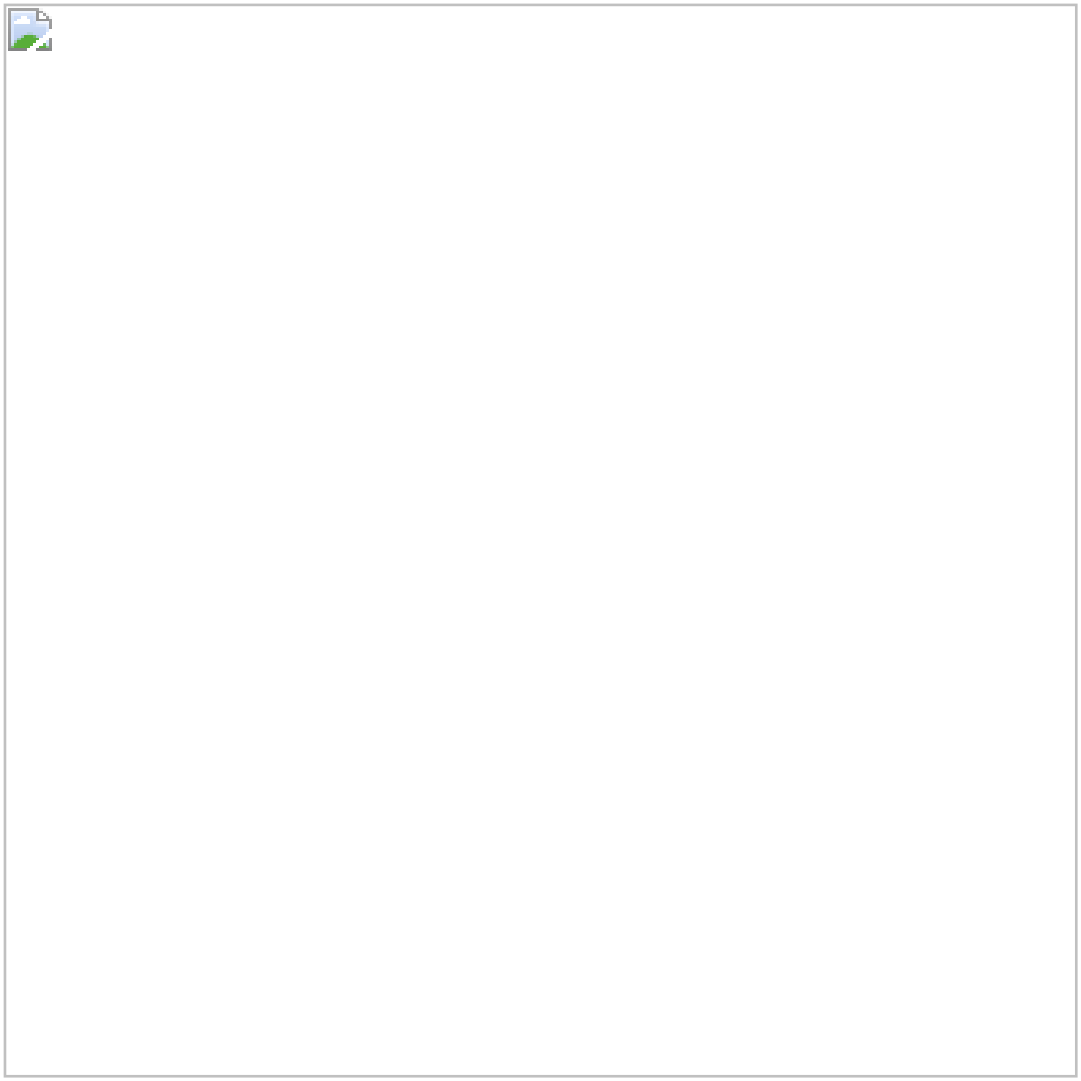

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DIAGNOSTIC CRITERIA FOR SELENIUM TOXICOSIS IN AQUATIC BIRDS: HISTOLOGIC LESIONS

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ABSTRACT: Chronic selenium toxicosis was induced in 1-yr-old male mallard ducks (*Anas platyrhynchos*) by feeding selenium, as seleno-DL-methionine, in amounts of 0, 10, 20, 40, and 80 parts per million (ppm) to five groups of 21 ducks each for 16 wk during March to July 1988. All mallards in the 80 ppm group, three in the 40 ppm group, and one in the 20 ppm group died. Histologic lesions in mallards that died of selenosis were hepatocellular vacuolar degeneration progressing to centrolobular and panlobular necrosis, nephrosis, apoptosis of pancreatic exocrine cells, hypermaturity and avascularity of contour feathers of the head with atrophy of feather follicles, lymphocytic necrosis and atrophy of lymphoid organs (spleen, gut-associated lymphoid tissue, and lumbar lymph nodes), and severe atrophy and degeneration of fat. Histologic lesions in surviving mallards in the 40 ppm group, which had tissue residues of selenium comparable to mallards that died, were fewer and much milder than mallards that died; lesions consisted of atrophy of lymphoid tissue, hyalinogranular swelling of hepatocytes, atrophy of seminiferous tubules, and senescence of feathers. No significant histologic lesions were detected in euthanized mallards in the 0, 10 and 20 ppm groups. Based on tissue residues and histologic findings, primarily in the liver, there was a threshold of selenium accumulation above which pathophysiologic changes were rapid and fatal. Pathognomonic histologic lesions of fatal and nonfatal selenosis were not detected. Criteria for diagnosis of fatal selenosis in aquatic birds include consistent histologic lesions in the liver, kidneys, and organs of the immune system. Although histologic changes were present in cases of chronic non-fatal selenosis, these were inconsistent. Consistent features of fatal and non-fatal chronic selenosis were marked weight loss and elevated concentrations of selenium in organs.

Key words: Selenium intoxication, mallard ducks (*Anas platyrhynchos*), cachexia, depterylation (feather loss), liver necrosis, lymphoid organ atrophy, nephrosis.

INTRODUCTION

Deficiency or excess of selenium (Se) in feed is a cause of health problems in invertebrates and vertebrates (Eisler, 1985; Ohlendorf, 1989). Selenium intoxication (selenosis) is a recognized cause of morbidity, mortality, reduced egg production and hatchability, and teratogenesis in domestic fowl and wild aquatic birds (Rosenfeld and Beath, 1964; Ohlendorf et al., 1986; Hoffman and Heinz, 1988). Selenosis of livestock has been attributed to consumption of seleniferous forage from regions with high-Se soils, although some cases of selenosis may lack a demonstrable source of Se (National Research Council, 1980; Traub-Dargatz and Hamar, 1986). Elevated concentrations of Se in irrigation drainwater, aquatic plants, invertebrates, and fish have adversely affected the health and reproduction of free-ranging aquatic birds (Presser and Ohlendorf, 1987; Ohlendorf, 1989). The effects of Se accumulation in other classes of wild vertebrates also are being studied (Clark, 1987; Ohlendorf, 1989).

Ohlendorf et al. (1986) first documented selenosis as a cause of embryonic death and general nesting failure in wild aquatic birds from the Kesterson National Wildlife Refuge in central California (USA). Several anomalies were detected in bird embryos, including absence or marked reduction in size of eyes, legs, toes, beaks and vertebrae; heart and liver defects also were found. Abnormalities observed in adult dead or killed American coots (*Fulica americana*) and pied-billed grebes (*Podilymbus podiceps*) were emaciation, pul-

monary edema, fibrinogelatinous hydrocoelom (ascites), feather loss from the head, splenomegaly, and nodular hepatomegaly (Ohlendorf et al., 1988). Histologically, the grebes and coots had hepatic necrosis with occasional biliary hyperplasia, amyloidosis, fibrosis, and regenerative nodules; the only other described histologic lesion was myocytic atrophy of breast muscles. The abnormalities of feather loss, splenomegaly, pulmonary edema, and hydrocoelom were not addressed histologically.

Experimental studies of chronic selenosis in mallard ducks (*Anas platyrhynchos*) either resulted in few deaths at the highest levels of Se added to feed (Hoffman et al., 1991a), deaths that were described in terms of gross abnormalities (Heinz and Fitzgerald, 1993; Heinz, 1993), or developmental anomalies in embryos (Hoffman and Heinz, 1988). Tissue or organ assessment of experimental chronic selenosis in mallard ducklings was either lacking (Heinz et al., 1988) or limited to histologic evaluation of a few internal organs (Hoffman et al., 1991b). Further, Se toxicosis in wildlife and livestock can be complicated by the presence of high concentrations of other metals and metalloids (Ohlendorf, 1989; Fairbrother et al., 1994) and by phytotoxins in Se-laden plants (James et al., 1983; O'Toole et al., 1996).

Our purposes in this study were to (1) characterize histologically the gross lesions of chronic selenosis in mallards uncomplicated by simultaneous ingestion of other metals or phytotoxins, (2) compare the histologic lesions of chronic selenosis in mallards with reported lesions in aquatic birds, livestock, and primates, (3) contrast the lesions of acute and chronic selenosis in aquatic birds, and (4) establish a set of histologic lesions that are diagnostic of chronic selenosis in aquatic birds.

The effects of ingested Se on body weight, plumage, organs, and Se concentrations in tissues of mallard ducks were reported in a companion paper (Albers et al., 1996). Gross abnormalities and tissue concentrations of Se were used to propose criteria for the diagnosis of Se toxicosis in dead and living aquatic birds. These criteria are now supplemented with histologic findings.

MATERIALS AND METHODS

One hundred twenty-five adult male mallards (Frost Game Farm, Coloma, Wisconsin, USA), approximately 14 mo old, were banded with numbered leg bands and randomly assigned three per cage, after a quarantine period of 2 wk at the Patuxent Environmental Science Center, Laurel, Maryland (USA). Seven randomly selected cages (21 birds) were assigned to each of five experimental groups. A biologically available form of selenium, seleno-DL-methionine (SeM) (98% pure; Bachem, Inc., Torrance, California), was mixed into a duck developer mash (Beacon Feeds, Cayuga, New York, USA) to produce diets containing 0, 10, 20, 40 or 80 parts per million (ppm) of Se (wet weight). Ducks were fed the treated diets (average moisture of 10.5%) for 16 wk from late March to mid-July 1988. Four mallards were randomly selected during the quarantine period, euthanized in a CO_2-O_2 chamber, and examined serologically, macroscopically, and histologically; all were considered free of infectious diseases and were generally healthy. Details on cages, feed preparation, feeding regimens, and clinical observations during the study were presented in Albers et al. (1996).

Dead mallards were stored at 4 to 6 C, then necropsied within 24 hr of death. During the course of the study, six non-experimental mallards were euthanized to monitor background health and tissue and organ appearance. Mallards that survived the 16 wk period were euthanized in a CO_2-O_2 chamber (built on site) one at a time, and then immediately necropsied. For additional details see Albers et al. (1996). Tissues were immersed in 10% buffered neutral formalin (Mollig's solution); formalin solutions were changed once 2 to 4 days after necropsy.

After at least 2 wk of fixation, tissues were trimmed, placed into plastic cassettes and held in Mollig's solution prior to processing. Tissues were embedded in paraffin and sectioned at 6 μm. Slides were stained with Harris' hematoxylin and eosin Y (H&E). Histologic slides were examined on a Leitz-Wetzlar binocular light microscope (Wetzlar Scientific Instruments, Elmsford, New York) with polarizer and micrometer.

Seventy mallards from five groups were examined histologically. The following tissues and

organs were examined from all 70 ducks: brain (right cerebrum, optic lobes, cerebellum, medulla), spinal cord (at least four cross sections each of cervical cord at segments 1 to 4 and 10 to 14, and lumbosacral cord at the level of the glycogen body), liver, pancreas, kidney, adrenal, testis, heart, breast muscle, spleen, skin of cheek, ventriculus (gizzard), three toes, mandible, cervical and lumbosacral vertebrae, and bone marrow. Histologic sections of the following organs were examined from over 75% of the 70 mallards: esophagus, proventriculus, duodenum, ileum, ceca, colon, and sublumbar lymph nodes. Generally, birds lack lymph nodes, but waterfowl have lymph nodes in two locations: the paired (sub-) lumbar lymph nodes and cervical lymph nodes (King and McLelland, 1984). In occasional ducks (10 to 50%), the following organs were examined: thyroid, thymus, bursa of Fabricius, jejunum, cloaca, and eyelid.

The severity of histologic lesions was rated subjectively as follows: none (0), very minimal (1), minimal (2), minimal to mild (3), mild (4), mild to moderate (5), moderate (6), moderate to severe (7), and severe (8). Distribution of lesions was rated as none (0.0), focal (0.2), two or three foci (0.3), more than three foci (multifocal) (0.4), multifocal to disseminated (0.5), disseminated (0.6), disseminated to diffuse (0.7), and diffuse (0.8). Hence, a mild diffuse lesion was rated 4.8 and a severe disseminated lesion was 8.6. A variety of tissue structures were measured by a micrometer or counted in order to evaluate the effect of Se on size, frequency of occurrence, or condition of the tissue. Counts of tissue structures were the actual numbers found in the tissue section. Certain tissues from some mallards had more than one section; when this occurred, the total number of lesions or structures was divided by the number of sections. It was assumed that variation in the size of some tissue sections was unrelated to the experimental group. The values of the two largest lymphoid germinal centers, the percentage values for feathers and feather follicles, and the diameter of the largest seminiferous tubule are examples of measurements that were independent of the amount of tissue in the sections.

A one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test for pairwise comparisons (SAS Institute, 1988a) were used to compare histopathological differences among experimental groups. Data sets were tested for normality with the W statistic (SAS Institute, 1988b) and for homogeneity of variances with Bartlett's test (Snedecor and Cochran, 1969). We used $\log_e$ and arcsin transformations to improve the distribution of data and reduce the inequality among variances. The Jonckheere-Terpstra test (Lehman, 1975) was employed to compare lesions among experimental groups according to degrees of severity and distribution within tissues. The alpha level was 0.05 for parametric and nonparametric tests.

All mallards that died (n = 25), regardless of the dose of selenium in their feed, were placed in one group for comparison to four groups of surviving mallards. Surviving mallards were the 0 ppm, 10 ppm, 20 ppm, and 40 ppm groups. Histologic examinations were done on 10 randomly selected mallards from each of the 0 ppm, 10 ppm, and 20 ppm groups. All 15 surviving mallards were in the 40 ppm group.

RESULTS

Macroscopic abnormalities in mallards from all experimental groups were examined histologically. Fifteen histologic characteristics were quantified by numerical count, measurement of diameters, or percentage of affected structures, and were found to be different among experimental groups (Table 1). Twelve categorical histologic lesions of the liver, pancreas, skin and adnexa, lymphoid organs (or tissues), and bone marrow also were described and found to be different among experimental groups (Table 2). In both the quantitative and categorical comparisons, the pairwise comparisons did not always identify differences among treatment groups (Tables 1 and 2).

Feathers and feather follicles of the head (cheek) of mallards that died had arrested development, loss of vascularity, absence of pinfeathers (blood feathers), hypermaturity (senescence), increased percentage of atrophied and featherless follicles, and subtle reduction in numbers (Table 1, Fig. 1). With Tukey's pairwise comparisons, we failed to detect significant differences among groups despite overall significance by ANOVA for numbers of granulocytic (heterophilic) cells.

The scaled skin of the digits (toes) had parabasal microvesiculation in about half of mallards that died; however, with paired statistical comparisons we failed to identify group differences; in part this was because

TABLE 1. Quantified histologic differences in tissues of mallard ducks on diets supplemented with 0, 10, 20, 40, and 80 ppm selenium as seleno-DL-methionine. All of the ducks in the 80 ppm group died before the end of the 16-wk experiment.

Group (ppm selenium)	Sample size	Analysis of variance: Mean ± S.E.	*P*	Tukey's[a]
Skin & feathers: Number of feather follicles in cheek skin				
0	10	58.00[b] ± 5.76	<0.01	A
10	10	60.50 ± 4.00		A
20	10	72.00 ± 6.48		A
40	15	59.67 ± 4.82		A
Died[c]	25	28.20 ± 1.28		B
Skin & feathers: Percentage of atrophied feather follicles				
0	10	16.50[d] ± 2.71	<0.01[e]	A
10	10	12.10 ± 2.15		A
20	10	16.80 ± 1.18		A
40	15	19.20 ± 3.63		A
Died	25	51.36 ± 3.54		B
Skin & feathers: Percentage of feathers with avascular (empty) shafts[f]				
0	10	14.30[d] ± 1.92	<0.01[e]	A
10	10	9.70 ± 1.19		A
20	10	11.67 ± 1.73		A
40	15	12.67 ± 2.11		A
Died	25	36.24 ± 2.63		B
Skin & feathers: Percentage of feathers with vascular shafts[f]				
0	10	61.80[d] ± 2.77	<0.01[e]	A
10	10	61.20 ± 4.04		A
20	10	52.80 ± 2.62		A
40	15	44.93 ± 4.11		A
Died	25	5.80 ± 1.55		B
Skin & feathers: Percentage of feathers with ≥5 heterophils in cross section of shaft[f]				
0	10	0.50[d] ± 0.27	0.05[e]	A
10	10	0.30 ± 0.21		A
20	10	0.60 ± 0.27		A
40	15	8.00 ± 3.94		A
Died	25	16.36 ± 4.88		A
Kidney: Number of cytoplasmic droplets in proximal convoluted tubules				
0	10	24.60[g] ± 5.79	<0.01	A
10	10	24.70 ± 7.35		A
20	10	23.50 ± 9.04		A
40	15	48.40 ± 17.04		A
Died	25	121.20 ± 21.07		B

TABLE 1. Continued

Group (ppm selenium)	Sample size	Analysis of variance: Mean ± S.E.	*P*	Tukey's[a]
Spleen: Number of germinal centers				
0	10	30.80[b] ± 4.32	<0.01	A
10	9	28.44 ± 5.24		A
20	10	32.50 ± 6.32		A
40	15	17.47 ± 3.87		A
Died	24	3.58 ± 1.43		B
Spleen: Diameters of the two largest germinal centers				
0	10	168.70[h] ± 22.77	<0.01[e]	A
10	8	185.50 ± 26.01		A
20	10	156.70 ± 23.44		A
40	14	144.14 ± 18.05		A
Died	17	79.12 ± 6.01		B
Sublumbar lymph nodes: Maximum dorso-ventral diameter				
0	10	1,566.00[h] ± 96.53	<0.01	A
10	9	1,521.33 ± 90.80		A
20	9	1,433.33 ± 184.38		A
40	14	1,373.57 ± 138.37		A
Died	22	587.73 ± 52.64		B
Esophagus: Number of lymphoid nodules in mucosa				
0	10	7.45[b] ± 1.90	<0.01	A
10	10	7.97 ± 1.46		A
20	8	7.87 ± 1.37		A
40	14	5.61 ± 1.04		A
Died	19	1.50 ± 0.32		B
Esophagus: Number of germinal centers in mucosa				
0	10	5.55[b] ± 1.22	<0.01	A
10	10	4.48 ± 1.59		A
20	8	5.06 ± 1.51		A
40	14	4.82 ± 1.25		A
Died	19	0.50 ± 0.18		B
Esophagus: Diameters of the two largest germinal centers				
0	10	281.00[h] ± 29.29	<0.01	A
10	10	207.00 ± 16.60		A
20	6	307.33 ± 41.21		A
40	12	209.33 ± 23.33		A
Died	7	85.83 ± 13.17		B
Ceca: Diameters of the two largest germinal centers				
0	10	284.50[h] ± 27.30	<0.01	A
10	9	263.67 ± 40.53		A
20	10	299.00 ± 24.17		A
40	12	242.17 ± 21.86		A
Died	12	132.83 ± 13.96		B

TABLE 1. Continued

Group (ppm selenium)	Sample size	Analysis of variance		
		Mean ± S.E.	*P*	Tukey's[a]
Liver: Number of lymphoid nodules				
0	10	5.00[b] ± 0.67	<0.01[c]	AB
10	10	5.70 ± 1.11		A
20	10	4.80 ± 1.45		AB
40	15	2.00 ± 0.56		BC
Died	25	1.48 ± 0.53		C
Testes: Maximum diameter of seminiferous tubules				
0	10	179.50[b] ± 16.27	<0.01[c]	AB
10	10	193.50 ± 22.82		B
20	10	139.00 ± 5.36		BC
40	15	109.67 ± 11.97		C
Died	25	133.00 ± 11.72		AC

[a] Means that do not share a letter in common are different ($P < 0.05$) based on Tukey's Honestly Significant Difference (HSD) test.

[b] Mean of the counted numbers of the structure in histologic sections.

[c] The 25 mallards that died include three ducks from the 40 ppm group and one duck from the 20 ppm group.

[d] Mean of the percentages of feathers with the specified condition in two sections of cheek skin taken adjacent to the ear opening.

[e] Based on an analysis of variance and Tukey's HSD test performed on $\log_e$ transformed values. Means shown are arithmetic means.

[f] Only the calamus portion of the feather shaft was used. Percentages were calculated by dividing by the total of follicles and feather shafts (above and below the skin) in the cross section.

[g] Mean of the total number of cytoplasmic droplets and necrotic tubular cells in five randomly selected high-power-fields (400×) of mostly proximal convoluted tubules.

[h] All diameters are in microns. For germinal centers, the mean was calculated for the averages of the two largest germinal centers in histologic sections. When zero or one germinal center were found in the organ, the bird was excluded from the group.

one 0 ppm mallard also had vesicles of unknown etiology (Table 2). Vesicles occurred in the parabasal and stratum lucidum layers and were entirely microscopic. Vesicles were preceded by edema and ballooning degeneration that progressed to cell lysis and formation of microscopic horizontal vesicles (Fig. 2). Most vesicles were bland, but occasionally a few heterophils infiltrated the epidermis and subjacent dermis; no inclusions or organisms were associated with any vesicles. Vesicles and ballooning degeneration rarely were detected in the feathered epidermis.

Some claws had onychitis or sloughing (onychoptosis), or both; but using paired statistical comparisons, we were unable to identify group differences (Table 2). Onychitis was primarily an acute heterophilic inflammation in which occasional coccal bacteria were found. Onychitis occurred predominantly in digits with onychoptosis, but it was not determined whether coccal onychitis induced the onychoptosis, or occurred secondarily or opportunistically. Onychoptosis without heterophilic coccal onychitis also occurred. Histologically, no distinctive features of onychoptosis were found; it was characterized by loss of the hard keratin of the dorsal and ventral plates. The epidermis of the claw separated in the intermediate layer (of the germinative layer) and spared the columnar cells of the basal layer. Remaining basal cells occasionally had segments of ballooning degeneration or vacuolation of cell apices, but there was no evidence of cell necrosis except when accompanied by heterophilic coccal onychitis.

All mallards that died and five (33%) of the 40 ppm mallards had prominent loss of fat in the skin (Fig. 1). Remaining lipocytes had marked edema, mucinous degeneration, and necrosis. Pectoral (breast) myocytes were atrophied in the mallards that died and 40 ppm groups but rarely had degenerative or necrotic changes. No mallards in the 20, 10 and 0 ppm groups had fat or muscle changes.

Livers of all mallards that died and several survivors in the 40 ppm group had prominent histologic changes (Table 2). The earliest lesion was hyalinogranular swelling of periportal hepatocytes; hepatocellular cytoplasm was homogeneously waxy to granular, eosinophilic, and increased in amount (Fig. 3). The hyalinogranular change progressed to foamy vacuolation of cytoplasm; vacuoles initially were minute and numerous (Fig. 4). Coarsely vacuolated hepatocytes had fewer

TABLE 2. Categorical differences in histologic lesions among mallard ducks on diets supplemented with 0, 10, 20, 40, or 80 ppm selenium as seleno-DL-methionine. All of the ducks in the 80 ppm group died before the end of the 16-wk experiment.

Group (ppm selenium)	Sample size	Frequency of lesion	Jonckheere-Terpstra analysis[a]				
				Overall		Pairwise	
				S[b]	D[c]	S	D
Skin: Scaled skin of toes: Micro-vesiculation							
0	10	1	$JT(x)$[d]:	1179	1177	A	A
10	10	0	P:	<0.01	<0.01	A	A
20	10	2				A	A
40	15	3				A	A
Died[e]	25	12				A	A
Claws: Onychitis							
0	10	0	$JT(x)$:	1144	1126	A	A
10	10	0	P:	<0.01	<0.01	A	A
20	10	1				A	A
40	15	5				A	A
Died	25	9				A	A
Fat cells of bone marrow (toes): Necrosis or mucinous degeneration							
0	10	0	$JT(x)$:	1465		A	
10	10	0	P:	<0.01		A	
20	10	0				A	
40	15	1				A	
Died	25	23				B	
Liver: Foamy vacuolation of hepatocytes							
0	10	0	$JT(x)$:	1469	1455	A	A
10	10	0	P:	<0.01	<0.01	A	A
20	10	0				A	A
40	15	3				A	A
Died	25	22				B	B
Liver: Hyalinogranular swelling of hepatocytes							
0	10	8	$JT(x)$:	1401	1142	A	A
10	10	4	P:	<0.01	<0.02	A	B
20	10	5				A	BC
40	15	13				A	AC
Died	24	24				B	A
Liver: Apoptosis of hepatocytes							
0	10	0	$JT(x)$:	1337	1325	A	A
10	10	5	P:	<0.01	<0.01	A	A
20	10	2				A	A
40	15	4				A	A
Died	21[f]	21				B	B
Liver: Centrolobular or panlobular necrosis of hepatocytes							
0	10	0	$JT(x)$:	1412	1413	A	A
10	10	0	P:	<0.01	<0.01	A	A
20	10	0				A	A
40	15	2				A	A
Died	25	20				B	B
Liver: Bile duct hyperplasia							
0	10	0	$JT(x)$:	1163		A	
10	10	0	P:	<0.01		A	
20	10	0				A	
40	15	0				A	
Died	25	10				A	

TABLE 2. Continued

Group (ppm selenium)	Sample size	Frequency of lesion	Jonckheere-Terpstra analysis[a]				
			Overall			Pairwise	
				S[b]	D[c]	S	D
			Liver: Hemosiderin-laden Kupffer cells				
0	10	0	*JT*(*x*):	1193	1194	A	A
10	10	0	*P*:	<0.01	<0.01	A	A
20	10	0				A	A
40	15	1				A	A
Died	22	14				B	B
			Pancreas: Apoptosis of exocrine cells				
0	10	0	*JT*(*x*):	1289	1296	A	A
10	10	0	*P*:	<0.01	<0.01	A	A
20	10	0				A	A
40	14	1				A	A
Died	24	18				B	B
			Spleen: Depletion of lymphocytes				
0	10	4	*JT*(*x*):	996		A	
10	9	1	*P*:	<0.01		A	
20	10	5				A	
40	15	8				A	
Died	24	24				B	
			Sublumbar lymph node: Depletion of lymphocytes				
0	10	0	*JT*(*x*):	1283		A	
10	9	1	*P*:	<0.01		A	
20	9	2				A	
40	14	4				A	
Died	22	22				B	

[a] $P \leq 0.05$ for overall comparison [$JT(x)$] and $P \leq 0.005$ as a Bonferroni adjustment to maintain the overall alpha level at 0.05 for pairwise comparison; means that do not share a letter in common are significantly different. Actual *P* values estimated by Monte Carlo simulation at the 99% level of confidence.

[b] Severity of the lesion: none, very minimal, minimal, minimal to mild, mild, mild to moderate, moderate, moderate to severe, severe.

[c] Distribution of the lesion: none, focal, two or three foci, multifocal (> three foci), multifocal to disseminated, disseminated, disseminated to diffuse, diffuse.

[d] The Jonckheere-Terpstra test (Lehman, 1975) tests for trend in a contingency table. The null hypothesis is no difference among proportions in categories of severity or distribution, whereas the alternative hypothesis is either an increasing or decreasing trend of proportions in the contingency table. Thus, the alternative hypothesis is that there is a definite order to proportions in categories of severity or distribution.

[e] The 25 mallards that died include three ducks from the 40 ppm group and one duck from the 20 ppm group.

[f] In four other mallards, lobular necrosis of hepatocytes was so severe that apoptosis could not be evaluated.

and larger vacuoles, and were considered to be in advanced degeneration and necrosis. Necrosis was first detected around central veins and then expanded to involve midzonal regions. A honeycomb pattern of necrosis (Fig. 5) was observed at low magnifications; the holes in the honeycomb corresponding to the spared periportal regions and the walls of the honeycomb were necrotic centrolobular and midzonal regions. Centrolobular necrosis progressed to panlobular necrosis (Fig. 6). Other features of liver necrosis were serum-like droplets, Councilman-like bodies, and pooling of blood in sinusoids and empty hepatic cords (ex vacuo hemorrhage). A second form of hepatocellular necrosis, apoptosis, involved hepatocytes in regions of hyalinogranular and foamy vacuolar degeneration. Minimal or mild bile duct hyperplasia (Fig. 6) was detected in 10 (40%) of 25 mallards that died. Bile duct "hyper-

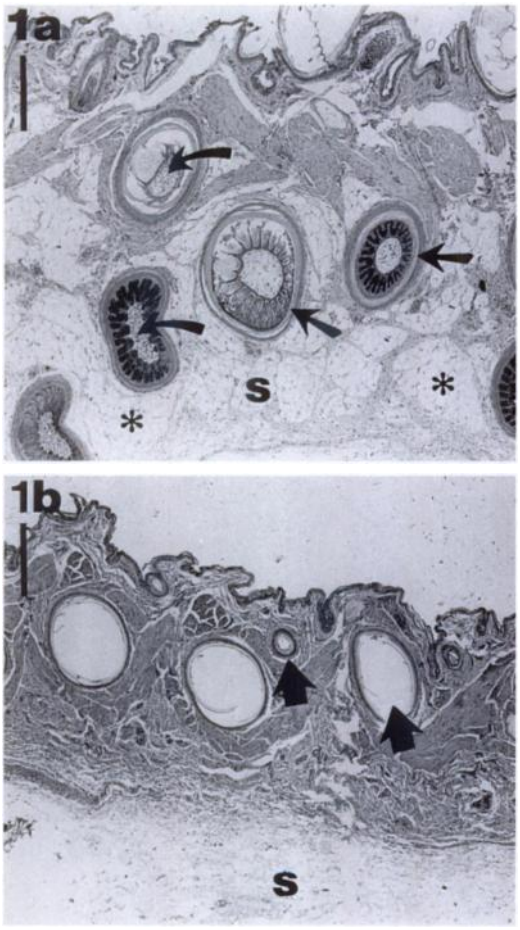

FIGURE 1. Feathered skin of cheek. (a) Zero ppm selenium mallard (No. 891). Note abundant lipocytes (*) in the subcutis. Developing feathers (arrows) are numerous; most feathers have prominent vascular cores (curved arrows). (b) Mallard (No. 805) in the 80 ppm selenium group. Note the complete absence of lipocytes in the subcutis and absence of growing, vascular feathers. Remaining feathers (arrows) are senescent feathers that lack growing barbules and vascular cores. S, subcutis. H&E stain. Bars = 300 μm.

plasia" in many foci was considered, in part, secondary to extensive necrosis and disappearance of hepatocytes with collapse of lobules and clustering of the spared ducts. Bile duct hyperplasia was absent in all mallards that survived the study period. All livers were remarkable for the lack of hepatocellular hemosiderosis, fibrosis, and regenerative nodules. However, hemosiderin-laden Kupffer cells became more numerous and swollen in mallards with necrosis of hepatocytes (Table 2).

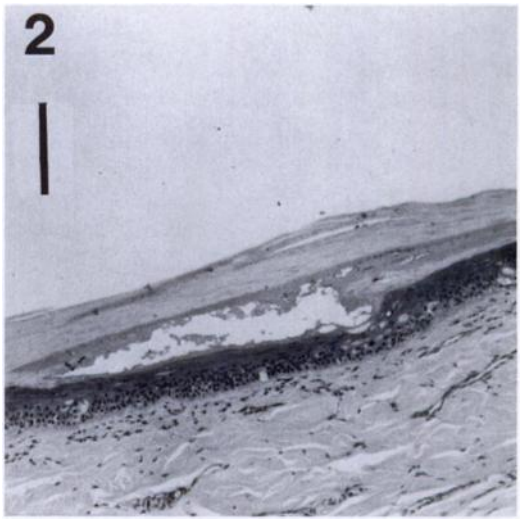

FIGURE 2. Scaled skin of dorsal surface of toe. Mallard (No. 686) in the 80 ppm selenium group. Note the horizontal parabasal vesicle within the epidermis. Vesicles were only detected histologically. H&E stain. Bar = 300 μm.

Severity and distribution of hepatocellular lesions, except for hyalinogranular swelling (Table 2), were greater in mallards that died than surviving mallards. Centrolobular or panlobular necrosis was

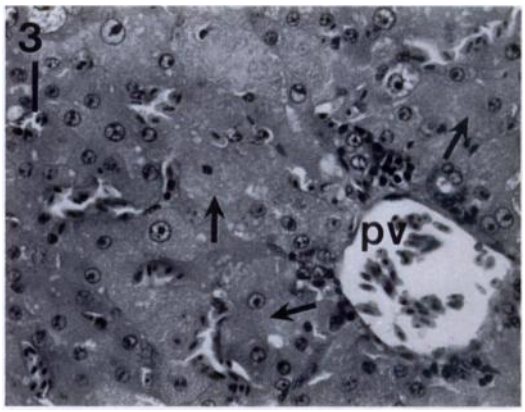

FIGURE 3. Liver of mallard (No. 815) in the 80 ppm selenium group. Note the numerous swollen hepatocytes (arrows) which have waxy-to-hyalinogranular cytoplasm. Minimal infiltrates of lymphocytes adjacent to the triad were considered non-specific leukocytic infiltrates. pv, portal vein of the hepatic triad. H&E stain. Bar = 20 μm.

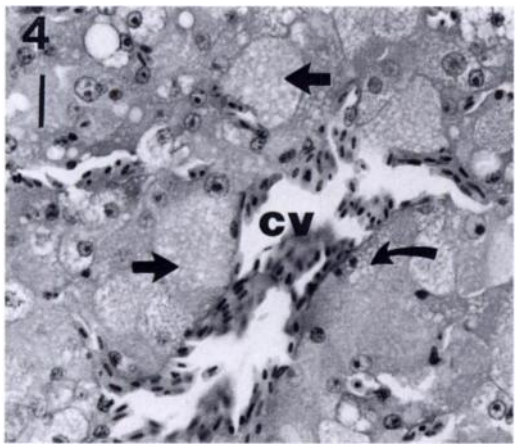

FIGURE 4. Liver of mallard (No. 811) in the 80 ppm selenium group. Swollen hepatocytes have finely vacuolated cytoplasm (arrows). Hepatocytes with waxy-to-hyalinogranular cytoplasm have a few small peripheral vacuoles (curved arrow). cv, central vein. H&E stain. Bar = 20 μm.

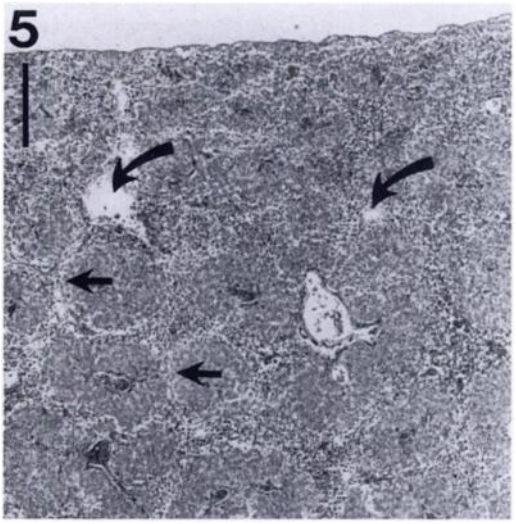

FIGURE 5. Liver of mallard (No. 802) in the 80 ppm selenium group. Bridging centrolobular necrosis of hepatocytes (arrows) imparting a "honeycomb" pattern. Curved arrows are central veins. H&E stain. Bar = 300 μm.

rated as mild to severe in 19 (76%) mallards that died. Minimal centrolobular necrosis was detected in one additional mallard in the group that died, and two (13%) survivors in the 40 ppm group. Mild to severe apoptosis occurred in most mallards that died and was associated with foamy vacuolation or centrolobular necrosis; minimal apoptosis and apoptosis not associated with foamy vacuolation were considered insignificant in all mallards. In four mallards that died, panlobular necrosis was so severe that apoptosis could not be assessed.

Pancreases had mild apoptosis of exocrine (acinar) cells in 18 (75%) of 24 mallards that died and one (7%) mallard in the 40 ppm group (Table 2). At low magnification, apoptosis was clearly evident as small, single cell-sized clear spaces. Necrosis of scattered single exocrine cells was evident in many forms: pyknosis of nuclei with basophilic condensation of cytoplasm, karyorrhexis with acidophilic fragmentation of cytoplasm, complete acidophilic necrosis with shrinkage and a distinct clear halo, and complete disappearance of cells.

Kidney lesions were mildly complicated by autolysis in mallards that died. Never-

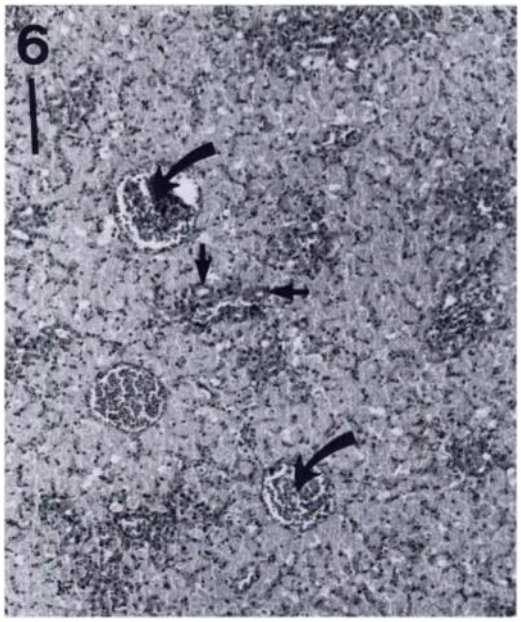

FIGURE 6. Liver of mallard (No. 885) in the 80 ppm selenium group. Acute panlobular necrosis of hepatocytes with very mild bile duct hyperplasia (small arrows). All hepatocytes in the photo have pyknotic nuclei and their cytoplasm has coagulative (acidophilic) necrosis. Curved arrows are dilated central veins. H&E stain. Bar = 100 μm.

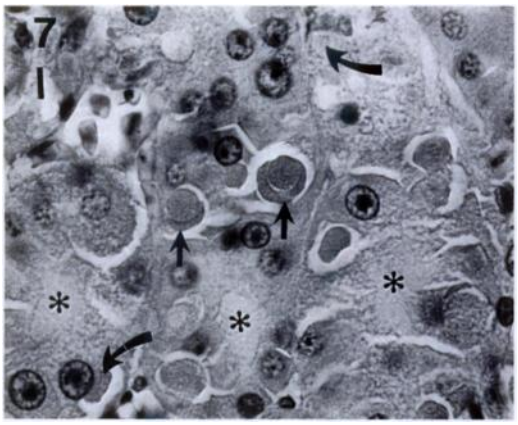

FIGURE 7. Kidney of mallard (No. 811) in the 80 ppm selenium group. Field of view consists mostly of proximal convoluted tubes (* marks lumina of proximal convoluted tubules). Two tubular cells have swollen, karyolytic eosinophilic nuclei with partial rims of coagulated cytoplasm imparting a laminated appearance to the necrotic cells (small arrows). Paranuclear droplets of coagulated cytoplasm (curved arrows) appear in other tubular cells. H&E stain. Bar = 10 μm.

theless, striking histologic changes were numerous cytoplasmic droplets and nephrosis of proximal convoluted tubules (Table 1). Cytoplasmic droplets were highly variable in size (3 to 15 μm in diameter) and shape (round, ovoid, and polygonal). Some droplets were distinctly intracytoplasmic, while many appeared to be an entire shrunken tubular cell with a distinct encircling clear halo. Cytoplasmic droplets were predominantly eosinophilic (red); many were the same color as normal-appearing cytoplasm, some were very pale and wispy, and some were distinctly dark red to magenta. Many droplets had thin, subtle basophilic rims with surrounding clear haloes (Fig. 7). In many affected proximal convoluted tubule cells, the nucleus was absent, which may mean darker droplets represented nuclear karyolysis; these larger droplets appeared to form from combined nuclear and cytoplasmic debris. Hence, larger droplets which spanned the basement membrane to tubular lumen were interpreted as necrotic cells (nephrosis). Most smaller and mid-sized droplets were clearly intra-cytoplasmic and paranuclear (Fig. 7). In cross sections of proximal convoluted tubules, one cell to 75% of cells were necrotic. Surviving mallards in the 20, 10, and 0 ppm groups, had proximal convoluted tubule cells with droplets that were much fewer, roundish, very pale (eosinophilic) or hydropic; nephrosis was not found in any surviving ducks (Table 1). Intraluminal cellular debris and sloughed epithelial cells were present in mild amounts in distal convoluted tubules and collecting ducts of mallards that died.

Spleens of mallards that died had decreased numbers of germinal centers compared to survivors (Table 1). A marked decrease in the number of splenic germinal centers (defined as <10; the fewest in any control duck was 11) occurred in 23 (96%) of 24 mallards that died. In the 40, 20 and 10 ppm groups, fewer than 10 splenic germinal centers were found in six (40%), two (20%) and one (13%) mallards, respectively. No germinal centers were present in the spleens of seven (29%) mallards that died and one (7%) mallard in the 40 ppm group. The splenic germinal centers in mallards that died were smaller in diameter (Table 1), occasionally had mild active lymphorrhexis, and had prominent depletion (loss) of lymphocytes (Table 2) compared to surviving mallards.

The thymus was not detected at necropsy in any mallards that died, but was incidentally detected by microscopic examination in one duck. The thymus of this one mallard that died was severely atrophied and severely depleted of cortical lymphocytes.

Located between the kidneys and ventral to the lumbar spinal column, the lumbar lymph nodes were examined in 64 (91%) of 70 mallards. Lymphocytic depletion (Table 2), occasionally with mild acute lymphorrhexis, was rated moderate to severe in 17 (77% of 22) mallards that died and one (7%) 40 ppm mallard (Fig. 8). Lymphocytic depletion was rated as mild in an additional three (14%) mallards that died, one (7%) 40 ppm, and one (11%) 20

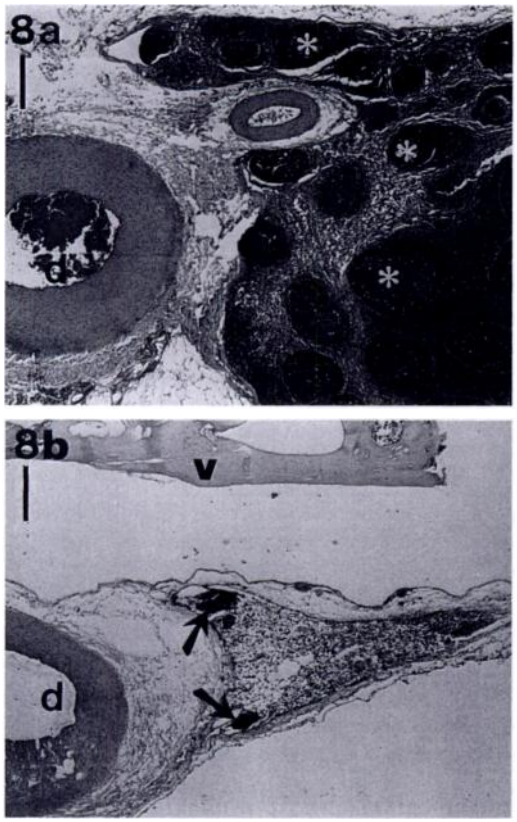

FIGURE 8. Lumbar lymph nodes. (a) Mallard (No. 648) received 0 ppm selenium and has a normal, cellular lymph node with prominent active germinal centers (*). (b) Mallard (No. 657) in the 80 ppm selenium group has severe atrophy of the lymph node with severe depletion of lymphocytes. Two very small germinal centers remain (arrows), both of which are inactive and contain necrotic lymphocytes. d, lumen of the descending aorta; v, lumbar vertebrae. H&E stain. Bars = 250 μm.

ppm mallards. The maximum dorso-ventral thickness of the lumbar lymph nodes was significantly reduced in mallards that died compared to survivors (Table 1, Fig. 8).

The gut-associated lymphoid tissue (GALT) in the submucosa of the esophagus was greatly reduced or absent in mallards that died (Table 1). When nodules of GALT were found in the esophagus of mallards that died, they were invariably small, hypocellular and lacked recognizable germinal centers. No GALT was detected in the esophagus of four (21%) mallards that died, whereas, at least one nodule of GALT was found in all other mallards. Germinal centers were not evident in the esophageal GALT of 12 (63%) mallards that died, two (14%) 40 ppm, and two (25%) 20 ppm mallards. Marked hypocellularity and reduced size of germinal centers in the cecal GALT were evident in mallards that died (Table 1). Seven mallards in four study groups had oro-esophageal capillariasis; two mallards that died had esophageal capillarid infections, but both lacked GALT in the submucosa.

The number of lymphoid nodules in the liver also was different among groups (Table 1). Mallards that died had fewer hepatic lymphoid nodules than mallards in the 0, 10, or 20 ppm groups. Mallards in the 40 ppm group had fewer nodules than mallards in the 10 ppm group.

Evaluation of phalangeal, cervical, and lumbosacral bone marrow was hampered by wash out of cells during processing. Thus, comparisons were not attempted; but, generally, no differences in hematopoietic cellularity were observed among groups. However, necrosis and mucinous degeneration of fat cells in digital bone marrow was greater in mallards that died than in surviving mallards (Table 2).

Testicles of all mallards became smaller during the course of the study compared to a group of quality control ducks that were killed in late March at the start of the experiment (data not shown). The seminiferous tubules of mallards that died were smaller in diameter and contained fewer sperm cells than mallards in the 10 ppm group; mallards in the 40 ppm group had smaller and less cellular seminiferous tubules than mallards in the 0 and 10 ppm groups (Table 1).

No lesions were detected in the central, autonomic, and peripheral nervous systems. Other organs that were considered histologically normal were bones, thyroids, parathyroids, adrenals, stomachs, and intestines. There was no difference among groups in the prevalence of incidental infections and parasitisms. The following

TABLE 3. Key histologic lesions of mallard ducks that died from diets supplemented with 20, 40, or 80 ppm selenium, as seleno-DL-methionine.

Organs	Lesions
Liver:	Hepatocellular foamy vacuolation, fine or coarse; centrolobular hepatocellular necrosis; panlobular hepatocellular necrosis
Pancreas:	Apoptosis of exocrine cells
Kidney:	Nephrosis: acidophilic necrosis of proximal convoluted tubular cells; acidophilic to magenta cytoplasmic droplets; karyolysis
Lymphoid organs and tissues:	
Spleen:	Absence or very reduced numbers of germinal centers; atrophy and hypocellularity of remaining germinal centers
Lumbar lymph nodes:	Atrophy and hypocellularity
Gut-associated lymphoid tissue:	Absence or very reduced numbers of germinal centers in esophageal and cecal mucosa; atrophy and hypocellularity of remaining lymphoid nodules
Bone marrow:	Mucinous degeneration and necrosis of fat
Skin and adnexa:	
Feathers:	Increased percentage of avascular (senescent) feather shafts; increased percentage of atrophied and featherless follicles
Fat (lipocytes):	Severe atrophy, degeneration, and necrosis

parasites were detected in mallards: intestinal tapeworms (n = 1 mallard), oroesophageal capillarid worms (n = 7), and skeletal muscular sarcosporidiosis (n = 1). Amyloidosis was detected in minimal to moderate degrees in the livers, spleens, adrenals, kidneys and islets of Langerhans in five mallards from all groups. Two mallards that died (one each in the 10 and 40 ppm groups) and four mallards that were euthanized at the end of the feeding period were deleted from the study because of extraneous serious diseases (gizzard abscesses, bacterial peritonitis, polycystic kidney disease, and hepatosplenomegaly of undetermined cause).

DISCUSSION

Feather loss on the head, emaciation (with myocytic atrophy), liver necrosis, and mild nephrosis were lesions common to adult American coots, pied-billed grebes, and mallards (Ohlendorf et al., 1988; Albers et al., 1996). Pathognomonic lesions of fatal selenosis were not detected grossly (Albers et al., 1996) or microscopically in mallards of this study, however, several abnormalities were unusual in their organ tropisms and intra-organ topistic effects. Twenty-seven tissue measurements or lesions were statistically different among experimental groups and most were different in mallards that died compared to surviving mallards (Tables 1 and 2). Although some of these lesions have not been reported in birds with toxic, infectious, or nutritional diseases, we are unaware of studies in which tissues have been examined in a comparable manner. Several lesions of selenosis, as described herein, potentially may be pathognomonic. For the present, however, combinations of gross and histologic lesions are preferable for diagnosing selenosis. Chronic selenosis should be a leading etiologic diagnosis when macroscopic abnormalities are present (Albers et al., 1996) with three of the six major categories of histologic lesions (Table 3).

Integumentary lesions in the feathers and claws of these mallards are analogous to reported changes in the hair, nails and hooves of Se-intoxicated mammals (Yager et al., 1993). Hair loss (depilation) is a key feature of selenosis of horses (Traub-Dargatz and Hamar, 1986), cattle (National Research Council, 1983), non-human primates (Loew et al., 1975) and humans

(Jensen et al., 1984) and may be considered analogous to the feather loss (depterylation) observed in mallards of this study. Depterylation on the head was observed in wild American coots with selenosis but it was not reported in pied-billed grebes with selenosis (Ohlendorf et al., 1988), and was not reported in avocet chicks (*Recurvirostra americana*) exposed in ovo to elevated concentrations of Se, arsenic, and boron (Fairbrother et al., 1994). Recently hatched chickens with in ovo selenosis had malformed down feathers characterized as "wire-like" (Poley and Moxon, 1938), but feather abnormalities have not been reported in young chickens fed toxic levels of Se (El-Begearmi and Combs, 1982; DaFalla and Adam, 1986), nor have histologic features of these selenosis-associated feather abnormalities been described. Although depterylation in adult American coots was marked and had 100% prevalence, the abnormality was not studied histologically (Ohlendorf et al., 1988); thus, comparisons are not possible.

Depterylation was observed only in post-molt mallards of the 40 ppm group that survived the full 16 wk; all 80 ppm mallards were dead between weeks 10 and 14, before the post-breeding molt was visually evident (Albers et al., 1996). Despite this striking gross lesion in some survivors in the 40 ppm group, no statistically significant or characteristic histologic lesions were detected in their feathers, skin, and follicles (Tables 1 and 2). Given the marked histologic evidence of abnormal feather development in mallards that died, we conclude that depterylation in mallards occurs only if the bird passes through a molt while exposed continuously to high levels of Se. Normally, when a feather is molted or plucked, the feather follicle begins formation of a new feather in 1 wk (Lucas and Stettenheim, 1972). With careful histologic examinations of follicles in mallards that died, lesions of marked atrophy, hypocellularity, avascularity, and lack of new feather formation were identified. These histologic features were nonspecific changes; thus the pathophysiologic change may be at the molecular level. The pathogenesis and histologic features of hair loss in livestock and primates remain unknown, but the continuously growing hair of Se-intoxicated livestock (tails and manes) and humans (scalp) are selectively vulnerable. One theory of hair loss and, by analogy, the feather loss is that selenium replaces sulfur molecules in cross-linking sulfur-bearing amino acids in keratin, resulting in dysfunctional keratin (dyskeratosis) (Fishbein, 1977). No attempt was made at the initiation of this study to follow the development of pinfeathers in the mallards. However, a striking paucity of pinfeathers in mallards that died, immediately preceding a molt, is evidence that selenosis not only results in formation of abnormal keratin in growing feathers, but also inhibits production of new feathers in empty feather follicles by a second pathophysiological mechanism that cannot be explained histologically.

Sloughing of avian claws (onychoptosis) may be considered analogous to sloughing of hooves in horses (Traub-Dargatz and Hamar, 1986) and cattle (Yager et al., 1993), and loss of finger nails in non-human primates and humans (Loew et al., 1975; Yang et al., 1983). Onychoptosis of Se-intoxicated birds has not been noted previously in water birds (Ohlendorf et al., 1988; Fairbrother et al., 1994) or poultry (Poley and Moxon, 1938; DaFalla and Adam, 1986), nor were claws examined histologically in these previous studies. The low incidence of sloughed claws in our mallards (Albers et al., 1996) makes this lesion an unreliable feature of selenosis in waterfowl unless large numbers of stricken or dead birds are examined. Other than separation and detachment of the hard keratin plates of the claws which leaves only the basal cell layer intact, the precise histologic lesion associated with or inducing the onychoptosis remains unclear. Specific histologic lesions of claws, nails, and hooves of mammals also are unreported. Failure to detect specific histo-

logic lesions in the claw germinativum supports the theory that the lesion, as in feathers and hair, may be at the molecular level. Hoof lesions in livestock include deep grooving and cracking; each groove reflects a peak of ingested Se (Yager et al., 1993); thus, grooved claws were not detected and were not expected in these mallards because ingested levels of Se were constant. Grooving of the claws, however, is a potential lesion worthy of evaluation in wild water birds that ingest greatly fluctuating amounts of Se.

The liver was affected consistently by Se toxicosis in these mallards and in other animals (Harr et al., 1967; Fishbein, 1977; National Research Council, 1983; Ohlendorf et al., 1988; Yager et al., 1993). The incidence and severity of hepatic lesions were greatest in mallards that died (Tables 1 and 2; Albers et al., 1996). Hepatocellular necrosis and degeneration were not detected in 2-yr-old male mallards fed selenomethionine for 14 wk, but in the highest dosage group (32 ppm) one (10%) mallard died (Hoffman et al., 1991a); this was comparable to our results wherein three (15%) of 20 1-yr-old male mallards died on 40 ppm. However, only mild gross and histologic lesions in the livers of surviving mallards in the 40 ppm group were observed, even though residue levels were nearly the same as mallards that died (Albers et al., 1996). Livers of survivors usually lacked evidence of centrolobular and panlobular necrosis; thus, hepatocellular necrosis may develop rapidly when a threshold concentration of Se occurs in hepatocytes. Centrolobular and panlobular liver necrosis corresponded to the grossly observed regions of marked yellow liver necrosis, and this necrosis may be a rapidly progressive event analogous to hepatic copper toxicosis of sheep (Kelly, 1993). Indeed, the liver lesions of fatal selenosis in mallards have similarities to ovine chronic copper poisoning. We conclude from our histologic examinations that liver necrosis, when present, was the principal lesion that resulted in the deaths of mallards.

Hepatic hemosiderosis, fibrosis, and bile duct hyperplasia have been reported in cases of experimental and natural selenosis of birds, but are considered non-specific lesions. Hoffman et al. (1991a) described hepatocellular hemosiderosis in two of 10 mallards fed 32 ppm. Hemosiderin accumulation in our study was limited to phagocytic Kupffer cells in 14 (64%) of 22 mallards that died, and one (7%) of 15 mallards in the 40 ppm group. Much of the hemosiderosis in mallards of this study was associated with ex vacuo hemorrhage in regions of hepatocellular necrosis. Similarly, bile duct hyperplasia in this study was strongly correlated with hepatocellular necrosis. Hoffman et al. (1991a) detected biliary hyperplasia in one (10%) of 10 mallards receiving 32 ppm, and Ohlendorf et al. (1988) found biliary hyperplasia in one of 10 Se-intoxicated American coots. In our study, 40% of mallards that died and none of the surviving mallards had biliary hyperplasia. The low incidence of biliary hyperplasia and hemosiderosis in surviving mallards and wild American coots was similar to the rare reports of biliary hyperplasia in selenosis of swine, cattle, or horses. In field cases, phytotoxins from ingested Se-accumulating plants could contribute to development of liver lesions (James et al., 1983; National Research Council, 1983). Hence, biliary hyperplasia in this study was considered secondary to the liver necrosis and not a primary reaction to rising Se concentrations in the liver. Hepatic fibrosis was not detected in our study but has been reported in spontaneous selenosis of coots, horses, sheep, and cattle (National Research Council, 1983; Jones and Hunt, 1983; Ohlendorf et al., 1988). Absence of hepatic fibrosis in our study was evidence that fluctuating levels of Se may be necessary, fibrosis may take longer than 16 wk of constant ingestion of Se, or some cases of natural selenosis were complicated by ingestion of other toxins, such as phytotoxins in Se-accumulating plants (James et al., 1983). Among the aquatic and shoreline plants analyzed for Se con-

tent at Kesterson National Wildlife Refuge (NWR) and elsewhere (Ohlendorf, 1989), none are known to contain hepatotoxic phytotoxins (Kingsburry, 1964; Jones and Hunt, 1983). Not all hepatotoxic plants can be ruled out as contributing to hepatic fibrosis in wild coots at Kesterson NWR (Short and Edwards, 1990); however, we propose that hepatic fibrosis represents a healing stage between episodes of high Se ingestion.

Kidney lesions of degeneration and nephrosis of proximal convoluted tubules are a consistent finding in acute and chronic selenosis of birds and mammals (Harr and Muth, 1972; Ohlendorf et al., 1988; Ahmed et al., 1990). Although nephrosis is a non-specific lesion, some aspects of Se-induced nephrosis were unusual. Acidophilic to magenta cytoplasmic coagulation affected a portion of or whole renal tubular cells. Larger droplets of coagulation appeared to accumulate additional portions of cytoplasm and nuclei in crescentic or semi-laminated patterns, and the magenta staining affinity of some droplets was attributed to karyolysis. Renal fibrosis was reported in natural cases of Se intoxication (Ohlendorf et al., 1988), but was not detected in our mallards. As with the liver, absence of fibrosis in the kidneys may be attributable to ingestion of constant levels of Se with no periods of respite to allow healing (scarring) and regeneration.

The pathogenesis of emaciation in dead mallards with selenosis was not determined histologically. It remains unresolved whether emaciation was directly attributable to toxic levels of selenium, or whether mallards found the feed unpalatable, ceased eating, and starved. Since all mallards that died in this study were emaciated and had toxic levels of selenium in multiple organs (Albers et al., 1996), the mallards were consuming feed. Emaciation is a common finding in many species with selenosis (Harr and Muth, 1972; National Research Council, 1983; Traub-Dargatz and Hamar, 1986; Ohlendorf et al., 1988). Although the question of palatability of selenium-spiked feed to these mallards cannot be resolved, anorexia and emaciation have been reported in horses, swine, sheep, and cattle administered sublethal doses of selenium by injection or drenching (National Research Council, 1980). Hence, we conclude that emaciation in these mallards was a form of cachexia principally due to chronic selenosis.

Lesions in testes, pancreas, fat, and lympho-hematopoietic tissues resembled the atrophy associated with emaciation. Necrosis (apoptosis) of the pancreas was observed in many mallards that died, and also has been reported in many species with Se deficiency disease (National Research Council, 1983). Hence, pancreatic apoptosis occurs in Se deficiency disease and selenosis. Evaluation of testicular changes was complicated by normal seasonal (post-breeding) involution which occurred during the study period.

Based on our quantitative and qualitative evaluations of lymphoid organs, we propose selenosis in mallards could cause profound immunologic inhibition or suppression. The lymphoid tissues of mallards that died had generalized hypocellularity, atrophy, and mild active necrosis consistent with an immunotoxic effect. Immunoglobulin-associated regions (germinal centers) of the spleen, lumbar lymph nodes, and GALT were consistently atrophied, hypocellular or absent in mallards that died. Thus, Se-intoxicated mallards may have had a compromised ability to process antigens and generate an humoral immune response. Compromised immunity in wild birds may be manifested as increased susceptibility to and prevalence of infectious diseases, leukopenia (specifically lymphopenia), reduced levels of serum immunoglobulins, or impaired function of leukocytes. Fairbrother et al. (1994) observed lymphopenia and reduced phagocytosis by macrophages in avocet chicks from regions with high levels of Se, arsenic, and boron; Fairbrother and Fowles (1990) detected impaired cell-mediated immunity in mallards experimentally fed

Se; and, Goldberg et al. (1990) found immunosuppression increased mortalities in mallards challenged with duck plague virus. In our study, no increased prevalence of infectious disease was detected among high dose mallards; the occurrence of gizzard abscesses, dermatitis, peritonitis, and helminthic parasitisms appeared random in all study groups. Although susceptibility to infectious diseases upon exposure to a microbial agent was not assessed in this study, the severity of atrophy in the spleens, thymuses, lumbar lymph nodes, and germinal centers of the GALT are evidence that, prior to death, mallards that died may have had an impaired ability to respond to opportunistic infections.

The immunotoxic effect of Se warrants serious consideration of concurrent selenosis when epizootics occur among waterfowl in geographic regions having elevated Se in the soil or Se-accumulating plants that are used as forage by water birds. Further studies of selenosis in wild water birds are warranted to verify the macroscopic (Albers et al., 1996) and histologic lesions of these studies, even though it may be very difficult to determine whether wild water birds with elevated residues of selenium have died of opportunistic infections, steady chronic selenosis, or sublethal chronic selenosis with an acute overwhelming episode of selenium ingestion.

Spinal poliomalacia is commonly seen with chronic natural and experimental selenosis in swine (Wilson et al., 1983). No evidence of spinal malacia was detected in four to 12 sections each of the cervical and lumbar intumences of each mallard. Hence, selenosis-induced malacia of the central nervous system remains a described lesion of the porcine species only.

Differential diagnoses of chronic selenosis in birds

Many toxic, infectious, traumatic, and nutritional deficiency diseases may produce histologic lesions similar to those found in mallards with selenosis. Centrolobular and panlobular necrosis are nonspecific lesions which have been associated with other intoxications of birds. Other agents that may cause severe vacuolar degeneration with centrolobular or panlobular necrosis include bishop's weed (*Ammi majus*) (Egyed et al., 1974; Shlosberg et al., 1974), crotolaria (*Crotolaria spp.*) (Alfonso et al., 1993), castor beans (*Ricinus communis*) (Jensen and Allen, 1981), blue-green algae (such as *Microcystis aeruginosa*) (Short and Edwards, 1990), aflatoxicosis (Robinson et al., 1982), and white phosphorus (P. Klein, pers. comm.). Numerous other hepatotoxic substances have been reported in poultry (Peckham, 1984) but have not been encountered or reported in water birds. In none of these intoxications were the other histologic lesions (Table 3) of Se intoxication reported.

Several viral infections also may cause acute liver necrosis in waterfowl, including duck (herpes-) viral enteritis (DVE), duck hepatitis virus, adenoviruses, some strains of avian influenza, and goose coronavirus; most of these infections principally affect very young waterfowl, kill birds rapidly before emaciation develops, sometimes produce characteristic inclusion bodies in the liver, and have not been reported to cause other lesions of selenosis in the pancreas, kidney, and integument (Wobeser, 1981; Calneck et al., 1991). Because many viral infections also may cause necrosis and atrophy of lymphoid organs, multiple organs besides liver and lymphoid tissues must be examined for features of selenosis.

Many bacterial, protozoal and helminthic infections may cause liver necrosis (Albers et al., 1996, Table 7), but most of these infections are accompanied by inflammatory cells, detectable organisms, enlarged spleens, abscesses or granulomas, or the organisms may be isolated in cultures.

Although depterylation and feather abnormalities may be common in birds (Roy et al., 1986), such conditions are uncommonly observed or reported in wild birds, except for normal seasonal molts, trauma, and self-mutilation of breast feathers dur-

ing nesting. Further, wild birds with pronounced gross lesions of depterylation have not been examined histologically (Roy et al., 1986; Ohlendorf et al., 1988). Among caged birds, circoviruses and polyomaviruses may produce feather loss and have been well studied (Gaskin, 1989; Latimer et al., 1991). In these virus infections, characteristic viral inclusion bodies are found in the feathers and other organs, but atrophy of feather follicles and loss of vascularity of feathers are not reported. Neither of these viruses have been reported to infect aquatic birds, and the patterns of feather loss rarely involve the head. Traumatic feather loss often is asymmetrical and usually involves other injuries (bite wounds, bone fractures) and should be readily distinguishable from the symmetric feather loss in Se-intoxicated mallards and American coots (Ohlendorf et al., 1988; Albers et al., 1996). Ectoparasites, such as depluming mites and lice (Loomis, 1984), and nutritional deficiencies (Austic and Scott, 1984; Wobeser and Kost, 1992) may cause depterylation in wild and domestic birds, but not with the cephalic patterns seen in Se-intoxicated mallards and coots.

Proposed diagnostic criteria for Se toxicosis

Diagnostically useful histologic lesions of fatal chronic selenosis in mallards occurred in the epidermal adnexa (feathers, feather follicles, and scaled skin), lymphoid organs (spleens, lumbar lymph nodes, and GALT), liver, pancreas, and kidney (Table 3). Although many of these lesions have never been reported in water birds, these lesions cannot be considered pathognomonic in view of the paucity of comparable histologic studies of many diseases and intoxications of water birds. We propose that a combination of three or more histologic lesions among the six major categories (Table 3) is sufficient for a presumptive diagnosis of fatal chronic selenosis. These histologic lesions, in combination with gross abnormalities and tissue residue analyses (Albers et al., 1996), are sufficient for a confirmed diagnosis of fatal chronic selenosis.

Diagnostically useful histologic lesions of non-fatal chronic selenosis in mallards were not found. Although as a group, the 40 ppm survivors had lesions intermediate between mallards that died of selenosis and control ducks, these lesions were mild, low in prevalence, and non-specific. High tissue concentrations of Se combined with all of the following gross abnormalities (Albers et al., 1996) are diagnostic of non-fatal chronic selenosis: a) significant weight loss or emaciation; b) depterylation of the head with poor plumage characterized primarily by frayed and worn feathers, or delayed or impaired molt; and, c) loss of claws (onychoptosis).

While our study of chronic selenosis in mallards was not designed to investigate acute selenosis, we propose that some lesions associated with selenosis in wild coots, grebes, stilts and other birds are due to ingestion of large amounts of Se in a short period of time. Pulmonary edema, fibrinogelatinous hydrocoelom, and splenomegaly were observed in wild aquatic birds (Ohlendorf et al., 1988; 1990) but in none of our experimental mallards. Effusions into body cavities and pulmonary edema have been reported in experimental acute Se intoxication of cattle (Shortridge et al., 1971), sheep (Lambourne and Mason, 1969; Morrow, 1968), and swine (Herigstad et al., 1973). Splenic swelling and congestion, as seen in coots, are nonspecific lesions associated with many diseases and deaths associated with stress and shock and, therefore, could be additional features of acute selenosis. Microscopic lesions of acute selenosis, as reported in livestock, were not detected in these mallards. Although these abnormalities may have multiple etiologies, including numerous other toxic and infectious agents, we propose that these abnormalities, in the absence of microbial agents, are produced by overwhelming acute selenosis with or without concurrent chronic selenosis.

Recently, it was suggested that the evi-

dence that selenosis was responsible for the deaths and developmental abnormalities observed in wild water birds at Kesterson NWR was weakened by the "paucity of corroborative morphological and microbiological studies" (O'Toole et al., 1996). The similarity of gross lesions, tissue selenium residues (Albers et al., 1996), and these histologic findings in experimental mallards, compared to the abnormalities and residues in wild water birds at Kesterson NWR (Ohlendorf et al., 1988; 1990), provide strong evidence that selenosis was the principal and probably sole factor responsible for morbidities and mortalities among the wild water birds.

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LITERATURE CITED

AHMED, K. E., S. E. I. ADAM, O. F. IDRILL, AND A. A. WAHBI. 1990. Experimental selenium poisoning in Nubian goats. Veterinary and Human Toxicology 32: 249–251.

ALBERS, P. H., D. E. GREEN, AND C. J. SANDERSON. 1996. Diagnostic criteria for selenium toxicosis in birds. Dietary exposure, tissue concentrations, and macroscopic effects. Journal of Wildlife Diseases 32: 468–485.

ALFONSO, H. A., L. M. SANCHEZ, M. A. FIGUERDO, AND B. C. GOMEZ. 1993. Intoxication due to *Crotalaria retusa* and *C. spectabilis* in chickens and geese. Veterinary and Human Toxicology 35: 539.

AUSTIC, R. E., AND M. L. SCOTT. 1984. Nutritional deficiency diseases. *In* Diseases of poultry, 8th ed., M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr. (eds). Iowa State University Press, Ames, Iowa, pp. 38–64.

CALNEK, B. W., H. J. BARNES, C. W. BEARD, W. M. REID, AND H. W. YODER, JR. 1991. Diseases of poultry, 9th ed. Iowa State University Press, Ames, Iowa, 929 pp.

CLARK, D. R. 1987. Selenium accumulation in mammals exposed to contaminated California irrigation drainwater. Science of the Total Environment 66: 147–168.

DAFALLA, R., AND S. E. I. ADAM. 1986. Effects of various levels of dietary selenium on Hybro-type chicks. Veterinary and Human Toxicology 28: 105–108.

EGYED, M. N., M. MALKINSON, AND A. SHLOSBERG. 1974. Observations on the experimental poisoning of young geese with *Ammi majus*. Avian Pathology 3: 79–87.

EISLER, R. 1985. Selenium hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85(1.5). U.S. Fish and Wildlife Service, Washington D.C., 57 pp.

EL-BEGEARMI, M. M., AND G. F. COMBS. 1982. Dietary effects on selenite toxicity in the chick. Poultry Science 61: 770–776.

FAIRBROTHER, A., AND J. FOWLES. 1990. Subchronic effects of Na selenite and SeM on immune functions of the mallard. Archives of Environmental Contamination and Toxicology 19: 836–844.

———, M. FIX, T. O'HARA, AND C. A. RIBIC. 1994. Impairment of growth and immune function of avocet chicks from sites with elevated selenium, arsenic, and boron. Journal of Wildlife Diseases 30: 222–233.

FISHBEIN, L. 1977. Toxicology of selenium and tellurium. *In* Advances in modern toxicology, Vol. 2: Toxicology of trace elements. R. A. Goyer, and M. A. Mehlman (eds.). John Wiley and Sons, New York, New York, pp. 191–240.

GASKIN, J. M. 1989. Psittacine viral diseases: A perspective. Journal of Zoo and Wildlife Medicine 20: 249–264.

GOLDBERG, D. R., T. M. YUILL, AND E. C. BURGESS. 1990. Mortality from duck plague virus in immunosuppressed adult mallard ducks. Journal of Wildlife Diseases 26: 299–306.

HARR, J. R., AND O. H. MUTH. 1972. Selenium poisoning in domestic animals and its relationship to man. Clinical Toxicology 5: 175–186.

———, J. F. BONE, I. J. TINSLEY, P. H. WESWIG, AND R. S. YAMAMOTO. 1967. Selenium toxicity in rats. II. Histopathology. *In* Selenium in biomedicine, O. H. Muth (ed.), AVI Publishing, Westport, Connecticut, pp. 153–178.

HEINZ, G. H. 1993. Re-exposure of mallards to selenium after chronic exposure. Environmental Toxicology and Chemistry 12: 1691–1694.

———, AND M. A. FITZGERALD. 1993. Overwinter survival of mallards fed selenium. Archives of Environmental Contamination and Toxicology 25: 90–94.

———, D. J. HOFFMAN, AND L. G. GOLD. 1988. Toxicity of organic and inorganic selenium to mallard ducklings. Archives of Environmental Contamination and Toxicology 17: 561–568.

HERIGSTAD, R. R., C. K. WHITEHAIR, AND O. E. OLSON. 1973. Inorganic and organic selenium toxicosis in young swine: Comparison of pathologic

changes with those in swine with vitamin E-selenium deficiency. American Journal of Veterinary Research 34: 1227–1238.

HOFFMAN, D. J., AND G. H. HEINZ. 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. Journal of Toxicology and Environmental Health 24: 263–271.

———, ———, L. J. LECAPTAIN, C. M. BUNCK, AND D. E. GREEN. 1991a. Subchronic hepatotoxicity of selenomethionine ingestion in mallard ducks. Journal of Toxicology and Environmental Health 32: 449–464.

———, C. J. SANDERSON, L. J. LECAPTAIN, E. CROMARTIE, AND G. W. PENDLETON. 1991b. Interactive effects of boron, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. Archives of Environmental Contamination and Toxicology 20: 288–294.

JAMES, L. F., K. V. VAN KAMPEN, AND W. J. HARTLEY. 1983. *Astragalus bisulcatus*—a cause of selenium or locoweed poisoning? Veterinary and Human Toxicology 25: 86–89.

JENSEN, D. O., W. CLOSSON, AND R. ROTHENBERG. 1984. Selenium intoxication—New York. Morbidity and Mortality Weekly Report 33: 157–158.

JENSEN, W. I., AND J. P. ALLEN. 1981. Naturally occurring and experimentally induced castor bean (*Ricinus communis*) poisoning in ducks. Avian Diseases 25: 184–194.

JONES, T. C., AND R. D. HUNT. 1983. Veterinary pathology, 5th ed. Lea and Febiger, Philadelphia, Pennsylvania, pp. 901–1030.

KELLY, W. R. 1993. The liver and biliary system. *In* Pathology of domestic animals, Vol. 2, 4th ed. K. V. F. Jubb, P. C. Kennedy, and N. Palmer (eds.). Academic Press, San Diego, California, pp. 398–402.

KING, A. S., AND J. MCLELLAND. 1984. Birds: Their structure and function, 2nd ed. Bailliere Tindall, Philadelphia, Pennsylvania, 334 pp.

KINGSBURY, J. M. 1964. Poisonous plants of the United States and Canada. Prentice-Hall, Englewood Cliffs, New Jersey, 626 pp.

LAMBOURNE, D. A., AND R. W. MASON. 1969. Mortality in lambs following overdosing with sodium selenite. Australian Veterinary Journal 45: 208–210.

LATIMER, K. S., P. M. RAKICH, F. D. HIAGRO, B. W. RITCHIE, W. L. STEFFENS, R. P. CAMPAGNOLI, D. A. PESTI, AND P. D. LUKERT. 1991. An updated review of psittacine beak and feather disease. Journal of the Association of Avian Veterinarians 5: 211–220.

LEHMAN, E. L. 1975. Nonparametrics: Statistical methods based on ranks. Holden-Day, San Francisco, California, 457 pp.

LOEW, F. M., E. D. OLFERT, AND B. SCHIEFER. 1975. Chronic selenium toxicosis in cynomolgus monkeys. Laboratory Primate Newsletter 14: 7–14.

LOOMIS, E. C. 1984. External parasites. *In* Diseases of poultry, 8th ed. M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr. (eds.). Iowa State University Press, Ames, Iowa, pp. 586–613.

LUCAS, A. M., AND P. R. STETTENHEIM. 1972. Avian anatomy: Integument, Part II. Agriculture Handbook 362, U.S. Government Printing Office, Washington, D.C., pp. 602–609.

MORROW, D. A. 1968. Acute selenite toxicosis in lambs. Journal of the American Veterinary Medical Association 152: 1625–1629.

NATIONAL RESEARCH COUNCIL. 1980. Selenium. *In* Mineral tolerance of domestic animals. National Academy Press, Washington, D.C., pp. 392–420.

———. 1983. Selenium in nutrition, Revised edition. National Academy Press, Washington, D.C., 174 pp.

OHLENDORF, H. M. 1989. Bioaccumulation and effects of selenium in wildlife. *In* Selenium in agriculture and the environment, Special Publication Number 23, Soil Science Society of America, Madison, Wisconsin, pp. 133–177.

———, D. J. HOFFMAN, M. K. SAIKI, AND T. W. ALDRICH. 1986. Embryonic mortality and abnormalities of aquatic birds: Apparent impacts by selenium from irrigation drainwater. Science of the Total Environment 52: 49–63.

———, A. W. KILNESS, J. L. SIMMONS, R. K. STROUD, D. J. HOFFMAN, AND J. F. MOORE. 1988. Selenium toxicosis in wild aquatic birds. Journal of Toxicology and Environmental Health 24: 67–92.

———, R. L. HOTHEM, C. M. BUNCK, AND K. C. MAROIS. 1990. Bioaccumulation of selenium in birds at Kesterson Reservoir, California. Archives of Environmental Contamination and Toxicology 19: 495–507.

O'TOOLE, D., M. RAISBECK, J. C. CASE, AND T. D. WHITSON. 1996. Selenium-induced "blind staggers" and related myths. A commentary on the extent of historical livestock losses attributed to selenosis on Western US rangelands. Veterinary Pathology 33: 104–116.

PECKHAM, M. C. 1984. Poisons and toxins. *In* Diseases of poultry, 8th ed., M. S. Hofstad, H. J. Barnes, B. W. Calneck, W. M. Reid, and H. W. Yoder (eds.). Iowa State University Press, Ames, Iowa, pp. 783–818.

POLEY, W. E., AND A. L. MOXON. 1938. Tolerance levels of seleniferous grains in laying rations. Poultry Science 17: 72–76.

PRESSER, T. S., AND H. M. OHLENDORF. 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. Environmental Management 11: 805–821.

ROBINSON, R. M., A. C. RAY, J. C. REAGOR, AND L. A. HOLLAND. 1982. Waterfowl mortality caused by aflatoxicosis in Texas. Journal of Wildlife Diseases 18: 311–313.

ROSENFELD, I., AND O. A. BEATH. 1964. Selenium. Geobotany, biochemistry, toxicity, and nutrition. Academic Press, New York, New York, 411 pp.

ROY, N. A., W. THRELFALL, AND T. A. WHEELER. 1986. Feather loss of unknown etiology in a gull colony in Newfoundland, Canada. Journal of Wildlife Diseases 22: 591–594.

SAS INSTITUTE. 1988a. SAS/STAT user's guide (release 6.03 edition). SAS Institute, Inc., Cary, North Carolina, 1028 pp.

———. 1988b. SAS procedures guide (release 6.03 edition). SAS Institute, Inc., Cary, North Carolina, 441 pp.

SHLOSBERG, A, M. N. EGYED, AND A. EILAT. 1974. The comparative photosensitization properties of *Ammi majus* and *Ammi visnaga* in goslings. Avian Diseases 18: 544–550.

SHORT, S. B., AND W. C. EDWARDS. 1990. Blue-green algae toxicoses in Oklahoma. Veterinary and Human Toxicology 32: 558–560.

SHORTRIDGE, E. H., P. J. O'HARA, AND P. M. MARSHALL. 1971. Acute selenium poisoning in cattle. New Zealand Veterinary Journal 19: 47.

SNEDECOR, G. W., AND W. G. COCHRAN. 1969. Statistical methods, 6th ed. Iowa State University Press, Ames, Iowa, 593 pp.

TRAUB-DARGATZ, J. L., AND D. W. HAMAR. 1986. Selenium toxicity in horses. Compendium on Continuing Education for the Practicing Veterinarian 8: 771–776.

WILSON, T. M., R. W. SCHOLZ, T. R. DRAKE. 1983. Selenium toxicity and porcine focal symmetrical poliomyelomalacia: Description of a field outbreak and experimental reproduction. Canadian Journal of Comparative Medicine 47: 412–421.

WOBESER, G., AND W. KOST. 1992. Starvation, staphylococcosis, and vitamin A deficiency among mallards overwintering in Saskatchewan. Journal of Wildlife Diseases 28: 215–222.

WOBESER, G. A. 1981. Diseases of wild waterfowl. Plenum Press, New York, New York, 300 pp.

YAGER, J. A., D. W. SCOTT, AND B. P. WILCOCK. 1993. The skin and appendages. *In* Pathology of domestic animals, Vol. 1, 4th ed., K. V. F. Jubb, P. C. Kennedy, and N. Palmer (eds.). Academic Press, San Diego, California, pp. 587–589.

YANG, G., S. WANG, R. ZHOU, AND A. SUN. 1983. Endemic selenium intoxication of humans in China. American Journal of Clinical Nutrition 37: 872–881.

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