Eggshell Thinning in Japanese Quail Fed Mercuric Chloride

Abstract. *The eggs produced by developing Japanese quail* (Coturnix coturnix japonica) *fed 1 to 8 parts of mercury per million as mercuric chloride for 10 weeks have thinned shells. Total amounts of mercury in tissues were quite proportional to the dosage and were higher in males than in females. Methylation of mercury was not observed.*

Poor reproductive efficiency in wood pigeons (*1*) and decreased hatchability of pheasant eggs (*2*) have been associated with relatively high amounts of dietary mercury. The methylation of mercury has been reported (*3*) for eggs and tissues of hens fed mercuric nitrate. The objectives of this study were (i) to examine egg production, shell thickness, and reproductive efficiency; (ii) to determine if mercury was methylated; and (iii) to measure the deposition and excretion of mercury in developing Japanese quail fed low amounts of mercuric chloride.

Day-old Japanese quail (*Coturnix coturnix japonica*) were used. This species is small but matures rapidly. The birds were divided into five duplicated groups of 20 birds each housed in a commercial brood unit, and fed partially purified cornstarch with isolated soybean protein diets (*4*) containing 0, 1, 2, 4, or 8 parts of mercury per million (ppm) added to each diet as HgCl$_2$. At 3 weeks of age the birds were debeaked, sexed, and segregated so that there were four males and six females per group. During the 9th and 10th weeks eggs were collected from each group and either incubated or freeze-dried for mercury analysis. Twenty eggs from the groups on each diet were longitudinally cut with iris scissors; the membranes were washed out, and the shells were dried at 50°C for 2 hours. The shells were then measured with a Starrett micrometer at four median points for each egg. At 10 weeks of age all birds were killed, and tissues were taken for mercury analysis.

A 24-hour sample of excreta was obtained during the last week and freeze-dried for analysis. From 0.5 to 1 g of each sample (liver, kidney, brain, testes, eggs, muscle, skin, feathers, and excreta) was analyzed for the total amount of mercury by means of oxygen-flask combustion (*5*) and flameless atomic absorption analysis (*6*). Depending on the amount of sample available, 1 to 10 g of kidney, muscle, eggs, and feathers were analyzed for methylmercury by means of the isolation procedure of Westöö (*7, 8*). They were analyzed by gas chromatography with a microwave-powered emission detector that specifically measured the 2537-Å atomic mercury line (*9*). Other samples (liver, brain, testes, skin, and excreta) could not be analyzed for methylmercury because the quantity of sample was insufficient. Recovery of 0.1 to 0.5 ppm of mercury and methylmercury added to control samples ranged from 80 to 100 percent and 63 to 84 percent, respectively. The methods were sensitive to about 0.005 and 0.05 ppm of mercury and methylmercury, respectively.

Growth and food intake of the quail on all treatments were similar. Although egg production, hatchability, and fertility were unaffected, eggshell thickness diminished as the concentration of HgCl$_2$ in the diet increased. This relationship is graphically illustrated in Fig. 1. The plot is strikingly similar to that published (*10*) for eggshells of mallard ducks that had been fed dieldrin. Analysis of mean differences according to Duncan's new multiple range test (*11*) indicates significant ($P \le .05$) decrease in the eggshell thickness of quail consuming 8

Table 1. Residues of total mercury in tissues, eggs, and excreta of Japanese quail fed mercuric chloride at several concentrations for 10 weeks.

| Sex	Mercury (ppm) in quail fed HgCl$_2$ at				
	0 ppm	1 ppm	2 ppm	4 ppm	8 ppm
Liver					
M	.006	.050	.049	.070	.134
F	.012	.017	.022	.061	.088
Kidney					
M	.017	.290	.380	.630	1.150
F	.008	.070	.110	.220	.400
Muscle					
M	.008	.009	.012	.014	.020
F	.005	.007	.008	.011	.019
Brain					
M	.007	.007	.007	.010	.015
F	.004	.004	.006	.008	.011
Testes					
M	.008	.010	.016	.031	.047
Eggs					
F	<.02	.06	.12	.30	.70
Skin					
M	.022	.060	.078	.082	.152
F	.014	.030	.040	.102	.136
Feathers, 4 weeks					
M	.06	.10	.12	.26	.42
F	.04	.10	.12	.16	.42
Feathers, 6 weeks					
M	.04	.10	.20	.28	.54
F	.04	.14	.16		.78
Feathers, 10 weeks					
M	.06	.20	.28	.56	.96
F	.08	.22	.34	.58	1.12
Excreta					
	.04	3.56	6.04	12.00	28.00
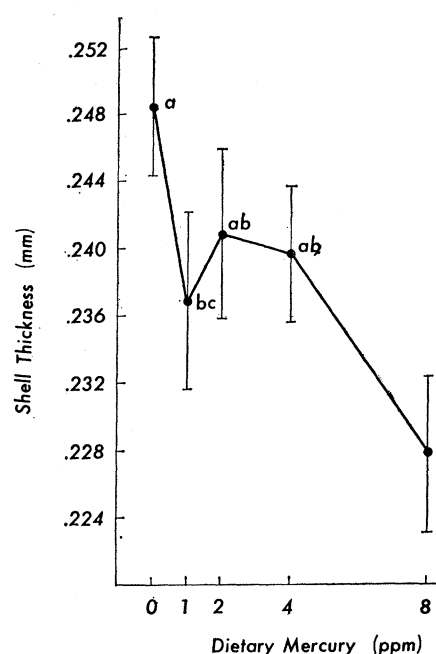
Fig. 1. Eggshell thickness of Japanese quail fed 0, 1, 2, 4, and 8 ppm of dietary mercury as mercuric chloride. Plotted values are means and standard errors. Different letters after the means indicate significant differences ($P \le .05$) as analyzed according to Duncan's new multiple range test (*11*).

ppm of mercury. Fimreite *et al.* (*12*) did not observe eggshell thinning in field studies of prairie falcon eggs.

Methylmercury was absent in kidney, muscle, eggs, and feathers analyzed in replicate. Kiwimae *et al.* (*3*) found methylmercury in tissues of chickens fed inorganic mercury in the diet. The metabolism of mercury is affected by strain differences in birds (*13*), and probably intestinal microorganisms in different avian species variously affect the methylation reaction.

Residues of total mercury in tissues, eggs, and excreta are listed in Table 1. Total amounts of mercury in the tissues and excreta were generally proportional to the amount of dietary mercury. The concentrations found in the males, especially those in the kidneys and liver, were most often higher than those in females. Female organs may have lower amounts because mercury is stored in the eggs (*14*) and ovaries (*15*). Storage of mercury in feathers was substantial and progressively increased with age. The amount of mercury in pooled excreta was very high. Concentrations of mercury in the brain, testes, muscle, and skin were comparatively low.

It is difficult to speculate to what extent mercury may be responsible for the decrease of certain avian species in the environment as a result of eggshell thinning. In the future it will be of interest to learn of other birds which may methylate mercury.

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Sporozoite-Induced Infections of Plasmodium berghei Administered by the Oral Route

Abstract. Administration of suspensions of Plasmodium berghei *sporozoites by the oral route to groups of susceptible animals (A/J mice, young rats, hamsters, and* Thamnomys*) resulted in high rates of infection (66 to 100 percent). Control animals each given a suspension of 200,000 to 500,000 erythrocytes infected with* P. berghei *by the same oral route did not develop parasitemia. Direct intubation of sporozoite suspensions into the stomach failed to produce parasitemia. In vitro studies showed that sporozoites kept in medium M 199 acidified to pH 3.6 (the acid environment of the rat stomach) for 5 to 15 minutes lost their viability and infectivity. We believe that sporozoites of* P. berghei *find their way to the bloodstream during their brief sojourn in the esophagus.*

Inoculation of viable sporozoites of *Plasmodium berghei* into susceptible hosts (*Thamnomys*, young albino rats, hamsters, and A/J mice) intravenously, intraperitoneally, or intramuscularly usually results in a malarial infection and parasitemia within 3 to 5 days. The incubation period for sporozoite-induced infections of rodent malaria, like that of other mammalian malarias, is directly dependent on the length of the primary exoerythrocytic growth cycle in the liver. It is also influenced by the innate susceptibilty of the host species (*1*) or genetic strain (*2*), the age of the animals, the number of viable sporozoites, and the route of their administration (*3*). It has generally been assumed that administration of sporozoites by the oral route would fail to produce patent malarial infections owing to the inimical environment of the digestive tract and the inability of sporozoites to reach a blood or lymph vessel, whence they could be carried to the liver.

In the course of a study on the movements of sporozoites of *P. berghei* in vivo, we administered sporozoite suspensions orally to groups of susceptible animals. We were surprised at the constant and high rates of infection

Table 1. Results of oral administration of *P. berghei* sporozoites to susceptible hosts. The sporozoite suspension administered orally to each animal was contained in 0.2 ml of fluid. The control A/J mice received sporozoites by intravenous inoculation.

Age of animals (weeks)	Sporozoites administered (No.)	Patent (No.)/ total infected	Prepatent period (days)	
			Average	Range
Rats				
4	100,000	6/7	5.3	4–7
3	100,000	7/10	4.9	4–6
3	30,000	8/8	4.1	4–5
3	10,000	4/6	5.7	5–6
3½	60,000	10/10	4	3–5
Hamsters				
4	100,000	6/7	5.6	4–6
3	100,000	5/6	5	4–6
3	25,000	4/6	5.2	5–6
15	300,000	2/2	5.5	5–6
A/J mice				
8	100,000	9/9	4.8	4–6
9	40,000	6/6	4.8	4–5
9	10,000	3/4	5	4–6
8	25,000	8/8	3.6	3–4
8	50,000	6/6	4.6	4–5
Thamnomys				
32	1 million	2/2	4	3–5
Control A/J mice				
8–9	10,000–20,000	11/12 (91)	3.8	3–5