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Histopathology of Mallards Dosed With Lead and Selected Substitute Shot[1]

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ABSTRACT

The histopathological response of male game farm mallards fed lead, three types of plastic-coated lead, two lead-magnesium alloys, iron, copper, zinc-coated iron, and molybdenum-coated iron shot was studied. Mallards fed lead, plastic-coated lead, or lead-magnesium alloy shot developed a similar pathological response, including the formation of acid-fast intranuclear inclusion bodies in the kidneys. Birds fed iron or molybdenum-coated iron shot developed hemosiderosis of the liver. Two of four mallards fed zinc-coated iron shot also developed hemosiderosis of the liver. No lesions were found in mallards fed copper shot.

Mortality of waterfowl due to the ingestion of spent lead shot has been recognized in North America since 1894 and has been reported from every major North American flyway. Since the studies of Wetmore[10] and Jordan and Bellrose[7], who demonstrated that lead was the chemical primarily responsible for losses, several efforts have been made to develop a nontoxic shot.

Basically, three approaches have been used: (1) developing a disintegratable lead shot which would fragment in water and thus become unavailable; (2) coating the lead shot to prevent the absorption of lead; and (3) replacing the lead with a less toxic metal or alloy. Although several investigators have worked with various substitute shot, little has been published on the pathological effects of these shot.

This paper reports certain pathological observations made during the course of our studies conducted in 1965 on various types of substitute shot. Data on toxicity and on survival of treated birds have been reported in a previous paper[6].

Materials and Methods

Possible variations in toxicity of lead to mallards due to age and sex were carefully controlled in this initial test. The test ducks were pen-reared mallard drakes (*Anas platyrhynchos*) near one-year-old which had been kept in a common holding pen for 5 months prior to the start of the tests. They were fed commercial duck pellets throughout this period. At the start of the tests their weights ranged from 900 to 1600 grams, with an average of 1140 grams.

During the last week in March 1965, the ducks were randomly divided into groups of eight and placed in 9 x 5 foot tank cages[3]. There were 3 groups of 8 ducks on each treatment, making a total of 24 birds per treatment.

Three types of plastic coated lead shot were received too late for inclusion in the above tests. Each of these types was tested

[1] Presented in part at the Wildlife Disease Association Annual Meeting, College Park, Maryland, August 1966.

with 10 ducks held in a common holding pen along with 10 other control ducks.

The types and sources of shot tested are listed in Table I.

The test dose consisted of eight pellets (each roughly equal to a #6 lead shot in size). These were given by means of a flexible plastic tube which was passed down the bird's esophagus until it reached the gizzard. Following the dosing, the ducks were maintained on a whole corn diet with quartz grit offered *ad libitum*. Where corn-fed wildfowl have easy access to heavy accumulations of lead shot, serious lead poisoning outbreaks are most common but such die-offs by no means are restricted to this combination of food and shot.

All ducks were examined by fluoroscopy about 2 weeks after dosing, thereafter, randomly selected ducks were examined at intervals of not less than one week to ascertain evidence of shot retention and elimination. The value of fluoroscopy data was questionable in certain instances. The number of shot present could not always be determined, and in a few cases shot were present but were not observed. Histories of treatment, weight, fluoroscopy, and mortality were recorded for each duck. .

Ducks which died during the experimental period were necropsied immediately or frozen for later examination. All surviving ducks were sacrificed 60 days after dosage and selected tissues were saved for chemical and microscopic examination.

Supplementary studies of the effects of iron shot were conducted in September 1965 and again in the spring of 1966.

Groups of mallards were dosed with one, four, or eight iron shot to obtain additional data on the deposition of hemosiderin in duck livers.

Chemical analyses were done in accordance with the Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer Corporation, Norwalk, Connecticut. The Perkin-Elmer atomic absorption spectrophotometer, Model 303, was used for all analyses.

Tissues for microscopic examination were fixed in 4% formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin, and selected tissues were also stained with the periodic-acid Schiff, Ziehl-Neelsen acid-fast, Prussian blue, Wolbach's Giemsa, or with the Brown and Brenn modification of the Gram stain.

RESULTS

Lead Shot

Twenty-three of the 24 mallards fed commercial lead shot developed typical signs of lead intoxication and died before the end of the 60 day test. Most died during the second and third week; the average day of death was 17. Typical lesions of lead poisoning were present in all 23 birds succumbing. Mallards fed eight commercial lead shot developed lesions typical of lead poisoning: anemia, marked emaciation with muscular atrophy and a prominent keel (so-called "razor-keel" or "hatchet-breast"), serous atrophy of adipose tissue, and liver atrophy. Gall bladders were usually enlarged (2 - 3X) and distended with bile. Gizzard linings were stained a blackish-green color by regurgitated bile.

Microscopically, there was necrosis of the liver cells, with marked hemosiderosis and destruction of the kidney tubule cells. Acid-fast intranuclear inclusion bodies were found in the cells of the proximal convoluted tubules. These have been described elsewhere[8]. Enlarged nuclei were frequently seen in the cells of the proximal convoluted tubules[5].

TABLE 1. *Types and Sources of Shot administered*

Shot Type	*Source*
Lead	Winchester-Western
Lead (plastic coated-46A)	3M Company
Lead (plastic coated-46B)	3M Company
Lead (plastic coated-46C)	3M Company
Lead-magnesium (T6)	Dow Chemical Company
Lead-magnesium (F)	Dow Chemical Company
Iron	Wheelabrator Company
Iron (zinc-coated)	Wheelabrator Company
Iron (molybdenum-coated)	Wheelabrator Company
Copper	Belmont Smelting & Refining Works, Inc.

Plastic-coated Lead

Ducks dosed with the three types of plastic-coated lead shot developed typical signs of lead poisoning and all but two died before the end of the test. Most died during the second and third weeks of the test.

Tissues from only one of the birds fed plastic-coated lead were available for microscopic examination. The carcass of this bird had been stored at 14° F for 10 months prior to examination. Typical acid-fast intranuclear inclusion bodies were found in the kidneys of this mallard.

Lead-Magnesium Alloy

Twenty-eight of the drakes dosed with the lead-magnesium alloy shot developed typical signs of lead intoxication and died. Several of the surviving 20 developed signs of lead intoxication, but recovered. Testes of four surviving mallards were examined histologically and found to be normal. Normal, motile sperm were present in testicular impression smears of three of the four birds. The testes of the fourth, though normal, was in an earlier stage of spermatogenesis and no sperm were present.

The livers of five mallards surviving the lead-magnesium dosage were examined microscopically. The livers of three of the five drakes surviving the test contained moderate amounts (2+) of hemosiderin. The liver of one duck had only a few widely scattered clumps of hemosiderin, while the fifth duck had no hemosiderin deposits.

Acid-fast intranuclear inclusion bodies were found in the kidneys of three of these five birds. The kidneys of two contained less than 20 affected nuclei (19 and 10 respectively) in 100 consecutive oil immersion fields. The third duck had 80 nuclei containing acid-fast inclusions in 25 oil immersion fields.

Copper Shot

None of the mallards fed copper shot developed any signs of illness, and the one duck that died was lost due to other causes. Tissues from four drakes killed at the end of the 60 day test were examined histologically and no lesions were found. Spermatogenesis was normal in all four drakes, and normal, motile sperm were present in the seminal fluid. No evidence of kidney damage or hemolytic anemia was found. No acid-fast inclusions were found in the kidney tubules, and no hemosiderin was found in the livers.

Uncoated and Coated Iron Shot

Tissues from four drakes fed uncoated iron shot and from four dosed with each of the coated iron shot were selected for microscopic examination. The ducks fed uncoated iron shot had retained all or some of their shot, but none of the drakes fed coated iron shot had retained any shot at necropsy. Later, liver sections from an additional six drakes fed uncoated iron shot, and a fifth drake fed molybdenum-coated iron shot were selected for microscopic study.

Microscopically the testes of all drakes given uncoated iron shot, zinc-coated iron shot, or molybdenum-coated iron shot exhibited active spermatogenesis. Normal motile sperm were present in smears of seminal fluid made at the time of necropsy.

The livers of 10 drakes dosed with uncoated iron shot, 5 dosed with molybdenum-coated iron shot, and 2 of 4 dosed with zinc-coated iron shot contained an intracellular, yellow-amber, granular pigment, which was primarily distributed along the biliary canaliculi. In drakes fed uncoated iron shot, the accumulations of pigment were frequently so great as to obscure cellular detail. This pigment stained an intense dark blue with the Prussian blue stain and was identified as hemosiderin.

An additional four mallards were dosed with eight uncoated iron shot on September 9 and were necropsied after 45 days on test. Massive hemosiderosis

of the liver occurred in each of these ducks. The results of the microscopic examinations and the chemical analyses of the livers of these four birds are summarized in Table 2. The level of iron in the livers of these mallards is much higher than that in the livers of those fed 8 uncoated iron shot in the original test. Iron levels in the latter ranged 918 to 2394 ppm with an average of 1530 ppm. Livers of control ducks had an average of 676 ppm iron, and ranged from 367 - 952 ppm.

The spleens of the mallards fed eight uncoated iron shot also contained increased amounts of hemosiderin; however, none was found in the kidney, brain, or heart.

In March 1966, tests were initiated to determine if hemosiderosis of the liver would occur in male mallards fed one or four uncoated iron shot. Hemosiderosis occurred in three of four ducks fed four iron shot, but was less intense than that which occurred in ducks fed eight iron shot. The fourth mallard had occasional scattered clumps of hemosiderin in the Kupffer's cells, and in this regard resembled the controls. This latter duck had lost all four shot by the time the test was concluded, 60 days after treatment, while the others had retained one or more shot. The ducks fed one iron shot and the undosed controls had occasional scattered clumps of Prussian blue-positive pigment in the liver.

DISCUSSION

Although several metals and alloys have been proposed at one time or another for use as a substitute for lead, very little work has been done to adequately evaluate the safety of these proposed substitutes. The use of iron shot as a possible substitute for lead has been urged by several investigators and the sporting arms companies have conducted a considerable amount of research on the ballistics of iron shot[1]. However, the massive accumulations of hemosiderin in the livers of our drakes fed eight uncoated iron shot suggests that additional tests be conducted to determine (1) if such an accumulation of hemosiderin interferes with liver function, and (2) the turnover rate of such iron pigments.

The accumulation of hemosiderin in the livers of mallards dosed with uncoated iron shot is suggestive of the liver accumulation of hemosiderin which Gillman et al[4] produced in rats fed on iron-enriched, maize-meal diet. Dietary siderosis was recently reported in Bantu tribesmen by Bothwell and Isaacson[2] and these authors suggested that such massive deposits of iron pigment may not be completely harmless. Theron et al[9] reported that when rats were fed a high iron-maize-meal diet there was marked siderosis of the liver, characterized by an increased uptake of iron by the hepatic cells with initial localization of the iron in the lysosomes. Degenerative changes occurred in the liver cell mitochondria with release of ferritin. Additional laboratory studies are needed to determine if iron-overloading of the mallard liver interferes with normal functioning of this organ.

Microscopically, mallards fed lead-magnesium and plastic-coated lead shot

TABLE 2. *Iron Accumulation in Livers of Mallards Fed Eight Iron Shot**

Number	Shot Recovered	Iron, ppm In Liver	Degree of Hemosiderosis
432	3	3,185	4+
433	0	6,131	4+
434	0	4,470	4+
435	No data	4,882	4+

* Necropsy 45 days after dosing.

tended to have the same pathological picture as mallards fed commercial lead shot — destruction of kidney tubules, acid-fast intranuclear inclusion bodies in the cells of the proximal convoluted tubules, and necrosis and hemosiderosis in the liver.

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BOOK REVIEW

HOFFMAN, G. L. *Parasites of North American Freshwater Fishes.* University of California Press, Berkeley and Los Angeles, 1967, i-viii, 486 pp.

Hoffman has written a general account of the taxonomy and distribution of parasites of freshwater fishes. Valuable features are a bibliography of 60 pages, and a host-parasite checklist of 83 pages. The remainder of the volume is composed of keys to, and diagnoses of, genera of Protozoa (pp. 21-69); Monogenea (71-104); adult Digenea (105-160); metacercariae (161-202); Cestoda (203-240); Nematoda (241-269); Acanthocephala (270-287); Hirudinea (289-298); and Copepoda (299-315). Species from freshwater fishes in each genus are listed with references. The author is apparently unfamiliar with nematode taxonomy since he prefers a classification more than 50 years out of date! Consultation with a nematologist would improve subsequent editions. Illustrations are kept to a minimum and are nicely reproduced. Disease aspects are not considered and there is little or no detailed information on transmission. However, this work is a valuable contribution to a difficult and important subject - the diagnosis of parasitic infections in fishes - and will undoubtedly be consulted widely by scientists concerned with diseases of fishes.

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