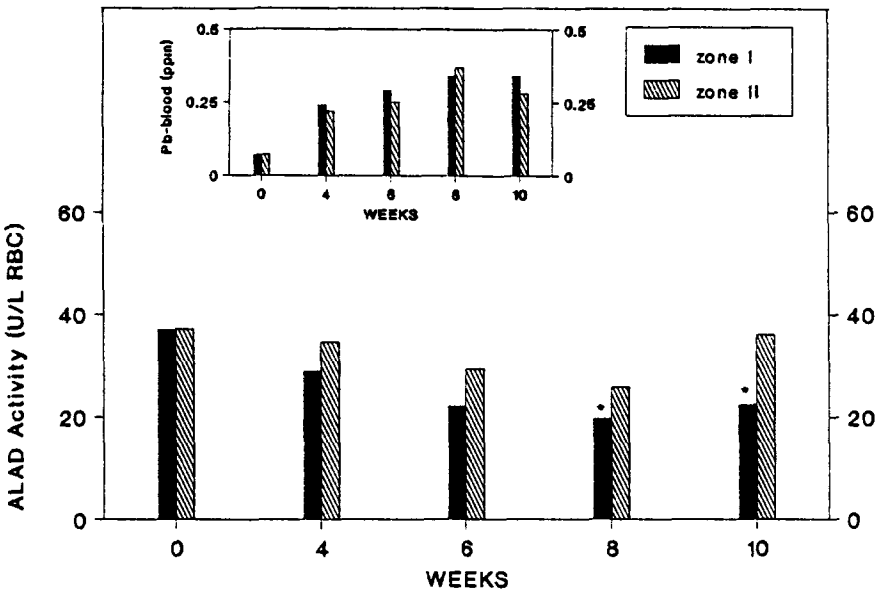

Figure 4. Blood δ-ALAD activity during exposition of male rock doves at environment of Alcalá de Henares City, Superscripts denote statistical differences between each exposed group and control: *p<0.05.

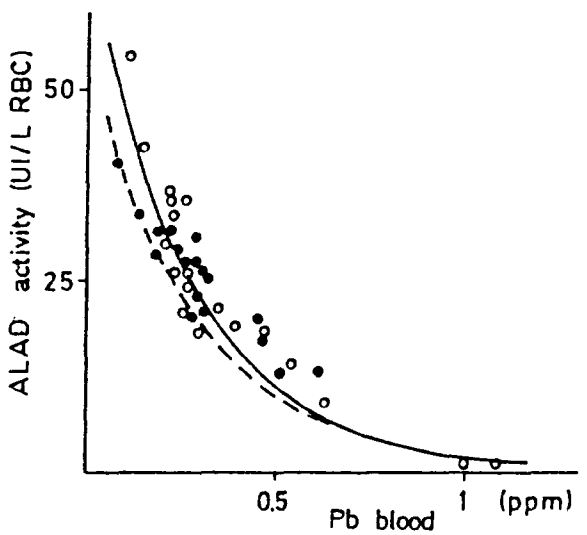

Figure 5. Correlation between blood δ-ALAD activity and blood lead levels in exposed and treated rock doves. The regression curves were: $\delta\text{-}ALAD = 52.48\ e^{-3.3[Pb]}$ (– – – , treated) and $\delta\text{-}ALAD = 68.20\ e^{-3.7[Pb]}$ (——— , exposed). Individual points are shown for exposed doves in Zone I (●) and Zone II (o).

in this latter studies of doves living in freedom with access to contamined foods and exposed for longer periods.

The correlation between blood δ-ALAD activity and blood lead levels of male doves was established (Fig. 5). Both exposed and treated rock doves present δ-ALAD activity in RBC exponentially decreasing while blood lead levels increased. But this δ-ALAD inhibition by lead is not uniform in all situations, since according to our results, it depends on dove sex and the conditions of exposure to lead. So, correlation curves for treated and exposed doves diverge for blood lead levels below 0.6 ppm (Fig. 5). It is possible that, when the contaminat impact is persistent, the inhibitory effect and the inductive response by the organism overlap and the general validity of δ-ALAD as a lead contamination indicator becomes conditioned. Consequently, we concur with Hutton's (1983) and Jaffe (1991) suggestion that δ-ALAD inhibition in the RBC of an organism is not an accurate bioindicator for lead levels in the habitat.

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