The Influence of Dieldrin on Tibial Bone Calcium Deposition in Domestic Chicks

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RATCLIFFE (1967) presented the first evidence correlating the decline of raptor (e.g., hawks, eagles, falcons) populations in Western Europe with increasing exposure to persistent organic pesticides. This investigation demonstrated a drastic decline in eggshell thickness which coincided with an increased incidence of egg breakage and consequent poor reproductive success of the peregrine falcon, Falco peregrinus; sparrow hawk, Accipiter nisus; golden eagle, Aquila chrysaetos; and other "raptoral birds" in the years studied.

Chlorinated hydrocarbons have been found to accumulate mainly in the kidney and liver fat of monogastrics (HAYES and CURLEY, 1968; JUDAH, 1949; STADELMAN et al., 1964). KORTE and ARENT (1965) found dieldrin was converted to hydrophilic products by rat liver homogenates. A dieldrin toxicity study by TINSLEY (1966) revealed an interaction involving the metabolism of polyunsaturated fatty acids. In female rats, dieldrin stimulated the transformation of oleic acid to 5, 8, 11-eicosatrienoic acid, an accumulation of which reduced metabolic efficiency. MENGE, et al., (1965) observed that a similar accumulation of 5, 8, 11-eicosatrienoic acid in linoleic acid deficient laying hens occurred. In both cases (MENGE et al., 1965; PEAKALL,1970) reproductive parameters (i.e., egg production, egg size, and fertility) were depressed. In ringdoves fed DDT, followed by an oral dose of radioactive calcium, PEAKALL (1970) observed an appreciable rise in liver enzyme activity prior to and after clutch completion. Significantly

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less labeled calcium was stored in the bone marrow of the DDT-treated birds than in the marrow of controls fed only labeled calcium. PEAKALL (1970) also reported that eggs laid by DDT-treated birds had significantly thinner shells than did those of controls. When dieldrin was fed to calcium-labeled ringdoves, liver enzyme activity increased with consequent reductions in blood estrogen levels. However, there was no effect on shell thickness. PORTER and WIEMEYER (1969) found a decrease in shell thickness in eggs of both dieldrin- and DDT-treated sparrow hawks.

It has been hypothesized (PEAKALL, 1970; RATCLIFFE, 1967) that chlorinated hydrocarbons, (i.e., DDT, dieldrin, and their metabolites), exhibit their most pronounced effects by altering hormonal balance through the induction of liver enzyme activity. Calcium, *per se*, does not appear to be affected by DDT or dieldrin; however, the effects of dieldrin and DDT on calcium metabolism during eggshell formation are incompletely understood.

Since an interaction of dieldrin with calcium can be experimentally induced and measured by decreased shell thickness, the question arises, does a similar interaction occur with calcium during bone formation? This study was designed to determine if subtoxic levels of dieldrin would influence skeletal calcium deposition in young, rapidly growing, birds maintained on marginal dietary calcium levels.

METHODS AND MATERIALS

Experiments were initiated with newly hatched Single Comb White Leghorn (SCWL) and broiler chicks. All chicks were wing-banded, weighed, and randomly divided into groups of 9 or 10 birds per pen. The Leghorns and broilers were reared in separate battery brooders and served as separate experiments but were maintained under the same conditions. Each experiment was repeated in two trials. Treatments were replicated twice in Trial I and three times in Trial II.

All experiments were conducted in wire floored, electrically heated, battery brooders. The birds received continuous incandescent light. Feed and water were supplied *ad libitum*. Feed consumption was recorded, per week, per replicate.

Diets. Broilers received 23.5% protein rations calculated to contain 0.6% calcium and 0.83% phosphorus. Leghorns received 0.5% calcium and 0.76% phosphorus. DAMRON and HARMS (1968) determined the marginal calcium and phosphorus levels for broiler chicks with a calcium phosphate source to be 0.58% calcium and 0.44% inorganic phosphorus. Since SCWL chicks have a slower rate of gain than broilers, their marginal calcium requirement was considered to be less than that of broilers. THE NATIONAL RESEARCH COUNCIL (NRC), (1971) recommendations for chickens 0-8 weeks of age were 1.0%

calcium and 0.5% inorganic phosphorus, irrespective of type, e.g., broiler or Leghorn. All other dietary components were calculated to meet NRC standards (1971).

Dieldrin Treatments. All birds received their respective control rations until seven days of age when dieldrin treatments were initiated. Within each replicate of each trial, for each type of bird, two groups received dieldrin in the diet, two other groups received individual capsules of dieldrin and a fifth group remained on the control ration. Dieldrin was administered in the feed at either 5.0 or 10.0 mg/kg feed. Control rations were fed to dieldrin capsulated groups, as well as controls. Dieldrin in gelatin capsules was administered orally at seven days of age; treatments of 5 mg/kg body weight received one capsule, and treatments of 10 mg/kg body weight received two capsules.

Body Weights. Each bird was individually weighed at 1, 7, 14, 21, 28 and 35 days of age. Rates of gain were determined for each successive 7 day period.

Calcium Determination. All birds were sacrificed by cervical dislocation at 5 weeks of age. The left tibia was removed from each bird, cleaned, marked for identification, individually wrapped in a plastic bag and stored at -23°C.

Calcium determinations were conducted on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer according to Perkin-Elmer ANALYTICAL METHODS FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY (1968). Standard absorption curves were plotted, and calcium concentrations for the bone samples were extrapolated in parts per million from these standard curves. Each calcium determination was then converted to a percentage on a fat-free, dry bone basis.

Calcium percentages were analyzed by standard analysis of variance procedures (SNEDECOR, 1967).

RESULTS AND DISCUSSION

Since toxicity would have confounded mineral metabolism, the pesticide levels employed were below reported toxicity levels (20 mg/kg/day capsules, 50 mg/kg/day mash) (EDEN, 1951). The levels administered did not produce any of the toxicity symptoms reported by ARENA (1963), for this pesticide.

Weight Gains. There were no significant differences in weekly weight gains between control and dieldrin treated groups in either SCWL or broiler chicks in either Trial I or Trial II. These results are in accord with those of EDEN (1951) who reported growth depression only at higher levels (100-200 p.p.m.).

Tibial Bone Calcium (Fat-free, Dry Bone Basis). No significant differences in calcium percentages, expressed on a fat-free, dry bone basis, between controls and dieldrin treated groups were found in either SCWL or broiler chicks (Table 1). Significant differences were found between Trials I and II in the SCWL chicks (Table 1) but not in broiler chicks; however, no significant differences between treatments were found within either trial. KIENHOLZ and WOOKEY (1971) reported a control mean of 17.0% calcium in the fat-free, dry tibiae of broiler chicks 4 weeks old fed an adequate starter diet containing 1.1% calcium. SCWL chicks on the same calcium level should yield approximately the same fat-free dry tibial calcium content as those found in broilers. The marginal calcium levels fed the SCWL and broiler chicks (0.5% and 0.6%, respectively) apparently had a depressing effect on the calcium content of fat-free dry tibiae, in both SCWL and broiler chicks, at 5 weeks of age.

TABLE 1

The effect of marginal dietary calcium[1] and dieldrin supplementation on percentage of calcium in fat-free, dry tibial bones of SCWL and broiler chicks at 35 days of age following 4 weeks of treatment.

Treatment	SCWL		Broiler	
	Trial 1*[2]	Trial II[3]	Trial I[2]	Trial II[3]
Control	9.34	12.84	10.85	11.12
5 mg/kg feed	11.74	12.70	12.15	12.28
10 mg/kg feed	11.62	12.06	12.08	12.74
5 mg/kg wt.-caps.	10.90	11.69	11.55	13.12
10 mg/kg wt.-caps.	10.06	12.63	11.33	11.87

[1]SCWL, 0.5% dietary calcium and 0.76% phosphorus. Broiler, 0.6% dietary calcium and 0.83% phosphorus.

[2]Unweighted mean of two replicates of 9 or 10 chicks each.

[3]Unweighted mean of three replicates of 9 or 10 chicks each.

*SCWL Trial I was significantly ($P<0.05$) lower in percent calcium than SCWL Trial II without regard to treatments. Standard error of the mean for Trials I and II = $\pm$ .165.

The effect of the marginal calcium levels is evidenced by the tibial calcium percentages (Table 1) which were considerably lower than those reported by KIENHOLZ and WOOKEY (1971). The data in all trials were consistent; no significant ($P<0.05$) differences were noted in tibial calcium percentages between controls and dieldrin-supplemented chicks. Dieldrin, at the levels employed, did not elicit an effect on tibial calcium deposition in either SCWL or broiler type chicks under the conditions employed.

The alteration of calcium metabolism when chlorinated hydrocarbons are fed, as reported for some laying birds, was evidently not discernible in young growing chicks when subtoxic levels of dieldrin were ingested.

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