showed an almost complete inhibition of immune response to *Salmonella pullorum* and of the lymphoid follicle formation in the bursa even by using the cyclophosphamide treatment during the postnatal period. Moreover, the disappearance of the beta$_2$-globulin peak in the electrophoresis of serum from E5 ducks may show a suppression of gammaglobulin production; this fact may endorse the functional elimination of antibody producing-capability in cyclophosphamide-treated ducks. These results may indicate that early treatment using cyclophosphamide damages the B-lymphocytes; the B-lymphocytes are damaged more than the T-lymphocytes by this agent. This may be due to the difference in cellular maturation (Peterson and Good, 1965).

From these results, early cyclophosphamide treatment in ducks may be more useful in chemical bursectomy of ducks than other chemical substances, such as testosterone.

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Sexual Maturation and Productivity of Japanese Quail Fed Graded Concentrations of Mercuric Chloride[1]

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ABSTRACT Japanese quail (*Coturnix c. japonica*) were fed 0, 2, 4, 8, 16, and 32 p.p.m. Hg as mercuric chloride ($HgCl_2$) from the time of hatching up to the age of 1 year. None of the birds manifested any gross signs of mercury poisoning. Food consumption, growth rate, and weight maintenance were unaffected. Initial oviposition tended to occur at a younger age as dietary mercuric chloride increased, e.g., the median age at which egg laying began among hens fed 32 p.p.m. Hg was 6 days younger than for controls. The average rate of egg production was positively related to the concentration of mercuric chloride with the most pronounced differences between treatments occurring among young (<9-week-old) hens. Beyond 9 weeks of age egg production was more uniform among the treatments, but even after 1 year hens on 32 p.p.m. Hg were laying an average of 13.5% more eggs than controls. Rate of egg fertilization was generally depressed for all Hg-treatments above 4 p.p.m. Hatchability of fertilized eggs and eggshell thickness appeared unaffected by mercuric chloride.

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INTRODUCTION

MERCURY (Hg) in trace amounts is a natural environmental component (Jonasson and Boyle, 1971), but the elevated levels sometimes associated with industrial or agricultural usage have proven toxic to a variety of life forms, including humans. Both the occurrence and deleterious effects of mercury have been well-documented and are summarized in various reviews (e.g., Friberg and Vostol, 1971; Nelson *et al.*, 1971; Wallace *et al.*, 1971; and Lambou, 1972).

Organic mercury, particularly some alkyl forms, has been of especial environmental importance to animals because it is virtually ubiquitous, is readily absorbed from the gastrointestinal tract, and can be quite toxic (Borg *et al.*, 1969; Lofroth, 1969; and Nelson *et al.*, 1971). Inorganic mercurials are usually less hazardous than organic forms, but since they can be microbially methylated in aquatic medium (Jensen and Jernelov, 1969; and Tonomura *et al.*, 1972) the eventual hazards are similar. Thus, organic mercury has been widely studied as an environmental pollutant while inorganic mercury has received less attention. Only a few studies of inorganic mercury have been reported for birds. Stoewsand *et al.* (1971) fed Japanese quail (*Coturnix c. japonica*) 1 to 8 p.p.m. Hg as mercuric chloride ($HgCl_2$) from hatching for 10 weeks and demonstrated egg shell thinning. Law *et al.* (1974) fed two generations of Japanese quail 1 p.p.m. of mercuric chloride without affecting egg shell thickness, but reported reduced hatchability. Their results are not convincing, however, because they also reported uncharacteristic embryonic mortality (i.e., first week deaths exceeded

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third week deaths by more than 2-fold) among all groups, including controls. Although there are normally two peaks of embryonic mortality during incubation, the late peak is well-known to be most pronounced. Dieter (1974) fed 2 to 8 p.p.m. of mercuric chloride to adult male Japanese quail for 12 weeks and reported decreased hemoglobin concentration and cholinesterase activity, but increased lactate dehydrogenase activity. Another series of experiments with mercuric chloride used drinking water as the vehicle for exposure. In those tests, 5 to 500 p.p.m. Hg were presented to young chickens (*Gallus gallus*) for 16 weeks. Negative results predominated up to the level of 125 p.p.m. Hg. Principal conclusions were as follows: no influences on water or feed consumption, growth, survival (Parkhurst and Thaxton, 1973), organ weights (Thaxton and Parkhurst, 1973b), immunological responsiveness (Thaxton and Parkhurst, 1973c), or selected blood chemistry parameters (Thaxton *et al.*, 1973). Similarly, water treated with mercuric chloride at 125 p.p.m. was fed to Japanese quail from hatching for 8 weeks and caused gonadal atresia and reduced numbers of mating attempts (Thaxton and Parkhurst, 1973a). In a 3-week study of mercury (II) nitrate [$Hg(NO_3)_2$], 16 p.p.m. caused slight weight loss among young Leghorn cocks (Swenson and Ulfvarson, 1969).

Because so little is known about the effects of long-term low-level exposure of birds to inorganic mercury, we conducted two Japanese quail reproduction experiments with mercuric chloride. The first experiment was conducted at the Patuxent Wildlife Research Center (P.W.R.C.) and the second, designed to amplify or substantiate the first with a second stock of birds, was carried out at the University of Maryland (U.M.D.). Feed consumption, growth rate, age at first oviposition, egg production, egg weight, egg shell thickness, fertility, and hatchability were studied.

PROCEDURES

Experiment 1. P.W.R.C. Japanese quail were fed *ad libitum* diets containing 0, 2, 4, and 8 p.p.m. Hg (as 0, 2.7, 5.4, and 10.8 p.p.m. $HgCl_2$) from hatching for 20 weeks. Initially 10 groups of 10 hatchlings per treatment were randomly established. At 3 weeks of age each group was reduced to 1 male and 3 females and was transferred to breeding batteries.

Each bird was weighed at 2-week intervals until 8 weeks of age, then monthly. Egg production was recorded daily. Egg shell thickness was determined for the first egg from each group and for one randomly selected egg from each group at 10-day intervals thereafter (air-dried egg shells, including membranes, were measured at the equator with a micrometer graduated in units of 10 μ.). Eggs from three 10-day collections (starting at 54, 74, and 124 days) were incubated to check fertility, embryonic development, hatchability, and chick survival. Gross necropsy was periodically performed on several birds from each treatment.

Statistical evaluations were by standard parametric methods (ANOVA, r, r^2) or the nonparametric Wilcoxon rank sum test, 2-sided, (Wilcoxon and Wilcox, 1964), as appropriate. Mean separations for significant F tests were by Duncan's (1955) procedure. The 5% level of significance was used, but values approaching significance are also indicated, e.g., $0.05 < P < 0.10$. The pen of birds was the basic sampling unit.

Breeding pens, constructed of wire-mesh (floors and doors) and galvanized sheeting (ceilings and partitions), measured 66 × 66 × 24.5 cm. high. The light regimen was 14L:10D. Ambient temperature was between 18 and 25° C. Feed and water were provided *ad libitum*. Eggs were incubated for 18 days at 37.5° C. and 60% relative humidity (R.H.). Maximum pre-incubation egg storage was for 10 days at 16° C. and 55% R.H.

Diets were prepared by mixing a solution of $HgCl_2$ and propylene glycol into turkey mash at the ratio of 1:99, by weight. Starter mash was provided until the birds were 6 weeks old, breeder mash was used thereafter. Control diets contained propylene glycol at the ratio of 1:99.

Experiment 2. Experiment 2 was similar to Experiment 1; therefore, only procedural differences are indicated herein.

U.M.D. Japanese quail were fed diets containing 0, 8, 16, and 32 p.p.m. Hg (as 0, 10.8, 21.7, and 43.3 p.p.m. $HgCl_2$) from hatching for 1 year. After 4 weeks of colonial maintenance, 35 breeding pairs were established in battery cages measuring 19.6 × 17.8 × 20.3 cm. high. Pair replication per treatment was as follows; controls 10; 8 p.p.m. Hg, 9; 16 and 32 p.p.m., 8 each. Bird weights and feed consumption were determined periodically to 16 weeks of age. During the same period, all eggs laid were weighed and checked for evidence of embryonation. Egg shell thickness was measured biweekly. Hatchability was determined for one 14-day egg collection starting at 70 days.

RESULTS AND DISCUSSION

No gross signs of intoxication were observed for Japanese quail fed mercuric chloride from hatching to 1 year of age. Mortality averaged less than 7% during brooding in both studies. There was no apparent relationship between deaths and treatment. After brooding (i.e., 3rd week), 6 of 160 birds died during Experiment 1 and 9 of 70 during Experiment 2. Birds that died in Experiment 2 were from all treatments and most were over 6 months old. Accidental strangulation by defective feeder guards was the principal cause of death. Feed consumption, growth, and weight maintenance were not affected by any of the treatments.

Hen maturation rates appeared to be correlated with the level of dietary mercuric chloride in both studies (Table 1). In Experiment 1, hens fed 8 p.p.m. Hg were younger on the average at first oviposition than the controls ($P < 0.05$) or hens fed lower levels of mercuric chloride ($P < 0.01$). Median and mean ages at first oviposition were related to the concentration of mercuric chloride as follows: 8 p.p.m. Hg < 4 p.p.m. Hg < 2 p.p.m. Hg. Because there were 3 hens in each pen, only the first egg in a pen was

Table 1.—*Age of Japanese quail hens at earliest oviposition (puberty egg) when fed $HgCl_2$ from hatching*

Treatment	Hens (n)	Age (days)		
		Median	Mean[1]	Extremes
Experiment 1 (P.W.R.C.):				
Control	30[2]	52	57.2a	43–133
2 p.p.m. Hg	30[2]	56	60.0aa	45–133
4 p.p.m. Hg	30	50	58.4aa	36–101
8 p.p.m. Hg	30	48	49.4A	35–75
Experiment 2 (U.M.D.):				
Control	9	50	49.6	42–56
8 p.p.m. Hg	7	53	52.7	46–63
16 p.p.m. Hg	7	49	47.3	36–52
32 p.p.m. Hg	7	44	46.3	36–60

[1]Means with letter A differ statistically from a ($P < 0.05$) and aa ($P < 0.01$).

[2]Two of 30 hens failed to lay during the experiment and are omitted from mean computations and ANOVA, but are included in median determinations.

TABLE 2.—*Egg production by P.W.R.C. Japanese quail fed $HgCl_2$ from hatching (Experiment 1)*

Treatment	Statistic[1,2]	Eggs per hen-day[3]	
		Adolescent (<64 days old)	Young-adult (64–123 days old)
Control	Mean	0.41aa	0.62a
	Extremes	0.31–0.54	0.48–0.84
2 p.p.m. Hg	Mean	0.43aa	0.59a
	Extremes	0.31–0.57	0.38–0.85
4 p.p.m. Hg	Mean	0.48a	0.68
	Extremes	0.29–0.69	0.47–0.88
8 p.p.m. Hg	Mean	0.66A	0.76A
	Extremes	0.40–0.97	0.60–0.91

[1]Statistics are based on 10 replicates (3 hens/replicate) per treatment.
[2]Means with letter A differ statistically from a ($P < 0.05$) and aa ($P < 0.01$) within a given column.
[3]Eggs per hen-day are derived by dividing total eggs laid per pen by total hen-days (days × hens) from first oviposition to 63 days of age or from 64 to 123 days of age.

definitely a puberty egg; therefore, puberty ages were assigned to the two remaining hens in a pen by the date that 2 or 3 eggs first appeared in their pen simultaneously. The estimated puberty ages of these latter hens may be slightly exaggerated because some puberty eggs were probably laid while another hen was pausing between clutches. In Experiment 2 median and mean puberty ages were again younger as the concentration of mercury increased. Comparison of control birds between the experiments showed the median age at puberty was 2 days younger in Experiment 2 than in Experiment 1. In contrast, hens fed 8 p.p.m. Hg in Experiment 2 were 5 days older than in Experiment 1. Overall, hens fed 32 p.p.m. Hg were youngest at first oviposition.

The highest rates of egg production were consistently by hens fed mercuric chloride (Tables 2 and 3). Usually increased production was positively correlated with increased levels of mercury. The differences were most pronounced during adolescence [period from sexual maturity (puberty egg) to physical maturity (full growth, ≈9 weeks of age)]. In Experiment 1 the rate during adolescence was greatest ($P < 0.05$ or $P < 0.01$) for hens

TABLE 3.—*Egg production by U.M.D. Japanese quail fed $HgCl_2$ from hatching for 1 year (Experiment 2)*

Treatment	Statistic[1,2]	Eggs					
		Adolescent[3]	Young-adult	Adult			
		(< 64)	(64–123)	(124–183)	(184–243)	(244–303)	(304–363)
Control	Mean	0.80aa	0.82	0.84b	0.82	0.80	0.72
	Extremes	0.43–0.92	0.53–0.95	0.67–0.97	0.62–0.97	0.65–0.97	0.60–0.97
8 p.p.m. Hg	Mean	0.76	0.82a	0.80a	0.84	0.72b	0.70
	Extremes	0.36–1.00	0.71–0.90	0.73–0.90	0.73–0.95	0.57–0.84	0.50–0.83
16 p.p.m. Hg	Mean	0.86	0.88	0.90A	0.86	0.80	0.80
	Extremes	0.75–1.06	0.73–0.98	0.82–0.97	0.63–0.93	0.70–0.90	0.63–0.85
32 p.p.m. Hg	Mean	0.93A	0.90A	0.93A,B	0.90	0.87B	0.84
	Extremes	0.83–1.00	0.82–0.97	0.73–1.03	0.78–0.98	0.77–0.97	0.60–0.98

[1]Statistics are based on individually housed hens. Replication at various parenthesized age periods for control, 8, 16, and 32 p.p.m. Hg, respectively, was: ≤63 days—8, 6, 7, and 6; 64 days—9, 7, 7, and 7; 184 days—8, 7, 6, and 7; 244 days—8, 6, 5, and 7; 304 days—5, 5, 5, and 6.
[1]Means with letter A differ statistically from a ($P < 0.05$) and aa ($P < 0.01$) within a given column; letter B approaches statistical differences from b ($0.05 < P < 0.10$).
[2]Eggs per hen-day were derived by dividing eggs laid by total days from first oviposition to 63 days of age (only hens laying ≥9 days are included).

fed 8 p.p.m. Hg (Table 2). Those hens averaged 61.0% more eggs than the control hens. After 9 weeks of age laying had increased for all groups, but the positive correlation between rate and dietary mercury persisted. Throughout Experiment 1 the least productive pen of hens fed 8 p.p.m. Hg laid at a rate nearly equal to the average rate for controls. In Experiment 2 the patterns of egg production were similar to those for Experiment 1, except the magnitude of differences between treatments was different (Table 3). For example, during adolescence the least productive hen (0.83 egg/hen-day) fed 32 p.p.m. Hg actually laid at a higher rate than the average control hen (0.80 egg/hen-day). Positive correlation between the rate of laying and increased dietary mercury continued throughout the entire 1-year study, but treatment differences were statistically inseparable after 6 months. Comparison of hens fed 8 p.p.m. Hg during both studies showed those in Experiment 2 did not perform as well in relation to the controls as those did in Experiment 1.

Egg fertility and the ultimate numbers of hatchlings decreased with increased exposure to mercuric chloride, even though egg production increased. This trend occurred with increased levels of mercury and with time. Egg fertilization decreased consistently from the first to last collection for birds fed 2 and 8 p.p.m. Hg during Experiment 1 (Table 4). Associated coefficients of variation (CV)

TABLE 4.—*Reproductivity of adolescent (<64 days old) and young-adult P.W.R.C. Japanese quail fed $HgCl_2$ from hatching (Experiment 1)*

Age (days)	Treatment	Replicates[1]	Statistic[2,3]	Eggs per hen-day	Percent fertilized	Percent hatchability	
						Fertile eggs	All eggs
54–63	Control	8	Mean	0.39aa	83.6	85.2	71.7A
			Extremes	0.05–0.57	64.3–100.0	72.7–91.7	50.0–90.0
	2 p.p.m. Hg	9	Mean	0.43aa	77.5	86.4	67.6B
			Extremes	0.30–0.60	61.5–100.0	71.4–100.0	38.5–90.0
	4 p.p.m. Hg	9	Mean	0.50aa	85.4	83.7	71.4A
			Extremes	0.13–0.73	68.8–100.0	50.0–100.0	42.1–100.0
	8 p.p.m. Hg	10	Mean	0.71A	70.1	76.7	51.3a,b
			Extremes	0.40–1.00	42.3–95.7	46.7–93.3	31.2–82.4
74–83	Control	8	Mean	0.60a	80.0	92.2	75.9
			Extremes	0.25–0.97	63.6–100.0	76.9–100.0	58.8–100.0
	2 p.p.m. Hg	9	Mean	0.63	74.1	90.6	68.8
			Extremes	0.15–0.93	66.3–100.0	68.8–100.0	6.3–100.0
	4 p.p.m. Hg	10	Mean	0.69	93.1B	90.2	85.0B
			Extremes	0.47–0.93	72.7–100.0	75.0–100.0	54.5–100.0
	8 p.p.m. Hg	10	Mean	0.77A	65.0b	91.1	56.6b
			Extremes	0.53–0.93	3.8–100.0	88.0–95.8	0.0–95.8
124–133	Control	7	Mean	0.60	81.1B	89.7	74.0A
			Extremes	0.27–0.93	60.0–100.0	71.4–100.0	53.3–100.0
	2 p.p.m. Hg	8	Mean	0.69	54.9b	87.7	49.8a,b
			Extremes	0.33–0.90	22.2–100.0	71.4–100.0	22.2–84.6
	4 p.p.m. Hg	9	Mean	0.73	83.8B	89.5	73.8B
			Extremes	0.37–0.90	68.2–92.3	62.5–100.0	55.6–92.3
	8 p.p.m. Hg	8	Mean	0.75	58.9b	86.2	51.5
			Extremes	0.30–1.00	9.1–93.8	68.4–92.3	0.0–87.5

[1]Eggs per hen-day are based on 10 replicates per treatment; where fewer than 10 eggs were laid, incubation was not attempted and replication was reduced accordingly for fertility and hatchability determinations.
[2]Statistics are based on 2- or 3-hen replicates; where hens per replicate are unequal, statistics are weighted.
[3]Means with letter A differ statistically from a ($P < 0.05$); superscript B approaches statistical difference from b ($0.05 < P < 0.10$) within a given column.

varied from 20.1 to 47.0% for the former group and 31.2 to 64.8% for the latter. In contrast, birds fed 4 p.p.m. Hg consistently had the highest rate of fertilization and the smallest CV's (<12.1%) of all groups, including controls. Egg fertility of controls was relatively uniform during the experiment as all CV's were less than 20%. Mercuric chloride, per se, did not appear to affect hatchability of fertilized eggs, but when the accumulative effects of factors affecting reproduction were combined (i.e., basing hatchability on all eggs incubated rather than just fertile eggs) statistical separation of treatments was possible.

Table 5 shows egg fertility and hatchability for a 14-day collection during Experiment 2. The collection coincides with the 74- to 83-day collection shown in Table 4. A general depression in the rate of egg fertilization and overall hatchability was correlated with increased dietary mercuric chloride. Hatchability of fertile eggs appeared unaffected by the treatments. Compared to controls, overall hatchability was depressed by 16.1, 25.2, and 25.9% for 8, 16, and 32 p.p.m. Hg groups, respectively. Though the magnitude of these differences appear large, statistical separation could not be made.

Comparing Experiments 1 and 2 by the performance of controls shows hatchability was lower during Experiment 2, even though the rate of fertilization was higher. An average of 92.2% of the fertile eggs from controls hatched in Experiment 1 (74–83 days), but only 77.2% in Experiment 2. As

TABLE 5.—*Reproductivity of young-adult U.M.D. Japanese quail fed $HgCl_2$ from hatching (Experiment 2)*

Age (days)	Treatment	Hens (n)	Statistic[1]	Eggs per hen-day	Percent fertilized	Percent hatchability[2] Fertile eggs	Percent hatchability[2] All eggs
70–83	Control	9	Mean	0.83	92.7B	77.2	74.9
			Extremes	0.43–1.00	57.1–100.0	25.0–100.0	14.3–90.0
	8 p.p.m. Hg	7	Mean	0.77	84.7	85.0	62.8
			Extremes	0.50–0.93	42.9–100.0	61.5–100.0	0.0–100.0
	16 p.p.m. Hg	7	Mean	0.90	80.0	78.1	56.0
			Extremes	0.70–1.00	30.0–100.0	53.8–91.7	0.0–91.7
	32 p.p.m. Hg	7	Mean	0.92	73.0b	76.4	55.5
			Extremes	0.86–1.00	0.0–100.0	41.7–100.0	0.0–100.0

[1]Means with letter B approach statistical difference from b ($0.05 < P < 0.10$).
[2]Samples containing fewer than 7 fertile eggs are omitted from hatchability computations.

TABLE 6.—*Egg fertilization by paired U.M.D. Japanese quail fed $HgCl_2$ from hatching (Experiment 2)*

Treatment	Hens	Statistic[1]	Percent fertilized[2] Adolescent (<64 days)	Young-adult (64–78 days)	Young-adult (79–93 days)	Young-adult (94–108 days)
Control	9	Mean	62.4A	84.8B	89.6A	87.3A,B
		Extremes	17.6–100.0	61.5–100.0	23.1–100.0	30.8–100.0
8 p.p.m. Hg	7	Mean	29.5a	86.0	76.2	55.1a
		Extremes	12.5–66.7	33.3–100.0	15.4–100.0	35.7–85.7
16 p.p.m. Hg	7	Mean	46.1B	86.6	77.0	66.2b
		Extremes	10.5–73.1	50.0–100.0	40.0–100.0	0.0–100.0
32 p.p.m. Hg	7	Mean	23.2a,b	73.4b	73.9a	58.1a
		Extremes	0.0–52.0	35.7–100.0	23.1–100.0	0.0–100.0

[1]Means with letter A differ statistically from a ($P < 0.05$) within a given column; letter B approaches statistical significance from b ($0.05 < P < 0.10$).
[2]Percent fertilized is derived by dividing fertile eggs by total normal eggs. Fertility was determined by dissection after 3 days of incubation.

TABLE 7.—*Hatchlings per hen-day for Japanese quail fed $HgCl_2$ from hatching*

Statistic[1]	Treatment	Experiment 1 (P.W.R.C.) Rep.[1]	Age (days) 54–63	Rep.[1]	74–83	Rep.[1]	124–133	Experiment 2 (U.M.D.) Treatment	Hens	Age (days) 70–83
Mean	Control	8	0.31	8	0.47	7	0.45	Control	9	0.61
Extremes			0.22–0.45		0.19–0.63		0.27–0.74			0.14–0.79
Mean	2 p.p.m. Hg	9	0.28	9	0.46	8	0.38	8 p.p.m. Hg	7	0.49
Extremes			0.19–0.43		0.04–0.79		0.07–0.68			0.00–0.93
Mean	4 p.p.m. Hg	9	0.37	10	0.59	9	0.52	16 p.p.m. Hg	7	0.52
Extremes			0.24–0.60		0.26–0.82		0.20–0.68			0.00–0.85
Mean	8 p.p.m. Hg	10	0.36	10	0.45	8	0.43	32 p.p.m. Hg	7	0.51
Extremes			0.15–0.51		0.00–0.83		0.00–0.88			0.00–0.93

[1]Statistics are based on 2- or 3-hen replicates; where hens per replicate are unequal, statistics are weighted.

storage and incubation facilities were the same for both studies, duration of egg storage probably contributed to the differences in hatchability. In Experiment 1 maximum storage was 10 days pre-incubation and in Experiment 2 it was 14 days. Mirosh and Becker (1974) report that best results are obtained with Japanese quail eggs stored for 1 week or less. This has also been shown for domestic fowl (Landauer, 1967).

Because egg fertilization decreased with increased levels of mercuric chloride in Experiment 1, embryonation was checked for all eggs laid through 108 days of age in Experiment 2. Table 6 shows increased levels of mercury were associated with depressed egg fertilization for pairs fed 8 and 32 p.p.m. Hg. The depression was most pronounced during adolescence. At that time egg fertilization for hens fed 32 p.p.m. Hg was 62.8% below that of the controls ($P < 0.05$). Fertility then increased for all treatments between 64 and 78 days of age. Thereafter, only the fertility of control eggs remained high (>85%) and that for mercury-treated groups decreased linearly. The depression in fertility was severe between 94 and 108 days of age.

Hatchlings per hen-day, representing total reproductiveness, were not statistically separable among treatments during either experiment (Table 7). That differences were not separable is important because it demonstrates that the positive influence of mercuric chloride on egg production was offset by reduced egg fertilization. The groups fed 4 p.p.m. Hg during Experiment 1 were an exception to this counterbalance. They averaged about 19.5% more hatchlings per hen-day than any other group, including controls. In Experiment 2 hatchling production was essentially the same for all mercury treatments. Again, this was because egg production was positively related to the level of mercury, and egg fertility was negatively related. Controls in Experiment 2 averaged 20.5% more hatchlings per hen-day than the

TABLE 8.—*Weight (g.) of eggs from U.M.D. Japanese quail at various ages after being fed $HgCl_2$ from hatching (Experiment 2)*

Variate	Statistic[1,2]	Treatment			
		Control	8 p.p.m. Hg	16 p.p.m. Hg	32 p.p.m. Hg
First egg[3]	No. hens	7	7	7	6
	Mean	7.9B	7.3	7.1	6.9b
	Extremes	6.6-8.8	3.5-9.7	4.2-8.9	4.8-8.9
	r^2	0.106	0.376	0.612*	0.880*
Fifth egg[4]	Mean	8.7	8.5	8.0	8.1
	Extremes	7.7-9.5	7.2-9.5	6.2-9.2	6.5-9.1
	r^2	0.017	0.386	0.634*	0.704*
Tenth egg[5]	Mean	9.2A	9.1B,C	7.9a,c	8.2a,b
	Extremes	7.1-10.2	7.9-10.6	6.7-9.4	7.1-9.3
	r^2	0.001	0.561	0.176	0.501
64 days old	Mean	9.0	8.9	8.3	8.6
	Extremes	7.6-10.3	7.7-9.9	7.2-9.2	7.9-9.8
79 days old	Mean	9.5	9.4	8.9	9.1
	Extremes	8.2-11.2	7.9-10.7	8.3-9.9	8.5-10.0
94 days old	Mean	10.0	9.3	9.3	9.4
	Extremes	8.5-11.4	8.0-10.6	8.2-10.1	8.8-9.8
109 days old	Mean	10.6A,B	9.7b	9.2aa	9.6a
	Extremes	8.9-11.7	8.3-11.0	8.4-10.6	8.8-10.7

[1]Statistics are based on individually housed hens. Coefficient of determination (r^2) is derived from regression analysis of the weight (y) of the 1st, 5th, and 10th egg on the age of the hen (x) when the egg was laid. Statistical significance is for r (*$P < 0.05$, **$P < 0.01$).

[2]Means with letter A differ statistically from a ($P < 0.05$) and aa with ($P < 0.01$) within a given row and C from c ($P < 0.05$); letter B approaches statistical significance from b ($0.05 < P < 0.01$).

[3]Age (mean, extremes) at first egg: control (49, 42-56); 8 p.p.m. Hg (53, 46-63); 16 p.p.m. Hg (47, 36-52); and 32 p.p.m. Hg (46, 36-60).

[4]Age at fifth egg: control (55, 46-72); 8 p.p.m. Hg (59, 52-71); 16 p.p.m. Hg (52, 41-59); and 32 p.p.m. Hg (52, 40-71).

[5]Age at tenth egg: control (61, 51-68); 8 p.p.m. Hg (65, 57-76); 16 p.p.m. Hg (58, 47-64); and 32 p.p.m. Hg (57, 46-76).

mercury-treated birds.

Eggs were weighed during Experiment 2 to determine if their sizes were influenced by early laying. Weights of the first (puberty), fifth, and tenth eggs laid and those laid at 64, 75, 94, and 109 days of age are shown in Table 8. Puberty eggs tended to be lighter as the mercury level increased. The relationship was linear. The linearity between puberty egg weights and mercury levels was not evident by the fifth egg, but the eggs of control birds and those of hens on 8 p.p.m. Hg were clearly heavier than the eggs of hens on 16 and 32 p.p.m. Hg. Beyond 79 days of age, hens on all mercury diets were laying similarly sized eggs which averaged about 7% lighter than control eggs. By 109 days of age, the differences between egg weights for control hens were statistically separable from all mercuric chloride treatments.

Marked correlation between egg size and age occurred during adolescence for the earliest layers (16 and 32 p.p.m. Hg), as indicated by significant coefficients of determination (r^2) of 61 to 88%. By the tenth egg, r^2 was no longer significant for 16 and 32 p.p.m. Hg treated birds, but their eggs remained significantly lighter ($P < 0.05$) than control eggs. The persistency with which light eggs were laid by hens fed mercury was possibly due to their high rate of laying.

Egg shell thickness was not changed significantly by mercuric chloride in either study, nor was there consistent evidence of any dose-response relationship (Table 9). The maximum difference in thickness between egg shells from controls and hens fed mercury was 6.1%. Stoewsand *et al.* (1971) reported statistically significant egg shell thinning ($\bar{x}$ = 8.1%) for 8- to 10-week-old Japanese quail after being fed mercuric chloride (8 p.p.m. Hg) from hatching. Our studies did not confirm their findings (cf. 64 days of age).

We have shown that Japanese quail reproduction can be influenced by mercuric chloride when fed *ad libitum* from hatching at relatively low (4-32 p.p.m. Hg) concentrations. The effects, most pronounced through young adulthood, were contradictory: some were stimulatory (onset and rate of oviposition), others were depressive (egg size, fertility, and hatchability).

The general reproductive responses of Japanese quail to mercuric chloride were related to mercury concentration as follows: 2 p.p.m. Hg, generally comparable to controls; 4 p.p.m. Hg, generally stimulatory; and 8 to 32 p.p.m. Hg, significantly stimulatory or significantly depressive with one effect counterbalancing the other.

Net reproductivity (healthy hatchlings/hen-day) is the result of ultimate concern. It integrates all events from ovulation to production of viable progeny. With this in mind, it appears that mercuric chloride was both helpful and harmful. Positive influences were associated with 4 p.p.m. Hg because at that concentration no toxic signs were observed in the parent colony, the rate of egg production was high, egg fertility was excellent, and normal hatchability followed. Because no medicament was incorporated into the diet (to avoid possible potentiation), and because mercuric chloride has bacteriostatic and fungicidal activity (Esplin, 1970), it is possible that 4 p.p.m. Hg provided an effective antibiotic action, thereby enhancing the health of the birds. However, it is also well-known that small doses of certain highly toxic compounds can be stimu-

TABLE 9.—*Shell thickness (μ.) of eggs from Japanese quail fed $HgCl_2$ from hatching*

Treatment	n	Statistic	Age (days)			
			First egg	64	94	124
Experiment 1 (P.W.R.C.):						
Control	10[1]	Mean	207	211	206	198
		Extremes	190-220	190-230	190-240	170-230
2 p.p.m. Hg	10	Mean	208	204	207	195
		Extremes	190-230	190-220	190-230	170-210
4 p.p.m. Hg	10	Mean	212	214	207	194
		Extremes	180-240	190-250	180-220	160-220
8 p.p.m. Hg	10	Mean	210	206	199	186
		Extremes	190-240	180-230	180-220	160-210
Experiment 2 (U.M.D.):						
Control	9[2]	Mean	200	218	216	—
		Extremes	160-210	203-240	197-240	
8 p.p.m. Hg	7	Mean	199	220	223	—
		Extremes	160-240	203-247	200-267	
16 p.p.m. Hg	7	Mean	201	220	214	—
		Extremes	180-230	200-237	197-230	
32 p.p.m. Hg	7	Mean	200	210	205	—
		Extremes	170-220	180-230	177-230	

[1]Statistics based on 1 randomly selected egg from each of 10 replicates (3 hens/replicate).

[2]Statistics for 64- and 94-day-old hens based on mean of 3 sequential eggs from each hen.

latory or even essential to an organism's survival, e.g., selenium. The concept of stimulation by small doses has been discussed extensively by Townsend and Luckey (1960), Smyth (1967), and Dinman (1972).

Depressed net reproductivity was associated with 8 to 32 p.p.m. Hg, even though egg production was stimulated. Inability to fertilize eggs was the principal deleterious event associated with the depression. Because unrestricted natural breeding was allowed and hatchability of fertilized eggs was unaffected, it is likely that the principal harmful effect of mercuric chloride was interference with spermatogenesis, sperm survival, or mating behavior. These parameters were not measured, but no testicular anomalies were seen during necropsy.

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The True Metabolizable Energy Values of Several Feedingstuffs Measured with Roosters, Laying Hens, Turkeys and Broiler Hens

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ABSTRACT Three experiments were made to measure the effect of the type of the assay bird on the true metabolizable energy (T.M.E.) values of certain feedingstuffs. In the first experiment wheat and oats were assayed with S.C.W.L. roosters and hens of different strains. The grains differed in T.M.E. content but the values obtained with roosters and laying hens were not different. In the second experiment yellow corn, soybean meal, wheat shorts and fish meal were assayed using S.C.W.L. roosters, S.C.W.L. laying hens of a different strain, and adult Small White turkey hens. The T.M.E. value of the soybean meal was greater ($P < 0.01$) for the turkeys than for the chickens; no other differences between birds was observed. In the final experiment a laying hen diet and wheat bran were assayed using broiler hens in addition to the three types of birds used in the second experiment. No differences attributable to the type of assay bird were observed.

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INTRODUCTION

THE apparent metabolizable energy (A.M.E.) values of feedingstuffs may vary with the type of bird used in the bioassay. Differences between the A.M.E. values obtained with chicks and turkeys have been reported by Slinger *et al.* (1964), Bayley *et al.* (1968), Fisher and Shannon (1973), and Leeson *et al.* (1974). A comparison between bantam chickens and blue-winged teal has also revealed species differences (Sugden, 1974). However, experiments with quail and chickens have failed to show significant species effects (Begin, 1968; Hoshii *et al.*, 1970). Small differences between the A.M.E. values obtained with various strains of chickens have been reported by Sibbald and Slinger (1963), Slinger *et al.* (1964), Foster (1968a, b), Proudman *et al.* (1970), and March and Biely (1971); however, the strain differences observed by Stutz and Matterson (1963) and Begin (1967, 1969) were not significant. The age of the assay bird may also have an effect upon observed A.M.E. values (Renner and Hill, 1960; Lockhart *et al.*, 1963; Bayley *et al.*, 1968; Zelenka, 1968; Lodhi *et al.*, 1969, 1970; Rao and Clandinin, 1970). Work with chicks has failed to reveal differences attributable to sex (Sibbald and Slinger, 1963; Begin, 1967).

Variation in A.M.E. values associated with the type of assay bird is usually small; however, it is a cause for concern because

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