EFFECTS OF CHEMICALS ON EGG SHELL FORMATION

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The recent decline in certain species of wild birds, which is often accompanied by a decrease in shell thickness, has led to an increased interest in shell formation and the effects of pesticides and other chemicals on this process. In addition, there is a growing awareness among investigators that the intense and rapid mineral metabolism during shell formation offers unique opportunities for studying basic aspects of calcification, ion transport, and skeletal metabolism. The purpose of this review is to gather the widely scattered information on the effects of chemicals on shell strength and to relate these effects, wherever possible, to the processes involved in shell formation. The ability of birds to form strong shells has been assessed with a variety of different measurements (1), including shell thickness, specific gravity of the egg, and shell weight as percentage of egg weight. Since the correlation among these three methods is relatively high, we will use the general term "shell thickness" in most of our discussion. An effort has been made to deal with all avian species. However, because of the economic importance of egg breakage to the poultry industry, much of the research has been carried out with *Gallus domesticus* and, unless mentioned otherwise, the studies summarized below have been carried out with this species.

Current knowledge of shell formation and structure has been described in detail elsewhere (2–4). Nevertheless, it seems essential to begin with a summary of those aspects that are needed for an understanding of the mechanisms of action of different substances. In this summary, references to original papers will only be given if they can not be found in the reviews or if necessary for clarity.

EGG SHELL FORMATION AND STRUCTURE

General

Although there are some differences in shell structure among species, all avian egg shells contain the following layers from the inside to the outside: (*a*) two mem-

branes, containing a keratin-like protein, (*b*) the true shell, and (*c*) a proteinaceous cuticle. The true shell, which is the subject of this review, accounts for about 80% of the thickness of the shell in the chicken. It consists of about 98% crystalline calcium carbonate (calcite) and is permeated by an organic matrix. The matrix is a glycoprotein with an amino acid composition similar to that of cartilage; the polysaccharide moiety contains 35% chondroitin sulfate. Because the calcium, carbonate, and matrix content of the true shell is relatively constant and because a defect in the deposition of any of these three components results in thin shells, it seems that all three components are essential for shell formation.

The nuclei of the calcite crystals are deposited on the outer shell membrane in the isthmus region of the oviduct (5). The remainder of the shell is formed in the next section, the shell gland, where the egg remains for about 20 hr in chickens. During the first 5 hr, the egg is "plumped" by diffusion of water and certain electrolytes through the shell membranes into the albumen. Initially, calcium carbonate deposition is slow, but then it increases gradually to a rate of 330 mg/hr that remains constant during the last 15 hr of shell formation.

Calcium Deposition

Because the shell gland does not store significant quantities of calcium, this ion has to be extracted continuously from blood. The mechanisms by which calcium is translocated across the shell gland mucosa are not clear, although active transport may be involved (for review see 6). In the laying hen, the total amount of blood calcium is about 20–30 mg, so that with an extraction rate of 130 mg/hr, calcium would be cleared in 9 to 14 min. Thus, blood calcium has to be replenished continuously. It has been shown that intestinal calcium absorption increases nearly twofold during shell formation (7). Another important source is medullary bone (for review see 4) which supplies 30–40% of the egg shell calcium in chickens, even if calcium intake is adequate. This bone occurs naturally only in female birds and derives its name from the fact that it is most easily observed in the medulla of the femur and tibia. Medullary bone formation, which begins about 10 days before the first ovulation, as well as its maintenance are controlled primarily by the synergistic action of estrogens and androgens. Its mobilization during shell formation is accompanied by an increase in osteoclastic activity and increased phosphate excretion.

Carbonate Deposition

It has not been proven conclusively in what form the carbonate radical of the egg shell mineral is secreted into the shell gland lumen and what mechanisms are involved in its synthesis and/or transport (for a discussion of different models see 8–10). Experiments with ^{14}C-bicarbonate have shown that the arteriovenous bicarbonate gradient across the shell gland is practically zero during shell calcification (11). Thus it is likely that, similarly to the mammalian pancreas (12), most of the bicarbonate is derived from metabolic CO_2 produced by the shell gland, rather than from serum bicarbonate. The hydration of metabolic CO_2 to bicarbonate is probably catalyzed by carbonic anhydrase which has been localized in the shell gland mucosa by histochemical (13), autoradiographic (14), and fluorescent antibody techniques

(15). From the rate of shell formation it can be estimated that the rate of bicarbonate formation is about 56 μmol/min. Maximum inhibition of carbonic anhydrase in the hen with 12 to 25 mg/kg acetazolamide causes a reduction in shell weight of about 80% (16), yielding an uncatalyzed rate of 11 μmol/min.
concentration of shell gland fluid is three to four times greater than that of serum (16, 17) and because the potential gradient from the serosal side to the shell gland lumen is negative or zero (6, 18) it seems possible that bicarbonate is secreted by an active process. Precipitation of calcium carbonate on the shell may be facilitated by release of NH_3 gas from the forming egg (19) and/or the high affinity of the organic matrix for cations (20).

The formation of calcium carbonate, either from metabolic CO_2 or from bicarbonate, results in the release of two protons. Egg shell formation in laying hens is accompanied by metabolic acidosis which reaches a maximum when the egg has been in the shell gland for about 16 hr (21). The acidosis is partially compensated for by an increase in respiratory rate (22) and urinary acidity (23). That the shell gland is the source of at least part of the acid is indicated by the finding of Hodges (24) that the arteriovenous pH gradient across the shell gland increases during the first 15 hr of shell formation. This is accompanied by a decrease in the intracellular pH of shell gland tissue, which is abolished if carbonic anhydrase is inhibited with acetazolamide (25). Other investigators (26) have been unable to find any difference in systemic pH between laying and nonlaying quail and have concluded that the pattern is diurnal and not associated with shell formation.

Oviposition

The expulsion of the egg from the shell gland seems to be under both neural and hormonal control, although other factors may also be involved (for reviews see 2, 27). Premature oviposition can be induced by stimulation of the preoptic hypothalamus and administration of substances such as anesthetics, acetylcholine, histamine, oxytocin, and arginine vasotocin. Oviposition is delayed by stimulation of the telencephalon and by epinephrine, ephedrine, spironolactone (28), and possibly certain sex hormones and desoxycorticosterone. Shell-less or thin-shelled eggs may be due to either premature expulsion of the egg from the shell gland or a decreased rate of shell deposition. Unfortunately, many investigators have failed to consider the first possibility.

SUBSTANCES THAT MAY AFFECT MATRIX FORMATION

Lathrogens

Interest in lathrogens is related to the fact that these substances produce skeletal defects in many species. Inclusion of 0.03 to 0.06% of the lathrogen β-aminoproprionitrile (BAPN) in the diet of hens resulted in reduced egg production, hatchability, and the laying of many shell-less and malformed eggs (29). The malformed eggs were wrinkled, checked, and ridged. Although it has been demonstrated that there is an interaction between calcium and BAPN (30), the primary effect of lathrogens appears to be on the crosslinking of connective tissue proteins such as collagen and

elastin (31). The protein of the shell membranes resembles collagen in that it contains the unique amino acid hydroxylysine (32). In another study (33), feeding the lathrogen semicarbazide to laying hens had no effect on shell thickness, but the shell membranes were thicker and less pigmented.

Manganese

Shortly after the discovery that manganese prevents a skeletal abnormality called perosis in young chicks (34), it was reported that manganese-deficient hens produce thin, rough, and translucent shells (35). More recent studies (36) confirmed this observation and showed that there was a decrease in shell matrix hexosamine content. These results are consistent with the effects of manganese deficiency on the mucopolysaccharide content of cartilage (37). Thus, the effect of manganese on shell formation is probably related to the role of this element in the synthesis of the polysaccharide component of the shell matrix.

SUBSTANCES THAT AFFECT CALCIFICATION

Calcium

In view of the high calcium requirement for shell formation, it is not surprising that egg shells become thinner when calcium intake is inadequate (for references on this and the effects of different calcium salts see 38). What is unexpected is that hens fed calcium-free diets stop laying after producing about six shells of decreasing thickness rather than continuing to lay eggs with uncalcified shells. The cessation of egg production is apparently due to inadequate gonadotrophin secretion, because hens continue to lay on calcium-deficient diets if they are injected daily with extracts of the avian anterior pituitary (39).

Phosphorus

Egg shell thinning, decreased breaking strength, and an increase in the number of shell-less eggs are some of the effects noted when hens receive an inadequate supply of phosphorus (40–43). Because the phosphorus content of the shell is very low (44), it is likely that phosphorus exerts its effect on shell formation by affecting bone mineral metabolism. Excessive levels of phosphorus have also been observed to cause a reduction in shell thickness (45, 46). Although it seemed likely that excess phosphorus was interfering with calcium metabolism, little evidence was obtained to support this hypothesis.

Magnesium

Magnesium might be expected to influence shell formation because it is second in abundance among the cations found in the shell mineral. However, the magnesium content of the shell is low (0.59%) relative to calcium (44). Severe magnesium deficiency in the laying hen results in reduced egg production and blood magnesium content. The thickness and magnesium content of the shell are also reduced under these conditions (47). Because some dolomitic limestones contain substantial quantities of magnesium, interest has also been shown in the effects of high dietary levels

of this element. Studies of magnesium tolerance by the laying hen indicate that egg production, egg weight, and shell thickness are reduced if the diet contains more than 1.2% magnesium (48).

Strontium

The feeding of strontium to laying hens at levels up to 5% of the diet resulted in a progressive increase in strontium content of the shell (49). There was a concomitant decrease in shell calcium content. The chemical form of strontium in the shell could not be identified, but it did not appear to be either $SrCO_3$ or $Sr_3(PO_4)_2$. Levels of strontium above 3% resulted in a significant decrease in shell thickness.

Vitamin D

In view of the well-known effects of Vitamin D on calcium metabolism it is not surprising that thin egg shells are one of the symptoms of vitamin D deficiency in laying hens (50, 51). Vitamin D is necessary for the production of a calcium-binding protein in the intestinal (52) and shell gland (53) mucosa. Studies with laying hens have shown that the amount of intestinal binding protein is responsive to physiological needs, while the amount of shell gland protein remains relatively constant (54). From these findings and other experimental evidence, it was concluded that the vitamin D-dependent calcium-binding protein was not playing a key role in the transfer of calcium across the shell gland mucosa.

SUBSTANCES THAT MAY AFFECT CARBONATE DEPOSITION

Carbonic Anhydrase Inhibitors

Hinshaw & McNeil's observation (55) that turkeys and chickens lay soft-shelled eggs after sulfanilamide administration has been confirmed in a number of studies (56–61). Benesch et al (57) were the first to ascribe this effect to carbonic anhydrase inhibition. This conclusion was based on the finding that sulfapyridine, in which substitution of the sulfonamide group abolishes inhibition, was without effect. Furthermore, the laying of shell-less or thin-shelled eggs coincided with the time when sulfanilamide (80% in the acetylated form) was present in the egg contents. Subsequent studies (58) showed that diets containing 0.3 to 0.5% unsubstituted sulfonamides, such as Soluseptazine and Neoprontosil, caused thin shells, while similar levels of the substituted sulfonamides, sulfathiazole, sulfaguanidine, sulfamerazine, and sulfadiazine had negligible effects on shell thickness. The only exception was sulfapyridine which, in contrast to the previous study (57), caused thin shells for 3 days after a single oral dose of 200 mg/kg. A single intramuscular injection of 12 to 25 mg/kg acetazolamide causes a decrease in shell weight of 70 to 80% (16); oral administration seems less effective, with a reduction in shell thickness of 32 and 43% for dosages of 50 and 100 mg, respectively (62). On an $-SO_2NH_2$ basis, benzenesulfonamide is about as effective as acetazolamide (62).

The relationship between the percentage of decrease in shell thickness and the sulfanilamide content of the diet is linear for concentrations from 0.02 to 0.50%, with a maximum reduction of about 40% (56). A similar relationship was found if

2 to 12 mg/kg acetazolamide was injected before the egg reached the shell gland (unpublished data). Higher dosages caused no further reduction if the egg was laid at the expected time. However about 45 and 70% of the eggs laid after injection of 50 or 100 mg/kg acetazolamide were expelled prematurely, while lower dosages only rarely had this effect. Premature oviposition has also been observed in turkeys (55) and hens (55, 56) fed diets containing 60 to 357 mg/kg sulfanilamide.

The best evidence for a direct effect of sulfonamides on the secretory activity of the shell gland seems to be provided by the changes in intracellular pH (63) and acid-base balance of shell gland fluid (16) after acetazolamide administration. However, carbonic anhydrase inhibitors have a number of effects, besides premature oviposition, whose contribution to egg shell thinning is uncertain. Oral doses of 60 but not of 30 mg acetazolamide per hen per day reduce serum calcium levels (64), and intravenous injection of 300 or 600 mg inhibits the hypercalcemic response to parathyroid hormone (65). An effect on calcium metabolism is also suggested by the finding that hens are in negative calcium balance for about 5 weeks after a diet containing 0.03% sulfanilamide has been withdrawn and shell thickness has returned to normal (66). Since avian osteoclasts contain significant amounts of carbonic anhydrase (67), it is possible that some of these effects are due to inhibition of bone resorption. Acetazolamide also decreases the excretion of ammonia and titrable acidity in laying hens (68), but because the acid-base balance of blood is not affected (16, 69) it is unlikely that this factor plays a major role.

Zinc

This element might be expected to affect shell formation because carbonic anhydrase is a zinc-containing enzyme. Although zinc deficiency reduced the carbonic anhydrase activity of blood in the calf (70) and decreased egg production in laying hens, it had no effect on shell formation (71).

Alterations of Acid-Base Balance

Much of the interest in the relationship between acid-base balance and egg shell formation dates back to the report of Hall & Helbacka (72) that inclusion of 2% ammonium chloride or of 0.74% HCl in the diet of laying hens decreases shell thickness by about 10%. Later studies have confirmed this finding (16, 73) and also indicate that 1% NH_4Cl is only marginally effective (72, 73). It is likely that the inhibition of shell formation by NH_4Cl is due in large part to metabolic acidosis (16, 73, 74) which may reduce the bicarbonate concentration in the secretory cells of the shell gland by proton capture (12) and/or counteract the uptake into blood of protons released during carbonate deposition. It has been shown that NH_4Cl causes a significant reduction in the pH and bicarbonate content of shell gland fluid (16) as well as changes in the Na, K, Ca, and Cl content of the fluid which is added to the albumen in the shell gland (75). The effect of acidifying substances on shell formation may be modified by the accompanying anion. Dietary $(NH_4)_2SO_4$ and H_2SO_4 cause a smaller decrease in shell thickness and a less severe acidosis than equivalent quantities of H^+ in the form of NH_4Cl or HCl, possibly because the anion influences the renal excretion of H^+ (74). If the dietary chloride concentration is

increased from 0.5 to 3.0% by addition of NaCl and KCl (Na/K = 0.2), shell thickness and strength decrease progressively (76). This effect may be due to a concomitant reduction of plasma bicarbonate, although the authors (76) ascribe it to carbonic anhydrase inhibition.

Attempts to increase shell thickness through feeding of sodium bicarbonate have had variable results (77–79), although metabolic alkalosis should promote shell formation by increasing the intracellular concentration of hydroxyl and bicarbonate ions (12). The variable results have been attributed to differences in dietary chloride (80), but further investigation is needed.

The effects of respiratory disturbances on shell formation are more consistent and shed additional light on the importance of acid-base balance in this process. Exposure to high environmental temperatures causes respiratory alkalosis and shifts in the acid-base balance of shell gland fluid accompanied by a reduction in shell thickness (16). A rather complete analysis of the effects of hypercapnia on shell formation in the hen has been carried out by Simkiss & Hunt (81, 82) and Sauveur & Mongin (83). Exposing hens to 7 or 20% CO_2 for about 12 hr (82) or to 2–5% for 12 to 54 hr (84), i.e. acute hypercapnia, caused a decrease in the base excess of serum (82) and formation of thin shells. If the exposure to CO_2 was prolonged for several weeks (chronic hypercapnia) blood bicarbonate increased, while blood pH decreased (85) and the egg shells became thicker than normal (77, 86). Similar changes in the acid-base balance of blood during hypercapnia have been observed in mammals (87) and were attributed to a delayed increase in renal bicarbonate reabsorption.

Hunt & Simkiss (82) suggested that the CO_3^{2-} concentration of plasma should be considered as an index for the ability of the shell gland to form $CaCO_3$, an idea further elaborated by Sauveur & Mongin (83). This concept seems particularly useful at the level of the shell gland, because it can be calculated from earlier data (16) that the thinning of egg shells after administration of NH_4Cl, acetazolamide, spironolactone, and exposure to high temperature was always accompanied by a decrease in the CO_3^{2-} concentration of shell gland fluid. The only substance for which this relationship did not hold was theophylline ethylenediamine which decreased shell thickness but caused an increase in CO_3^{2-} concentration.

PESTICIDES

Only the effects of pesticides on shell thickness and the processes involved in shell formation will be discussed; the general effects on birds (88) and chickens (89) have been reviewed earlier.

DDT

The argument that pesticides, particularly DDT and its metabolites, cause egg shell thinning and thereby interfere with the reproductive success of certain species of wild birds is based on three lines of evidence. First, an examination of museum eggs in Britain (90) and the U.S. (91) indicates a marked decrease in shell thickness of raptor eggs between 1940 and 1950. Second, studies show a negative correlation

Table 1 Effect of DDT on shell thickness

Species[1]	Pesticide	Dosage[2]	Percent change in shell quality[3]	Significance[4]	Ref.
Ringdove	*p,p'*-DDT	10 ppm	10–12[a]	5	102
Ringdove	*p,p'*-DDE	150 mg/kg i.p.	23[a]	1	102
Bobwhite quail	DDT (tech)	10 or 20 ppm	(2)–4[b]	nt	103
Mallard duck	DDT (tech)	10 or 20 ppm	1–6[b]	nt	103
Mallard duck	DDT (tech)	1000 mg/kg o	18 – 28[b]	nt	103
Mallard duck	*p,p'*-DDT	2.5 ppm	5[b]	ns	104
Mallard duck	*p,p'*-DDT	10 ppm	8[b]	ns	104
Mallard duck	*p,p'*-DDT	25 ppm	13[b]	1	104
Mallard duck	*p,p'*-DDE	10 or 40 ppm	8–13[b]	5	104
Mallard duck	DDD	10 or 40 ppm	3–5[b]	ns	104
Bengalese finch	*p,p'*-DDT	0–300 μg/day o	(7)[c]	1	105
Japanese quail	*o,p'*-DDT	100 ppm[a]	4[b]	0.1	106
Japanese quail	*p,p'*-DDT	100 ppm[a]	6[b]	0.1	106
Japanese quail	*p,p'*-DDT	100 ppm[b]	0[b]	ns	107
Japanese quail	*p,p'*-DDE	100 ppm[b]	2[b]	ns	107
Chicken[a]	DDT (tech)	10 or 50 ppm	increase[b]	5	108
Chicken[b]	DDT (tech)	10 or 50 ppm	decrease[b]	5	108
Chicken[a]	*p,p'*-DDT	5 to 300 ppm	no effect[b]		109
Chicken[a]	*o,p'*-DDT	5 to 300 ppm	no effect[b]		109
Chicken[a]	*p,p'*-DDE	5 to 300 ppm	no effect[b]		109
Chicken[a]	*p,p'*-DDT	100 or 200 ppm	0[b]		110
Chicken[a]	DDT (tech)	0.1 to 10 ppm	6[b]	5	111
Chicken[b]	DDT (tech)	1 to 7.5 ppm	3–6[b]	ns	112
Chicken[b]	DDT (tech)	10 ppm	9[b]	5	112

[1] Letter superscripts indicate age of hens: [a]: first year of egg production; [b]: second year of egg production.

[2] i.p.: single intraperitoneal injection. o: oral dose. In all other experiments the pesticide was added to the feed. Superscripts indicate diet concentration of calcium [a]: 0.56% Ca; [b]: 2.7% Ca.

[3] Changes relative to control group(s). Numbers in parentheses indicate an increase; all other values are either a decrease or no change. When the results were presented as graphs, the words increase, decrease, and no effect are used to describe the effect of DDT. Superscripts show the method used to measure shell quality: [a]: shell weight; [b]: shell thickness; [c]: shell weight as percentage of egg weight.

[4] Figures indicate level of significance for differences between control and treatment in percent. nt: significance not tested; ns: difference not significant at 5% level.

Table 2 Identity of pesticides mentioned in this review

Common name	Chemical name
p,p'-DDT	1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane
o,p'-DDT	1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl) ethane
p,p'-DDE	1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene
o,p'-DDE	1,1-dichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl) ethylene
DDD	2,2-bis (*p*-chlorophenyl) ethane
Methoxychlor	1,1,1-trichloro-2,2-bis (*p*-methoxy-phenyl)-1,1-dichloroethane
Lindane	99.5% ϒ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
Aroclors®	mixtures of polychlorinated biphenyl isomers
Dieldrin	1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene
Parathion	O,O-diethyl-O-*p*-nitrophenyl phosphorothioate
Malathion	O,O-dimethyl S-(1,2-dicarbethoxyethyl) dithiophosphate
Chlordecone	decachloro-octahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalen-2-one

between the concentration of DDT residues in eggs and various measures of shell thickness for herring gulls (91), peregrine falcons (92), prairie falcons (93, 94), double crested cormorants (95), and brown pelicans (96). The only exception seems to be a study with common terns in Alberta, where no correlation was found, despite low reproductive success of the colony and high DDE concentrations relative to other aquatic species of the area (97). The validity of both types of studies has been questioned, primarily with respect to sampling procedures. A good summary of the principal criticisms and their rebuttal can be found in a series of papers and letters (96, 98–101).

The third line of evidence is based on controlled studies of the effect of DDT on shell thickness (Table 1) and shell formation. These experiments were carried out with different isomers of DDT or DDE (Table 2) and with technical DDT. The isomer *p,p'*-DDE is the principal metabolite of DDT and accounts for 50 to 100% of the DDT residues in body tissues and eggs of wild birds (109). Technical DDT contains about 80% *p,p'*-DDT and 15–20% *o,p'*-DDT as well as other isomers and related compounds.

In general, the decrease in shell thickness is greater in wild populations, where thinning in excess of 50% has occurred (103), than in controlled experiments, even if the concentration of DDT residues is similar. The degree of shell thinning caused by DDT varies considerably among species (Table 1). Ringdoves (102) and mallard ducks (103, 104) seem to be relatively sensitive, while Japanese quail (106, 107), bobwhite quail (103), and particularly chickens (108–112) are relatively resistant. In an experiment with Bengalese finches (105) and in one experiment with chickens during their first year of lay (108) DDT caused a significant increase in shell thickness.

There is some evidence that the effect of DDT depends on the method of administration. In Japanese quail, 100 ppm *p,p'*-DDT caused significant shell thinning if the diet was deficient in calcium (106) but not if the calcium level was adequate (107). Tucker & Haegele (103) concluded that a single dose of DDT was more effective than continuous administration, a conclusion that could also be drawn from data obtained for ringdoves (102). However, in both instances the single dose was rather massive, so that the difference in response may have been due to the larger amount of DDT rather than to the method of administration. The response to DDT may also depend on the age of the bird. With one exception (111), administration of DDT to chickens during their first year of lay caused no change (109, 110) or increased shell thickness (108), while during the second year of production, levels in excess of 10 ppm caused shell thinning (108, 112). Another factor may be the position of the egg in the clutch, where a clutch is a sequence of eggs laid on successive days and two clutches are separated by one or more days when no egg is laid. In Japanese quail fed 0.56% calcium, DDT had little effect on the shell of the first egg in the clutch, while later eggs showed a progressive decline in shell calcium (106). Susceptibility may also vary among individual birds. When Japanese quail were fed DDT, about 80% of the broken eggs were produced by one quarter of the birds (107).

Other Pesticides

Feeding of 25 to 5000 ppm methoxychlor, a pesticide whose use has increased since the banning of DDT, had no effect on shell thickness in laying hens (113). Similar results have been obtained for lindane at a concentration of 100 ppm (114).

Although polychlorinated biphenyls may affect a number of reproductive traits, shell formation is usually not inhibited. Negative results have been obtained in studies with ringdoves (115), Japanese quail (116), mallards (117), bobwhite quail (117), and chickens (118, 119).

A number of experiments have also been carried out with dieldrin. Feeding 20 ppm (110) or administering 6 oral doses of 3 mg each over an 11 day period (120) had no effect on shell thickness in laying hens. In one study with mallard ducks, dietary levels of 1.6, 4, and 10 ppm dieldrin caused significant shell thinning (121), while in another experiment 4 ppm had no such effect (122). Prairie falcons that ate starlings fed a diet containing 10 ppm dieldrin showed a significant reduction of shell thickness, if the dieldrin content of their eggs was more than 20 ppm, but not if it was less (94).

Parathion at a concentration of 10 ppm caused significant shell thinning when it was fed to mallard ducks (122).

Effect

The similarity in configuration of DDT to the synthetic estrogen diethylstilbestrol has stimulated investigation of the estrogenic activity of DDT in mammals and birds (for references see papers cited below). The isomer *o,p'*-DDT was found to be estrogenic in chickens and quail, stimulating growth and glycogen deposition in the

oviduct, while *p,p'*-DDT was only weakly estrogenic (123). There are a number of other effects of DDT which suggest that it may interfere with the reproductive endocrinology of birds. DDT delayed sexual maturity and ovulation in Bengalese finches (105), Japanese quail (106, 107), and ringdoves (102). Both technical DDT (10 ppm) and dieldrin (2 ppm) increased the breakdown of testosterone and progesterone by liver microsomes to polar metabolites in king pigeons (124), and *p,p'*-DDT (10 ppm) reduced the concentration of estradiol in the blood of ringdoves (102). One mechanism through which these effects might interfere with shell formation is by inhibition of medullary bone formation. Such inhibition was observed when 100 ppm *p,p'*-DDT was fed to king pigeons (125). It has also been reported that 10 ppm *p,p'*-DDTdecreased the accumulation of ^{45}Ca in bone during the prelaying period of ringdoves (102). Other investigators (106) found no effect of DDT on medullary bone. It can also be argued that any decrease in bone calcium stores would be compensated for by increased utilization of dietary calcium, particularly in raptorial birds that have a relatively high calcium intake. On the other hand, it is possible that the greater sensitivity of Japanese quail on low calcium diets (106, 107) is due to increased dependence on medullary bone calcium.

Jefferies and co-workers have suggested that the effect of DDT on shell thickness may be due to altered thyroid function. In Bengalese finches, *p,p'*-DDT caused hyperthyroidism and increased shell thickness (105, 126), while pigeons became hypothyroid at dose rates in excess of 3 mg/kg/day (126, 127) and produced shells of lower weight (102). Besides accounting for one species difference in the response to DDT, these findings are also in concert with some studies on the effect of thyroid function on shell thickness (see below).

A number of recent investigations have focused on the effects of pesticides on the shell-forming process itself. Studies with the scanning electron microscope (128) have shown that feeding technical DDT (225 ppm) or Chlordecone (5 to 225 ppm) causes marked changes of shell structure in Japanese quail that cannot be explained completely by shell thinning alone.

There is controversy with regard to the effects of DDT on carbonic anhydrase. In one study with Japanese quail, diets containing 100 ppm *p,p'*-DDT or *p,p'*-DDE reduced the carbonic anhydrase activity of blood by 22 and 44% (129), while in another experiment with 100 ppm technical DDT there was no such effect (130). Inhibition of carbonic anhydrase has also been reported for quail shell glands after feeding of *p,p'*-DDT or *o,p'*-DDT (129) and for the oviduct of ringdoves after a single injection of 150 mg/kg *p,p'*-DDE but not of dieldrin (102).

Several authors have claimed that these results may be artifacts. Dvorchick et al (131) found no inhibition of human red cell or purified bovine carbonic anhydrase in vitro at *p,p'*-DDT or *p,p'*-DDE concentrations of 50 to 100 μg/ml. Pocker et al (132) confirmed these results and suggested that the reduction of catalytic efficiency by DDT may be due to precipitation of carbonic anhydrase. However, Serine & Schraer (133) have shown that both *o,p'*-DDT and *p,p'*-DDT inhibit purified chicken carbonic anhydrase in buffers containing 34% dimethylformamide which prevents coprecipitation of the enzyme and DDT (132).

OTHER SUBSTANCES

Mercury

The interest in environmental contamination from mercury has led to two investigations of the effect of this element on egg shell thickness. In experiments with Japanese quail (134), feeding from 1 to 8 ppm mercury as mercuric chloride produced a progressive decrease in egg shell thickness. Tissue mercury content was proportional to dosage. Methyl mercury was not detected in the tissues of this species. Contrasting results were obtained with methylmercury administration to ringdoves and American kestrels (135). This form is thought to be an important source of mercury for predatory birds. In these studies, injection or oral dosing with amounts equivalent to a dietary intake of 10 ppm had no significant effect on egg shell thickness.

Lithium

Inclusion of lithium carbonate at levels of 282 to 685 ppm lithium in the diet produced diarrhea, excessive salivation, regurgitation, and shell-less eggs as early as 24 hr after initiation of treatment (136). The mode of action of lithium on shell formation is unknown. Although lithium resulted in reduced serum calcium levels, soft-shelled eggs were produced before this change took place.

Hormones

The endocrine control of shell formation is poorly understood. Administration of hormones to laying hens often reduces egg production and egg size, resulting in a decreased need for calcium which may indirectly increase shell thickness.

It has been shown that medullary bone formation and maintenance depend on the synergistic action of estrogens and androgens, but other endocrine glands such as the thyroids and adrenals may also be involved (4). Little is known about the mechanisms that synchronize the metabolic activity of bone with the different stages of egg formation, although it has been proposed that either parathyroid hormone or estrogen is the regulatory factor (4). Administration of female sex hormones to laying hens has no effect on shell thickness, while testosterone may cause a slight improvement (for references see 38).

The calcemic and phosphatemic response of laying hens to parathyroid hormone is considerably larger, more rapid, and more transient than that of mammals (137), while administration of calcitonin has generally no effect (138). To our knowledge the effect of parathyroid hormone on shell formation has not been studied. When 10 MRC units/kg porcine calcitonin was injected intramuscularly at the onset of shell calcification, shell thickness was not affected although the interval between successive ovipositions was significantly shortened (139).

The effects of thyroprotein (iodinated casein) and of thiouracil have been studied rather extensively in laying hens (for references see 38). Although the results are conflicting, taken together, they suggest that shell thickness may be improved by iodinated casein and decreased by thiouracil, at least under certain conditions.

Thyroidectomy decreased egg production, egg weight, and shell thickness in chickens (140).

Cortisone, which is not known to occur in the fowl, increased shell thickness in one experiment (141) but not in another (142). When the aldosterone anatagonist spironolactone was injected daily at dosages of 4 to 9 mg per hen, shell weight decreased initially by 12 to 24% but then returned gradually to control values (28).

Injection of 0.8 mg/kg glucagon (138), 300 mg/kg imidazole (138), or 6 mg/hen theophylline ethylenediamine (16) at the onset of shell calcification reduced shell thickness without affecting the time of oviposition.

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