Toxicity of Metals to Chick Embryos

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It is apparent from numerous investigations that appreciable levels of metallic toxicants are included in the diets of many natural and domestic avian species (FIMREITE 1971, MILLER AND BERG 1969). Natural avian populations are subject to a wide variety of metal contaminants which are distributed to all facets of their ecosystem--air, water and soil. Automobile emissions, smelting, metal plating and use of fossil fuels are but a few of the many sources of metallic pollutants which may accumulate in natural food chains. Numerous reports indicate that such metals as cadmium and mercury reach concentrations of 0.01-1.0 ppm in various fruits, grains and animal tissues which serve as natural food sources for avian species (WALLACE et al. 1971, D'ITRI 1972, FRIBERG et al. 1971). Wheat grown on soil containing 0.001-0.003% cadmium has been shown to concentrate this metal to levels of 8-15 ppm (FRIBERG et al. 1971). The extent of metal accumulation in animal tissues (WALLACE et al. 1971, PLUMMER AND BARTLETT 1975) suggests that the use of fish, bone and whale meals used as feed supplements may further increase dietary metal intake for poultry and other domestic avian species.

It has been well established that subacute exposure to certain metallic toxicants adversely affects reproduction in birds. Methyl and inorganic mercury administered to feed grains may reach 1 ppm or more in eggs, markedly reducing hatchability (TEJNING 1967, BACKSTROM 1969, FIMREITE 1971). Administering mercurial compounds subcutaneously to laying hens, KUWAHARA (1970) found that approximately 60-80% of the mercury was accumulated in the eggs. BACKSTROM (1969) treated quail with intravenous doses of methyl mercuric nitrate, recovering 40-53% of the mercury from the eggs. Selenium also is known to accumulate in chicken eggs at levels which reduce hatchability (FRANKE et al. 1936). Considering the tendency of birds to concentrate ingested metals in eggs, and the high sensitivity of avian embryos to trace metals (RIDGWAY AND KARNOFSKY 1952, BIRGE AND JUST 1974), it appears likely that numerous metallic toxicants may adversely affect avian reproduction. The principal objective of the present study is to provide a comparative index to the embryopathic effects of a number of metals which may appear as trace contaminants in avian food sources.

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MATERIALS AND METHODS

Chicken eggs (White Plymouth Rock strain) were obtained from the Poultry Department, University of Kentucky. Immediately prior to incubation, eggs were treated by yolk injection with sodium arsenite, sodium selenate and chloride salts of cadmium, lead, mercury, methyl mercury and zinc. Metals were administered in 0.1 ml aliquots of distilled, deionized water at concentrations calculated to dilute egg yolk volume to test concentrations ranging from 1.0 ppb to 50.0 ppm. Based on measurements of 200 eggs, mean yolk mass and standard error were 18.41 ±0.10 gm. Concentrations of test solutions were based on metal content and were confirmed analytically using a Perkin-Elmer atomic absorption spectrophotometer (model 503). Total mercury was determined by the cold vapor technique, and other metals were analyzed by flameless AAS, using a model HGA-2100 graphite furnace (PERKIN-ELMER 1973).

Prior to yolk injection eggs were positioned horizontally for 24 hours, allowing the embryonic blastodisc to orient away from the point of needle entry. The test aliquot administered to each egg was deposited in a needle track extending through the diameter of the egg yolk, using a 27 gauge, 1¼ inch hypodermic needle. The needle was inserted through the blunt end of the egg after cleansing with an alcohol swab. Subsequent to injection, the entry point was sealed with paraffin. Eggs were then transferred to a forced-draft incubator maintained at a temperature of 38° C and a relative humidity of 60-65%. During incubation, eggs were rotated 4 times daily.

As previously confirmed by Anger scintillation photographic analysis of ^{203}Hg-injected eggs, the needle track procedure greatly facilitates the uniform distribution of the toxicant throughout the egg yolk (BIRGE AND JUST 1974). Also, distilled water is the preferred carrier fluid for administering metallic toxicants to avian eggs (BIRGE AND JUST 1974). It does not affect hatchability adversely and it provides the least change in the composition of the egg yolk. As the injection does not directly involve the embryonic blastodisc, there is no problem with osmotic shock.

All determinations were based on a sample size of 200 eggs, combining data for three replicates. Control eggs were injected with 0.1 ml aliquots of distilled, deionized water. Except for the omission of test metals, they received identical treatment as given the experimentals. Percent survival was determined as hatchability in experimental populations/hatchability of controls. Control hatchability averaged 81%, with an overall range of 75-86%. Chicks which survived the hatching process were screened for teratogenic effects.

RESULTS

The effects of metal treatment on the chick embryo are summarized in Table 1. Selenium, arsenic and cadmium were extremely toxic, giving survival rates (hatchability) of 64-66% at a concentration of 0.001 ppm. Selenium and arsenic produced complete lethality at 1.0 ppm. Approximate TL_{50} values for selenium, arsenic and cadmium were 0.01, 0.05, and 0.05 ppm, respectively.

Lead, methyl mercury, inorganic mercury and zinc were moderately less toxic to chick embryos, producing survival rates of 74-83% when distributed in the egg yolk at a concentration of 0.001 ppm. The TL_{50} values were 1.0 ppm for lead, inorganic mercury and zinc, and 0.1-0.5 ppm for methyl mercury. As seen in Table 1, these metals displayed a more extended dose/survival response than obtained for selenium, arsenic and cadmium. Concentrations of 10-50 ppm were required to produce complete lethality.

All metals produced appreciable percentages of teratogenic or defective survivors when administered at or above their TL_{50} concentrations. These values are given parenthetically in Table 1. Observations were restricted to major defects at hatching, including brain deficiencies, absent eyes, skeletal anomalies, unabsorbed yolk sacs, and severe motor impairment. The types of defects did not vary substantially for the different metals. Locomotor impairment was the most common affliction. More extreme anomalies (e.g., hydrocephaly, acephaly, absent beak) generally were not encountered among survivors, as grossly defective embryos seldom live to complete the hatching process.

In all instances, the frequencies of metal-induced teratogenesis showed striking concentration dependence, correlating inversely with survival. The percentages of anomalous survivors decreased with decreasing concentration for all metals, reaching a threshold for teratogenic effects at 0.01-0.05 ppm. The greatest percentages of defective survivors were always at the highest test concentrations which permitted survival, ranging from 0.5 ppm for selenium and arsenic to 10.0 ppm for mercury and zinc.

DISCUSSION

Assessing the effects of environmental contaminants upon avian reproduction depends largely on 1) levels of contamination in avian food sources, 2) the rates at which toxicants consumed by laying females are incorporated into eggs, and 3) the effects of egg-stored toxicants upon fecundity, fertilization, and embryonic development. Studies with mercury clearly indicate that, concerning the effects of environmental contamination on avian populations, reproduction is the critical, sensitive link

(TEJNING 1967, BACKSTROM 1969, FIMREITE 1971, BIRGE AND JUST 1974). The data presented in Table 1 indicate that numerous metallic contaminants are highly toxic to avian development, which could represent serious hazards to avian reproduction. The sensitivity of the chick embryo to metallic toxicants further substantiates the contention that concentrations of trace metals which are not acutely toxic to adult birds may prove lethal to avian embryos. While metal-induced embryonic mortality may preclude or diminish egg hatchability, teratogenic development may further reduce reproductive potential by producing appreciable frequencies of defective offspring which are unlikely to survive. It seems evident that more attention should be given to the levels of metallic toxicants stored in avian eggs.

Results of this study further indicate that the needle-track procedure of yolk injection produces dose/survival responses quite similar to those obtained when metallic contaminants accumulate in avian eggs as a result of exposure to laying hens. Treating adult hens with methyl mercuric chloride and mercuric chloride, KUWAHARA (1970) recovered high levels of mercury in the eggs, with methyl and inorganic mercury tending to concentrate in albumin and yolk, respectively. Nevertheless, both forms of mercury inhibited hatchability to similar extents, producing embryonic mortality. When egg concentrations reached 5 ppm, hatching was less than 50% and no hatching occurred at 20 ppm. These results correlate closely with the mercury data presented in Table 1. Also, in controlled feeding experiments with methyl mercury-treated grains, FIMREITE (1971) showed that pheasant eggs containing 0.5-1.5 ppm mercury displayed significant loss of hatchability, averaging 46% over a 12-week period for hens which received the highest of 3 treatment levels. After 8 weeks of continuous feeding, hatchability for all treatment levels dropped to approximately 50% or less of that obtained for control populations (FIMREITE 1971, Figure 6). The egg mercury levels reported fall at or close to the TL_{50} range of mercurial compounds given in Table 1.

Still closer correlation exists between our hatchability data and that presented by TEJNING (1967), who used methyl mercury in conducting feeding experiments with the domestic fowl. When mercury content ranged from 0.20-0.35 mg/egg, hatchability was reduced to an average of 16.9%. Eggs containing 0.36-0.56 mg mercury gave 0% hatchability in three samples and 30% in a 4th,for an overall average of 10%. The mercury levels reported by TEJNING (0.20-0.56 mg Hg/egg)obviously fall at or very close to the threshold for 0% hatchability. By comparison, using a sample size of 200, we obtained 10% survival when methyl mercury was administered at 10 ppm (0.184 mg/egg) and 0% hatchability at 50 ppm (0.92 mg/egg).

Both TEJNING (1967) and FIMREITE (1971) noted hatchability to decrease with the duration of exposure to the laying hens, suggesting some damage to spermatozoa and/or fertilization

TABLE 1

TOXICITY OF METALS TO CHICK EMBRYOS

CONCENTRATION[2] (ppm)	PERCENT SURVIVAL[1]						
	Se^{+6}	As^{+3}	Cd^{++}	Pb^{++}	CH_3Hg^{+}	Hg^{++}	Zn^{++}
0.001	64	65	66	74	78	78	83
0.010	43	54(2)	55	73(1)	71	73	75
0.050	38(7)	47(6)	48	74(7)	65(4)	64	69
0.100	30(9)	43(17)	41(6)	63(10)	54(7)	61(2)	64(2)
0.500	24(11)	18(29)	25(15)	57(16)	45(11)	56(3)	58(9)
1.0	0	0	8(33)	52(14)	26(15)	51(8)	49(8)
5.0	0	0	0	23(24)	23(22)	37(12)	35(17)
10.0	0	0	0	0	10(25)	8(33)	9(29)
50.0	0	0	0	0	0	0	0

[1] Each percentage represents frequency of hatching for experimental eggs/controls, with a sample size of 200. Percentages of surviving embryos possessing gross teratologies are given parenthetically.

[2] Metals were administered by yolk sac injection in amounts calculated to dilute egg yolk to specified concentrations.

presumably due to increased mercury levels in the oviduct. Such effects would not be reflected in our data, where mercury was administered just prior to onset of incubation. In addition, we should note that results from feeding experiments are rather limited in terms of mercury treatment levels and egg sample numbers used in hatchability determinations. Also, in feeding experiments, mercury levels of eggs used for hatchability tests are determined indirectly by analyses of independent subsamples. However, the correlations cited above provide substantial support for our contention that the needle track yolk injection procedure may be used to supplement toxicity data obtained from feeding experiments. In addition, this procedure affords a simple and economical bioassay system with which to evaluate and compare the embryopathic effects of metallic toxicants.

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REFERENCES

BACKSTROM, J. Acta Pharm. Toxicol. 27, Suppl. 3 (1969).
BIRGE, W.J. and J.J. JUST. U.S. Dept. Interior, Res. Report #71 (1974).
D'ITRI, F.M. The Environmental Mercury Problem. The Chemical Rubber Co. Press, Cleveland (1972).
FIMREITE, N. Can. Wild. Ser., Occasional Paper #9 (1971).
FRANKE, K.W., A.L. MOXON, W.E. POLEY and W.C. TULLY. Anat. Rec. 65, 15 (1936).
FRIBERG, L., M. PISCATOR and G. NORDBERG. Cadmium in the Environment. The Chemical Rubber Co. Press, Cleveland (1971).
KUWAHARA, S. J. Kumamoto Med. Soc. 44, 90 (1970).
MILLER, M.W. and G.G. BERG. Chemical Fallout. Charles C. Thomas, Springfield, Illinois (1969).
PERKIN-ELMER MANUAL. Analytical Methods for Atomic Absorption Spectrophotometry. The Perkin-Elmer Co., Norwalk, Conn. (1973).
PLUMMER, F.R. and B.E. BARTLETT. Bull. Environ. Contam. Toxicol. 13, 324 (1975).
RIDGWAY, L. and D.A. KARNOFSKY. Ann. N.Y. Acad. Sci. 55, 203 (1952).
TEJNING, S. Oikos 8 (Supplement),(1967).
WALLACE, R.A., W. FULKERSON, W.D. SHULTS and W.S. LYON. Mercury in the Environment. Oak Ridge National Lab., ORNL-NSF Environmental Program (1971).