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REPRODUCTION IN MALLARDS FED SELENIUM

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Abstract—Mallards (*Anas platyrhynchos*) were fed diets containing 1, 5, 10, 25 or 100 ppm selenium as sodium selenite, a diet containing 10 ppm selenium as seleno-DL-methionine or a control diet. There were no effects of 1, 5 or 10 ppm selenium as sodium selenite on either weight or survival of adults or on reproductive success, and there did not appear to be a dose-response relationship at these lower levels. The 100 ppm selenium diet killed 11 of 12 adults; one adult male fed 25 ppm selenium died. Selenium at 25 and 100 ppm caused weight loss in adults. Females fed 25 ppm selenium took longer to begin laying eggs and intervals between eggs were longer than in females in other treatment groups. Hatching success appeared to be reduced in birds fed 10 ppm selenium as selenomethionine, but the reduction was not statistically significant. The survival of ducklings and the mean number of 21-d-old ducklings produced per female were reduced in the 25 ppm selenium as sodium selenite group and the 10 ppm selenium as selenomethionine group. Egg weights were not affected by any selenium treatment, but 25 ppm selenium lowered the Ratcliffe Index. Duckling weights at hatching and at 21 d of age were reduced 28 and 36%, respectively, in birds fed 25 ppm selenium, as compared with controls. Body weights measured on day 21 were lower for ducklings fed 10 ppm selenium as selenomethionine than in some other groups. Selenium in concentrations of 10 and 25 ppm as sodium selenite caused mainly embryotoxic effects, whereas 10 ppm as selenomethionine was more teratogenic, causing hydrocephaly, bill defects, eye defects (microphthalmia and anophthalmia) and foot and toe defects, including ectrodactyly. Selenomethionine was much more readily taken up by mallards and passed into their eggs than was sodium selenite, and a greater proportion of the selenium in the eggs ended up in the white when selenomethionine was fed. Adult males accumulated more selenium than did females, probably because of the females' ability to eliminate selenium in their eggs.

Keywords—Mallards Selenium Reproduction Toxicity test Teratogenic effects Selenomethionine Sodium selenite

INTRODUCTION

In 1983, reproductive success was poor and there was a high rate of deformities in embryos and hatchlings of mallards (*Anas platyrhynchos*), American coots (*Fulica americana*), eared grebes (*Podiceps nigricollis*) and black-necked stilts (*Himantopus mexicanus*) at Kesterson National Wildlife Refuge in California [1]. Contamination of the refuge's ponds by selenium in irrigation drainwater was believed to be the cause of the problem.

Previously, ducks and other aquatic birds had not been reported to be harmed by selenium, but reports of adverse effects in chickens fed grains grown in soils high in selenium date back to the 1930s [2–5]. In addition to the accumulation of harmful levels of selenium in some plants grown in soils naturally high in selenium, other sources of selenium elevate selenium levels in birds. Seven species of wintering shorebirds at Corpus Christi, Texas, had mean selenium levels in their kidneys of 1.77 to 5.62 ppm, the source possibly being smelting operations along the coast [6]. Selenium also has been reported at high levels in fly ash from coal burning [7] and in sewage sludge [8]; the disposal of both fly ash and sludge could contaminate areas occupied by aquatic birds.

The objective of this study was to determine whether environmentally realistic dietary levels of selenium affected the condition of or reproduction in mallards.

METHODS

Animals and treatments

One-year-old game farm mallard drakes were assigned randomly to outdoor holding pens, each containing 5 birds. Two-year-old mallard hens were assigned randomly to indoor pens, each containing 10 birds. In the indoor pens, 8 h of light

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was provided. One week later, both sexes were randomly assigned to the following dietary concentrations of selenium in the form of sodium selenite: 10 pairs were fed 1, 5, 10 or 25 ppm, and 5 pairs were fed 100 ppm. Another 5 pairs were fed 10 ppm selenium in the form of seleno-DL-methionine. Thirteen pairs were fed a control diet. The sodium selenite was 97% pure (Sigma Chemical Company, St. Louis, MO); the seleno-DL-methionine was 98 to 99% pure (Behring Diagnostics, San Diego, CA). Additional pairs of mallards were fed selenium diets, one pair for each dietary concentration except 0 ppm. From the hens of each of these pairs, the eggs numbered 1, 5, 9, 13, 17, 21, 25, 29 and 33 were saved to check for variability in selenium concentration with egg sequence.

To make an approximate conversion from dietary concentration to milligrams selenium per kilogram of body weight, we estimate that a 1,000-g mallard consumes about 100 g of feed per day; this is an estimate based on our experience. We used three types of commercially available duck mash: developer, breeder and starter. Developer mash contained about 14.5% protein, 2.75% crude fat and 7% crude fiber. Breeder mash contained about 17, 8 and 6% of the same constituents, and starter mash contained about 22, 3 and 4% of these constituents. The dry mashes contained about 7 to 10% water.

The diets were prepared by dissolving the selenium compounds in distilled, deionized water and then mixing the solutions into a commercial duck developer mash so that the mixed feed contained 1% added water. Controls received 1% untreated added water in their diet. A sample of feed from each dietary concentration was saved for selenium analysis. Each sample was a composite of 20-g aliquots of feed reserved each time a particular diet was mixed. Selenium recovery averaged 80.4% of the calculated concentrations. Diets containing sodium selenite were mixed biweekly, and the single diet containing selenomethionine was mixed weekly.

After three weeks on the treated diets, female mallards were randomly assigned to outdoor breeding pens that measured 1 m^2 and were equipped with a feeder, water pan and nest box. At that time, the dietary concentrations of selenium were mixed into a breeder mash. By keeping the females indoors under only 8 h of light each day and on a maintenance diet, we were able to prevent them from laying eggs until they had been on the treated diets for at least four weeks. After the females had been in the breeding pens for 4 d, a randoml selected male on the same diet as the female wa moved into each pen. The adults were weighed o the day dietary treatments were started, on the da the males were paired with the females and on th day when each pair was killed.

Collection of reproductive data

Eggs were collected each day, labeled according to pen and stored at 10 to 13°C. The tenth egg laid by each hen was saved for residue analysis and measurement of egg weight and Ratcliffe Index (the weight of the dried shell in milligrams divided by the product of the length and width of the egg in millimeters) [9]. For hens that did not lay at least 10 eggs, the last egg laid was saved. At weekly intervals, the eggs were set in an incubator maintained at 37.6°C. Every third egg incubated from each hen was removed after 18 d of incubation and the embryo was examined for abnormalities. Eggs were collected from each hen until 10 eggs had been set aside for the 18-d-old embryo examination and another 20 had been set for the full incubation period. All eggs were candled twice weekly to check for fertility and embryo deaths. Dead embryos were examined for abnormalities, as were embryos in eggs that failed to hatch.

Hatchlings were weighed, banded and examined for abnormalities. Healthy hatchlings were reared for three weeks on a diet of duck starter mash containing the same concentration of selenium that their parents had been fed. All ducklings were weighed and killed at three weeks of age.

Adult pairs were killed after the hen had provided all the eggs needed for the study (1 for residue analysis, 10 for 18-d-old embryo analysis and 20 for full incubation). Cracked eggs were discarded. The study was terminated on 24 May for all but four hens that had not produced the full number of eggs. Pieces of breast muscle and liver were saved for selenium analysis from five pairs in each treatment.

To learn about the loss of selenium from tissues and eggs, four pairs of ducks were switched to a control diet after being fed a diet containing 10 ppm selenium as sodium selenite for 78 d. Two of the pairs were sacrificed one week later and the liver and breast muscle from each bird were analyzed for selenium; each of these females laid an egg 5 d into the control diet period that was analyzed for selenium. The other two pairs were killed after two weeks on a control diet, and the liver and breast muscle were analyzed for selenium; an egg

that each female laid after 14 d on the control diet was analyzed for selenium.

Statistical analyses

All statistical comparisons among treatments were done at a significance level of $\alpha = 0.05$. When analysis of variance was used and a probability of 0.05 or less was achieved, the means were separated by Duncan's multiple range test [10]. Percentage data were subjected to angular transformation before statistical analysis. Chi-square tests were used to compare treatments for the proportion of hens that laid eggs and the proportion of embryos that survived to at least 7 d and that were abnormal. All of the following measurements were tested by analysis of variance: (a) number of days on treatment until the first egg was laid, (b) average number of days between eggs, (c) percent fertility of eggs, (d) percent hatch of fertile eggs, (e) percent survival of healthy hatchlings to 21 d of age, (f) number of 21-d-old ducklings produced, (g) weight of tenth egg, (h) Ratcliffe Index of eggshell quality, (i) weights of adults when treatments started, when pairs were formed and at sacrifice and (j) weights of ducklings at hatching and at 21 d of age, based on ducklings that survived 21 d.

To check for differences in the distribution of selenium in the yolk versus the white of eggs, we saved two additional eggs (the sixteenth and twenty-seventh) from one female fed 10 ppm selenium as sodium selenite and one female fed 10 ppm selenium as selenomethionine. The yolk was separated from the white for selenium analyses.

Chemical analyses

All samples for selenium analysis were homogenized using a Virtis blender. A 1-g portion of homogenate (except for feed for which a 0.2-g portion was used) was digested with 20 ml of concentrated nitric acid in an Erlenmeyer flask. The acid–sample mixture was concentrated to 2 ml and then diluted to 25 ml with distilled, deionized water. Selenium determinations were made using graphite furnace atomic absorption spectrophotometry on a Perkin-Elmer Zeeman 5000 equipped with an HGA 500 furnace, an AST-40 autosampler and a data system 10. The stabilized temperature platform furnace technique of Slavin et al. [11], Zeeman effect background correction and a matrix modifier containing 1.25 g/L Cu and 0.5 g/L Fe^{2+} were used. A selenium electrodeless discharge lamp was used at 196.0 nm with a spectral slit width of 2.0 nm. The furnace program was similar to that proposed by Welz et al. [12]. Recoveries from spiked chicken eggs and livers, and from National Bureau of Standards reference material averaged 101%. The lower limit of reportable residue was 0.05 ppm wet weight. For statistical analyses, tissue and egg samples that contained no reportable level of selenium were assigned a value of 0.025, which is one-half the reportable limit. The selenium concentrations in mallard eggs and tissues are reported on a wet-weight basis. Should a reader want to convert the concentrations to a dry-weight basis, the approximate moisture contents were 71% for eggs, 71% for liver and 74% for breast muscle.

RESULTS

All six female and five of six male mallards fed 100 ppm selenium died within 16 to 39 d, including the extra pair set aside to check for variation in selenium concentrations in every fourth egg. One male fed 25 ppm selenium died after 57 d. The selenium concentrations in the liver and breast muscle of the dead birds are listed in Table 1.

There were no differences among the treatment groups in the weights of males or females at the onset of treatment (Table 2). However, about three weeks later, when the sexes were paired, the weights of both males and females fed 25 or 100 ppm selenium were significantly depressed. Two of the 13 pairs of controls were eliminated from the study, one because the male had a severe case of bumblefoot and the other because the female had yolk peritonitis.

None of the hens fed 100 ppm selenium laid eggs before they died (Table 3). There were no differences among any of the other treatment groups in the proportion of hens that laid eggs. Hens fed 25 ppm selenium took longer to begin laying eggs and laid less frequently than did hens in any of the other groups. Neither percent fertility nor hatchability of eggs differed significantly among any of the groups, but eggs from females fed 10 ppm selenium as selenomethionine had very low hatching success. Survival of ducklings in the 25 ppm selenium as sodium selenite and 10 ppm selenium as selenomethionine groups was lower than in the control group or in the group fed 5 ppm selenium. The number of 21-d-old ducklings produced per hen was significantly lower for birds fed 25 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine.

In control mallards, abnormal embryo growth or development occurred in 4.2% of the eggs in which embryos survived at least 7 d. One of these embryos had a bill defect, but most abnormalities

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Table 1. Selenium concentrations (mean ± SE) in liver and breast muscle of mallards that died after being fed a diet containing 25 or 100 ppm selenium as sodium selenite

Selenium added to diet (ppm)	Selenium (ppm, wet wt.)			
	Liver		Breast muscle	
	Female	Male	Female	Male
25	—	6.1 (1)	—	0.73 (1)
100	5.6 ± 0.31 (6)	8.3 ± 2.97(5)	1.4 ± 0.03 (3)	1.3 ± 0.18 (4)

Sample size in parentheses.

Table 2. Weights (mean ± SE) of adult mallards fed diets containing 1, 5, 10, 25 or 100 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine or a control diet

Selenium added to diet (ppm)	n	Weight (g)					
		Males			Females		
		At onset of treatment	When birds were paired	At sacrifice or death	At onset of treatment	When birds were paired	At sacrifice or death
0	11	1,125 ± 38.2 A	1,105 ± 37.1 A	1,046 ± 29.0 A	959 ± 21.1 A	971 ± 15.6 A	1,141 ± 26.7 A
1	10	1,122 ± 38.0 A	1,146 ± 32.3 A	1,110 ± 40.7 A	990 ± 40.0 A	997 ± 37.1 A	1,193 ± 58.7 A
5	10[a]	1,086 ± 31.4 A	1,119 ± 27.9 A	1,064 ± 23.7 A	932 ± 19.1 A	924 ± 15.8 A	1,090 ± 41.5 A
10	10	1,090 ± 34.5 A	1,104 ± 42.1 A	1,081 ± 27.7 A	947 ± 23.4 A	966 ± 20.4 A	1,088 ± 35.6 A
25	10	1,097 ± 14.5 A	906 ± 31.6 B	913 ± 55.2 B	972 ± 23.3 A	837 ± 27.8 B	902 ± 26.0 B
100	5[b]	1,168 ± 28.0 A	692 ± 37.3 C	564 ± 23.6 C	876 ± 22.9 A	557 ± 40.6 C	451 ± 11.6 C
10 (as selenomethionine)	5	1,250 ± 55.4 A	1,234 ± 44.2 A	1,120 ± 36.7 A	932 ± 5.8 A	956 ± 17.8 A	1,114 ± 65.0 A

Means in the same column that do not have a letter in common were found to be significantly different at $\alpha = 0.05$ by analysis of variance followed by Duncan's multiple range test.
[a] $n = 9$ for females at sacrifice or death.
[b] $n = 3$ for females when pairs were formed.

were limited to stunted growth and swollen necks. In mallards fed 1 and 5 ppm selenium as sodium selenite, 3.1 and 4.5%, respectively, of the eggs produced abnormal embryos, which was not significantly different from the defect rate in controls. In mallards fed 10 and 25 ppm selenium as sodium selenite, and 10 ppm as selenomethionine, 11.2 and 22.2, and 18.3%, respectively, of the eggs produced abnormal embryos. These rates were all significantly greater than that in the control, 1 or 5 ppm selenium groups by chi-square analysis, but were not significantly different from each other. Most abnormal embryos from females fed 10 or 25 ppm selenium as sodium selenite exhibited manifestations of embryotoxicity rather than teratogenicity. These embryotoxic effects included stunted growth, swollen necks, edema and fewer than normal feathers. However, 1 of 10 females fed 10 ppm selenium as sodium selenite produced some embryos with multiple malformations, including hydrocephaly, small eyes (microphthalmia) and shortened toes. In contrast, selenomethionine was quite teratogenic and 3 of 5 females fed 10 ppm selenium as selenomethionine produced at least one embryo that exhibited multiple malformations, including hydrocephaly, bill defects (frequently of the lower bill), eye defects (anophthalmia and microphthalmia), twisted legs and foot defects with missing toes (ectrodactyly).

There were no differences among groups in whole egg weight, but shell quality as measured by the Ratcliffe Index was poorer in birds fed 25 ppm selenium (Table 4). Duckling weights at hatching and at 21 d of age were 28 and 36% lower, respectively, for birds in the 25 ppm selenium treatment, as compared with controls (Table 5). At 21 d of age, the diet of 10 ppm selenium as selenomethionine depressed duckling weight, as compared with some other treatments.

The selenium concentrations in eggs, liver and

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Table 3. Reproductive success of mallards fed diets containing 1, 5, 10, 25 or 100 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine or a control diet

	Selenium added to the diet (ppm)						
	As sodium selenite						As selenomethionine
	0	1	5	10	25	100	10
Pairs of breeders	11	10	10	10	10	5	5
Hens laying eggs	11	9	8	10	9	0	5
Days on treatment until first egg	38.8 ± 0.85 A [11]	37.1 ± 1.92 A [9]	41.0 ± 1.90 A [8]	42.1 ± 1.59 A [10]	57.0 ± 3.70 B [9]	—	41.2 ± 4.21 A [5]
Days between eggs	1.3 ± 0.09 A [11]	1.3 ± 0.17 A [9]	1.2 ± 0.08 A [8]	1.4 ± 0.11 A [10]	2.5 ± 0.21 B [7][a]	—	1.2 ± 0.07 A [5]
Percent fertility of eggs	99.2 A (94.4–100) [11]	99.6 A (97.7–100) [9]	98.3 A (90.0–100) [8]	96.2 A (84.7–100) [10]	88.5 A (52.2–100) [7][a]	—	100 A (100–100) [5]
Percent hatch of fertile eggs	65.7 A (51.1–78.9) [11]	73.3 A (57.7–86.4) [9]	69.0 A (52.2–83.5) [8]	61.9 A (31.0–88.3) [10]	53.4 A (8.4–95.0) [7][a]	—	30.9 A (0–86.1) [5]
Percent survival of healthy hatchlings to 21 d of age[b]	98.7 A (90.6–100) [11]	91.9 AB (69.9–100) [9]	99.2 A (94.7–100) [8]	96.4 AB (86.5–100) [9]	50.0 B (0–100) [2]	—	50.0 B (0–100) [5]
Number of 21-d-old ducklings produced per hen[c]	9.7 ± 1.39 A [11]	9.2 ± 2.02 A [10]	7.7 ± 1.61 A [10]	8.1 ± 1.39 A [10]	0.2 ± 0.22 B [9]	—	2.0 ± 1.55 B [5]

Means in the same row that do not have a letter in common were significantly different at $\alpha = 0.05$ by analysis of variance followed by Duncan's multiple range test. Sample size in brackets.

[a]Excludes a female that laid only one egg and another that laid only two eggs.

[b]Excludes one hen fed 10 ppm selenium as sodium selenite, seven hens fed 25 ppm selenium and two hens fed 10 ppm selenium as selenomethionine; these hens had no ducklings available for the study of survival to 21 d of age.

[c]Based on all hens in the study, except one hen fed 25 ppm selenium whose mate died.

Table 4. Whole egg weight and Ratcliffe Index for eggs laid by mallards fed diets containing 1, 5, 10 or 25 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine or a control diet

Selenium added to diet (ppm)	n^a	Whole egg weight (g)	Ratcliffe Index[b]
0	11	56.4 ± 1.67 A	2.16 ± 0.034 A
1	9	60.9 ± 2.30 A	2.12 ± 0.049 A
5	8	55.5 ± 1.34 A	2.05 ± 0.047 AB
10	10	55.6 ± 1.10 A	2.03 ± 0.067 AB
25	7	51.6 ± 3.75 A	1.90 ± 0.056 B
10 (as selenomethionine)	5	56.1 ± 2.24 A	2.13 ± 0.053 A

Values are means ± SE.
Means that do not have a letter in common were significantly different at $\alpha = 0.05$ by analysis of variance followed by Duncan's multiple range test.
[a]Number of hens. The tenth egg laid by each hen was measured unless a hen did not lay as many as 10 eggs, in which case the last egg was used.
[b]Equals the weight of the dried eggshell (mg) divided by the product of the egg length and width (mm) [9].

Table 5. Weights (mean ± SE) of mallard ducklings fed diets containing 1, 5, 10 or 25 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine or a control diet

Selenium added to diet (ppm)	n^a	Weight (g) At hatching	Weight (g) At 21 d old
0	11	36 ± 0.9 A	371 ± 12.3 AB
1	9	37 ± 1.6 A	398 ± 15.9 A
5	8	35 ± 0.5 A	390 ± 20.6 A
10	9	34 ± 0.9 A	406 ± 19.4 A
25	1	26 B	236 C
10 (as selenomethionine)	2	36 ± 0.0 A	297 ± 31.0 BC

Means in the same column that do not have a letter in common were significantly different at $\alpha = 0.05$ by analysis of variance followed by Duncan's multiple range test.
[a]Number of hens with ducklings. A mean weight for all ducklings from the same hen was calculated; these means were then averaged to produce the means given in the table.

breast muscle are listed in Table 6. There were no temporal trends in selenium levels in every fourth egg laid by one female fed each diet, although selenium levels in eggs from the female fed 10 ppm selenium as selenomethionine varied more than in the females fed sodium selenite (Fig. 1). The selenium levels in eggs, liver and breast muscle of mallards switched from a 10 ppm selenium diet to a control diet are given in Table 7. When sodium selenite was fed, more selenium was deposited in the egg yolk than in the white, but the reverse was true when selenomethionine was fed (Table 8).

DISCUSSION

Toxicity of selenomethionine versus sodium selenite

Little is known about the toxicity of selenomethionine to birds, even in chickens, in which selenium toxicity has been studied extensively. A diet containing 10 ppm selenium from selenium-rich grain reduced the hatching success of chicken eggs to zero [4]. Because the most prevalent form of selenium in cereal grains has been reported to be selenomethionine [13], it is likely that much of the selenium in the 10 ppm selenium diet fed the chickens was in that form. A diet containing 6 ppm selenium as selenomethionine had no effect on adult chickens or on their egg production, but hatching success was not measured [14]. When injected into the air cell of chicken eggs containing 4-d-old embryos, the LD50 for selenomethionine was 0.13 ppm selenium in the egg contents, whereas for sodium selenite the LD50 was 0.3 ppm selenium [15].

In our study, the survival of ducklings and the number of 21-d-old ducklings produced were significantly lower in mallards fed 25 ppm selenium as sodium selenite and 10 ppm selenium as selenomethionine, but not in birds fed 10 ppm selenium as sodium selenite. In addition, the hatching success of fertile eggs was only 30.9% for hens fed 10 ppm selenium as selenomethionine, compared with 65.7% for controls and 61.9% for hens fed 10 ppm selenium as sodium selenite. Probably because we had only five pens of mallards fed the selenomethionine diet, this decrease in hatching success was not statistically significant, but it does suggest a harmful effect. Selenomethionine did not appear to have any effect on egg laying, egg weights, Ratcliffe Index or fertility; nor were adult weights affected, but duckling growth was retarded. Selenomethionine has been reported to be the predominant form of selenium in plants [13,16] and may, therefore, pose a hazard to waterfowl that consume plants high in selenium.

Selenomethionine may have caused greater reproductive effects in mallards than did sodium selenite because of the former's much greater accumulation in eggs. The diet of 10 ppm selenium as selenomethionine resulted in nearly 10 times as

Table 6. Selenium concentration (mean ± SE) in eggs, liver and breast muscle of mallards fed diets containing 1, 5, 10, 25 or 100 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine or a control diet

Selenium added to diet (ppm)	Selenium (ppm, wet wt.)							
	Eggs		Liver			Breast muscle		
	n[a]	Concn.	n	Female	Male	n	Female	Male
0	5	0.05 ± 0.022	5	0.15 ± 0.022	0.54 ± 0.079[b]	5	0.17 ± 0.031	0.20 ± 0.011
1	9	0.03 ± 0.005	5	0.31 ± 0.020	0.94 ± 0.204[b]	5	0.15 ± 0.007	0.23 ± 0.004[b]
5	8	0.18 ± 0.044	5	0.51 ± 0.092	2.0 ± 0.53[b]	5	0.21 ± 0.111	0.28 ± 0.014[b]
10	10	0.53 ± 0.048	5	1.0 ± 0.25	2.9 ± 0.72[b]	5	0.28 ± 0.024	0.29 ± 0.011
25	8	1.3 ± 0.35	5	2.6 ± 0.20	5.0 ± 0.53[b]	5	0.42 ± 0.029	0.51 ± 0.045
100	0	—	1	—	12	1	—	0.65
10 (as selenomethionine)	5	4.6 ± 0.51	5	4.7 ± 0.75	8.6 ± 0.97[b]	5	4.9 ± 0.70	3.1 ± 0.17[b]

[a]The tenth egg from each hen was analyzed, except for five hens fed 25 ppm selenium that did not lay 10 eggs; for these hens, the last egg laid was analyzed.
[b]Values for males were significantly different from those for females for the same treatment and tissue at $\alpha = 0.05$ by a t-test.

Table 7. Selenium concentration in eggs, liver and breast muscle of mallards switched from a diet containing 10 ppm selenium as sodium selenite to a control diet

Weeks fed control diet	Pair number	Selenium (ppm, wet wt.)				
			Liver		Breast muscle	
		Egg	Female	Male	Female	Male
1	1	0.17[a]	0.61	1.6	0.18	0.23
1	2	0.19[a]	0.44	0.90	0.15	0.18
2	1	0.056[b]	0.38	2.4	0.18	0.21
2	2	ND[b]	0.27	1.6	0.14	0.18

ND, not detected at a level of 0.05 ppm or more.
[a]Laid 5 d after the female had been switched to a control diet.
[b]Laid 14 d after the female had been switched to a control diet.

much selenium in the eggs as did the diet 10 ppm as sodium selenite. Chickens fed 10 ppm selenium in grain (with the predominant form of selenium probably being selenomethionine) produced eggs with 6.4 ppm selenium in the white and 3.9 ppm in the yolk [17], which is a little higher than the levels we found in mallard eggs. Moksnes [14] fed chickens 6 ppm selenium as selenomethionine and reported levels of 4.4 to 6.2 ppm selenium in whole eggs. Chickens, like mallards, store selenomethionine more readily than sodium selenite in eggs and adult tissues [14]. Scott and Thompson [18] hypothesized that inorganic selenium cannot be stored in the body above certain levels because of the lack of binding sites, whereas organic forms may be deposited in tissues along with sulfur amino acids. The incorporation of selenomethionine into proteins was suggested by McConnell and Hoffman [19]. More recently, Beilstein et al. [20] suggested that, in mammals, selenomethionine is converted to selenocysteine, which is then incorporated into proteins. In contrast, selenite is reported to be protein-bound as well, but not through peptide bonds [21]. It is possible, therefore, that the greater toxicity of selenomethionine that we observed was due not only to greater accumulation in eggs but to a different chemical form and binding site in the eggs.

Although our sample was small, we showed that when a selenomethionine diet was fed mallards, more selenium was deposited in the egg white than in the yolk. The opposite was true when a sodium selenite diet was fed. Similar findings have been reported in chickens [22]. This dif-

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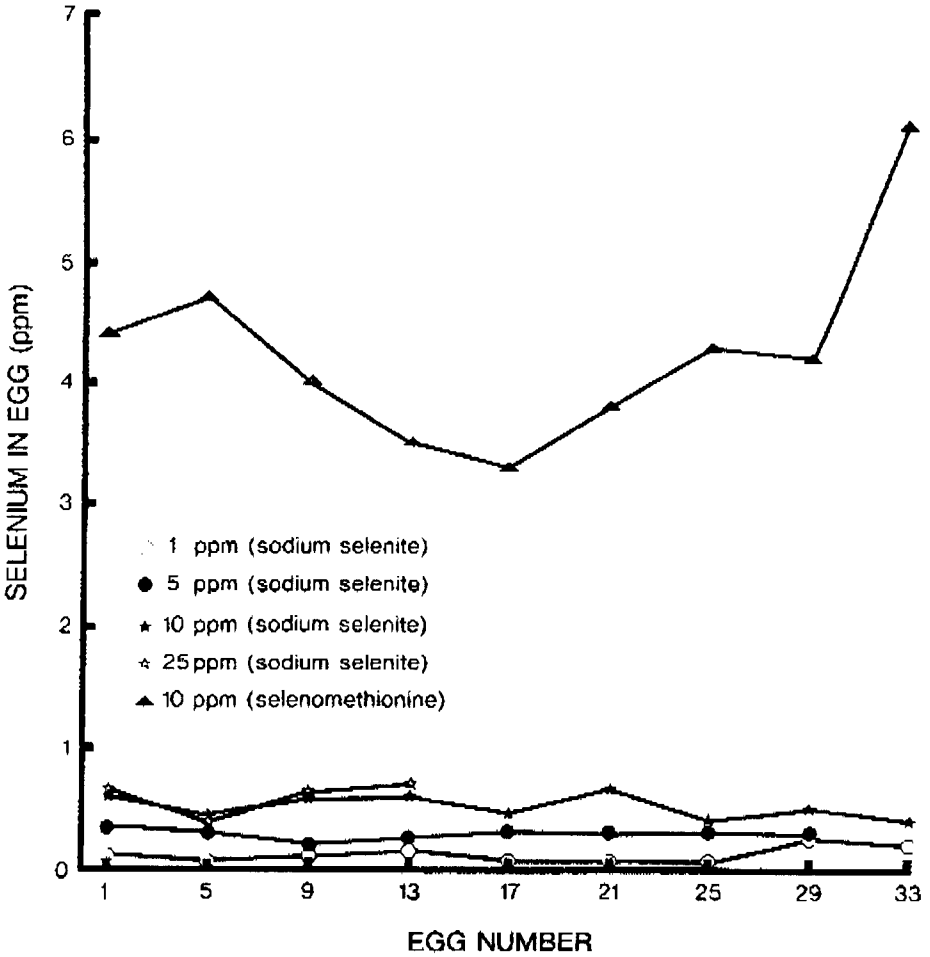

Fig. 1. Selenium concentration in every fourth egg laid by females fed 1, 5, 10 or 25 ppm selenium as sodium selenite or 10 ppm selenium as selenomethionine. The female fed 5 ppm selenium laid only 29 eggs and the female fed 25 ppm selenium laid only 13 eggs.

Table 8. Selenium concentration in yolk and white of eggs from a female fed 10 ppm selenium as sodium selenite and from a female fed 10 ppm selenium as selenomethionine

Selenium added to diet (ppm)	Egg number	Selenium (ppm, wet wt.)	
		Yolk	White
10 (as sodium selenite)	16	0.62	0.31
	27	0.56	0.35
10 (as selenomethionine)	16	2.5	6.1
	27	2.9	7.9

ference in the distribution of selenium in the egg also could contribute to differences in the toxicity of the two forms of selenium.

Sodium selenite toxicity in mallards versus chickens and Japanese quail

Most avian toxicity tests with sodium selenite have used chickens and Japanese quail (*Coturnix japonica*). Our mallards appeared to be less sensitive to sodium selenite than either of those species. According to Ort and Latshaw [23], 5 ppm selenium as sodium selenite is "borderline toxic" to chickens when the hatching success of fertile eggs—

the most sensitive measure of a reproductive effect of selenium in chickens—is considered. At 7, 8 and 9 ppm selenium, a definite decrease in hatching success has been observed in chickens [23,24]. A diet containing 6 or 12 ppm selenium as sodium selenite reduced hatchability in Japanese quail [25]. In our study, a diet of 10 ppm selenium as sodium selenite did not affect the hatching success of fertile eggs and, although there was a suggestion that 25 ppm selenium reduced the hatching success of fertile eggs, that reduction was not statistically significant.

In chickens, 8 and 9 ppm selenium as sodium selenite lowered egg production [23,24]; in Japanese quail, 12 ppm did the same [25]. However, we found egg laying to be depressed only in the 25 ppm treatment group. As little as 7 ppm selenium has been reported to decrease egg weight in chickens [23], but there were no significant effects of sodium selenite in mallards, even at 25 ppm selenium. Arnold et al. [24] reported that 8 ppm selenium as sodium selenite had no effect on eggshell thickness in chickens, but we found that 25 ppm reduced shell quality as measured by the Ratcliffe Index. The reduction in shell quality we observed may have been due more to the generally poor condition of the females fed 25 ppm selenium than to some inherent shell-thinning property of selenium.

Even fairly high levels of selenium do not appear to affect the fertility of eggs. There was no effect in the eggs of chickens fed 8 ppm selenium [24] and only at 25 ppm selenium in our study was there any suggestion of depressed fertility, although it was not a statistically significant effect.

The survival of Japanese quail chicks was reduced by a diet containing 8 ppm selenium [25]; in chickens, 8 and 20 ppm had no effect, though 40 ppm did reduce survival [24,26]. The survival of our mallards was not much different; 25 ppm selenium reduced survival of young birds. Dean and Combs [27] fed mallard ducklings 1 ppm selenium as sodium selenite and, as might be expected, observed no effect on survival.

In our study, there was a significant reduction in duckling growth in birds fed 25 ppm selenium, although this result must be interpreted with caution because it is based on only a few ducklings from one hen. Jensen [26] reported reduced growth in young chickens fed 5 ppm selenium, and Sell and Horani [28] found decreased growth in chickens fed 8 ppm selenium from 1 to 28 d of life. In contrast, Arnold et al. [24] reported no effect on the growth of chickens fed 8 ppm selenium and a temporary increase in growth in those fed a diet containing 2 ppm. The growth of Japanese quail chicks was not affected by 8 or 12 ppm selenium [25,28].

One reason that reproduction in our mallards fed sodium selenite was not affected as was reproduction in chickens may be that mallards do not accumulate as much selenium in their tissues and eggs as do chickens. Our female mallards had 0.31 ppm selenium in liver, 0.15 ppm in breast muscle and 0.03 ppm in eggs when fed 1 ppm selenium as sodium selenite. In contrast, Moksnes and Norheim [29] reported levels of 0.67 ppm selenium in liver, 0.20 ppm in breast muscle and 0.35 ppm in the eggs of chickens fed 1 ppm selenium as sodium selenite. At 5 ppm selenium in the diet, our female mallards had 0.51 ppm selenium in liver, 0.21 ppm in breast muscle and 0.18 ppm in eggs, whereas chickens fed 5 ppm selenium for two or more weeks had 2.42 ppm selenium in liver, 0.21 ppm in breast muscle and 0.78 to 0.85 ppm in egg white and 2.31 to 2.75 ppm in egg yolk [23]. At 10 ppm selenium in the diet, our female mallards had 1.0 ppm selenium in liver, 0.28 ppm in breast muscle and 0.53 ppm in eggs, whereas chickens fed 9 ppm selenium for 16 weeks had 3.03 ppm in liver, 0.20 ppm in breast muscle and 1.23 ppm in egg white and 3.62 ppm in egg yolk [23]. When we fed 25 ppm selenium to mallards, the eggs contained 1.3 ppm selenium, a level reached in chickens at approximately 5 ppm selenium as sodium selenite in the diet.

The differences in species' sensitivities to selenium poisoning in the laboratory are important to understand because they probably exist in the field and thus should influence the way we assess the hazard of selenium to wild birds. Although mallards appear to be less sensitive to sodium selenite than are chickens and Japanese quail, we know virtually nothing about the sensitivities of other wild birds, some of which may be even more sensitive than chickens and Japanese quail.

Other findings

In nearly all cases in which there was a difference in tissue selenium concentrations between the sexes, males had the higher levels. The one exception was the higher selenium level in the breast muscle of females fed 10 ppm selenium as selenomethionine. Male Japanese quail fed 3.4 ppm selenium in wheat had more selenium in their livers than did females fed the same concentration, a result attributed to the loss of selenium through egg laying [30]. It is likely that the elimination of

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selenium through eggs was at least partly responsible for the lower selenium levels in our female mallards, as compared with males.

When we stopped sodium selenite treatment at 10 ppm selenium, the selenium level in eggs dropped to the control level after two weeks. Selenium levels in liver and breast muscle of males and females also dropped, but not to control levels. After two weeks on a control diet, the selenium concentration in the eggs of chickens fed 8 ppm selenium as sodium selenite for 104 weeks was not higher than that in controls [24]. Ort and Latshaw [23] fed chickens 9 ppm selenium as sodium selenite for 16 weeks, at which time the selenium concentration reached 1.23 ppm in egg white and 3.62 ppm in yolk; after two weeks on a control diet, the levels dropped to 0.16 and 0.55 ppm and after four weeks to 0.09 and 0.36 ppm, levels similar to control levels.

Our study was not designed to determine concentrations of selenium in tissues associated with death, but there was no indication in the tissues taken from dead birds that selenium concentrations differed from those in survivors. The selenium levels in the liver and breast muscle of the one male that died in the 25 ppm selenium treatment group was only slightly higher than those in surviving males. All females and all but one male fed 100 ppm selenium died. The single surviving male had higher selenium levels in liver and lower levels in breast muscle than the mean values for the dead males.

We observed an increased incidence of abnormal embryos in the groups fed 10 and 25 ppm selenium as sodium selenite and 10 ppm as selenomethionine. Both forms have been reported to be embryotoxic and teratogenic in chickens. Seven to nine parts per million selenium as sodium selenite caused edema in the heads and necks of chicken embryos [23,24]. When chickens were fed 15 ppm selenium in seleniferous grain (which contains selenomethionine as the major form of selenium), the embryos manifested edema, as well as twisted necks and legs, missing or fused toes, missing or protruding eyes, deformed beaks and missing wings [3,5]. When sodium selenite was injected into the air cell of chicken eggs, it caused similar embryo deformities [31–33], but Palmer et al. [15] found that of several selenium compounds, including sodium selenite, selenomethionine caused the most teratogenic effects. In our study, selenomethionine caused the worst deformities. The deformities we saw were similar to those reported in aquatic birds at the Kesterson National Wildlife Refuge in California [1]. The forms of selenium in the foods of aquatic birds at Kesterson have not been determined, but at least some is probably in the selenomethionine form.

Our findings demonstrate that the levels of selenium known to exist in nature, such as at the Kesterson National Wildlife Refuge in California, could harm the adult health and reproductive success of waterfowl such as mallards, especially if a high percentage of the selenium is in the selenomethionine form.

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