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# Comparison between Lead Levels in Blood and Bone Tissue of Rock Doves (*Columba livia*) Treated with Lead Acetate or Exposed to the Environment of Alcalá de Henares

M. C. Tejedor and M. Gonzalez

Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá de Henares, 28871 Alcalá de Henares (Madrid), Spain

Although rising public awareness of the problem in advanced societies has recently begun to cause a decrease in the release of pollutants into different habitats, the increase in the amount of lead released into the environment in developed countries during the last two to three decades has resulted in a significant increase in levels of this metal in organisms from completely different environments, whether rural, urban or industrial (Albaiges et al. 1987; Kaye et al. 1987; Hernández et al. 1987; Rincon-Leon et al. 1988).

Several surveys have been made in urban areas with high traffic densities in an attempt to identify plant (Hernandez et al. 1987) and animal species (Hutton and Goodman 1980) that might reflect environmental metal concentrations so that those species could be used as sensitive biological indicators of heavy metal contamination. Studies of lead accumulation in U.K. rock doves (Johnson et al. 1982) imply that use of this species as a pollution indicator would facilitate periodic monitoring of chronic lead exposure conditions in the urban environment. Laboratory investigations cannot readily reflect environmental conditions since the validity of extrapolating laboratory results, where high doses are administered over short-time periods, to the natural environment has been seriously questioned (Hutton 1982).

The present study was made on four rock dove (*Columba livia*) populations: two groups (males and females) were dosed with lead acetate in the laboratory and two groups of males were housed in different parts of the city of Alcalá de Henares. Data on lead bioacumulation were collected in two situations: The first was in a laboratory with controlled amounts of lead, while in the second situation the amounts reflected the actual environmental levels in Alcalá de Henares. Lead levels were determined in two tissues: blood, which is the target of first impact in possible acute situations; and bone, which is the main tissue where lead accumulates and, therefore, very important during chronic exposure. The study focused on the following three items: (1) lead

Send reprint request to M.C. Tejedor at the above address.

tissue distribution (2) variation with habitat, and (3) an evaluation of the levels of lead contamination in the city of Alcalá de Henares.

## 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 body weight) was administered orally once a week, for one, two, three or four weeks. One week after the last dose, blood and bone samples were removed and frozen until lead analyses.

Total lead analyses were performed on thawed tissue. Duplicate analyses were carried out on all samples. 50 µL of whole blood were treated with 1 mL 1% Triton X-100 (Fernández 1975). Bone samples were digested in nitric acid; diluted to a known volume with deionized water and transferred to acid-washed test tubes with Teflon screw-on caps. In both resultant solutions, the Pb concentrations were determinated by atomic absorption spectrophotometry using graphite tube atomization (Perkin-Elmer Model 2380 with HGA-400 graphite tube). The detection limit was 0.02 ppm. Lead concentrations were calculated from the bone weight or blood volume and the results expressed as µg/g (ppm). Geometric means were used to express the lead levels. Student's t-test was used to determine statistical significance.

## RESULTS AND DISCUSSION

Lead concentrations in blood and bone of adult male and female rock doves were determined before and after lead administration to determine the patterns of lead distribution (Figure 1). Lead concentrations in blood and bone of doves increased with the number of doses administered, indicating a bioaccumulation process in both tissues.

Lead concentration in blood was relatively high as compared with the controls from the first dose (Fig. 1, filled bars). Blood lead in male rock doves reached as high as 0.927 ppm in the third dose, with a slight decrease thereafter. Blood lead concentrations in female doves increased continuously, reaching 2 ppm after the fourth dose. The maximum lead concentration in blood (2 ppm) was close to the range of 1.5-1.9 ppm or 150-190 µg/dL, which has been considered non toxic to doves (Ohi et al. 1980). Moreover, lead levels obtained from the blood of female doves were always comparatively higher than those of male doves. This important

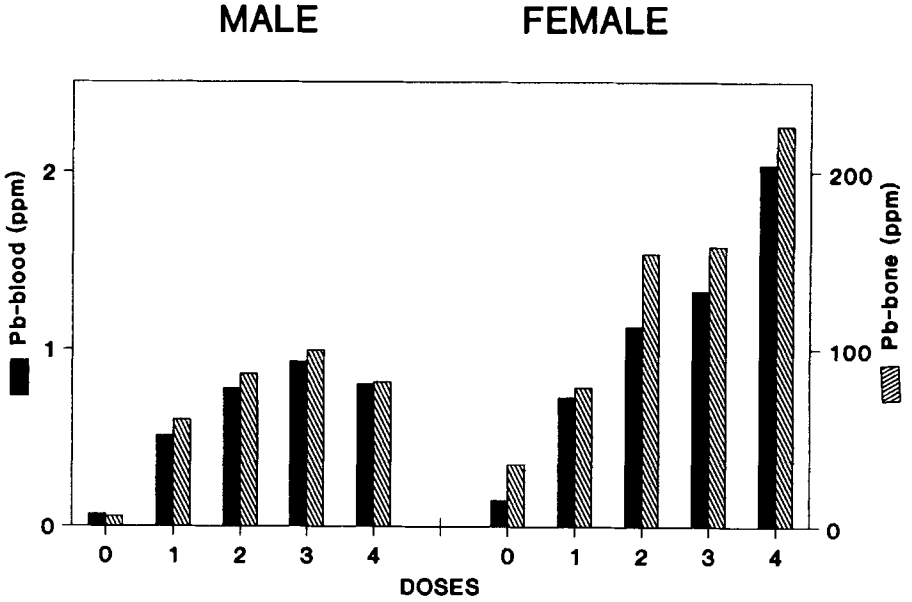


**Figure 1**. Lead concentrations (ppm) in blood and bone of male and female rock doves administered lead orally. $P < 0.01$ between treated and control doves; $P < 0.05$ between male and female doves, for blood and bone samples.

difference in lead bioacumulation processes between sexes will be considered later.

Lead levels in bone were compared to those in blood after each dose (Fig. 1). Lead concentrations in bone (hactched bars) were always about one hundred times higher than those in blood (filled bars) (notice the difference between both scales). Concentrations in bone were also higher than those which would result from even distribution of the each administered dose over the whole body (2.77 ppm). This bioconcentration of lead in bone may indicate that most of the metal is transferred from the blood to the bones. Distribution levels in these tissues are similar to those shown for lead and other metals in terrestrial and aquatic birds whether environmentally exposed (Kendall and Scanlon 1982; Newland and Dawn 1982) or laboratory-dosed (Latta et al. 1986; Marn et al. 1988). Newland and Dawn (1982) describe the capacity of bone to bioconcentrate lead and propose that total body lead is contained in two general pools, with the hard tissues being the most important pool as regards total lead.

To establish the relative bioconcentration capacity of these two tissues, the ratio between blood or bone lead concentrations and the amount of lead administered was calculated, and the bioconcentration factor values were obtained (Table 1). The ratios in blood were below 1 ,indicating that the bioconcentration process did not take place in this tissue, although there was a

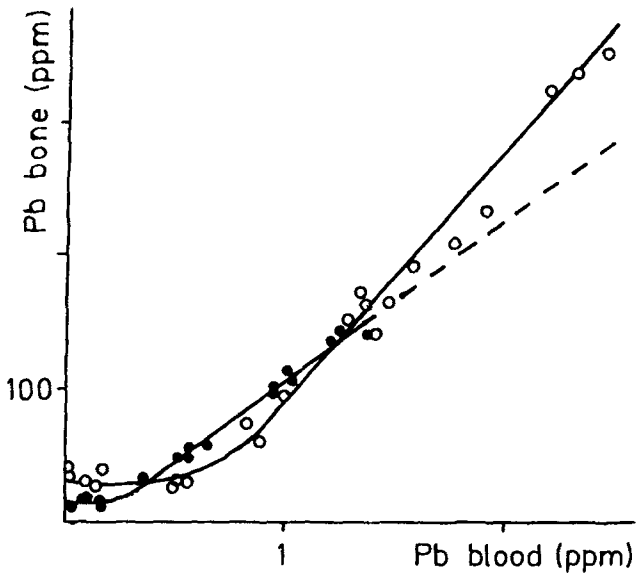


**Figure 2.** Linear regression of lead concentration in bone on lead concentration in blood of rock doves. Females (○), n=22, y=-101+188x. Males (●), n=18, y=-21+123x. Correlation index > 0.98.

**Table 1.** Bioconcentration factors of lead in dove blood and bone at different times; calculated from the amount of lead accumulated in each tissue in function of the amount of lead administered.

| | MALE | | FEMALE | |
|---|---|---|---|---|
| | Blood | Bone | Blood | Bone |
| 1 dose | 0.16 | 19.9 | 0.24 | 15.6 |
| 2 doses | 0.13 | 14.6 | 0.19 | 21.6 |
| 3 doses | 0.10 | 11.3 | 0.15 | 14.9 |
| 4 doses | 0.06 | 6.9 | 0.17 | 21.7 |

continuous accumulation process which did not reach saturation during this period. The markedly high values for the bone tissue ratios (nearly twenty) are indicative of a strong bioconcentration process. A similar finding was reported in doves (Marn et al. 1988) and feral urban pigeons (Hutton and Goodman 1980; Johnson et al. 1982).

The lead bioconcentration capacity of dove bone was also evaluated by means of a scatter plot of bone versus blood lead concentrations (ppm) in two groups of male and female doves (Fig. 2). In view of the curved slope, it is possible to affirm that, in

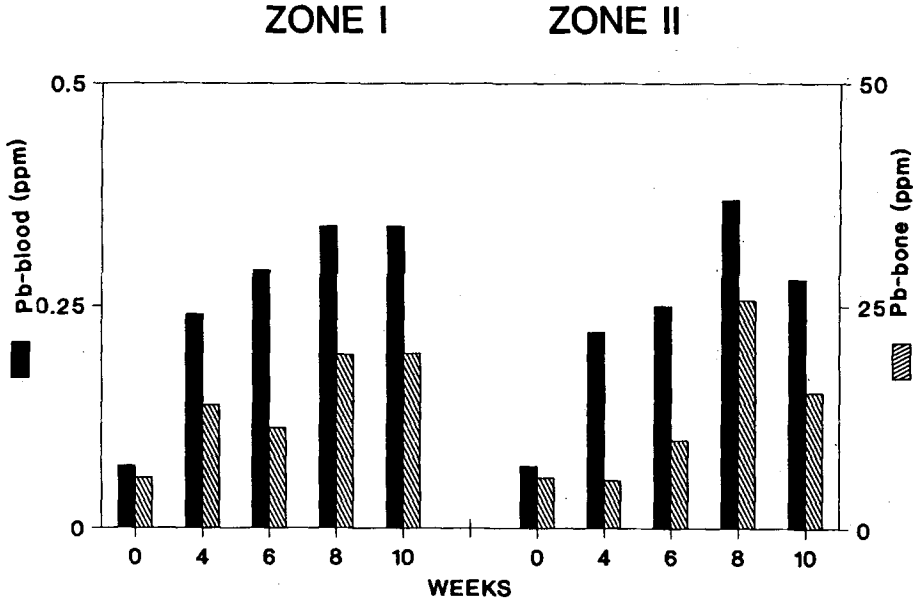


**Figure 3.** Lead concentrations (ppm) in blood and bone of male rock doves exposed to different urban areas of Alcalá de Henares. $P < 0.1$ for blood samples between both zones; $P > 0.1$ for bone samples between both zones.

male doves, an increase in blood lead of one ppm corresponds to an increase of 123 ppm in bone, thus confirming the capacity of bone tissue to bioconcentrate lead. In female doves, the slope of the curve is even steeper (188). Moreover, since the blood lead concentration at which lead accumulation in the bone begins is higher in female than in male doves. It may be deduced that lead bioconcentration is a sex-dependent process.

This sex difference is probably associated with the increase in the requirement for calcium during egg-shell formation in female birds. Intestinal calcium absorption is related to parathyroid hormone action and in avian species there is a marked increase in absorption during egg-shell formation. Diets low in calcium may allow more lead absorption from the gastrointestinal tract, as indicated by studies of intestinal calcium-binding proteins in chickens (Fullmer et al. 1985). Therefore, since a higher mobility of calcium is maintained in female birds, the xenobiotic ion ($Pb^{2+}$) may interfere with the biotic ion ($Ca^{2+}$) metabolism to a higher extent (Minnema et al. 1988). Lead accumulation may be produced in a similar way to $Ca^{2+}$ accumulation during the ossification process (Honda et al. 1987).

To evaluate lead contamination levels in the environment of Alcalá de Henares, two groups of adult males from a rural area were housed in two different sites in the city of Alcalá. Blood and bone lead concentrations were calculated five times over ten weeks

(Fig. 3). During the first four weeks the blood lead concentration rose three-fold (from approximately 0.07 ppm to 0.24 ppm) in both zones. In the second phase this accumulation was slower (from 0.24 ppm to 0.34 ppm in Zone I and from 0.22 ppm to 0.28 ppm in Zone II). In Zone I, which was in the center of the City, lead levels rose for 8 weeks and then remained stationary. In Zone II, which was a more congested area, the levels peaked at week 8 and at week 10 returned to about the level of week 6; the peak may possibly indicate a period of heavier traffic. Blood lead levels in doves exposed to other urban environments with high traffic density (Hutton 1982; Johnson et al. 1982; Kendall and Scallon 1982; Hernandez et al. 1987; Marn et al. 1988) are very similar to the samples taken from doves exposed to the Alcalá de Henares environment during the spring of 1989. All these authors conclude that there is a significant difference between the lead concentration in the blood of doves exposed to urban or to rural environments, so that Alcalá de Henares, a satellite-city east of Madrid, has an environment like that of other urban areas in different countries.

Comparing these results with those obtained from doves exposed in the laboratory, the lead concentration in the blood of exposed doves during three months was always lower than the lead accumulated from one oral dose (Fig. 1). The environmental lead level in Alcalá was persistent enough to produce a continuous increase of lead in dove blood since blood lead levels did not significantly decrease during the period studied. It may be concluded, therefore, that although the level of lead contamination in Alcalá de Henares is not excessively high, it is significant because the blood lead concentrations reached during exposure for 6 to 8 weeks are similar to the maximum permissible lead level for human blood (0.30 ppm) allowed by different international organizations (DHSS, 1980, EEC, 1982 , NCPCC, 1982). Levels of acute lead poisoning were not reached in any case (lead concentration > 300 µg/100 mL blood).

Lead levels in bone did not increase significantly during the period of exposure (Fig. 3). A process of lead bioconcentration takes place in bone tissue, but the intensity is lower for exposed doves than for treated ones. This difference may be due to environmental conditions such as lower amounts of lead from sources in the environment or possible metabolic alterations produced by habitat situation. Assuming that the final concentrations were in the same range and that the blood levels increased almost in parallel, it may be considered that the contamination level in the two zones was similar, maybe slightly lower in Zone II despite the peak at week 8. Consequently, lead levels reached in the blood and bone of doves exposed to the Alcalá de Henares environment indicate a city with important and constant lead enmission levels, a characteristic of an urban environment.

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