SHELL THINNING IN AVIAN EGGS BY ENVIRONMENTAL POLLUTANTS

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ABSTRACT

Literature has been reviewed concerning shell thinning in avian eggs by environmental pollutants. Field evidence indicates that the declines in shell thickness observed in certain species in North America and Great Britain since the Second World War have been largely caused by residues of pp'-DDE or other compounds or metabolites of the DDT group. In North America, polychlorinated biphenyls and cyclodiene insecticides have played no more than minor roles, although in Britain cyclodienes have probably made a significant contribution. Mercury compounds are not apparently associated with the shell thickness declines.

Results from controlled experiments, in which laying birds have been exposed to pollutants, generally support these suggestions. Laboratory investigations indicate an interspecific difference in shell thinning response, gallinaceous species tending to be the most resistant and falcons the most susceptible.

In a laying bird, organochlorine residues affect many biochemical mechanisms known to be essential for proper shell formation and the extent of the contribution of each affected mechanism towards decreasing shell thickness probably depends on variables such as species and environmental conditions. There is no evidence to suggest that one mechanism is always dominant irrespective of the conditions.

In North America shell thinning has often been associated with population decreases, but in Britain declines in shell thickness are not thought to be responsible for the population decreases observed in certain raptor species.

INTRODUCTION

Since Ratcliffe first reported thinner shells in raptor eggs in 1967 and suggested that organochlorine insecticides might be responsible, there has been considerable

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interest in shell thinning observed in the field in Europe and North America. More recently, there have been suggestions that polychlorinated biphenyls (PCBs), which are industrial pollutants, and organomercurial fungicides and other mercury compounds might also be implicated in this phenomenon. Concomitant laboratory investigations have assisted in determining the effects of these pollutants on many aspects of avian reproduction, including the production of abnormally thin shells.

There have, however, been few reviews on the subject of thin shells. Shell thinning effects in the field in Britain have been discussed by Ratcliffe (1970). In addition, Peakall (1970*a*) has presented a popular discussion and Talekar (1971) has considered some aspects, but the only detailed review of possible mechanisms responsible for shell thinning is that of Risebrough *et al.* (1970). It is unfortunate that, being published in symposium proceedings, such an important contribution may be unknown to many interested workers. In the short time since these reviews were written, many relevant research findings have been published, several of which have necessitated considerable revision and re-examination of current theories and concepts. The present paper is an attempt to review the available literature on the effects of pesticides and PCBs on shell thickness, both in the field and in the laboratory, and to assess mechanisms by which thin shells might be formed.

THE PRODUCTION OF EGGS WITH THIN SHELLS BY AVIAN POPULATIONS IN THE FIELD

After noting an increased incidence of egg destruction amongst peregrines *Falco peregrinus*, sparrowhawks *Accipiter nisus* and golden eagles *Aquila chrysaetos* in Britain dating from about 1950, Ratcliffe (1967, 1970) examined egg shells of known age from museums and private collections, and reported that, regionally or nationally, the shells of all three species significantly declined in thickness during the period 1945–50. The interest aroused by the former paper is reflected by the number of investigations into similar phenomena that have since been reported, particularly from the United States and Canada. Ratcliffe (1967) devised an index,

$$\frac{\text{weight of shell (mg)}}{\text{length of shell (mm)} \times \text{breadth (mm)}}$$

that facilitates a comparison of the thickness of whole shells without breaking them. This index has been adopted by many other workers. In North America recent decreases in thickness index, thickness, or shell weight, have been reported for populations of the following: peregrine (Hickey & Anderson, 1968; Berger *et al.*, 1970; Cade *et al.*, 1971); bald eagle *Haliaeetus leucocephalus* (Hickey & Anderson, 1968); prairie falcon *Falco mexicanus* (Fyfe *et al.*, 1969; Enderson & Berger, 1970); osprey *Pandion haliaetus* (Hickey & Anderson, 1968); red-tailed hawk *Buteo jamaicensis* (Seidensticker & Reynolds, 1971); merlin *Falco columbarius* (Fox,

1971); white pelican *Pelecanus erythrorhynchos* (Anderson *et al.*, 1969); brown pelican *Pelecanus occidentalis* (Anderson & Hickey, 1970; Keith *et al.*, 1970; Risebrough *et al.*, 1970; Blus, 1970; Risebrough *et al.*, 1971); double-crested cormorant *Phalacrocorax auritus* (Anderson *et al.*, 1969; Risebrough *et al.*, 1970); common egret *Casmerodius albus* (Faber *et al.*, 1972); great blue heron *Ardea herodius* (Faber *et al.*, 1972); guillemot *Uria aalge* (Gress *et al.*, 1971); herring gull *Larus argentatus* (Hickey & Anderson, 1968); ashy petrel *Oceanodroma homochroa* (Risebrough *et al.*, 1970).

On the other hand, no change has been reported for the following: golden eagle *Aquila chrysaetos* (Hickey & Anderson, 1968); great horned owl *Bubo virginianus* (Hickey & Anderson, 1968; Seidensticker & Reynolds, 1971); gyr falcon *Falco rusticolus* (Cade *et al.*, 1971); rough-legged hawk *Buteo lagopus* (Cade *et al.*, 1971); whooping crane *Grus americana* (Anderson & Kreitzer, 1971); mourning dove *Zenaidura macroura* (Kreitzer, 1971), or for some regional populations of several of the species in the first list. In Britain other species for which shell thickness has declined are the heron *Ardea cinerea* (Prestt, 1970) and the kestrel *Falco tinnunculus*, merlin, hobby *Falco subbuteo*, osprey, rook *Corvus frugilegus*, carrion crow *Corvus corone* and shag *Phalacrocorax aristotelis* (Ratcliffe, 1970). The last writer reported no change for the raven *Corvus corax*, golden plover *Charadrius apricarius*, greenshank *Tringa nebularia*, black-headed gull *Larus ridibundus*, kittiwake *Rissa tridactyla*, razorbill *Alco torda*, or guillemot. The largest recorded decrease was in a brown pelican colony on Anacapa Island off the Californian coast in 1969 (Risebrough *et al.*, 1971), the average decrease in thickness being 50% when compared with the pre-1943 data of Anderson & Hickey (1970). Many of these pelicans laid eggs with soft shells (Keith *et al.*, 1970; Risebrough *et al.*, 1971), as did common egrets in a Californian colony (Faber *et al.*, 1972). Reductions in shell thickness exceeding 20% have been reported for other species by Hickey & Anderson (1968), Berger *et al.* (1970), Fox (1971) and Cade *et al.* (1971).

In addition to the British populations of the peregrine, sparrowhawk and golden eagle for which shell thickness decreased between 1945 and 1950 (Ratcliffe, 1967, 1970), the date of the thickness decrease has been reported for the following:

In California:	peregrine, 1947–52	Hickey & Anderson (1968)
In Britain:	kestrel, started 1946 merlin, started 1951 hobby, started 1952	Ratcliffe (1970)
In Sweden and Finland:	osprey, started 1947–49	Odsjo (1971)

Other researchers reporting declines have compared the recently-laid thin shells with shells from eggs laid before 1940 or 1950. Care must be taken when selecting old shells for comparison with recent ones. Blus (1970) noted that before 1947 the eastern subspecies of the brown pelican laid eggs with significantly heavier and

thicker shells on the Gulf Coast of Florida than on the Atlantic Coast of the same State. The cause of these differences remains unknown. Tuck (1960), referring to Uspenski's work, pointed out that shell thickness of eggs of Brunnich's guillemot *Uria lomvia* can change by a factor of two, depending on the nature of the ground upon which they are laid.

Since the initial declines in Britain, thickness of the peregrine and sparrowhawk shells has remained more or less steady at the reduced level, while golden eagle shells have significantly increased in thickness recently but have not yet regained pre-1945 thickness (Ratcliffe, 1970). Shells of osprey eggs laid in Sweden and Finland have progressively declined in thickness since the late 1940s (Odsjo, 1971).

The factor or factors responsible for shell thinning in British raptors were discussed by Ratcliffe, briefly in 1967 and at length in 1970. He concluded in 1970 that (1) DDT and γBHC used as domestic, veterinary, horticultural and agricultural insecticides were probably implicated in the initial decrease in shell thickness; (2) cyclodiene insecticides contributed to the thickness decline after their widespread introduction into agriculture in the mid 1950s; and (3) PCBs might also be implicated. Disease, other forms of pollution and various factors responsible for poultry laying eggs with thin shells were not considered important. The evidence upon which Ratcliffe's assertions were made is presented below, together with more recent evidence that generally supports his views.

(1) Organochlorine insecticides and their metabolites occur in much higher residues in the tissues of predatory birds than in granivorous species (*see* Moore, 1965), particularly high residues being found in raptors taking birds or freshwater fish. Thus, if organochlorines have had any harmful effects on birds in the field, birds of prey might be the most likely group to be affected.

(2) There have been many temporal and geographical associations between insecticide use and birds of prey producing thin shells. For instance, the decrease in shell thickness for the British species sufficiently well studied coincided with the introduction of DDT. Egg shells of the golden eagle only became thinner in those parts of Scotland where the birds fed mainly on sheep carrion and so exposed themselves to insecticides in the sheep dip. Since the ban on the use of dieldrin dips (Cook, 1964) was implemented in 1966, shell thickness index in the affected regions has increased significantly (Ratcliffe, 1970). Several other examples of such relationships are quoted by Ratcliffe (1970).

Odsjo (1971) reported that shells of osprey eggs were significantly thinner in Sweden and Finland during the period 1939–46 than during 1934–38, a change attributed to PCB pollution. However, this would seem to be an instance of the facts being made to fit the hypothesis, rather than the other way round. Compared with pre-1934 egg shells, those laid during 1934–38 were, on average, thicker by 0·08 mg/mm^2, while those laid during 1939–46 were thinner by only 0·04 mg/mm^2. Thus, the question that should have been asked was: Why were the 1934–38 shells thicker?

(3) Ratcliffe (1970) showed that, for 14 species, those whose eggs contained higher residues had proportionately thinner shells. Amongst brown pelican colonies in America, those birds with the highest DDE residues have been laying eggs with the thinnest shells (Keith *et al.*, 1970). For field material the decline in egg shell thickness or thickness index has frequently been demonstrated to be related to pollutant content in the egg, either on an individual egg basis or on a colony basis. The pollutant in the egg has not been suggested as the cause of the thin shell, but only as an indicator of the level in the laying bird. Cecil *et al.* (1972) considered that, in laboratory experiments, DDT-type residues in egg lipid are usually similar to those in body lipid. Ratcliffe (1970) reported a relationship between shell thickness index and total organochlorine content for British peregrine eggs. From North America a flood of papers has recently demonstrated inverse relationships between shell thickness or thickness index and DDE content of the egg for the peregrine (Cade *et al.*, 1971), prairie falcon (Fyfe *et al.*, 1969; Enderson & Berger, 1970; Fimreite *et al.*, 1970), great blue heron (Vermeer & Reynolds, 1970), white pelican (index only, not thickness; Anderson *et al.*, 1969), brown pelican (Risebrough *et al.*, 1970; Blus *et al.*, 1971, 1972) and double-crested cormorant (Anderson *et al.*, 1969), but not for the common tern *Sterna hirundo* (Switzer *et al.*, 1971). Some of these workers have confidently presented significant correlation coefficients despite the values for egg residues clearly not being normally distributed, but even in these instances the general trend that highly contaminated eggs have thin shells is apparent. Unfortunately, interpretation of the data is hindered by the presence of other contaminants in the eggs. Within a locality, tissue concentrations of PCBs often tend to be positively correlated with DDE concentrations (Risebrough *et al.*, 1968; Peakall & Lincer, 1970*a*) so it is difficult to determine the significance of the reports of egg PCB content being related to the degree of shell thinning for pelicans (Anderson *et al.*, 1969; Risebrough *et al.*, 1970). A similar relationship was not found for eggs of the double-crested cormorant (Anderson *et al.*, 1969) or the great blue heron (Vermeer & Reynolds, 1970). In prairie falcon eggs both dieldrin and DDE levels were related to the decrease in shell thickness index in Wyoming and Colorado (Enderson & Berger, 1970), but no such relationship existed for mercury levels in Canadian eggs (Fimreite *et al.*, 1970). Many writers who analysed for DDE did not report the concentration of other pollutants. For the brown pelican, Blus *et al.* (1971) reported significant negative relationships between shell thickness and egg residues of pp'-DDE, pp'-DDT, pp'-TDE, dieldrin and PCBs, but further statistical analysis revealed pp'-DDE to be the only residue that was consistently associated with significant shell thinning. The contamination pattern of mercury in the egg samples was different to that of the other pollutants, a positive relationship between mercury content and shell thinning being found. A positive correlation between egg content of pp'-DDE and shell thickness has been observed for the eggs of the moorhen *Gallinula chloropus* (Fowler *et al.*, 1971).

The techniques and conclusions of Blus *et al.* (1971, 1972) have come under severe attack from Hazeltine (1972). The first objection was that data from three colonies and two subspecies were grouped together by Blus *et al.* (1972) and the negative relationships between shell thickness and DDE content only existed on an inter-colony, and not an intra-colony, basis. Hazeltine (1972) seems rather confused over the word 'colony', since Blus *et al.* (1971, 1972) clearly stated that eggs were collected from twelve (not three) colonies in three different states. Lack of significance for 'within-state' data, as calculated by Hazeltine (1972), was perhaps due to the small sample sizes. The Florida data of Blus *et al.* (1972), which may indeed show a significant negative relationship, were not examined by Hazeltine (1972). Two other objections on statistical grounds included, once again, misuse of the correlation coefficient for data not normally distributed (*see* above). Hazeltine's fourth objection was that Blus's eggs were apparently often partially incubated, sometimes containing cheeping embryos. Blus *et al.* (1971, 1972) made no mention of using incubated eggs, referring only to fresh and addled eggs. 'Addled' has several meanings, but it is never applied to eggs with living embryos. When working with part-incubated eggs, Hazeltine (1972) recommended that ppm DDE in egg lipid would be a suitable measure of initial pollutant concentration in the egg, and he found a highly significant *positive* relationship between ppm DDE in the lipid and shell thickness for nine pelican eggs (four part-incubated) collected from Anacapa Island. Certainly, for these eggs, the rate of disappearance of DDE during incubation was similar to the rate of loss of egg lipid. If some of Blus's eggs were partially incubated, the errors should not be too serious since residues in total egg contents (not in the yolk, as stated by Hazeltine, 1972) have apparently been determined, and the relative loss in weight of egg contents would be expected to be similar to Hazeltine's figures for DDE loss. Nevertheless, until more is known about the dynamics of egg residues in the species under study, it is dangerous to include part-incubated eggs in investigations of this nature. An added complication is that shell thickness changes slightly during incubation (Vanderstoep & Richards, 1971). In contrast to his own Anacapa data, when Hazeltine (1972) examined data supplied for sixty-five Anacapa eggs by R. W. Risebrough, there was a highly significant *negative* relationship between lipid DDE content and shell thickness. Hazeltine (1972) alleged that conflicting evidence may have been suppressed by some scientists intent on having DDT banned, a misguided action that can never be justified, but for which he has presented no conclusive evidence.

(4) Just as field workers were stimulated into action by Ratcliffe's first paper (1967), so toxicologists began deliberately exposing captive birds to organochlorines in order to (a) examine eggs for decreases in shell thickness and/or (b) study their tissues to determine possible biochemical mechanisms to explain this phenomenon. These experiments and their implications are discussed at length in the next two sections. As is stressed later, however, the mechanism(s) responsible for shell thinning still require(s) much research. The widely-quoted theory at the end of the

1960s was that organochlorines increased sex hormone metabolism and so decreased medullary bone deposition (Peakall, 1967; Risebrough *et al.*, 1968), medullary bone being a labile store of egg shell calcium. Although this mechanism is now thought to play no more than a minor role in decreasing shell thickness, it was generally accepted as a satisfactory hypothesis when Ratcliffe presented his conclusions in 1970. Despite the fact that the reasons for shell thinning are now apparently less clearly defined than they seemed to be two or three years ago, during this time there have been several more laboratory experiments that have strengthened the case for organochlorines being to blame by demonstrating that these compounds can cause captive birds to lay eggs with thin shells.

Hickey & Anderson (1968) were the first workers in North America to follow up the egg shell effects reported by Ratcliffe (1967) and they concluded, on the strength of the evidence available, that 'the onset of the calcium change one year after the introduction of chlorinated hydrocarbons into general usage was not a random circumstance'. More recently, Peakall (1970*a*) and Risebrough *et al.* (1970) discussed the evidence for organochlorines being the cause of shell thinning. Sometimes researchers studying thin shells have perhaps not been sufficiently cautious or critical about new evidence blaming organochlorines. Some of the laboratory evidence has been misleading and much of the field evidence is circumstantial. As Robinson (1970) has pointed out, 'inferences based on circumstantial evidence may be regarded as doubtful, probable or demonstrated beyond reasonable doubt, according to the weight of the evidence'. Placing too much faith in chronological and geographical associations can be dangerous. For example, such associations exist between the decline of the frog *Rana temporaria* in Britain and cyclodiene insecticide usage, although insecticides have probably only played a minor part in the animal's decline, the relationships being coincidental (Cooke, 1972). Nevertheless, there are now so many well-documented temporal and spatial relationships for decreases in shell thickness and organochlorine contamination of the environment and/or affected species that even a sceptic must admit that organochlorines are the probable cause. The recent reports of high amounts of organochlorines in eggs with thin shells add yet more weight to the argument and, although the presence of more than one contaminant often complicates the issue, the relationship between DDE content and decrease in shell thickness certainly suggests that one or more of the DDT group of compounds is affecting the laying

bird. pp'-DDE is not necessarily the molecule responsible since high DDE concentrations might simply indicate harmful levels of a water-soluble metabolite of pp'-DDT that is lost during conventional organochlorine analysis. The recent field evidence, coupled with the many toxicological reports, would seem to put the causal link 'beyond reasonable doubt'. Perhaps the only reason preventing total acceptance of the theory is the failure to elucidate with any degree of certainty the mechanism(s) by which organochlorines cause thin shells. However, taking into account our present lack of knowledge of some of the basic principles of egg shell formation, inability to find a mechanism is perhaps not surprising. While elucidation of the mechanism(s) would strengthen the case against organochlorines, failure to elucidate does not weaken the case. Armed with the many recent pieces of evidence, there is no reason to doubt any of the concluding remarks of Ratcliffe (1970), except perhaps that γBHC was involved at all, and only if a hitherto unexpected and unstudied factor is linked with egg shell thinning is the pesticide theory likely to be displaced. Talekar (1971) is one writer who has expressed concern that uninvestigated industrial pollutants may be at least partially responsible for the shell thinning phenomenon.

One should be cautious about blaming pollution every time shell thinning is observed in the field, since other environmental variables can affect shell thickness (Tuck, 1960; Anderson *et al.*, 1970; Blus, 1970; Bjerk & Holt, 1971).

EXPERIMENTS TO INVESTIGATE THE EFFECTS ON SHELL THICKNESS OF DELIBERATE EXPOSURE OF LAYING BIRDS TO ORGANOCHLORINES AND OTHER POLLUTANTS

Details of thirteen experiments in which organochlorine insecticides were reported as changing shell thickness are given in Table 1. Experiments in which no change in shell thickness was observed are referred to later.

Methods for determining shell thickness were reviewed by Tyler & Geake (1961). If a micrometer is used, a large number of measurements is required to produce an accurate value of mean shell thickness, since, longitudinally, shell thickness can vary considerably (Tyler, 1961). For chicken eggs, Tyler (1961) recommended twelve measurements per shell in a longitudinal line from broad cap to narrow cap, but the writers listed in Table 1 who used a micrometer made four or less measurements per shell, usually at the waist. Although thickness at the waist often differs greatly from mean shell thickness (Tyler 1961), providing an adequate number of shells has been measured, results and conclusions should be satisfactory since information is required on differences between control and treatment groups of shells, rather than on absolute values for individual shells. Both Ratcliffe's index (1967) and a direct measure of shell weight per unit area (Tyler & Geake, 1961) permit an accurate, rapid assessment of shell thickness, overcoming the tedious replication of measurements at different points on the same shell.

An increase in thickness was reported in only one of the experiments shown in Table 1 (Jefferies, 1969), but in this experiment egg weight decreased for the dosed birds and there was, in fact, no difference between the weight of shell material laid down by the control and dosed groups, suggesting no effect on shell formation.

In eleven of the twelve experiments reporting shell thinning, insecticide was administered in the diet, and of these only in the experiment on wild falcons described by Enderson & Berger (1970) was alternative uncontaminated food available. Jefferies (1967, 1969) and Prestt *et al.* (1970) recognised that partial starvation due to dietary rejection after the addition of organochlorines might mask any direct effects the ingested organochlorines were having on the birds under study. They took precautions against this by presenting untreated seed to Bengalese finches *Lonchura striata* for 21 h/day and the treated diet for only 2 h. Stephen *et al.* (1970) fed chickens, *Gallus domesticus*, on diets containing 20 ppm DDT + 20 ppm DDE and considered that any decreases in percentage shell calcium were due to decreased calcium consumption, rather than to a direct effect of the insecticide. In none of the ten experiments in which thin shells were observed after birds had been fed solely on diets with added insecticide were figures for food consumption given. The experimental design is open to criticism (*e.g.* by Davison & Sell, 1972) when the additive under investigation might lead to partial rejection of the diet and so cause a bird to lay eggs with thin shells because of reduced food intake. The effect of withholding food on shell thickness can be illustrated by an experiment I carried out on twelve chickens. The birds were fed normally for five days, food was then withheld on the sixth and seventh days, and the usual diet was resumed on the eighth day. Mean shell thickness for the group decreased by 16% on day 7 and by 22% on day 8, but recovered to the normal value by day 12.

Apart from Stephen *et al.* (1970), however, writers have only reported decreased consumption of DDT-contaminated diets when the DDT level was high. No rejection was reported at 250 ppm by chickens (Noakes & Benfield, 1965), at 300 ppm by chickens (Lillie *et al.*, 1972), at 400 ppm by pheasants *Phasianus colchicus* (Genelly & Rudd, 1956) or at 500 ppm by bobwhite quail *Colinus virginianus* (Linduska & Springer, 1951), but a diet containing 700 ppm DDT was rejected by Japanese quail *Coturnix coturnix japonica* (Cross *et al.*, 1962). In contrast, a diet containing only 25 ppm dieldrin was rejected by pheasants (Genelly & Rudd, 1956), food consumption being only 86% that of birds on a control diet. 50 ppm dieldrin lowered food consumption to 71%, and a direct relationship between consumption and egg production was apparent. On the other hand, Gillett & Arscott (1969) noted that young Japanese quail on a diet containing 5 ppm dieldrin consumed more than those on the basal diet. Atkins & Linder (1967) observed decreased food consumption after pheasants had been given dieldrin in capsules for thirteen weeks (6 mg/bird/week, an intake equivalent to 16 ppm in the diet). Here the dieldrin cannot have affected the palatability of the diet, yet food intake

TABLE 1

EXPERIMENTS IN WHICH EGG SHELL THICKNESS HAS BEEN AFFECTED AFTER EXPOSURE OF THE LAYING BIRD TO ORGANOCHLORINE INSECTICIDES

Author(s)	*Species*	*Pollutant(s)*	*Exposure*	*Mean egg content (ppm)*	*Shell measurement*	*Effect on shell thickness*
Bitman *et al.* (1969)	Japanese quail *C. coturnix japonica*	pp′-DDT or op′-DDT	100 ppm in low Ca diet for 45 days	520 / 27 total residues of DDT type compounds	Thickness at 3 points around waist of shell without membranes†	Decrease, 6%‡ / Decrease, 4%‡ Compared with controls on low Ca diet
Lehner & Egbert (1969)	Mallard *Anas platyrhynchos*	Dieldrin	Up to 10 ppm* in diet for 1–2 years	Up to 44	Thickness at broad pole and waist of shell plus outer membrane†	Decrease, up to 4%‡
Porter & Wiemeyer (1969)	American Kestrel *Falco sparverius*	pp′-DDT plus dieldrin	Up to 15 ppm pp′-DDT plus 3 ppm dieldrin* in diet for 1–2 years	Not given	Thickness at 4 points of shell plus membranes†	Decrease, up to 17%‡
Heath *et al.* (1969) (*see* also Heath *et al.*, in press)	Mallard	pp′-DDE or pp′-DDT	Up to 40 ppm* in diet for between 1 and 2 years	Not given	Thickness of shell plus membrane†	Decrease, up to 13%‡
Jefferies (1969)	Bengalese finch *Lonchura striata*	pp′-DDT	Up to 300 μg/day* in diet for about 15 weeks	Not given	Shell weight/egg weight	Increase by average of 7%
Enderson & Berger (1970)	Prairie falcon *Falco mexicanus*	Dieldrin	Wild birds fed on up to 12 tethered starlings previously dosed with dieldrin	40 –	Thickness index	Decrease of 9% (compared with controls) for 7 most contaminated eggs

was reduced. Unfortunately, these writers did not measure shell thickness. Jefferies & French (1971) noted a similar loss of appetite when pigeons were treated with encapsulated pp'-DDT at the much higher level of 54 mg/kg/day. In addition, the food consumption of young cockerels on a diet containing 500 ppm DDT began to drop after the birds had been subjected to the treatment for about six weeks (Srebocan *et al.*, 1971). Body residues of organochlorines must have been responsible for this partial loss of appetite. The longer a bird is fed on a contaminated diet, so the likelihood is increased of tissue residues building up to an effective level, unless this is above the plateau level. For chickens being fed DDT, this critical effective level seems to be very high. Feeding birds on a diet containing 25 ppm pp'-DDT for twenty-eight weeks followed by 300 ppm for twelve weeks led to small, but non-significant, decreases in food consumption (Lillie *et al.*, 1972) although the mean content in the abdominal fat of the birds at the end of treatment was 2,300 ppm (Cecil *et al.*, 1972). The chicken is, however, an atypical species, tending to be resistant to DDT (*see* Noakes & Benfield, 1965; Lillie *et al.*, 1972; and later in this and in other sections). Other species may start to show a loss of appetite at much lower tissue concentrations. Nevertheless, while it is possible that reduced food intake has contributed to shell thinning in some of the experiments in Table 1, the food rejection/appetite loss data available at present indicate that reduced food intake cannot account for the great majority of observed decreases in thickness summarised in the table.

In the two experiments in which food intake has been controlled or determined, however, the administered pesticide has failed to cause shell thinning (Davison & Sell, 1972; Cecil *et al.*, 1972). These two experiments contrast markedly with those of Smith *et al.* (1970) and Sauter & Steele (1972). In each experiment, White Leghorn chickens were treated by adding DDT to the diet. Significant shell thinning was reported by Smith *et al.* (1970) after feeding 10 ppm technical DDT for two months and by Sauter & Steele (1972) after feeding 0·1 ppm technical DDT for ten weeks. On the other hand, no shell thinning was found by Davison & Sell (1972) after feeding 200 ppm pp'-DDT for twelve weeks or by Cecil *et al.* (1972) after feeding 50 ppm pp'-DDT or op'-DDT for twenty-eight weeks, followed by 300 ppm for twelve weeks. It could be argued that the compound responsible for shell thinning is a component (or its metabolite) of technical DDT, other than op'- or pp'-DDT. Since pp'-TDE can probably be ruled out as well (Heath *et al.*, 1969), the dietary level of this proposed compound in the experiment of Sauter & Steele (1972) must have been less than 0·004 ppm, the concentration of any other constituent in technical DDT being less than 4% (Metcalf, 1955). The purity of the pp'-DDT used by Davison & Sell (1972) was 99%, so their 200 ppm pp'-DDT diet might be expected to have contained a higher level of the proposed active compound than the 0·1 ppm technical DDT diet of Sauter & Steele (1972). Further work is needed before one can even consider ascribing potent shell thinning properties to a minor component of technical DDT. Other differences exist between

Peakall (1970*b*)	Ring dove *Streptopelia risoria*	pp′-DDE	Single intraperitoneal injection of 150 mg/kg	80	Weight	Decrease by 23%
Wiemeyer & Porter (1970)	American kestrel	pp′-DDE	10 ppm in diet for more than 1 year	32	Thickness at 4 points of shell plus membranes†	Decrease by 10%
Smith *et al.* (1970)	Chicken *Gallus domesticus*	Technical DDT	Up to 10 ppm* in diet for 2 months	Up to 6 ppm in yolk	Not given	Decrease, up to 11%‡
Stickel & Rhodes (1970)	Japanese quail	pp′-DDT	Up to 25 ppm* in diet for 26 weeks	Not given	Not given	Decrease, up to 7%‡
Tucker & Haegele (1970)	Bobwhite quail *Colinus virginianus*	Technical DDT	Up to 30 ppm* in diet for 96 days	Not given	Thickness at 4 points around waist†	↓ Decrease, up to 4%‡ ↓ Decrease, up to 5%‡
	Mallard	Technical DDT	Single oral dose 1000 mg/kg. Food withheld for 2 days after treatment	Not given	As above	Decrease by 9% over 16 days
	Mallard					
Longcore *et al.* (1971*b*)	Black duck *Anas rubripes*	pp′-DDE	Up to 30 ppm* in diet for 6 months	Up to 135	Thickness at waist and both poles of shell plus membranes†	Decrease, up to 38%‡
Sauter & Steele (1972)	Chicken	Technical DDT or lindane	Up to 10 ppm* in diet for 10 weeks	Not given	Not given	Decrease, up to 9%‡ after treatment period

* Highest treatment dose
† Measured with a micrometer
‡ Mean thickness change for most affected treatment group

observations made in these four experiments, which may simply illustrate how similarly-designed studies can sometimes produce totally different results.

Several other experiments have been reported in which organochlorines have had no effect on shell thickness. Cecil *et al.* (1971*a*) repeated the work of Bitman *et al.* (1969) except that (1) quail were exposed to pp'-DDE and not op'-DDT, (2) exposure time was increased from forty-five to seventy-four days and (3) the level of dietary calcium was adequate and not deficient as before. No change in shell thickness was found. Assuming there is a misprint in the shell thickness units in the table of Cecil *et al.* (1971*a*), the 0·6% low calcium diet of Bitman *et al.* (1969) appears to have contributed to shell thinning to a much greater extent than the 100 ppm DDT added to the diet. Heath *et al.* (1969) reported no significant change in shell thickness after 40 ppm TDE was added to the diet of mallards. Bobwhite quail fed on a diet containing 30 ppm DDE throughout a laying season did not produce thin-shelled eggs, unlike mallards on a 10 ppm DDE diet for two seasons (Heath *et al.*, in press; this being a similar experiment to that of Heath *et al.*, 1969). DDE had no marked effect on shell thickness in chickens at dictary levels sufficient to produce concentrations in the abdominal fat of greater than 2000 ppm (Cecil *et al.*, 1972). Dieldrin has been found to have no effect on shells laid by ring doves *Streptopelia risoria* (Peakall, 1970*b*), pheasants (Dahlgren & Linder, 1970), chickens (Robinson, 1970; Davison & Sell, 1972) or mallards (Muller, 1971*a*). Whitehead *et al.* (1972) administered encapsulated lindane to chickens at a level equivalent to about 100 mg/kg diet, but shells were of normal thickness. This is in sharp contrast to the study of Sauter & Steele (1972) in which chickens exposed to dietary levels of 0·1 mg lindane/kg diet laid eggs with thin shells.

Earlier studies on the effects of insecticides on avian reproduction and survival (*e.g.* Genelly & Rudd, 1956; Cross *et al.*, 1962) were published before the recent upsurge of interest in shell thickness. Since no mention was made in these papers of thickness changes, obvious changes are unlikely to have occurred. A decrease in shell thickness of even 10% would probably have been missed unless the shells were weighed or the thickness measured. One can only speculate at the number of experiments that yielded negative results, which were not considered worth publishing.

Single oral doses of one of the commercial PCB mixtures, Aroclor 1254, sufficient to disrupt and then terminate laying in Japanese quail and mallard, produced thin shells in the few eggs laid before complete termination (Tucker's experiment referred to by Peakall & Lincer, 1970*a*). However, Tucker & Haegele (1970) reported that mallards, similarly treated with Aroclor 1268 and then starved for three days, laid eggs with shells of approximately normal thickness. In addition, ring doves, fed 10 ppm Aroclor 1254 in their diet for six months or injected intraperitoneally with 160 mg/kg Aroclor 1254, laid eggs with normal shells (Peakall, 1971). Similarly, administration of enough encapsulated Aroclor 1254 to adult pheasants to decrease egg production and hatchability had no effect on shell

thickness (Dahlgren & Linder, 1971). Neither mallards fed on (1) a diet containing 500 ppm Aroclor 1254 for thirty-nine days or (2) 25 ppm for two laying seasons, nor bobwhite quail fed on a diet containing 50 ppm for a single season, laid eggs with thin shells (Heath *et al.*, in press). Chickens maintained on diets containing 100 ppm Aroclor 1242 or 1254 laid eggs with shells thinner by 17 and 24% respectively, but dietary consumption, reduced by 37 and 33% respectively (Monsanto Company, 1971), could have accounted for this. Mean shell thickness of a group fed 100 ppm Aroclor 1260 was only 2% below that of the control group, food consumption being reduced by 6%. In a second experiment using Aroclor 1242, neither food consumption nor shell thickness was affected (Monsanto Company, 1971).

Risebrough *et al.* (1970) observed that gallinaceous species tend to be more resistant than birds in other orders to decreases in shell thickness after exposure to organochlorine compounds. The trend is still apparent when later evidence is considered. Bobwhite and Japanese quail seem rather resistant, no changes in shell thickness being reported after treatment by Cecil *et al.* (1971*a*) and Heath *et al.* (in press) and small changes or changes only after severe treatment were reported by Bitman *et al.* (1969), Stickel & Rhodes (1970), Peakall & Lincer (1970*a*) and Tucker & Haegele (1970). In contrast to these experiments, Japanese quail maintained for 21 days on a diet containing 225 ppm technical DDT laid eggs with shells of greatly reduced thickness both during and soon after treatment, the maximum thickness reduction for a shell being 60% (McFarland *et al.*, 1971). Although chickens have been observed to lay eggs with thin shells following organochlorine treatment (Smith *et al.*, 1970; Sauter & Steele, 1972), they usually show no response or else a response only under severe dietary exposure (Robinson, 1970; Stephen *et al.*, 1970; Cecil *et al.*, 1972; Davison & Sell, 1972; Heath *et al.*, in press). Similarly pheasants are unaffected by dieldrin (Dahlgren & Linder, 1970) or PCBs (Dahlgren & Linder, 1971). On the other hand, deliberately exposed falcons have suffered quite substantial decreases in shell thickness (Porter & Wiemeyer, 1969; Enderson & Berger, 1970; Wiemeyer & Porter, 1970). Japanese quail eggs containing 520 ppm total residues of pp'-DDT and its metabolites had shells only 6% thinner than controls (Bitman *et al.*, 1969) but eggs from American kestrels *Falco sparverius* containing only 32 ppm DDE had shells 10% thinner than controls (Wiemeyer & Porter, 1970). Ring doves and mallards, the other species used in these experiments, seem intermediate in their response, sometimes producing thin shells (Lehner & Egbert, 1969; Heath *et al.*, 1969; Peakall & Lincer, 1970*a*; Peakall, 1970*b*; Longcore *et al.*, 1971*b*) and sometimes not (Heath *et al.*, 1969, in press; Peakall, 1970*b*, 1971; Muller, 1971*a*). So, if it is possible to generalise, bearing in mind the very diverse sets of conditions that different experimenters have employed, gallinaceous species tend to show less of a response in terms of reduced shell thickness than ring doves and mallards and these, in turn, tend to be more resistant than falcons. Since birds of prey are more susceptible in the laboratory and their tissues

tend to contain higher residues in the field, the fact that they are the species most affected in the field further supports the theory that pesticides are responsible.

It is, of course, not surprising that such taxonomic differences should occur when one is considering the response to a group of compounds, the organochlorine insecticides and the PCBs, that are closely related both physically and chemically. In contrast, Tucker & Haegele (1971), who examined the acute oral toxicity to six avian species of sixteen pesticides, including carbamate and organophosphate insecticides, dieldrin and strychnine, reported that the average sensitivity of any one species to this broad spectrum of compounds was not statistically different from that of any of the other five species. Nevertheless, it is interesting that the six species in order of increasing resistance were the house sparrow *Passer domesticus*, mallard, pigeon *Columba livia*, pheasant, Japanese quail and chukar partridge *Alectoris graeca*, the three gallinaceous species tending to be more resistant.

Compounds of the DDT group or their metabolites usually reduced shell thickness of captive birds to a greater extent than dieldrin or PCBs. For example, doves injected intraperitoneally just before laying with single doses of 160 mg/kg Aroclor 1254 (Peakall, 1971) or 30 mg/kg dieldrin (Peakall, 1970*b*) laid eggs with normal shells, while those similarly injected with 150 mg/kg pp'-DDE laid eggs with shells 23% thinner than normal (Peakall, 1970*b*). Mallards maintained for two seasons on a diet containing 10 ppm DDE laid eggs with thin shells, while those on a diet containing 25 ppm Aroclor 1254 produced normal shelled eggs (Heath *et al.*, in press). This, coupled with the field evidence presented in the previous section, suggests that DDT-type compounds have a greater effect on shell thickness than the other organochlorines so far studied.

Captive birds have also been used to study the effects of mercury on shell thickness. Japanese quail fed on diets containing up to 8 ppm mercuric chloride (Stoewsand *et al.*, 1971) laid eggs with thin shells. Feeding chickens (Tejning, 1967) or pheasants (Fimreite, 1971) on methylmercury-treated grain led to a marked increase in soft shelled eggs. Although Fimreite (1971) noted a significant reduction in food intake for birds at the highest dietary mercury levels, significantly more soft-shelled eggs were also laid by pheasants on the lower levels, demonstrating that the ingested mercury must be affecting shell formation. There is, however, evidence indicating that mercury-induced shell thinning does not occur in the field (Fimreite *et al.*, 1970; Blus *et al.*, 1971).

Regarding the organophosphate insecticides, Sauter & Steele (1972) reported that diazinon and malathion caused egg shell thinning in chickens, and Muller (1971*a*, *b*) observed that parathion had a similar effect on captive mallard and partridge *Perdix perdix*.

There are now more than twenty reports of organochlorine or organophosphate insecticides, PCBs or mercury compounds causing thin shells in experimental birds. Although several experimental designs or techniques are open to criticism and some nagging doubts about reduced food consumption still remain, the experiments

nevertheless appear to demonstrate positive shell thinning due to treatment. Sometimes severe treatment conditions were required to produce even only slight shell thinning. For example, Stickel & Rhodes (1970) reduced the light period from 14 to 8 h at the end of treatment and several birds died from DDT poisoning. Bitman *et al.*, (1969) reported that after pp'-DDT treatment, their quail contained on average 1560 ppm pp'-DDT + metabolites in the fat and 240 ppm in the liver. Levels as high as this are only very rarely reported in field samples (*e.g.* Risebrough *et al.*, 1968; Prestt, 1970; Keith *et al.*, 1970). Of the remaining experiments on captive birds referred to in Table 1, only in the papers by Peakall (1970*b*) and Smith *et al.* (1970) are tissue analyses given. In the former paper, an average of 77 ppm DDT in the shell gland tissue of ring doves was associated with shell thinning of 23%, and in the latter 117 ppm DDT plus metabolites in the abdominal fat of chickens was associated with shell thinning of 11%. No doubt at least some of the experiments yielding negative results failed because conditions were not severe enough. The severity of the treatment should depend on what the experimenter is trying to determine. If he is attempting to elucidate the mechanism for shell thinning then he is justified in using more extreme conditions in order to produce measurable biochemical or physiological changes than if he simply wishes to reproduce field effects. So far these controlled experiments have failed to reach the 50% *average* reduction in shell thickness reported for the brown pelicans on Anacapa (Risebrough *et al.*, 1971), although reductions exceeding 20% have been reported (Peakall, 1970*b*; Longcore *et al.*, 1971*b*). Tucker & Haegele (1970) observed that greater decreases in shell thickness in captive mallard were produced by acute doses of DDT than by chronic doses, and they pointed out that, in the field, fasting and stress during the reproductive period might mobilise sufficient pesticide residues to cause shell thinning.

POSSIBLE MECHANISMS FOR THIN EGG SHELL FORMATION

The case against organochlorines would be considerably strengthened if mechanisms by which they cause thin shells could be precisely elucidated. This would then permit a clearer assessment of the importance of other environmental variables and it would also become possible to evaluate the effect of residues at the site of action.

Normal egg shell formation

Before discussing possible mechanisms for thin egg shell formation after exposure of a bird to organochlorines, it is necessary to outline what is known about the process of shell formation. Research on this topic has been largely carried out on chickens and this atypical species must therefore serve as a model for all species. For most aspects of shell formation, reviews are quoted which discuss the subject in much greater detail than is permitted here.

Egg formation was reviewed by Gilbert (1967). Ovulation is controlled by pituitary gonadotrophin secretion (reviewed by Gilbert, 1971*a*) and the egg is formed in the oviduct (Fig. 1). Oviducal structure and function have been reviewed by Aitken (1971). In most avian species, including the chicken, only the left oviduct usually develops and is functional. The ovum passes via the infundibulum to the magnum where albumen is secreted by the epithelial cells; then to the isthmus region where shell membrane material, the first egg shell component, is laid down. Shell formation has recently been reviewed by Simkiss (1968), Wilbur & Simkiss (1968), Taylor (1970) and Simkiss & Taylor (1971). Recent evidence (Robinson *et al.*, 1966, 1968) indicates that shell calcification is initiated in the isthmus and although this is generally accepted (Cooke & Balch, 1970*a*; Simkiss & Taylor, 1971), Fujii & Tamura (1970) have described shell initiation occurring in the next region, the shell gland. Certainly the main part of the shell is laid down in the shell gland, where an egg usually remains for 16–20 h. About 95% of the shell is mineral matter (water content <2%) and of this more than 95% is calcium carbonate (Romanoff & Romanoff, 1949) in its most stable polymorphic state, calcite.

The calcium metabolism of the laying bird has been reviewed by Taylor & Stringer (1965) and Simkiss (1967) and has been discussed in a popular article by Taylor (1970). Calcium that is eventually incorporated into the shell comes initially, of course, from the diet. Transport of calcium across the intestinal wall has been reviewed by Simkiss (1967) and Wasserman (1968). Plasma calcium is either ionic or protein-bound and the two forms are believed to be in equilibrium. About 25–40% of the calcium incorporated into the shell does not come directly from the diet (Comar & Driggers, 1949; Driggers & Comar, 1949), but is stored in the form of medullary bone (Kyes & Potter, 1934), spicular growth that occurs inside well-vasculated bones (Taylor & Moore, 1953, 1956) and is most obvious in the medullary cavities of the limb bones. Oestrogens and androgens act synergistically to control medullary bone deposition (Taylor & Stringer, 1965). Calcium in the medullary bone is mobilised and carried via the blood to the shell gland. The role of the parathyroid and ultimobranchial secretions in regulating mobilisation has been discussed by Taylor (1966, 1970) and by Simkiss & Dacke (1971) respectively. It is generally accepted that parathormone is responsible for the osteoclastic activity in medullary bone when the shell is being calcified. Probably it is the ionic calcium fraction that is depleted as the blood passes through the shell gland (Simkiss & Taylor, 1971). Calcium is accumulated in the ciliated epithelial cells of the shell gland (Gay & Schraer, 1967) and is actively transported into the shell gland lumen (Schraer *et al.*, 1965; Schraer & Schraer, 1970), perhaps bound to a carrier protein (Corradino *et al.*, 1968).

The origin of the shell carbonate fraction is discussed at some length below.

Egg shell nomenclature can be very confusing since various writers use the same word or phase to describe different parts of the shell, and some of the old phrases, such as 'spongy layer', can be misleading. A new nomenclature system proposed

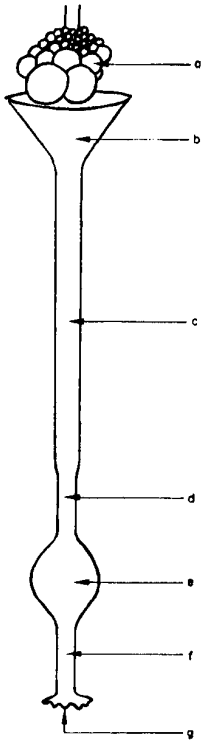

Fig. 1. Diagram of a chicken's ovary and oviduct; a: ovary; b: infundibulum; c: magnum; d: isthmus; e: shell gland containing a calcifying egg; f: vagina; g: opening to cloaca.

by Schmidt (1957, 1962*a*, *b*) and Tyler (1965) is shown in Fig. 2 and will be used in this paper. The term 'mammillary layer' is retained to describe the cone layer plus basal caps.

The mineralised shell begins as spherulitic crystal growth around mucoprotein concentrations on the outside of the outer shell membrane (Fig. 2). These organic aggregations with deposited calcite crystals are called mammillary cores, and the nature of the mucoprotein has been investigated histochemically (Robinson & King, 1968) and biochemically (Cooke & Balch, 1970*a*). The organic cores initiate

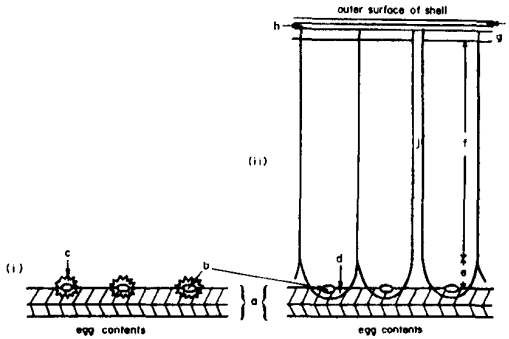

Fig. 2. Diagrams of radial sections of shells to show (i) spherulitic growth on the surface of the shell membranes and (ii) the structures that can occur in a fully-formed shell. Nomenclature after Schmidt (1957, 1962*a*, *b*) and Tyler (1965): a: shell membranes; b: mammillary core; c: spherulitic growth around the organic mammillary core; d: basal cap; e: cone layer; f: palisade layer; g: surface crystal layer; h: cuticle; i: cover; j: pore. The surface crystal layer and the cover are absent from the shells of many species.

crystal growth (Fujii & Tamura, 1970), perhaps by providing a convenient lattice for calcite formation or by binding mineral ions either electrostatically or by chelation, thereby exceeding the solubility product of calcium carbonate and so achieving precipitation (Wilbur & Simkiss, 1968; Cooke & Balch, 1970*a*). Inward growth of the spherulites is inhibited by the presence of the shell membranes, so the crystals tend to develop outwards. They coalesce and the growth is then columnar, forming the palisade layer. In some places coalescence does not occur, possibly because of liquid diffusing through the shell during formation (Tyler, 1965) and pores are formed. Running throughout the calcified shell is a continuous organic phase, the matrix, which again is largely mucoprotein (Baker & Balch, 1962; Cooke & Balch, 1970*b*). This organic matter probably plays an intimate role

in shell development since electron micrographs show that matrix deposition precedes crystallisation (Simons, 1971). Around the outside of the shell is an organic cuticle.

Possible factors controlling oviposition have been reviewed by Sturkie (1965) and Gilbert (1967, 1971*b*). In a modern breed of chicken, oviposition occurs about 24 h after ovulation.

Mechanisms by which pesticides might cause thin shells

The poultry industry has been concerned for many years with possible methods of reducing loss due to thin shells. Although many workers have studied shell formation and treatments that cause shell thickness to change, our knowledge of these subjects is still sketchy and incomplete. Nevertheless, literature on thin shells, experimentally induced in chickens, is freely referred to in this section in an attempt to find similarities between the action of various treatments known to cause thin shells and the action of pesticides.

Basically, possible mechanisms can be divided into four categories: (1) those reducing the availability of the calcium ions, (2) those reducing the availability of the carbonate ions, (3) those affecting other shell constituents upon which proper shell formation depends, and (4) miscellaneous mechanisms producing disruptive changes. Each will be discussed in turn.

1. *Reduction of calcium availability*

Between entering the gut and becoming incorporated in the shell, calcium might be blocked by the action of organochlorines during five different stages: (a) absorption from the gut, (b) deposition of medullary bone, (c) mobilisation of medullary bone, (d) transport in the blood and (e) transport from the blood to the developing shell.

1(*a*) *Absorption from the gut:* Most of the mechanisms involved in the transport of calcium from the intestinal lumen to the blood are dependent on the steriod vitamin D (reviewed by Simkiss, 1967; Wasserman, 1968). An active form was shown by Blunt *et al.* (1968) to be the 25-hydroxy derivative of vitamin D_3 (cholecalciferol), and Anderson *et al.* (1969) suggested that since organochlorines can induce hydroxylation of steroids (*see* section 1(*b*) below), exposure to insecticide might lead to the production of inactive molecules hydroxylated on the wrong carbon atom. Furthermore, Risebrough *et al.* (1970) have argued that organochlorines might affect egg shell formation by inhibiting ATPases involved in active calcium transport across the intestinal wall or shell gland, pointing out that DDT inhibits the *in vitro* incorporation of labelled phosphorus into ATP in insect respiratory particles (Avi-Dor & Gonda, 1959). Koch *et al.* (1969) have reported inhibition *in vitro* of insect nerve, muscle and brain ATPases by pp'-DDT and by some of its metabolites.

Experiments designed to investigate whether pesticides affect calcium uptake from the gut have, however, had conflicting results. Peakall (1969) observed that DDT failed to reduce the uptake of labelled calcium in the intestine of the zebra finch *Poephila guttata*. On the other hand, Nowicki *et al.* (1972*b*) reported that, in rachitic chicks treated with vitamin D_3, DDT decreased the normal rise in calcium absorption from the gut. A companion study was carried out to determine whether detrimental effects on vitamin D_3 metabolism were associated with this change in calcium absorption (Nowicki *et al.*, 1972*a*). Recently, the most active metabolite of vitamin D_3 in the intestine was found by Norman and his co-workers to be 1,25-dihydrocycholecalciferol. In the rachitic chicks, Nowicki *et al.* (1972*a*) observed no detrimental effects on hepatic or renal conversion of vitamin D_3 to its active 25- and 1,25-hydroxylated derivatives. Peakall (1969) reported that, in excised liver, the production of polar metabolites from vitamin D is unaffected by DDT. Stephen *et al.* (1970) fed chickens on diets containing DDT plus DDE, some diets being supplemented with sixty-five times the normal requirement of vitamin D_3 in order to negate possible increased steroid metabolism. Shell calcium tended to decline for all groups except the controls, and these decreases were attributed to decreased food consumption.

Thus, further work is required (*1*) to explain the conflicting observations of Peakall (1969) and Nowicki *et al.* (1972*b*) on calcium absorption, and (2) to investigate other mechanisms that may be involved, such as inhibition of ATPases (Risebrough *et al.*, 1970).

1(*b*) *Deposition of medullary bone:* Medullary bone deposition is controlled by the synergistic action of oestrogens and androgens (Taylor & Stringer, 1965). Organochlorine insecticides increase the activity of hepatic microsomal enzymes involved in the oxidative metabolism of drugs and steroids. The earlier work on this subject has been reviewed by Kupfer (1967). In mammals organochlorines were observed to increase the activity of enzymes responsible for hydroxylating the sex hormones (Kuntzman *et al.*, 1964; Conney *et al.*, 1967), and this led Peakall (1967) to demonstrate with pigeon livers that the rate of production of polar metabolites from testosterone and progesterone increased if the birds had been treated with dieldrin or DDT. A similar experiment was carried out by Risebrough *et al.*, (1968), who found increased breakdown of oestradiol after administration of pp'-DDT, pp'-DDE or PCBs. Although the pigeons used by Peakall (1967) were fed for seven days on a diet contaminated with only 10 ppm DDT, to achieve a significant increase in the production of polar metabolites of oestrogen in chicken liver, birds had to be pre-treated with a diet containing 500 ppm DDT for eighteen days (Britton, 1970). Thus, there is a considerable interspecific difference in response. A PCB level of 250 ppm in the diet was sufficient to suppress comb growth of young cockerels, but this anti-androgenic-type effect may have been due, at least in part, to decreased food consumption (Platonow & Funnell, 1971). Risebrough *et al.* (1968) were the first to propose that organochlorines might cause thin

shells by increasing the rate of metabolism of the sex hormones, thereby reducing medullary bone deposition. This idea received wide acceptance. Peakall (1970*b*) subsequently demonstrated that pp'-DDT decreased both blood levels of oestradiol and medullary bone deposition in the ring dove early in the breeding cycle, and Oestreicher *et al.* (1971) reported that dietary DDE significantly depressed medullary bone formation in pigeons. Other evidence, however, does not support this hypothesis.

(i) In three experiments thin shells have been laid without an accompanying anomalous change in medullary bone (Bitman *et al.*, 1969; Peakall, 1970*b*; Risebrough *et al.*, 1970). In addition, Muller & Lockman (1971) found that feeding young chicks on a diet containing as much as 10 ppm dieldrin for four weeks had no effect on calcium deposition in the tibia. It is perhaps significant that the experiments demonstrating medullary bone depletion have been carried out on pigeons and doves. The X-ray studies of Schraer & Schraer (1970) show that there is a pronounced rhythmic rise and fall in 'bone mass' for the pigeon in phase with egg laying, while, in the chicken, a species that lays continuously instead of in two-egg clutches, such cyclic changes are not apparent.

(ii) Several experiments have failed to demonstrate positive microsomal oxidative enzyme induction in birds treated with organochlorines. Stephen *et al.* (1970) could not produce consistent enzyme induction in chickens with DDT or dieldrin. When young Japanese quail were treated with dieldrin, microsomal aldrin epoxidase activity was increased, but DDT depressed the activity in adult quail (Gillett & Arscott, 1969). These writers also found that in pheasants caught in an alfalfa-seed growing area with a history of severe treatment with DDT and other insecticides, aldrin epoxidase activity was lower than normal. After treating Japanese quail with the op'- or pp'- isomers of DDT, DDE or TDE, Bitman *et al.* (1971) reported an increase in pentobarbital sleep time, indicating a slower breakdown of the drug in the liver. Later, Sell *et al.* (1972) showed that DDT caused a reduction in hepatic oxidase activity in the same species.

Cytochrome P_{450} is thought to be involved in these microsomal oxidative reactions (*see* Kupfer, 1967) but attempts in the above experiments to relate changes in the level of this cytochrome with changes in enzyme activity have not been successful (Gillett & Arscott, 1969; Stephen *et al.*, 1970; Sell *et al.*, 1972).

It is interesting to note that in all these experiments resistant gallinaceous species have been used.

(iii) The enzyme induction work of Risebrough *et al.* (1968) indicates that PCBs are more efficient inducers than DDE and it is thought that PCBs act in the same manner (Lincer & Peakall, 1970; *see* also Kufper, 1967). However, both in the field (Anderson *et al.*, 1969; Blus *et al.*, 1971) and in experiments on captive birds (*see* previous section) DDE has apparently had a greater effect on shell thickness.

(iv) Eggs collected in the field that contain only small amounts of DDE often display considerable shell thinning (Anderson *et al.*, 1969; Ratcliffe, 1970; Peakall,

1970*a*; Blus *et al.*, 1972). If thin shells were caused by increased hormone metabolism, at these low DDE levels a feedback mechanism would be expected to maintain sex hormone levels via increased gonadotrophin secretion.

(v) Lower levels of circulatory oestrogen might not have been caused by increased formation of hydroxylated derivatives. In the laboratory organochlorines have been found to delay ovulation (Jefferies, 1967; Bitman *et al.*, 1969; Peakall, 1970*a*; Cecil *et al.*, 1971*a*), and in the field spermatogenesis in wood-pigeons *Columba palumbus* was retarded in Cambridgeshire in 1961, perhaps because of sublethal insecticide residues (Lofts & Murton, 1966). Peakall (1970*a*) stated that the delay in ovulation, 'evidently was caused by the depression of the estrogen level resulting from the induction of liver enzymes by the pesticide'. Reduced secretion of gonadotrophins from the pituitary, however, seems a more plausible explanation of delayed ovulation and reduced oestrogen levels. In order to explain how increased metabolism of sex hormones could result in low oestrogen levels being maintained, one has to assume disruption of the feedback mechanism (*see* Sturkie, 1965) that would *normally correct low oestrogen levels by increasing gonadotrophin secretion.* Thus, it seems likely that organochlorines have a direct or indirect effect on the pituitary.

The evidence suggests that although impairment of medullary bone deposition has been reported for pigeons and doves, this mechanism is unlikely to play any part in shell thinning observed in gallinaceous species or perhaps in other species.

1(*c*), (*d*) *Mobilisation from the medullary bone and transport in the blood:* Medullary bone mobilisation is probably controlled by parathormone (Taylor, 1966, 1970), while calcitonin, a hormone secreted by the ultimobranchial glands, is probably responsible for limiting the extent of the bone resorption (Simkiss & Dacke, 1971). Hypostimulation of the parathyroids or hyperstimulation of the ultimobranchials might lead to hypocalcaemia but the effect of pesticides on these glands remains unknown. Nowicki *et al.* (1972*b*) demonstrated that, in rachitic chicks, the vitamin D_3-mediated rise in serum calcium due to increased bone resorption could be inhibited by pre-treatment with pp'-DDT. Inhibition was not caused by impairment of the production of hydroxylated vitamin D_3 metabolites in the liver or the kidney (Nowicki *et al.*, 1972*a*) and an alternative mechanism has not yet been suggested.

Since serum oestrogen levels can be decreased either directly or indirectly by pp'-DDT (Peakall, 1970*b*) and since this sex hormone regulates the synthesis in the liver of phosvitin, a protein concerned with the transport of much of the calcium in the blood (Taylor, 1970), DDT might restrict transport of serum calcium. Although phosvitin is directly concerned with the provision of calcium for the egg yolk (Taylor, 1970), a reduction in protein-bound calcium will reduce the amount of calcium available to the shell gland. However, Bitman *et al.* (1969) reported that neither op'- nor pp'-DDT caused a reduction in the serum calcium of laying quail on calcium-deficient diets. Further studies are needed to decide whether pesticides have significant effects on bone resorption or calcium transport in the blood.

1(*c*) *Transport from the blood to the shell:* The ciliated epithelial cells of the shell gland are thought to be able to transport calcium ions from the blood to the shell gland lumen (Hohman & Schraer, 1966). These workers studied the distribution of labelled calcium amongst cell organelles and their results indicated that calcium ions are stored in mitochondria and are transported via endoplasmic reticulum. More recently, Corradino *et al.* (1968) demonstrated that vitamin D_3 increased calcium-binding by the shell gland of laying chickens and these workers isolated a calcium-binding protein that was electrophoretically indistinguishable from the protein involved in calcium transport in chicks' intestines (Wasserman & Taylor, 1966). Since this protein is capable of removing calcium from mitochondria (Hamilton & Holdsworth, 1969), it is likely to be implicated in shell formation.

Just as with absorption mechanisms in the gut, it has been proposed that DDT may reduce calcium transport by affecting hydroxylation of vitamin D or by inhibiting ATPases concerned with active transport. As Risebrough *et al.* (1970) point out, in the Anacapa pelicans (a) enzyme inhibition is more likely to be the shell thinning mechanism than increased breakdown of hormones because of lack of a rectifying feedback at low pollutant concentrations (*see* objection (iv) to the decreased deposition of medullary bone theory); and (b) the site of action is probably in the shell gland since apparently healthy pelicans laid eggs with soft shells (Risebrough *et al.*, 1971).

There is some evidence that organochlorines can selectively reduce calcium transport to the shell gland lumen. Bitman *et al.* (1969) reported that quail on a low calcium diet laid eggs with thinner shells and with a lower 'percentage of shell calcium' when fed op'- or pp'-DDT. 'Percentage shell calcium' was the weight of calcium in the shell (× 100) divided by the weight of the fresh *egg*, and, since pesticides also decreased egg weight, interpretation of the results of these workers in terms of percentage of calcium in the shell is rather difficult. However, it can be inferred that pesticides decreased the weight of calcium per unit area of shell. More recently, Cecil *et al.* (1971*a*) reported that both pp'-DDE and pp'-DDT in a diet containing adequate calcium decreased 'percentage shell calcium', but had no effect on shell thickness. No details of egg weights were given. Comparing the results from the two experiments, the effect of the low calcium diet on 'percentage shell calcium' was between three and six times greater than that of the pesticide treatments. Longcore *et al.* (1971*a*) reported a decrease in calcium content from 35·2 to 34·0% in the shells of mallards that had been fed on a diet containing 10 ppm DDE, a diet known to cause a significant drop in shell thickness (Heath *et al.*, 1969). Longcore *et al.* (1971*a*) also observed a significant increase in magnesium content for the shells of both the mallards and black duck *Anas rubripes* (fed up to 30 ppm pp'-DDE), as well as a reduction in the strontium content of the black duck shells. A shift in the proportions of cations present in the shell may point to the partial inhibition of the passage of some metal cations from the blood to the shell gland lumen, so allowing other metals to take their places in the crystal lattice of

the developing shell. It is, however, possible that this simply reflects a minor disturbance in calcium metabolism outside the shell gland, and might indeed be a consequence of reduced deposition of medullary bone. Snetsinger *et al.* (1966) reported that increasing dietary magnesium or strontium resulted in higher levels of these elements in the shell. The fact that Drori *et al.* (1964) reported that the barrier between the blood and the developing shell has a Sr^{89}/Ca^{45} discrimination factor of 0·93 led Simkiss (1968) and Simkiss & Taylor (1971) to doubt the existence of a specific calcium pump in the shell gland.

In support of a partial blockage of calcium ion movement from the blood to the shell, a reduction was noted by Peakall & Lincer (1970*b*) in the active transport of calcium ions across sections of shell gland (not magnum; Peakall, personal communication) removed from the oviducts of ring doves after the birds had been given either PCBs orally or DDE by injection. Later experiments with PCBs were inconclusive, but reduced transport with DDE was again observed (Peakall, personal communication).

However, Stephen *et al.* (1970) reported that 20 ppm pp'-DDT + 20 ppm pp'-DDE in the diets of chickens did not significantly affect the percentage of egg shell calcium (compared with egg weight). Similarly, Davison & Sell (1972) found no change in shell thickness or shell calcium (compared with shell weight) when chickens were exposed to dietary levels of 20 ppm dieldrin or 200 ppm DDT. Thus, although small changes in egg shell calcium have been observed and DDE has been shown to reduce calcium transport, changes have only been slight, and available evidence suggests that inhibition of calcium transport from the blood to the shell gland lumen is not the major mechanism by which organochlorines cause thin shells.

2. *Reduction in carbonate availability*

The origin of shell carbonate has received the attention of many workers and has recently been reviewed by Simkiss (1968). In some suggested mechanisms, it has been proposed that blood bicarbonate is transported either to the shell gland epithelium (Gutowska & Mitchell, 1945), or directly to the sites of shell development (Robinson & King, 1963). However, Hodges & Lörcher (1967) demonstrated that very little labelled bicarbonate injected into the blood becomes incorporated into the shell, and the theory of Simkiss (1961) that the source of the shell carbonate fraction is metabolic CO_2 is now widely accepted. In all proposed schemes the enzyme carbonic anhydrase (CA) is thought to play an important part by maintaining the equilibrium between CO_2 and carbonic acid, so ensuring a supply of anions for shell formation.

Sulphanilamide added to a chicken's diet immediately reduces shell thickness (Scott *et al.*, 1944) and since sulphanilamide is a powerful CA inhibitor (Mann & Keilin, 1940), Gutowska & Mitchell (1945) suggested that it produces thin shells by enzyme inhibition. These last workers reported that the activity of CA in the

shell gland epithelium is greater in good laying birds than in poor ones, but Mueller (1962) was unable to repeat this observation and suggested that sulphanilamide may reduce shell thickness because of its diuretic properties. However, Opel (1965) and Cooke (1968) have since demonstrated that diuresis does not cause thin shells. More recently, Mueller *et al.* (1969) showed that acetazolamide, another sulphonamide drug known to inhibit CA, caused a significant drop in bicarbonate and CO_2 levels in the shell gland lumen, and CA is still generally believed to play an intimate part in shell formation. According to the current theory of carbonate ion production, protons are released by the dissociation of water molecules in shell gland tissue, so that observation of a fall in intracellular pH during egg shell formation (Simkiss, 1969) supports the theory. Birds fed acetazolamide do not exhibit this drop in pH (Simkiss, 1970).

No correlation between shell strength and shell gland CA content was found by Heald *et al.* (1968), who concluded that supplies of enzyme are normally in excess. CA is found in the tubular glands of the shell gland (Diamantstein & Schluens, 1964) while calcium tends to be accumulated in the ciliated epithelial cells (Gay & Schraer, 1967; Schraer & Schraer, 1970), so it is likely that the two main ionic components of the shell are secreted by different sets of cells (Simkiss & Taylor, 1971). The site of action of CA is not necessarily restricted to shell gland tissue since it has been reported in lumen fluid (Bernstein & Schraer, 1969) and in the mammillary cores (Robinson & King, 1963, *see* 3(*a*)).

Theories for organochlorines affecting the availability of the carbonate moiety of the shell have centred on possible CA inhibition. It is difficult to envisage how the activity of water soluble proteins can be inhibited by fat soluble organochlorines, since they would be expected to be concentrated in different parts of the cell. In the chicken, CA is found in the soluble fraction of shell gland homogenates (Bernstein *et al.*, 1968) and organochlorines would be expected to be associated with intracellular membranes. This objection was considered by Risebrough *et al.* (1970) who pointed out that both pp'-DDE and pp'-DDT can bind with soluble proteins (Brunnert & Matsumura, 1969). Risebrough *et al.* (1970) also suggested that the enzyme might possibly be membrane-bound in sensitive species.

Literature on whether DDT can inhibit CA is conflicting. While Torda & Wolff (1949) and Keller (1952) reported apparent inhibition, Anderson & March (1956) and Wistrand (quoted in Maren, 1967) found no inhibition. Risebrough *et al.* (1970) carried out a series of *in vitro* experiments and reported that dieldrin, Aroclor 1254 and pp'-DDE all inhibited CA activity. pp'-DDE was the most potent inhibitor, being nearly as powerful as sulphanilamide. These workers, however, considered inhibition might be due to non-specific adsorption and expressed some doubt as to whether inhibition could occur *in vivo*.

Two experiments have been reported in which organochlorines have been administered to birds and then reductions in carbonic anhydrase activity have been found. Peakall (1970*b*) injected ring doves with pp'-DDE within a day of the first egg of

the two-egg clutch being laid. The second eggs had thin shells (by 23%) and the birds were killed after laying. CA activity of the shell gland (not magnum; Peakall, personal communication) was reduced by 60%. Similar injections of dieldrin had no effect on shell thickness or CA activity (Peakall, 1970*b*). In the second experiment, Bitman *et al.* (1970) added pp'-DDE or pp'-DDT to low and normal calcium diets being fed to Japanese quail over a period of three months. Birds were killed with an egg in the shell gland and lower CA activity was found both in the blood (22–44%) and in the shell gland (16–19%). These workers recognised that their *in vitro* demonstration did not 'preclude normal functioning of the CA enzymatic machinery in the intact tissue *in vivo*'. However, results for the two different calcium diets were not separated and it was not stated whether the eggs laid by the quail did, in fact, have thin shells. In similar experiments, Bitman *et al.* (1969) reported thinner shells after feeding a low calcium diet containing pp'-DDT, but Cecil *et al.* (1971*a*) reported no change with pp'-DDE or pp'-DDT in normal diets. Thus it is not clear whether the 16 to 19% decrease in CA activity in the quail shell glands, as recorded by Bitman *et al.* (1970), was associated with thin shells being laid. Indeed, it was pointed out by Dvorchik *et al.* (1971) that the degree of reduction in enzyme activity reported by Peakall (1970*b*) and Bitman *et al.* (1970) was probably insufficient to cause physiological inhibition. It is thought that under normal conditions CA activity in the shell gland exceeds the physiological requirement and is not rate limiting (Heald *et al.*, 1968). In chickens, intramuscular injection of sufficient acetazolamide to reduce shell weight by 41% caused CA activity in shell gland scrapings to be zero or 'extremely low' (Bernstein *et al.*, 1968). Thus, CA inhibition would not be expected to cause shell thinning after slight contamination with pesticide (Risebrough *et al.*, 1970) as has often been reported. When, however, chickens are exposed to different levels of sulphanilamide in the diet, there is a linear relationship between the decrease in shell thickness and the logarithm of the dietary concentration of sulphanilamide (Scott *et al.*, 1944), strikingly reminiscent of the relationship between shell thickness and DDE content of pelicans' eggs from different colonies as observed by Blus *et al.* (1972).

Dvorchik *et al.* (1971) criticised the experimental technique of Peakall (1970*b*) and Bitman *et al.* (1970), and gave an account of their own *in vitro* experiments, which suggested that DDT should not affect CA activity at concentrations found in tissues in the field. Inhibition was only noted when high DDT concentrations resulted in precipitation. Since then, Pocker *et al.* (1971) have demonstrated that pesticides are not true inhibitors of CA. Apparent inhibition occurs in *in vitro* tests only when a pesticide precipitate forms and occludes CA from solution.

Thus, inhibition is unlikely to be caused directly by pp'-DDT, pp'-DDE or dieldrin (Dvorchik *et al.*, 1971; Pocker *et al.*, 1971), but inhibition by metabolites of these compounds, or a reduction in enzyme synthesis (suggested by Kenny & Dacke, personal communication) cannot be ruled out. Inhibition by a water soluble

metabolite is an attractive theory, since interaction with the soluble enzyme (Bernstein *et al.*, 1968) then becomes more plausible.

Before leaving CA inhibition, the 'salt gland theory' of Risebrough *et al.* (1970) should be mentioned. CA is found in many places in the body including, in birds, the salt gland, and effects noted in the shell gland might possibly only be a consequence of primary effects elsewhere. The early work on the avian salt gland was carried out by Schmidt-Nielsen and his co-workers in the late 1950s. They found that the salt (or nasal) glands of marine species excrete a highly concentrated salt solution, thereby ridding the bird extrarenally of unwanted salt. Brown pelicans were injected intravenously with salt solution by Schmidt-Nielsen & Fange (1958) who found that the salt gland excreted about four times as much salt as the kidney. CA is essential for proper functioning of the salt gland and sulphonamides can prevent fluid excretion by the gland (*see* Maren, 1967). Since an excess of chloride in the diet causes chickens to lay eggs with thin shells (Hall & Helbacka, 1959; Hunt & Aitken, 1962*a*, *b*; Lörcher *et al.*, 1964), Risebrough *et al.* (1970) suggested that pesticides may inhibit proper functioning of the salt gland in marine species, so reducing chloride excretion and causing a decrease in shell thickness. They also pointed out that this would explain why some species, such as the brown pelican, which largely rely on extrarenal means for chloride excretion, should be particularly affected. This is an interesting theory and affords yet another site of action for a CA inhibitor. It is, however, still only a tentative theory. One objection is that chloride salts in the diet do not consistently reduce shell thickness. Although Lörcher *et al.* (1964) reported shell thinning when sodium chloride and potassium chloride were added together to the rations of chickens, Hunt & Aitken (1962*b*) reported that neither calcium nor potassium chloride reduced shell thickness. The latter writers considered that the common characteristics of salts causing reduced shell thickness were (a) an acidic anion, not readily removed from the blood, and (b) a readily-metabolisable cation. Thus, it is uncertain whether circulatory excess of sodium chlorine, the main chloride to which the marine species will be exposed, can cause significant shell thinning.

It is well proven that an alteration in the acid-base balance of the blood can lead to changes in shell thickness. For instance, supplementing the diet of laying chickens with 1–3% ammonium chloride leads to metabolic acidosis, characterised by a decrease in blood pH and bicarbonate concentration and by the production of eggs with thin shells (Hall & Helbacka, 1959; Hunt & Aitken, 1962*b*). That high environmental temperatures cause birds to lay eggs with thin shells has long been known. This effect is probably due to the birds hyperventilating, so that blood pCO_2 is decreased and the bicarbonate level drops (Mongin & Lacassagne, 1966; Simkiss, 1968). Such birds are in a state of respiratory alkalosis, blood pH being raised. The opposite, respiratory acidosis, can be achieved by exposing a bird to high levels of atmospheric CO_2. Helbacka *et al.* (1963) and Hunt & Simkiss (1967) reported that, after acute exposure, birds in a state of respiratory acidosis laid eggs

with thin shells, but after a longer study, Frank & Burger (1965) reported that thicker shelled eggs were laid. These discrepancies may be due to renal compensation, leading to an increase in blood bicarbonate and carbonate levels during chronic exposure (Simkiss, 1968). Studies on the effect of organochlorines on blood pH, pCO_2, etc. would seem to be particularly worthwhile.

3. *Effects on other shell constituents*

Most workers studying mechanisms by which pesticides cause thin shells have devoted their attentions to the laying birds' tissues. The thin shells, apart from having their thickness measured, have apparently been discarded. There have, however, been two scanning electron microscope studies of thin shells caused by pesticides. Erben & Krampitz (1971) examined the fine structure of two very thin shells collected from the brown pelican colony on Anacapa Island. They reported that the palisade layer decreased in thickness and the number of globular inclusions in this layer increased. Pelican shells have a calcareous cover (Tyler, 1969; *see* Fig. 2) and Erben & Krampitz (1971) noted that in the cover of the thin shells were numerous abnormal granules. Although the shells were much thinner than a control shell (0·20 and 0·36 mm compared with 0·59 mm), there was apparently no loss of matrix protein. There were, however, marked differences in the amino acid patterns of both the membranes and the matrix. In the second investigation, quail were dosed with technical DDT by McFarland *et al.* (1971). Thin shells laid by the quail showed changes in mineral deposition and porosity in the mammillary layer, but the overall decreases in thickness could usually be accounted for by decreases in the palisade layer. One shell that was 40% thinner than normal had a palisade layer reduced in thickness by 60%. The surface crystal layer changed considerably, sometimes being thicker and sometimes being virtually absent. Shells often had a rippled surface with enlarged pore channels. Such gross defects in shell structure suggest that changes in other shell constituents, apart from the calcium and carbonate moieties, may be markedly impairing normal shell formation. Factors to be considered are (a) disruption of the sites of initiation of crystal growth, (b) deleterious effects on shell constituents essential for proper shell formation, (c) enhancement of the action of constituents responsible for inhibition of shell growth, and (d) premature induction of the shell constituents or factors controlling termination of shell growth.

3(*a*) *Disruption of initiation sites:* The presence of CA in the mammillary cores, the initiation sites, was reported by Robinson & King (1963). Although this has since been refuted by Diamantstein (1966), it is worth remembering that if pesticides do reduce the activity of this enzyme, the effect is not necessarily restricted to shell gland tissue (or the salt gland).

Calcification is initiated by the deposition of calcite on preformed organic mammillary cores (Fujii & Tamura, 1970). In a collection of chicken shells examined by Robinson & King (1970), while the cores in shells of average strength

were always found to be normal, more than half of the weak and thin shells had abnormal mammillary cores. The abnormality was characterised by the organic cores being irregular and diffuse, and sometimes small dense organic particles were present on the membrane surface. Shells with surface concretions were found to be completely devoid of organic mammillary cores beneath the concretions. Overlying the membrane fibres in such shells were aggregations of organic material, considered by Robinson & King (1970) to be an extreme form of the mammillary core abnormality. Thus, here is an association between weak, thin and malformed shells and abnormal mammillary cores.

The organic cores are probably composed of modified shell membrane material (Robinson & King, 1968; Robinson *et al.*, 1968). There are modifications in structure (Simons & Wiertz, 1963) and in carbohydrate content (Cooke & Balch, 1970*a*), but no marked differences in amino acid content have so far been found (Cooke & Balch, 1970*a*). Erben & Krampitz (1971) reported a change in the amino acid content of shell membranes on the thin, pelican shells from Anacapa. If this is indicative of a similar change in amino acids in the mammillary cores, then initiation of crystallisation could be affected. This is only speculation, but in the shells of quail treated with DDT by McFarland *et al.* (1971), there were gaps between normal basal caps occupied by calcite spherulites about 10μ in diameter. These may have been sites where crystallisation failed soon after initiation. The densely staining particles reported by Robinson & King (1970) on the membranes of chicken shells with abnormal cores may have been the organic material in such failed centres that was left attached to membrane fibres after the preparations were decalcified. These particles were up to 15μ in diameter. The calcite spherulites are unlikely to have been caused by a calcium deficiency, since thin shells from chickens on a low calcium diet have structurally normal mammillary layers (Cooke, 1968). Irregularities have been observed in the mammillary layers of shells laid by chickens exposed to high temperatures (El-Boushy *et al.*, 1968). In the shells examined by Erben & Krampitz (1971) and McFarland *et al.* (1971), however, the palisade layer seems to have been affected more than the mammillary layer, indicating less disruption during initiation of shell growth than during later stages of calcification.

3(*b*) ***Deleterious effects on shell matrix:*** Nothing is known about essential inorganic constituents (apart from calcium carbonate) so this discussion is limited to possible effects on shell matrix. Since no researchers into pesticide effects on egg shells have so far discussed the importance of matrix, the subject is reviewed in detail here.

The matrix is a continuous organic phase found throughout the calcified shell and was included in an electron microscope study of shell components by Simons & Wiertz (1963). It is mainly composed of protein (Baker & Balch, 1962), with 10–11% carbohydrate (dry weight; Baker & Balch, 1962; Cooke, 1968) and fat has been detected histochemically (Simkiss & Tyler, 1957). Analytical studies

(Baker & Balch, 1962; Cooke, 1968; Cooke & Balch, 1970*b*) reveal that the mucopolysaccharide moieties bound to the protein are chondroitin sulphates A and B (*see* Brimacombe & Webber, 1964) plus at least two neutral polysaccharides. Degradative acid hydrolysis (Cooke, 1968) indicates that the neutral polysaccharides have the typical structure (Dische, 1965) of a backbone of hexosamine and hexose units with short side chains terminating in fucose or sialic acid.

Matrices from thin shells have already been examined in detail (Cooke, 1968). The treatment regimes of one experiment involving nine chickens are shown in Table 2. Treatments selected to cause thin shells were high temperature (34°C);

TABLE 2

TREATMENT REGIMES USED IN AN EXPERIMENT TO STUDY THE ORGANIC MATTER IN THICK AND THIN SHELLS LAID BY CHICKENS *Gallus domesticus* (COOKE, 1968)

	Control	*Room temperature treatment* (13–19°C)	*Recovery*	*High temperature treatment* (34°C)	*Recovery*
	2 weeks	3 weeks	3 weeks	3 weeks	3 weeks
3 birds	B.D.	B.D.	B.D.	B.D.	B.D.
3 birds	B.D.	Supplement, 0·03% sulphanilamide	B.D.	Supplement, 0·03% sulphanilamide	B.D.
3 birds	B.D.	Supplement, 2% ammonium chloride	B.D.	Supplement, 2% ammonium chloride	B.D.

B.D. = basal diet.

0·03% sulphanilamide in the diet at room temperature (13–19°C) or high temperature; and 2% ammonium chloride in the diet at room or high temperature. After removal of the membranes and the cuticle, shell thickness was determined (mg/cm^2) and bulked samples of shells from the last two weeks of each period were decalcified. The matrix suspension was centrifuged. Then the matrix was dried, weighed and analysed for sialic acid and uronic acid, which were chosen because they contain acidic groups and are constituent units of matrix mucopolysaccharides. These are believed to be involved in stabilising the matrix (Baker, 1959; Simkiss & Tyler, 1959) and/or in binding calcium to the developing shell lattice either ionically (Baker, 1959; Cooke & Balch, 1970*b*) or by chelation (Simkiss & Tyler, 1958, 1959).

The experiment yielded thirty samples from birds on a control diet and fourteen samples of abnormal thin shells laid under the various treatment conditions (there should have been fifteen, but one bird on the ammonium chloride treatment at room temperature ceased laying for three weeks). Birds on a control diet will, of course, produce shells with a wide range of thicknesses, and to find out whether differences exist between the organic matter in 'normal thick' and 'normal thin' shells, the bulked samples from control and recovery periods have been divided into two groups:

(i) 'normal thick' shells, samples with mean shell thickness > 70 mg/cm^2 ($n = 16$);

(ii) 'normal thin' shells, samples with mean shell thickness < 70 mg/cm^2 ($n = 14$)

Results are shown in Table 3. In these and in other normal shell samples, the amount of matrix present was significantly correlated with shell thickness ($r = 0{\cdot}560$, d.f. = 37, $P < 0{\cdot}001$; Cooke, 1968). In Table 3, there is significantly less matrix (mg/cm^3) in the 'normal thin' shells than in the 'normal thick' shells and there is a tendency for thinner shells to have a lower matrix content (mg/g). Although the difference in thickness between the 'abnormal thin' shells and the 'normal thin' shells shown in Table 3 is twice that between the two normal groups, the 'abnormal thin' shells do, in fact, contain as much matrix (mg/cm^2) as the 'normal thin' shells. Because of this, their matrix content (mg/g) is significantly higher. The two types of thin shells contained significantly less sialic acid ($\mu g/m^2$), but their matrix sialic acid content ($\mu g/mg$) is similar to that of 'normal thick' shell matrix. The matrix in 'abnormal thin' shells is, however, considerably deficient in uronic acid ($\mu g/mg$) and, furthermore, a shift in the ratio of the constituent uronic acids has often been found (Cooke, 1968). 'Abnormal thin' shell matrix is relatively richer in glucuronic acid (present in chondroitin sulphate A) and poorer in iduronic acid (present in chondroitin sulphate B).

In order to make a convenient comparison of matrix composition, all the 'abnormal thin' shells have been grouped together. There were, in fact, some differences in shell matrix composition from the various treatments, but an increase in matrix content and a decrease in uronic acid were consistently found for these 'abnormal thin' shell samples, irrespective of treatment conditions (Cooke, 1968). Similarly, Longstaff & Hill (1972) recently reported that in thin-shelled eggs laid by pullets on manganese deficient diets, shell matrix content increased and the uronic acid content of the matrix decreased. Nevertheless, this cannot be taken as a general rule for the matrices of all 'abnormal thin' shells, since a bird maintained on a strontium-rich diet laid eggs with thin shells, but they contained less matrix (mg/g) and the matrix contained unusually high amounts of uronic acid (Cooke, 1968). The shift towards glucuronic acid was, however, still evident. Frank *et al.* (1965) reported that in a group of twenty-nine shells the weaker shells contained a higher percentage of protein. Deciding the relevance of changes in matrix composition in thin shells is rendered considerably more difficult by the fact that matrix distribution throughout the depth of a normal shell is not uniform (Cooke & Balch, 1970*b*). Thus it is not possible to draw up a simple formula for calculating the matrix requirement for proper shell formation.

The observed changes in the matrix of 'abnormal thin' shells are not, of course, necessarily the cause of shell thinning. The usually-accepted theories for explaining why high temperature, ammonium chloride or sulphanilamide treatment cause thin shells revolve around reducing the availability of the carbonate fraction. These treatments all produce significant decreases in bicarbonate and CO_2 levels in shell gland lumen fluid (Mueller *et al.*, 1969), but the reasons for shell thinning are liable to be more complex than simple reductions in the available carbonate. Sulphonamides, for instance, depress calcium retention from the food (Tyler, 1950),

reduce blood calcium levels (Siegmund & Dulce, 1960) and can reduce egg size (Cooke, 1970*a*). High temperature reduces food consumption and, therefore, calcium intake, but retention is increased (Mueller, 1959). Despite this, serum calcium levels are reduced (Mueller, 1959) and incorporation into the shell of labelled calcium, given orally, is significantly decreased at elevated temperatures (Bragg *et al.*, 1971), indicating impairment of calcium metabolism. Ammonium chloride may reduce blood calcium (Siegmund & Dulce, 1960).

Questions that need answering are: (a) how essential is the matrix for proper shell formation and (b) can subtle changes in composition or structure contribute to shell thinning after a bird has been exposed to sulphonamides, ammonium chloride, high temperature or pesticides? Electron micrographs of developing shells taken by Simons (1971) clearly demonstrate that deposition of organic matter precedes crystallisation. Like egg shell matrix, molluscan shell matrix is largely protein in nature with smaller amounts of neutral and acidic mucopolysaccharides and traces of fat (Wilbur & Simkiss, 1968). Mollusc shell matrices have the ability to exert some degree of control over which polymorph of calcium carbonate is formed (Watabe & Wilbur, 1960; Simkiss, 1965). These writers found that a matrix, removed by decalcification from a calcite mollusc shell and placed in a solution that normally precipitated calcium carbonate as aragonite, often caused calcite to be deposited instead. Wilbur & Watabe (1967) concluded that molluscan matrix probably 'provides a substratum on which crystals nucleate, orient and grow'. Since the matrix is apparently vital for proper shell formation, deficiencies or changes in matrix composition might indeed result in crystal disruption or termination in both mollusc shells and avian egg shells. Whilst on the subject of molluscan shell deposition, E. Pollard and I recently exposed snails, *Helix pomatia*, to pp'-DDT during their first growing season. Preliminary analysis of the results indicates a significant retardation of shell growth at exposure levels that had no effect on body growth.

The structure of thin shells caused by subjecting chickens to high temperatures was examined by El-Boushy *et al.* (1968), who reported that a rise in temperature from 13° to 29°C resulted in normal columnar crystal orientation being severely disrupted, less secure attachment of the membranes to the mammillary layer and cavities in the palisade layer. Electron micrographs of decalcified sections showed that the vesicles in the matrix in the palisade layer were abnormally large and cavities in the matrix were also recorded. The organic matrix showed physical deformations corresponding to those in the mineralised shell (Wiertz & Simons, personal communication), mineral matter disruption apparently being caused by an abnormal matrix.

There are similarities between the high temperature thin shells examined by El-Boushy *et al.* (1968) and the DDT-induced thin shells examined by McFarland *et al.* (1971), who reported defects where the membrane fibres joined the shell and cavities penetrating the palisade layer. Crystal structure was not so disrupted in

TABLE 3

THE AMOUNT OF MATRIX, SIALIC ACID AND URONIC ACID IN THICK AND THIN SHELLS LAID BY CHICKENS *Gallus domesticus*. FOR DETAILS SEE TEXT (ADAPTED FROM COOKE, 1968)

	No. of bulked samples[*]	*True shell thickness* (mg/cm^2)	*Matrix*		*Sialic acid*		*Uronic acid*	
			(mg/cm^2)	(mg/g *shell*)	($\mu g/cm^2$)	($\mu g/mg$ *matrix*)	($\mu g/cm^2$)	($\mu g/mg$ *matrix*)
'Normal thick' shells, thickness > 70 mg/cm^2	16	73·7 ± 0·5	1·09 ± 0·05[a]	14·8 ± 0·5[a]	9·3 ± 0·2[a]	8·6 ± 0·3[a]	14·4 ± 0·3[a]	13·3 ± 0·4[a]
'Normal thin' shells, thickness < 70 mg/cm^2	14	66.2 ± 0·5	0·90 ± 0·04[c]	13·5 ± 0·5[a]	8·1 ± 0·2[d]	9·2 ± 0·3[a]	12·5 ± 0·6[c]	13·6 ± 0·3[a]
'Abnormal thin' shells	14	52·7 ± 1·3	0·90 ± 0·04[c]	17·2 ± 1·0[b]‡	7·7 ± 0·3[d]	8·7 ± 0·3[a]	10·6 ± 0·4[d]	11·9 ± 0·4[b]‡

± S.E.

[*] Samples contained segments from 3–13 shells

[a, b, c, d] For a pair of means differing by one letter, $P < 0.05$; by two letters, $P < 0.01$; by three letters, $P < 0.001$.

‡ Compared with 'normal thin' shells, $P < 0.01$

the shells investigated by Erben & Krampitz (1971), but an abnormal number of globular inclusions were noted in the palisade layer. In addition, Erben & Krampitz (1971) found a threefold increase in organic matter content of the shell, expressed in μmoles of amino acid/100 mg shell. Nothing was stated about the amount of matrix *per shell*, although Erben and Krampitz's figures suggest that little, if any, was missing compared with normal shells. At this point it is worthwhile remembering that the 'abnormal thin' shells (including the high temperature shells) shown in Table 3 contained as much matrix per shell as the 'normal thin' shells although they were, on average, more than 20% thinner. Changes in matrix composition have been noted for both pesticide-induced and high temperature thin shells, Erben & Krampitz (1971) reporting amino acid changes in the former and Cooke (1968) uronic acid changes in the latter. Thus, if shell disruption in high temperature thin shells is caused by changes in the physical and chemical structure of the matrix, as has been proposed, shell disruption in pesticide-induced thin shells may be similarly caused.

Having reached this tentative conclusion, it should be stressed that matrix disruption is only part of the story. The chemically and physically changed matrix can only affect the deposition of those lattice ions that are available in the lumen during shell formation, so that, if a blockage in either calcium or carbonate ions occurs, the shell will be thin. If the matrix is abnormal, then the mineralised shell is also likely to have an abnormal structure, but can essential shell organic material be so changed that, even though sufficient mineral ions are present in the lumen, the shell is thin, due to low incorporation of minerals into the shell? Since high temperature, sulphonamides and ammonium chloride, which all produce changes in matrix composition (Cooke, 1968), also all lower the bicarbonate concentration of shell gland lumen fluid, and since this parameter is known to be significantly correlated with shell thickness (Mueller *et al.*, 1969), organic matter disruption is most unlikely to be the primary cause of thin shells. Longstaff & Hill (1972) reached a similar conclusion after studying shells produced by manganese-deficient birds. However, an enhancement of shell thinning due to changes in organic matter cannot be ruled out, particularly since Robinson & King (1970) reported an association between physical and histochemical abnormalities in mammillary cores and thin, weak shells. Matrix may influence shell strength. Simons (1971) examined two of the shells studied by Robinson & King (1970) and found that, in a shell that was unusually weak for its thickness, the number of vesicles in the matrix was greatly increased. Treatment of shells with sodium sulphide solution at 95°C does not affect the mineral matter, but shells become weaker because of destruction of matrix (Simons *et al.*, 1966). Frank *et al.* (1965) were, however, unable to detect significant changes in the amino acid patterns of matrices in abnormally weak or strong shells.

Why matrix composition should be changed by certain treatments is not known. It may be essentially a secondary effect due to reduced incorporation of minerals

into the shell or there may be some direct effect on the matrix. A dietary lack of manganese is thought to have a direct effect on glucosamine metabolism (*see* Longstaff & Hill, 1971). Glucosamine, a hexosamine, is present in egg shell membranes, mammillary cores, matrix and cuticle (Baker & Balch, 1962; Cooke & Balch, 1970 *a*, *b*). A deficiency of manganese in the diets of pullets just beginning to lay leads to a reduction in the glucosamine content of the isthmus and shell gland and can also cause the production of weak egg shells (Longstaff & Hill, 1971). Matrix in these shells contains decreased amounts of hexosamine and uronic acid, apparently because of direct action on mucopolysaccharide metabolism (Longstaff & Hill, 1972).

In the shell gland both the epithelial cells and the tubular glands secrete acid mucin fractions, the more strongly acidic mucins being in the epithelial cells (Robinson *et al.*, 1968). Some shifts in the matrix composition in thin shells might be explained by relative changes in activity in the two types of secretory tissue. From the observed changes in matrix composition in 'abnormal thin' shells (Cooke, 1968), it would appear that the epithelial cells, which are thought to be involved in secreting calcium ions (Gay & Schraer, 1967; Schraer & Schraer, 1970) are affected more than the tubular glands.

3(*c*) *Enhancement of shell growth inhibitors:* The results obtained by Brooks & Hale (1955), Itoh & Hatano (1964) and Snetsinger *et al.* (1966) show that, in shells produced by chickens, the higher levels of phosphorus, magnesium and strontium occur towards the outside, *i.e.* in those parts of the shell that are the last to be formed. Simkiss (1964) reported that phosphate ions can inhibit calcite crystal growth, and Sobel & Hanok (1952) observed that strontium ions inhibit the calcification of rachitic bone *in vitro*, while magnesium ions enhance this effect. These ions, therefore, may play some part in the inhibition of shell growth. The only experiment designed to study changes in shell mineral constituents other than calcium after administration of organochlorines is that of Longcore *et al.* (1971*a*). In shells laid by ducks being fed a diet containing DDE, there was a decrease in strontium content and an increase in magnesium, but no precise data were given for phosphorus. Thus there is no evidence to suggest that the effect of these known inhibitors may be enhanced in shells from treated birds.

3(*d*) *Premature termination:* Since shell growth is normally terminated quite suddenly in the chicken oviduct (Cooke, 1968), it is unlikely that increasing amounts of the inhibitors significantly contribute to the process of termination. The concentration of calcium ions in the shell gland lumen falls during the last 2–4 h of calcification, but the level when mineralisation ceases is still higher than during the early stages of calcification (Mongin & Saveur, 1970). Termination may be brought about by secretion of cuticular material, which, if secreted quickly, could blanket the shell and prevent inorganic ions reaching the lattice. The basic amino acids, arginine and lysine, are present in high concentrations in the cuticle (Baker & Balch, 1962) and may compete with calcium ions for negative sites on the matrix during cuticle secretion. Thus, premature secretion of the cuticle might

produce shells with the outer layers missing. Gross analysis of shells laid by hens treated with sulphanilamide at room temperature suggests they may, indeed, be lacking the outer layers (Cooke, 1968). Whether one is justified in comparing thin shells with the inner levels of normal, naturally-laid shells is doubtful. Although (a) matrix content is non-uniform, increasing from the inside of the shell, then decreasing over the outer third of the shell's thickness (Cooke & Balch, 1970*b*) and (b) Robinson *et al.* (1968) noted a decrease in staining for acid mucins in shell gland tissue during the final stages of calcification, nevertheless, the rate of incorporation of organic material into the shell seems to be linearly related to shell growth (Cooke, 1968; Longstaff & Hill, 1972). These apparently contradictory observations can largely be reconciled by assuming that incorporation of fresh matrix into the shell occurs not only on the developing surface but also in channels radiating out from the pores. A matrix fraction readily attacked by alkali is thought to occur in such a location (Tyler & Simkiss, 1958). This means that a shell developing in the oviduct might contain less organic matter than the corresponding inner layers of a completed shell.

The thin shells structurally investigated by McFarland *et al.* (1971) certainly do not seem to have been caused by premature termination, since the surface crystal layer just below the cuticle (*see* Fig. 2) was sometimes of increased thickness in thin shells. A pelican shell does not have a surface crystal layer (Tyler, 1969), and it is not possible to rule out premature termination as the cause of the thin shells examined by Erben & Krampitz (1971). Indeed, if premature termination by cuticular secretion produced these thin shells, the deficient palisade layer, reported by Erben & Krampitz (1971), would be expected. Moreover, these workers also observed irregular calcareous granules in the cover (*see* Fig. 2) which might be shell material that would have normally been deposited before (and not after) the cuticle. On the surface of some chicken shells, Tyler & Simkiss (1959) have found abnormal calcified globules that apparently contained normal shell matrix. On the other hand, aberrant spheres of shell material were also occasionally noted on the surface of thin shells examined by McFarland *et al.* (1971) and these shells were apparently not caused by premature termination.

Pigment is present in the outer shell layers and in the cuticle, and lack of pigment could be indicative of premature termination, premature oviposition or reduced incorporation of pigment into the developing shell. Ratcliffe (1970) pointed out that since 1947 peregrine shells have become less richly marked, and, similarly, Fimreite (1971) noted that pheasants fed on organomercury-treated grain often laid eggs with abnormally light-coloured shells. Premature oviposition is unlikely to be involved in shell thinning (*see* below) so these poorly pigmented shells are likely to have been caused by premature termination, by a reduction in available pigment or by reduced binding of pigment to shell components. Poorly pigmented shells are also produced by other treatments that cause thin shells, such as sulphanilamide (Scott *et al.*, 1944).

4. *Changes in other factors involved in egg shell formation*

4(*a*) *Premature oviposition:* The possibilities of neural or hormonal control of oviposition have been discussed by Sturkie (1965) and Gilbert (1967, 1971*b*). Eggs laid prematurely will lack the outer levels of shell and will also be without a cuticle. Chickens in a state of shock will sometimes lay prematurely.

Premature oviposition has been tentatively suggested as the possible cause of thin shells induced by pesticides (*e.g.* Bitman *et al.*, 1969; Ratcliffe, 1970), but the evidence now points towards this being very improbable. In the pesticide-induced thin shells that have been studied (Erben & Krampitz, 1971; McFarland *et al.*, 1971), the shell components deposited during the final stages of formation, the cuticle and the cover, were present.

Sulphanilamide, ammonium chloride or high temperature conditions are also unlikely to act in this manner, since none of these treatments affects either the time the egg remains in the shell gland or the amount of cuticular material deposited (Cooke, 1968). Similarly, cuticles are present on thin-shelled eggs laid by pullets on manganese-deficient diets (Longstaff & Hill, 1972).

4(*b*) *Decreased food consumption:* It is quite feasible that the residues in the laying bird could (a) have a detrimental effect on the bird's hunting ability (as suggested by Ratcliffe, 1970), perhaps by impairing vision (suspected in pigeons sublethally poisoned with endrin; Revzin, 1966) or (b) cause a loss of appetite. In this way pesticides might have an indirect effect on shell thickness via reduced food intake. Administration in capsules of either dieldrin to pheasants (Atkins & Linder, 1967) or pp'-DDT to pigeons (Jefferies & French, 1971) resulted in loss of appetite. Jefferies & French (1971) reported significantly lower hepatic levels of vitamin A in their birds and suggested that a reduction in vitamin A might be responsible for appetite loss (*see* Moore, 1967). Against a theory of decreased food consumption, laboratory birds have consumed, at a normal rate over periods of more than one month, diets containing 250–500 ppm DDT (Linduska & Springer, 1951; Genelly & Rudd, 1956; Noakes & Benfield, 1965; Davison & Sell, 1972; Lillie *et al.*, 1972).

4(*c*) *Thyroid effects:* Thyroidectomy in chickens causes severe effects on growth and reproduction (*see* Sturkie, 1965), so it is perhaps not surprising to find that it also causes shell thinning. Taylor & Burmester (1940) reported a 9% reduction in percentage shell after the operation, although they expressed concern that unintentional removal of the parathyroids may have been at least partially responsible for the effect. Other reproductive changes were a 3% reduction in egg weight and a decrease in egg production of about 70%. Egg production and egg weight were again decreased by the addition of 0·1% thiouracil to the diet (Berg & Bearse, 1951), shell thickness being either unchanged (Berg & Bearse, 1951) or reduced (Gabuten & Shaffner, 1954). Thiouracil causes a bird to become hypothyroid by blocking thyroxine synthesis (*see* Sturkie, 1965). In thyroidectomised drakes, the rate of induction of medullary bone by oestrogen was retarded (Benoit & Clavert, 1947).

This bone was found to be osteoporotic, attempts to repeat this observation using thyroid inhibitors failed (Taylor & Stringer, 1965). The oestrogen-induced rise in blood calcium can be inhibited by both thyroxine and thiouracil (Höhn, 1961). Jefferies & French (1971) and Jefferies *et al.* (1971) observed that pigeons became hyperthyroid after being fed low doses of DDT in the diet, while those fed higher doses were hypothyroid. This led these workers to suggest that in the field birds of prey laying thin-shelled eggs were hypothyroid. Loss of colloid from the follicles of the thyroid, indicating either hyper- or hypothyroidism, was also noted after pigeons were treated with pp'-DDE or dieldrin (Jefferies & French, 1972). Richert & Prahlad (1972) reported that histological sections of thyroids taken from Japanese quail previously treated with DDE or DDT indicated hypofunctioning of the glands. In addition, DDE doubled the weight of the thyroids and halved iodine uptake per unit weight (the absolute uptake of iodine therefore apparently being unaffected). DDA had no significant effect on the gland.

Hyperthyroidism resulting from eating a diet containing iodinated casein or thyroprotein caused chickens to lay eggs with thicker shells (Berg & Bearse, 1951; Gabuten & Shaffner, 1954). In a similar experiment, Wilson (1949) also reported that thicker shells were sometimes laid, but since egg weight decreased, shell weight was probably unaffected. Asmundson & Pinsky (1935) found that hyperthyroidism caused a slight decrease in egg weight and a slight increase in shell weight. Conditions used by these workers were sufficient to reduce or terminate egg laying. Bengalese finches subjected to DDT treatment by Jefferies (1969) were apparently in a hyperthyroid state. Egg weight was reduced, but shell weight was unchanged, so that the shells were thicker.

To explain hyper- and hypothyroidism in their pigeons, Jefferies *et al.* (1971) suggested that pesticides might have two effects, stimulation of the thyroid, which is probably mediated via the pituitary, and competition with thyroxine for binding sites on the transport proteins in the serum. There is evidence that op'-TDE can lower serum protein-bound iodine in humans by competing for sites on thyroxine binding globulin (Marshall & Tomkins, 1968) although op'-TDE administration did not cause hypothyroid symptoms and the effect could not be demonstrated *in vitro*. The last fact may indicate that a metabolite was the active compound rather than op'-TDE itself. In birds treated with DDT, the thyroid gland is considered by Jefferies (personal communication) to be permanently hyperactive, but the metabolic activity of the bird, as reflected by pulse rate, body temperature, etc., might be indicative of hypo- or hyperthyroidism, depending on whether competition for binding sites overrides stimulation. Variations in response between mammals and birds, and between different species of birds could be due to differences in serum binding proteins (Jefferies *et al.*, 1971). Certainly, thyroxine is bound more strongly in human serum than in duck or chicken serum (Sturkie, 1965).

Jefferies *et al.* (1971) have suggested that a thyroid defect with an accompanying change in vitamin A metabolism might be responsible for many of the observed

sublethal effects of organochlorines, *e.g.* changes in liver and gonad weight. Can hypothyroidism, however, explain the thin shells observed in the field and in the laboratory? Effects on egg shells in the experiments quoted above have been slight, if indeed they have occurred at all, and other reproductive effects, such as reduced egg production, were often much more marked. It should be remembered, however, that most of these tests were carried out on chickens, a species that is probably more resistant to shell thickness changes than those species affected in the field (*see* the previous section). Although, with caged pigeons, hypothyroid birds cannot be distinguished from normal birds by their external appearance (Jefferies, personal communication), if the pelicans on Anacapa were in such a hypothyroid condition as to reduce egg shell thickness by 50%, other changes in reproductive processes or in behaviour might have been expected. On the island in 1969, a minimum of 1272 nests were built and eggs were probably laid in at least 75% of these nests, but shells collapsed during incubation and not more than four chicks were raised (Risebrough *et al.*, 1971). Nest-building, mating, ovulation and brooding were apparently normal, but shell thickness was, on average, halved. The birds might have been in a hypothyroid state but it seems likely that thyroid malfunction reflects a general pesticide effect on the bird, particularly if the activity of the pituitary is altered (*see* section 1(*b*)) rather than the binding of pesticide on to thyroxine transport proteins being the primary lesion responsible for egg shell thinning.

Possible effects of hypothyroidism on reproduction have concerned poultry keepers for many years, since a decreased thyroid secretion rate has been suggested as a possible cause of the usual decline in egg production and shell thickness during the summer months (Thornton & Moreng, 1959; Tyler & Geake, 1960; Sturkie, 1965). This is of particular interest to the present discussion, since it offers a further connection between the effects of pesticides and the effects of high temperature on shell formation. Attempts to increase egg production in chickens by feeding thyroprotein or iodinated casein have met with some (Turner *et al.*, 1945) or no success (Wilson, 1949). The last writer also reported that iodinated casein failed to correct shell weight at high temperatures. Vitamin C has also been proposed as a dietary additive to correct shell thinning due to high temperature, but attempts to achieve corrective shell thickening with this vitamin have usually been unsuccessful (*see* El-Boushy *et al.*, 1968).

4(*d*) *Adrenal effects:* The adrenals of pigeons dosed with pp'-DDT increased in weight (Jefferies *et al.*, 1971) and these writers suggested that the thyroid may directly control such changes, since both hypothyroidism and marked hyperthyroidism cause adrenal hypertrophy in the chicken (*see* Sturkie, 1965). pp'-DDE, but not dieldrin, causes similar increases in adrenal weight (Jefferies & French, 1972). Srebocan *et al.* (1971) reported reduced concentrations of corticosteroids in the adrenals and in the plasma after young cockerels had been treated with technical DDT or pp'-DDT.

Administration of adrenal corticosteroids to a laying bird can affect shell deposition. Decreases in shell weight after cortisone treatment have been noted by Urist & Deutsch (1960) and Tyler (personal communication), although intramuscular injections of 10–30 mg cortisone acetate/day cause chickens to lay eggs with thicker shells before the treatment terminates laying (Cooke, 1968). Cortisone causes a type of osteoporosis and hypercalcaemia (Urist & Deutsch, 1960) and marked decreases in body weight (Cooke, 1968), so it would appear that its action is to mobilise skeletal calcium. Thicker shells might be formed because of a short-term rise in available calcium before a calcium deficiency terminates ovulation (Taylor *et al.*, 1962). Alternatively, Simkiss (1961) has suggested that cortisone might inhibit the 3-phosphoadenosine 5-phosphosulphate system (Whitehouse & Lash, 1961) which is involved in the sulphation of acid mucopolysaccharides (Suzuki & Stromінger, 1959, 1960*a*, *b*, *c*), so reducing the synthesis of shell organic matter essential for proper shell development. It was proposed that this reaction would occur in the isthmus region of the oviduct, but since shell organic material secreted by isthmal cells does not apparently contain acid mucopolysaccharide (Robinson *et al.*, 1968; Cooke & Balch, 1970*a*), this explanation seems unlikely. Nevertheless, an effect on acid mucopolysaccharide availability in the oviduct certainly cannot be dismissed, as daily intramuscular injections of 10 mg cortisone acetate cause a considerable shift in the ratio of glucuronic and iduronic acid in the shell (Cooke, 1968). Glucuronic acid is a constituent unit of chondroitin sulphate A (*see* Brimacombe & Webber, 1964), the main acid mucopolysaccharide in egg shell matrix (Baker & Balch, 1962; Cooke, 1968), while iduronic acid, an epimer of glucuronic acid, is a constituent unit of chondroitin sulphate B, which is also found in the matrix (Baker & Balch, 1962; Cooke, 1968). Three birds subjected to this cortisone acetate treatment for three weeks showed increases in glucuronic acid in the shell of 52, 44 and 73% and decreases in iduronic acid of 37, 62 and 59% respectively (Cooke, 1968). Thus cortisone, and to a lesser extent other treatments (*see* section 3(*b*)), can somehow reduce the relative availability of chondroitin sulphate B to the developing shell.

There has been one experiment linking pesticides, corticosteroids and carbohydrate metabolism. After treating cockerels with DDT, Srebocan *et al.* (1971) found a decrease in liver glycogen synthesis associated with a decrease in adrenal and blood corticosteroid levels. Decreased corticosteroid levels might result in thin shells being laid, but supporting evidence is lacking.

4(*e*) *Hormone-type effects:* Levin *et al.* (1968) reported that technical DDT increased uterine wet weight in rats, the active constituent being op'-DDT, which, they found, competed with labelled oestradiol for binding sites on the uterus. At about the same time, oestrogen-like responses after op'-DDT treatment were reported in weight and glycogen content of chicken and quail oviducts and rat uteri (Bitman *et al.*, 1968). op'-DDT given orally to quail produced, however, no marked changes in serum calcium levels (Bitman *et al.*, 1969; Cooke, 1970*b*) or

in the mating behaviour of males (Cooke, 1970*b*). Since op'-DDT is readily lost by birds (French & Jefferies, 1969; Bitman *et al.*, 1971; Cecil *et al.*, 1972) and is only very rarely detected in wildlife samples, the intake of this isomer by predatory birds is probably negligible. Even if birds in the field are exposed to technical DDT, they are likely to be killed by pp'-DDT before op'-DDT has any significant sublethal effect (Cooke, 1970*b*).

Nevertheless, that op'-DDT should exhibit oestrogenic-type properties is of great interest and researchers have turned their attentions to its effects on mammals (Welch *et al.*, 1969; Singhal *et al.*, 1970; Cecil *et al.*, 1971*b*; Heinrichs *et al.*, 1971; Wrenn *et al.*, 1971). The relative oestrogenicity of op'-DDT is about one ten thousandth (Cecil *et al.*, 1971*b*) or one hundred thousandth (Singhal *et al.*, 1970) that of oestradiol. Although op'-DDT has certain oestrogenic properties, it does not inhibit pregnancy in the rat, unlike oestrone (Duby *et al.*, 1971), and at a dietary level of 10 ppm has no oestrogenic action on ewes (Wrenn *et al.*, 1971). Bitman & Cecil (1970) extended the study to include other organochlorines and their results indicated that halide or alkyl groups in the para positions on the basic diphenylalkane structure rendered the compounds inactive, while -H, -OH or $-OCH_3$ in these positions rendered them active. Some PCBs were also active. Objections on steric grounds as to why DDT and similar molecules should be oestrogenic (Fisher *et al.*, 1952) have been overcome by Bitman & Cecil (1970).

Apparent hormone mimicry is not limited to sex hormone-type effects. It is believed that op'-TDE can compete with thyroxine for protein binding sites in human serum (*see* section 4(*e*)) and thereby lower protein bound iodine (Marshall & Tomkins, 1968).

With so many egg formation mechanisms under hormonal control, it is not possible to rule out hormone mimicry having effects on egg shell formation.

An overall assessment of possible mechanisms

So far each mechanism has been discussed individually and an assessment, based on available information, is presented in Table 4. There could be several reasons for the lack of an obvious mechanism.

(1) Most of the discussions in this section have been based on knowledge derived from experiments on the chicken, a species relatively resistant to shell thinning by organochlorines. It is quite likely that the physiological and biochemical processes involved in egg shell formation in species that have been observed to lay thin shells in the field differ considerably in degree or nature from those in the chicken. For an example of an interspecific difference see the hepatic metabolism data for the chicken and pigeon referred to in section 1(*b*). There is, however, little information on non-domestic species, and even for the chicken our knowledge of shell formation is very incomplete.

(2) The list of factors discussed is unlikely to be exhaustive and shell thinning may be due to a factor not considered.

(3) Pesticides produce such a broad spectrum of effects in an organism, perhaps due to action on the endocrine system, that deciding whether changes in a physiological process merely reflect this broad effect on the bird or whether they are responsible for reducing shell thickness is difficult.

(4) Pesticides may cause thin shells because of effects on several (probably interrelated) mechanisms and various factors such as species and environmental conditions might affect which mechanism or mechanisms are dominant in any given situation.

TABLE 4

AN ASSESSMENT OF POSSIBLE MECHANISMS BY WHICH ORGANOCHLORINES CAUSE EGG SHELL THINNING

Mechanism	*Evidence For*	*Against*
1. Reduced availability of calcium ions		
(a) Absorption from gut	+	–
(b) Deposition of medullary bone	+ + +	– –
(c) Mobilisation of medullary bone	+	–
(d) Transport in blood		–
(e) Transport from blood to shell	+ +	–
2. Reduced availability of carbonate ions	+ + +	– –
3. *Effects on other shell constituents*		
(a) Initiation sites	+*	–
(b) Matrix	+*	–
(c) Shell growth inhibitors		–
(d) Premature termination	+ +	
4. *Other factors*		
(a) Premature oviposition		–
(b) Decreased food consumption	+	–
(c) Thyroid defects	+ +	–
(d) Stimulation of adrenals		
(e) *Hormone-type effects*		

+ or – indicates a little evidence available;
+ + or – –, more evidence;
+ + + or – – –, good evidence.
* There is reasonable evidence for these factors being responsible for crystal disruption rather than shell thinning.

Some researchers probably already have information that would assist in deciding on likely mechanisms. For instance, it would help to know the pattern of shell thickness plotted against time when birds are subjected to continuous exposure of pesticide. For the chicken, a low calcium diet, sulphanilamide in the diet and perhaps high temperature all produce characteristic patterns (Fig. 3). The first two patterns are well known and the compensatory rise in thickness at high temperature has been observed before (B. A. Morris, personal communication). Unfortunately, there is no definite information on the pattern produced by pesticides. Information from Bitman *et al.* (1969) suggests it may be of the low calcium type, but their controls in this experiment were fed a calcium deficient diet and did not lay to this pattern. The results of Stickel & Rhodes (1970) indicate it may be a sulphanilamide-type pattern. On the other hand, shell thickness, relative to egg residues, of eggs laid by North American cormorants in 1965 was greater in the second clutch

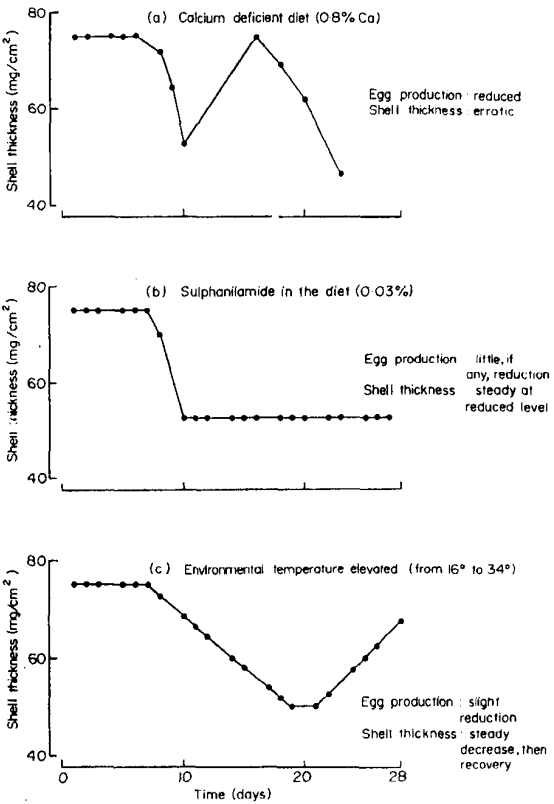

Fig. 3. Type of response in shell thickness shown by chickens to (a) a calcium deficient diet, (b) sulphanilamide in the diet, or (c) elevated environmental temperature. Birds were subjected to the treatment from day 8 to day 28. These curves, which show the salient characteristics of the responses, have been constructed from the results of Cooke (1968).

than in the first (Anderson *et al.*, 1969), and this may indicate operation of a compensating mechanism (Risebrough *et al.*, 1970) as has been observed at elevated temperatures (Fig. 3).

The effects on the bird and on the egg of other treatments known to cause thin shells have been frequently referred to in an attempt to find corresponding effects to those produced by pesticides. These effects are summarised in Table 5. The one recurring theme throughout this section has been the similarity in many respects between the effects of high temperature and the effects of pesticides. High temperature is believed to reduce shell thickness because blood pCO_2 is depressed when the birds hyperventilate (Mongin & Lacassagne, 1966; Simkiss, 1968) and it would be interesting to know whether organochlorines have a similar effect on blood pCO_2. There could be an effect via thyroid-mediated changes in breathing. In pigeons treated with high doses of DDT, Jefferies & French (1971) noted reduced oxygen consumption and body temperature, which may indicate hypoventilation and an accompanying elevation of blood pCO_2. This state of respiratory acidosis is, however, the reverse of the alkalosis produced by high environmental temperatures, and the effect of an increased level of atmospheric CO_2 (to increase blood pCO_2) on shell thickness is not entirely clear. Thinner shells have been observed after acute exposure (Helbacka *et al.*, 1963; Hunt & Simkiss, 1967) and thicker shells after chronic exposure (Frank & Burger, 1965), the difference apparently being due to renal compensation raising blood bicarbonate levels during long-term studies (Simkiss, 1968).

While it is possible that inhibition of thyroid function can contribute indirectly to some observed changes in shell thickness, blockage of sites on thyroxine-binding globulin seems unlikely to be the primary lesion involved in thin shell formation by pesticides. Undoubtedly changes in the thyroids and the adrenals can occur when birds are exposed to organochlorine insecticides, and since DDT can virtually completely inhibit thyroid function (Jefferies, personal communication), the effects on the well-being of the bird must be considerable. Such changes might indeed be responsible for many of the frequently-observed sublethal effects, such as reduced fertility, hatchability, egg weight, chick weight, delay in ovulation, etc. (Jefferies *et al.*, 1971) and they would therefore be expected to exert some influence on essential mechanisms in shell formation. It is, however, hard to reconcile this broad-based effect due to direct or indirect action on thyroid function with the apparently relatively specific effect of pesticides on shell formation sometimes observed in the field and in the laboratory. Although several mechanisms are probably affected by pesticides when thin shells are produced (*see* below), present evidence from laboratory experiments suggests that thyroid malfunction has relatively little effect on shell deposition. With respect to thyroid changes, pesticides may again be similar to high environmental temperature, which is known to cause the thyroid secretion rate to fall (*see* Sturkie, 1965), but this is seemingly unconnected with the mechanism causing the shells to become thin (*see* Simkiss, 1968).

TABLE 5

A COMPARISON OF THE EFFECTS ON THE BIRD AND ON ITS EGGS OF PESTICIDES, SULPHONAMIDES, AMMONIUM CHLORIDE OR A DEFICIENCY OF CALCIUM IN THE DIET OR HIGH ENVIRONMENTAL TEMPERATURE

	Pesticides and PCBs	*High temperature*	*Sulphonamide*	*Ammonium chloride*	*Calcium deficient diet*
Effects on the bird					
(a) Blood calcium	No change: Bitman *et al.* (1969), Cooke (1970*b*), Whitehead *et al.* (1972)	Decrease: Mueller (1959)	Decrease: Siegmund & Dulce (1960) No change: Scott *et al.* (1944)	Decrease: Siegmund & Dulce (1960) No change: Hunt & Aitken (1962*b*)	Decrease: Taylor & Hertelendy (1961)
(b) Other changes in calcium metabolism	Reduction in medullary bone: Peakall (1970*b*), Oestreicher *et al.* (1971) Dieldrin no change on bone deposition: Mueller & Lockman (1971) Effects on vitamin D_3 mediated changes: Nowicki *et al.* (1972*b*)	Retention from food increased: Muller (1959) Incorporation into shell reduced: Bragg *et al.* (1971)	Retention decreased: Tyler (1950,1954)	—	Disrupted: *e.g.* Hurwitz (1970), Taylor (1970), Bragg *et al.* (1971)
(c) Possible connection with thyroid malfunction	Jefferies (1969), Jefferies & French (1969, 1971), Jefferies *et al.* (1971) Richert & Prahlad (1972)	Reviewed by Sturkie (1965)	—	—	—
(d) Carbonic anhydrase inhibition	Reduction in shell gland: Peakall (1970*b*), Bitman *et al.* (1970). No *in vitro* inhibition: Dvorchik *et al.* (1971), Pocker *et al.* (1971)	—	*e.g.* Mann & Keilin (1940)	Unconvincing: *see* Hunt & Aitken (1962*b*)	—
Effects on the egg					
(a) Egg production	DDT decrease: Cross *et al.* (1962), Stickel & Rhodes (1970), Sauter & Steele (1972), Lillie *et al.* (1972). DDT no change: Genelly & Rudd (1956), Tucker & Haegele (1970), Hurst *et al.* (1971), Davison & Sell. (1972) Dieldrin decrease: Genelly & Rudd (1956), Atkins & Linder (1967) Dieldrin no change:	Decrease: Sturkie (1965), Cooke (1968), Bragg *et al.* (1971)	Decrease at high dose rate: Scott *et al.* (1944) No change: Tyler (1950, 1954), Cooke (1968)	Decrease: Hunt & Aitken (1962*a*) No change: Hunt & Aitken (1962*b*), Cooke (1968)	Decrease: *e.g.* Cooke (1968) Bragg *et al.* (1971)

TABLE 1

EXPERIMENTS IN WHICH EGG SHELL THICKNESS HAS BEEN AFFECTED AFTER EXPOSURE OF THE LAYING BIRD TO ORGANOCHLORINE INSECTICIDES

Author(s)	*Species*	*Pollutant(s)*	*Exposure*	*Mean egg content (ppm)*	*Shell measurement*	*Effect on shell thickness*
Bitman *et al.* (1969)	Japanese quail *C. coturnix japonica*	pp'-DDT or op'-DDT	100 ppm in low Ca diet for 45 days	520 / 27 total residues of DDT type compounds	Thickness at 3 points around waist of shell without membranes†	Decrease, 6% / Decrease, 4% Compared with controls on low Ca diet
Lehner & Egbert (1969)	Mallard *Anas platyrhynchos*	Dieldrin	Up to 10 ppm* in diet for 1–2 years	Up to 44	Thickness at broad pole and waist of shell plus outer membrane†	Decrease, up to 4%‡
Porter & Wiemeyer (1969)	American Kestrel *Falco sparverius*	pp'-DDT plus dieldrin	Up to 15 ppm pp'-DDT plus 3 ppm dieldrin* in diet for 1–2 years	Not given	Thickness at 4 points of shell plus membranes†	Decrease, up to 17%‡
Heath *et al.* (1969) (*see* also Heath *et al.*, in press)	Mallard	pp'-DDE or pp'-DDT	Up to 40 ppm* in diet for between 1 and 2 years	Not given	Thickness of shell plus membrane†	Decrease, up to 13%‡
Jefferies (1969)	Bengalese finch *Lonchura striata*	pp'-DDT	Up to 300 μg/day* in diet for about 15 weeks	Not given	Shell weight/egg weight	Increase by average of 7%
Enderson & Berger (1970)	Prairie falcon *Falco mexicanus*	Dieldrin	Wild birds fed on up to 12 tethered starlings previously dosed with dieldrin	40+	Thickness index	Decrease of 9% (compared with controls) for 7 most contaminated eggs

Peakall (1970b)	Ring dove *Streptopelia risoria*	pp'-DDE	Single intraperitoneal injection of 150 mg/kg	80	Weight	Decrease by 23%
Wiemeyer & Porter (1970)	American kestrel	pp'-DDE	10 ppm in diet for more than 1 year	32	Thickness at 4 points of shell plus membranes†	Decrease by 10%
Smith *et al.* (1970)	Chicken *Gallus domesticus*	Technical DDT	Up to 10 ppm* in diet for 2 months	Up to 6 ppm in yolk	Not given	Decrease, up to 11%‡
Stickel & Rhodes (1970)	Japanese quail	pp'-DDT	Up to 25 ppm* in diet for 26 weeks	Not given	Not given	Decrease, up to 7%‡
Tucker & Haegele (1970)	Bobwhite quail *Colinus virginianus*	Technical DDT	Up to 30 ppm* in diet for 96 days	Not given	Thickness at 4 points around waist†	Decrease, up to 4%‡
	Mallard	Technical DDT	Single oral dose 1000 mg/kg. Food withheld for 2 days after treatment	Not given	As above	Decrease, up to 5%‡ Decrease by 9% over 16 days
Longcore *et al.* (1971b)	Black duck *Anas rubripes*	pp'-DDE	Up to 30 ppm* in diet for 6 months	Up to 135	Thickness at waist and both poles of shell plus membranes†	Decrease, up to 38%‡
Sauter & Steele (1972)	Chicken	Technical DDT or lindane	Up to 10 ppm* in diet for 10 weeks	Not given	Not given	Decrease, up to 9%‡ after treatment period

* Highest treatment dose
† Measured with a micrometer
‡ Mean thickness change for most affected treatment group

TABLE 3

THE AMOUNT OF MATRIX, SIALIC ACID AND URONIC ACID IN THICK AND THIN SHELLS LAID BY CHICKENS *Gallus domesticus*. FOR DETAILS SEE TEXT (ADAPTED FROM COOKE, 1968)

	No. of bulked samples[a]	*True shell thickness* (mg/cm^2)	*Matrix*		*Sialic acid*		*Uronic acid*	
			(mg/cm^2)	(*mg/g shell*)	($\mu g/cm^2$)	(*μg/mg matrix*)	($\mu g/cm^2$)	(*μg/mg matrix*)
'Normal thick' shells, thickness > 70 mg/cm^2	16	73·7 ± 0·5	1·09 ± 0·05[a]	14·8 ± 0·5[a]	9·3 ± 0·2[a]	8·6 ± 0·3[a]	14·4 ± 0·3[a]	13·3 ± 0·4[a]
'Normal thin' shells, thickness < 70 mg/cm^2	14	66·2 ± 0·5	0·90 ± 0·04[c]	13·5 ± 0·5[a]	8·1 ± 0·2[d]	9·2 ± 0·3[a]	12·5 ± 0·6[c]	13·6 ± 0·3[a]
'Abnormal thin' shells	14	52·7 ± 1·3	0·90 ± 0·04[c]	17·2 ± 1·0[b]‡	7·7 ± 0·3[d]	8·7 ± 0·3[a]	10·6 ± 0·4[d]	11·9 ± 0·4[b]‡

± S.E.

[a] Samples contained segments from 3–13 shells

[a, b, c, d] For a pair of means differing by one letter, $P < 0.05$; by two letters, $P < 0.01$; by three letters, $P < 0.001$.

‡ Compared with 'normal thin' shells, $P < 0.01$

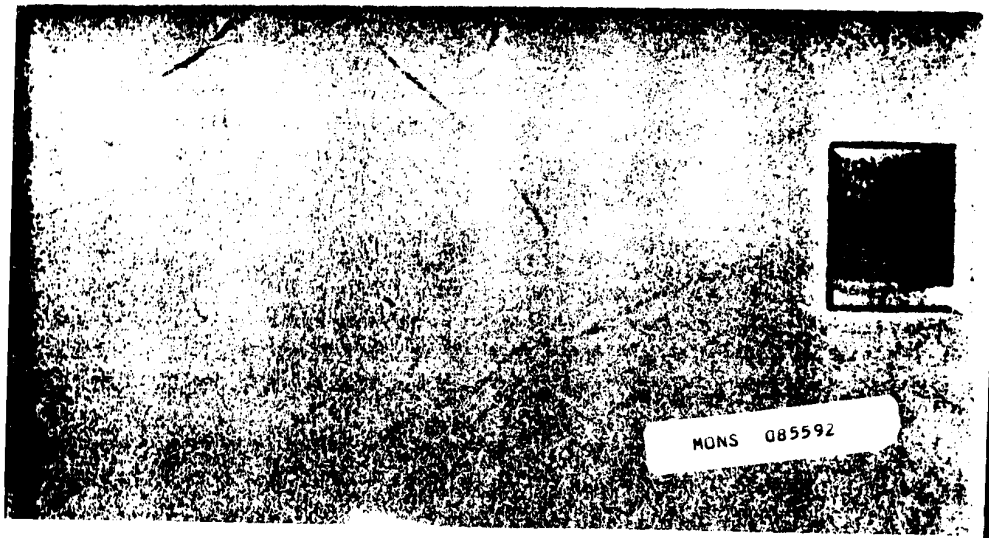

TABLE 5

A COMPARISON OF THE EFFECTS ON THE BIRD AND ON ITS EGGS OF PESTICIDES, SULPHONAMIDES, AMMONIUM CHLORIDE OR A DEFICIENCY OF CALCIUM IN THE DIET OR HIGH ENVIRONMENTAL TEMPERATURE

	Pesticides and PCBs	*High temperature*	*Sulphonamide*	*Ammonium chloride*	*Calcium deficient diet*
Effects on the bird					
(a) Blood calcium	No change: Bitman *et al.* (1969), Cooke (1970*b*), Whitehead *et al.* (1972)	Decrease: Mueller (1959)	Decrease: Siegmund & Dulce (1960) No change: Scott *et al.* (1944)	Decrease: Siegmund & Dulce (1960) No change: Hunt & Aitken (1962*b*)	Decrease: Taylor & Hertelendy (1961)
(b) Other changes in calcium metabolism	Reduction in medullary bone: Peakall (1970*b*), Oestreicher *et al.* (1971) Dieldrin no change on bone deposition: Mueller & Lockman (1971) Effects on vitamin D_3 mediated changes: Nowicki *et al.* (1972*b*)	Retention from food increased: Muller (1959) Incorporation into shell reduced: Bragg *et al.* (1971)	Retention decreased: Tyler (1950,1954)	—	Disrupted: *e.g.* Hurwitz (1970), Taylor (1970), Bragg *et al.* (1971)
(c) Possible connection with thyroid malfunction	Jefferies (1969), Jefferies & French (1969, 1971), Jefferies *et al.* (1971) Richert & Prahlad (1972)	Reviewed by Sturkie (1965)	—	—	—
(d) Carbonic anhydrase inhibition	Reduction in shell gland: Peakall (1970*b*), Bitman *et al.* (1970). No *in vitro* inhibition: Dvorchik *et al.* (1971), Pocker *et al.* (1971)	—	*e.g.* Mann & Keilin (1940)	Unconvincing: *see* Hunt & Aitken (1962*b*)	—
Effects on the egg					
(a) Egg production	DDT decrease: Cross *et al.* (1962), Stickel & Rhodes (1970), Sauter & Steele (1972), Lillie *et al.* (1972), DDT no change: Genelly & Rudd (1956), Tucker & Haegele (1970), Hurst *et al.* (1971), Davison & Sell. (1972) Dieldrin decrease: Genelly & Rudd (1956), Atkins & Linder (1967) Dieldrin no change: Davison & Sell. (1972) PCB decrease: Hurst *et al.* (1971) Dahlgren & Linder (1971) Mercury decrease: Fimreite (1971) Mercury no change: Stoewsand *et al.* (1971)	Decrease: Sturkie (1965), Cooke (1968), Bragg *et al.* (1971)	Decrease at high dose rate: Scott *et al.* (1944) No change: Tyler (1950, 1954), Cooke (1968)	Decrease: Hunt & Aitken (1962*a*) No change: Hunt & Aitken (1962*b*), Cooke (1968)	Decrease: *e.g.* Cooke (1968) Bragg *et al.* (1971)
(b) Egg size or weight	DDT decrease: Jefferies (1969), Bitman *et al.* (1969) DDT no change: Davison & Sell (1972), Cecil *et al.* (1972) Dieldrin increase: Atkins & Linder (1967) Dieldrin no change: Davison & Sell (1972) Hexachlorobenzene decrease: Vos *et al.* (1971) Mercury decrease: Fimreite (1971)	Decrease: Cooke (1968)	Sometimes decrease: Cooke (1968, 1970*a*)	Decrease: Hall & Helbacka (1959) No change: Cooke (1968)	Decrease: Bragg *et al.* (1971)
(c) Yolk size	DDT decrease: suggested by Jefferies (1971) Lindane no change: Whitehead *et al.* (1972)	Decrease: Sturkie (1965)	—	—	
(d) Apparent ability to cause soft shelled eggs	Risebrough *et al.* (1971), Keith *et al.* (1970), Faber *et al.* (1972)	Occasionally: Cooke (1968)	*e.g.* Tyler (1950, 1954)	Not recorded by authors who have used this treatment	Occasionally, but cut-out mechanisms should operate: Taylor *et al.* (1962), Taylor (1970)
(e) Compensatory rise in shell thickness (*see* text)	Suggested by Risebrough *et al.* (1970) to explain results of Anderson *et al.* (1969)	Cooke, (1968), B.A. Morris (personal comm.)	No: Cooke (1968)	No: Cooke (1968)	No: Cooke (1968)
(f) Defects in shell structure in mammillary or palisade layer or on surface	Various defects: Erben & Krampitz (1971) McFarland *et al.* (1971)	Widespread defects: El-Boushy *et al.* (1968)	Granular surface: Scott *et al.* (1944) Structure not examined	—	Normal mammillary layer but palisade layer defective
(g) Calcium: percentage in shell	Decrease: Longcore *et al.* (1971*a*), DDT and	—			

	Davison & Sell, (1972) PCB decrease: Hurst *et al.* (1971) Dahlgren & Linder (1971) Mercury decrease: Fimreite (1971) Mercury no change: Stoewsand *et al.* (1971)				
(b) Egg size or weight	DDT decrease: Jefferies (1969), Bitman *et al.* (1969) DDT no change: Davison & Sell (1972), Cecil *et al.* (1972) Dieldrin increase: Atkins' & Linder (1967) Dieldrin no change: Davison & Sell (1972) Hexachlorobenzene decrease: Vos *et al.* (1971) Mercury decrease: Fimreite (1971)	Decrease: Cooke (1968)	Sometimes decrease: Cooke (1968, 1970*a*)	Decrease: Hall & Helbacka (1959) No change: Cooke (1968)	Decrease: Bragg *et al.* (1971)
(c) Yolk size	DDT decrease: suggested by Jefferies (1971) Lindane no change: Whitehead *et al.* (1972)	Decrease: Sturkie (1965)	—	—	—
(d) Apparent ability to cause soft shelled eggs	Risebrough *et al.* (1971), Keith *et al.* (1970), Faber *et al.* (1972)	Occasionally: Cooke (1968)	*e.g.* Tyler (1950, 1954)	Not recorded by authors who have used this treatment	Occasionally, but cut-out mechanisms should operate: Taylor *et al.* (1962), Taylor (1970)
(e) Compensatory rise in shell thickness (*see* text)	Suggested by Risebrough *et al.* (1970) to explain results of Anderson *et al.* (1969)	Cooke, (1968), B.A. Morris (personal comm.)	No: Cooke (1968)	No: Cooke (1968)	No: Cooke (1968)
(f) Defects in shell structure in mammillary or palisade layer or on surface	Various defects: Erben & Krampitz (1971) McFarland *et al.* (1971)	Widespread defects: El-Boushy *et al.* (1968)	Granular surface: Scott *et al.* (1944) Structure not examined	—	Normal mammillary layer but palisade layer deficient: Cooke (1968)
(g) Calcium: percentage in shell	Decrease: Longcore *et al.* (1971*a*). DDT and dieldrin no change: Davison & Sell (1972)	—	No change: Tyler (1950)	—	No change: Bragg *et al.* (1971)
(h) Matrix: increase in percentage in shell or change in composition	Erben & Krampitz (1971)	Cooke (1968)	Cooke (1968)	Cooke (1968)	—

Failure to find the primary cause of shell thinning is hardly surprising since, in the past, investigations into mechanisms for thin shell formation have rarely led to unquestionable conclusions and often seemingly well-proven and widely-accepted theories have fallen from favour. The mode of action of sulphanilamide on chickens seemed so simple in the 1940s. Gutowska & Mitchell (1945) observed that the CA content of the shell glands of good layers was greater than that of shell glands of poor layers, and knowing that sulphanilamide, a CA inhibitor (Mann & Keilin, 1940), caused thin shells (Scott *et al.*, 1944), their theory that enzyme inhibition produced the thin shells seemed completely sound. The first seeds of doubt were sown when Tyler (1950, 1954) showed that sulphanilamide caused changes in calcium retention that could not be explained as secondary effects arising from inhibition in the shell gland. Then reduction of blood calcium levels by acetazolamide was reported by Siegmund & Dulce (1960), although Scott *et al.* (1944) had previously found that sulphanilamide did not affect blood calcium. In 1962 Mueller proposed that sulphanilamide caused thin shells because it was a diuretic. Even though Heald *et al.* (1968) demonstrated that CA content of the shell gland is unrelated to shell thickness, sulphonamides are now once again thought to cause thin shells by CA inhibition in the shell gland (Bernstein *et al.*, 1968), although it is generally appreciated that enzyme inhibition cannot be the complete answer since both metabolic and respiratory acidosis occur after treatment (Mongin, 1970). This and other information given in this section suggests that sulphanilamide, ammonium chloride and high temperature affect, in chickens, a variety of mechanisms involved in shell formation, but shell thinning is largely brought about by a change in a single vital mechanism.

Treatment of birds with organochlorines has been shown to bring about detrimental changes in many processes involved in egg shell formation (Table 4). None of these changes can alone account for the observed decreases in shell thickness, and it is likely that each suggested mechanism plays an appreciable part in some situations but a negligible part in others. For instance, if medullary bone deposition is reduced by increased hepatic metabolism of the sex hormones, then this should have a lesser role in shell thinning in gallinaceous species than in pigeons and doves (*see* section 1(*b*)). The evidence suggests that, in shell thinning by organochlorines, no one mechanism is dominant, irrespective of conditions. However, when very thin shells are formed after exposure to organochlorines, only one mechanism is likely to have been markedly affected or else each major affected mechanism must be very close to the site of shell formation, since a general effect of all mechanisms would be expected to produce other symptoms such as prevention of ovulation or changes in behaviour.

The brown pelican colony present on Anacapa Island in 1969 can be taken as an example, since, although the birds were atypical as regards the extent of shell thinning, their reproductive failure has been well documented. Their egg shells had relatively normal mammillary layers but deficient palisade layers (Erben &

(b) Egg size or weight	Davison & Sell. (1972) PCB decrease: Hurst *et al.* (1971) Dahlgren & Linder (1971) Mercury decrease: Fimreite (1971) Mercury no change: Stoewsand *et al.* (1971) DDT decrease: Jefferies (1969), Bitman *et al.* (1969) DDT no change: Davison & Sell (1972), Cecil *et al.* (1972) Dieldrin increase: Atkins & Linder (1967) Dieldrin no change: Davison & Sell (1972) Hexachlorobenzene decrease: Vos *et al.* (1971) Mercury decrease: Fimreite (1971)	Decrease: Cooke (1968)	Sometimes decrease: Cooke (1968, 1970*a*)	Decrease: Hall & Helbacka (1959) No change: Cooke (1968)	Decrease: Bragg *et al.* (1971)
(c) Yolk size	DDT decrease: suggested by Jefferies (1971) Lindane no change: Whitehead *et al.* (1972)	Decrease: Sturkie (1965)	—	—	—
(d) Apparent ability to cause soft shelled eggs	Risebrough *et al.* (1971), Keith *et al.* (1970), Faber *et al.* (1972)	Occasionally: Cooke (1968)	*e.g.* Tyler (1950, 1954)	Not recorded by authors who have used this treatment	Occasionally, but cut-out mechanisms should operate: Taylor *et al.* (1962), Taylor (1970)
(e) Compensatory rise in shell thickness (*see* text)	Suggested by Risebrough *et al.* (1970) to explain results of Anderson *et al.* (1969)	Cooke, (1968), B.A. Morris (personal comm.)	No: Cooke (1968)	No: Cooke (1968)	No: Cooke (1968)
(f) Defects in shell structure *in mammillary* or palisade layer or on surface	Various defects: Erben & Krampitz (1971) McFarland *et al.* (1971)	Widespread defects: El-Boushy *et al.* (1968)	Granular surface: Scott *et al.* (1944) Structure not examined	—	Normal mammillary layer but palisade layer deficient: Cooke (1968)
(g) Calcium: percentage in shell	Decrease: Longcore *et al.* (1971*a*). DDT and dieldrin no change: Davison & Sell (1972)	—	No change: Tyler (1950)	—	No change: Bragg *et al.* (1971)
(h) Matrix: increase in percentage in shell or change in composition	Erben & Krampitz (1971)	Cooke (1968)	Cooke (1968)	Cooke (1963)	—

Krampitz, 1971), evidence consistent with a general calcium deficiency being responsible (Cooke, 1968). This is not supported by other evidence, however. The average decrease in shell thickness was 50% and many birds laid eggs with soft shells (Risebrough *et al.*, 1971). Calcium deficient poultry do not usually lay eggs with soft shells, they stop laying instead. This is thought to be due to low blood calcium levels triggering a cut-out mechanism that prevents further secretion of gonadotrophins from the pituitary (Taylor *et al.*, 1962). In this way birds are protected from utilising too much skeletal calcium. That the pelicans laid eggs with soft shells suggests that a general calcium deficiency was not to blame (assuming that pelicans also have a cut-out mechanism) and mechanisms 1(*a*)–(*d*) can be ruled out. Although evidence implicating reduced transport of calcium from the blood to the shell gland (1(*e*)) is not sufficiently convincing to suggest that here is the cause of a 50% reduction in shell material, it should be remembered that again the supporting experiments have been carried out on laboratory species. Since movement of calcium ions across the shell gland wall can be reduced by prior treatment with DDE (Peakall & Lincer, 1970*b*; Peakall, personal communication), a reduction of available calcium because of reduced transport may have been responsible, at least in part, for the thin shells. Mechanisms such as effects on thyroid or adrenal function or decreases in food consumption that would be expected to cause other obvious symptoms can probably be discounted. There is no evidence to suggest that the change in the amino acid composition of shell matrix noted by Erben & Krampitz (1971) can have played more than a very minor role in producing the thin shells. This leaves premature termination and carbonate deficiency to be assessed. The former is feasible, at least for these pelican eggs (*see* section 3(*d*)), but, as with all the suspected mechanisms, further research is needed. A reduction in carbonate availability can lead to the laying of eggs with soft shells. It has long been known that sulphanilamide treatment can reduce shell thickness to such an extent, as can changes in the acid-base balance of the blood, since the maximum reduction in thickness for a shell laid by any of the three control birds at high temperature (Table 2) was 75%. Attempts to explain how pesticides might reduce carbonate availability have mainly rested on CA inhibition. Failure to inhibit CA *in vitro* (Dvorchik *et al.*, 1971; Pocker *et al.*, 1971) can be overcome by assuming a metabolite to be the active compound. There is a marked similarity between the shell thinning response of chickens to various levels of dietary sulphanilamide (Scott *et al.*, 1944) and the response of brown pelicans from twelve American colonies to environmental DDE levels, as reflected by egg residues (Blus *et al.*, 1972). Inhibition of CA or possible change in the acid-base balance of the blood must at present, however, remain speculation. Nevertheless, the evidence points towards enzyme inhibition close to the site of shell formation being the dominant affected mechanism, at least in this particular instance (Risebrough *et al.*, 1970). As suggested by these writers, mechanisms that might be involved are (a) inhibition of CA leading to a reduction in carbonate availability and (b) inhibition of ATPases in

the shell gland resulting in reduced calcium ion concentration in the lumen. If the action of the factor reponsible for premature termination is confined to the shell gland, then premature termination could be at least a contributory mechanism. Premature termination certainly cannot be implicated in all situations, since this was not responsible for the thin shells examined by McFarland *et al.* (1971).

Finally, something should be said about the accusations (*e.g.* by Rogers, 1972) that disturbances by visiting biologists during 1969 and 1970 contributed to shell thinning and breeding failure on Anacapa. Disturbance of the breeding birds sufficient to increase shell breakage during incubation or adversely affect breeding in any other way is to be deplored. However, shells of eggs laid prematurely by poultry in a state of shock are characterised by a lack of the outer shell components, and the two thin shells, produced by Anacapa pelicans, that were examined by Erben & Krampitz (1971) both possessed the outermost component, the calcareous cover. There is no reason to doubt that these shells were typical of the thin shells laid by this pelican colony. Shell thinning appears to have been caused by a biochemical, rather than an emotional, disturbance.

THE SIGNIFICANCE OF THIN SHELLS TO AVIAN POPULATIONS IN THE FIELD

That a decrease in shell thickness has occurred for certain populations of birds of prey since the Second World War has seldom, unlike its cause, been disputed. It is important to determine what effect this has had on population levels. At the beginning of the first section of this paper it was pointed out that Ratcliffe initially studied shell thickness because of an increased incidence of egg breakage in the nest. He suspected that thin shells were responsible for increased shell breakage (Ratcliffe, 1967, 1970), and Prestt (1970) reported that, in the three heron colonies he studied in Eastern England, virtually every female laid eggs with abnormally thin shells and egg shell breakage was practised by about one third of the pairs. In North America an association between decreased shell thickness and increased shell breakage has been reported by Hickey & Anderson (1968) for herring gulls in Wisconsin, by Berger *et al.* (1970) for peregrines in Quebec, and by Keith *et al.* (1970) and Risebrough *et al.* (1970) for the Anacapa Island pelicans. Similarly, an association between a decrease in shell thickness and a decrease in population has been observed for the prairie falcon (Fyfe *et al.*, 1969), merlin (Fox, 1971), double-crested cormorant (Anderson *et al.*, 1969), brown pelican (Keith *et al.*, 1970; Risebrough *et al.*, 1970, 1971; Blus, 1970), bald eagle, osprey and peregrine (Hickey & Anderson, 1968). Although common egrets in the Audubon Canyon Ranch colony in California did not decline between 1967 and 1970, a breeding failure associated with thin shells was observed (Faber *et al.*, 1972). Some of the birds produced eggs with soft shells, and, in 1970, 54% of their attempts to nest suffered shell breakage during incubation. The fledging success of prairie falcons in Wyoming

and Colorado in 1967 and 1968 was directly related to shell thickness (Enderson & Berger, 1970). Twenty-one pairs laying eggs with relatively thick shells had a fledging rate of 2·6 young per pair, while ten pairs producing thin shells fledged only three young between them. No declines occurred for regional populations of several raptor species that had not suffered shell thinning (Hickey & Anderson, 1968). In Alaska, however, where peregrines have laid eggs with thin shells for about two decades, breeding populations have remained steady although breeding failures have been observed recently (Cade *et al.*, 1971). This is similar to the situation in Britain, where the golden eagle (Ratcliffe, 1970) and the heron (Prestt, 1970) have been observed to lay eggs with thin shells in some regions, but breeding populations have not declined. Theoretically a population of a long-lived, slow-breeding bird is much more quickly reduced by the death of adults than by the breeding failure of those adults (Young, 1968). Thus, although thin shells due to organochlorine insecticides have probably often caused breeding failure amongst British raptors, adult mortality due to cyclodienes is a more likely reason for the population crashes of the peregrine (Ratcliffe, 1970) and sparrowhawk (Prestt, 1965; Jefferies & Prestt, 1966). Indeed, during the first decade after the onset of shell thinning, the peregrine increased in several regions after being reduced by controlled killing during the war (Ratcliffe, 1970). It is interesting to note that although in the Californian egret colony referred to above (Faber *et al.*, 1972) DDE levels in adults' tissues and in eggs were comparable with those in other species laying eggs with thin shells, dead and dying adults were found apparently poisoned by the dieldrin also present in their tissues. Although reduced breeding success may not have caused population crashes in Britain, it has probably retarded subsequent recoveries (Jefferies & Prestt, 1966; Ratcliffe, 1970).

An egg laid by a bird previously exposed to organochlorine insecticides might be of reduced viability for several reasons. If the shell is thin it is more likely to be cracked when laid (McNally, 1965). When captive ducks were exposed to DDE (Heath *et al.*, 1969; Longcore *et al.*, 1971*b*) shell thickness was reduced and there was an increased incidence of cracked shells. Cracks elevate the risk of bacterial infection (Romanoff & Romanoff, 1949) so causing embryonic mortality. Heath *et al.* (1969) reported that hairline cracks in the shell halved the hatchability of mallard eggs while all eggs with shell fractures failed to hatch. Dead embryos were found by Berger *et al.* (1970) in six falcon eggs with cracked shells collected from the field. The eggs contained about 13 ppm DDE (wet weight).

Ratcliffe (1970) proposed that brooding adults might respond to a damaged shell by destroying the egg, often eating it. He suggested that egg eating may be a normal adaptive response to certain conditions that reduce breeding success, and insecticide residues in the bird might elicit a similar response. Parental destruction of an egg with a damaged shell or destruction of an infertile or addled egg will not reduce reproductive success, whereas deliberate destruction of an egg with a thin but uncracked shell might kill an embryo that would otherwise have survived.

It has been tentatively suggested that egg shell breakage amongst herring gulls in a Lake Michigan colony (Ludwig & Tomoff, 1966) and in a Wisconsin colony (Keith, 1966) may have been due to sublethal doses of organochlorines causing aberrant behaviour in the brooding adults. Recently, Kenny & Dacke (personal communication) have demonstrated that Japanese quail maintained on a calcium deficient diet tend to destroy and eat many eggs immediately after oviposition, and addition of commercial DDT to the diet usually significantly increases the proportion lost. This could account, at least partially, for an observed decrease in egg production by captive birds being dosed with pp'-DDT (Stickel & Rhodes, 1970; Lillie *et al.*, 1972) or PCBs (Dahlgren & Linder, 1971).

The likelihood of shells being eaten in the field because of 'calcium hunger' was discussed by Ratcliffe (1970), but he concluded that, since birds of prey have sometimes been observed to throw eggs out of the nest or eat the contents and leave the shell, 'it is therefore possible that egg eating is simply a behavioural response to the internal state of the bird, with no nutritional significance'. In the peregrine, egg loss has been most frequently observed during the later stages of incubation, although it can occur at any time after the first egg has been laid (Ratcliffe, 1970). This contrasts with the experiment of Kenny & Dacke (personal communication) in which many eggs known by palpation to be calcifying were never seen and were presumed to have been eaten soon after being laid. If this occurred in the field it is improbable that the birds would even be suspected of having laid.

After inducing birds to lay thin-shelled eggs on a diet contaminated with insecticide, several workers have carried their experiments one stage further, either by allowing natural incubation (Porter & Wiemeyer, 1969) or else by artificially incubating the eggs (Heath *et al.*, 1969; Stickel & Rhodes, 1970; Sauter & Steele, 1972). Porter & Wiemeyer (1969) reported that the factor mainly responsible for reproductive failure amongst their dosed American kestrels was the disappearance of eggs during incubation plus, perhaps, some disappearance of newly hatched young. This was attributed to the egg or young bird being eaten by the adults after (accidental?) shell breakage, due to abnormally thin shells.

Data on artificial incubation of eggs with thin shells are valuable because such eggs are no longer vulnerable to fits of aberrant behaviour by the adult bird. Reduced breeding success from eggs with thin, but sound, shells need not solely be due to parental breakage. The thin shells themselves might not be such a hazard to the developing embryo as the pesticide in the egg contents and indeed reduced shell thickness might sometimes merely be an indicator of harmful pesticide residues in the yolk. Mortality would be expected mainly (a) during the later stages of incubation due to increased rate of uptake (Guthrie & Donaldson, 1970; Cooke, 1971) or (b) during the first few days of life due to absorption of yolk sac material (Koeman *et al.*, 1967) coupled with mobilisation of fat reserves (Cooke, 1971). Increased mortality at these stages, probably due to pesticide, has been observed both in the laboratory and in the field.

(a) After treating mallards with DDE, Heath *et al.* (1969) artificially incubated the eggs with sound shells and found that eggs with thin shells from dosed birds had reduced hatchability, mainly because of increased embryo mortality during the final stages of incubation. Eggs with thin shells laid by chickens treated with technical DDT or lindane by Sauter & Steele (1972) also had reduced hatchability, but increased embryonic mortality occurred throughout the incubation period. Stickel & Rhodes (1970) incubated eggs with thin shells laid by groups of quail on a series of pp'-DDT-treated diets. There was a tendency for eggs from the highest dosed group to have reduced hatchability, but the difference was not significant. Dahlgren & Linder (1971) reported increased mortality amongst pheasant chicks during the hatching stage after adults had been treated with Aroclor 1254. In this experiment PCBs did not cause thin shells and this is presumably an example of mobilisation of egg residues rather than fragile shells having a detrimental effect on chick survival. In the field dead, almost fully-developed, embryos have been found in contaminated falcon eggs (Fyfe *et al.*, 1969; Berger *et al.*, 1970), but since there will, of course, also be some embryonic mortality amongst uncontaminated eggs, dead-in-shell embryos in the field cannot be regarded as more than supporting evidence. A report by Peakall (1970*a*) of an interesting experiment carried out by Spitzer suggests that breeding failure amongst ospreys in the United States might be due to egg residues causing embryonic mortality. Eggs were exchanged between nests in a failing New England colony and a flourishing colony in Chesapeake Bay. The New England eggs still suffered a low hatching success in the Chesapeake Bay nests, while the Chesapeake Bay eggs hatched quite normally in the ailing New England colony.

(b) Young Sandwich terns, *Sterna sandvicensis*, in a Dutch colony have been observed dying in convulsions after hatching from eggs with high organochlorine residues (Koeman *et al.*, 1967). Feeding pheasants on diets containing DDT or dieldrin resulted in decreased survival of young during the first two weeks of life, particularly during the first day after hatching (Genelly & Rudd, 1956). Injection of eggs with DDT or dieldrin solutions insufficient to cause embryonic mortality can kill young chicks soon after hatching if food is withheld (Koeman *et al.*, 1967; Dunachie & Fletcher, 1969). The disappearance of American kestrel chicks after hatching (Porter & Wiemeyer, 1969) could be accounted for by death due to an internal post-hatch release of pesticide followed by being eaten by the adult.

Fimreite (1971) fed pheasants with methylmercury-treated grain and noted a significant increase in unfertilised eggs and/or in embryonic mortality early in the (artificial) incubation period. As yet no one has investigated the rate at which mercury in the egg becomes incorporated in the embryo's tissues.

Thus, to summarise, there are probably three main reasons why a raptor population may suffer loss of embryos or young chicks as a consequence of prior exposure to organochlorine insecticides.

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(1) Thin shells may be cracked during laying or become cracked later and the embryos die. Parental destruction of such eggs may occur before or after death of the embryos.

(2) Eggs, embryos or chicks may be destroyed or eaten because of aberrant behaviour by the adults.

(3) Embryos or young chicks might die because of residues mobilised from the egg contents and again this may be followed by being eaten.

GENERAL CONCLUSIONS

For many species of birds, particularly raptors, in Europe and North America, egg shell thickness has decreased since the Second World War. The species most affected are usually those with the highest levels of organochlorine insecticide residues in their tissues. Often, spatial and temporal associations have been noted between shell thickness declines and organochlorine insecticide usage, and relationships have frequently been observed between the decrease in shell thickness and levels of pp'-DDE in the parent bird or in the egg. The DDE in the laying hen may be the residue directly responsible for shell thinning, but it may simply be indicative of a more harmful compound (or compounds). If this proposed compound is not in the DDT group, its physical and chemical properties must closely resemble those of pp'-DDE in order to account for the above relationships. PCBs and other organochlorine insecticides have similar properties, and within a locality residue levels of PCBs and organochlorine insecticides in living organisms are related. Present field evidence suggests that in North America these compounds have probably played no more than a minor role, although in Britain the cyclodienes have made a significant contribution towards the reduction of egg shell thickness in certain species exposed to relatively high environmental levels. The possibility cannot be dismissed of an unstudied pollutant with similar properties being responsible. There is at present no evidence to implicate mercury in these widespread declines in shell thickness.

Findings from experiments on captive birds generally support the conclusions from field evidence. There are now more than 20 reports of organochlorine or organophosphate insecticides, PCBs or mercury compounds causing thin shells in experimental birds. Unfortunately, in the great majority of these experiments the pollutant was administered via the diet and food consumption was neither controlled nor monitored. Decreased food consumption, either by partial rejection of a diet of reduced palatability or by loss of appetite through the action of tissue residues, could lead to the production of thin shells. Available evidence on the consumption of treated diets does, however, indicate that decreased food consumption is unlikely to have been serious in most of the experiments. There are many reports of pesticides and PCBs having no effect on shell thickness, and some marked

differences between very similarly-designed experiments, some reporting no change and some a significant decrease, cannot be satisfactorily explained.

The main conclusions from the controlled experiments are as follows.

(1) Some experiments demonstrate that environmental pollutants can cause shell thinning in captive birds.

(2) DDT-type compounds generally produce a greater response than other organochlorine insecticides or PCBs.

(3) Interspecific differences in response exist, gallinaceous species tending to be the most resistant. Falcons seem to be the most susceptible birds so far studied.

These conclusions support the field evidence set out above.

Several experiments in which shell thickness has been reduced by organophosphate insecticides or mercury compounds suggest that, should birds come into contact with high levels of these compounds in the field, then shell thickness could be reduced.

Much work has been carried out in an attempt to elucidate the mechanisms involved in the reduction of shell thickness by organochlorines. Effects have been observed on many mechanisms known to be essential for proper shell formation. Birds laying eggs with thin shells after treatment with an organochlorine insecticide might suffer from (a) a general impairment of calcium metabolism, (b) a reduction in available carbonate in the shell gland lumen, (c) a deleterious effect on both the thyroids and the adrenals, and (d) an alteration in organic matter being incorporated into developing shells. Each of these detrimental changes is probably potentially capable of thinning the shell or disrupting shell structure, and the extent of the contribution of each mechanism is likely to be governed by variables such as species, condition of the bird and environmental conditions. When very thin shells are formed after exposure to organochlorines, only one mechanism is likely to have been markedly affected, or, alternatively, each major mechanism affected must be close to, or in, the shell gland, since a general effect on all mechanisms would be expected to produce other responses such as prevention of ovulation or a change in behaviour. Other factors that cause shell thinning in chickens, such as sulphanilamide, ammonium chloride or high environmental temperature, also affect a variety of mechanisms involved in shell formation, but shell thinning is thought to be brought about mainly by a change in a single mechanism. There is no evidence to suggest that in shell thinning mediated by organochlorines the same mechanism is always dominant, irrespective of the circumstances. Similarities have been noticed between the effects of organochlorines and high environmental temperature on the bird and on the egg. In the case of the brown pelicans breeding on Anacapa Island, evidence points towards shell thinning being caused by one or more of the following:

(1) a reduction in available carbonate, perhaps by CA inhibition;

(2) reduced transport of calcium from the blood to the shell gland lumen; and

(3) premature termination of shell growth.

In both Britain and North America, eggs with thin shells have led to breeding failure amongst predatory birds. In North America thin shells have often been associated with population declines, but in Britain population declines are thought to have been due to increased adult mortality because of cyclodiene poisoning.

An egg with a thin shell is more liable to be cracked and this may lead to death of the embryo because of bacterial infection or by creating a stimulus for destruction by the brooding parent. An egg with a thin but sound shell might fail to produce a healthy chick because of (a) aberrant behaviour by the parents suffering from sublethal poisoning, resulting in destruction of the egg or chick, or (b) lethal residues mobilised from the yolk lipid.

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NOTE ADDED IN PROOF

Since this paper was submitted for publication, there has been a heated debate on the thin egg shell problem in the journal *Nature*. Switzer *et al.* (1972) (in addition to Hazeltine, 1972; *see* p. 90) have been critical of some of the laboratory and field evidence, while Wiemeyer & Porter (1972), Risebrough (1972) and Blus *et al.* (1972*a*) have answered these criticisms.

Switzer *et al.* (1972) maintained that Wiemeyer & Porter (1970) had not, after all, demonstrated that DDE caused egg shell thinning in the American kestrel. They also claimed that these kestrels did not contain residue levels typical of those found in the field. Both of these objections were answered satisfactorily by Wiemeyer & Porter (1972). Residues in the birds or the eggs in most of the controlled experiments shown in Table 1 of the main text were probably comparable to residues found in some predatory species in Britain or North America (*e.g. see* Ludwig & Tomoff, 1966; Risebrough *et al.*, 1968; Anderson *et al.*, 1969; Fyfe *et al.*, 1969; Keith *et al.*, 1970; Prestt, 1970; Ratcliffe, 1970; Vermeer & Reynolds, 1970; Cade *et al.*, 1971). As pointed out on p. 100, even the high tissue levels induced in quail by Bitman *et al.* (1969) are sometimes encountered in the field. Switzer *et al.* (1972), like Hazeltine (1972), were critical of the misuse of the correlation coefficient by Blus *et al.* (1972) but this does not invalidate Blus' conclusions.

Replying to Hazeltine (1972) and Switzer *et al.* (1972), Blus *et al.* (1972*a*) pointed out that, for their 49 brown pelican eggs collected from Florida, there was, indeed, a highly significant negative relationship between DDE content and shell thickness. Previously, after examining the South Carolina and California data of Blus *et al.* (1972), but for some reason completely ignoring the Florida data, Hazeltine (1972) had stated that significant within-state relationships could not be found.

Switzer *et al.* (1972) believed that comparing museum egg shells with shells collected recently from the field could be misleading, since oologists may have favoured eggs with thicker shells. This, they suggested, may be why pelican eggs collected recently in South Carolina and Florida have thinner shells than museum eggs. However, despite using a sampling technique that introduced bias towards the collection of thicker shells, Schreiber & Risebrough (1972) found that eggs in Florida colonies in 1969 and 1970 had shells 9% thinner on average than those taken prior to 1943 (data of Anderson & Hickey, 1970). Blus *et al.* (1972*a*) also pointed out that two investigations (Ratcliffe, 1967; Hickey & Anderson, 1968) have depended entirely upon the measurement of shells in museums or private collections.

The part-incubated eggs upon which Hazeltine (1972) based much of his attack on Blus *et al.* (1972) were apparently not incubated after all. Blus *et al.* (1972*a*) quoted J. O. Keith as stating that, in each of the eggs Hazeltine believed to be

incubated, the vitelline membrane around the yolk had burst when the fresh egg was deep frozen and the yolk had become contaminated with albumen. In a short amendment Hazeltine (1972*a*) was less explicit about his mistake: 'Recently disclosed errors in the original laboratory reports invalidate the conclusions of the ten-fold lipid and DDE residue decline with incubation'. He then continued that the positive relationship between lipid DDE and shell thickness was still valid, which is true. It is, however, difficult to understand why he then claims that this 'refutes the contention of cause and effect between egg residue and shell thickness'. His data, based on only nine eggs, may be inconsistent with the theory, but they certainly do not refute it. Earlier he had analysed Risebrough's data, based on 65 eggs from Anacapa, and had found a significant negative relationship between lipid DDE and shell thickness (Hazeltine, 1972). He did not comment on this finding, which contradicted his own views, and he also failed to point out that his eggs, which contained on average 1040 ppm DDE in the yolk lipid, had shells on average 34% thinner than pre-1943 Californian shells examined by Anderson & Hickey (1970). Risebrough (1972) and Blus *et al.* (1972*a*) presented additional information which should convince anyone making an objective assessment of the problem that a relationship exists between thinning in brown pelican egg shells and egg DDE content. Blus *et al.* (1971, 1972) did in fact use part-incubated eggs in their studies (*see* p. 90). However, since a correction was made for the loss in weight of egg contents during incubation and since DDE loss as the eggs were incubated was likely to be negligible (Abou-Donia & Menzel, 1968; Guthrie & Donaldson, 1970; Cooke, 1971), the estimated DDE content of the egg must have been close to the actual pre-incubation content.

Switzer *et al.* (1972) have been accused by Wiemeyer & Porter (1972) of choosing 'to ignore obvious evidence' and Risebrough (1972) has made similar criticisms of Hazeltine (1972). Indeed, Risebrough (1972) was sufficiently exasperated to comment, 'One begins to suspect intrigue and conspiracy'. The effect of papers such as Hazeltine's (1972) on public or even informed opinion should not be underestimated. On 18 October 1972, five days after Hazeltine's article was published, it was featured by *The Times* newspaper under the heading 'Pesticides: exploding the DDE myth'. The article began, 'The belief that DDT residues make birds' egg shells dangerously thin is a myth'. In fact none of these papers changes in any way the conclusions listed at the end of the main text (pp. 138 to 140).

Gunn (1972) deduced from the figures of Ratcliffe (1967) that the shell thinning phase was completed for the peregrine by 1947 and for the sparrowhawk by 1948. Since Gunn's researches indicated that the use of DDT was very restricted at this time, he concluded that DDT could not have been responsible for shell thinning. Why he did not examine Ratcliffe's later, more comprehensive, graphs (1970) instead of those given in the 1967 paper is not clear, the later paper being included in Gunn's reference list but not in his text. In fact shell thickness reached a 'typical' low level for the peregrine in 1948 and for the sparrowhawk in 1949 or 1950,

although shells of the latter species were at their thinnest in 1954 and 1955. After the war, 1950 was the first year in which every sparrowhawk clutch collected contained thin shells (Ratcliffe, 1970). Ratcliffe (1970) discussed the early uses of DDT at great length and concluded that, in the peregrine, DDT sprayed or dusted to control ectoparasites on homing pigeons, the peregrine's principal prey south of the Scottish Highlands, could have caused shell thinning as early as 1946. This particular use was not mentioned by Gunn (1972). The early contamination of sparrowhawks is thought to have resulted from horticultural uses of DDT (Ratcliffe, 1970).

There was an intensive survey of the British peregrine population in 1971 (Ratcliffe, 1972). An increase has occurred since 1963, but the population is still well below the pre-war level. The main cause of breeding failure was clutch depletion, thought to be due to deliberate shell breakage by the sublethally poisoned parents rather than to accidental breakage of the thin shells (*see* p. 135).

The Montrose Chemical Corporation, the only manufacturer of DDT in the United States, has made a significant contribution to DDT pollution in Californian coastal waters by discharging effluent *via* the Los Angeles County sewer into the sea (Burnett, 1971). This effluent is believed to be the main source of the residues accumulated by the Anacapa pelicans (Schreiber & Risebrough, 1972).

Several more laboratory studies have been published recently. Foster *et al.* (1972) fed White Leghorn chickens for six weeks on a diet containing 0·1 ppm technical DDT + 0·1 ppm chlordane + 0·1 ppm lindane + 0·5 ppm ethion + 0·5 ppm atrazine + 0·5 ppm linuron. In contrast to the observations of Sauter & Steele (1972; *see* pp. 96 and 97), shell thickness was found to be unaffected.

Screech owls *Otus asio* fed on a diet containing 10 ppm DDE (dry weight) laid eggs with shells 13% thinner than those of control owls (McLane & Hall, 1972). Thus this seems to be one of the most sensitive species yet tested (*see* Table 1).

Methyl mercury injected or given orally to ring doves (10 mg/kg) or fed to American kestrels (100 ppm in diet) had no effect on shell thickness (Peakall & Lincer, 1972). Previously mercury compounds given to gallinaceous species have been found to reduce shell thickness or increase the incidence of soft shelled eggs (Tejning, 1967; Fimreite, 1971; Stoewsand *et al.*, 1971).

Whitehead *et al.* (1972*a*) have extended their γBHC studies (Whitehead *et al.*, 1972). A level of 200 mg/kg in the diet of chickens reduced egg production by 30%, but the shells were again of normal thickness and scanning electron microscopy revealed no structural abnormalities.

The X-ray diffraction study of Gould (1972) demonstrated no obvious differences in the relative amounts of the calcium carbonate polymorphs present in brown pelican shells of different thicknesses. Although there were appreciable amounts of aragonite and vaterite in most of the pelican shells examined, the mineral phase in chicken shells is almost entirely calcite (Cain & Heyn, 1964), showing once again the gulf that exists between chickens and field species.

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