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Hematological Evaluation of Lead Intoxication in Mallards

Michele Mautino and John U. Bell

Department of Physiological Sciences, Box J-137, JHMHC, University of Florida, Gainesville, FL 32610-0137

Environmental lead exposure is a significant contributor to waterfowl mortality with an estimated 1.6 to 2.4 million ducks dying in North America each year (U. S. Fish and Wildlife Service, 1976). The high mortality rates have resulted in the implementation of lead shot restrictions in some areas of North America, yet the long term effects of these restrictions have not been determined. Many areas have not been restricted to the use of steel shot and remain sources of lead exposure for several species of waterfowl. Future waterfowl management decisions must be based on reliable indices of environmental lead exposure and an understanding of the impact of spent lead shot on waterfowl populations.

The measurement of blood lead has been the most widely accepted laboratory test for monitoring environmental lead exposure. Many technical difficulties are inherent in the precise quantification of blood lead at low concentrations and a margin of error of fifteen percent is not uncommon (Keppler et al. 1970). Additionally, difficuties arise when attempts are made to use blood lead concentration, which quantitates the intensity of recent environmental lead exposure, as a diagnostic indicator of lead intoxication. Measurement of critical biochemical alterations resulting from lead exposure may provide a better indication of the toxic response of an individual than the actual measurement of blood lead.

Lead exposure alters a number of enzymes resulting in impaired heme synthesis and an elevation or excretion of intermediates. Although the determination of blood lead has been the reference standard for monitoring lead exposure, the measurement of delta-aminolevulinic acid dehydratase activity (delta-ALAD) and free erythrocyte protoporphyrin concentrations (FEP) have been utilized to evaluate the extent of lead exposure in several species. In humans, there is a good correlation between both delta-ALAD and FEP and the deleterious effects seen following lead exposure (Blumberg et al., 1977; Labreche and Pan, 1982). In avian species, investigators have measured elevated lead concentrations and an inhibition of delta-ALAD activity in lead-dosed mallards (Finley and Dieter, 1976; Finley et al.,

1976), canvasbacks (Dieter et al., 1976), quail (Stone et al., 1977) kestrels (Franson et al., 1983) and eagles (Hoffman et al. 1981). Elevated concentrations of FEP have also been measured in small experimental populations of lead-dosed mallards using a direct fluorometric technique (Roscoe and Nielson, 1979). Delta-ALAD and FEP have each been proposed as potential biochemical indicators of lead intoxication in populations of wild waterfowl (Roscoe and Nielsen, 1979; Finley et al. 1976); however, no comparison of the two techniques has been made. This paper reports the results of the determination of delta-ALAD and FEP in experimentally lead-dosed mallard ducks and the regression comparison of these measurements with blood lead concentrations. The potential utility of each technique to assess lead intoxication in waterfowl is explored.

MATERIALS AND METHODS

Mallard ducks (Anas platyrhynchos) obtained through conventional trapping methods were maintained in large pens with natural forage and small ponds. Commercial game bird mash and green forage were provided ad libitum. After an acclimation period of several weeks, all birds were weighed, fluoroscoped and bled at regular weekly intervals for three weeks prior to dosing and for seven weeks after dosing. Individual birds with hematological values which deviated excessively from normal, or birds which exhibited lead shot in their gastrointestinal tract upon fluoroscopy, were eliminated from the experiment prior to dosing.

Experimental birds were randomly divided into dosed and undosed groups and housed in separate pens. Baseline hematological values (blood lead, delta-ALAD, FEP and hematocrit) were obtained weekly for a period of three weeks prior to dosing. Six undosed mallard ducks were maintained as controls, while seventeen mallards were dosed with two #4 lead shot (average weight 219 mg) orally via a flexible plastic tube inserted to the proventriculus. This is equivalent to an average elemental lead dosage of 423.8 mg/kg body weight. Weekly fluoroscopy insured that all dosed birds retained lead shot administered to them. Approximately 3 ml of blood were collected via jugular venipuncture into freshly-heparinized tubes at weekly intervals for 7 weeks post-dosing. At the conclusion of the 10 week experimental period, all birds were euthanized with an iv administration of pentobarbitol (Burns-Biotech) and necropsied. Kidney, liver and bone samples were removed and analyzed for lead utilizing atomic absorption spectrophotometry.

Lead concentrations in fresh blood and frozen tissues were determined by electrothermal atomic absorption spectrophotometry using a model 2380 atomic absorption spectrophotometer equipped with an HGA 400 graphite furnace and a deuterium arc background corrector (Perkin-Elmer Corp., Norwalk, CT.). Tissue wet weights were determined prior to ashing at 450°C for twelve hours in a muffle furnace and the ashed tissues were dissolved in hot nitric acid (Instra-analyzed, Fisher Scientific Co.) and brought up to volume with hydrochloric acid (Instra-analyzed, Fisher Scientific

Co.). Blood samples were wet digested (1:4) with nitric acid (Instra-analyzed Fisher Scientific Co.) at 65°C for twelve hours in stoppered glass tubes prior to lead analysis. Samples were calibrated against certified commercially-available standards (Fisher Scientific Co.) and results expressed as ug of lead per gram of tissue or per ml of whole blood.

Heparinized blood was analyzed for delta-ALAD activity within 12 hours of collection utilizing the spectrophotometric technique of Burch and Siegel (1971). In this technique, the pH of the incubation mixture is maintained at an optimum (6.2-6.7) during incubation by the use of a phosphate-citrate buffer (Wigfield and Farant, 1981). Enzymatic activity was measured in duplicate using a Gilford model 250 spectrophotometer and the results were expressed as units of delta-ALAD activity (change in absorbance at 555 nm per ml of erythrocytes per hour at 35°C).

Free erythrocyte protoporphyrin (FEP) concentrations were determined in fresh heparinized blood utilizing the extraction technique developed by Piomelli (1977). The extraction of protoporphyrin from whole blood avoids hemoglobin interference inherent in the direct fluorometric determination of FEP. FEP was extracted using an ethyl acetate-acetic acid mixture (4:1) and was then transferred to 1.5 M hydrochloric acid. Samples were analyzed in duplicate using a spectrofluorometer at an excitation wavelength of 405 nm and an emission wavelength of 595 nm and the fluorescence compared with that of a commercially-available standard (Porphyrin Products; Logan, UT). Results were expressed as mg of free erythrocyte protoporphyrin per dl of whole blood.

Non-parametric procedures in the Statistical Analysis System (SAS) were used to evaluate the three parameters measured during the dosing experiment. Analysis of variance was performed with the Wilson-Wilcoxon procedures (NPAR1WAY). Differences between dosed and undosed birds at a given time interval were analyzed for significance using the Wilson-Wilcoxon signed rank procedure, while differences between the time intervals were analyzed using the Wilson-Wilcoxon ranked sum procedure. Linear regression analysis of the data was performed utilizing SAS (REG). The response variable (blood lead) was predicted by regression variables delta-ALAD, FEP, time post-dosing and pre-dose blood lead concentrations. Each of these variables was then removed to determine independent effects.

RESULTS and DISCUSSION

Mallard ducks dosed with lead shot demonstrated acute neurological signs of lead toxicosis within twenty-four hours of dosing. Fourteen of the seventeen dosed mallards exhibited varying degrees of paralysis, kinetic ataxia or abnormal locomotor function. These neurological signs of lead intoxication gradually abated and by day eight post-dosing all mallards appeared normal. The dosed birds manifested no further signs of lead toxicosis during the seven week experimental period and were grossly indistinguishable

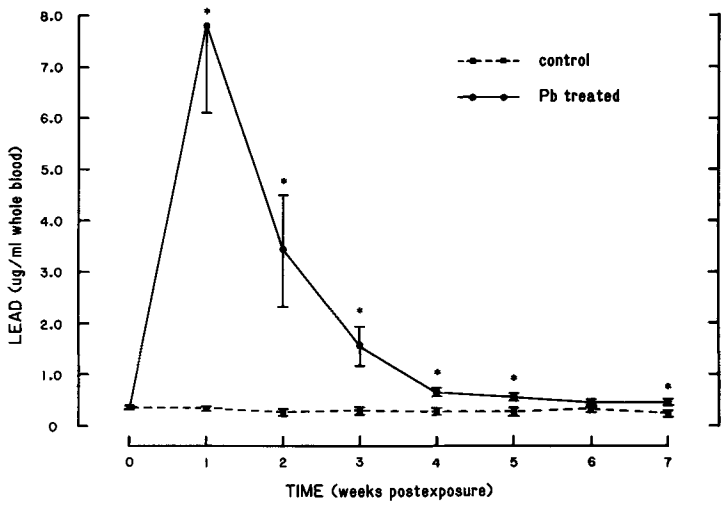

Figure 1. Concentrations of lead in the blood of dosed and undosed mallards (Anas platyrhynchos). Time 0 represents an average predosing value and 1 through 7 represent weeks postdosing. Vertical lines indicate standard errors and asterisks (*) represent time periods where dosed and undosed values are significantly different ($p<0.001$).

from the undosed control birds. Concentrations of lead in the blood of dosed birds were highest one week after dosing (7.8 ug/ml) and differed significantly from undosed birds until week 6 post-dosing (Fig. 1.). Blood lead concentrations in undosed birds remained at pre-dose levels throughout the seven week experimental period. The hematocrits of both dosed and undosed birds remained at normal levels. No deaths occurred in either the control group or the dosed group of mallards. Necropsies conducted seven weeks after dosing revealed no apparent gross lesions and all birds were of normal appearance and body weight.

Attempts to evaluate hematological changes in waterfowl as indices of lead exposure have been contingent upon the development and refinement of techniques measuring these changes in mammalian species. It is unquestionable that both delta-ALAD and FEP are reliable indices of lead exposure; however, each measures a different hematopoietic alteration and each appears to be suited for different applications.

The measurement of delta-ALAD has proven to be the most sensitive indicator of sub-clinical low level lead exposure in humans (Nieberg et al. 1974, Burch and Siegel 1971). The effect of lead exposure on delta-ALAD activity is shown in Figure 2 for mallards. Delta-ALAD activity was maximally inhibited in dosed birds at week one post-dosing (80 % inhibition) and gradually returned to near normal levels by week seven of the experiment. In

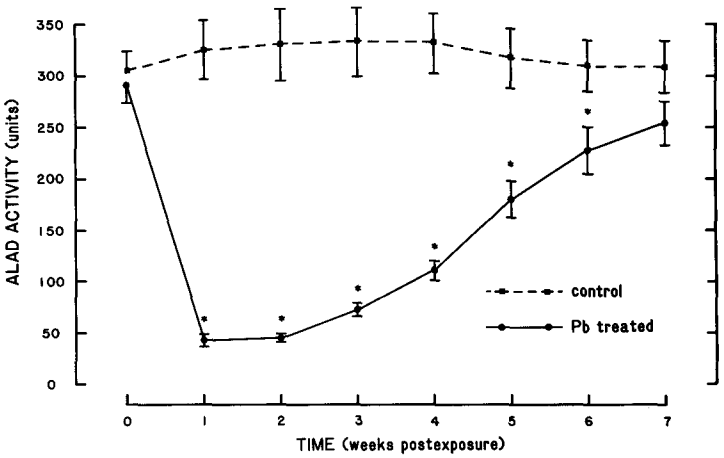

Figure 2. Delta-aminolevulinic acid dehydratase (ALAD) activity in lead dosed and control mallards (Anas platyrhynchos) at various times after exposure. Vertical lines indicate standard errors and asterisks (*) denote time intervals where ALAD activities of dosed and undosed birds are significantly different ($p<0.001$).

undosed birds, delta-ALAD activity remained at normal levels throughout the experimental period. Statistical regression analysis indicated that the log of delta-ALAD activity was inversely correlated with the concentration of lead in the blood ($r=-0.79$, $p<0.01$). The analysis of this enzyme has some important advantages: it is inactivated by lead over a range corresponding to subclinical lead intoxication (Haeger-Aronson et al. 1971, Weissberg et al. 1971); the interaction of lead with delta-ALAD is direct, thus the assay does not rely on the detection of an accumulated metabolite as in FEP measurement; and inhibition of this enzyme reflects both acute and chronic lead exposure although they cannot be differentiated. Unfortunately, delta-ALAD is inhibited to a low and constant level when blood lead is equal to or greater than 0.02 uM, so elevated concentrations of blood lead beyond this threshold cannot be distinguished. These characteristics of the response of this enzyme to lead exposure contribute to the suitability of this measurement for use as a screening technique to detect low level lead exposure.

Increases in FEP concentration only become significant when the blood lead concentration exceeds 2.90 uM, thus changes in this hematopoietic intermediate are much less sensitive to low levels of lead exposure. The concentration of FEP in lead-dosed mallard ducks was highest one week after dosing (255 ug/dl) (Fig. 3). Blood concentrations of this intermediate in heme synthesis gradually decreased until no significant difference was noted when

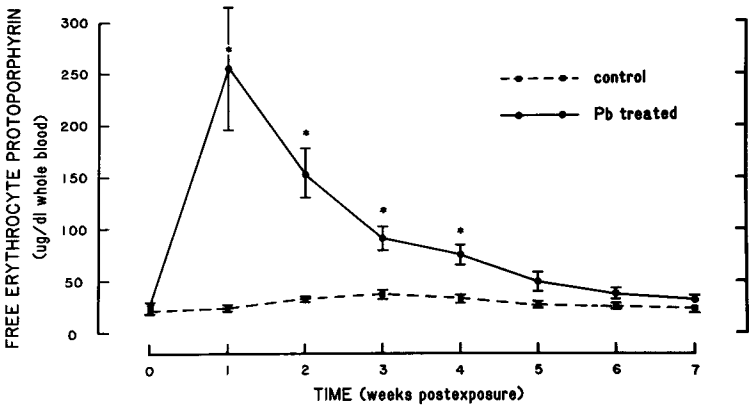

Figure 3. Free erythrocyte protoporphyrin (FEP) concentrations in lead-dosed and control mallards (Anas platyrhynchos) at various times after dosing. Vertical lines indicate standard errors and asterisks (*) denote time intervals where FEP concentrations of dosed and undosed birds are significantly different from each other ($p<0.001$).

compared with undosed birds at week five post-dosing. The log of FEP concentration showed a stronger positive correlation with blood lead concentration ($r=0.84$, $p<0.01$) than did delta-ALAD.

Field survey research has demonstrated that the accumulation of lead in tissues correlates with the presence of lead shot in the gizzard (Hall and Fisher 1985). The concentrations of lead in bone, liver and kidney measured at the end of this seven week experiment were significantly higher in dosed birds than in undosed birds (Table 1). The partitioning of lead among target organs and the concentration of diffusible lead in the blood are directly responsible for the occurrence of clinical signs of lead toxicity. Comparing the concentrations of lead in the three tissues revealed that lead concentrations were highest in bone, intermediate in kidney and lowest in liver. These findings were in

Table 1. Concentration of lead (ug/g wet weight) in tissues of dosed and undosed mallards.

	Bone	Liver	Kidney
Undosed Birds	11.69 ± 3.44	0.22 ± 0.02	0.69 ± 0.08
Dosed Birds	20.25 ± 2.51	0.62 ± 0.06	1.30 ± 0.17

agreement with previously published values (Chasko et al., 1984). Thus these birds, demonstrating no clinical signs of lead intoxication, were carrying heavy body lead burdens sequestered in bone. It must be emphasized that the experimental population utilized in this investigation was composed of wild-caught birds, thus the individual history of lead exposure is unknown and interpretation of tissue lead concentrations must be undertaken with some degree of caution.

Previous waterfowl management practices have determined species at high risk for lead intoxication by the concentration of lead in bone tissue (Baker and Thompson 1979). Although the concentration of lead in bone is a reliable index of total lead body burden, this measurement may not be the best assessment of lead intoxication. Research on human lead toxicity has demonstrated that the first measurable adverse effect occurring after lead exposure is the enzymatic alteration of heme synthesis; this is the most sensitive and specific clinical sign of lead toxicity (Chisolm et al. 1975). Demonstration of strong correlations between delta-ALAD, FEP and blood lead concentration support the proposal that alterations in heme synthesis may provide a better indicator of the toxic response to environmental lead exposure than the measurement of the concentration of lead in blood or bone.

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REFERENCES

Baker JL, Thompson RL (1979) Shot ingestion by waterfowl on national wildlife refuges in Florida. Proc Ann Conf SE Assoc Fish Wildl Agr 32:256-262.

Blumberg WE, Eisinger J, Lamola AA, Zuckerman DM (1977) Zinc protoporphyrin level in blood determined by a portable hemato-fluorometer: a screening device for lead poisoning. J Lab Clin Med 84:713-723.

Burch HB, Siegel, AL (1971) Improved method for measurement of delta-ALAD activity of human erythrocytes. Clin Chem 17:1038-1041.

Chisolm JJ, Barrett MB, Mellits, ED (1975) Dose effect and dose response relationships for lead in children. J Pediat 87(6):1152-1160.

Dieter MP, Perry MC, Mulhern, BM (1976) Lead and PCB's in Canvasbacks: Relationship between enzymes and residues. Arch Environ Contam Toxicol 5:1-13.

Finley MT, Dieter MP (1976) Sublethal effects of chronic lead ingestion in mallard ducks J Toxicol Environ Health 1:929-937.

Finley MT, Dieter MP, Locke LN (1976) Delta-aminolevulinic acid dehydratase: inhibition in ducks dosed with lead shot. Environ Res 12:243-249.

Franson JC, Sileo L, Pattee OH, More JF (1983) Effect of chronic dietary lead in American kestrels. J Wildl Dis 19(2):110-115.

Haeger-Aronsen B, Abdulla, M, Fristedt, L (1971) Effect of lead on delta-aminolevulinic acid dehydratase activity in red blood cells. Arch Environ Health 23:440-444.

Hall SL, Fisher FM (1985) Heavy metal concentrations of duck tissues in relation to ingestion of spent shot. Bull Environ Contam Toxicol 35:163-172.

Hoffman DJ, Pattee OH, Weimeyer SN, Mulhern B (1981) Effects of lead shot ingestion on delta-aminolevulinic acid dehydratase activity, hemoglobin concentration and serum chemistry in bald eagles. J Wildl Dis 17(3):423-431.

Keppler JF, Maxfield MF, Mors WD, Tietjen G, Linch AL (1970) Interlaboratory evaluation of the reliability of blood lead analysis. Amer J Indust Hyg Assoc 31:412-415.

Labreche F, Pan A (1982) Relationships between three indicators of lead exposure in workers: blood lead, delta-aminolevulinic acid dehydratase and free erythrocyte protoporphyrin. Int Arch Occup Environ Health 5:35-44.

Nieberg PI, Weiner LS, Oski BF, Oski FA (1974) Red blood cell delta-aminolevulinic acid dehydratase activity: an index of body lead burden. Amer J Dis Child 127:348-350.

Piomelli S. (1977) Free erythrocyte protoporphyrin in detection of undue absorption of lead and iron deficiency. Clin Chem 23:264-269.

Roscoe DE, Nielsen SW (1979) A simple quantitative test for red blood cell protoporphyrin in lead poisoned ducks. J Wildl Dis 15:127-136.

Stone CL, Fox MRS, Jones AL, Mahaffey, KR (1977) Delta-aminolevulinic acid dehydratase: a sensitive indicator of lead exposure in Japanese quail. Poultry Science 57(3):811-813.

U.S. Fish and Wildlife Service (1976) Final environmental statement on the proposed use of steel shot for hunting waterfowl in the U.S. U.S. Government Printing Office. Washington, D.C.

Weissberg JB, Lipschutz F, Oski F (1971) Delta-aminolevulinic acid dehydratase activity in circulatory red blood cells: a sensitive laboratory test for the detection of childhood lead poisoning. N Eng J Med 284:565-569.

Wigfield DC, Farant J, Goldberg C, MacKeen JE (1981) Interferences in the delta-aminolevulinic acid dehydratase (ALA-D) assay. J Anal Toxicol 5:57-61.

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