

Chlorinated Hydrocarbons and Eggshell Changes in Raptorial and Fish-Eating Birds

Abstract. Catastrophic declines of three raptorial species in the United States have been accompanied by decreases in eggshell thickness that began in 1947, have amounted to 19 percent or more, and were identical to phenomena reported in Britain. In 1967, shell thickness in herring gull eggs from five states decreased with increases in chlorinated hydrocarbon residues.

New perspectives on the role of chlorinated hydrocarbon insecticides in our environment have come into focus in recent years. Successive discoveries have demonstrated that these compounds are systematically concentrated in the upper trophic layers of animal pyramids (1). Raptorial bird populations have simultaneously suffered severe population crashes in the United States and Western Europe (2, 3, 4). These involve reproductive failures which, at least in Britain, are characterized by changes in calcium metabolism and by a decrease in eggshell thickness resulting in the parent birds' breaking and eating their own eggs (4, 5, 6). Such a derangement of calcium metabolism or mobilization perhaps could result from breakdown of steroids by hepatic microsomal enzymes induced by exposure to low dietary levels of chlorinated hydrocarbons (7).

We have examined the possibility that the eggshell changes reported in Britain (6) have also occurred in the United States and that the raptor population crashes in Europe and North America may have had a common physiological mechanism. The population changes are without parallel in the recent history of bird populations (8). They include the pending extirpation of the peregrine falcon (*Falco peregrinus*) in northwestern Europe, the complete extirpation of the nesting population of this species in the eastern half of the United States, and simultaneous declines among other bird- and fish-eating raptors on both sides of the Atlantic.

We examined 1729 blown eggs in 39 museum and private collections. Shells were weighed to the nearest hundredth of a gram. In 29 percent of these, we were able to insert a micrometer through the hole drilled by the collector at the girth of the shell and to take four measurements of thickness 7 mm from the edge of the blow hole; these were then averaged to the nearest 0.01 mm for each shell. Thickness in each case then represented the shell itself plus the dried egg membranes. Peregrine falcons, bald eagles (*Haliaeetus leucocephalus*), and ospreys (*Pandion haliaetus*) were selected as having one or more regionally

declining populations; golden eagles (*Aquila chrysaetos*), red-tailed hawks (*Buteo jamaicensis*), and great horned owls (*Bubo virginianus*) were selected as representative of reasonably stationary populations that may be slowly declining as their habitats are gradually destroyed by man, but for which widespread reproductive failures are currently unknown. In addition, 57 eggs of the herring gull (*Larus argentatus*) were collected from five colonies in 1967. The shells of these were dried at room temperature for 4 months before being measured, and residues of the entire egg contents were analyzed by the Wisconsin Alumni Research Foundation for chlorinated hydrocarbons but not for polychlorinated biphenyls. Analytical procedure followed that outlined by the U.S. Food and Drug Administration

(9). Analyses were conducted on a gas chromatograph (Barber Coleman, model GC 5000, and Jarrell-Ash, model 28-700) with electron-capture detectors. The glass column (0.6 cm by 1.2 m) was packed with 5 percent DC 200 (12,500) on Chromport XXX. The column temperature was 210°C, and the nitrogen flow rate was 75 cm³/min. Each portion of the ground and dried samples was extracted for 8 hours or more in a Soxhlet apparatus with a mixture of ether and petroleum ether (70:170). Portions of the extracts were further purified by putting them through a Florisil column.

In California, where the peregrine falcon population is in "a serious condition" (10), a change of 18.8 percent in shell weight occurred from 1947 to 1952. Ratcliffe (6) found a corresponding decrease of 18.9 percent in Britain. The change in California involved a decrease in shell thickness and had no precedent in the previous 57-year recorded history of the peregrine in that state (Fig. 1). In the eastern United States, where the nesting population of peregrines has now been wiped out (3), fragmentary data indicate that the same change took place (Table 1). Broken

Table 1. Weights of raptor eggshells in museum and private collections. Citations (23-25) refer to the data for the population trend; S.E., standard error of the mean.

Region	Period	No.	Weight (g)		Population trend (or reproduction)
			Mean $\pm$ S.E.	Change (%)	
<i>Red-tailed hawk</i>					
Calif. (23)	1885-1937	386	6.32 $\pm$ 0.032		
	1943-44	6	6.09 $\pm$ 0.237	- 3.6	Stationary
	1953-67	8	6.49 $\pm$ 0.214	+ 2.7	Stationary
<i>Golden eagle</i>					
Calif. (23)	1889-1939	278	13.03 $\pm$ 0.083		
	1940-46	28	12.70 $\pm$ 0.161	- 2.5	Stationary
	1947-65	33	13.41 $\pm$ 0.232	+ 2.9	Stationary
<i>Bald eagle (24a)</i>					
Brevard Co., Fla.	1886-1939	56	12.15 $\pm$ 0.127		
	1947-62	12	9.96 $\pm$ 0.280	- 18.0	Declining
Osceola Co., Fla.	1901-44	25	12.32 $\pm$ 0.240		
	1959-62	8	9.88 $\pm$ 0.140	- 19.8	Declining
<i>Osprey (24b)</i>					
Md.-Va.	1890-1938	152	7.05 $\pm$ 0.054		
	1940-46	21	6.91 $\pm$ 0.164	- 2.0	Stationary
	1955	3	6.85	- 2.8	Stationary
N.J.	1880-1938	117	7.08 $\pm$ 0.069		
	1957	6	5.30 $\pm$ 0.446	- 25.1	Declining
<i>Peregrine (25)</i>					
B.C.	1915-37	19	4.24 $\pm$ 0.061		
	1947-53	15	4.18 $\pm$ 0.081	- 1.4	Stationary
Calif. (23)	1895-1939	235	4.20 $\pm$ 0.031		
	1940-46	49	4.07 $\pm$ 0.038	- 3.1	No data
	1947-52	31	3.41 $\pm$ 0.084	- 18.8	Declining
N.H. to N.J.*	1888-1932	56	4.38 $\pm$ 0.034		
	Vt.	3	4.30	- 1.8	Stationary
	Mam.	3	3.47	- 20.8	Extirpated
	N.J.	3	3.24	- 26.0	Extirpated
<i>Great horned owl</i>					
Calif. (23)	1886-1936	154	4.50 $\pm$ 0.033		
	1948-50	12	4.62 $\pm$ 0.119	+ 2.4	Stationary

* Including Vermont and Massachusetts.

cyrie were observed for the first time in 1947 by J. A. Hagar 60 miles (9.6 km) from the Massachusetts eyrie cited in this table (11). They were next inferred in Quebec in 1948 when egg-eating was observed at the same site in 1949 (12), and were observed in Pennsylvania in 1949 and 1950 (13). Chlorinated hydrocarbon data for this now-extinct regional population are completely absent. For nine surviving adult peregrines in Canada's Northwest Territories in 1966, the data are reported to have averaged 369 parts per million (ppm) (fresh weight) in fat (14). For four adults in another migratory population in northern Alaska, values were even higher (15).

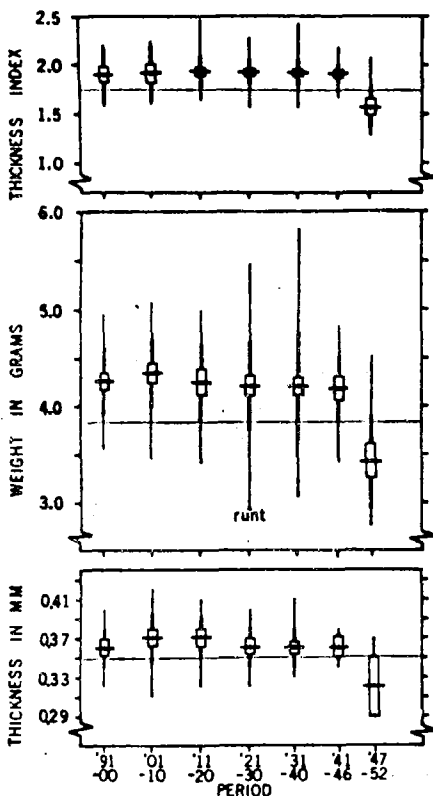


Fig. 1. Measurements of 614 California peregrine eggshells collected since 1891. The dotted horizontal line is the midpoint between the 95 percent confidence limits for 1947-52 and the lowest of any preceding group. Solid horizontal bars are means; rectangles, 95 percent confidence limits; heavy vertical lines, standard deviations; narrow vertical lines, range in sample. The thickness index, calculated as ten times the weight divided by the product of the length and breadth (in mm) of each egg, was devised by Ratcliffe (6) for the study of museum eggs and appears here to be a meaningful statistic. The sizes of samples for measurement of weight and of thickness index for the periods were 71, 49, 36, 85, 155, 30, and 31, respectively; and the sizes of the samples for measurement of thickness were 24, 31, 29, 23, 37, 7, and 6.

have studied, the data do not permit a precise delineation of the onset of the change in calcium metabolism or mobilization, but the decrease (Table 1) in shell weight (and hence thickness) has involved only declining populations and not stationary ones. Change in shell thickness occurs in poultry as a result of dietary deficiencies and age (16, 17). This phenomenon would probably not occur simultaneously on two continents 1 year after the chlorinated hydrocarbon insecticides came into general usage. Other chemicals affect shell thickness in poultry (17), but the finding of high concentrations of chlorinated hydrocarbons in the eggs of wild populations of raptors and the time correlation of shell changes with the introduction of DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] tend strongly to suggest that chlorinated hydrocarbons are the major contributing cause, although it is not unlikely that other chemicals could be contributory.

In order to test the hypothesis that these recent changes of thickness in raptor eggshells were the result of differences in exposure to chlorinated hydrocarbons we analyzed 10 to 14 eggs taken in 1967 from each of five colonies of the herring gull (*Larus argentatus*). Mean shell weight and thickness in 55 eggs collected in the same five states prior to 1947 disclosed no geographic gradients or significant differences. The 1967 mean thicknesses for each colony were therefore compared to mean levels of residual DDE [1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene] on a fresh-weight basis, with the result shown in Fig. 2, the r value being significant, with $P = .001$. The residues of polychlorinated biphenyls (18) have not been studied in these ecosystems, but DDE has been consistently high in the Lake Michigan birds, averaging (fresh weight) 1925 ppm (S.E. 274) in the fat of 12 healthy adults collected in 1963-64 (19).

Reproduction in these gull colonies was generally normal in 1967 except perhaps in Wisconsin. At the latter colony where an 11 percent mean decrease in shell thickness occurred, some egg breakage and shell flaking was evident in 1967, although not at the frequency seen in previous years. Excessive reproductive failure occurred at this site in 1964 when about 18 percent of the eggs lost about one-third of the shell due to flaking, when clutch size decreased and embryonic mortality was high, and when DDE residues averaged

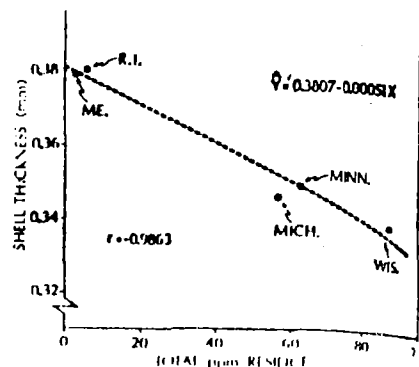


Fig. 2. Variation in shell thickness and DDE concentrations in the eggs of herring gulls in 1967. The eggs were taken at Block Island, R.I.; Green Island in Penobscot Bay, Me.; Rogers City, Mich., on Lake Huron; near Knife River, Minn., on Lake Superior; and the Sister Islands in Green Bay, Wis. Some polychlorinated biphenyls probably occurred in these eggs but they have not yet been identified.

202 ppm (S.E. 34) in nine eggs (20). (If linear extrapolation of the 1967 values is carried out to 202 ppm, the shell thickness in 1964 could be estimated as having decreased by about 2 percent.) The effectiveness of DDE in the enzymatic metabolism of aminopyrine has been reported by Hart and Fouts (21), and our data suggest this compound, because of its prevalence, has played a major role in inducing the hepatic microsomal metabolism of steroids that in turn resulted in the eggshell changes we have encountered in museum collections. Without doubt DDE is the commonest insecticide analog now being found in avian tissues (22). In 1966, it was found to average 284 ppm (S.E. 62) in the fat of nine arctic-breeding peregrine falcons (14) and about 414 ppm in falcons on a wet-weight basis (15). Concentrations of this compound and other chlorinated hydrocarbons in the peregrine populations that crashed farther south can be assumed to have been high—and they may have been much higher.

From the above evidence and that accumulated by others (2, 4, 6, 8), we have reached these conclusions: (i) many of the recent and spectacular raptor population crashes in both the United States and Western Europe had a common physiological basis; (ii) eggshell breakage has been widespread but largely overlooked in North America; (iii) significant decreases in shell thickness and weight are characteristic of the unprecedented reproductive failures of raptor populations in certain

set of the intracarbon random existing insidious birds a ecosystem.

Department of University

1. E. G. Gamble, *Pesticide of Wisconsin*, 248-264.
2. C. Den Linkola, *P. Linkola*, *Ibid.*, 23, 80 (1964).
3. D. D. Gamble, *Their B.* (Univ. of p. 165).
4. D. A. R.
5. —
6. —
7. D. B. P. Hart, R. Appl. Ph. Kupfer.
8. J. J. Fouts, *Wisconsin*.
9. U.S. Food and Health, *Publ.* (19).
10. B. Gladir.
11. J. A. Ha.
12. G. H. Ha.
13. J. N. Ric.
14. J. H. En.
15. T. J. Cade.
16. A. L. Ro.
17. T. G. Tay.
18. D. C. Hol.
19. J. J. Hicke.
20. J. A. Keit.
21. L. G. Har.
22. E. H. Dus.
23. J. B. Dixoi.
24. A. Sprunt.
25. W. A. Stic.
26. —
27. —
28. —
29. —
30. —
31. —
32. —
33. —
34. —
35. —
36. —
37. —
38. —
39. —
40. —
41. —
42. —
43. —
44. —
45. —
46. —
47. —
48. —
49. —
50. —
51. —
52. —
53. —
54. —
55. —
56. —
57. —
58. —
59. —
60. —
61. —
62. —
63. —
64. —
65. —
66. —
67. —
68. —
69. —
70. —
71. —
72. —
73. —
74. —
75. —
76. —
77. —
78. —
79. —
80. —
81. —
82. —
83. —
84. —
85. —
86. —
87. —
88. —
89. —
90. —
91. —
92. —
93. —
94. —
95. —
96. —
97. —
98. —
99. —
100. —

set of the calcium change 1 year after the introduction of chlorinated hydrocarbons into general usage was not a random circumstance; and (v) these persisting compounds are having a serious insidious effect on certain species of birds at the tops of contaminated ecosystems.

JOSEPH J. HICKEY
DANIEL W. ANDERSON

Department of Wildlife Ecology,
University of Wisconsin, Madison

References and Notes

1. E. G. Hunt and A. I. Bischoff, *Calif. Fish Game* 46, 91 (1960); review in R. L. Rudd, *Pesticides and the Living Landscape* (Univ. of Wisconsin Press, Madison, 1964), pp. 248-264.
2. C. Demandt, *Ornithol. Mitt.* 7, 5 (1955); P. Linkola, *Suomen Luonto* 18, 3, 34 (1959); P. Linkola, *ibid.* 19, 20 (1960); P. Linkola, *ibid.* 23, 5 (1964); K. Kleinsthuber, *Falke* 10, 80 (1963); C. Kruythoff, in *Working Conference on Birds of Prey and Owls* (International Council Bird Protection, London, 1964), p. 70; J.-F. Terrasse, *ibid.*, p. 73.
3. D. D. Berger, C. R. Sindelar, Jr., K. E. Gamble, in *Peregrine Falcon Populations: Their Biology and Decline*, J. J. Hickey, Ed. (Univ. of Wisconsin Press, Madison, 1968), p. 165.
4. D. A. Ratcliffe, *Bird Study* 10, 56 (1963).
5. —, *Brit. Birds* 51, 23 (1958).
6. —, *Nature* 215, 208 (1967).
7. D. B. Penkall, *ibid.* 216, 505 (1967); L. G. Hart, R. W. Shulties, J. R. Fouts, *Toxicol. Appl. Pharmacol.* 5, 371 (1963); A. H. Connors, *Pharmacol. Rev.* 19, 317 (1967); D. Kupfer, *Residue Rev.* 19, 11 (1967).
8. J. J. Hickey, Ed., *Peregrine Falcon Populations: Their Biology and Decline* (Univ. of Wisconsin Press, Madison, 1968).
9. U.S. Food and Drug Administration, *Pesticide Analytical Manual*, vol. 1 [U.S. Dept. of Health, Education, and Welfare, FDA Adm. Publ. (1963, revised 1964 and 1965)].
10. B. Glading, in Hickey (8), p. 96.
11. J. A. Hagar, in Hickey (8), p. 123.
12. G. H. Hall, *Brit. Birds* 51, 402 (1958).
13. J. N. Rice, in Hickey (8), p. 155.
14. J. H. Enderson and D. D. Berger, *Condor* 70, 149 (1968).
15. I. J. Cade, C. M. White, J. R. Haugh, *ibid.*, p. 170.
16. A. L. Romanoff and A. J. Romanoff, *The Avian Egg* (Wiley, New York, 1949), pp. 154-157.
17. T. G. Taylor and D. A. Stringer, in *Avian Physiology*, P. D. Sturkie, Ed. (Cornell Univ. Press, Ithaca, N.Y., ed. 2, 1963), p. 486.
18. D. C. Holmes, J. H. Simmons, J. O'O. Tanton, *Nature* 216, 227 (1967).
19. J. J. Hickey, J. A. Kekh, F. B. Coon, *J. Appl. Ecol.* 3 (suppl.), 141 (1966).
20. J. A. Keith, *J. Appl. Ecol.* 3 (suppl.), 57 (1966).
21. I. G. Hart and J. R. Fouts, *Arch. Exp. Pathol. Pharmacol.* 249, 486 (1965).
22. H. Dustman and L. F. Stickel, *Amer. Soc. Aeron. Spec. Publ.* 8, 109 (1966).
23. J. B. Dixon, B. Glading, W. C. Hanna, E. N. Harrison, S. B. Peyton, personal communication.
24. A. Sprunt, IV, personal communication.
25. W. A. Stickel, in Hickey (8), p. 337.
26. Except for the California data (23) the data on population trends are given in (8) by F. E. Beche for British Columbia; by W. R. Seaford for Vermont; by J. A. Hagar for Massachusetts; and by D. D. Berger et al. for New Jersey.
27. Research carried out under contract with the Bureau of Sport Fisheries and Wildlife, Fish and Wildlife Service, U.S. Dept. of Interior.
28. H. Drury, J. T. Emlen, and M. E. Slate incubated gull eggs for analysis. E. N. Harrison, W. C. Hanna, and many other oologists greatly facilitated our measurements of egg-chicks. We thank D. A. Ratcliffe for advice throughout the entire study.

© 1968

OCTOBER 1968

Phagocytosis by Polymorphonuclear Leukocytes

Abstract. *In vitro*, the human polymorphonuclear leukocyte can recognize and ingest the free forms of Plasmodium falciparum. Digestive vacuoles form about the engulfed organisms. Neither the polymorphonuclear leukocyte nor the monocyte can recognize the parasitized red cell. Phagocytosis of normal or parasitized red cells was not observed.

Both cellular and humoral elements and events are involved in malaria immunity. The presence of pigment and red cells in the reticuloendothelial cells of the spleen and bone marrow led in-

vestigators to consider phagocytosis a most important factor in removing parasites from the blood (1). Huff and Weatherby (2) first observed phagocytosis of a free schizont of avian malaria



Fig. 1. Sequence of frames is from top down and from left to right. Arrow points to parasitized red cell. In the left column, a monocyte passes the parasitized red cell. In the remaining frames a polymorphonuclear leukocyte moves by the cell. Neither white cell recognizes the diseased red cell (about $\times 1000$).

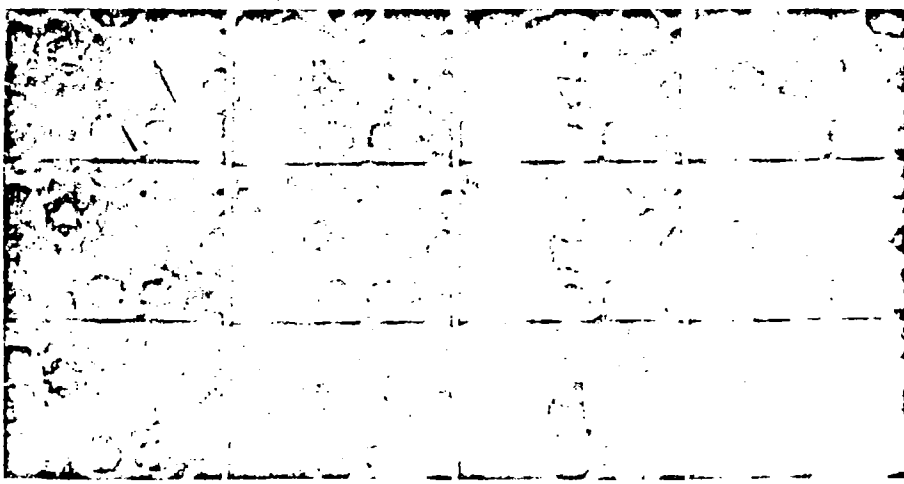


Fig. 2. Sequence of frames is from top down and from left to right. In the top frame (left column) the merozoites escape from lysing red cell (arrow) and immediately attract the polymorphonuclear leukocyte which engulfs and digests them (about $\times 1000$).