DDE, PTH and Eggshell Thinning In Mallard, Pheasant and Ring Dove

by

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The application of DDT to limited areas of the earth's land surface has resulted in widespread contamination of the atmosphere and the earth's land, sea, and air masses (ANAS and WILSON 1970, RISEBROUGH et al. 1968, SLADEN et al. 1966). This contamination is due to the long half-life of DDT and its metabolites in the atmosphere (ABBOTT et al. 1966), soil (NASH and WOOLSON 1967), and ocean (MOORE and TATTON 1965), to the evaporative characteristics of the chemical (LLOYD-JONES 1971), and to its solubility in lipid substances, which allows its incorporation and transport within the food chain (DUSTMAN and STICKEL 1969, HARRISON et al. 1970, HOLDEN 1962).

Accumulation of DDT in the food chain has led to the investigation of the physiological effects that DDT or its metabolites may have on various organisms. One of the DDT effects which could result in lowered population recruitment is eggshell thinning, demonstrated in controlled studies of DDT and DDE ingestion by several species of birds (HEATH et al. 1969, LONGCORE et al. 1971, PORTER and WIEMEYER 1969). Theories on the physiological mechanisms of eggshell thinning center around either a decreased supply of calcium to the eggshell gland or decreased use of calcium by the gland (COOKE 1973). Supply of calcium to the gland may come from absorption across the gut under the primary influence of vitamin D and increased by parathyroid hormone, and from the mobilization of stored calcium deposited in medullary bone under the influence of estrogens and mobilized by PTH, or both. Use of calcium by the gland in eggshell formation is thought to be mediated by carbonic anhydrase. These four factors, vitamin D, PTH, estrogens, and carbonic anhydrase, appear to be involved in shell calcification.

PEAKALL (1969) observed no effect of dietary DDT in zebra finches on gut absorption of calcium or vitamin D

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metabolism in ring doves. Although o,p' DDT and p,p' DDT do block some carbonic anhydrase action (16-19%) in the shell gland of Japanese quail (BITMAN et al. 1969), DVORCHIK et al. (1971) concluded that inhibition by p,p' DDT or p,p' DDE would be insignificant at biological concentrations of carbonic anhydrase in the shell gland. POCKER et al. (1971) have demonstrated that neither DDT nor DDE are true inhibitors of carbonic anhydrase. DDT does affect the metabolism of steriod compounds through induction of hepatic microsomal enzyme systems (PEAKALL 1967) and reduces circulating estradiol and stored calcium in medullary bone during egg-laying (PEAKALL 1970). Injection of p,p' DDE 20 hr. prior to egg-laying also induces eggshell thinning (PEAKALL 1970) and this rapid reaction rules out reduced deposition of calcium in medullary bone as the only factor influencing eggshell thinning. Species which have not shown an eggshell thinning response to DDT as pheasant (HUNT 1966, HUNT et al. 1969) and chickens (BALASUBRAMANIAM 1972), recover spontaneously from parathyroidectomy, while mallard and ring doves, species which are affected by eggshell thinning, are extremely sensitive to parathyroid gland removal. This relationship suggested to us the parathyroid gland as a target organ for DDT effects on eggshell thinning.

METHODS AND MATERIALS

Hen mallards and pheasants were caged individually and the experimentals given a 10 ppm p,p' DDE (99.9%) diet, the mallards of cracked corn and Purina Layena and the pheasants of Purina Game Bird Layena (Purina Co., St. Louis, Mo.). Ring doves were caged in pairs and the experimentals fed a 40 ppm p,p' DDE (99.9%) diet of Purina Checkers Pigeon Chow. The DDE was mixed 1:99 with corn oil and food was added to the right proportion. Control birds received food treated with corn oil. All birds had free access to food and water. All birds were on their experimental diet at least three weeks before egg-laying began and received a 16 hr. light-8 hr. dark light cycle.

Blood samples were drawn from the ulnar artery of pheasant and mallards into a 5 ml. heparinized syringe and the serum spun off for total calcium analysis on an atomic absorption spectrophotometer (Perkin-Elmer Model 303). Blood samples from the brachial vein of ring doves were taken mid-way between the laying of the two eggs in the clutch by puncture with a lancet and collection in a heparinized capillary tube. The serum was spun off and total calcium was analyzed by a Coleman 21 flame photometer in conjunction with a Coleman Junior spectrophotometer using the advanced method for calcium analysis.

Mallard and pheasant were injected with 30 U.S.P. units of Lille PTH injection and blood samples were taken for calcium analysis every 15 minutes up to 4 hrs. later. The eggs laid within 24 hrs. of this injection were noted for contrast with other eggs in the clutch. Ring doves were injected with 100 U.S.P. units/kg at the midpoint in the egg cycle to determine effect on the eggshell thickness. Blood samples were also taken 2 or 4 hrs. after a similar injection for calcium determination. Eggshell thickness in all species was determined by averaging twelve micrometer readings. Statistical analyses of between group differences on mallard and pheasant data utilizing student's t-test and Mann-Whitney u-test, both yielded similar results. Ring dove data was tested in a nested analysis of variance.

RESULTS AND DISCUSSION

Mallard: (Table 1) Mallards were held through a five month refractory laying condition. Few birds entered laying condition and eggs were sparse (45 total), however, the DDE treated birds showed a 20.41% decrease in eggshell thickness below controls ($p<0.01$), and depressed serum calcium (12.39 mg% for DDE birds vs. 15.06 mg% for control birds, $p<0.05$). No eggs were collected after PTH injection for determination of its effect on eggshell thickness. Mallards showed a significant short-lived increase in serum calcium values from 2 to 3 hrs. after PTH injection. Injection of PTH also abolished the difference in serum calcium values of control and DDE birds for up to 4 hrs. post injection.

Ring doves: (Table 2) Data from ring doves, which lay a two egg clutch, was tested in a nested analysis of variance. Variation in eggshell thickness as a result of treatment, individual bird and 1st and 2nd egg could be assessed. All factors varied significantly as determined by F values. If the thickness of all eggs produced by each treatment was compared to values for other treatments, control birds produced significantly thicker ($p < 0.05$) eggs than DDE-fed or DDE-fed plus PTH injected birds. If only the 1st egg of each clutch is considered in the analyses, there are no significant differences between groups, but if only the thickness of the 2nd egg is analyzed, control birds produced significantly thicker eggs ($p < 0.05$), than the DDE or PTH groups. Serum calcium values in DDE treated ring doves were significantly depressed ($p < 0.01$) at the midpoint in the clutch cycle, and serum calcium values were elevated an average of 2.61 mg% 2 hr. after PTH injection and 4.36 mg% 4 hr. after PTH injection. This resulted in equal serum calcium values of control and DDE birds.

Pheasant (Table 3) There was no significant difference between the eggshell thickness of control and DDE pheasants whether they were injected with PTH or

TABLE 1

Mallard Eggshell Thickness and
Serum Calcium Data

	Control	DDE 10.0ppm	% Difference	Significance
Eggshell Thickness (mm)				(DDE vs Control)
Mean (N)*	0.431(6)	0.343(6)	20.41	$p < 0.01$
St. Dev.	±0.0803	±0.0660		
Random Serum Ca^{++} Values (mg%)				
Mean (N)[1]	15.06(30)	12.39(30)	17.06	$p < 0.10$
St. Dev.	±8.69	±6.25		
Serum Ca^{++} Values After PTH Inj.				
Mean (N)[1]-mg%				
1-2 hr.	13.55(5)	14.60(5)		None
2-3 hr.	18.98(30)	18.97(26)		None
3-4 hr.	13.22(20)	12.85(25)		None
T-test, Serum Ca^{++} After PTH Inj.				
1st vs. 2nd hr.	$p < 0.10$	$p < 0.02$		
2nd vs. 3rd hr.	$p < 0.01$	$p < 0.01$		
1st vs. 3rd hr.	None	None		

* (N) = Number of birds in the experiment. Six hens in each experiment laid eggs, the hen was used as the experimental unit; df = 10. A total of 45 eggs was measured.

1.(N) = Individual serum samples analysed.

TABLE 2 Ring Dove Eggshell Thickness and Serum Calcium Data

	Control	DDE 40 ppm	DDE & PTH
Eggshell Thickness (mm)			
All Eggs - Mean (N)*[1]	0.1354(90)	0.1290(52)	0.1296(52)
St. E.	0.00075	0.00119	0.00100
1st Egg - Mean (N)*	0.1387(45)	0.1335(33)	0.1331(34)
St. E.	0.0010	0.0013	0.0012
2nd Egg - Mean (N)*[1]	0.1322(45)	0.1233(19)	0.1261(18)
St. E.	0.0010	0.0019	0.0016
Serum Ca^{++} at midpoint of cycle - mg%			
Mean (N)[2]	26.45(15)	20.02(20)	
St. Dev.	±5.96	±6.05	
Serum Ca^{++} Increase			
2 hr. post PTH			
Mean (N)[2] mg%	2.611 (22)		
St. Dev.	±1.945		
4 hr. post PTH			
Mean (N)[2] mg%	4.36(10)		
St. Dev.	±2.265		

* (N) = Number of eggs in experiment.
1. Means significantly different ($p < 0.05$), nested analysis of variance, critical difference, t test.
2. (N) = Individual serum samples analysed.

not. There is, however, a significant increase ($p < 0.01$) in the eggshell thickness of birds treated with PTH (8 times during the 2 month laying period); both DDE and control birds were affected. This long-term effect of PTH on eggshell thickness does not appear to be operating through elevated serum calcium levels, for effects on calcium levels were varied. Control bird values decreased 0-4 hrs. before oviposition with PTH injection, while DDE-fed birds serum calcium values increased 9-14 hrs. before oviposition and more than 22 hrs. before oviposition with PTH injection. The significance of serum calcium values at varying times after injection was abolished because serum calcium varied as much as 15 mg% in a normal cycle. Birds never became synchronous layers. They were taken out of laying condition to prevent serum Ca^{++} fluctuation and no increase in serum calcium was found up to 4 hrs. post PTH injection.

TABLE 3

Pheasant Eggshell Thickness and Serum Calcium Data

	Control	DDE 10 ppm	Significance
Eggshell Thickness (mm)			
w/o PTH - Mean (N)*	0.490(5)	0.475(10)	None
St. Dev.	±0.0128	±0.0285	
w/PTH - Mean (N)*	0.536(11)	0.528(11)	None
St. Dev.	±0.0170	±0.0158	

	PTH Birds	Non-PTH Birds	Significance
Eggshell Thickness (mm)			
All birds Mean (N)*	0.532(22)	0.480(15)	$p < 0.01$
St. Dev.	±0.0166	±0.0243	

	Control	DDE 10 ppm	Significance
Serum Ca^{++} Values w/o PTH - mg%			
Hours Before Egg (N)[1]			
0-4	23.98(10)	27.19(13)	None
5-8	25.95(6)	30.05(17)	None
9-14	32.33(10)	22.71(5)	p 0.02
22-	27.45(2)	26.30(11)	None
Serum Ca^{++} Values w/PTH - mg%			
Hours Before Egg (N)[1]			
0-4	25.18(9)	26.75(9)	None
5-8	27.58(11)	26.53(9)	None
9-14	28.50(2)	32.51(5)	None
22+	27.71(5)	34.14(7)	None
Serum Ca^{++} Values after PTH Injection			
(Non-laying Birds) - mg% Ca^{++}			
Mean (N)[1]			
No - PTH	10.94(9)	11.99(10)	None
1-2 hrs.	11.025(6)	10.787(6)	None
2-3 hrs.	10.920(5)	10.680(5)	None
3-4 hrs.	11.910(5)	11.600(5)	None

T-test - Serum Ca^{++} Values after PTH Injection

	Control	DDE
Non-PTH vs. 1st hr.	None	None
Non-PTH vs. 2nd hr.	None	None
Non-PTH vs. 3rd hr.	None	None

* (N) = Number of birds in the experiment; an average value for the clutch of each bird was used in statistical analysis. A total number of 779 eggs was measured.

1.(N) = Individual serum samples analysed.

Doves and mallards both showed eggshell thinning and depressed serum calcium with DDE treatment, and increased serum calcium after PTH injection. Pheasants showed no eggshell thinning and no clear pattern of depressed serum calcium as a result of DDE treatment, and no increase in serum calcium after PTH injection. Pheasants did show a long-term effect of eggshell thickening with PTH injection. Larger or repeated doses of purified or avian PTH in mallard and dove, which would elevate serum calcium for the total time the egg is in the eggshell gland, might result in eliminating the thinning effect of DDE.

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