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LEAD SHOT POISONING IN RED-TAILED HAWKS

By

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ABSTRACT

LEAD SHOT POISONING IN RED-TAILED HAWKS

By

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Research was conducted to duplicate lead poisoning as seen in living birds of prey. Nine birds were divided into 3 groups. Group 1 consisted of control birds which received no lead. Birds in the second group received 4 sequential doses of 10 number 5 lead shot. Individuals in the third group were dosed 4 times with 30 number 5 shot.

Birds were evaluated for clinical evidence of lead poisoning, blood and tissue lead levels, total leukocyte numbers, hemoglobin, hematocrit, osmotic erythrocyte fragility, and gross and microscopic lesions.

Apparent clinical signs were not observed in any bird on experiment. Analyses of repeated blood samples from all dosed birds were indicative of acute exposure to lead. Tissue lead levels did not indicate toxicosis. Blood parameters monitored in this study were found not to be predictable for lead intoxication. Spontaneous erythrocyte fluorescence occurred on a non-uniform basis in birds from groups 2 and 3. Gross observation and microscopic examination of selected tissues revealed no pathological changes associated with plumbism.

Birds of prey which were able to egest lead shot showed neither gross nor microscopic evidence of lead poisoning but, in fact, did absorb lead during instances of acute exposure.

".....The greatest importance of lead poisoning under natural conditions may not be direct mortality, but subclinical disease and its subsequent effect on susceptibility to disease, predators, and natural elements as well as its effect on reproduction."

- R. S. Cook and D. O. Trainer,
Journal of Wildlife Management,
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INTRODUCTION

Lead is a recognized constituent of the food chain of all birds. Sublethal exposure by ingestion of plants and other living tissues produces natural background lead levels in raptors and non-raptors alike (Bagley et al., 1967).

Excessive ingestion of lead, especially in the form of spent shot, has been a major factor in North American waterfowl poisonings since the late 1800's (Cook et al., 1966). These migratory individuals with ingested lead shot and high tissue lead levels are often food sources for predatory birds.

Non-digested material forms a pellet in the muscular stomach of the raptor. This material is periodically egested in the normal individual (Duke, 1978). Digestible muscle and viscera from crippled ducks and small mammals with high tissue lead levels would not be egested and may provide a means of secondary toxicosis.

The quantity of lead ingested, the duration, and the frequency of exposure to lead required to produce toxicosis are unknown in the raptor, although well documented in waterfowl (Coburn et al., 1951).

Plumbism in raptors has been diagnosed at necropsy since severely debilitated birds die shortly after presentation.

The objectives of this research were to attempt to duplicate the natural occurrence of lead poisoning in raptors using red-tailed hawks, and to evaluate the clinical and subclinical changes in the experimental birds.

LITERATURE REVIEW

Lead poisoning has been noted as a factor in waterfowl mortality since 1874 (Phillips and Lincoln, 1930). Grinnell (1901) observed physical signs of plumbism in ducks, geese, and swans naturally poisoned on the North Carolina coast during the winter of 1893-4. Bowles (1908) reported a condition in mallards found in marshy areas on the Puget Sound. The birds had no wounds or visible trauma, but contained quantities of lead shot in the stomach. The causes of death were not positively determined as many of these animals had recently been eaten by hawks or owls.

The possibility of death due to ingestion of spent shot has been recorded in scaled and bobwhite quail (Campbell, 1950; Westemeier, 1965) and mourning doves (Locke et al., 1967).

Birds affected by lead show pronounced weight loss, difficulty in flying and walking, and severe muscle degeneration (Linduska, United States Department of the Interior, 1964). The inability for flight escape enables these birds to become sitting ducks for hunting raptors.

The dietary preferences of raptors vary. Red-tailed hawks, for example, survive on rodents, rabbits, insects, and snakes, as well as medium or large birds caught on the ground (Brown et al., 1968). Research on captive birds of prey indicates superior performance and maintenance when a diet of larger mammals is supplemented with smaller mammals and birds (Mendelssohn et al., 1970). Red-tails in harsh winter climates expand their diets to include poultry, game birds and

song birds (Hausman, 1948). The golden eagle, another raptor, feeds on ducks, geese and other waterfowl, grouse, wild turkeys, herons, other birds, mammals and carrion. The sea eagle has a predilection for a seafowl diet in addition to mammals. Other raptors fond of waterfowl are the black and white gyrfalcon, peregrine falcon, and the large white snowy owl (Hausman, 1948). Studies by Craighead and Craighead in 1942 and 1948 of raptor winter diets in southern Michigan showed that the red-tailed hawk was more flexible in its tastes than some of the other buteos. A red-tailed hawk's summer diet in Superior Township of Michigan in 1942 and 1948 included adult ring-necked pheasants, as did that of Cooper's hawks and horned owls (Craighead and Craighead, 1956).

Benson et al. (1974) reported on the death of a prairie falcon fed ground duck heads which had detectable lead levels. The author felt these levels were high enough to cause plumbism and subsequent death. This method of acquired lead toxicosis falls into a classification of secondary poisoning (e.g., lead poisoning due to the ingestion of tissues from an animal sick or dead from lead poisoning). Jacobson et al. (1977) described a case of lead poisoning in an immature bald eagle which on postmortem had a total of 75 lead shot in the gastro-intestinal tract. Redig (1979) diagnosed clinical lead poisoning in 2 prairie falcons and 1 goshawk. He also suspected lead as being the primary cause of death in 1 prairie falcon, 1 bald eagle, and 2 red-tailed hawks.

Unpublished information from the logs of the Michigan State University Animal Health Diagnostic Laboratory lists 32 avians as having died

since 1968 either from lead poisoning alone or in conjunction with other disease processes. Fourteen of these 32 birds (ranging from ducks to bustards) still retained eroded lead shot in the gizzard.

Clinical Signs and Gross Lesions

Grinnell (1901) in his observations of waterfowl in Texas and North Carolina made note of the clinical signs of lead poisoning. He found birds free from external lesions, but unable to fly. His initial descriptions were of a "croupy" (his terminology) condition with the bird having labored, rattling respirations and a yellow discharge from the bill. His dissection of an affected goose revealed particles of pitted lead shot in the gizzard. The gizzard had a decayed, greenish inner membrane not seen in a healthy individual. There was marked inflammation of the intestine and the rectum. One liver lobe was discolored. This animal had a healthy external appearance. Similar lesions appeared in a swan necropsied by Grinnell a few days later.

Additional information in 1950 (Jordon et al.) included severe weight loss as a common finding in lead poisoned ducks.

Robert Stroud, writing from Alcatraz in 1939, observed awkward movements, blindness and digestive disorders in some of his domestic fowl which he later concluded had lead toxicosis. His microscopic evaluations of livers and kidneys from poisoned juvenile birds revealed "lipid degeneration" (his terminology). There were no macroscopic lesions in these tissues. Another bird had jerky movements, vocalized, and eventually convulsed and died. The kidneys again appeared grossly normal and healthy, although the liver was discolored and yellow and the spleen shrunken. Beer and Stanley (1964) necropsied 74

birds (swans, ducks, geese) suspected of having lead poisoning. Two-thirds of these birds were emaciated, having little or no subcutaneous or visceral fat. Other findings noted were gizzard impaction, anemia, enlarged gallbladders, hepatic atrophy and deterioration. At least 63 percent of these wildfowl had recognizable lead in the gizzard. Bellrose, in a 1964 United States Department of the Interior publication, described lead poisoning symptoms. He believed the initial sign of toxicosis was inappetence due to partial paralysis of the gizzard musculature. His view was that birds with prolonged cases of 2 or 3 weeks' duration either recovered or died after impaction of the stomach. The critical time period for retaining or voiding this shot was 20 days (Bellrose, 1964). Birds did not linger in acute toxicity cases, but succumbed in 1 to 2 weeks depending upon the quantity of lead ingested (Bellrose, 1964). Chronic victims had atrophied flight muscles and green-stained vents. They were less likely to escape hunters and predators.

The average period of shot retention in mallards was 18 days (Bellrose, 1964). Waterfowl eliminated the shot by defecation. Raptors had a much slower course of disease (Redig, 1979). The clinical signs were not pronounced and sometimes were nothing more than depression or weakness. The mutes often became runny as the disease progressed (Redig, 1979).

Raptors had an additional mechanism for eliminating non-digestible material. Raptors egested this material in the form of a pellet. This regurgitation was considered a physiologic requirement for birds of prey (Mendelssohn, et al., 1970). Predicting the regularity of this egestion was rather difficult. The time period from eating to

egestion for owls, for example, was about 12 hours. Hawks had an interval of 19.5 hours to 23.5 hours, supposedly triggered by light (Duke et al., 1976). The quantity of food consumed influenced the rate of egestion in owls. Meal size apparently did not have the same effect in hawks. Duke calculated that hawks cast pellets from approximately 80% of their meals. The specific diet, as well as the health of the raptor, apparently influenced the egestion rate (Duke et al., 1976). Consumption of lead shot, or of prey with high tissue lead levels, did not result in the same rate of casting for owls as for hawks. Hawks continued to eat with retained stomach pellets, while owls did not (Duke et al., 1976).

The number of retained lead shot fatal to birds varied among individuals (Bellrose, 1964). The variability in the number of shot required for poisoning and the course of disease were influenced by the type and quantity of food consumed. Birds with ingested shot, continuing to eat as they normally would, had excellent chances for survival (Bellrose, 1964). The gizzard in these birds also continued to function as did the egestion, or casting, mechanism.

Coburn et al. (1951) dosed wild caught mallards with lead to simulate field poisonings and to clinically observe the birds. These birds did not demonstrate the classic mammalian signs seen with lead poisoning. They initially displayed lethargy and weakness. Progressive paralysis of leg and wing musculature was observed in most birds. Green bile staining the vent area was a common finding. The gross lesions included emaciation, liver atrophy, gizzard atrophy, and flabby heart muscles. Roscoe et al. (1977) found ruffled feathers, head pressing, and diarrhea in addition to previously observed clinical

signs. His gross lesions duplicated those previously mentioned.

Published descriptions of the clinical signs of lead poisoning in specific birds of prey mentioned incoordination in flight, ataxia, apparent problems in visual perception (Benson et al., 1974), head tilts, and yellowish material lining the oral cavity (Jacobson et al., 1977).

Microscopic Lesions

Buck et al. (1973) indicated that certain changes were consistent with exposure to excessive lead. Acute exposures resulted in mild gastritis, pale livers with centrilobular degeneration, occasional acid-fast intranuclear inclusions in the renal tubular epithelium, renal hemorrhage, and degeneration or necrosis of the renal tubular epithelium. A thickened glomerular capsule was often present.

Stroud (1939) investigated microscopic sections of liver and kidney which grossly appeared unaffected, but had marked "lipoid degeneration" (his terminology).

Canada geese fatally dosed with number 4 lead pellets had such lesions as hepatic congestion, brownish pigment in the hepatic and Kupffer cells, necrosis of the renal tubular epithelial cells, myocardial necrosis, hyperemia and hemorrhage in the lungs, muscle necrosis in the gizzard, and irregular necrotic areas in the pancreas (Cook and Trainer, 1965). Locke and Bagley (1967) likewise found focal hepatic necrosis and pigment deposition in lead poisoned mourning doves. They saw acid-fast intranuclear inclusion bodies in the epithelium of the proximal convoluted tubules of the kidney as did Locke et al. (1966) and Grandy et al. (1968). Canada geese dying from

lead intoxication did not consistently have acid-fast intranuclear inclusion bodies in the renal tubular epithelium (Bagley and Locke, 1967).

Roscoe et al. (1977) examined tissues from mallards dosed with predetermined numbers of number 4 lead shot. There were acid-fast intranuclear inclusions in the proximal convoluted tubular epithelium of the kidney in 70% of the birds that died during the study. Other lesions included bile pigment accumulation in the hepatocytes, degeneration of the proventricular plica, edema of the gizzard mucosa, and lymphocytic and heterophilic infiltration into the gizzard mucosa.

The histopathologic changes seen in the tissues of lead poisoned birds necropsied since 1968 at the Michigan State University Animal Health Diagnostic Laboratory ranged from hepatic hydropic degeneration and fatty metamorphosis (cases 103557-560, 119047, 111423) to mild toxic tubular nephrosis and renal tubular epithelial degeneration (cases 103557-60, 111243-4, 111423, 119047, 135898).

Histopathologic changes in various raptors with lead poisoning included degenerative changes in the renal tubular epithelial cells, acid-fast intranuclear inclusion bodies in those same cells, and cloudy swelling of hepatocytes (Locke et al., 1969). Examination of tissues from an affected bald eagle revealed focal hepatic necrosis, pulmonary congestion, mild catarrhal enteritis, and mild interstitial nephritis. The bald eagle had no lead inclusions in the renal tubular epithelium (Jacobson et al., 1977).

Diagnostic Techniques

A blood lead determination has been the best antemortem diagnostic technique for evaluating acute lead poisoning in domestic mammals (Hernberg et al., 1970; Buck et al., 1973). Values for blood lead levels in the chicken ranged from 8 parts per million (ppm) without clinical manifestations of poisoning to 13 ppm just before death (personal communication of Buck et al. to V. E. Vengris, College of Veterinary Medicine, Iowa State University, 1972). Cattle, horses, waterfowl, and dogs apparently are more frequently affected by lead poisoning, while goats, chickens, and pigs are less susceptible (Buck et al., 1973).

Cook and Trainer (1966) dosed Canada geese with 2, 5, 10, 25, 50 and 100 number 4 lead pellets. All geese had rapid rises in blood lead levels within 3 days which reflected a direct relationship between the quantity of lead ingested and the blood lead levels. Predosing blood lead levels ranged from .018 milligrams per 100 grams (mg/100 gm) to .037 mg/100 gm. These were normal values for geese. Postdosing levels ranged from .081 mg/100 gm to 1.020 mg/100 gm.

Barrett and Karstad (1971) monitored 6 mallard ducks receiving 8 number 6 lead shot. The ducks had pretrial blood lead levels ranging from .6 ppm to 1.8 ppm. The lead levels ranged from 7 ppm to 19.1 ppm by the third day after dosing. Another group of mallards receiving 6, 12, and 18 pellets had markedly increased blood lead levels by 10 hours after dosing. The data generated by Barrett and Karstad did not reflect a direct relationship between the quantity of lead shot ingested and the resulting blood lead levels. Additional birds receiving 15 or 25 pellets had elevated blood lead levels by the first day after

exposure.

Longcore et al. (1974) dosed each of 100 four-month-old drake mallards with a single number 4 lead shot. Blood lead levels ranged from 2.7 ppm to 20 ppm in the birds that died. Birds euthanized during the study had blood lead levels from 3.7 ppm to 22.9 ppm. A blood lead level of .02 ppm was considered a normal background level for wild mallards (Finley and Locke, 1976).

A study by Finley et al. (1976) in which six-month-old mallards were dosed with a single number 4 lead shot had the following results. The first day after dosing the mean blood lead level reached 1.66 ppm. The blood lead level peaked at 1 week postdosing at 2.36 ppm and remained elevated at .64 ppm through the fourth week. Additional birds treated in a similar fashion averaged .64 ppm of blood lead over a 4 week period. Control birds had blood lead levels of .05 ppm during the same period. Ten ppm has been considered indicative of an acute exposure to lead despite the wide range of blood lead levels obtained by various authors (Longcore et al., 1974).

Clinical cases of apparent lead poisoning in 2 Florida cranes and 2 greater sandhill cranes were reported in 1977 by Kennedy et al. The blood lead levels in these cranes ranged from 1.46 ppm to 3.78 ppm before treatment was initiated.

Other Blood Values

Kennedy (1977) determined no clear relationship between blood lead levels and leukocyte numbers in lead poisoned cranes. The hematocrits of these cranes did not reflect depressed red cell counts although 3 of the 4 cranes appeared clinically anemic.

Redig (1979) stated that a lead poisoned raptor usually will have a pronounced leukopenia, but insignificant changes in total protein and hematocrit.

A group of mallards given 8 number 4 lead pellets by Roscoe et al. (1976) exhibited an average mean corpuscular hemoglobin concentration of 26 grams percent (g%) as compared to 31g% seen in control birds 8 days after poisoning. Coburn et al. (1951) dosed 12 mallards with 8 and 12 milligrams of lead per kilogram of body weight over periods ranging from 19 days to 41 days. They found consistent decreases in hemoglobin values (e.g., average initial hemoglobin value for all birds was 107.9% and final average hemoglobin value for all birds was 47.6%). The red cell counts dropped in most of Coburn's experimental individuals during the first 10 to 14 days of lead administration. The total leukocyte counts varied from predosage counts of 19,000 to 90,000.

Changes in the hemoglobin levels and erythrocyte numbers were further substantiated by de Bruin (1971). He rationalized that lead was partial to and interacted with the erythrocyte membrane. The erythrocyte then lost potassium and water and had an altered phosphorous uptake. These changes accounted for the shortened lifespan of circulating red blood cells. At the same time hemoglobin synthesis was occurring primarily in the erythroblasts of the bone marrow. Several of the enzymes in the hemoglobin synthesis pathway were inhibited due to lead's affinity for sulfhydryl groups. These negative influences on circulating erythrocytes and erythroid elements in the bone marrow perhaps accounted for the shortened lifespan of erythrocytes in lead poisoned individuals (de Bruin, 1971).

Erythrocyte Fragility

Griggs (1964) found the red cells from lead poisoned human subjects increasingly resistant to hypotonic sodium chloride solutions. Incubation of the collected samples further increased the osmotic erythrocyte resistance. Griggs concluded that as a result of lead poisoning the red blood cell shrank, or lost corpuscular volume, and consequently swelled to a greater critical size in hypotonic solution before lysis occurred. Wintrobe et al. (1974), in discussing the anemia of lead poisoning, also made mention of the decreased osmotic fragility and the increased mechanical fragility of the erythrocytes in affected humans.

Data summarized and presented by Aub et al. (1925) indicated a species variation in erythrocyte osmotic fragility after exposure to lead. Man, rabbits, guinea pigs and rats apparently had characteristic changes in red cell resistance while horses, chickens, dogs and cats did not display this phenomenon. Aub et al. (1925) specifically demonstrated that erythrocytes from lead poisoned rabbits had increased resistance to lysis in hypotonic saline solution. Schalm et al. (1975) concluded that the pronounced blood loss anemia in a lead poisoned heifer resulted from the increased erythrocyte destruction directly due to decreased osmotic resistance - in opposition to Aub's rabbit research. Frankel et al. (1970) stated that conditions causing spherocytosis of the erythrocytes (e.g., most hemolytic anemias) resulted in increased osmotic fragility while thin, flat target cells were less susceptible to hypotonic saline solutions.

Blood Values[1]

Lucas and Jamroz (1961) commented on the intrinsic value of monitoring hematologic parameters in birds. They concluded that total white cell counts and differential counts on a single bird were of limited value. Hemoglobin and hematocrit values, on the other hand, had a very limited range of variability and would be indicative of problems in an individual bird. They also recommended the minimum numbers of birds required for studying each blood parameter.

Tissue Lead Levels

Adler (1947) analyzed the tissue lead concentrations of 4 lead poisoned Canada geese. He concluded from his data that the liver was the organ of choice for chemical analysis to diagnose lead poisoning. This hepatic lead level represented an ongoing toxicosis, while an analysis of bone indicated chronic poisoning. His control birds had liver lead levels of 0 ppm to 1 ppm. The concentrations of lead in the livers of the 4 poisoned geese ranged from 9 ppm to 27 ppm. Kidney lead levels for the controls were 0 ppm to 6 ppm. The lead levels in kidney tissue from the poisoned birds ranged from 12 ppm to 57 ppm. Adler's 2 control birds had no measurable lead in the lung. Lead levels of 2 ppm to 9 ppm were found in the lungs of the affected geese. The heart muscles of the control birds had no detectable

[1]Alfred M. Lucas and Casimir Jamroz, Atlas of Avian Hematology (United States Department of Agriculture, 1961), p. 217.

quantities of lead upon analysis. Corresponding data for the poisoned geese was not available.

Buck et al. (1973) established liver lead levels of 10 ppm and kidney cortex levels of 15 ppm as consistent with lead poisoning. Control liver lead levels of less than 3 ppm (often ranging from .3 to 1.5 ppm) and control kidney lead levels of 1 ppm to 3 ppm were also established.

Coburn et al. (1951) determined that any one of liver, skeleton, or soft tissues might be used as field samples for chemical analyses to determine lead poisoning. He found average liver lead values 40 times greater in his poisoned mallards than in the corresponding control birds. The poisoned birds' livers had 12 to 36 ppm of lead. The rate of lead deposition in any tissue in this same experiment did not directly reflect the dosage level of lead nor the length of the test period.

A 1965 report by Hunter and Rosen on a lead poisoned wild pheasant stated that 42 ppm of lead were found in breast tissue and 168 ppm in liver tissue. Normal lead values were set at 1 ppm. Experimentally poisoned pheasants had breast and liver lead levels of 50 ppm and 143 ppm respectively. Cook and Trainer (1966) found liver lead levels ranging from 5 ppm to 32 ppm in experimentally poisoned Canada geese. These levels were compatible with those derived by Adler (1947) and Coburn (1951).

A mourning dove with clinical plumbism had lead concentrations of 72 ppm and 187 ppm in the liver and tibia respectively (Locke and Bagely, 1967). Further field investigations at this same time generated data on 40 dove livers. The lead content of these samples ranged

from .4 ppm to 14 ppm. The dove having a liver lead concentration of 14 ppm had 2 lead shot in its gizzard. Bagley and Locke (1967) analyzed samples from 483 Canada geese collected during a massive die-off on the Atlantic Coast which lasted from November, 1965, to April, 1966. Chemical analyses on the livers and tibias of 20 of these birds yielded the following data: livers had lead concentrations from 6 ppm to 53 ppm and tibias had lead levels from 12 ppm to 102 ppm. Kidneys from 7 geese had lead levels from 8 ppm to 32 ppm. Eleven control geese had liver lead levels of .4 ppm to .8 ppm. Bagley and Locke (1967) subsequently undertook a study of liver lead levels in 28 species of wild birds and bone lead levels in 13 species. They concluded that lead was a regular constitutent in the tissues of wild birds. Periods of active exposure were reflected by elevated liver lead concentrations, while bone lead levels were indicative of chronic toxicity. The danger of lead mobilization from bone sites during stress situations was also noted.

A clinically affected Andean condor was found to have 38 ppm of lead in its liver (Locke et al., 1969). No controls were available to evaluate the lead levels in livers of healthy condors, but this level was high enough to indicate lead poisoning. The condor had been fed hunter-killed carcasses and apparently had ingested some of the lead shot in the carcasses.

Longcore et al. (1974) compared tissue lead levels in mallards dosed with a single number 4 lead shot. He concluded that while a specific lead level was not diagnostic for plumbism, lead levels in the brain, liver, and kidneys were indicative of fatal lead poisoning. The lead levels in the tibias of euthanized birds in this study ranged

from 57 to 212 ppm. Birds that died as a result of lead poisoning had tibial lead levels of 87 to 209 ppm. Liver lead levels in euthanized birds were 26 to 65 ppm. Fatally affected birds had liver lead levels ranging from 32 to 83 ppm. The researchers concluded that lead levels from 6 to 20 ppm in the liver indicated acute lead intoxication. Kidney lead levels were measured at 53 to 408 ppm for mallards that died due to lead poisoning. Euthanized birds had kidney lead levels of 22 to 243 ppm. It was suggested that the kidney tissues might be vital indicators of lead toxicity inasmuch as they function to excrete body wastes and ingested poisons. Twenty ppm lead in the kidney tissues was indicative of a serious lead toxicosis. Heart muscle from euthanized mallards in this same study had .4 ppm to 46 ppm lead. Dying birds had cardiac lead levels of 1.3 to 4.8 ppm. Lastly, lead levels in the lungs of euthanized ducks ranged from 1.7 to 15 ppm. Ducks that died on experiment had lung lead levels of 2.5 to 8.2 ppm. The lung lead levels in euthanized birds were initially higher, but birds euthanized at longer intervals from the time of dosing had decreasing lung lead levels.

Longcore et al. (1974) concluded that the most reliable test for diagnosing lead poisoning was the analysis of soft tissues from affected birds. They also concluded that continuous oral ingestion of lead resulted in high residues in bones and lower residues in livers and kidneys. The smallest amounts of lead were accumulated by the heart, lung, muscle and brain. The authors speculated that tissues involved with circulatory function (e.g., heart, lung, and blood) might have lead levels which decreased with time since circulatory lead levels decreased as lead was stored in such other tissue sites as

bone.

A report of lead poisoning in a prairie falcon in 1974 (Benson et al.) cited the feeding of duck heads with high lead levels as the source of the poisoning. Lead residues in the affected falcon were 3.9 ppm in the heart muscle, 6.0 ppm in the kidney, 12.9 ppm in the brain tissue, 17.4 ppm in the liver, and 36.0 ppm in the humerus and femur. It was felt that the liver lead level of 17.4 ppm was highly significant and indicated fatal lead poisoning.

A bald eagle with numerous lead shot in the gizzard was submitted to the University of Maryland for clinical examination (Jacobson et al., 1977). The bird was presented alive and died shortly thereafter in acute respiratory distress. Chemical analyses of the liver and kidney yielded lead concentrations of 22.9 ppm and 11.3 ppm respectively. These values were considered to be consistent with lead toxicosis.

Analyses of the liver and kidney from a fatally affected greater sandhill crane resulted in lead levels of 29 ppm and 18.6 ppm respectively. It was noted that these tissue lead levels were significant for lead poisoning (Kennedy et al., 1977).

Birds presented to the Animal Health Diagnostic Laboratory at Michigan State University since May, 1968, had the following tissue lead levels:

1. Four pelicans had liver and kidney levels of 32 ppm. (Case 103557-560).
2. One swan had liver and kidney levels of 33.3 ppm. (Case 104655)

3. Two ducks had combined stomach contents, liver and kidney levels of 31 ppm. (Case 105917-919)
4. A bustard had liver and kidney levels of 100 ppm. (Case 107035)
5. Three pelicans had composite liver and kidney samples of 44 ppm. (Case 111243-4)
6. One duck had liver and kidney lead levels measured at 12.8 ppm. (Case 111423)
7. An Emperor penguin had liver levels of 85 ppm lead. (Case 113188)
8. Two ducks had liver lead levels of 95 ppm and kidney lead levels of 91 ppm. (Case 181748-51)

All of the above birds were reported as confirmed cases of lead poisoning either clinically affected or dead at presentation.

Spontaneous Erythrocyte Fluorescence

Low concentrations of lead inhibit several enzymes in heme synthesis. Measurement of heme precursors in the blood indicates the effects of lead absorption (Baloh, 1974). One step of heme synthesis impaired by lead is the insertion of iron into the protoporphyrin moiety (Sassa et al., 1972). The enzyme heme synthetase, which has a thiol (-SH) group, is needed for this step in the pathway (Buck et al., 1973). Lead apparently has an affinity for essential sulfhydryl or thiol groups (de Bruin, 1971). There is a subsequent accumulation of the hemoglobin precursors within the tissues and body fluids of individuals with adequate lead absorption (de Bruin, 1971). One such precursor, protoporphyrin, is formed in the mitochondria of the

immature erythrocyte during its stay in the bone marrow. Lead inhibits the use of this protoporphyrin, so as the maturing erythrocyte loses its mitochondria and enters the circulation, it has the same protoporphyrin content as it had in the bone marrow (Sassa et al., 1973). This increased protoporphyrin, also known as free erythrocyte protoporphyrin, enables erythrocytes to fluoresce red when excited by blue light (Vannotti et al., 1949).

Whitaker and Vietti in 1959, screened blood smears from several children with lead poisoning. They examined the slide preparations under ultraviolet light and found red fluorescence in 75% to 100% of the erythrocytes. They concluded that the fluorescence was apparently related to the quantities of free protoporphyrin in the erythrocytes. Nelson et al. (1968) examined blood smears from human patients with lead poisoning and estimated the number of fluorescing erythrocytes in 25 to 50 oil immersion fields viewed under a Leitz fluorescent microscope. The authors then quantitated erythrocyte free protoporphyrin levels and measured urinary lead excretion in their patients. They concluded that their "fluorescyte" preps had a high degree of correlation with the quantitated erythrocyte protoporphyrin concentrations and the urinary lead levels. Their findings confirmed the assumption that peripheral erythrocytes fluoresce due to increased protoporphyrin levels.

These increased levels of protoporphyrin, specifically protoporphyrin IX, were also noted in patients with iron deficiency anemia (Lamola et al., 1974). Lamola demonstrated that protoporphyrin in the blood of humans with lead poisoning, or severe iron deficiency anemia, was complexed with zinc and not truly free erythrocyte protoporphyrin.

Lamola et al. (1974) concluded that measuring whole blood for fluorescence at 594 nm was the simplest and most direct screening test for lead poisoning.

Chisolm (1973) noted that the fluorescence seen with accumulations of protoporphyrin during lead poisoning resulted from a delayed reaction due to interference with erythropoiesis. The fluorescence appeared at some later time after chronic lead absorption (Chisolm, 1973).

Barrett et al. (1971) decided to employ spontaneous erythrocyte fluorescence as a rapid test to diagnose lead poisoning and estimate its incidence in waterfowl. Mallard ducks and Canada geese were experimentally poisoned with various quantities of number 6 lead shot. Blood from the birds was collected in heparin. Each sample was screened for fluorescence within 48 hours of collection under 400x magnification by dark field microscopy. The combined ultraviolet and blue light illuminating the viewing field had a wavelength of 400 nm. One hundred and forty-one of the 176 blood samples fluoresced. The intensity of fluorescence varied from pale pink to bright red and lasted for from 5 seconds to several minutes. The longest and brightest fluorescence was observed in erythrocytes collected 3 to 9 days after the birds had been exposed to lead. Fluorescence diminished as each mallard duck and goose returned to a state of health (Barret et al., 1971).

It has been observed that other disease conditions, namely, severe iron-deficiency anemia, hemolytic anemia and pernicious anemia, may cause increases in protoporphyrin and perhaps spontaneous erythrocyte fluorescence; however, these conditions have been unknown in wild

waterfowl (Barret et al., 1971). The finding of fluorescent erythrocytes in blood smears from lead poisoned birds may not indicate fatal poisoning, but perhaps an increased vulnerability to predation and hunting (Barrett et al., 1971). Fewer than 1% fluorescytes on blood smears treated as previously described were assumed to be normal; more than 10% were abnormal (Nelson et al., 1968). Buck et al. (1973), using the 1971 data obtained by Barrett and Karstad, stated: "In waterfowl, a simple diagnostic test involving microscopic examination of red blood cells for red fluorescence has been shown to be of value."[2]

It was determined in 1976 that fluorescent erythrocytes from lead poisoned waterfowl have emission and excitation maxima of 635 nm and 408 nm respectively (Roscoe et al., 1976). In a study by Roscoe et al. (1976) 40 mallards had the maximum accumulation of protoporphyrin IX on day 8 after treatment with lead. This protoporphyrin IX had the same maxima as that previously determined for fluorescent erythrocytes (Lamola et al., 1975). Roscoe et al. (1976) also concluded that the protoporphyrin levels were related to the number of shot each bird ingested, and these levels, measured in micrograms per deciliter (µg/dl), were inversely proportional to the mean corpuscular hemoglobin concentrations. This group of investigators analyzed the

[2]William B. Buck, Gary D. Osweiler, and Gary A. Van Gelder: Clinical and Diagnostic Toxicology. Kendall Hunt Publishing Company, Dubuque, Iowa, (1973), p. 196.

spectra of blood samples from their research birds and determined that the fluorescing compound in lead poisoned birds was not complexed with zinc as in man. Further studies by Roscoe et al. (1977) in which mallard ducks were divided into an acutely poisoned group and a chronically poisoned group confirmed the previous findings. Roscoe et al. (1979), using a modified hematofluorometer, were able to measure protoporphyrin levels in lead poisoned mallards. The data collected demonstrated a lag period of approximately 6 days between peak blood lead concentrations and peak blood protoporphyrin IX concentrations. They speculated that an anemia resulting from lead intoxication would increase erythropoiesis and increase the synthesis of protoporphyrin IX by immature erythrocytes.

Summary of Literature Review

The literature cited in this section indicates that lead poisoning is a serious problem among wildfowl. There is no reason to believe that the inclusion of lead in the food chain of raptors is not also a very real threat. The clinical signs and gross and microscopic lesions characteristic of lead toxicosis have been documented at length. The analyses of selected tissues for lead concentrations serve as confirmation of other diagnostic methods (e.g., spontaneous erythrocyte fluorescence) which are gaining wider field acceptance. It is apparent that further investigations are needed to define the problem of lead toxicosis in those avian species feeding on poisoned birds or mammals.

OBJECTIVES

The objectives of this research were:

1. To reproduce lead poisoning, as seen in the wild, in a representative raptor - the red-tailed hawk (Buteo jamaicensis).

2. To define and enumerate clinical signs, if any, resulting from lead administration to these birds.

3. To define any gross or microscopic changes, in selected avian tissues, which were caused by the lead.

MATERIALS AND METHODS

This research was initiated in January 1978. The birds were maintained at the Rose Lake Wildlife Station, Stoll Road, East Lansing, Michigan. Analyses were conducted at the Department of Pathology, Michigan State University, East Fee Hall.

Sources and Care of Experimental Animals

Red-tailed hawks which had sustained injuries, and were unsuitable for release into the wild, were supplied by the Michigan Department of Natural Resources. All birds were maintained indoors in suitable caging and standardized on a diet of freshly killed laboratory mice (outbred Swiss-Webster ICR mice supplied by Spartan Research Animals, Haslett, MI). Birds were housed 1 to a cage and had no visual contact with one another. The cages were monitored daily and cleaned as needed. Fresh water in large crocks was available at all times.

Experimental Method

The birds were randomly divided into 3 representative groups after a standard period of acclimation to food and housing. One group of birds (birds 2, 6, and 8) were sequentially fed freshly killed mice with 10 number 5 lead shot hidden in the brain tissue. Another group of birds (birds 3, 7, and 9) received 30 number 5 lead pellets at each dosing. Birds 4, 5, and 10 were controls and as such received mice with no lead pellets.

Procedures

A feasibility study was conducted using a single red-tailed hawk. The diet, dosing schedule, blood collection techniques and methodology were standardized using this individual (bird 1).

On day 1 all birds were weighed and bled. Blood was obtained from the alar vein by fresh venipuncture with a 25 gauge 5/8 inch needle. Approximately 1.25 milliliters (ml) to 1.5 ml of blood were collected from each bird in a heparinized plastic syringe (Crisler et al., 1972). Three blood smears were immediately made from each sample and allowed to air dry. The remaining blood samples were transferred to sterile heparinized plastic tubes and refrigerated. On day 1 all birds were radiographed (Department of Radiology, Veterinary Clinical Center, Michigan State University) for the presence of any retained shot acquired in the wild. Birds were then offered the appropriately dosed freshly killed mice. On day 4 each bird was again weighed, bled, radiographed, and redosed with lead-primed mice. On day 7 all birds were again weighed, radiographed, and dosed. On day 10 birds were weighed, bled, and dosed. Birds were weighed, bled, and radiographed on day 17. On day 24 each individual was weighed, bled, and euthanized.

Each bird in the 3 replicate trials was observed daily for the following clinical signs:

1. lethargy
2. inappetence
3. green diarrhea
4. facial edema
5. wing droop

6. feather loss

7. death

A daily record was kept of the casts from each bird. The mutes were examined daily for any lead shot lost through the digestive system. All expelled casts were retrieved and examined. Lead pellets adhering to or housed inside cast material were salvaged.

Hematologic Procedures

An aliquot of blood was taken for microhematocrit and hemoglobin (AO hemoglobinometer) determination (Benjamin, 1961).

Blood smears were stained with Wright's stain while being processed through an automatic stainer (Ames Hema-Tek Slidestainer^{R}).

Microscopic examination of the prepared smears was performed to evaluate relative changes in the numbers of monocytes, heterophils, eosinophils, lymphocytes and basophils. Absolute leukocyte counts as well, were performed on blood obtained from birds 5, 6, 7, 8, 9, 10 (hemocytometer). A dilution factor of 200 was used for all white cell counts.

Spontaneous Erythrocyte Fluorescence

A drop of heparinized blood was placed on a microscope slide and coverslipped. The wet mount was examined at 400X magnification through a Zeiss binocular microscope equipped with a dark field condenser. A fluoroisothiocyanate (FITC) filter was used as the primary (excitation) filter. The wavelength of the excitation filter peaked at 495 nm. A secondary (barrier) filter of 530 nm wavelength was also used. An additonal filter, a BG 38 red-suppressor filter, excluded any red

light except that originating from the fluorescing erythrocytes. Fifty oil-immersion fields were examined on each slide. The number of fluorescing erythrocytes, the brilliance (pink to red), and the duration of fluorescence were noted for each slide.

Erythrocyte Fragility

The osmotic fragility of the erythrocytes was determined using the Dacie method.[3] A phosphate buffered stock solution osmotically equal to 10% saline was prepared. The stock sample was diluted 1:10 with normal saline to prepare a working solution. Dilutions ranging from .1% to .85% were made by adding calculated quantities of distilled water to the working solution. Units of .05 ml of heparinized venous blood were added to tubes containing 5 ml of each dilution. The solutions were mixed, incubated for one-half hour at room temperature, remixed, and centrifuged at 2000 rpm for 5 minutes. The supernatant from each solution was collected and inserted into a colorimeter (Perkin Elmer spectrophotometer) set at 545 nm to determine optical density. The .85% solution supernatant was used as a blank, and the .1% saline solution supernatant was used as an indicator of 100% hemolysis. The percent hemolysis was calculated from the highest theoretical optical density reading (.1% saline).

[3] Gradwohl's Clinical Laboratory Methods and Diagnosis. (ed. by S. Frankel, S. Reitman, and A. C. Sonnenwirth). C. V. Mosby Co., St. Louis, 1, (1970), p. 572-574.

Percent hemolysis in each tube plotted against the corresponding saline concentrations graphically depicted osmotic fragility.

Blood Lead Determinations

Blood was kept frozen prior to preparation for lead level determinations. All determinations were made on a Perkin Elmer 360 atomic absorption spectrophotometer set at 283.3 nm. The Delves technique was used to analyze the blood (Delves, 1970). Each sample of 10 micro-liters (µl) of whole heparinized blood was transferred to a nickel-alloy (Delves) cup for this technique. One set of standards containing .4 µg/ml lead and .8 µg/ml lead was used. All unknowns were analyzed in triplicate. The blood samples were dried at 140°C, and 20 µl of 30% hydrogen peroxide was added to each sample. The samples were then oxidized at 140°C for approximately 2 minutes. Each cooled sample was placed in the spectrophotometer, which had been refitted with a 3 slot burner head, and atomized in an air-acetylene flame. The air to acetylene ratio was 70 to 45. An attached strip chart recorder recorded peak heights. The following formulae were used to calculate blood lead concentrations:

$$\mu g\ Pb/ml\ of\ blood = \frac{\text{average sample peak height}}{\text{average standard peak height}} \times \text{concentration of standard}$$

$$\mu g\ Pb/dl\ of\ blood = \mu g\ Pb/ml \times 100$$

Tissue Handling

All birds were euthanized with carbon dioxide gas and necropsied at the termination of each trial. Selected tissues (e.g., gizzard, spleen, proventriculus, crop, kidney, lung, liver, bone, and heart)

were saved for chemical analysis and histopathologic preparation. Tissues for chemical analysis were weighed and frozen until required. Tissues for microscopic examination were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or Ziehl-Neilsen acid fast stains.

Tissue Lead Determinations[4]

All tissues were ashed with magnesium acetate to facilitate removal of organic material. A gram of each tissue, except bone, and 1 ml of magnesium acetate were dried in a 56°C oven overnight. The samples were then ashed in a muffle furnace at 450°C to 500°C for 4 to 5 hours. The ashed samples were dissolved in hydrochloric acid solution (2N HCl), complexed by the addition of ammonium pyrrolidine dithiocarbamate (APDC) and freed from any residual tissue matrices by partitioning into an organic solvent, methyl isobutyl ketone (MIBK). The organic supernatant was aspirated after centrifugation and analyzed against an MIBK blank.

The ashing process for bone material was done as follows. The right femur from each experimental bird was rinsed with distilled water and dried in a 100°C oven. Two milliliters of magnesium acetate were added to a 1 gram sample of bone. This mixture was then dried in

[4]W. Hyde, J. Kiesey, P. F. Ross, and H. M. Stahr: Analytical Toxicology Methods Manual. Iowa State University Press, Ames, Iowa, (1977), p. 42-44.

a 100°C oven. All material was ashed for 16 hours in a 550°C muffle furnace after drying. The residues were dissolved in 2N HCl and transferred to labelled tubes. The original crucibles were washed with 2 rinses of distilled water to insure the transfer of all ashed materials. The extraction procedure at this point continued as previously described.

RESULTS

Lead shot was successfully administered to the experimental birds. Overt clinical signs of lead poisoning were never noted in dosed individuals. The parameters measured were treated in two different fashions. Individual data were presented where appropriate, while other data were more readily understood if presented in a composite form as controls, 10 pellet dosed, or 30 pellet dosed birds.

Weight

All birds were weighed on days 1, 4, 7, 10, 17 and 24 of the research protocol. The weight graph in Figure 1 shows all 3 groups of birds having had a demonstrable weight gain after an initial period of stabilization. This period of gain was followed by a pronounced weight loss in all 3 groups. However, at the end of the experimental period all birds had a net weight gain.

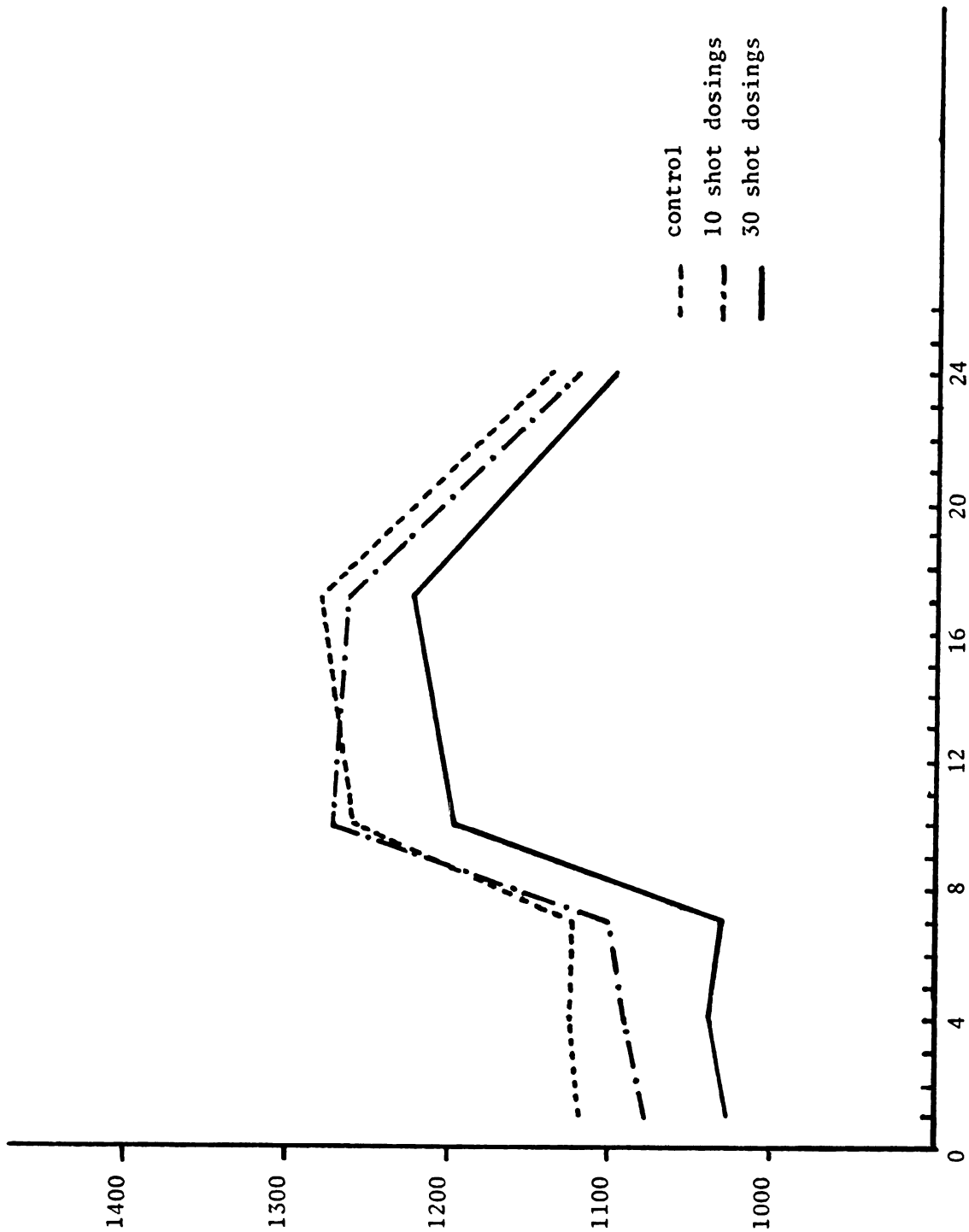

Figure 1. Body Weight

Blood Lead Levels

The average blood lead levels for each group of birds are presented in Table 1.

Table 1. Average Blood Lead Levels (ppm)

Days on Experiment	Control Birds	Birds Dosed With 10 Pellets	Birds Dosed With 30 Pellets
1 (Baseline Values)	.163 ppm	.820 ppm	.375 ppm
4	.170 ppm	2.053 ppm	2.767 ppm
10	.203 ppm	3.693 ppm	9.693 ppm
17	.203 ppm	2.963 ppm	8.960 ppm
24	.167 ppm	1.67 ppm	4.340 ppm

Each group of birds had increased blood lead levels. Machine variability or sensitivity may have accounted for changes in the control group. The standards used during blood analyses reflected very slight changes in mechanical operation of the atomic absorption spectrophotometer.

Table 2 shows the percentage increase from baseline, or original blood lead levels, in each group over the duration of the experiments.

Table 2. Changes in Blood Lead Levels (%)

Days on Experiment	Control Birds	Birds Dosed With 10 Pellets	Birds Dosed With 30 Pellets
1	Baseline 0	Baseline 0	Baseline 0
4	4%	150%	638%
10	24%	350%	2485%
17	24%	261%	2289%
24	2%	104%	1057%

The group of birds receiving 30 number 5 lead shot at each dosing demonstrated the most marked increases in blood lead levels. The hawks that received less lead had measurable increases in blood lead levels, but not as pronounced as the more heavily dosed individuals.

Hemoglobin, Hematocrit, and Mean Corpuscular Hemoglobin Concentration

The average composite values for hemoglobin (Hb), hematocrit (HCT) and mean corpuscular hemoglobin concentration (MCHC) are presented in Table 3.

Table 3. Hemoglobin (Hb), Hematocrit (HCT) and Mean Corpuscular Hemoglobin Concentrations (MCHC)

Days on experiment	Controls			Birds Dosed With 10 Pellets			Birds Dosed With 30 Pellets		
	HB gm/dl	HCT (%)	MCHC gm/dl	HB gm/dl	HCT (%)	MCHC gm/dl	Hb gm/dl	HCT (%)	MCHC gm/dl
1	11.0	33.2	33.2	12.0	35.5	33.8	11.2	35.7	32.5
4	13.7	39.3	34.9	10.8	34.0	31.6	11.6	34.7	33.5
10	12.2	38.8	31.3	10.0	31.5	31.8	10.2	33.0	30.8
17	12.0	36.3	33.1	10.9	35.7	30.5	9.9	32.5	30.4
24	12.4	37.7	32.8	15.8	37.5	42.2	11.6	34.5	33.6

No consistent conclusions were drawn from this data due to fluctuations in the values for Hb, HCT and thus the calculated MCHC. Graphic depictions of this same data did not present any uniform direction for each measurement and are therefore not included in the results.

Leukocytes

The total leukocyte counts, or total white cell counts, for each group of birds are presented in Table 4. The initial total white cell count on day 1 was taken as a zero point.

Table 4. Total Leukocyte Counts (mm^3)

Day	Control Birds	Birds Dosed With 10 Shot	Birds Dosed With 30 Shot
1	17, 139	33, 778	24, 556
4	16, 750	26, 222	21, 917
10	21, 167	25, 445	21, 278
17	35, 334	27, 111	28, 000
24	24, 278	22, 889	21, 566

Each group of birds had a decrease in total leukocyte numbers after the initial blood collection. The lead dosed groups had more marked declines in leukocyte numbers after the initial dosing than did the control group. The control group had a rise in total cell numbers at the time of the third bleeding. Birds in the lead dosed groups continued to experience declining cell numbers. Sample 4 on day 17 showed the control group responding with a dramatic increase in leukocyte numbers. The birds in the group receiving 30 lead pellets at each dosing had a marked rise in total leukocyte numbers, while the group receiving 10 pellets at each dosing also had a perceptible increase in cell numbers. This rebound in total leukocyte numbers occurred 1 week after the last lead was fed. None of the birds retained shot at the time of this blood collection. Blood lead levels had decreased by

day 17 as previously noted in Table 1. All 3 groups had decreased total white cell counts at the time of the last blood sampling.

Tissue Lead Levels

The average tissue lead levels for each group of birds are presented in Table 5.

Table 5. Average Tissue Lead Levels (ppm)

Tissue	Control Birds	Birds Dosed With 10 Pellets	Birds Dosed With 30 Pellets
kidney	.163 ppm	.420 ppm	.933 ppm
spleen	.300 ppm	.961 ppm	1.193 ppm
gizzard	.291 ppm	.665 ppm	1.332 ppm
proventriculus	.258 ppm	.521 ppm	.490 ppm
crop	.551 ppm	.521 ppm	.567 ppm
heart	.414 ppm	.644 ppm	.757 ppm
lung	.560 ppm	.840 ppm	1.990 ppm
liver	.350 ppm	.433 ppm	1.100 ppm
bone	.953 ppm	1.06 ppm	3.617 ppm

Table 6 presents the tissue lead values from the experimentally dosed birds as percent change from the baseline values from the control birds. These baseline values represent background lead levels which an individual could accumulate in his or her natural environment.

Table 6. Changes in Tissue Lead Levels (%)

Tissue	Birds Dosed With 10 Pellets	Birds Dosed With 30 Pellets
kidney	157.20%	471.42%
spleen	120.44%	397.77%
gizzard	136.80%	374.58%
proventriculus	102.37%	90.22%
crop	- 7.36%	2.91%
heart	55.61%	83.03%
lung	50.00%	255.36%
liver	23.8%	214.29%
bone	11.19%	279.38%

Figure 2 pictorially reflects these changes. The kidneys, gizzards and spleens from the dosed birds apparently concentrated the highest levels of lead when compared to the same tissues in undosed individuals. The gizzard, which concentrates the ingesta for cast formation and mechanically abrades the material, had a level of lead far above the control levels. The proventriculus and crop are essentially rapid transit areas for food in raptors. The proventricular lead levels were markedly higher than those of the control birds, but crop lead levels for the dosed groups were unremarkable. The birds in the group receiving 10 pellets at each dosing had a composite crop lead level below the control level. The heart, constantly in contact with the elevated blood lead, had measurable increases in ppm of lead per gram of muscle mass in both dosed groups. Birds receiving higher doses of lead had higher tissue lead levels than the group receiving

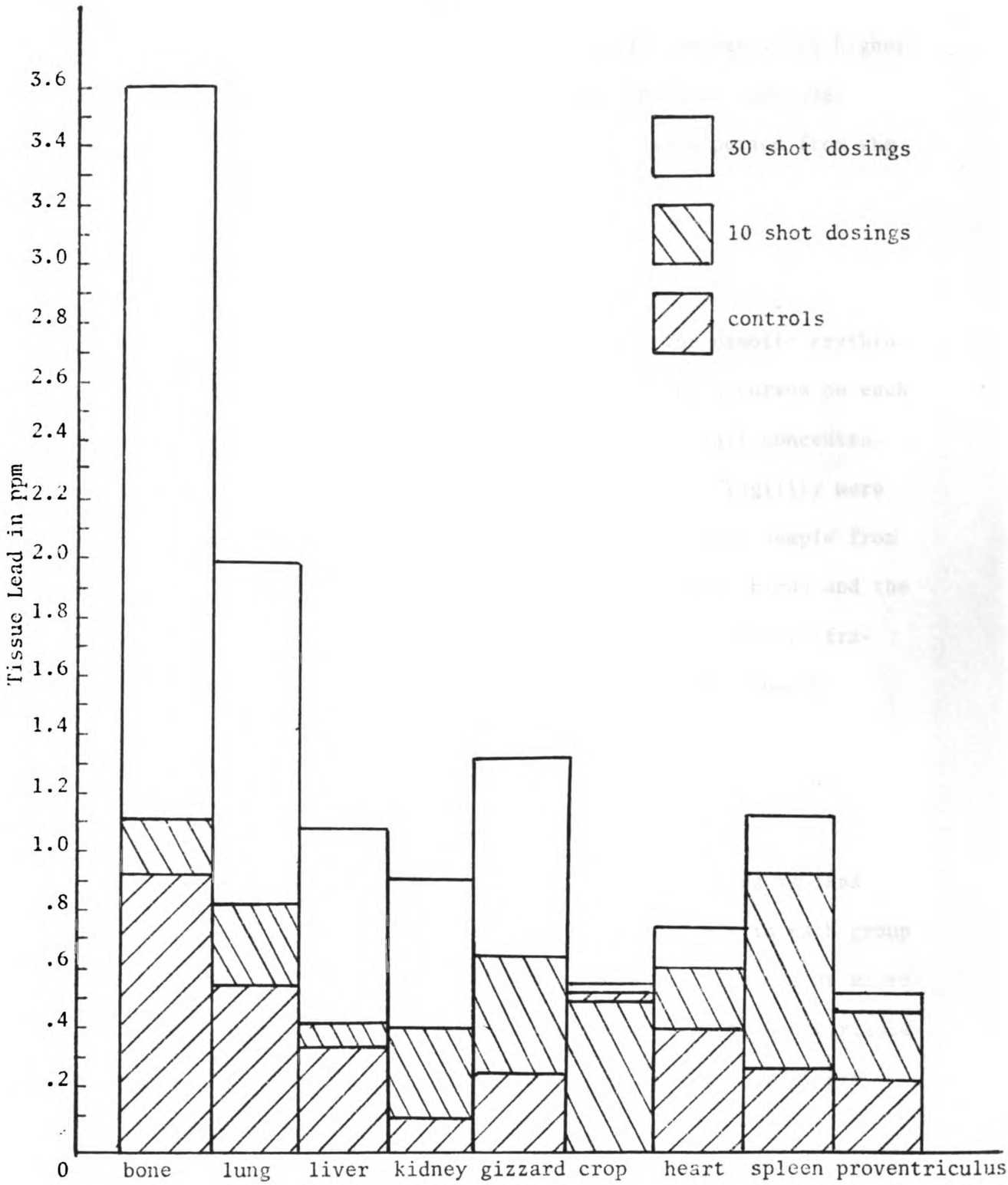

Figure 2. Tissue Lead Concentrations (ppm)

lower doses in almost all cases. All dosed birds similarly had higher tissue lead levels than undosed birds in every instance save one. Tissue lead levels remained elevated despite a 2 week period from the day of the last dosing to the day of euthanasia.

Erythrocyte Fragility

The 3 graphs in Figures 3, 4, and 5 represent the osmotic erythrocyte fragility curves for all 3 groups of birds. The 5 curves on each graph depict areas of maximum hemolysis occurring at salt concentrations between .15% and .25%. Slight rises in osmotic fragility were seen at salt solutions of .4% to 5.5% for the first blood sample from both control birds and heavily dosed birds. The control birds and the high dose group of birds again had slight increases in osmotic fragility between salt concentrations of .3% to .35% at the fourth bleeding.

Spontaneous Erythrocyte Fluorescence

Spontaneous erythrocyte fluorescence was only observed in lead dosed birds. The predose blood samples from individuals in each group contained no fluorescent erythrocytes when viewed by fluorescent microscopy. The quality and quantity of fluorescence were of such variable natures that individuals from each group are represented as single units in Figure 6.

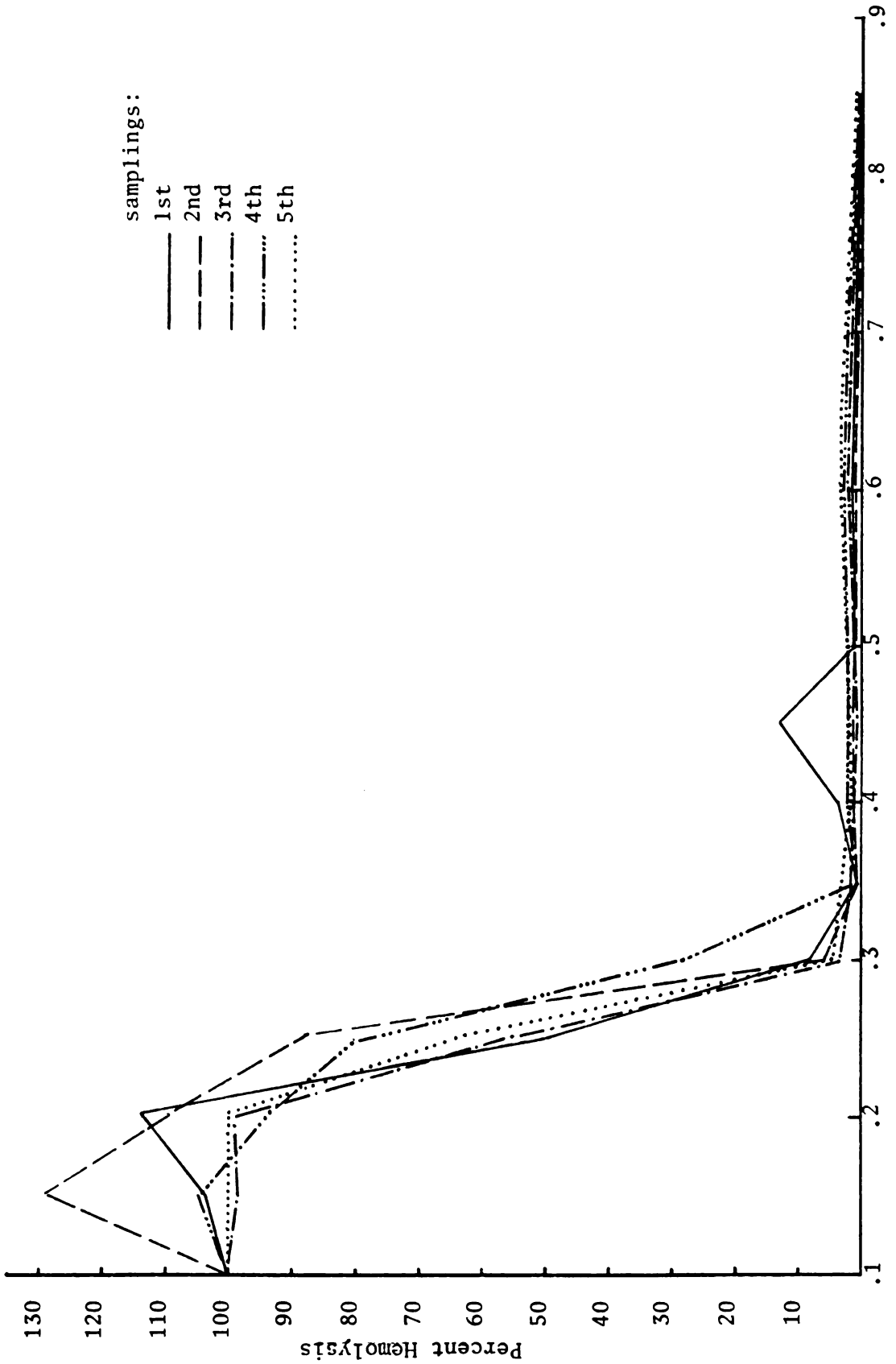

Figure 3. Osmotic Erythrocyte Fragility in Birds 4, 5, 10 (Controls)

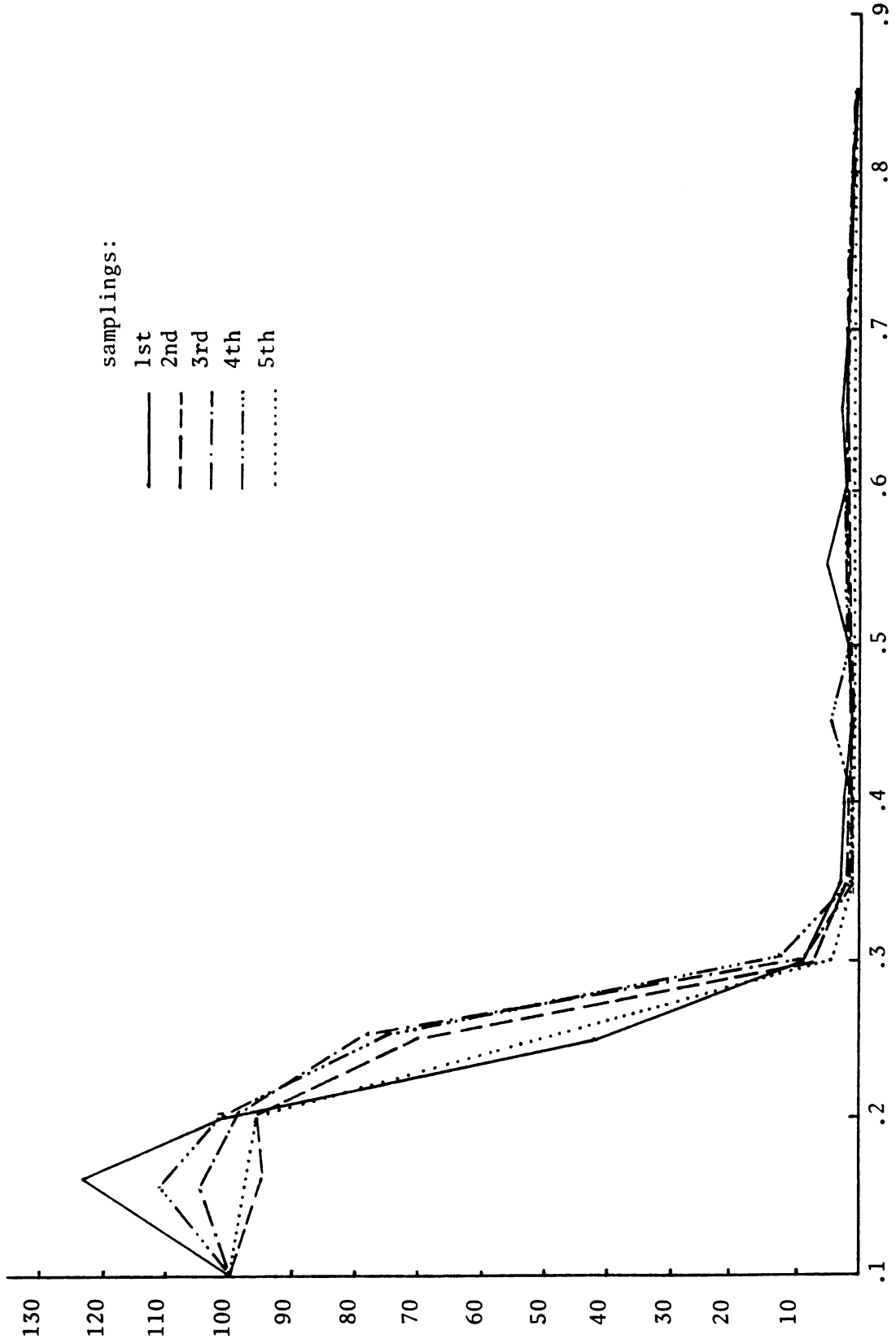

Figure 4. Osmotic Erythrocyte Fragility in Birds 2, 6, 8 (10 shot)

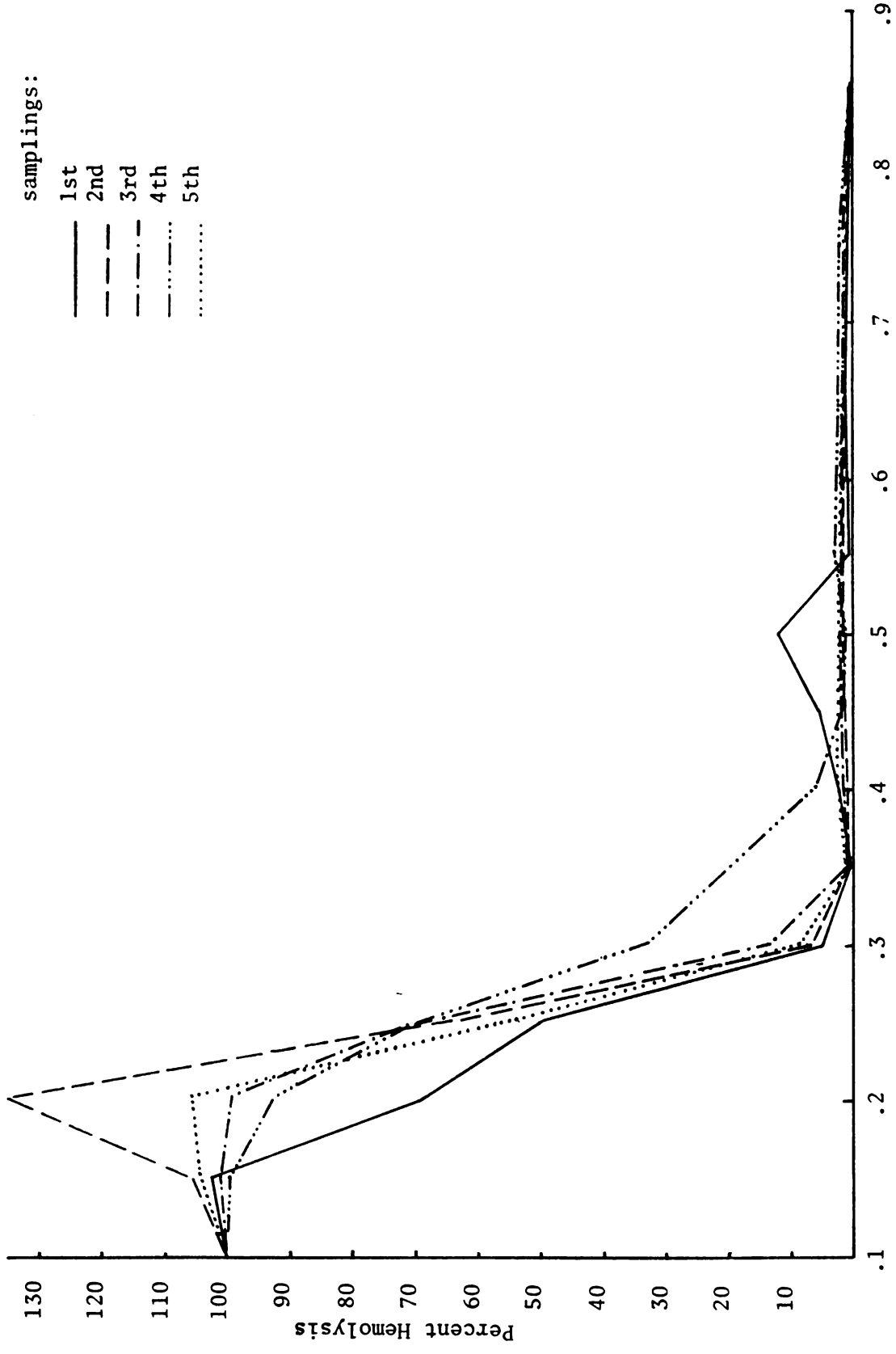

Figure 5. Osmotic Erythrocyte Fragility in Birds 3, 7, 9 (30 shot)

Bird 2 (10 pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 High Power Fields (hpf)	0	6	245	1718	2871
Color of Cells	-	faint pink	red and pink - faint	red and pink-mixed brightness	faint pink
Duration of Fluorescence	-	5 seconds	15 seconds	15 seconds	5 seconds

Bird 6 (10 Pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 hpf	0	0	6	5	0
Color of Cells	-	-	pale red	bright red	-
Duration of Fluorescence	-	-	5 seconds	12 seconds	

Bird 8 (10 Pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 hpf	0	0	0	0	0
Color of Cells	-	-	-	-	-
Duration of Fluorescence	-	-	-	-	-

Figure 6. Spontaneous Erythrocyte Fluorescence

Figure 6 (cont'd).

Bird 3 (30 Pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 hpf	0	7	184	0	357
Color of Cells	-	bright red	faint pink to bright red	-	faint pink to bright red
Duration of Fluorescence	-	5 seconds	10 seconds	-	10 seconds

Bird 7 (30 Pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 hpf	0	0	1	2	0
Color of Cells	-	-	faint red	faint red	-
Duration of Fluorescence	-	-	5 seconds	5 seconds	-

Bird 9 (30 Pellet)

Days	1	4	10	17	24
Number of Fluorescing Cells per 50 hpf	0	0	0	10	numerous
Color of Cells	-	-	-	very faint pink	faint pink
Duration of Fluorescence	-	-	-	2 seconds	too brief to count

Not all dosed birds developed fluorescing erythrocytes as seen from the above chart. The quantity of lead the bird received apparently did not correspond to the quantity or quality of erythrocyte

fluorescence. Bird 2, which received 10 lead pellets at each dosing, had the most fluorescing erythrocytes with the longest duration of fluorescence. Bird 8 never developed any fluorescing erythrocytes. Birds 2, 3 and 9 retained varying qualities of fluorescence despite being pellet-free since the 17th day on experiment. Birds 6 and 7 returned to a nonfluorescent state by the termination of the experiment. Examples of this fluorescence are seen in Figures 7 and 8.

Radiographic Findings and Shot Retention Times

Birds 2, 3, and 4 comprised the first experimental group. A preliminary radiograph was taken of each individual.

Bird 2

An intramedullary pin, which had not stabilized 2 fracture sites of the right humerus, was seen on the preliminary radiograph. Bird 2 was dosed with 10 number 5 lead shot on day 1 of which 8 were recovered from casts and mutes found on the cage floor on days 2 and 3. Radiographs taken on day 4 revealed 2 pellets remaining in the gizzard. The bird ate 10 more lead shot on day 4, for a total of 12 internally located lead shot. A cast egested on day 6 contained 7 pellets. Mutes and casts recovered on day 7 contained the 5 remaining shot and radiographs taken that same day revealed no retained lead pellets. The bird was again fed 10 shot on day 7, cast 7 of these shot by the following day, and retained 3 other shot. The bird was dosed on day 10 with 10 more pellets. Bird 2 eliminated all remaining 13 lead pellets by day 11. Bird 2 retained 2 lead shot for 7 days and 3 shot

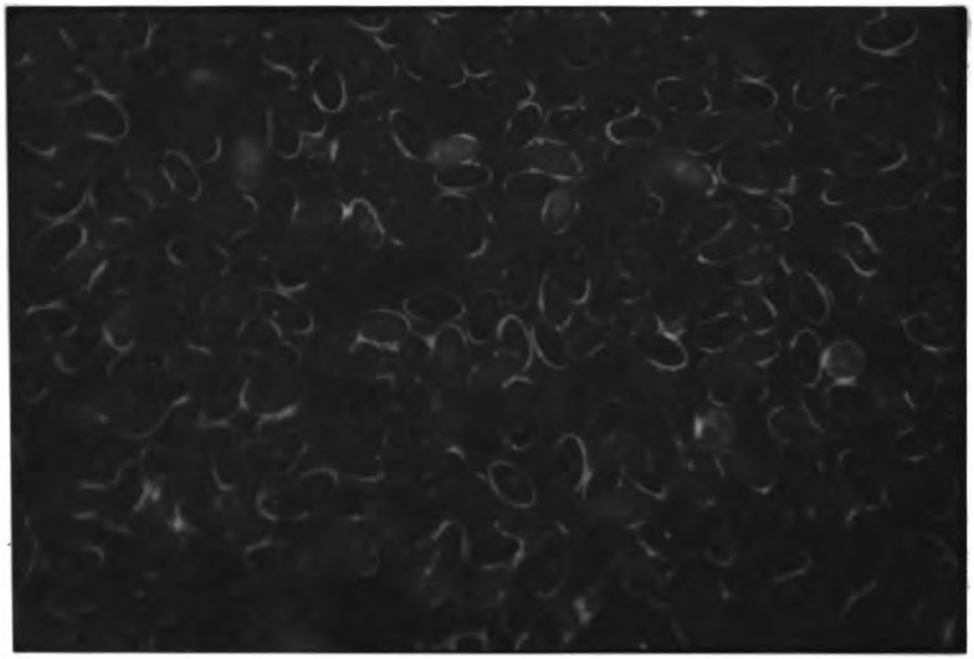

Figure 7. Spontaneous erythrocyte fluorescence in bird 2. Photographed 1 week after last dosing.

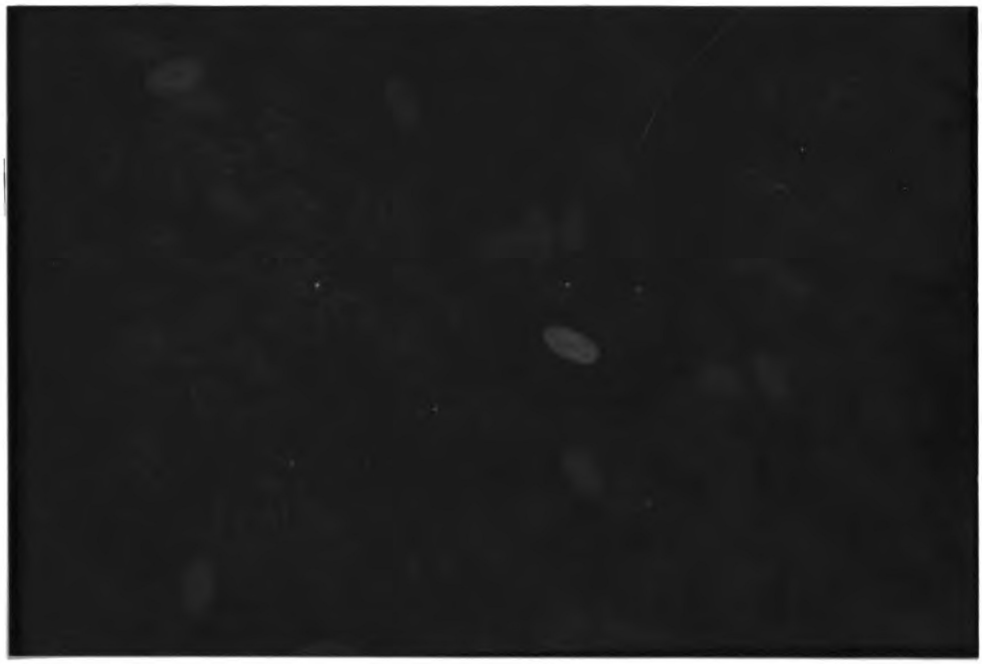

Figure 8. Spontaneous erythrocyte fluorescence in bird 3. Photographed 2 weeks after last dosing.

for 4 days.

Bird 3

Bird 3 had cerclage wires visible at 2 sites of a nonunion fracture of the left humerus on the preliminary radiograph. The bird was fed 30 number 5 lead shot in a mouse meal on day 1. Several shot were passed on day 2, and by day 3 all the pellets had been either egested or defecated by the bird. Radiographs on day 4 showed no retained lead shot, and the bird was redosed with an additional 30 pellets on that same day. He egested a cast containing 28 pellets on day 5 and passed an additional pellet on each of days 6 and 7. Radiographs on day 7 showed no pellets in the gastrointestinal tract, and the bird was redosed that same day. A cast containing 23 pellets was found on the cage floor on day 8. The remaining 7 lead shot were recovered from the cage floor on day 10, at which time the bird was radiographically free from lead shot. Bird 3 retained 7 lead shot for 3 days and 1 pellet for 3 days.

Bird 4

Bird 4 was the control bird for this group and received a mouse diet without lead shot. This bird was free from any previously ingested pellets, but had radiographic evidence of metallic foreign bodies in 5 locations. What appeared to be lead pellets were found in the muscle masses adjacent to both left and right tibias. Another pellet was superimposed on the pelvis. Two additional pellets were located near the left radius. The left proximal ulna had been

fractured as an apparent consequence of being shot. These pellets never changed position at any time in the sequence of radiographs.

The second group of experimental birds included birds 5, 6, and 7.

Bird 5

Bird 5 was the control individual in this group. Radiographs taken on days 1 and 4 showed no evidence of either ingested lead shot or pellets embedded in bone or soft tissue.

Bird 6

The initial radiograph of bird 6 showed no lead pellets in any location. This bird received 10 number 5 lead shot in a mouse meal on day 1. A single pellet was found in the cage on day 2, and 9 more lead shot were recovered from a cast egested on day 3. The bird was radiographically free from lead on day 4, and on that day was again fed 10 lead shot. Single shot were passed by the bird on days 5 and 6 with the 8 remaining pellets found in mutes and casts littering the cage floor on day 7. Radiographs on day 7 confirmed the abscence of retained lead shot. A 10 pellet dosing was repeated at that time. A cast egested on day 8 contained 3 of those pellets. One pellet was found on the cage bottom on day 9, and the remaining pellets were retrieved from a cast and mutes examined on day 10. A subsequent radiograph demonstrated the absence of lead in this bird on the same day just prior to redosing with 10 lead shot in a mouse meal. Three shot were found in the bird's water dish, 3 in a cast, and the remaining shot on the cage floor on day 11. The bird was radiographically free from lead pellets on that day and received no additional

shot. Bird 6 retained 9 lead shot, 8 shot, and 6 shot each for periods as long as 3 days.

Bird 7

The preliminary radiographs taken of bird 7 showed no lead pellets located either in the gastrointestinal tract or soft tissues. The bird was dosed on day 1 with 30 number 5 lead shot and cast 28 of these pellets on day 2. No pellets were egested on day 3. A single pellet was found in the cage on day 4, and radiographs taken on that same day showed a single shot retained in the gizzard. The bird ate another 30 pellets on day 4. Combined casts from days 6 and 7 contained all 31 pellets. Radiographs on day 7 demonstrated no lead shot in the bird. Thirty additional pellets were fed to the bird on day 7, and he egested 28 of these pellets on day 8. Two pellets were found on the cage floor on day 10, and radiographs taken on that day revealed no retained lead shot. The last dose of lead shot was administered at this time. Bird 7 cast 21 of these pellets on day 11, egested 2 lead shot on day 12, and egested the remaining 7 pellets on day 13. Bird 7 retained a single lead pellet for 6 days, 2 pellets for 3 days and 7 pellets for 3 days.

Birds 8, 9 and 10 completed the last replicate.

Bird 8

Bird 8 had no shot in any tissue prior to the start of this experiment as determined by radiographic technique. This bird ate 10 number 5 lead shot offered in a mouse meal on day 1. Undigested material cast late on day 2 and early on day 3 contained all 10 shot.

The bird was fed another 10 lead pellets on day 4, and 3 of these pellets were passed on day 5. A cast egested on day 6 contained 7 additional lead pellets. A radiograph taken on day 7 demonstrated no remaining lead shot, and an additional 10 pellets were consumed by the bird on that day. One lead shot was found in the mutes on day 8, and 9 more shot were cast by the bird on day 10. A radiograph taken on day 10 demonstrated no lead shot. This bird was dosed for the last time on day 10. Examination of the cage floor on day 11 yielded 9 of these 10 pellets. The last lead pellet was found in a cast on day 13 at which time a final radiograph showed the bird to be free from all fed lead shot. This bird retained 9 lead pellets for 3 days, and 1 lead pellet for 3 days.

Bird 9

An initial radiograph of bird 9 showed a metallic foreign object in the soft tissue immediately ventral to the left femur. The object appeared to be a lead pellet. This bird was fed 30 number 5 lead shot in a mouse meal on day 1. Twenty-six shot were recovered from material on the cage floor on day 2, 1 shot on day 3, and 3 shot on day 4. A radiograph taken on that day showed only the lead shot in the left thigh area. An additional 30 pellets were fed on day 4. One lead shot was recovered on day 5. A cast containing 18 pellets was retrieved from the cage on day 6 along with an additional 7 pellets recovered from the cage floor. A radiograph taken on day 7 showed 4 lead shot remaining in the gizzard. Thirty more pellets were consumed by bird 9 on day 7. Nine shot were removed from the floor of the cage on the following day, as well as a cast containing 22 more shot. A

single lead pellet was found on the cage floor on day 9. Radiographs on day 10 showed 2 lead shot remaining in the gizzard. The bird was dosed with lead shot for the last time on day 10. There were 4 pellets in the mutes and 28 in a cast from the bird on day 12. Radiographic examination of the bird at this time demonstrated only the initial lead pellet seen in the femoral area. This bird retained 3 lead shot in the gizzard for 4 days, 4 shot for 3 days, and 2 shot for 8 days. No other bird in the study retained lead shot for as long, however, as individual lead shot were not identified it was impossible to determine if the same shot were retained throughout the entire period.

Bird 10

Bird 10 was an undosed control bird. Radiographic examination showed no evidence of lead pellets in any tissues or organs.

Pathologic Changes - Gross and Microscopic Lesions

Bird 2

It was observed on gross examination that an intramedullary pin joined the fractured halves of the right ulna, but not the radius. Fetid green caseated material surrounded the fracture site. No additional gross lesions were observed in any other tissues. Inflammatory cells were seen on microscopic examination to have infiltrated the submucosal and subepithelial layers of the organs of the upper digestive tract (esophagus, crop, proventriculus). The esophagus appeared to be most markedly infiltrated by these cells which were primarily eosinophils and heterophils.

Bird 3

Gross examination of the left radius and ulna revealed midshaft fractures with pieces of cerclage wire attached to the distal portions of both bones. A hard yellowish-green mass surround the proximal ends of the fracture sites. No other gross or microscopic lesions were seen.

Bird 4

What resembled 2 number 5 lead shot were found on gross inspection. One pellet was harbored in an area of yellow, flaky fat in the omentum and the second was lodged in the right tibio-tarsal joint. No gross tissue proliferation had occurred around the shot in the joint. Microscopic examination of the tissues revealed no microscopic abnormality.

Bird 5

The only visible change at necropsy was what appeared to be a slight thickening of the proventricular mucosa. Microscopic examination of this area, as well as the other tissues collected, showed no significant pathologic changes. There was congestion and hemorrhage in the peribronchiolar areas of the lung which most likely resulted from death due to asphyxiation.

Bird 6

No significant gross lesions were found in this bird. Microscopic areas of peribronchiolar hemorrhage, most likely due to death by asphyxiation, were seen in the lungs.

Bird 7

This was a one-legged bird. Gross inspection of the internal structures showed a whitish area 3 millimeters wide on the ventral pole of the dorsal lobe of the right kidney. This proved to be an area of degeneration on histopathologic examination. The submucosa of the gizzard and especially the lamina propria of the crop had areas of heterophilic and eosinophilic infiltration when viewed microscopically. Bird 7 also had areas of peribronchiolar hemorrhage in the lung.

Bird 8

Small red foci less than 1 millimeter in diameter were grossly visible scattered throughout the pancreas. These spots appeared to be areas of congestion when viewed microscopically. The crop had marked eosinophilic infiltration into the lamina propria, noticeable vascular congestion, and mononuclear infiltration into the muscularis on microscopic examination. Several bi-operculate parasite ova had penetrated the epithelial layer of the crop. These ova could best be identified as Capillaria spp. A similar, but milder, cellular response was seen in the submucosa of the gizzard.

Bird 9

A single shot was lodged in the muscle mass of the left thigh immediately ventral to the femur. No gross tissue response to this foreign body was observed. Microscopic findings were as follows. The lungs had areas of vascular congestion and peribronchiolar hemorrhage. Sections of esophagus and crop had marked eosinophilic

infiltration into the lamina propria with parasitic ova similar to those seen in bird 8 present at the epithelial surface of the esophagus. Small collections of eosinophils and heterophils were seen in the lamina propria of the gizzard.

Bird 10

No gross lesions were observed in this bird. The lung was the only tissue with pronounced histopathologic changes, which included marked areas of peribronchiolar hemorrhage, vascular congestion, and edema. A granuloma with some accompanying heterophils was observed in this tissue. Acid-fast stains revealed no causative agent in or near the granuloma.

DISCUSSION

Ingested lead shot has been a mortality factor in upland game birds (Campbell, 1950; Westemeier, 1965), waterfowl (Phillips et al., 1930; Grinnell, 1901), and raptors (Locke et al., 1969; Jacobson et al., 1977; Redig, 1979).

Clinical signs and gross and microscopic pathology have been described by numerous investigators (Cook et al., 1966; Coburn et al., 1951; Bagley et al., 1967; Bellrose, 1964; Grinnell, 1901).

Acid-fast inclusion bodies in the epithelial cells of the proximal convoluted tubules have been reported in kidney sections from a variety of birds exposed to lead, including mourning doves (Locke et al., 1967), Canada geese (Bagley et al., 1967), ducks (Locke et al., 1966), and raptors (Locke et al., 1969). These inclusions have not been found consistently in every bird having lead poisoning.

The literature describes most birds dying from lead poisoning as having characteristic clinical signs and histopathologic lesions. None of the birds used in this study displayed either the gross signs or microscopic lesions associated with plumbism or died as a result of lead poisoning. Length of exposure to lead and opportunity for its prolonged absorption from the gastrointestinal tract apparently are important factors in fatal poisoning.

Tissue lead levels indicative of toxicosis have been established by previous investigators (Adler, 1974; Buck et al., 1973; Coburn et al., 1951; Longcore et al., 1974) and were markedly higher than those

for 4 days.

Bird 3

Bird 3 had cerclage wires visible at 2 sites of a nonunion fracture of the left humerus on the preliminary radiograph. The bird was fed 30 number 5 lead shot in a mouse meal on day 1. Several shot were passed on day 2, and by day 3 all the pellets had been either egested or defecated by the bird. Radiographs on day 4 showed no retained lead shot, and the bird was redosed with an additional 30 pellets on that same day. He egested a cast containing 28 pellets on day 5 and passed an additional pellet on each of days 6 and 7. Radiographs on day 7 showed no pellets in the gastrointestinal tract, and the bird was redosed that same day. A cast containing 23 pellets was found on the cage floor on day 8. The remaining 7 lead shot were recovered from the cage floor on day 10, at which time the bird was radiographically free from lead shot. Bird 3 retained 7 lead shot for 3 days and 1 pellet for 3 days.

Bird 4

Bird 4 was the control bird for this group and received a mouse diet without lead shot. This bird was free from any previously ingested pellets, but had radiographic evidence of metallic foreign bodies in 5 locations. What appeared to be lead pellets were found in the muscle masses adjacent to both left and right tibias. Another pellet was superimposed on the pelvis. Two additional pellets were located near the left radius. The left proximal ulna had been

fractured as an apparent consequence of being shot. These pellets never changed position at any time in the sequence of radiographs.

The second group of experimental birds included birds 5, 6, and 7.

Bird 5

Bird 5 was the control individual in this group. Radiographs taken on days 1 and 4 showed no evidence of either ingested lead shot or pellets embedded in bone or soft tissue.

Bird 6

The initial radiograph of bird 6 showed no lead pellets in any location. This bird received 10 number 5 lead shot in a mouse meal on day 1. A single pellet was found in the cage on day 2, and 9 more lead shot were recovered from a cast egested on day 3. The bird was radiographically free from lead on day 4, and on that day was again fed 10 lead shot. Single shot were passed by the bird on days 5 and 6 with the 8 remaining pellets found in mutes and casts littering the cage floor on day 7. Radiographs on day 7 confirmed the abscence of retained lead shot. A 10 pellet dosing was repeated at that time. A cast egested on day 8 contained 3 of those pellets. One pellet was found on the cage bottom on day 9, and the remaining pellets were retrieved from a cast and mutes examined on day 10. A subsequent radiograph demonstrated the absence of lead in this bird on the same day just prior to redosing with 10 lead shot in a mouse meal. Three shot were found in the bird's water dish, 3 in a cast, and the remaining shot on the cage floor on day 11. The bird was radiographically free from lead pellets on that day and received no additional

shot. Bird 6 retained 9 lead shot, 8 shot, and 6 shot each for periods as long as 3 days.

Bird 7

The preliminary radiographs taken of bird 7 showed no lead pellets located either in the gastrointestinal tract or soft tissues. The bird was dosed on day 1 with 30 number 5 lead shot and cast 28 of these pellets on day 2. No pellets were egested on day 3. A single pellet was found in the cage on day 4, and radiographs taken on that same day showed a single shot retained in the gizzard. The bird ate another 30 pellets on day 4. Combined casts from days 6 and 7 contained all 31 pellets. Radiographs on day 7 demonstrated no lead shot in the bird. Thirty additional pellets were fed to the bird on day 7, and he egested 28 of these pellets on day 8. Two pellets were found on the cage floor on day 10, and radiographs taken on that day revealed no retained lead shot. The last dose of lead shot was administered at this time. Bird 7 cast 21 of these pellets on day 11, egested 2 lead shot on day 12, and egested the remaining 7 pellets on day 13. Bird 7 retained a single lead pellet for 6 days, 2 pellets for 3 days and 7 pellets for 3 days.

Birds 8, 9 and 10 completed the last replicate.

Bird 8

Bird 8 had no shot in any tissue prior to the start of this experiment as determined by radiographic technique. This bird ate 10 number 5 lead shot offered in a mouse meal on day 1. Undigested material cast late on day 2 and early on day 3 contained all 10 shot.

The bird was fed another 10 lead pellets on day 4, and 3 of these pellets were passed on day 5. A cast egested on day 6 contained 7 additional lead pellets. A radiograph taken on day 7 demonstrated no remaining lead shot, and an additional 10 pellets were consumed by the bird on that day. One lead shot was found in the mutes on day 8, and 9 more shot were cast by the bird on day 10. A radiograph taken on day 10 demonstrated no lead shot. This bird was dosed for the last time on day 10. Examination of the cage floor on day 11 yielded 9 of these 10 pellets. The last lead pellet was found in a cast on day 13 at which time a final radiograph showed the bird to be free from all fed lead shot. This bird retained 9 lead pellets for 3 days, and 1 lead pellet for 3 days.

Bird 9

An initial radiograph of bird 9 showed a metallic foreign object in the soft tissue immediately ventral to the left femur. The object appeared to be a lead pellet. This bird was fed 30 number 5 lead shot in a mouse meal on day 1. Twenty-six shot were recovered from material on the cage floor on day 2, 1 shot on day 3, and 3 shot on day 4. A radiograph taken on that day showed only the lead shot in the left thigh area. An additional 30 pellets were fed on day 4. One lead shot was recovered on day 5. A cast containing 18 pellets was retrieved from the cage on day 6 along with an additional 7 pellets recovered from the cage floor. A radiograph taken on day 7 showed 4 lead shot remaining in the gizzard. Thirty more pellets were consumed by bird 9 on day 7. Nine shot were removed from the floor of the cage on the following day, as well as a cast containing 22 more shot. A

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Bird 10 was an undosed control bird. Radiographic examination showed no evidence of lead pellets in any tissues or organs.

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Bird 2

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Tissue lead levels indicative of toxicosis have been established by previous investigators (Adler, 1974; Buck et al., 1973; Coburn et al., 1951; Longcore et al., 1974) and were markedly higher than those

obtained in this study. There were increases in tissue lead levels over the control values obtained from the red-tailed hawks, but these were not indicative of fatal toxicosis.

Published blood lead levels in birds with fatal lead poisoning range from 2.7 to 20 ppm, with 10 ppm suggestive of acute exposure (Longcore et al., 1971). This correlates with the blood lead values of 1.67 to 9.69 ppm generated by this study.

There have been few published reports of hematologic observations on lead poisoned birds. Inconsistencies in total leukocyte numbers seen in lead poisoned birds, as previously reported, were also observed in this study. The only published information on the hematocrit in lead poisoned raptors described alterations in the hematocrit as being insignificant (Redig, 1979). Changes in hematocrit, hemoglobin values, and erythrocyte fragility were not useful in monitoring the progress of lead absorption in the red-tailed hawks. The insignificant changs in erythrocyte fragility were reflective of the material presented by Aub et al. (1925) in which species variation existed with regard to changes in red cell osmotic resistance. The chicken erythrocyte did not display any characteristic changes in osmotic resistance in Aub's work.

Spontaneous erythrocyte fluorescence was observed only in those birds receiving lead shot; however, not all birds receiving lead shot developed fluorescing erythrocytes. The number and intensity of the fluorescing erythrocytes did not directly correlate with either the quantity of lead ingested nor the retention time of the pellets in the birds. This fluorescent phenomenon may assist in the recognition of lead poisoned birds, although other diagnostic tests might provide more information.

It may be concluded from this research that repeated ingestion of lead shot by red-tailed hawks and other raptors may not produce clinical signs or result in fatal lead poisoning. The raptor's ability to egest lead pellets significantly decreases shot retention time in the bird, and apparently minimizes the opportunity for absorption of the toxic material from the gastrointestinal tract. Interference with this casting mechanism may be of primary importance in pellet retention, lead absorption, and fatal poisoning in birds of prey.

SUMMARY

Ten red-tailed hawks were used in this investigation. Research was conducted to duplicate lead poisoning as seen in free living birds of prey. The first bird was used for a feasibility study. The remaining 9 birds were divided into the following groups of 3 birds each:

(1) control, un-dosed birds, (2) birds receiving 4 sequential doses of 10 number 5 lead shot, and (3) birds receiving 4 sequential doses of 30 number 5 lead shot.

Birds were evaluated for clinical evidence of lead poisoning, blood and tissue lead levels, changes in total leukocyte numbers, hemoglobin, hematocrit, and osmotic erythrocyte fragility, and gross and microscopic lesions.

Clinical signs were not observed in any of the birds during the course of experimentation. Analyses of repeated blood samples from all dosed birds indicated increases in blood lead levels consistent with acute exposure to lead. Tissue lead levels, although elevated, were not indicative of toxicosis. Blood values monitored in this study were found not to be predictable for lead intoxication. Spontaneous erythrocyte fluorescence occurred on a nonuniform basis in lead dosed birds from groups (2) and (3). Gross observation and microscopic examination of selected tissues revealed no pathologic changes associated with plumbism.

Birds of prey which were able to egest lead shot showed neither gross nor microscopic evidence of lead poisoning, but did absorb lead during instances of acute exposure based on the findings under this protocol.

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