DPH decreased the duodenal absorption of the test dose of ^{47}Ca and duodenal CaBP to near zero (comparable to that in rickets) in a dose-related fashion. A depression in calcium binding activity, which reflects CaBP, was also noted.

Chicks on the higher dosage of D_3 (groups 5, 6, and 7) were affected by DPH but less so than those receiving 3 I.U. of D_3 per day. The ingestion of 2.5 g of DPH per kilogram of diet (group 7) resulted in a significant reduction in growth ($P < .03$), serum calcium ($P < .01$), and tibia ash ($P < .01$) (Table 1). Little or no effect of the lower level of DPH on these parameters was noted. Similarly, group 7 (6 I.U. of D_3; 2.5 g of DPH per kilogram of diet) displayed a reduction in ^{47}Ca absorption (Fig. 2A), vitamin D–induced CaBP (Fig. 2B), and calcium binding activity (Fig. 2C). At the lower intake of DPH (1 g per kilogram of diet), the only significant effect observed was a reduction in CaBP (Fig. 2).

Group 7 chicks maintained calcium absorption and CaBP levels equal to those of group 2, but were hypocalcemic. Bone analysis showed no significant difference between group 2 and group 7 ($P = .25$) (Table 1 and Fig. 2). Hypocalcemia without bone involvement has been noted clinically (*1*); no explanation is apparent at the present time.

Our data showing a depression in CaBP by DPH treatment is in disagreement with the reports of Koch *et al.* (*9*) and Caspary (*10*). These investigators assessed CaBP by a nonspecific calcium binding assay, using the ion exchange resin technique (*14*), which measures total calcium binding activity and not that due exclusively to CaBP. In our study, both the nonspecific ion exchange resin procedure and the specific radioimmunoassay indicated a depression in intestinal CaBP (Fig. 2). Since Koch *et al.* (*9*) and Caspary (*10*) used rats, there is a possibility of a species difference in responsiveness to DPH.

It is apparent that, in chicks, DPH administration causes readily demonstrable effects on calcium metabolism which provides support for clinical observations on epileptic patients given anticonvulsant therapy. The effects were dose dependent and related inversely to the amount of calciferol given. The fact that the vitamin D–induced CaBP also changed in proportion to the change in calcium absorption provides further support for the contention that the DPH-dependent defect is related in some way to the abnormal metabolism of or responsiveness to D_3. There is no evidence for a direct effect of DPH on the calcium absorption mechanisms per se, as thought to pertain to cortisone treatment (*19*). Our findings support suggestions that close attention should be paid to calciferol intake in patients requiring seizure therapy. They also indicate that the chick may be a useful model for testing drugs suspected of being calciferol antagonists.

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References and Notes

1. F. Schmid, *Fortschr. Med.* **9**, 381 (1967); R. Kruse, *Monatsschr. Kinderheilkd.* **116**, 378 (1968); A Richens and D. J. F. Rowe, *Br. Med. J.* **1970-IV**, 73 (1970); C. F. Dent, A. Richens, D. J. F. Rowe, T. C. B. Stamp, *ibid.*, p. 69; J. Hunter, J. D. Maxwell, D. A. Stewart, V. Parsons, R. Williams, *ibid.* **1971-IV**, 202 (1971); E. A. Sotaniemi, H. K. Hakkarainen, J. A. Puranen, R. O. Lahti, *Ann. Intern. Med.* **77**, 389 (1972); A. D. Borgstedt, M. F. Bryson, L. W. Young, G. B. Forbes, *J. Pediatr.* **81**, 9 (1972).
2. G. Ponchon and H. F. DeLuca, *J. Clin. Invest.* **48**, 1273 (1969); G. Ponchon, A. L. Kennan, H. F. DeLuca, *ibid.*, p. 2032; D. R. Fraser, and E. Kodicek, *Nature (Lond.)* **228**, 764 (1970); R. Gray, I. Boyle, H. F. DeLuca, *Science* **172**, 1232 (1971); A. W. Norman, R. J. Midgett, J. F. Myrtle, H. G. Nowicki, *Biochem. Biophys. Res. Commun.* **42**, 1082 (1971).
3. T. J. Hahn, B. A. Hendin, C. R. Scharp, J. G. Haddad, *N. Engl. Med. J.* **287**, 900 (1972).
4. T. J. Hahn, S. J. Birge, C. R. Scharp, L. V. Avioli, *J. Clin. Invest.* **51**, 741 (1972).
5. K. G. Tolman, W. Jubiz, H. F. DeLuca, J. W. Freston, *Clin. Res.* **20**, 414 (1972).
6. K. Schaefer, W. H. Flury, D. von Herrath, D. Kraft, R. Schweisgruber, *Schweiz. Med. Wochenschr.* **102**, 785 (1972).
7. J. Silver, G. Neale, D. Davies, A. Breckenbridge, G. R. Thompson, *Clin. Sci.* **42**, 12 (1972).
8. T. C. B. Stamp, J. M. Round, D. J. F. Rowe, J. G. Haddad, *Br. Med. J.* **1972-IV**, 9 (1972).
9. H. U. Koch, D. Kraft, D. von Herrath, K. Schaeffer, *Epilepsia* **13**, 509 (1972).
10. W. F. Caspary, *Arch. Pharmacol.* **274**, 146 (1972).
11. R. H. Wasserman and A. N. Taylor, *Science* **152**, 791 (1966); R. H. Wasserman, R. A. Corradino, A. N. Taylor, *J. Biol. Chem.* **243**, 3978 (1968); R. H. Wasserman and R. A. Corradino, *Annu. Rev. Biochem.* **40**, 501 (1971).
12. R. L. Clark, J. P. Kuhn, C. A. DuJovne, *Invest. Radiol.* **6**, 152 (1971).
13. R. A. Corradino and R. H. Wasserman, *Proc. Soc. Exp. Biol. Med.* **133**, 960 (1970); R. A. Corradino, J. G. Ebel, P. H. Craig, A. N. Taylor, R. H. Wasserman, *Calcif. Tissue Res.* **7**, 81 (1971).
14. R. H. Wasserman and A. N. Taylor, *Science* **152**, 791 (1966); R. H. Wasserman, R. A. Corradino, A. N. Taylor, *J. Biol. Chem.* **243**, 3978 (1968).
15. R. A. Corradino and R. H. Wasserman, *Science* **172**, 731 (1971).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, P. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
17. I. M. Bett and G. P. Fraser, *Biochem. J.* **68**, 13 (1958).
18. We thank Dr. Gerald E. Simon, Department of Clinical Pathology, Upstate Medical Center, who provided diphenylhydantoin analyses.
19. D. V. Kimberg, R. D. Baerg, E. Gershon, R. T. Graudusius, *J. Clin. Invest.* **50**, 1309 (1971).
20. Supported in part by NIH grant AM-04652.
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DDE: Its Presence in Peregrine Eggs in 1948

Abstract. *DDE has been eluted from the dried membranes of peregrine eggshells collected in California from 1948 to 1950, and identified by gas-liquid chromatography.*

The question of the involvement of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in the sudden decrease of eggshell thickness in many species of predatory birds starting around 1947 has been hotly debated (*1–4*). Ratcliffe (*1*) marshalled the evidence to show that enough DDT could have been present by 1947 to cause eggshell thinning, while Gunn (*4*) came to the opposite conclusion. It appears that the information available on the use of DDT at that time is not adequate to support a definite conclusion. Gunn (*4*) concluded by saying that "an effect occurring before its cause is utterly unacceptable." It is certainly correct that the decrease in eggshell thickness was abrupt and occurred very soon after the introduction of DDT as an insecticide in 1945. In Great Britain the annual mean shell thickness for the peregrine (*Falco peregrinus*), calculated from the data of Ratcliffe (*1*), decreased in 1946 and was significantly lower by 1947. Hickey and Anderson (*5*) found that peregrine eggshells from California were markedly lighter in weight from 1947 on. In recent years it has been possible to demonstrate eggshell thinning caused by DDT and its principal metabolite DDE [1,1-dichloro-

2,2-bis(*p*-chlorophenyl)ethylene] in many, but not all, species (*6*). A nonlinear relation between eggshell thickness and DDE residues has been demonstrated for the peregrine (*1*, *7*) and the brown pelican (*Pelicanus occidentalis*) (*2*, *8*). In both species the eggshell thickness initially decreased rapidly as DDE concentration increased, and this was followed by a more gradual decrease. Thus, a small amount of DDE is capable of exerting a marked effect on eggshell thickness. Since the contents of peregrine eggs are not available for the years before 1963 (*1*), direct proof of the involvement of DDT in the late 1940's has been lacking. The presence of DDE in the dried membranes of five eggshells collected in California from 1948 to 1950 is reported here.

A mixture of diethyl ether and petroleum ether (1 : 3 by volume) was injected into the egg through the hole made by the egg collector until the egg was filled with solvent. The egg and solvent were maintained at 45°C for 4 hours, more solvent being added as necessary. At the end of this period the solvent was removed from the egg with the same all-glass syringe, and and amounts of organochlorines were measured by gas-liquid chromatography (Fig. 1). Half of the solvent was evaporated to dryness at 45°C and the amount of extractable fat was determined by weighing the material that remained on a microbalance with a precision of 10^{-5} g. This extractable fat would consist of material dissolved from the membrane plus any yolk lipids that may have remained after the egg-blowing procedure. By this means it is possible to express the results in terms of parts per million on a lipid weight basis.

Both the dried eggshells and the pesticide residue values of the egg contents on a lipid weight basis are available for a series of peregrine eggs collected in Alaska in 1968; the data on eggshell thickness and DDE content have been published (*7*). By measuring the amount of DDE in the membranes of these eggshells, I was able to calculate the ratio of DDE in the egg contents to DDE in the membranes (see Table 1). Since the percentage of lipid in peregrine eggs is known (*9*) it is possible to calculate the original amount of DDE in the egg contents on a wet weight basis (Table 1) and thus compare the residue levels with shell thickness, as has been done for more recent specimens. The calculated values fit the relationship found by Cade *et al.* (*7*) for Alaskan eggs collected from 1967 to 1970. Thus, at least as early as 1948, DDE was present in peregrine eggs in sufficient concentration to account for eggshell thinning.

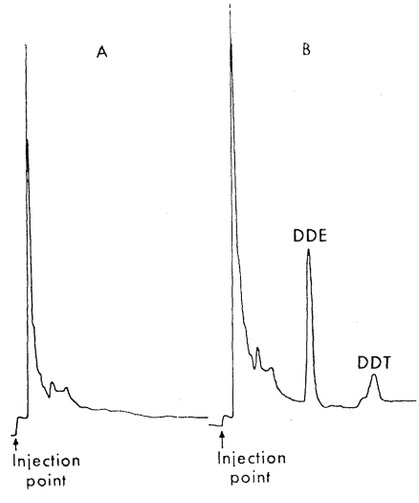

Fig. 1. Gas-liquid chromatograms of eluants from (A) 1935 and (B) 1948 peregrine eggshells. The liquid phase and solid support were 1 percent QF-1 on (40/50) Anakrom ABS. Samples were evaporated to 10 ml and injected without cleanup into a Varian Aerograph gas chromatograph equipped with ^{63}Ni electron capture detectors. The operating temperatures were: column, 206°C; inlet, 230°C; detector, 285°C. The carrier gas was filtered nitrogen. Confirmation was carried out under the same conditions with 1 percent XE-60 on (60/80) Chromosorb W.

Table. 1. Weight, thickness index, and extractable fat of peregrine eggshells and DDE residues in membranes and contents. The thickness index is the weight (milligrams) divided by the length times breadth (millimeters) (*1*). The weight and thickness index for 18 Alaskan eggs are from Cade *et al.* (*7*), who also determined DDE in the egg contents. From their values and the amount of DDE in the membranes of the Alaskan eggshells, I find that the ratio of DDE in the egg contents to DDE in the membranes is 1.83 to 5.66; the mean is 3.56. From this mean ratio for the Alaskan eggs and the ratio 1 : 24 for lipid to wet weight (*9*), I estimated the DDE in the contents of the California eggs. The eggs collected in 1948 to 1950 also contained DDT, approximately 10 percent as much as the DDE. Abbreviations: N.D., not detected; ppm, parts per million. The numbers in parentheses are mean values.

Year	Collection area	Weight (g)	Thickness index	Fat (mg)	DDE in dried membrane		DDE in egg contents (ppm, wet weight)
					μg	ppm lipid basis	
		California eggs					
1894	San Diego Co.	4.137	1.88	0.9	N.D.		
1924	San Luis Obispo Co.	4.062	1.98	1.1	N.D.		
1935	San Diego Co.	5.120	2.07	1.4	N.D.		
1940	San Luis Obispo Co.	4.454	2.10	1.3	N.D.		
1948	San Luis Obispo Co.	3.726	1.66	1.0	0.050	50	7
1948	San Luis Obispo Co.	3.964	1.87	1.8	0.040	22	3
1949	San Diego Co.	3.188	1.42	0.8	0.050	62	9
1950	San Diego Co.	3.213	1.46	1.6	0.320	200	30
1950	Monterey Co.	3.854	1.64	1.8	0.045	25	4
		Alaskan eggs					
1968	Yukon and Colville	2.595–3.820 (3.123)	1.32–1.64 (1.46)	0.9–2.8 (1.7)		41–1005 (225)	

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References and Notes

1. D. A. Ratcliffe, *J. Appl. Ecol.* **7**, 67 (1970).
2. L. J. Blus, C. D. Gish, A. A. Belisle, R. M. Prouty, *Nature* **235**, 376 (1972).
3. W. Hazeltine, *ibid.* **239**, 410 (1972); B. C. Switzer, F. H. Wolfe, V. Lewin, *ibid.* **240**, 162 (1972). Rebuttal letters: S. N. Wiemeyer and R. D. Porter, *ibid.*, p. 163; R. W. Risebrough, *ibid.*, p. 164; L. J. Blus, C. D. Gish, A. A. Belisle, R. M. Prouty, *ibid.*, p. 164.
4. D. L. Gunn, *Ann. Appl. Biol.* **72**, 105 (1972).
5. J. J. Hickey and D. W. Anderson, *Science* **162**, 271 (1968); D. W. Anderson and J. J. Hickey, in *Proceedings of the 15th International Ornithological Congress*, K. H. Voous, Ed. (Brill, Leiden, 1972), vol. 1, p. 514.
6. Reviewed → A. S. Cooke, *Environ. Pollut.* **4**, 85 (1973).
7. T. J. Cade, J. L. Lincer, C. M. White, D. G. Roseneau, L. G. Swartz, *Science* **172**, 955 (1971).
8. R. W. Risebrough, paper presented at the Sixth Berkeley Symposium on Mathematical Statistics and Probability, Berkeley, California, July 1971.
9. T. J. Cade, C. M. White, J. R. Haugh, *Condor* **70**, 170 (1968).

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