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HORMONAL CONTROL AND DEVELOPMENT OF THE INCUBATION PATCH IN ICTERIDS, WITH NOTES ON BEHAVIOR OF COWBIRDS

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In the course of an experiment on the effects of injection of estradiol, prolactin, and progesterone on the behavior of the Redwinged Blackbird (*Agelaius phoeniceus*) and the Brown-headed Cowbird (*Molothrus ater*), we noted development of incubation patches in both male and female redwings. This observation led to a second experiment designed to test further the effectiveness of these hormones, alone and in combination, in inducing incubation patches in the two species of icterids. Specific objectives of the study were: (1) to determine if an incubation patch could be induced in icterids by the same method used by Bailey (1952) in his work on fringillids, that is, by administration of estrogen or estrogen and prolactin; (2) to determine the effect of progesterone on incubation patch formation; (3) to measure histologic changes in the integument of the ventral apterium resulting from hormone treatment; (4) to compare artificially induced incubation patches with those of wild birds; (5) to determine the natural sequence of events in the formation of the incubation patch in an icterid and to compare this sequence with that in fringillids (Bailey, 1952; and Hinde, 1962) and swallows (Petersen, 1955); and (6) to ascertain if hormone treatment could induce response in the integument of the ventral apterium in parasitic cowbirds, which do not build nests or incubate.

In a previous study, Selander (1960) failed to induce incubation patches in a small sample of adult cowbirds by implanting estradiol pellets and later giving injections of prolactin. He concluded that parasitic cowbirds fail to develop incubation patches, not as a result of hormonal deficiency, but because of inability of the integument to respond to hormones. This conclusion was partly supported by Höhn's demonstration (1959) of prolactin in the pituitaries of breeding cowbirds. The logical inference was that the integument of the ventral apterium had become refractory to hormonal stimulation in the course of evolution of the parasitic habit. However, Selander's experiment did not provide conclusive support for this hypothesis, since it was not established that incubation patches can be induced in nonparasitic species of icterids by treatment with estrogen and prolactin. Additionally, although Selander had observed no gross change in the ventral apterium of cowbirds following hormone treatment, the possibility remained that histologic study would reveal slight but significant responses. Finally, neither Selander (1960) nor Bailey (1952) tested the effect on incubation patch formation of progesterone, a hormone which often acts synergistically with estrogen (Mason, 1952), and which has been demonstrated in the blood and ovaries of laying hens (Layne, *et al.*, 1957; Lytle and Lorenz, 1958; see additional references in Lehrman, 1961:1277–1278) and in extracts of metamorphosed testis tubules of birds (Lofts and Marshall, 1957: 625; 1959).

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METHODS

NONCAPTIVE BIRDS

Samples of breast integument from 27 redwings and 19 cowbirds were collected in the field in Texas and Louisiana between March 9 and June 15, 1962. The gonadal condition of the birds was noted, and the phase of the breeding cycle of females was estimated on the basis of numbers of collapsed ovarian follicles and size of developing follicles. Since the clutch size of the Redwinged Blackbird in Texas is three to five eggs (Simmons, 1925:174), birds having three or more collapsed follicles and no enlarged follicles were considered to have completed their clutches and were judged to be incubating. Males were designated as breeding or nonbreeding on the basis of the size of the testes.

To supplement information on the redwing, notes were made on the condition of the incubation patch in four female Common Grackles (*Quiscalus quiscula*) and a female Boat-tailed Grackle (*Cassidix major*) collected in Louisiana in April, 1962.

EXPERIMENTS

Birds were housed in metal cages measuring 63 × 46 × 46 cm. and containing two perches; water, grit, and mixed seeds were provided in containers attached externally to the fronts of the cages. Photoperiod in the laboratory was 10 hours per day, and air temperature was maintained at approximately 20°C.

All birds used in the experiments were trapped at Austin or Eagle Lake, Texas; most individuals were first-year birds.

Estradiol benzoate (Progynon B, Schering) and progesterone (Proluton, Schering) were injected in aqueous suspension; and prolactin (luteotrophic hormone, LTH; Panlitar, Armour) was injected in aqueous solution. Control birds were given injections of Ringer's solution (Abbott). Each injection was 0.1 cc. of fluid. Injections were given in the pectoral muscles (Experiment I) or leg muscles (Experiment II) three times weekly at two- or three-day intervals.

For the reader interested in calculating hormone dosage in terms of mg. of hormone per unit mass of bird, weights of birds collected in central Texas are given in table 1.

TABLE 1

WEIGHTS OF ICTERIDS COLLECTED IN CENTRAL TEXAS IN NOVEMBER

Species	Sex	Number of specimens	Weight (gm.) Mean	Weight (gm.) Range
Redwinged Blackbird	♂	71	60	52–75
	♀	59	44	36–52
Brown-headed Cowbird	♂	49	44	37–54
	♀	15	33	31–37

Experiment I.—As noted previously, this experiment was designed to test the influence of hormones on behavior; data on incubation patch formation were obtained only incidentally. On October 4, 1961, 20 redwings (10 males and 10 females) and 20 cowbirds were placed in individual cages. Injections were first given on October 25. For purposes of hormone treatment, the 20 birds of each species were placed in groups as follows:

Phase 1, October 25 through November 8, 7 injections. Group 1AB, estrogen, 4 birds received injections of 1.0 mg., 4 birds, 0.5 mg., and 4 birds, 0.25 mg. estradiol benzoate; Group 1C, estrogen and progesterone, 4 birds received 1.0 mg. estradiol benzoate and 0.25 mg. progesterone; Group 1D, controls, 4 birds received 0.1 cc. Ringer's solution.

Phase 2, November 11 through November 20, 4 injections. Group 2A, estrogen and prolactin, 6 redwings (4 from Group 1AB and 2 from Group 1C) and 4 cowbirds (2 from Group 1AB and 2 from Group 1C) received 1.0 mg. estradiol benzoate and 50 I.U. prolactin; Group 2B, prolactin, 4 redwings and 4 cowbirds from Group 1AB received 50 I.U. prolactin; Group 2C, progesterone, 4 redwings (3 from Group 1AB and 1 from Group 1C) and 5 cowbirds (3 from Group 1AB and 2 from Group 1C) received 0.42 mg. progesterone; Group 2D, controls, control birds of Phase 1 continued to receive 0.1 cc. Ringer's solution.

At the end of Phase 1, each bird was inspected to determine the degree of defeatherization and thickening and vascularity of the ventral apterium. At the end of Phase 2, all birds, except four cowbirds, were sacrificed and again examined, and a piece of integument from the center of the breast was fixed in Bouin's solution. Some of the tissues from the redwings were later sectioned at 10 μ and stained with Harris' hematoxylin and eosin for histologic study.

Experiment II.—The subjects were 24 redwings (18 males and 6 females) and 38 cowbirds (19 males and 19 females). On February 14, 1962, the cowbirds and 12 of the redwings were confined, a male and a female per cage, and the remaining redwings were caged individually. Beginning on February 16, each bird received a series of six injections given at two- or three-day intervals over a period of two weeks. The birds were divided into groups as follows:

Group 1, estrogen, 3 redwings and 6 cowbirds received injections of 1.0 mg. estradiol benzoate; Group 2, progesterone, 4 redwings and 6 cowbirds received 0.5 mg. progesterone; Group 3, prolactin, 4 redwings and 6 cowbirds received 50 I.U. prolactin; Group 4, estrogen and prolactin, 5 redwings and 8 cowbirds received 1.0 mg. estradiol benzoate and 50 I.U. prolactin; Group 5, estrogen and progesterone, 4 redwings and 6 cowbirds received 1.0 mg. estradiol benzoate and 0.5 mg. progesterone; Group 6, controls, 4 redwings and 6 cowbirds received 0.1 cc. Ringer's solution.

All birds were sacrificed on March 3, 1962, 15 days after the first injections were given. The ventral apterium was examined and samples of the breast integument were prepared for histologic study.

HISTOLOGIC STUDY

In histologic sections we measured the thickness of the stratum germinativum and the dermis and counted numbers of blood vessels and fat cells present per unit length (1 mm.) of section. From 20 to 30 sections of integument available for each bird, five were selected at random for measuring; and the measurements for individual birds in tables 2, 3, and 4 are averages for the five sections.

Although Bailey (1952), Hinde (1962), Selander (1960), and others have attributed the conspicuous thickening of the integument involved in incubation patch formation in passerines to "edema," it is apparent from our material that edema (which is defined as an excessive accumulation of watery fluid in intercellular tissue spaces) is but one of several factors responsible. Actually, the observed increase in thickness of the dermis results from an increase in number and diameter of blood vessels, an increase in number of connective tissue cells, and from edema (see fig. 1). Additionally, there is an increase in thickness of the epidermis resulting in large part from an increase in number of cells in the stratum germinativum. Höhn (1961:94) claims that hyperemia

TABLE 2

DEVELOPMENT OF INCUBATION PATCH IN WILD FEMALE REDWINGED BLACKBIRDS

Group and reference number	Condition of ovary	Stage of breeding cycle	Degree of feather loss	Thickness (in μ)		Blood vessels per mm.	Fat cells per mm.
				Stratum germinativum	Dermis		
Group I							
X12	Inactive (5 mm. in diameter)	Early prelaying	Few lost from center breast	4	91	2	10
X6	Inactive (5 mm. in diameter)	Early prelaying	Few lost from center breast	5	96	3	4
Group II							
X2	2 c.f.[1]	Laying	Complete	7	139	12	0
X10	0 c.f., 8 mm. follicle	Advanced prelaying[2]	Complete	8	178	11	3
AB4	0 c.f., 6 mm. follicle	Advanced prelaying	Complete	13	149	7	4
X3	0 c.f., follicles to 2 mm.	Advanced prelaying	Complete	10	144	13	0
X11	3 c.f., follicles to 3 mm.	Finishing laying	Complete	14	154	10	0
G3	0 c.f., large follicle	Advanced prelaying	Extensive but incomplete	23	139	12	0
AB2	0 c.f., 2 mm. follicle	Advanced prelaying	Complete	25	207	16	0
AB3	2 c.f., 2 mm. follicle	Laying	Extensive but incomplete	22	240	8	0
Group III							
SB1	Small	Incubating	Complete	12	284	25	0
G2	3 c.f., follicles to 2 mm.	Incubating	Complete	13	192	19	0
GD4	4 c.f., follicles small	Incubating	Complete	20	240	16	0
GD3	4 c.f., follicles small	Incubating	Complete	22	255	13	0
X1	"Several" c.f.	Incubating	Complete	23	289	16	0
AB5	Damaged by shot	Incubating	Not quite complete	25	250	8	0
G1	"Several" c.f., follicles 2 mm.	Incubating	Complete	25	433	19	0

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[1] c.f. = collapsed follicle(s).

[2] Bird would have laid in one or a few days.

of the integument also occurs. In view of these facts, the term "edematous" should not be used as a synonym of "thickened" in reference to the integument of the incubation patch area, which exhibits both edema and physiological hypertrophy. The relative contribution of these two factors (fluid imbibition and growth) could easily be determined by comparing wet and dry weights of the integument of breeding and nonbreeding females.

RESULTS

NONCAPTIVE BIRDS

Nonbreeding birds.—Measurements of the breast integument of nine nonbreeding male redwings and 16 nonbreeding cowbirds (12 males and 4 females) collected in

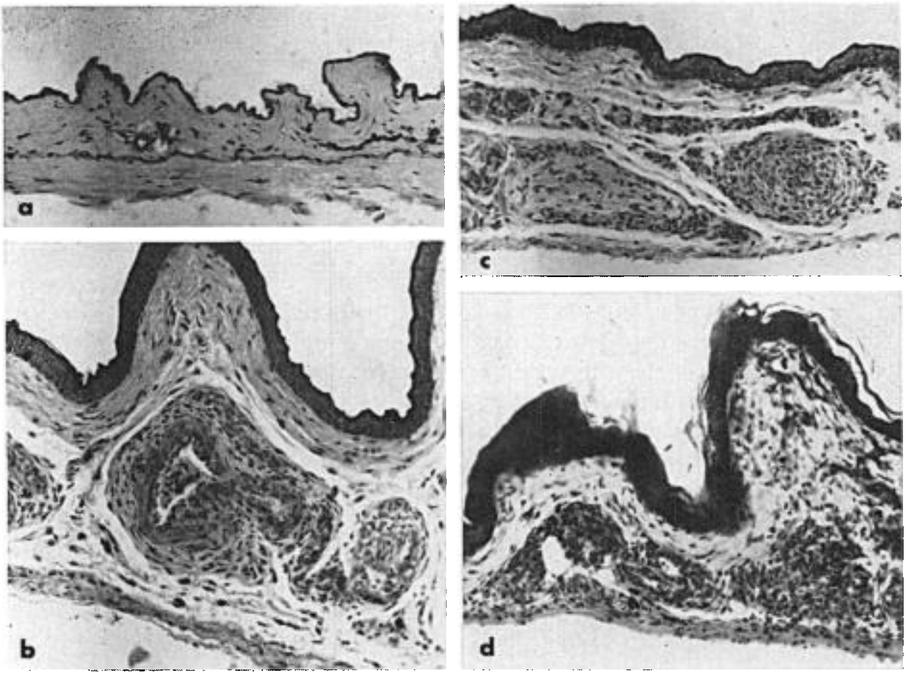

Fig. 1. Histologic sections of breast integument of icterids. a, Nonbreeding Redwinged Blackbird; b, wild female Redwinged Blackbird of Group III, incubating; c, wild female Redwinged Blackbird of Group II, laying; d, Redwinged Blackbird after injections of estrogen and progesterone, showing maximum level of response obtained in experimental birds.

March are given in tables 3 and 4. Comparing males and females from the nonbreeding and control groups, we failed to find in either species statistically significant sexual differences in thickness of the stratum germinativum and dermis or in number of blood vessels and fat cells. Comparing the two species, we found no difference in dermal thickness, but the stratum germinativum was significantly thinner, on the average, in redwings than in cowbirds, notwithstanding the fact that the former species is larger in body size (table 1). This is illustrated by the following measurements (mean ± standard error, in microns) of nonbreeding males of the two species:

Species	Number of specimens	Thickness of stratum germinativum
Redwinged Blackbird	9	4.55±0.22
Brown-headed Cowbird	15	5.74±0.33

Breeding birds.—Samples of breast integument of 17 noncaptive female redwings taken in April, May, and June were assigned to one of three groups reflecting progressive degrees of development of the incubation patch (table 2): Group I, early prelaying stage; Group II, advanced prelaying and laying stage; and Group III, incubating stage.

The condition of the ovary could be correlated with the degree of development of the incubation patch. In Group I (two birds), in which the ovaries were relatively small and inactive, the beginning of incubation patch formation was evidenced by the fact that a few feathers had been dropped from the center of the breast; but measurements of the integument and counts of blood vessels and fat cells differed slightly if at all from those of wild, nonbreeding individuals and laboratory control birds (table 3). Group II (eight birds) included individuals that were laying or would have laid in a few days. Five of the birds had no collapsed follicles (and, hence, had not ovulated), two had two collapsed follicles, and one had three collapsed follicles. Histologically, individuals of Group II were characterized by a thickening of the stratum germinativum (mean thickness, 15.3 μ) and dermis (mean, 168.8 μ) and by an increase in number (mean, 11.0 per mm.) and size of blood vessels and a decrease in number of fat cells (mean, 0.9 per mm.). The seven birds of Group III presumably were incubating. Measurements (table 3) showed further increase in thickness of both skin layers (means: stratum germinativum, 20.2 μ; dermis, 277.5 μ), an increase in vascularity (mean, 16.7 vessels per mm.), and a decrease in number of fat cells as compared with birds of Group II.

Our material shows that defeatherization is not completed before increasing vascularity and thickening of the integument are apparent, for both Group II and Group III included individuals with patches that were not completely defeathered. Probably some birds never completely defeather but, instead, retain a few down feathers near the margin of the ventral apterium even during incubation.

Additional evidence that dermal and epidermal thickening is evident in icterids before defeatherization is complete is provided by the following data obtained from prelaying female grackles. Common Grackle: Bird A (April 12); ventral apterium vascular and integument thickened; patch not completely defeathered, but with many loose feathers that dropped out when touched or rubbed lightly. Bird B (April 14); incubation patch just beginning to develop; only a few feathers lost but skin obviously thickened; ovarian follicles up to 4 mm. in diameter. Bird C (April 14); some feather loss

TABLE 3

BREAST INTEGUMENT OF REDWINGED BLACKBIRDS

Group	Number of birds[1]	Thickness (in μ) of stratum germinativum		Thickness (in μ) of dermis		Blood vessels per mm.		Fat cells per mm.	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Nonbreeding male	9	4.5	4–6	83.6	67–110	2.4	1–4	7.1	1–16
Breeding male	1	4		86		3		1	
Breeding female									
Group I	2	4.5	4–5	93.8	91–96	2.6	2–3	7.0	4–10
Group II	8	15.3	7–25	168.8	139–240	11.0	7–13	0.9	0–13
Group III	7	20.0	12–25	277.5	192–433	16.7	8–25	0.0	0–0
Experiment I									
Estrogen-estrogen and prolactin	4(3)	11.7	10–13	114.0	96–125	13.3	10–20	2.1	0–4
Estrogen and progesterone-estrogen and prolactin	1(1)	14		101		10		0	
Experiment II									
Control	4(1)	6.1	4–9	88.9	82–96	3.6	3–4	7.2	3–14
Progesterone	4(1)	6.5	5–8	82.9	63–91	1.7	1–2	3.2	1–6
Prolactin	4(1)	5.3	5–6	76.9	63–91	2.5	1–4	3.4	0–10
Estrogen	3(1)	14.8	12–19	113.8	101–135	10.3	8–13	3.7	1–8
Estrogen and prolactin	4(1)	13.7	10–21	113.0	86–135	7.4	4–10	5.0	1–13
Estrogen and progesterone	3(1)	18.8	11–28	128.2	106–159	8.8	7–10	5.4	0–11

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[1] Sexes combined; number of females indicated in parentheses.

TABLE 4

BREAST INTEGUMENT OF BROWN-HEADED COWBIRDS

Group	Number of birds[1]	Thickness (in μ) of stratum germinativum		Thickness (in μ) of dermis		Blood vessels per mm.		Fat cells per mm.	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Nonbreeding	16(4)	5.6	4–8	83.5	58–111	2.6	1–6	2.1	0–18
Breeding	3(1)	3.9	3–5	94.6	77–106	2.6	2–3	0.6	0–2
Controls	6(3)	5.4	4–6	83.3	63–106	2.1	1–3	3.7	1–7
Progesterone	5(2)	7.0	5–8	101.9	72–154	2.7	1–4	6.9	0–24
Prolactin	5(3)	5.3	4–7	92.3	77–120	2.3	1–3	2.1	0–9
Estrogen	6(3)	6.3	3–8	84.1	63–111	2.8	2–4	1.4	0–2
Estrogen and prolactin	6(3)	5.1	4–6	84.1	77–91	1.9	1–4	1.0	0–10
Estrogen and progesterone	5(3)	5.9	4–9	87.8	63–111	2.6	1–5	2.8	0–6

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[1] Sexes combined; number of females indicated in parentheses.

evident on breast and abdomen; skin somewhat thickened; ovary with enlarged follicles. Bird D (April 14); skin vascular and thickened but defeatherization only three-fourths to four-fifths complete; ovarian follicles to 12 mm. in diameter. Boat-tailed Grackle: Bird A (April 12); skin thick and moderately edematous but ventral apterium only one-third to one-half defeathered; ovarian follicles to 3 mm. in diameter.

Measurements of the breast integument of a breeding male redwing (table 3) and of breeding male and female cowbirds (table 4) show no significant differences from those of nonbreeding birds of the same species, except for a reduction in number of fat cells.

EXPERIMENT I

Redwinged Blackbird.—All redwings, both males and females, receiving hormones showed some defeatherization in Phase 1 of the experiment. Four birds experienced extensive feather loss within a week of the first injection, while in six birds only moderate defeatherization was apparent after a week and a half of treatment. Rate of defeatherization was thus variable individually and there was no apparent difference among groups. The degree of defeatherization attained by the end of the experiment varied from two-thirds to complete loss of feathers from the ventral apterium. In all birds which completely defeathered, this condition was achieved by the 12th day of the experiment; and the shortest time required for complete defeatherization was nine days, which was recorded for a female of Group 1AB. All redwings receiving estrogen exhibited increased vascularity, with the average number of blood vessels ranging from 3.2 to 12.0 per mm. above the mean for nonbreeding birds. All but two birds showed moderate thickening of the stratum germinativum and dermis.

From Phase 2 of the experiment we obtained evidence that a more prolonged period of hormonal treatment does not enhance development of the incubation patch beyond the level obtained within a two-week injection period, which was the length of Phase 1 of Experiment I and also the length of Experiment II (see beyond). Measurements of the integument of a male and three females which received estrogen for two weeks in Phase 1 and estrogen and prolactin for another week in Phase 2 were similar to those of birds in Experiment II (table 3) receiving estrogen or estrogen and prolactin for only two weeks. And the patch was no better developed in a female in Experiment I which received injections of 1.0 mg. estradiol benzoate and 0.25 mg. progesterone in Phase 1 and injections of 1.0 mg. estradiol benzoate and 50 I.U. prolactin in Phase 2 (table 3).

Defeatherization and vascularity appeared in variable sequence. Vascularity was noted before feather loss in four birds, at the time of first feather loss in six birds, and not until after defeatherization was in progress in three individuals. Thickening of the integument was noted before feather loss in three birds, at the time of first feather loss in eight birds, and later in two birds. In two individuals receiving estrogen and progesterone in Phase I and progesterone (Bird 22) or estrogen and prolactin (Bird 36) in Phase 2, the integument became only slightly thickened, although it showed increased vascularity and a moderate degree of feather loss.

Incubation patches were not developed by control redwings.

Brown-headed Cowbird.—There were no visible changes in the ventral apterium of any of the cowbirds.

In summary, the results of Experiment I showed that incubation patches can be induced in male and female Redwinged Blackbirds, but not in Brown-headed Cowbirds, by injecting estradiol benzoate; but the induced patches were not identical with those of incubating birds, the most notable difference being a lesser degree of dermal thickening. The temporal relationship of defeatherization and increased vascularity and thickening of the integument in artificially induced patches was highly variable individually. The experiment also suggested that treatment with estrogen for two weeks and with estrogen and prolactin for an additional week did not enhance development of the induced patch. Finally, the experiment demonstrated that the integument of the ventral apterium of redwings is responsive to hormonal stimulation by the time the birds are five or six months old.

Summary of Observations on Response of Cowbirds to Nests Under Conditions of Experiment I

In the course of Experiment I, each redwing and cowbird was on several occasions presented with nesting material (grass) and an unfinished redwing nest containing eggs of its own species; the reactions of the birds were recorded in detail by observers viewing the cages through a one-way mirror. Following are accounts of observations of six cowbirds which responded by settling in the cup of the nest.

Female number 17. Treatment: Phase I (Oct. 25–Nov. 10), Group 1AB (0.5 mg. estradiol benzoate); Phase 2 (Nov. 11–22), Group 2C (progesterone). Nov. 6, presented with nest and nest material (25-minute test-period): settled in nest cup 5 times for periods from 2 to 5 seconds in length. Nov. 9, presented with nest containing 4 cowbird eggs (15 minutes): settled in nest cup for periods from 5 to 90 seconds. Nov. 16, presented with empty nest (10 minutes): settled twice for 2 seconds. Nov. 22, presented with nest containing 2 cowbird eggs (4 minutes): settled once for 2 seconds. Nov. 29 (9 days after cessation of hormone treatment), presented with empty nest (13 minutes): crouched in nest cup 4 times for periods ranging from 3 to 20 seconds but did not settle down fully in cup.

Female number 19. Treatment: Phase 1, Group 1AB (0.25 mg. estradiol benzoate); Phase 2, Group 2A (estrogen and prolactin). Nov. 6, presented with nest and nest material (20 minutes): crouched in cup for a few seconds and later settled for 1 second. Nov. 10, presented with nest containing 4 eggs (12 minutes): did not visit nest. Nov. 16, presented with empty nest (10 minutes): did not visit nest. Nov. 22, presented with nest containing 2 eggs (5 minutes): flew to nest and pecked at eggs for 15 seconds; nest and eggs removed from cage and observation discontinued.

Female number 27. Treatment: Phase 1, Group 1C (estrogen and progesterone); Phase 2, Group 2A (estrogen and prolactin). Nov. 6, presented with nest and nest material (20 minutes): made strong intention movements to settle in cup and later settled for 60 seconds, stood up for a moment, and resettled for 20 seconds. Nov. 11, presented with empty nest (15 minutes): made intention movement ("half-crouch") to settle in cup. Nov. 22, presented with nest containing 4 eggs: immediately flew to nest and pecked at eggs; nest and eggs removed from cage and observation discontinued. Nov. 30 (10 days after cessation of hormone treatment), presented with empty nest (20 minutes): spent a total of 4 minutes and 17 seconds standing in or scratching at bottom of nest cup; made two intention movements to settle in cup.

Female number 21. Treatment: Phase 1, Group 1AB (0.25 mg. estradiol benzoate); Phase 2, Group 2C (progesterone). Nov. 8, presented with nest and nest material (20 minutes): made four intention movements ("crouched") to settle and once settled in cup for a few seconds. Nov. 10, presented with nest containing 4 eggs: immediately flew to nest and pecked at eggs; after 1 minute nest and eggs removed from cage and observation discontinued. Nov. 16, presented with empty nest (10 minutes): half-crouched in cup for a few seconds and later settled for a few seconds. Nov. 22, presented with empty nest (10 minutes): spent much time perched on rim of nest but did not stand or settle in cup. Nov. 29 (9 days after cessation of hormone treatment), presented with empty nest (13 minutes): crouched in nest cup for 10 seconds, then settled down in cup but almost immediately left nest when alarmed by outside noise.

Female number 39. Treatment: Phases 1 and 2, groups 1D and 2D (control). Nov. 9, presented with nest and nest material (20 minutes): spent much time perched on rim of nest but did not enter cup. Nov. 16, presented with empty nest (15 minutes): crouched in nest for 30 seconds, then began foraging while standing in cup and crouched in cup for 2 seconds. Nov. 30, presented with empty nest (20 minutes): crouched in cup 3 times and settled in cup 7 times for periods ranging from 1 to 12 seconds.

Male number 5. Treatment: Phase 1, Group 1AB (0.5 mg. estradiol benzoate); died of undetermined cause on Nov. 8. Nov. 2, presented with nest and nest material (30 minutes): immediately hopped to rim of nest, stepped into cup, and settled there for 3 seconds; repeated this pattern of behavior 3 times again; later stepped into cup for a second but did not crouch or settle.

Comments.—A bird was recorded as "settling" in the nest cup when the body was lowered to a point at which the breast feathers made contact with the nest. In a "crouch" the body was lowered to some degree but not enough to bring the feathers into contact with the nest. An "intention movement" to settle was simply a crouch of short duration.

In the light of the observations detailed above, we are now convinced that the reported (Selander, 1960) "nest-molding movements" of an estrogen-treated female cowbird in a wire feeding cup were motions involved in an attempt by the bird to settle in the cup. None of the cowbirds observed by us has performed motor patterns clearly corresponding to the nest-building movements of passerines (Van Dobben, 1949, and Marler, 1956:127–129). Not infrequently, four of the six cowbirds scratched with their feet on the bottom of the nest cup before crouching or settling. But this was not the "scrabbling" movement of nest building described by Marler (1956:128) for the Chaffinch (*Fringilla coelebs*); rather, it seemed to be identical to a scratching movement made by cowbirds when foraging on the ground (Selander, MS).

Our notes on the behavior of female number 39 indicate that the settling response to a nest may be elicited in a bird that has not received hormone injections. We also call attention to the fact that female number 21 settled in the nest cup in a test conducted nine days after the last injection of hormone (progesterone) was administered. In fact, we are not entirely convinced that responsiveness to the nest is at all enhanced by hormone treatment.

EXPERIMENT II

Redwinged Blackbird.—There was no loss of feathers in birds of the prolactin, progesterone, and control groups. The thickness of the stratum germinativum and dermis of the prolactin group and the progesterone group was not different from that of the control group or of nonbreeding birds (table 3). Additionally, numbers of blood vessels were similar in the prolactin, progesterone, and control groups and in the sample of nonbreeding birds.

Degree of defeatherization was individually variable within the estrogen group. One male lost all feathers from the ventral apterium, while the other male of this group retained five feathers on the center of the breast and many along the margin of the apterium. The lone female of the estrogen group retained three feathers in the center of the breast and also a few marginally. All four birds of the estrogen-prolactin group showed complete defeatherization; but the estrogen-progesterone group showed the same degree of variation as the estrogen group, with one bird becoming completely defeathered, one retaining a few marginal feathers, and one retaining 15 feathers.

Size and number of blood vessels were roughly equivalent in the estrogen, estrogen-prolactin, and estrogen-progesterone groups but greater than in the controls and in the sample of nonbreeding birds (table 3). Compared with breeding birds, individuals of these groups showed an increase in vascularity approximately equal to that seen in individuals in the advanced prelaying and laying stage (Group II).

The thickness of the stratum germinativum in the estrogen and the estrogen-prolactin groups (means, 14.8 and 13.7 μ, respectively) was similar to that of redwings of Group II, which were approaching laying and laying; and the stratum germinativum in the estrogen-progesterone group (mean, 18.8 μ) was even thicker, being similar to that of breeding females of Group III (table 3).

The dermis in the estrogen group (mean, 113.8 μ) and in the estrogen-prolactin group (mean, 113.0 μ) was thicker than in either the controls or nonbreeding birds, but it was not as thick as that of any of the breeding female birds (groups II and III). In the estrogen-progesterone group, the dermis appeared to be thicker than in either the estrogen group or the estrogen-prolactin group but was not equivalent in this respect to that of breeding birds in the advanced prelaying and laying stage (Group II). In none of the experimental birds was the dermis as thick as in incubating birds (Group III); see figure 1.

Brown-headed Cowbird.—None of the 33 cowbirds surviving to the end of the experiment showed defeatherization, thickening of the stratum germinativum or dermis, or increase in number of blood vessels; and no significant variation in number of fat cells was noted. The dermis of one female of the progesterone group was unusually thick (154 μ), but the measurement of this bird probably falls within the range of variation expected in a large sample of untreated cowbirds (see table 4).

To summarize, the results of Experiment II again showed that the integument of the ventral apterium of cowbirds fails to respond to hormonal treatment. In redwings, prolactin or progesterone alone produced no effect. When compared with breeding redwings in the advanced prelaying and laying stage (Group II), individual redwings of the estrogen group and the estrogen-prolactin group showed similar degrees of defeatherization, vascularity, and thickness of the stratum germinativum; but they failed to develop equivalent thickening of the dermis. The estrogen-progesterone group showed development more nearly equal to that of breeding birds in Group II, the dermis being slightly thicker than in birds of the estrogen and the estrogen-prolactin groups.

DISCUSSION

Formation of the incubation patch.—Evidence from wild Redwinged Blackbirds indicates that defeatherization of the ventral apterium is usually complete before egg laying begins. Of the five breeding redwings of Group II that were approaching laying, four were completely defeathered. This finding agrees with those of Bailey (1952), Petersen (1955), Brackbill (1958), and Hinde (1962) for other species of passerines.

The time required by wild birds for defeatherization was not determined, but in experimental birds complete feather loss was obtained 9 to 12 days after injections were started. In domestic canaries, the defeatherization process lasts from 13 to 26 days (Hinde, 1962); and Petersen (1955:271) reported that "several days" are required for defeatherization in the Bank Swallow (*Riparia riparia*). Bailey (1955:302) noted gradual defeatherization in the tinamou (*Nothoprocta ornata*) and in galliform birds, but actual lengths of the periods for these birds were not reported. In view of these findings, Bailey's report (1952:125) of complete defeatherization in wild White-crowned Sparrows (*Zonotrichia leucophrys*) and Oregon Juncos (*Junco oreganus*) occurring precipitously within a 24-hour period, four or five days before the first egg is laid, requires confirmation. Rate of defeatherization may in fact be relatively rapid in some finches, for Bailey also reported (1952:130) that defeatherization was accomplished in three days in House Finches (*Carpodacus mexicanus*) treated with estrogen.

In icterids an increase in vascularity and some thickening of the epidermis and dermis occur before egg laying and coincident with feather loss. This is apparent in five noncaptive, breeding redwings of Group II that had not ovulated but showed significant increases in integumentary thickness and vascularity. Also, in Experiment I increased vascularity was apparent to the naked eye in the period of defeatherization in at least ten birds. And we have already cited data on female grackles which had vascular and thickened integument although defeatherization was still in progress. Similarly, Hinde (1962) found vascularization occurring synchronously with defeatherization in canaries. These findings are at variance with Bailey's report (1952) that defeatherization in wild passerines is completed before the structural changes of thickening and vascularity are apparent. Moreover, as noted by Eisner (1960:161), in Bailey's experimental birds vascularity was reported to have appeared coincident with, if not before, defeatherization.

By the end of the egg-laying period in White-crowned Sparrows, Bailey (1952) found well developed vascularity but only slight thickening of the integument; and, also, he noted an increase in vascularity and, especially, dermal "edema" during incubation. Comparison of measurements of breeding female redwings of groups II and III with those of nonbreeding birds supports Bailey's findings. The mean number of blood vessels in birds of Group II was greater than that in nonbreeding birds, but it was less than in birds of Group III (table 3). This indicates that the greatest increase in vascularity occurs in the egg-laying period and that there is further increase during incubation. Birds approaching laying or laying (Group II) showed a mean dermal thickness about twice that of nonbreeding birds, which would indicate that a considerable degree of thickening is already developed at the time of laying. In incubating birds (Group III) mean thickness of the dermis was more than three times as great as in nonbreeding birds. Our data (table 2) also show that the stratum germinativum is much thicker in laying than in nonbreeding birds and undergoes further hypertrophy during incubation. The fact that incubating redwings had only about five more blood vessels per millimeter of section than did laying birds supports Bailey's conclusion (1952) that vascularization reaches maximum development earlier than does integumentary "edema."

Feather follicles, but not feather papillae, remain visible in the full incubation patch of redwings, although they may degenerate from the nonbreeding condition, as indicated by the absence of a lumen from the follicles of some incubating birds in our sample. Bailey (1952) did not mention whether or not follicles persist in fringillid incubation patches, but he reported that the feather papillae degenerate.

Because our study was terminated before the end of the breeding season, we failed to obtain data on refeathering and other aspects of the recovery phase of the incubation patch. In other birds it is reported that thickening and vascularity begin to diminish several days before the eggs hatch (Petersen, 1955:271); and the skin has "almost returned to normal" as regards vascularity and thickness by the time the young are ready to fledge (Bailey, 1952:127). Bailey (*loc. cit.*) believed that the ventral apterium remains unfeathered until the postnuptial molt. However, Hinde (1962:94–95) found refeathering in canaries beginning in the absence of a molt within ten days after eggs were removed from an incubating bird; and one female showed feather growth on the ventral apterium during incubation. In the Boat-tailed Grackle, Selander and Nicholson (1962:85–86) found that the incubation patch of autumnal breeding females is refeathered following incubation although the birds do not experience a postbreeding molt.

Hormonal control of the incubation patch.—This study has shown that incubation patches can be induced equally well in male and female Redwinged Blackbirds by treatment with estrogen, alone or in combination with prolactin or progesterone; no response is obtained by injecting either prolactin or progesterone alone. All processes involved in formation of the incubation patch (defeatherization, increase in vascularity, and hypertrophy and edema of the integument) appear to be dependent upon a high level of estrogen; and we may conclude that hormonal control of the incubation patch in icterids is similar, at least in this respect, to that of fringillids (Bailey, 1952; Hinde, 1962). Bailey's work (1952) with hypophysectomized fringillids suggests that defeatherization and the development of dermal "edema" depends upon the production of prolactin by the pituitary in response to estrogen stimulation, but the present study was not designed to investigate this aspect of the problem. However, it should be noted that the mode of action of prolactin on the integument of the incubation patch remains to be determined; possibly the action is direct, but prolactin may also have its effect by stimulating secretion of progesterone by the gonads. In mammals, prolactin is presumed to be essential for progesterone synthesis by luteal tissue (Gorbman and Bern, 1962: 242). Also, it is somewhat surprising that edema did not occur in Bailey's hypophysectomized birds receiving estrogen, since edema is a common response of tissues to estrogen administration (F. L. Hisaw, Jr., 1959).

In redwings there were no differences between incubation patches induced by the combination of estradiol and prolactin and those produced by injections of estradiol alone, except that an average greater degree of defeatherization was achieved by birds receiving both hormones; thus, in Experiment II defeatherization was complete in all four individuals receiving estrogen and prolactin but in only one of four birds receiving estrogen alone. This result suggests that prolactin acts with estrogen to influence defeatherization, a conclusion previously reached by Bailey (1952) in his work with fringillids.

Although progesterone secretion is thought to occur, at least in some birds, during nest building (Lehrman, 1958; 1961:1293–1294) and at approximately the same time as defeatherization, this hormone does not appear to affect the defeatherization process in experimentally induced incubation patches. Of three redwings receiving estrogen and progesterone, only one showed complete defeatherization; and this result is similar to that obtained from the group receiving estrogen alone.

The stratum germinativum and dermis were slightly thicker on the average in the estrogen-progesterone group than in either the estrogen or the estrogen-prolactin groups, suggesting that progesterone augments development of edema and other factors producing integumentary thickening. In Experiment II, birds of the estrogen-progesterone group developed patches most nearly equivalent to those of laying wild redwings. These results are actually not unexpected, since progesterone commonly acts synergistically with estrogens and other steroids (Mason, 1952; Lehrman and Brody, 1957; Turner, 1960).

If it is true that nest-building behavior induces progesterone secretion in passerines, as in doves (Lehrman, 1958), it may be that progesterone, in synergism with estrogen, initiates hypertrophy of the ventral apterium. Prolactin, the level of which is presumably high during incubation (Eisner, 1960), may in turn be responsible for enhancing and maintaining this response.

The results of this study strongly suggest that an incubation patch equivalent in all respects to that of an incubating wild bird cannot be induced in redwings by injection of estrogen, alone or with either prolactin or progesterone. By treating House Finches with estradiol and prolactin, Bailey (1952:131) produced in nine days incubation patches which "would have been impossible to distinguish . . . from that of a breeding female." But it is possible that histologic measurements would have revealed differences comparable to those found in the present study. In any event, although incubation patches induced in redwings may be fully defeathered, they differ from those of incubating birds in showing lesser dermal thickening, a lower degree of vascularity, and greater retention of fat cells (table 3). This applies not only to birds in Experiment II, in which there was no opportunity for estrogen "priming" of tissues before progesterone or prolactin was administered, but also to those individuals in Group 2A of Experiment I, which received seven injections of estrogen before receiving prolactin. These findings lead to the hypothesis that full development of dermal edema and hypertrophy and of vascularity depends upon some special sequential action of estrogen, progesterone, and prolactin, perhaps operating over a longer period than that provided in the experiments. Another hypothesis is that tactile stimulation of the ventral apterium, such as that provided by eggs during incubation, may be essential for full development of the incubation patch. There is also the possibility that the superphysiological doses of hormones administered in the experiments adversely affected processes related to normal incubation patch formation; and there is some chance that other estrogens or other hormones not yet tested experimentally are involved in the normal physiological control of the incubation patch.

LOSS OF RESPONSE TO HORMONES IN PARASITIC COWBIRDS

This study demonstrates that none of the processes involved in formation of the incubation patch can be induced to any degree in parasitic cowbirds by administration of estradiol benzoate or of prolactin or progesterone, alone or in combination with estradiol benzoate. These findings extend those of Selander (1960) and support the conclusion that failure of parasitic cowbirds to develop incubation patches is due to insensitivity of the integument to those hormones which mediate formation of the patch in nonparasitic species. This finding is consistent with the generalization that the evolution of endocrine mechanisms "is not an evolution of hormones but an evolution of the uses to which they are put; an evolution . . . of reactivities, reaction patterns and tissue competences" (Medawar, 1953:334; see also Zuckerman, 1955; Hamilton, 1962; and exceptions to this generalization discussed by F. L. Hisaw, 1959, and Gorbman and Bern, 1962). The principle applies not only to endocrine-controlled morphologic responses but to behavioral mechanisms as well.

Some aspects of the basis for this generalization are apparent. Aside from limitations in evolutionary possibilities at the biochemical level (details of which must await further knowledge of the biochemical action of hormones), there would seem to be limitations imposed by the fact that a given hormone may have several actions (morphologic, physiologic, or metabolic) affecting a variety of "targets" and thus may influence a large number of characters subject to natural selection. If one character loses adaptive significance, selection will operate to eliminate the action of the hormone on the particular target concerned; but this cannot readily be achieved by changes in hormone titers or in molecular structure of the hormone, for such changes would be selected against as a result of their nonadaptive effects on other targets.

In the course of evolution of brood parasitism of cowbirds, we presume that the ventral apterium became increasingly refractory to hormonal stimulation and thus failed to develop an incubation patch. Theoretically, it might be argued that disappearance of the patch led to a failure to incubate, but we think it unlikely that loss of the patch was causally related to evolution of brood parasitism. Rather, loss of this structure may be viewed as a natural consequence of selection once birds were in an environmental situation in which failure to incubate was adaptive. Refractoriness of the integument would then mean a saving of energy otherwise expended on a nonadaptive structure; and, although incubation behavior in birds is not strictly dependent upon the presence of an incubation patch (Eisner, 1960:163), absence of the patch would doubtless also be adaptive in reducing the probability of a bird responding to eggs by incubating.

In much the same way as the integument of the ventral apterium became unresponsive to estrogen and other hormones which normally control formation of the incubation patch, it seems probable that hormone-sensitive neural and other mechanisms mediating nest building, incubation, and parental behavior lost their responsiveness to hormonal stimulation, thereby contributing to the elimination of behavior patterns that were no longer adaptive. For reasons already discussed in relation to the evolutionary loss of the incubation patch, we are inclined to reject the hypothesis (Friedmann, 1960:37) that "endocrine imbalance or change, may have brought about the loss of broodiness and ushered in the parasitic mode of reproduction." Causal factors underlying the evolution of brood parasitism may, in our opinion, more profitably be sought in ecologic areas; and postulation of endocrine "imbalance," "lag," or "changes" as factors of this type does not seem justified.

As a result of our recent unsuccessful attempt to elicit nest-building and incubation behavior in Brown-headed Cowbirds by injecting hormones and observing responses to nesting material and to nests and eggs, we are now strongly inclined to believe that it will not be possible by hormonal manipulation to induce cowbirds to perform behavior that is not part of the normal behavioral repertoire of the species. The redwings and cowbirds used in Experiment I were presented with nesting material (grass), an unfinished nest, or a nest and eggs. In the redwings, behavior that was clearly part of reproductive patterns was shown by only three birds (a female receiving injections of estrogen and progesterone and a male and a female receiving estrogen), which occasionally carried grass to unfinished nests but dropped the material without making nest-building motions. We attribute our almost complete failure to elicit nesting or incubation behavior in this species in part to the fact that the birds were extremely wild and frightened in captivity; escape reactions, which in some individuals became habitual, stereotyped patterns, dominated the behavior of the birds and presumably inhibited responses to nests and eggs that might otherwise have been forthcoming.

Notwithstanding the general failure of redwings to respond to the stimuli presented, we are inclined to attribute some significance to the failure of cowbirds to respond, since individuals of this species are relatively tractable in captivity, and many of our experimental birds showed no evidence of fear during the observation periods. None of the cowbirds carried nest material or performed nest-building motions; but, when presented with an empty redwing nest or with a nest containing cowbird eggs, five females and one male responded by settling in the cup of the nest and remaining there for periods ranging from 1 to 90 seconds. (See p. 80 for an account of the experimental conditions and the responses of these individuals.)

On first consideration, it might appear that the six cowbirds had been induced to incubate, that is, to perform a pattern of behavior not manifested by the species but which has nonetheless remained latent. More probably the birds were simply exhibiting a behavior pattern normally performed by female cowbirds when they are laying eggs in the nests of hosts. Mayfield (1960) reports that female cowbirds visiting nests of the Kirtland Warbler (*Dendroica kirtlandi*) remained on the nests for periods up to 25 seconds; and Howell (1914) saw a female cowbird remain on the nest of a Red-eyed Vireo (*Vireo olivaceus*) for "about two minutes," in which period the bird apparently laid an egg. A cowbird watched by Friedmann (1929:185) at the nest of an American Robin (*Turdus migratorius*) "lit on the Robin's nest, looked around a little, and then settled on it. She shifted her position three times, a little bit each time and then settled down in the same way that a Robin does on the nest." Hann's photographs (1941:figs. 1, 2) of female cowbirds on nests of the Ovenbird (*Seiurus aurocapillus*) show the birds in postures similar to those assumed by our experimental birds responding to nests placed in their cages.

The fact that cowbirds, but not redwings, sometimes responded to nests by settling in the nest cup may reflect nothing more than specific differences in adjustment to captivity, as mentioned previously. It is also possible that the cowbird, which parasitizes a large variety of bird species having diverse nest types (Friedmann, 1929), is for adaptive reasons capable of being stimulated by more "generalized" features of nest structure than is the redwing. (In this connection, it is worth noting the report of F. L. Rand [*in* Friedmann, 1929:184], who induced a captive female cowbird to lay 13 eggs in a variety of nests containing candy eggs that were placed about the room of a house.) Moreover, in a colonial species like the redwing, we might expect especially strong selection against tendencies toward indiscriminate responsiveness to nests because of the danger of a bird being stimulated to perform reproductive activities at nests other than its own.

Although nesting and incubation behavior does not appear to have remained latent in Brown-headed Cowbirds, it has been reported (Bonwell, 1895; Forbush, 1927; Fletcher, 1925) that female cowbirds occasionally respond to juvenal cowbirds by feeding them. That this parental response has not been completely lost in the evolution of brood parasitism in cowbirds is perhaps understandable on the basis of the fact that, by failing to perform nesting and incubation behavior, cowbirds rarely encounter stimulus situations requisite for elicitation of the parental behavior of feeding, and thus there is no selection against it. Also, occasional feeding of young cowbirds is probably less maladaptive for a parasitic bird than is wasting time and effort in desultory nest building and incubation. Finally, cowbirds would most likely encounter young after their own reproductive period, when the feeding response would not interfere directly with their reproductive efforts and would not, on this basis, be selected against.

In closing this discussion, we wish to mention the possibility of ontogenetic changes in degree of refractoriness of cowbirds to hormonal stimulation, both as regards the integument of the ventral apterium and nesting and incubation behavior. Additional testing seems warranted on the hypothesis that younger birds may be more responsive than the individuals used in the present study, all of which were five or more months old.

SUMMARY

This study has been a combined field and experimental investigation of the development and hormonal control of the incubation patch in icterids.

In development of the incubation patch, increase in thickness of the integument of the ventral apterium results from a combination of factors, including dermal edema and increase in number and size of blood vessels, number of dermal connective tissue cells, and number of cell layers in the stratum germinativum. In wild female Redwinged Blackbirds and grackles, as well as in male and female redwings receiving hormone treatment in the laboratory, integumental thickening and increasing vascularity are apparent before defeatherization of the apterium is completed; in some birds increased thickening and vascularity may be evident before feather loss begins.

In wild icterids, defeatherization is usually completed before egg laying begins. The greatest increase in vascularity occurs in the egg-laying period, but there is further increase during incubation. Birds ready to lay or laying show a mean dermal thickness about twice that of nonbreeding birds; and in incubating birds the dermis is more than three times as thick as in nonbreeding birds. Similarly, the stratum germinativum is thicker in laying birds than in nonbreeding individuals and undergoes further hypertrophy during incubation. Vascularization reaches maximum development earlier than does dermal edema.

By injecting estradiol benzoate, either alone or in combination with prolactin or progesterone, incubation patches were induced equally well in male and female redwings. In experimental birds, prolactin apparently enhanced the average degree of defeatherization achieved but did not increase dermal or epidermal thickness or degree of dermal vascularity. Birds receiving estrogen and progesterone developed patches most nearly equivalent to those of laying wild redwings, but none of the experimental birds (including four that received estrogen for two weeks and estrogen and prolactin for an additional week and one female that received estrogen and progesterone for two weeks and estrogen and prolactin for an additional week) developed incubation patches equivalent in integumentary thickness and degree of vascularity to those of incubating birds. It is suggested that full development of the patch may depend upon some special sequential action of estrogen, progesterone, and prolactin or upon tactile stimulation of the integument of the ventral apterium. Redwings injected with progesterone or prolactin alone failed to show any of the processes involved in development of the incubation patch.

Gross and histologic examination of the integument of Brown-headed Cowbirds which had received injections of estrogen, progesterone, and prolactin, alone or in combination, failed to reveal any response. The results of the study confirm an earlier conclusion (Selander, 1960) that the integument of the cowbird is refractory to hormonal stimulation.

An attempt to induce nest building and incubation behavior in captive cowbirds proved unsuccessful. Six individuals responded to nests or nests containing cowbird eggs by settling in the nest cups. This behavior is interpreted not as induced incubation, but as a pattern normally performed by female cowbirds when they lay eggs in the nests of hosts; possibly the response to nests is independent of hormone treatment.

Some aspects of loss of the incubation patch and of nesting and incubation behavior in parasitic cowbirds are discussed in relation to the Medawar-Hisaw generalization concerning the evolution of endocrine systems. Refractoriness of the integument and of presumed neural mechanisms mediating nesting and incubation behavior to hormones is regarded as a consequence of natural selection once birds are in an environmental

situation in which failure to incubate is adaptive. The hypothesis that "endocrine imbalance" is a causal factor in the evolution of brood parasitism is rejected.

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