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Authors: G. L. MARCH, T. M. JOHN, B. A. McKEOWN, L. Sileo, and J. C. GEORGE

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## THE EFFECTS OF LEAD POISONING ON VARIOUS PLASMA CONSTITUENTS IN THE CANADA GOOSE\*

G. L. MARCH\*\*, T. M. JOHN<sup>1</sup>, B. A. McKEOWN<sup>1</sup>, L. SILEO<sup>2</sup> and J. C. GEORGE<sup>1</sup>

**Abstract:** Plasma glucose, free fatty acid and uric acid levels were measured in lead-poisoned Canada geese (*Branta canadensis*). Although plasma glucose levels were only slightly elevated, uric acid was significantly higher and free fatty acids were significantly lower. Altered plasma levels were attributed to increased protein catabolism and perhaps renal disfunction. Plasma level of growth hormone and prolactin was assessed by radioimmunoassay. Growth hormone remained unchanged while prolactin was unusually high. The increased prolactin levels may reflect an effort to stabilize free fatty acids.

### INTRODUCTION

Lead poisoning is a significant mortality factor in a variety of vertebrate species, and is presently considered to be a major cause of waterfowl mortality in eastern and central North America.<sup>4,25,27,29</sup> The increasing amount of lead added to wetland areas not only has caused serious loss of waterfowl but understandably may have an effect on other marsh dwelling species.<sup>2,4,6,28</sup> The pathologic effects of lead on waterfowl have been described by Trainer & Hunt,<sup>28</sup> Bagley *et al.*,<sup>3</sup> and more recently by Karstad.<sup>16</sup>

Migratory birds increase rapidly in weight and deposit considerable fat just prior to migration. Any interference with this phenomenon would be extremely critical since lipids are the major source of energy for avian flight.<sup>8</sup> Lead-poisoned Canada geese are often severely emaciated and have a conspicuous cephalic edema.

A number of hormones are involved in lipid metabolism. In birds prolactin influences both lipid synthesis and utilization, depending on the time of day the hormone is administered. Injections of

prolactin, when given early in a 16 h photoperiod, suppress body weight and lipid deposition.<sup>22</sup> Contrarily, injections given late in the photoperiod promote lipid synthesis and deposition. Recent work has implicated prolactin as one of the hormones capable of elevating plasma levels of free fatty acid (FFA) and in this respect closely resembles the activity of growth hormone.<sup>30</sup>

Since lead-poisoned birds experience various degrees of emaciation<sup>13</sup> during the migratory period, a time when energy utilization is critical, the blood parameters associated with metabolism were investigated. Consideration was given to glucose, FFA, uric acid, growth hormone and prolactin.

### MATERIALS AND METHODS

Eight moribund Canada geese were captured during December 1973 in a large marsh area along the southeastern side of Lake St. Clair, Ontario. Under licence from the Canadian Wildlife Service, eight free flying healthy Canada geese were obtained from the Kortright Waterfowl Research Center, and used

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\*\*Present address: G. L. March, 5th floor, 1111 West Georgia St., Vancouver, B.C. V6E 3G7.

<sup>1</sup> Department of Zoology, and <sup>2</sup> Department of Pathology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

for controls. All birds were killed by decapitation at approximately the same time of day (1000 to 1200) to avoid possible fluctuation of certain metabolites attributable to circadian rhythm.

Blood was collected in oxalated centrifuge tubes, centrifuged, and plasma was stored frozen for later analysis. Necropsies were performed on the moribund geese immediately following decapitation. Selected tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5  $\mu$ m and stained with Hematoxylin and Eosin. In some cases the Ziehl-Neelson acid-fast method was used.<sup>18</sup> Plasma lead levels were determined by atomic absorption spectrophotometry.

The radioimmunoassay for both growth hormone and prolactin was the solid phase method of Catt and Tregear,<sup>5</sup> with slight modifications described earlier for avian prolactin,<sup>19</sup> and avian growth hormone.<sup>20</sup> Each plasma sample was assayed at three dilutions (1:200, 1:400, 1:800) in 0.15 M NaCl. The antiserum was diluted 1:300 for growth hormone and 1:1000 for prolactin. Since purified avian growth hormone or prolactin was unavailable, standard curves showing absolute hormone concentrations could not be produced. However, one plasma sample was used to produce a "standard curve" and all

other plasma samples were compared to this curve to express plasma hormone concentrations as a relative percent difference. Each sample was assayed separately and a group average was calculated.

The plasma FFA levels were determined by using the semiautomated colorimetric method of Antonis,<sup>1</sup> carried out on the Technicon Autoanalyser system. Uric acid was assayed using The Sigma Chemical Co. method (Technical Bulletin #292-UV). Glucose was assayed by the glucostat method of Worthington Biochemical Corporation; New Jersey, U.S.A.

The means of all measurements were compared statistically by the Student's *t* test.

## RESULTS

Results of post-mortem and microscopic examinations were indicative of lead poisoning and are summarized in Table 1. Lead levels in a pooled plasma sample of all the lead-poisoned birds were found to be higher by a factor of three than the plasma lead levels of the controls (1.2 ppm compared with 2.9 ppm).

Data on plasma levels of glucose, FFA and uric acid are given in Table 2. Plasma glucose did not change, however uric acid levels were significantly higher

TABLE 1. Post-mortem results of eight lead-poisoned Canada geese killed by decapitation.

No. of Birds	Gross Lesions
2	Emaciation
2	Esophageal impaction
6	Lead shot in gizzard
4	Bile-stained liver
Microscopic Lesions	
3	Fibrinoid degeneration of arteries
2	Skeletal muscle necrosis
2	Myocardial necrosis
4	Hepatic hemosiderosis
2	Acid fast intranuclear inclusions (renal tubule epithelium)

TABLE 2. Plasma levels of glucose, FFA and uric acid in lead-poisoned and control Canada geese. (A = Adult; HY = Hatch year; M = Male; F = Female; Sex and age of number 8 not recorded).

Bird No.	Age and Sex	Lead-poisoned			Normal			
		Glucose mg/100 ml	FFA $\mu$ eq/litre	Uric Acid mg/100 ml	Bird No.	Glucose mg/100 ml	FFA $\mu$ eq/litre	Uric Acid mg/100 ml
1	HYM	293	438	16.25	1	297	446	7.45
2	HYM	291	635	6.05	2	340	660	8.20
3	HYM	290	660	13.38	3	325	500	4.75
4	HYF	430	510	13.63	4	227	1165	3.38
5	AF	510	560	39.15	5	277	725	4.85
6	AF	353	200	13.65	6	337	725	8.25
7	HYF	370	480	13.70	7	250	543	6.95
8	—	318	110	11.40	8	290	1057	5.85
Mean		356	449	15.90		292	727	6.21
± SEM		26	65	3.26		13	85	0.59

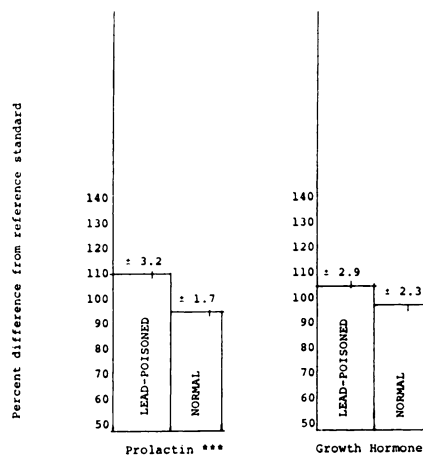


FIGURE 1. Plasma growth hormone and prolactin concentrations in lead-poisoned Canada geese: (\*\*\*)  $p < 0.001$ .

( $P < 0.05$ ) in the lead-poisoned birds compared with the controls. Plasma FFA levels were markedly decreased in lead-poisoned birds ( $P < 0.05$ ).

Results obtained from the radioimmunoassay of prolactin and growth hormone in lead-poisoned birds indicate a significantly higher level of prolactin ( $P < 0.001$ ) but no change in growth hormone (Fig. 1).

#### DISCUSSION

Previous research indicates that lead-poisoned birds have acute anorexia, resulting in severe emaciation.<sup>16,3</sup> Thus geese moribund from lead poisoning should experience considerable loss of weight and have blood plasma parameters consistent with starvation. In most mammals and immature chickens deprivation of food reduces plasma glucose and increases FFA.<sup>17</sup> However, in lead-poisoned geese the situation is reversed in that the level of plasma glucose is

slightly elevated while FFA is significantly lower. This agrees with work done by Heald and Rookledge<sup>10</sup> on starving mature laying hens and is also compatible with results obtained by McKeown *et al.*<sup>21</sup> for the kokanee salmon, *Oncorhynchus nerka*. Plasma levels of FFA in lead-poisoned geese indicate severe starvation and, in addition, uric acid is unusually high, again suggesting increased protein catabolism and perhaps renal disfunction as well.

The plasma levels of growth hormone would be expected to increase when FFA level is low, especially if there is demand for more FFA for oxidation by the muscles, since growth hormone has been shown to have a strong adipokinetic effect in both mammals<sup>11</sup> and birds.<sup>14</sup> The apparent lack of any significant changes in circulating levels of growth hormone may indicate little demand for FFA, possibly due to low oxidative metabolism, thereby producing a feedback reduction in growth hormone release. The fact that these geese were moribund and could not maintain sustained flight suggests their metabolism is more glycolytic than normal geese, which are highly aerobic and mainly utilize fat as a source of muscular energy. Moreover, increased lead levels in swine cause structural degradation of the cristae in the mitochondria.<sup>20</sup> More recently, using beef mitochondria, Scott *et al.*<sup>20</sup> have shown that succinoxidase activity is inhibited by Pb<sup>2+</sup>. The primary site of inhibition is in the succinic dehydrogenase step. Lead also has been shown to activate the energy-linked uptake of ions by the mitochondria with the result that lead accumulates by an energy-dependent reaction, a feature lead shares with Ca<sup>2+</sup>. Therefore the inference is that in these birds glycolytic pathways should be preferred over utilization of lipids, which necessarily require an efficient mitochondrial system.

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Prolactin, also implicated in energy metabolism, was markedly increased. Injections of this hormone in pigeons causes an immediate increase in the activity of those enzymes involved with lipogenesis,<sup>9</sup> and also it has shown that labelled precursor substances rapidly appear in liver FFA. Winkler *et al.*<sup>20</sup> have concluded from their studies on the dog that prolactin is directly involved in the increase of plasma FFA, an effect similar to that produced by growth hormone. Possibly the increased prolactin secretions reflects an effort to stabilize FFA levels.

Jordan and Bellrose<sup>15</sup> compared the physiologic conditions of normal and lead-poisoned ducks provided with identical diets and reported decreased appetite in birds poisoned with lead. Apparently many of the characteristics of lead poisoning, such as weight loss curves, signs and time to death, were indistinguishable from those of starvation. Lead interacts physiologically with calcium in many cases,<sup>7</sup> and can replace calcium in certain blood proteins.<sup>24</sup> Since it has been reported that increased amounts of dietary calcium depress food intake and produce marked retardation in body and weight gain,<sup>12</sup> lead may have a coincidental effect and produce results similar to calcium. Work by Bellrose<sup>4</sup> indicates that possibility of calcium-lead interaction with respect to food intake.

Considerable evidence suggests many of the pathologic effects of lead poisoning could be terminal.<sup>3,16</sup> However, some birds have recovered completely following experimental lead poisoning and necropsies revealed repair of damaged tissue.<sup>3</sup> This information, when combined with results from plasma analysis, suggests the possibility of both food deprivation and mitochondrial damage playing a major role in terminal lead poisoning.

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