EFFECT OF SELENIUM AND MERCURY ON SURVIVAL OF CHICK EMBROYS

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ABSTRACT Factorial experiments were arranged in a completely randomized or randomized block design. The factors included: selenium and day of injection; mercury and day of injection; selenium and mercury; and selenium, mercury and day of injection. Each treatment factor consisted of several levels, selenium ranged from 0.00 p.p.m. to 0.05 p.p.m., mercury from 0.00 p.p.m. to 0.30 p.p.m., and injection was performed on day-3, 9, and 15 of incubation. Babcock-300, and White Leghorn × New Hampshire cross eggs were obtained from 13-15 month old hens. Mercury was injected into the air cell at 4 or 24 hours after selenium injection.

Analysis of variance on arcsine transformed data showed that selenium significantly decreased survival at all 3 injection times ($P < 0.01$). Survival was significantly greater with increasing age at injection ($P < 0.01$).

Survival of embryos significantly decreased ($P < 0.01$) with increasing levels of mercury from 0.00 p.p.m. to 0.20 p.p.m. injected into eggs on day-3 of incubation. Survival of embryos injected at later stages was less than that of controls but not significantly less. Injection of low levels of selenium, 0.01 p.p.m. or 0.02 p.p.m., to mercury treated eggs tended to improve the survival of embryos as compared to treatment with mercury alone, although individual differences were not significant. At higher levels, selenium accentuated the toxicity of mercury.

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INTRODUCTION

REVIEWS of selenium poisoning in poultry and domestic animals have been given by Moxon (1937), Moxon and Rhian (1943), Frost (1972), and Harr and Muth (1972).

The survival rate of embryos was low when eggs were injected with less than one p.p.m. selenium as sodium selenite (Franke *et al.*, 1936; Moxon and Poley, 1938).

The estimated LD_{50} value for sodium selenite injected into the air cell prior to incubation was 0.7 p.p.m. (Franke *et al.*, 1936). By the injection of sodium selenite into the air cell on day-4 of incubation the LD_{50} value was found to be 0.3 p.p.m. (Palmer *et al.*, 1973). The value obtained was based on observation at the end of the incubation period and birds at the live-pip stage were counted as live chicks. At a level of 0.1 mg./egg (approximately 2 p.p.m.) injected into the yolk sac prior to incubation, no embryos developed to more than day-5 (McLaughlin, 1963).

The survival rate of embryos has also been studied by the injection of potassium selenite into the air cell on day-14 of incubation and the eggs were broken 64-68 hours after the injection (Halverson *et al.*, 1965). The ratio between the number of survivors and the number of eggs injected decreased from 15/16 to 1/16 when the doses increased from 0.3 to 0.8 p.p.m. The LD_{50} value was found to be 0.5 p.p.m.

The LD_{50} values of selenium as selenious acid injected into the yolk sac on day-4 and

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day-8 of incubation were found to be 0.3-0.4 p.p.m., and one p.p.m., respectively (Ridgeway and Karnofsky, 1952). The LD_{50} value was about 0.2-0.3 p.p.m. when it was injected into the chorioallantoic membrane on day-8 of incubation.

McLaughlin *et al.* (1963) who injected mercuric chloride into the yolk sac of eggs prior to incubation reported that at 0.5 mg./egg (approximately 10 p.p.m.), all embryos died at the beginning of incubation.

Ridgeway and Karnofsky (1952) studied the toxicity and teratogenicity of metals by the injection into the yolk sac on day-4 and day-8 of incubation. On the basis of these studies they did not classify mercury as teratogenic in chick embryos. They believed, however, that mercury is not completely free of teratogenic activity.

Parizek and Ostadalova (1967) were the first who showed that in rats, sodium selenite has a protective effect against renal necrosis caused by simultaneous injection of mercuric chloride. When subcutaneous injection of 0.02 mmole mercuric chloride per kilogram body weight was followed by injection of 0.03 mmole sodium selenite per kilogram body weight after a one hour interval, the survival observed on day-7 after the selenite treatment was 97.5 percent as compared to 3.3 percent for rats receiving only mercuric chloride.

Ganther *et al.* (1972) found that Japanese quail (*Coturnix coturnix japonica*) fed 20 p.p.m. mercury in a cornsoya diet were intoxicated at 4 weeks and had a 52 percent mortality in the 4-6 weeks period. However, those fed the same concentration of mercury in a diet containing 17 percent of tuna fish were free of symptoms of intoxication for a longer time and only 7 percent died in the same period. Tuna fish contains enough selenium (2-3 p.p.m.) to reduce the toxic level of methyl mercury. A progress report presented by Ganther and Sunde (1974) indicated that one p.p.m. methyl mercury in the tuna diet did not impair egg production or hatchability, or cause malformation of embryos. Evidence that selenium protected against methyl mercury intoxication in quail has also been reported by Stoewsand *et al.* (1974). El-Begearmi and Sunde (1974) found that a combination of selenium, arsenic, and methionine decreased mercury toxicity in quail. Mercury tends to reduce the toxic effect of selenium. The reversal effect of mercury on selenium has been demonstrated by Parizek *et al.* (1971), Groth *et al.* (1973), Hill (1974), and El-Begearmi *et al.* (1973).

The objectives of the present study were to measure toxicity of selenium and mercury injected into eggs at different stages of incubation, and to learn whether either of these elements, when injected into the egg, counteracts the toxic effect of the other in the same manner as when both are provided in the diet.

MATERIALS AND METHODS

The experiments were conducted at the Faculty of Veterinary Medicine and the Faculty of Animal Husbandry of the Bogor Agricultural University, and at the Department of Poultry Science, University of Wisconsin—Madison.

Babcock-300 and White Leghorn × New Hampshire cross eggs from 13-15 month old hens were obtained from P. T. Prihadi Farm and Hatchery, and the Poultry Research Laboratory of the University of Wisconsin, respectively. The eggs were incubated in forced-draft incubators. During these periods they were kept under proper conditions with reference to temperature, humidity, air ventilation, and turning the eggs.

Factorial experiments in this study were arranged in a completely randomized or randomized complete block design. The factors included: selenium and day of injection; mercury and day of injection; selenium and mercury; and selenium, mercury and day of

TABLE 1.—*Plan of experiments 3 to 6*

Experiment	Strain	Eggs injected no.	Se levels no.	Hg levels no.	Day of injection	Time between Se and Hg injection, hours	Location
3	Babcock 300	2520	6	7	3	24	Bogor
4	N.H. × S.C.W.L.	540	3	3	3	24	Madison
5	N.H. × S.C.W.L.	540	3	3	3	4	Madison
6	Babcock 300	2880	4	4	3,9,15	24	Bogor

injection. Each factor consisted of several levels; selenium ranged from 0.00 to 0.05 p.p.m., mercury from 0.00 to 0.30 p.p.m., and injections were performed on day-3, 9, and 15 of incubation.

The procedure of injection was as follows: the fertile eggs, placed on egg trays with the blunt end up, were swabbed with 70 percent ethyl-alcohol before being punched with a sharp probe. The solution was layered on the surface of the inner shell membrane with a plastic tuberculin syringe. The hole in the egg shell was sealed with collodion. The injection was undertaken randomly for each dose and under aseptic conditions insofar as was possible.

The strengths of the solutions expressed in parts per million (p.p.m.) were based on the average weight of albumen and yolk. The volume of solution of different concentrations to be injected into eggs was kept constant at 0.10 ml. The eggs of the control groups were injected with 0.10 ml. physiologic salt solution, similar to those of treated groups. The solutions, sterilized by steam heat, were made just before they were used. Selenium was in the form of sodium selenite (Nutritional Biochemical Corporation, Cleveland, Ohio), and mercury was in the form of mercuric chloride (Merck and Co., Rahway, N.J.).

The injected eggs were candled on alternate days after the injection, up to hatching time, and the dead embryos were collected.

Table 1 summarizes information on location, materials, and procedures for Experiments 3 to 6. Similar details for experiments 1 and 2 are presented with the experimental results in following tables. Experiments 1 and 2 were conducted with Babcock-300 embryos at Bogor.

The LD_{50} values were calculated according to Goulden (1961). Data on embryos surviving and chicks hatched were subjected to analysis of variance to evaluate the effects of selenium, mercury and/or day of injection and their interactions. The data in percent were transformed into arcsin before they were analyzed (Snedecor and Cochran, 1968). Only if several linear relationships of the simple effects of mercury within various doses of selenium, and conversely, several linear effects of selenium over all levels of mercury were significant, were the data further analyzed by the use of the covariance method to evaluate the respective coefficients of regression. Duncan's multiple range test was used to compare the treatment means when the slopes of the regression of various simple effects were the same.

RESULTS AND DISCUSSION

Experiment 1. The survival data are summarized in Table 2. The analysis of variance showed that the main effects of day of injection, selenium, and their interactions are significant. The survival of embryos significantly improved ($P < 0.01$) in a linear trend with increasing age at treatment. Conversely,

TABLE 2.—*Mean survival of embryos to hatching after injection of selenium on day-3, 9 or 15 of incubation (%). Experiment 1*

Selenium (p.p.m.)	Day of injection		
	Day-3	Day-9	Day-15
0.00	66.66	83.33	90.00
0.01	46.66	80.00	83.33
0.02	33.33	83.33	53.33
0.03	16.66	30.00	36.66
0.04	23.33	20.00	13.33

the injection of selenium into eggs at various ages significantly decreased ($P < 0.01$) the survival of embryos.

The LD_{50} values of the chemical injected on day-3, 9, and 15 of incubation approximated 0.03 p.p.m. It has been reported that the LD_{50} of selenium injected at day-4 of incubation was 0.30 p.p.m. (Palmer *et al.*, 1973), but in that study the live-pip chicks were counted as alive. In this study they were counted as dead.

Experiment 2. In this experiment the chicks hatched were kept for one month for survival study. Survival of embryos is summarized in Table 3, survival of chicks to 30 days post-hatching in Table 4. The survival of embryos significantly decreased ($P < 0.01$) in linear trend with increasing levels of mercury from 0.00 to 0.20 p.p.m. injected on day-3, but the survival of the older embryos was not significantly affected (Table 3). The survivors of 0.20 p.p.m. mercury showed a quadratic trend ($P < 0.01$) with increasing age of embryos.

TABLE 3.—*Mean survival of embryos to hatching after injections of mercury on day-3, 9 or 15 of incubation (%). Experiment 2*

Mercury (p.p.m.)	Day of incubation		
	Day-3	Day-9	Day-15
0.00	93.33	86.66	90.00
0.05	60.00	66.66	70.00
0.10	53.33	56.66	66.66
0.15	43.33	60.00	66.66
0.20	26.66	83.33	80.00

The LD_{50} values of mercury injected into eggs on day-3, 9, and 15 of incubation were 0.10, 0.35, and 0.81 p.p.m., respectively. The result was in good agreement with the report of Ridgeway and Karnofski (1952) who injected mercury as mercuric chloride into the yolk sac on day-4 and day-8 of incubation. It appeared that mercury toxicity in chick embryos showed a similar trend irrespective of the route of injection. Older embryos are definitely more tolerant. Survival of chicks from eggs injected on day-3 and day-9 of incubation was not significantly affected (Table 4).

Survival of chicks treated with mercury on day-15 of incubation showed a quadratic trend ($P < 0.01$) but its significance rests entirely on the response of one treatment group and would require further investigation before being accepted.

Experiments 3, 4, 5 and 6. For Experiment 3, the survival data are given in detail in Table 5. The main effects of selenium, mercury and their interactions were significant. The nature of the interaction was that higher levels of either element intensified the toxic effect of the other. There was no indication of a counteracting effect at higher levels.

In Table 6 are summarized the results of administration of low levels of selenium and mercury in the 4 experiments. There are 18 possible comparisons in which 0.01 or 0.02 p.p.m. of selenium was added to 0.05 or 0.10 p.p.m. of mercury. In 13 of the 18 comparisons the survival was higher with the added

TABLE 4.—*Mean survival to one month post hatching after injection of mercury on day-3, 9 or 15 of incubation (%). Experiment 2*

Mercury (p.p.m.)	Day of injection		
	Day-3	Day-9	Day-15
0.00	94.81	91.25	91.66
0.05	78.25	79.56	82.17
0.10	86.30	81.34	69.04
0.15	87.50	95.53	66.31
0.20	81.94	94.35	57.80

TABLE 5.—*Mean survival of embryos to hatching after injections of selenium and mercury (%). Experiment 3*

Selenium (p.p.m.)	Mercury (p.p.m.)						
	0.00	0.05	0.10	0.15	0.20	0.25	0.30
0.00	76.22	39.07	35.08	25.26	8.77	17.84	10.72
0.01	78.33	46.58	46.27	17.01	20.12	6.85	12.48
0.02	67.54	55.26	36.26	16.93	23.50	20.60	17.21
0.03	55.31	48.40	20.48	15.02	21.31	5.55	8.70
0.04	37.84	26.10	19.44	16.43	9.58	6.48	15.46
0.05	32.24	23.93	25.44	18.18	19.86	11.47	11.43

selenium. However, in individual comparisons the low levels of selenium did not significantly improve survival of mercury treated embryos. In Experiments 4 and 5 only relatively low levels of the two elements were fed. The statistical analyses showed no significant interaction.

The data in Table 6 show that selenium injected alone gave a different trend than selenium injected with mercury. In 5 of 6 comparisons survival was less with 0.02 p.p.m. of selenium than with no selenium, and in 3 of 6 comparisons it was less with 0.01 p.p.m. than with none. These data indicate that the untreated eggs were not selenium deficient.

Evidence that selenium protected against intoxication of mercury has been reported in quail (Ganther *et al.*, 1972), and in rats (Ganther *et al.*, 1972; and Iwata *et al.*, 1974). The reversal effects of mercury on selenium toxicity have also been described by several authors (Parizek *et al.*. 1971; Groth *et al.*, 1973; Hill, 1974; and El-Begearmi *et al.*, 1973). Our results with incubating eggs showed a trend in the same direction although individual differences were not significant. Possibly the interaction of low levels of the two elements was partially masked or prevented by absorption of one or both by the albumen or yolk.

In Experiment 6, higher levels of mercury

TABLE 6.—*Mean survival of embryos to hatching after injections of selenium and mercury (%)*

Experiment	Day of injection	Selenium (p.p.m.)	Mercury (p.p.m.)		
			0.00	0.05	0.10
3	3	0.00	76.22	39.07	35.08
		0.01	78.33	46.58	46.27
		0.02	67.54	55.26	36.26
		0.03	55.31	48.40	20.48
4	3	0.00	90.66	56.00	27.00
		0.01	86.33	54.00	41.66
		0.02	78.33	52.33	42.00
5	3	0.00	83.00	71.33	74.66
		0.01	92.33	82.33	67.66
		0.02	83.33	78.00	67.33
6	3	0.00	82.71		22.16
		0.01	70.72		30.70
		0.02	65.51		25.05
		0.03	59.68		31.67
6	9	0.00	78.33		78.33
		0.01	86.66		85.00
		0.02	70.00		63.25
		0.03	81.31		66.48
6	15	0.00	94.82		77 19
		0.01	93.33		91.66
		0.02	89.91		93.07
		0.03	89.82		88.24

were given, in addition to those shown in Table 6. A level of 0.20 p.p.m. mercury, with no selenium, injected on day 3, 9, or 15 of incubation, led to survivals of 10, 80, or 83 percent, respectively. A level of 0.30 p.p.m. mercury with no selenium on day 3, 9, or 15 led to survivals of 9, 73, or 79 percent, respectively. The main effects of day of injection, mercury level, and their interaction were significant in confirmation of the results of Experiment 2 (Table 3).

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