Scale Calcification In A Chrysophycean Alga: A Test System For The Effects of DDT On Biological Calcification[1]

J. H. Elder[2], C. A. Lembi, L. Anderson and D. J. Morre'

Department of Botany and Plant Pathology

Purdue University, Lafayette, Indiana 47907

Abstract

The chrysophycean alga, *Coccolithus huxleyi*, responds to the chlorinated hydrocarbon, DDT, by reduced calcification of surface scales. Cytological and biochemical findings show reduced calcium carbonate deposition on the scales and reduced calcium utilization by the organism.

Chlorinated hydrocarbons [particularly certain types of pesticides such as dieldrin, DDT, DDE (a stable metabolite of DDT) and the polychlorinated biphenyls (PCB's) used as plasticizers][3] at high levels in the diet, result in changes in calcium metabolism which may lead to eggshell thinning and reproductive failure in predators and raptorial birds near the top of the food chain (2, 5-13, 15, 16, 17, 19-21, 24). The mechanism by which these compounds reduce calcium carbonate deposition has not been established. One possibility is that carbonic anhydrase is inhibited (1, 16). This enzyme catalyzes the conversion of carbon dioxide to bicarbonate which then reacts with calcium to form calcium carbonate. Another possibility, frequently mentioned, is hormonal imbalance (1, 10-13, 15-17). Estrogen, for example influences calcium mobilization to the shell gland during eggshell formation. Because of the hormonal complexity of the avian calcification system, simpler systems were sought which might serve as models for the study of DDT effects on eggshell calcification.

The marine alga, *Coccolithus huxleyi*, secretes cell wall scales, certain of which are layered with calcium carbonate (14). The scales are produced within cisternae of the Golgi apparatus and transported to the cell surface via secretory vesicles (14). Calcification occurs during secretion. The purpose of this study was to determine whether DDT influenced calcification in the algal system.

Materials and Methods

Cultures of *Coccolithus huxleyi* (Lohm.) Kamptner were obtained from the Indiana University culture collection. Cells were grown on

---

[1]Supported in part by EP 00872-01 and GB 23183. Journal Paper Number 4603. Purdue University Agricultural Experiment Station.

[2]Recipient of a summer research fellowship sponsored by an award from the Research Grants Committee of the Indiana Academy of Science.

[3]DDT - 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane
DDE - 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl) ethane
Dieldrin - 1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-endo-5,8-dimethanonapthalene.

Erdschreiber Enrichment Medium (18), with and without technical grade DDT in a saturated solution, using acetone at a final concentration of 1% as a carrier. Control cells were grown in the presence of 1% acetone. Cultures were maintained under 100 ft-c at 23° C with a 16-hour photoperiod.

Cells were prepared for electron microscopy by fixation in 2% glutaraldehyde in 0.1 M potassum phosphate, pH 7.2, for 1 hour, post-fixation in 1% osmium tetroxide in 0.1 M potassium phosphate, pH 7.2, for 1 hour and dehydration through an acetone series. The tissue was embedded in Epon (23), sectioned and then observed with a Philips EM 300.

Carbonic anhydrase assays followed the procedure of Roughton and Booth (22). Calcium utilization was determined by adding 100 $\mu C^{45}Ca$ to 3 ml aliquots of cell suspensions grown in the presence and absence of DDT. After 2 hours, the cells were transferred to a solution containing unlabeled calcium for 30 min to remove exchangably bound

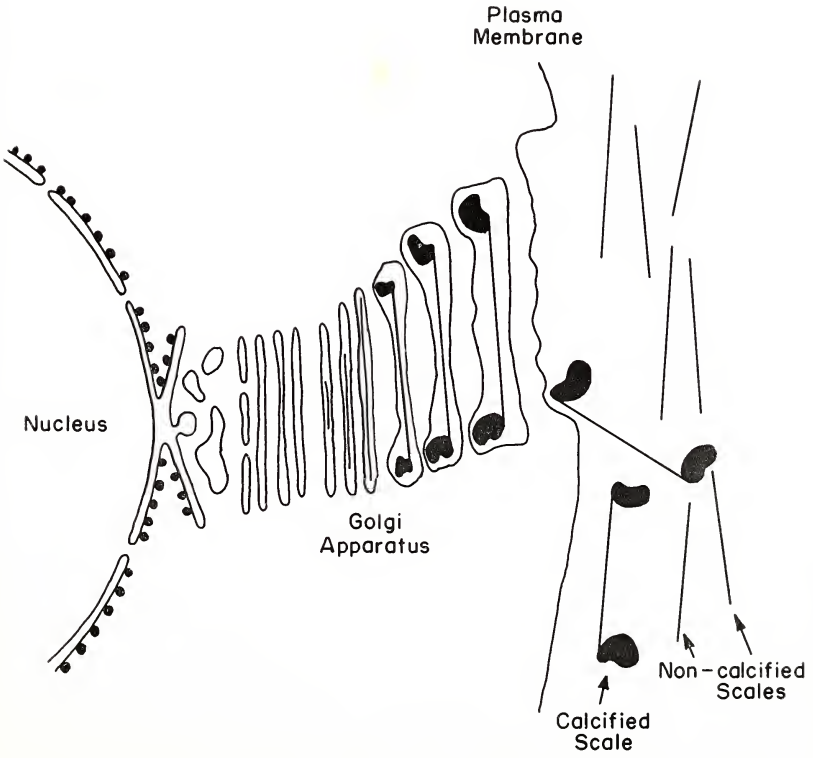

FIGURE 1. *Schematic diagram illustrating the process of scale formation in a chrysophycean alga. The scales are formed within cisternae of the Golgi apparatus. The cisternae of the Golgi apparatus separate from the stack and the cisternal membranes fuse with the plasma membrane during discharge of individual scales. Calcification occurs at the scale margin (solid black projections) prior to discharge of the scale to the cell surface. Adapted from Brown* et al. (4).

radioactivity. Radioactivity was measured with a Nuclear Chicago gas-flow monitoring system.

Results and Discussion

Coccolithus huxleyi secretes cellulosic scales into the cell wall (14). As with other Chrysophycean algae (3, 4), these scales are formed in the cisternae of the Golgi apparatus and are transported to the plasma membrane in secretory vesicles (14, Fig. 1). The scales are of two types that are secreted in alternating waves. Type I scales are small and non-calcified (Fig. 2A). Type II scales are larger and have a mineralized rim around their edges (Fig. 2B). Calcification takes place while the Type II scales are en route to the cell wall in the secretory vesicles.

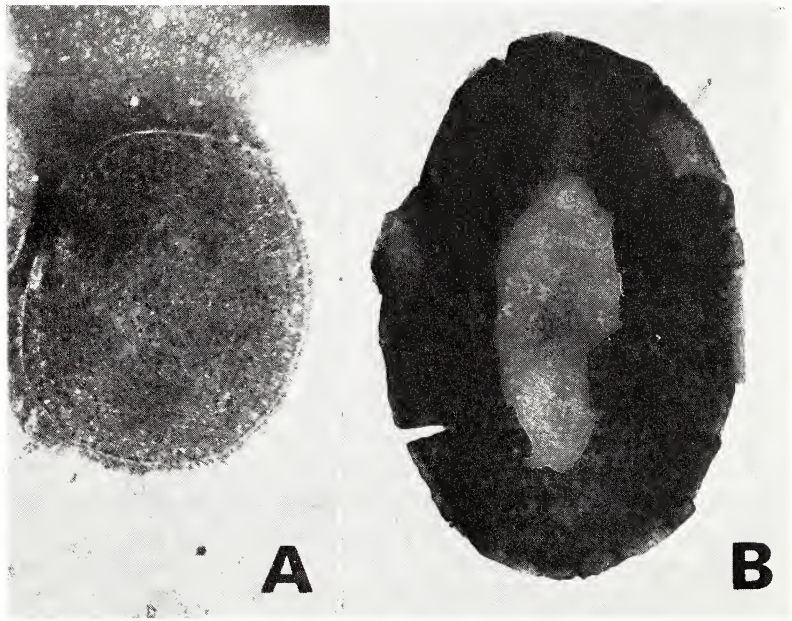

FIGURE 2. *Electron micrographs of isolated scales of* Coccolithus huxleyi *after negative staining with 1% potassium phosphotungstate, pH. 6.5* A. *Noncalcified (Type I) scale.* B. *Calcified (Type II) scale. The calcified rim appears in B as a wide electron dense margin at the periphery of the scale. X 33,000.*

A typical control cell is shown in Figure 3. The alternating waves of Type I and Type II scales are apparent within the cell. Where calcification is heavy on the Type II scales (scales normally calcified), the mineralized deposit is lost during specimen preparation and only a hole is left. Loss of the deposits from the section is a criterion for heavy calcification that was previously reported by Manton and Leedale (14).

When cells were grown in the presence of DDT, calcification in the wave of Type II scales being formed was markedly reduced (Fig. 4),

whereas the previous wave of Type II scales was normally calcified. Figure 5 shows that the scale and the matrix upon which the

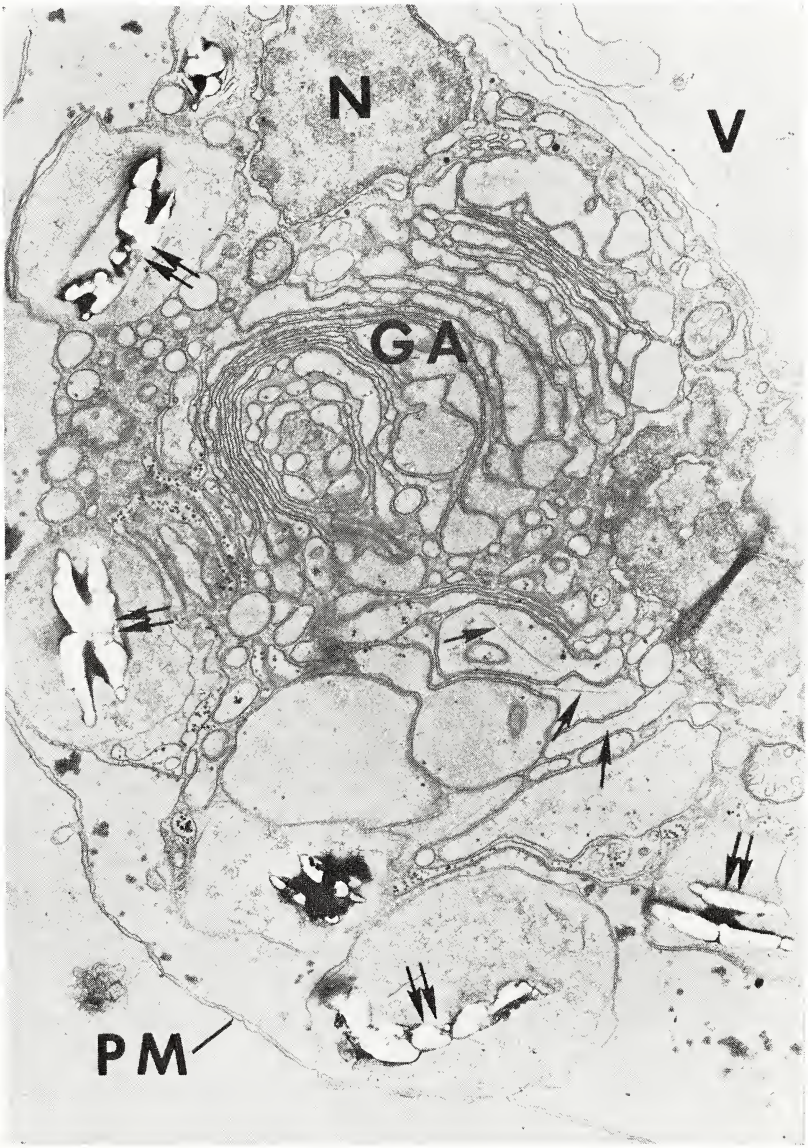

FIGURE 3. *Thin section of a control cell of* Coccolithus huxleyi. *Glutaraldehyde-osmium tetroxide fixation. Section post-stained with lead citrate. The outer wave of Type II scales (double arrows) emanating from the Golgi apparatus (GA) is heavily calcified as evidenced by the holes (double arrows) where the heavily mineralized deposits have dropped out during specimen preparation. The inner wave is of the Type I, non-calcified scales (single arrows). PM = plasma membrane. V = vacuole. N = nucleus. X 26,000.*

calcium carbonate is layered are formed normally in the DDT-treated cells, but the amount of calcium carbonate deposited is less than that for control cells.

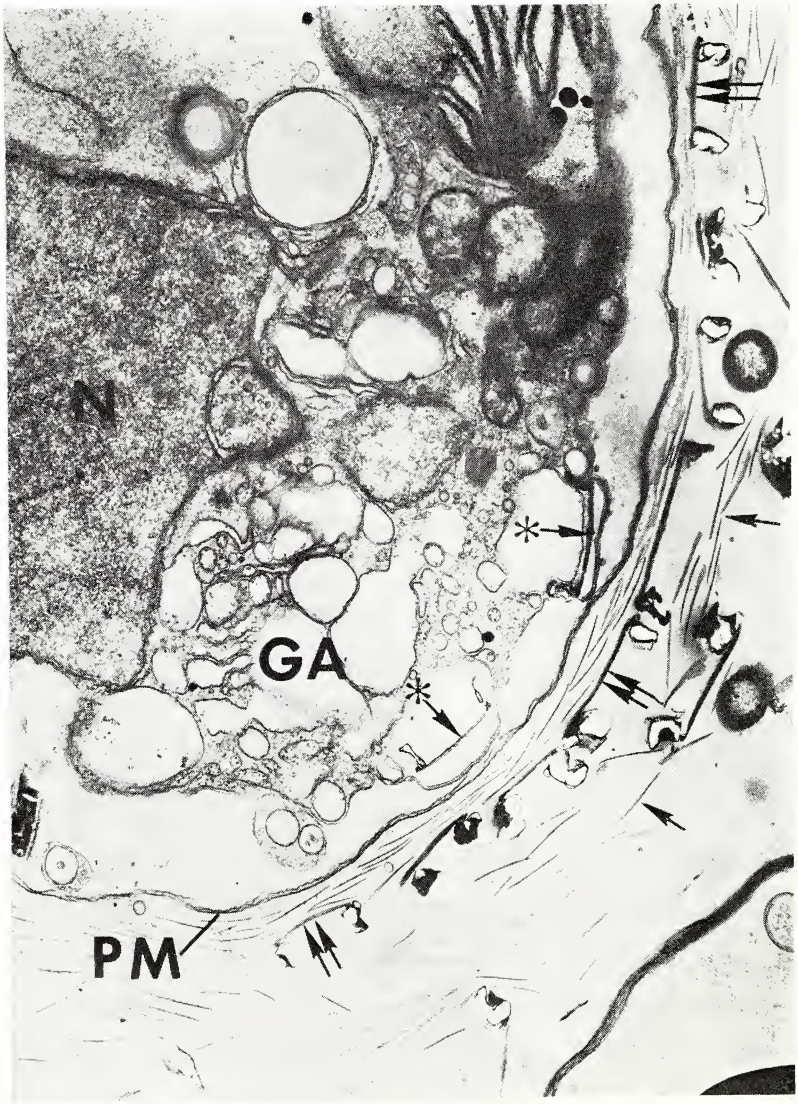

FIGURE 4. *Thin section of a DDT-grown cell of* Coccolithus huxleyi *prepared for electron microscopy as in Figure 3. The outer layer of calcified, Type II scales (double arrows) shows evidence of reduced calcification (compare with Figure 3), but the reduction in calcification is most marked in the inner wave of Type II scales (those still associated with Golgi apparatus cisternae within the cytoplasm; arrows with asterisks). Noncalcified, Type I scales (single arrows) appear normal. PM = plasma membrane. N = nucleus. X 16,000.*

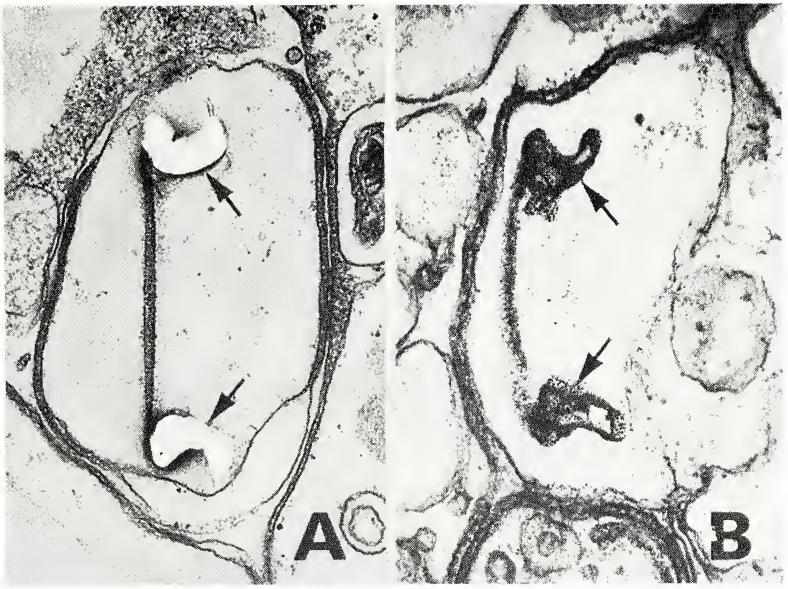

FIGURE 5. *Comparison of scales from control (A) and DDT-treated (B) cells of* Coccolithus huxleyi *as seen in thin section. The scale from the DDT-treated cell (B) has developed normally but calcification is reduced (arrows) as evidenced by the absence of the hole-forming mineralized deposits (see Fig. 3 and text) characteristic of control scales (A; arrows). X 40,000.*

Further evidence for a DDT effect on algal calcification was sought from determinations of carbonic anhydrase activity. Results summarized in Table 1 show inhibition of carbonic anhydrase from the alga by DDT, although the effect was much less dramatic than expected. The levels of DDT required to inhibit carbonic anhydrase seem high but concentrations of 2,500 ppm of DDT have been reported from thin-shelled eggs of fish-eating birds (16). In experiments with ^{45}Ca, calcium utilization was inhibited by 10 to 15% in the DDT-grown cells.

TABLE 1. *Effect of DDT on carbonic anhydrase from* Coccolithus huxleyi[1].

Extract From	DDT in Assay	Carbonic Anhydrase (Relative Specific Activity)
Experiment I		
Control Cells	None	0.45
	1000 μg	0.36
Experiment II		
Control Cells	None	0.22
DDT-Grown Cells	None	0.12

[1]Based on time required to lower the pH of veronal buffer from pH 8.15 to pH 6.30 (22).

The results show that DDT retards the calcification of scales in the alga *C. huxleyi* and that the organism merits further study as a test

system for DDT effects on biological calcification. The alga offers the advantage of a reproducible and inexpensive laboratory system that is free from the complicating hormonal influences found in birds.

Literature Cited

1. BITMAN, J., H. C. CECIL and G. F. FRIES. 1970. DDT-induced inhibition of avian shell gland carbonic anhydrase: A mechanism for thin eggshells. Science **168**:594-596.

2. BITMAN, J., H. C. CECIL, S. J. HARRIS, and G. F. FRIES. 1969. DDT induces a decrease in eggshell calcium. Nature **224**:44-46.

3. BROWN, R. M., W. W. FRANKE, H. KLEINIG, H. FALK, and P. SITTE. 1969. Cellulosic wall component produced by the Golgi apparatus of *Pleurochrysis Scherffelii*. Science **166**:894-896.

4. _____. 1970. Scale formation in Chrysopycean algae. J. Cell Biol. 45:246-271.

5. COOKE, A. S. 1970. The effect of o,p-DDT on Japanese quail. Bull. Environ. Cont. Toxicol. **5**:152-157.

6. HEATH, R. G., J. W. SPANN, and J. F. KREITZER. 1969. Marked DDE impairment of mallard reproduction in controlled studies. Nature **224**:47-48.

7. HICKEY, J. J. 1969. Peregrine falcon populations: Their biology and decline. Univ. Wis. Press, Madison, Wis. 567 p.

8. _____, and D. W. ANDERSON. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science **162**:271-273.

9. HILTON, B. D., and R. D. O'BRIEN. 1970. Antagonism by DDT of the effect of valinomycin on a synthetic membrane. Science **168**:841-843.

10. JEFFRIES, D. J. 1967. The delay in ovulation produced by p,p'-DDT and its possible significance in the field. Nature **109**:266-272.

11. _____. 1969. Induction of apparent hyperthroidism in birds fed DDT. Nature **224**:578-579.

12. _____, and M. C. FRENCH. 1969. Avian thyroid: Effect of o,p'-DDT on size and activity. Science **166**:1278-1280.

13. KAGAN, Y S., S. I. FUDEL-OSSIPOVA, B. J. KHAIKINA, U. A. KUZMINSKAYA, and S. S. KOUTON. 1969. On the problem of the harmful effect of DDT and its mechanism of action. Residue Rev. **27**:43-138.

14. MANTON, I., and G. F. LEEDALE. 1969. Observations on the microanatomy of *Coccolithus pelagicus* and *Criscosphaera carterae*, wiith special reference to the origin and nature of coccoliths and scales. J. Marine Biol. Assn. U. K. **49**:1-16.

15. PEAKALL, D. B. 1967. Pesticide-induced enzyme breakdown of steroids in birds. Nature **216**:505-506.

16. _____. 1970. Pesticides and the reproduction of birds. Sci. Amer. **222**:73-78.

17. _____. 1970. pp'-DDT. Effect on calcium metabolism and concentration of estradiol in the blood. Science **168**:592-594.

18. PROVASOLI, L. 1968. Media and prospects for the cultivation of marine algae. p. 63-75. *In* A. Watanabe and A. Hattori (eds.). Cultures and collections of Algae. Proc. U.S.-Japan Conf. September 1966. Jap. Soc. Plant Physiol. Nakone, Japan.

19. RATCLIFFE, D. A. 1967. Decrease in eggshell weight in certain birds of prey. Nature **215**:208-210.

20. _____. 1970. Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. J. Appl. Ecol. 7:67-116.

21. RISEBROUGH, R. W., P. RIECHE, S. G. HERMAN, D. B. PEAKALL, and M. N. KIRVEN. 1968. Polychlorinated biphenyls in the global ecosystem. Nature 220: 1098-1102.

22. ROUGHTON, F. J. W., and V. H. BOOTH. 1946. The effect of substrate concentration, pH and other factors upon the activity of carbonic anhydrase. Biochem. J. 40:319-330.

23. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

24. WIEMEYER, S. N., and N. G. ANDERSON. 1970. DDE thins eggshells of captive American kestrels. Nature 227:737-738.