not synaptically related to the type 1 terminal. Since type 1 shows first a neurofilamentous degeneration reaction and then disappears after the optic nerve is cut, it apparently originates from the retinal ganglion cell. Since the other types of terminals are reduced in number after optic nerve section, there is a possibility some of them also are of retinal origin (*15*, *17*).

Electron microscopic radioautographs of LGN laminae receiving radioactive axons revealed that tritium-containing material did reach the synaptic terminals (Fig. 2). Of the three synaptic types, only type 1 was definitely labeled, containing 60 percent of the 139 silver grains counted in the LGN (Table 1). Three grains were found over the cell membranes of an adjacent type 1 and type 2 terminal, but since the resolution in these radioautographs is 2000 Å (*18*) there is an equal possibility that these grains originated from a radioactive decay occurring in either terminal. Within the type 1 terminals the greatest number of silver grains (33 percent) was over cytoplasmic areas rich in synaptic vesicles, but the resolution prevented actually localizing them to the vesicles themselves. Mitochondria also contained numerous grains (23 percent), whereas the bundles of neurofilaments within the terminal were infrequently labeled (4 percent).

Light microscopic radioautographs indicated that the numbers of silver grains over the LGN neuronal cell body did not vary between laminae receiving radioactive and nonradioactive axons. In electron microscopic radioautographs, although silver grains were found over synaptic vesicles adjacent to the membrane density that apparently marks the site of synaptic interaction, there was no concentration of grains over the dendrites opposite this density. As judged from grain counts, labeling of neurons (5 percent) was little different from that of glia (7 percent) in the LGN, which indicates that neuronal labeling is caused by blood-borne tritiated leucine and not by a transynaptic movement of axonal labeled materials. This is in contrast to a previous light microscopic radioautographic study of peripheral nerve which suggested a transynaptic movement of protein labeled with $C^{14}$-glycine from hypoglossal nerve into tongue muscle (*19*).

Both a "rapid" and a "slow" rate of axonal movement has been shown in the visual system (*2*, *4*, *5*, *12*). Since it has been reported that substances differing in particle size and chemical composition constitute these two components in the goldfish visual system (*5*), the distribution of label within the synaptic terminal might be different at shorter or longer survival times. The observation that regions rich in synaptic vesicles contain the greatest amount of label indicates that at least some component associated with these vesicles originates in the neuronal cell body. What axonal structures contain this labeled material and at what point the vesicles appear as morphological entities is still not clear. In this connection, the small amount of label over cytoplasmic regions containing neurofilaments within the terminal axon is interesting since both neurotubules and neurofilaments have been implicated in axonal transport (*20*). The observation that both axonal and terminal mitochondria contained silver grains is understandable since these organelles synthesize protein (*21*) and move between the cell body and the axon (*22*).

These data indicate that after an intraocular injection of tritiated leucine, tritiated material is contained within the axons originating from the injected eye and is distributed throughout their extent, including the synaptic terminal. In the LGN only the type 1 terminal is definitely labeled; thus it originates from the retina. The two nonlabeled synaptic terminal types apparently originate elsewhere. The labeled material does not cross the synaptic gap into dendrites; neither does it appear to cross an axo-axonal synapse.

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**References and Notes**

1. P. Weiss and A. Hiscoe, *J. Exp. Zool.* **107**, 315 (1948).
2. S. Ochs, J. Johnson, M.-H. Ng, *J. Neurochem.* **14**, 317 (1967).
3. R. P. Peterson, R. M. Herwitz, R. Lindsay, *Exp. Brain Res.* **4**, 138 (1967).
4. J. J. Bray and L. Austin, *J. Neurochem.* **15**, 731 (1968).
5. B. S. McEwen and B. Grafstein, *J. Cell Biol.* **38**, 494 (1968).
6. See references in B. S. McEwen and B. Grafstein, *ibid.*
7. S. Goldberg and M. Kotani, *Anat. Rec.* **158**, 325 (1967); R. Lasek, B. S. Joseph, D. C. Whitlock, *Brain Res.* **8**, 319 (1968).
8. E. G. Gray and R. W. Guillery, *Int. Rev. Cytol.* **19**, 111 (1966).
9. L. E. Westrum and R. D. Lund, *J. Cell Sci.* **1**, 229 (1966).
10. P. Granboulan, in *The Use of Radioautography in Investigating Protein Synthesis*, C. P. Leblond and K. Warren, Eds. (Academic Press, New York, 1965), p. 43.
11. E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
12. A. Hendrickson and J. deC. Downer, *Fed. Proc.* **27**, 234 (1968).
13. A. Hendrickson, in preparation.
14. W. E. LeGros Clark, *J. Anat. (London)* **75**, 225 (1941).
15. M. Colonier and R. W. Guillery, *Z. Zellforsch.* **62**, 333 (1964).
16. J. A. Campos-Ortega, P. Glees, V. Neuhoff, *ibid.* **87**, 82 (1968); J. Saavedra, O. Vaccarezza, T. Reader, *ibid.* **89**, 462 (1968).
17. R. W. Guillery, in *Degeneration Patterns in the Nervous System, Progress in Brain Research*, M. Singer and J. P. Schade, Eds. (Elsevier, Amsterdam, 1965), vol. 13, p. 1; P. Glees, K. Meller, J. Eschner, *Z. Zellforsch.* **41**, 29 (1966).
18. Determination of resolution in electron microscopic radioautographs is reviewed in A. Rogers, *Techniques of Radioautography* (Elsevier, Amsterdam, 1967), chap. 4. In this report the "50% circle" method of L. Bachman and M. M. Salpeter [*Lab. Invest.* **14**, 1041 (1965)] was used for the grain counts.
19. Q. M. Koor, P. N. Wilkinson, F. W. Chornock, *Science* **155**, 342 (1967).
20. B. Droz, *J. Microscop.* **6**, 201 (1967); A. P. DeIraldi and E. DeRobertis, *Z. Zellforsch.* **87**, 330 (1968).
21. S. H. Barondes, *J. Neurochem.* **13**, 721 (1966).
22. P. Weiss and A. Pillai, *Proc. Nat. Acad. Sci. U.S.* **54**, 48 (1965).
23. I thank Drs. C. Kupfer, S. Futterman, J. Downer, and D. Graney for their assistance; and the Department of Biological Structure for the use of the electron microscope. Supported by PHS special fellowship 1 F10NB 1924-01 VSN, PHS grants NB06952 and NB5594, and American Cancer Society grant IN261.

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## Hormonal Induction of Increased Zinc Uptake in Mammalian Cell Cultures: Requirement for RNA and Protein Synthesis

Abstract. *The zinc content of HeLa $S_3$ cells is markedly increased after growth in medium containing adrenal glucocorticoid hormones. Studies with inhibitors indicate that the synthesis of RNA and protein is required for enhanced zinc uptake. When protein synthesis is blocked in the presence of the steroid, an intermediate, presumably messenger RNA, which specifies enhanced zinc uptake accumulates and is expressed when the inhibition of protein synthesis is removed.*

Hormones have been implicated in the control of the ionic content of cells, and the ensuing alteration in the intracellular environment may explain, in part, the effects of a hormone on a particular cell or tissue (*1*). Adrenal glucocorticoid hormones increase the uptake of zinc in certain mammalian cell cultures (*2*). This enhanced accumulation of zinc is specific in that the uptake of other cations—for example, calcium and rubidium—is not altered and a number of other monovalent and divalent ions do not compete with zinc for up-

take. The accumulation of zinc is markedly depressed by low temperatures, by sulfhydryl blocking agents, and by simultaneous addition of inhibitors of glycolysis and oxidative metabolism. Kinetic studies show that cells must grow from 8 to 12 hours in the presence of the hormone before an increased accumulation of zinc is apparent (*2*).

I now present evidence that the induction of increased zinc accumulation by $\Delta^1$-hydrocortisone (prednisolone) depends upon the synthesis of RNA followed by the synthesis of protein necessary for enhanced transport or binding of zinc. An intermediate, presumably messenger RNA (mRNA), can accumulate in steroid-treated cells in the absence of protein synthesis. When the inhibition of protein synthesis is relieved, the accumulated mRNA is expressed without a lag. Therefore, the hormone appears to act either to increase the synthesis of mRNA or to decrease its degradation, and this mRNA specifies the protein involved in the increase of zinc uptake.

For these studies HeLa $S_3$ cells were grown in suspension culture in minimum essential medium containing calf serum (7 percent) and antibiotics (50 units of penicillin and 50 $\mu$g of streptomycin per milliliter) (*3*). The cell concentration was approximately 20 to $30 \times 10^4$ cell/ml. Samples of the cell suspension were placed in sterile 125-ml erlenmeyer flasks and were incubated in a New Brunswick gyratory shaker in 95 percent air and 5 percent $CO_2$; inducers and inhibitors were added to the culture, as described in Tables 1 and 2 and in the legend to Fig. 1. Certain experiments were carried out in two steps; an incubation with inducer or inhibitors, or both, for 16 hours, followed by washing of the cells and further incubation in medium with or without further additions (Table 2).

The nonradioactive zinc content of complete medium is approximately 0.9 $\mu$g/ml. Zinc-65 was obtained with a purity of greater than 99 percent (New England Nuclear Corporation) (specific activities varied from 2.30 to 3.87 mc/mg). Cultures were incubated for 3 hours with zinc-65 at a final concentration of 2.3 $\mu$c/ml. After the labeling experiments, zinc efflux was prevented by carrying out all procedures at 0°C. The cell suspension was harvested by centrifugation at 0°C, washed three times with ice-cold 0.9 percent saline, and lysed in 0.5 percent deoxycholate. Cell protein and radioactivity were determined as described (*2*). Results are expressed as radioactivity per milligram of cell protein. In certain experiments cultures were incubated for 3 hours with either 100 nc of leucine-1-$^{14}$C (31.0 mc/mmole) or 100 nc of uridine-2-$^{14}$C (55.2 mc/mmole) per milliliter. Radioactivity incorporated into material insoluble in trichloroacetic acid was assayed by scintillation counting (*4*).

Table 1. Effect of cycloheximide (1.0 $\mu$g/ml) and actinomycin D (0.1 $\mu$g/ml) on incorporation of leucine-1-$^{14}$C and uridine-2-$^{14}$C in HeLa $S_3$ suspension cultures grown with and without prednisolone (1.5 $\mu$g/ml). Values are averages and ranges of duplicate assays. Cultures were incubated for 3 hours with each isotope (100 nc/ml) before they were harvested; P, prednisolone; C, cycloheximide; A, actinomycin D.

| Incubation time (hr) with additions | | | Incorporation | | | |
|---|---|---|---|---|---|---|
| | | | Leucine-1-$^{14}$C | | Uridine-2-$^{14}$C | |
| P | C | A | $10^3$ count/min per milligram of protein | Percent of control | $10^3$ count/min per milligram of protein | Percent of control |
| 0 | 0 | 0 | 16.2 ± 1.3 | | 82.6 ± 5.6 | |
| 24 | 0 | 0 | 16.8 ± 1.8 | 104 | 81.8 ± 7.1 | 99 |
| 0 | 5 | 0 | 1.4 ± 0.3 | 9 | 84.6 ± 6.8 | 103 |
| 24 | 5 | 0 | 1.2 ± 0.2 | 7 | 87.2 ± 5.9 | 105 |
| 0 | 16 | 0 | 0.8 ± 0.3 | 5 | 26.0 ± 2.4 | 31 |
| 0 | 16* | 0 | 9.0 ± 1.2 | 55 | 50.5 ± 4.7 | 62 |
| 0 | 0 | 5 | 15.8 ± 0.8 | 97 | 16.6 ± 0.6 | 20 |
| 24 | 0 | 5 | 14.6 ± 1.6 | 90 | 15.8 ± 0.9 | 19 |

* Cultures were incubated in medium containing cycloheximide for 16 hours; then the cells were washed and reincubated for 6 hours in medium without added inhibitor.

The steroid-induced enhancement of zinc uptake in HeLa $S_3$ cultures could not be attributed to a general stimulation of protein synthesis accompanied by zinc binding, since incorporation of radioactive leucine was about the same in cells grown in the presence or absence of the hormone (Table 1). To determine whether the increased zinc accumulation mediated by the hormone required protein synthesis rather than an activation of a preexisting precursor, cycloheximide (1.0 $\mu$g/ml) was given at various times after addition of prednisolone (Fig. 1). This drug completely blocked the increased uptake of zinc even when it was added as much as 5 to 6 hours after addition of the hormone. If cycloheximide was added after enhanced zinc uptake became apparent, the inhibitor blocked further increase in zinc accumulation (Fig. 1). Cycloheximide concentrations of 1.0 $\mu$g per milliliter used in these experiments, inhibit incorporation of radioactive leucine by approximately 85 to 95 percent (Table 1).

In animals, hormones increase the

Table 2. Enhancement of zinc-65 uptake after removal of inhibitor of protein synthesis from hormone-treated cultures. The experiment was carried out in two steps. In the initial step, HeLa $S_3$ cells were incubated during the first 16 hours in medium with or without added prednisolone (P), cycloheximide (C), or actinomycin D (A). In the second step cells were washed and then incubated for the next 6 hours (16 to 22) in medium with or without added prednisolone or inhibitors. Values are the averages and ranges of duplicate assays. Cultures were then incubated for 3 hours with 2.3 $\mu$c of zinc-65 per milliliter before they were harvested.

| First step ($\mu$g/ml) | | | Second step ($\mu$g/ml) | | | Zinc-65 uptake ($10^3$ count/min per mg protein) |
|---|---|---|---|---|---|---|
| P | C | A | P | C | A | |
| 0 | 0 | 0 | 0 | 0 | 0 | 3.2 ± 0.4 |
| 0 | 1.0 | 0 | 0 | 1.0 | 0 | 3.7 ± 0.4 |
| 0 | 1.0 | 0 | 0 | 0 | 0 | 3.9 ± 0.5 |
| 0 | 1.0 | 0 | 1.5 | 0 | 0 | 4.1 ± 0.3 |
| 1.5 | 0 | 0 | 1.5 | 0 | 0 | 7.9 ± 0.7 |
| 1.5 | 1.0 | 0 | 1.5 | 1.0 | 0 | 3.4 ± 0.2 |
| 1.5 | 1.0 | 0 | 1.5 | 0 | 0 | 6.6 ± 0.4 |
| 1.5 | 1.0 | 0 | 0 | 0 | 0 | 6.4 ± 0.8 |
| 1.5 | 1.0 | 0 | 0 | 0 | 0.1 | 6.0 ± 0.2 |
| 1.5 | 1.0 | 0.1 | 1.5 | 0 | 0 | 3.5 ± 0.6 |

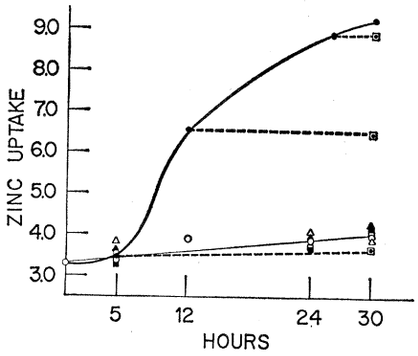


Fig. 1. Effect of cycloheximide and actinomycin D on HeLa $S_3$ cell suspension cultures grown in medium with and without prednisolone. Prednisolone and the inhibitors were added at zero hour. Zinc-65 uptake is shown as the number of counts per minute times $10^{-3}$ per milligram of cell protein after the cells were incubated for 3 hours with 2.3 $\mu$c of $^{65}$Zn per milliliter. ○——○, No addition; ●——●, prednisolone (1.5 $\mu$g/ml); □——□, cycloheximide (1.0 $\mu$g/ml); ■——■, cycloheximide (1.0 $\mu$g/ml) and prednisolone (1.5 $\mu$g/ml); △——△, actinomycin D (0.1 $\mu$g/ml); ▲——▲, actinomycin D (0.1 $\mu$g/ml) and prednisolone (1.5 $\mu$g/ml); ●----⊡, prednisolone (1.5 $\mu$g/ml) and at the time indicated by the broken line cycloheximide (1.0 $\mu$g/ml) was added.

total RNA content of certain target organs and increase the rate of synthesis of RNA in vivo, as measured by the incorporation of radioactive precursors (*5*). No increase in total RNA synthesis was observed in HeLa $S_3$ cells during growth in prednisolone (Table 1); similar findings in other tissue culture studies have been reported (*4, 6, 7*). Despite the absence of gross changes in RNA synthesis in HeLa $S_3$ cells during induction of enhanced zinc uptake by prednisolone, studies with inhibitors suggest that RNA synthesis is required for increased zinc accumulation. Actinomycin D (0.1 $\mu$g/ml) inhibits the prednisolone-mediated increase in zinc accumulation (*8*) (Fig. 1). This concentration of inhibitor reduces incorporation of $^{14}$C-uridine by 75 to 85 percent (Table 1). Although no gross changes in RNA synthesis were observed after growth of HeLa $S_3$ cells in the presence of the steroid, the effect of actinomycin D suggests the synthesis and accumulation of RNA which specifies protein or proteins necessary for enhanced uptake of zinc.

Actinomycin D even at low concentrations damages cells when used for more than a few hours; therefore, our experiments with this inhibitor cannot be interpreted with confidence. However, the findings in Table 2 provide support that RNA is a necessary intermediate for enhanced uptake of zinc, since in the presence of the steroid and when protein synthesis is blocked by cycloheximide the cells accumulate an intermediate (mRNA) which can express itself after the inhibitor is removed by washing (*9*). The enhanced accumulation of zinc occurs rapidly when the inhibition of protein synthesis is relieved without the usual 8- to 12-hour lag. Further support for the conclusion that hormone-mediated RNA synthesis is required for enhanced uptake of zinc is shown in Table 2. (i) The hormone must be present during the period of incubation with the inhibitor of protein synthesis, since incubation with cycloheximide alone produces no significant increase in zinc uptake after the inhibitor is removed. (ii) Although the hormone must be present during the incubation with cycloheximide, after removal of the inhibitor enhanced zinc uptake occurs with or without further addition of hormone. (iii) Addition of actinomycin D after removal of cycloheximide from hormone-treated cultures does not prevent the rapid increase in zinc accumulation, an indication that during the reincubation period RNA synthesis is not required for enhanced zinc uptake to occur. (iv) If actinomycin D is present with the cycloheximide during the initial incubation with hormone the increased zinc uptake is completely inhibited.

The mechanisms responsible for enhanced zinc uptake mediated by adrenal glucocorticoid hormones are not known. The results indicate that prednisolone does not modify directly preexisting cell membranes so as to increase selectively zinc accumulation, nor does it activate a precursor of the transport or binding molecules. Moreover, the hormone does not stimulate a general increase in RNA and protein synthesis, although the effects on zinc uptake do require RNA and protein synthesis. Our findings are consistent with the accumulation of an intermediate, presumably mRNA, which when translated specifies a mechanism for increased uptake of zinc.

Several steroid hormones are believed to exert their effects on RNA synthesis. Aldosterone appears to act by inducing *de novo* synthesis of proteins necessary for enhanced sodium transport (*10*). Evidence has been obtained to show that estrogen acts on the vagina and the uterus (*11*) and that testosterone acts on the testis by stimulating protein synthesis via an effect on nuclear synthesis of RNA (*12*). Experiments on uptake of zinc in cultured cells are similar to those reported by Tomkins and his collaborators on adrenal glucocorticoid hormone induction of tyrosine aminotransferase in Morris rat hepatoma cells in cultures (*7*). The induction of this enzyme in this cell line is due to the increased synthesis and accumulation of enzyme protein. The synthesis of the enzyme requires RNA synthesis and the tyrosine aminotransferase mRNA can accumulate in steroid-treated cells in the absence of protein synthesis (*13*). Hydrocortisone induction of glutamine synthetase in chick embryo retina also can stimulate the accumulation of enzyme-specific mRNA in the absence of protein synthesis (*14*). The marked similarity between experiments on hydrocortisone induction of enzymes in cell culture and the steroid's effects on zinc uptake in HeLa $S_3$ cells suggests that in mammalian cells the hormonal control of enzyme activities and regulation of concentrations of certain intracellular ions may be determined by similar mechanisms.

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### References and Notes

1. T. R. Riggs, in *Action of Hormones on Molecular Processes*, G. Litwack and D. Kritchevsky, Eds. (Wiley, New York, 1964), pp. 1–57; G. Lester and O. Hechter, *Proc. Nat. Acad. Sci. U.S.* **45**, 1792 (1959); P. Richards, K. Smith, A. Metcalfe-Gibson, O. Wrong, *Lancet* **1966-I**, 1099 (1966); P. L. Jørgensen, *Biochim. Biophys. Acta* **151**, 212 (1968).
2. R. P. Cox, *Mol. Pharmacol.* **4**, 510 (1968).
3. H. Eagle, *Science* **130**, 432 (1959).
4. M. J. Griffin and R. P. Cox, *J. Cell Biol.* **29**, 1 (1966).
5. L. R. Garren, R. R. Howell, G. M. Tomkins, *J. Mol. Biol.* **9**, 100 (1964); F. T. Kenny. W. Wick, D. Greenman, *J. Cell Comp. Physiol.* **66**, 125 (1965); J. W. Gorski, W. Noteboom, J. Nicolette, *ibid.*, p. 91; H. G. Williams-Ashman, *ibid.*, p. 111.
6. M. J. Griffin and R. P. Cox, *Proc. Nat. Acad. Sci. U.S.* **56**, 946 (1966).
7. G. M. Tomkins, E. B. Thompson, S. Hayashi, T. Gelehter, D. Granner, B. Peterkofsky, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 349 (1966).
8. The specific uptake of zinc-65 per milligram of cell protein in cultures treated with actinomycin D was occasionally increased, whether the hormone was present or not. Cells that were grown in actinomycin D for more than 8 hours show a decrease in cellular protein, and the elevated zinc uptake per milligram of cell protein occasionally observed in other experiments may be due to the relative stability of the zinc transport or binding protein when compared to the average cell protein. Since actinomycin D cannot be removed from cells by washing (*13*) the reversibility of these effects cannot be tested.
9. Table 1 shows that prolonged incubation of cells with cycloheximide inhibits uridine as well as leucine incorporation, and this inhibition is partially reversed by washing the cells to free them of inhibitor.
10. I. S. Edelman, R. Bogoroch, G. A. Porter, *Proc. Nat. Acad. Sci. U.S.* **50**, 1169 (1963); P.

DeWeer and J. Crabbé, *Biochim. Biophys. Acta* **155**, 280 (1968).
11. H. Ui and G. C. Mueller, *Proc. Nat. Acad. Sci. U.S.* **50**, 256 (1963); W. D. Noteboom and J. Gorski, *ibid.*, p. 250.
12. S. Liao and H. G. Williams-Ashman, *ibid.* **48**, 1956 (1962).
13. B. Peterkofsky and G. M. Tomkins, *ibid.* **60**, 222 (1968).
14. L. Reif-Leher and H. Amos, *Biochem. J.* **106**, 425 (1968).
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## Dieldrin and DDT: Effects on Sparrow Hawk Eggshells and Reproduction

Abstract. *Patterns of reproductive failure in declining populations of several European and North American raptorial species were duplicated experimentally with captive American sparrow hawks* Falco sparverius *that were given a diet containing two commonly used organochlorine insecticides. Major effects on reproduction were increased egg disappearance, increased egg destruction by parent birds, and reduced eggshell thickness.*

Marked declines in populations and reproductive success of several species of North American and European raptors have occurred during the past two decades (*1–3*). These declines have been attributed to effects of organochlorine insecticides which these birds obtained from their food and accumulated in their tissues (*1, 3–5*). Reproductive failures of some species were associated with significant decreases in eggshell thickness (*6, 7*) and, especially in British species, with marked increase in frequency of egg-eating and of egg breakage in the nests (*5, 8*). These changes were ascribed to alterations in calcium metabolism of adult birds (*7*).

We have investigated effects of two sublethal dietary levels of DDT and dieldrin (*9*), in combination, on reproductive success of captive American sparrow hawks *Falco sparverius* and the influence of these chemicals on eggshell thickness.

The sparrow hawk was selected because it had been bred successfully in captivity on a limited scale (*10*), was relatively abundant, was easily handled and sexed, and was closely related to the peregrine falcon *F. peregrinus*, a declining species of raptor (*1*).

The principal experimental group consisted of 27 pairs of hawks, all obtained as fledglings in the summer of 1964 from the Northeast and maintained as pairs since early in 1965. Nine pairs of these birds were randomly assigned to each of three treatments —control, low dosage, and high dosage (*11*). An additional group of nine pairs of hawks that had a heterogeneous history and were housed at a different location were randomly assigned, three pairs each, to the same treatments as the principal group. Females of this latter group were birds caught from the wild in Florida in the winter of 1965–66; males were produced by the parent colony in 1965 before dosage began on 11 March 1966.

Low dosage represented amounts equal to residues often found in raptor food items in the field (*12*). High dosage was calculated to be just short of lethal to adults and it was equivalent to that obtainable in the field, at least in some areas containing prey items with unusually high pesticide residues (*13*).

Birds of both sexes were carried over from one year of the experiment to the next. Females that died during the experiment were not replaced. Males that died during the experiment were replaced at the onset of each reproductive season. Dosed males that died were replaced with males of the same treatment when available; otherwise, they were replaced with nondosed males.

In 1968, reproduction of first-generation (yearling) hawks was investigated. These hawks were produced by the experimental colony in 1967 and were retained on the same diet as their parents. The 24 pairs of hawks used in this experiment were selected on the basis of body condition. In pairing them, the heaviest females were mated with the heaviest males to insure successful pairing. Siblings were not paired with each other. Dosages were randomly assigned to pens. In respect to age and history of pesticide exposure, yearling hawks were our most homogeneous group.

Table 1. Reproductive success of treated sparrow hawks. Data were analyzed by chi-square and presented as numbers of birds or eggs. Abbreviations: C, control; L, low dosage; H, high dosage (*11*).

| Category | Parental group | | | | | | | | | | | | Yearling group 1968 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Northeastern females | | | | | | Florida females | | | | | | | | |
| | 1967 | | | 1968 | | | 1967 | | | 1968 | | | | | |
| | C | L | H | C | L | H | C | L | H | C | L | H | C | L | H |
| Pairs (clutches) | 8 | 8 | 7 | 8 | 8 | 7 | 2 | 3 | 3 | 2 | 2 | 3 | 8 | 8 | 8 |
| Eggs laid | 40 | 40 | 33 | 39 | 40 | 32 | 10 | 14 | 13 | 10 | 10 | 13 | 41 | 33 | 42 |
| Eggs taken for study | 0 | 0 | 0 | 8 | 8 | 7 | 0 | 0 | 0 | 2 | 2 | 3 | 8 | 6 | 8 |
| Eggs incubated | 40 | 40 | 33 | 31 | 32 | 25 | 10 | 14 | 13 | 8 | 8 | 10 | 33 | 27 | 34 |
| Eggs disappeared* | 1 | 7† | 9† | 2 | 8† | 10† | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 7† | 4† |
| Eggs remaining | 39 | 33 | 24 | 29 | 24 | 15 | 10 | 12 | 11 | 8 | 8 | 10 | 33 | 20 | 30 |
| Infertile eggs‡ | 1 | 0 | 3 | 1 | 2 | 2 | 0 | 1 | 3 | 1 | 0 | 0 | 2 | 1 | 2 |
| Dead embryos | 6 | 9 | 2 | 13 | 6 | 3 | 0 | 2 | 0 | 2 | 5 | 6 | 3 | 0 | 9§ |
| Eggs hatched of eggs incubated | 32 | 24‖ | 19† | 15 | 16 | 10 | 10 | 9† | 8† | 5 | 3 | 4 | 28 | 19 | 19† |
| Young fledged of eggs incubated | 30 | 22¶ | 18¶ | 13 | 13 | 4§ | 7 | 8 | 7 | 4 | 3 | 4 | 28 | 19 | 13§ |
| of eggs hatched | 30 | 22 | 18 | 13 | 13 | 4§ | 7 | 8 | 7 | 4 | 3 | 4 | 28 | 19 | 13§ |

* May include disappearance of some young early in the post-hatching period. † Significant difference between dosed group and controls at $P < .05$. ‡ Refers to eggs without obvious embryonic development. § Significant difference between dosed group and controls and also from other dosed group at $P < .05$. ‖ Significant difference between dosed group and controls at $P \approx .06$. ¶ Significant difference between dosed group and controls at $P \approx .07$.