EFFECTS OF INTRAHYPOTHALAMIC IMPLANTATION OF TESTOSTERONE PROPIONATE AND OF CYPROTERONE (AN ANTIANDROGEN) ON THE TESTICULAR CYCLE OF THE TREE SPARROW (SPIZELLA ARBOREA)

by

EDWARD KENT CUSICK

B. A., University of Northern Colorado, 1968

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

BIOLOGY

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

Approved by:

Fred E. Wilson

Major Professor

TABLE OF CONTENTS

THIS BOOK CONTAINS NUMEROUS PAGES WITH THE PAGE NUMBER ON THE FRONT SIDE OF THE PAGE AND THE INFORMATION ON THE BACK SIDE OF THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.

Testicular development is seasonal and photoperiodically induced in many species of birds that breed in the North Temperate Zone. After reaching maturity, testes are maintained in full functional status for a short time; then they regress (for reviews, see King et al., 1966; Hamner, 1968). Regression begins spontaneously while days are long both in nature or under artificial lighting regimes.

The role of the hypothalamo-hypophysial axis in the control of annual gonadal cycles of birds has been investigated extensively during the past decade (for reviews, see Farner et al., 1967; Lofts et al., 1970). The tubero-infundibular region of the hypothalamus has been implicated in the control of pituitary gonadotropin secretion in several species by lesion techniques (Wilson and Farner, 1965; Lepkovsky and Yasuda, 1966; Graber et al., 1967; Koike and Lepkovsky, 1967; Wilson, 1967; Wilson and Hands, 1968; Sharp and Follett, 1969; Snapir et al., 1969; Stetson, 1969) and by implantation techniques shown sensitive (as revealed by reduced pituitary gonadotropic function) to increased local concentrations of androgen both in the domestic mallard (Gogan and Kordon, 1964; Kordon and Gogan, 1964; Gogan, 1968) and in the Tree Sparrow (Wilson, 1970). The latter finding, confirming the potential for inhibitory feedback of androgen on gonadotropin secretion, raises the possibility that feedback of androgen onto the central nervous system may be involved in the onset of testicular regression in some photoperiodic species (see Farner, 1961, 1964, 1967, 1970; Farner and Follett, 1966; Kobayashi and Farner, 1966; Wilson, 1970). To explore that possibility, testosterone propionate (TP) or the free alcohol of cyproterone [(6-chloro-17-hydroxy-1α,2α-methylenepregna-4,6-diene-3,20-dione) CYP], an androgen antagonist with little or no testosterone-like activity (Bloch and Davidson, 1967, 1971; von Berswordt-Wallrabe and

Neumann, 1967, 1968; Davidson, 1969a, 1969b; Davidson and Bloch, 1969; Stern and Eisenfeld, 1969; Goslar et al., 1970; Karfsmeier and Davidoff, 1970; Neumann et al., 1970), was implanted stereotaxically into the hypothalamo-hypophysial axes of Tree Sparrows (Spizella arborea). In one experiment, TP was implanted to see if testicular regression could be induced. In another, CYP was implanted to see if antiandrogen might prevent or retard spontaneous testicular regression in chronically photostimulated birds. Results of both experiments suggest that androgen plays an important role in the onset of spontaneous testicular regression in Tree Sparrows and, further, that the site of androgen sensitivity may be a relatively discrete region of the basal infundibular nucleus.

METHODS

GENERAL

Tree Sparrows captured with mist nets from wintering flocks near Manhattan, Kansas, between 19 December 1969 and 21 February 1970 and retained in walk-in aviaries or 3 to 5 per cage in small cages (26x27x50 cm) were held on 8-hour (0830-1630 CST) daily photoperiods until late summer when experiments began. Food (a vitamin- and mineral-enriched chick starter crumble mixed with parakeet food) and water were freely available. Temperature varied a few degrees about 22°C. At least 3 months prior to experiments, birds were sexed by laparotomy under Nembutal anesthesia (Donovan, 1958); only males were used.

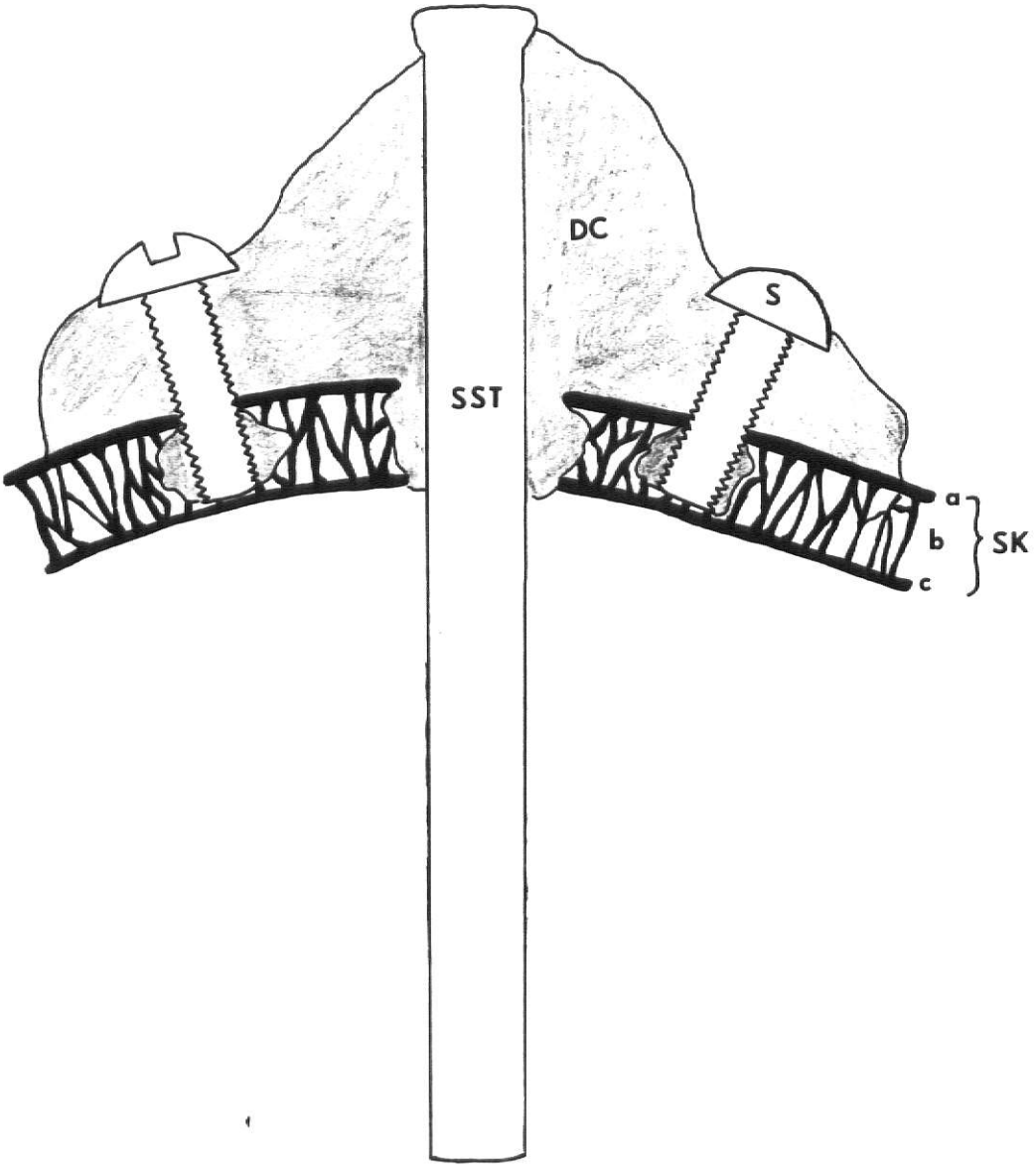
Stainless steel tubes [27-gage (OD = 0.41 mm; ID = 0.20 mm)] containing minute amounts of TP, CYP, or cholesterol (CH) mixed homogeneously with cocoa butter [CCB (2 parts steroid to 1 part CCB by weight)], or CCB alone, were implanted stereotaxically into the hypothalamo-pituitary axes of males (for procedure, see Wilson, 1967, 1970). Tubes were filled by tamping in mortars plus occasional packing of lumenal contents with a small wire; ends of filled tubes were dipped in saturated sucrose solution to protect against loss of contents. After implantation, tubes were fixed in place with Teets "Cold Cure" dental cement (Co-Oral-Ite Dental Mfg. Co., Box 793, Santa Monica, Ca. 90406). Small Screws (0-80 round head, C. L. Presser Hdw. Co., 3524 Market Street, Philadelphia, Pa. 19104) aided in adhesion of cement to skull (Fig. 1).

At the end of the experiments, birds were killed by decapitation. Brains were fixed 3 to 5 days in Bouin's fluid, dissected according to Oksche et al. (1959), embedded in Paraplast through dioxane, sectioned

Fig. 1. Sketch showing method of anchoring carrier tube. DC = dental cement; S = stainless steel round head screw; SST = 27-gage stainless steel tube; SK = skull; a = outer laminar layer; b = middle reticular layer; c = inner laminar layer. About 40 times natural size.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.


DC
S
SST
a
b
c
SK

parasagittally (10μ), and stained with paraldehyde-fuchsin for identification of implant centers. Testes and seminal sacs were fixed 5 days in AFA, transferred to 70% ethanol for 5 additional days, and then debrided and weighed on a torsion balance. Selected testes and seminal sacs were embedded in Paraplast through dioxane, sectioned (8μ), and stained with periodic acid Schiff's and hematoxylin. Student's t-test was employed to determine significance of differences in weights of testes and seminal sacs among the various experimental and control groups.

TESTOSTERONE PROPIONATE EXPERIMENT

Between 3 and 17 September 1970, 37 photosensitive males were transferred (3 to 11 per day) from short (8-hour) to long (20-hour) daily photoperiods (0830-0430 CST). Illumination was by fluorescent lamps at an intensity of at least 320 lux; daylight and extraneous light were excluded. To determine if TP could halt testicular growth and induce regression, 26 birds were subjected to intrahypothalamic implantation of TP (~ 250 μg) or CH (~ 260 μg) in CCB 3 weeks after transfer to long days. Five birds sacrificed after 3 weeks on long days plus 2 that died during surgery served as initial controls. As a test for systemic effects of TP, 4 birds received subcutaneous implants of 27-gage tubing (~1 cm) containing ~250 μg TP in CCB. All birds were sacrificed 28 days after implantation (i.e., 7 weeks after transfer to long days).

CYPROTERONE EXPERIMENT

Tubes containing CYP (~150 μg) or CH (~210 μg) in CCB, or CCB alone (~185 μg), were implanted stereotaxically between 7 August and 1 September 1970 into the hypothalamo-hypophysial axes of 91 photosensitive males held

on 8-hour daily photoperiods since capture. To observe the chronic effects of antiandrogen on spontaneous testicular regression, birds were transferred from short (8-hour) to long (20-hour) daily photoperiods (0830-0430 CST) on the first postoperative day and sacrificed 17 weeks later. Under the latter lighting regime, 12 to 15 weeks is usually sufficient to complete a gonadal cycle, i.e., growth, maintenance, and complete regression of testes. Source and intensity of light were as before. To see whether testicular weight changed during the 25-day implantation period, 5 birds were killed on 7 August and 4 on 1 September. These along with 7 that died during surgery or on the first postoperative day served as initial controls.

RESULTS

TESTOSTERONE PROPIONATE EXPERIMENT

As noted previously, birds for this study were subjected to implantation of TP or CH in CCB 3 weeks after transfer from 8- to 20-hour daily photoperiods. Mean testicular weight at the time of implantation was 141.0 mg (Group IT; Table 1). Testicular growth continued during the subsequent 4-week experimental period in all birds bearing CH implants (Group CT; Figs. 2 and 3; Tables 1 and 2) or subcutaneous TP implants (Group NT; Table 1). Although mean testicular weight of Group NT was significantly less ($P < .05$) than that of Group CT (Table 1), selected testes and seminal sacs of both groups showed full spermatogenic activity as assessed histologically. On the other hand, testicular regression was induced, at least partially, in all birds with TP implants in the basal infundibular nucleus (Fig. 2; Table 3). Nine birds (Fig. 2) had large testes ($\bar{x}$ = 287.3 mg) at sacrifice, but testes and seminal sacs showed histological signs of regression (Table 4; see legend for explanation of histological analyses). Testes of 6 others (Fig. 2) had regressed to or near the midwinter condition ($\bar{x}$ = 3.57 mg; Fig. 3; Table 4). Seminal sacs of these were small and inactive (Table 4). Four TP implants, none of which terminated in the basal infundibular nuclear region (Fig. 2), had no effect on the spermatogenic function of the testes (Table 4). However, with the exception of 1 bird in which the implant ended high along the border of the optic chiasm and infundibular nucleus, seminal sacs were relatively small (Fig. 2; Tables 3 and 4).

CYPROTERONE EXPERIMENT

All birds had been on 8-hour daily photoperiods for at least 5 months

TABLE 1

TESTICULAR WEIGHTS OF INITIAL AND TERMINAL CONTROL BIRDS
(TESTOSTERONE PROPIONATE EXPERIMENT)

Group[a]	Date of sacrifice	Days on 20L-4D	Weight of two testes (mg)[b]	Range (mg)
IT	24 Sept. to 5 Oct. 1970	21	141.0 ± 24.57 (7)[c]	75.0 - 230.2
CT	22 to 26 Oct. 1970	49	552.3 ± 30.07 (7)	416.1 - 682.0
NT	4 to 5 Nov. 1970	49	446.6 ± 24.42 (4)[d]	396.1 - 508.2

[a]See text for explanation of group designation.

[b]Mean ± SE. Number of birds in each group is indicated in parentheses.

[c]Significantly different ($P < .05$) from Groups CT and NT.

[d]Significantly different ($P < .05$) from Group CT.

TABLE 2

LOCATIONS OF CHOLESTEROL IMPLANTS IN TREE SPARROWS
(TESTOSTERONE PROPIONATE EXPERIMENT)

Bird no.	Direction and distance of implant center from midline (mm)	Location of implant center					Region[a]	Weight of two testes (mg)	Weight of two seminal sacs (mg)
		OCT	IN	ME	IR	ALP			
1268	R 0.07		*					456.7	171.1
1269	R 0.06		*					496.7	108.0
1314	R 0.03		*				A	682.0	151.1
1207	L 0.06		*					625.1	48.7
1301	R 0.05		*					516.1	148.6
1230	R 0.10	*					C	577.7	121.7
1421	R 0.12			*			E	511.6	75.0

OCT = optic chiasm-optic tract; IN = infundibular nucleus; ME = median eminence; IR = infundibular recess of third ventricle; ALP = anterior pituitary.

* = implant within structure specified.

[a]For comparison between Tables 2 and 3.

TABLE 3

LOCATIONS OF TESTOSTERONE PROPIONATE IMPLANTS IN TREE SPARROWS

Bird no.	Direction and distance of implant center from midline (mm)	Location of implant center					Region	Weight of two testes (mg)	Weight of two seminal sacs (mg)
		OCT	IN	ME	IR	ALP			
1234	L 0.08		*					3.54	2.70
1402	L 0.05		*					4.57	2.18
1264	L 0.08		*					4.73	1.86
1311	L 0.12		*					2.92	0.52
1310	L 0.19		*					2.81	1.14
1235	L 0.12		*					2.83	1.02
1354	L 0.07		*				A	70.0	17.00
1244	R 0.04		*					220.9	5.66
1386	R 0.13		*					417.0	73.5
1249	L 0.14		*					317.7	6.92
1240	L 0.16		*					558.2	12.17
1236	L 0.10		*					217.0	1.87
1228	L 0.24		*					173.2	8.21
1416	R 0.19		*					352.1	6.10
1403	L 0.24	+	+[a]					550.2	74.1
1401	L 0.15	+	+				B	259.7	5.17
1308	L 0.09	*					C	202.8	11.27
1279	R 0.28					*	D	504.4	14.40
1232	L 0.15			*			E	249.4	10.95

+...+ = implant at common border of structures specified. Other symbols and designations as in Table 2.

[a]Dorsal to basal infundibular region.

TABLE 4

HISTOLOGICAL CONDITION OF SELECTED TESTES[a] AND SEMINAL SACS[b]
(TESTOSTERONE PROPIONATE EXPERIMENT)

Bird no.	Combined testicular wt. (mg)	Histological condition of testes								Combined seminal sac wt. (mg)	Histological condition of seminal sacs			
		F1	F2	R1	R2	R3	R4	R5	R6		F	R1	R2	R3
TP-CCB implants: severe gonadosuppression (6 of 6)														
1235	2.83							13	7	1.02				6
1402	4.57							9	11	2.18				6
1311	2.92							8	12	0.52				6
1264	4.73							3	17	1.86				6
1310	2.81							2	18	1.14				6
1234	3.54							2	18	2.70				6
TP-CCB implants: moderate gonadosuppression (9 of 9)														
1240	558.2			15	5					12.7			6	
1228	173.2			8	12					8.21	2	4		
1386	417.0			7	13					73.5	2	4		
1244	220.9			2	18					5.66		6		
1249	317.7			1	19					6.92			6	
1416	352.1				20					6.10			3	3
1401	259.7				16	4				5.17			1	5
1236	217.0				14	6				1.87				6
1354	70.0						2	18		17.00				6
TP-CCB implants: slight or no gonadosuppression (4 of 4)														
1403	550.2	0	20							74.1	6			
1232	249.4	5	15							10.95	6			
1279	504.4	6	14							14.40	2	4		
1308	202.8	10	10							11.27		6		

CH-CCB implants: no gonadosuppression (3 of 7)					
1207	625.1	8	12	48.7	6
1268	456.7	5	15	171.1	6
1314	682.0	2	18	151.1	6
Subcutaneous TP-CCB implants: no gonadosuppression (2 of 4)					
1238	396.1	11	9	128.7	6
1245	508.2	16	4	122.0	6

[a]Regression was evidenced by dissolution and sloughing of tubular cells. Stage of regression was determined by observing five random tubular cross sections in four consecutive testis sections (20 observations) and tallying these in the following categories: F1 = full spermatogenesis with spermatids, but neither sperm nor evidence of degeneration; F2 = full spermatogenesis with spermatids and sperm, but no degeneration; R1 = degeneration with spermatids and few sperm; R2 = degeneration with many spermatids, but no sperm; R3 = degeneration with few spermatids; R4 = no spermatids, but degenerating spermatocytes plentiful; R5 = few degenerating spermatocytes; R6 = single layer of gonial cells.

[b]Regression was evidenced by decrease in tubular diameter and contents along with increase in intertubular tissue space. Stage of regression was determined by observing two random tubular cross sections in three consecutive seminal sac sections (6 observations) and tallying these in the following categories: F = fully active (for detailed description, see Bailey, 1953); R1 = sperm and slough cells, tubular lumenal diameter $\leq$ 0.186 mm; R2 = no sperm, few slough cells, and tubular diameter $\leq$ 0.124 mm; R3 = no sperm or slough cells, tubular lumenal diameter $\leq$ 0.062 mm.

Fig. 2. Schematic parasagittal sections through the hypothalamo-pituitary region showing locations of testosterone propionate and cholesterol implants and their effects on testes (ellipses) and seminal sacs (cones) of photostimulated Tree Sparrows. Size of ellipses and cones reflects relative intensity of gonadotropin release. (A) Testosterone propionate implants: moderate (open circles) to severe (closed circles) gonadosuppression (9 birds showed only histological evidence of testicular regression: combined testicular weights, 70.0-558.2 mg; combined seminal sac weights, 1.87-73.5 mg; 6 birds showed severe gonadosuppression: combined testicular weights, 2.81-4.73 mg; combined seminal sac weights, 0.52-2.70 mg). (B) Testosterone propionate implants: no gonadosuppression (half-closed circles) as evidenced by histological condition of seminiferous tubules (4 birds: combined testicular weights, 202.8-550.2 mg; combined seminal sac weights, 10.95-74.1 mg). (C) Cholesterol implants: no gonadosuppression (7 birds: combined testicular weights, 456.7-682.0 mg; combined seminal sac weights, 48.7-171.1 mg).

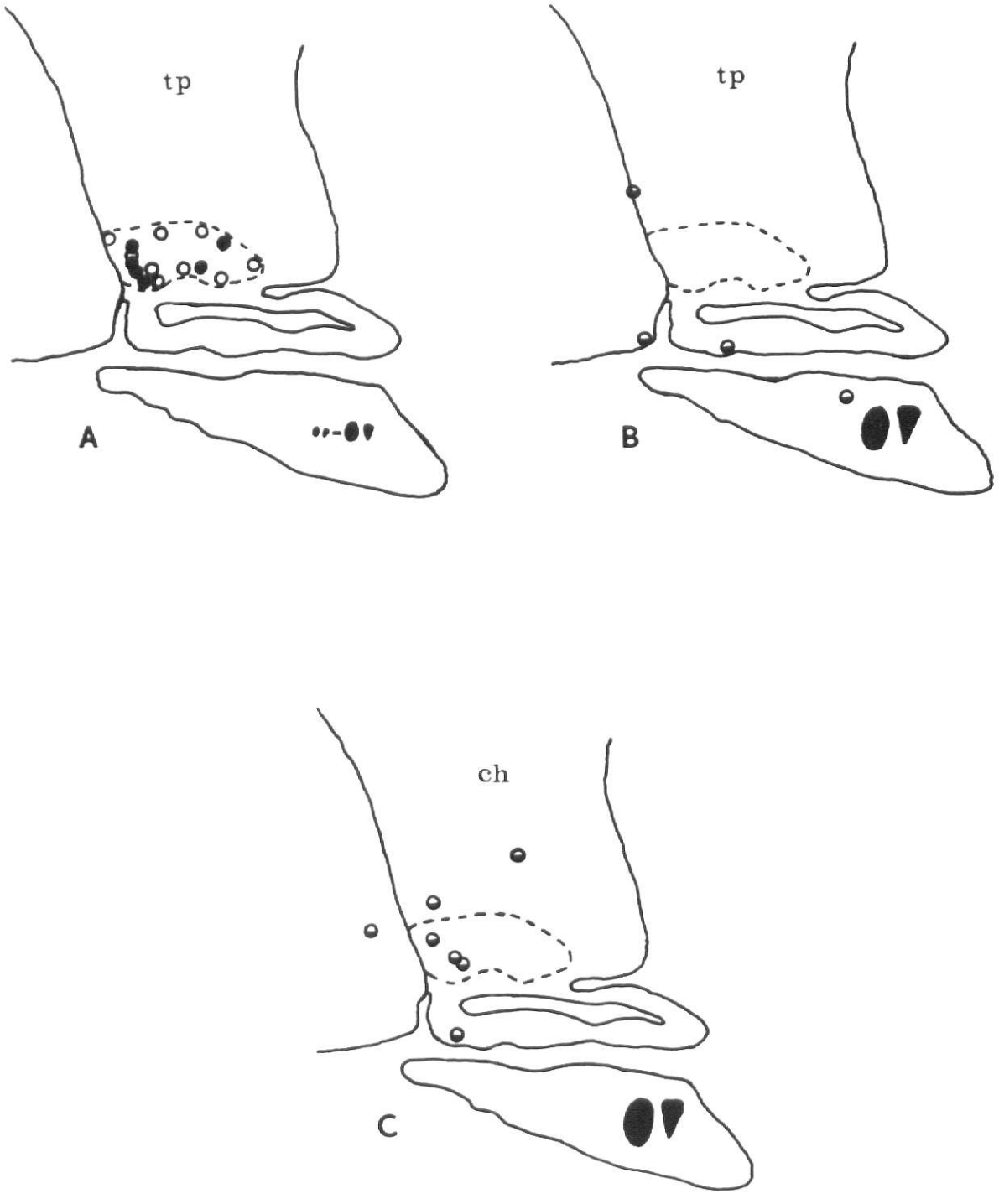
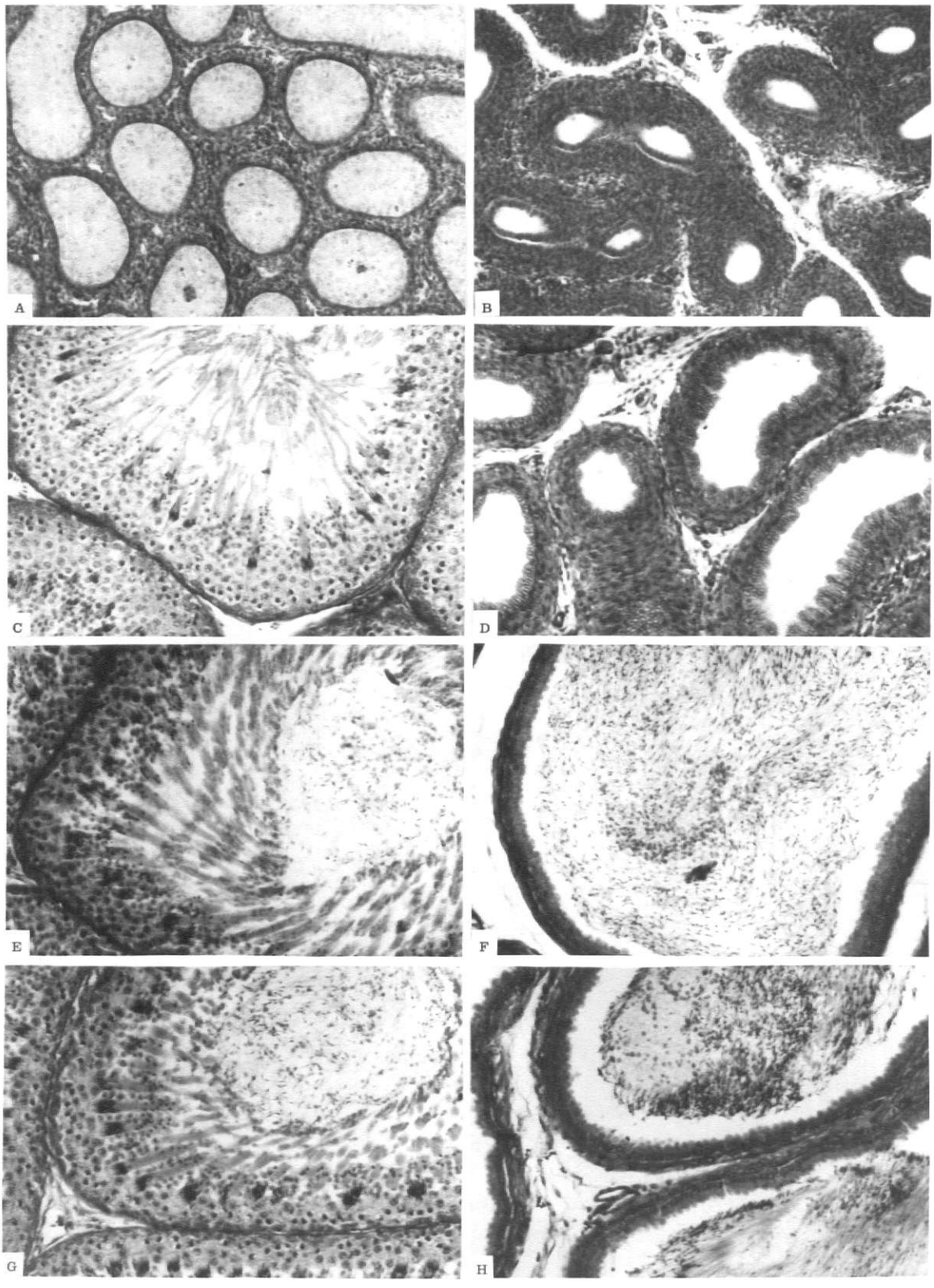

tp
A
tp
B
ch
C

Fig. 3. Transverse sections of testes (left column) and seminal sacs (right column) of birds bearing testosterone propionate (TP) or cholesterol (CH) implants at sacrifice, 4 weeks after implantation and 7 weeks after transfer to 20-hour daily photoperiods. (A,B) 1402; TP; combined testicular weight, 4.57 mg; combined seminal sac weight, 2.18 mg. (C,D) 1416; TP; combined testicular weight, 352.1 mg; combined seminal sac weight, 6.10 mg. Note absence of free sperm. (E,F) 1403; TP; combined testicular weight, 550.2 mg; combined seminal sac weight, 74.1 mg. (G,H) 1314; CH; combined testicular weight, 682.0 mg; combined seminal sac weight, 151.1 mg. (See Tables 2, 3, and 4.) AFA. Periodic acid Schiff's and hematoxylin. X172.

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

THIS IS AS RECEIVED FROM CUSTOMER.


A
B
C
D
E
F
G
H

when the experiment began. Five birds sacrificed on 7 August (Group ICBI) and 4 on 1 September 1970 (Group ICAI) served as initial controls. Testicular weights of Groups ICBI and ICAI were not significantly different ($P > .05$) from each other or from those of birds that died during surgery or on the first postoperative day (Group BDDI). Thus, the 3 groups were combined (Group ICB). Mean testicular weight at the time of implantation (1.68 mg; Table 5) approximated the midwinter value reported for Tree Sparrows by Wilson (1970).

Without exception, neither CH (Group CH-CCB; Fig. 4; Table 6) nor CCB (Group CCB; Fig. 4; Table 7) implants affected spontaneous testicular regression: after 17 weeks of long-day stimulation, testes and seminal sacs were at or near the midwinter level (Table 5). On the other hand, testicular regression was inhibited in 7 birds bearing CYP implants (Figs. 4 and 5). Magnitude of inhibition ranged from complete to moderate (Tables 8 and 9) as evidenced by both weight and histological criteria. Seminal sacs of birds with effective CYP implants ranged histologically from fully active to fully regressed and were heavier (though not always significantly so) than those of birds with ineffective CYP implants (Table 8; Fig. 6). Testes of birds with ineffective CYP implants or CH or CCB implants did not differ markedly in weight or histologically from those of initial controls.

CYP implants that prevented or retarded testicular regression ended, with 1 exception, in a relatively specific region (Fig. 4) of the basal infundibular nucleus (der Grundkern of Oehmke, 1968). Three effective implants were in complete contact with hypothalamic tissue whereas 3 others made only partial contact therewith (Fig. 5). The latter 3 also made contact with pars tuberalis cells and loose connective tissue adjacent to the hypothalamic border. Of the 6 implants in the CYP-sensitive

TABLE 5

TESTICULAR WEIGHTS OF INITIAL AND TERMINAL CONTROL BIRDS
(CYPROTERONE EXPERIMENT)

Group[a]	Date of sacrifice	Photoperiod at sacrifice	Days on photoperiod	Weight of two testes (mg)[b]	Range (mg)
ICBI	7 Aug. 1970	8L-16D	since capture	1.69 ± .25 (5)	0.85-2.31
ICAI	1 Sept. 1970	8L-16D	since capture	1.40 ± .10 (4)	1.23-1.60
BDDI	7 Aug. to 1 Sept. 1970	8L-16D	since capture	1.85 ± .15 (7)	1.31-2.60
ICB	7 Aug. to 1 Sept. 1970	8L-16D	since capture	1.68 ± .11 (16)	0.85-2.60
CH-CCB	15 to 22 Dec. 1970	20L-4D	119	1.76 ± .11 (8)	1.25-2.10
CCB[c]	8 to 24 Dec. 1970	20L-4D	119	2.72 ± .40 (10)	1.27-5.11

[a]See text for explanation of group designation.

[b]Mean ± SE. Number of birds in each group is indicated in parentheses.

[c]Excludes one bird from which a testis was lost.

TABLE 6

LOCATIONS OF CHOLESTEROL IMPLANTS IN TREE SPARROWS[a]
(CYPROTERONE EXPERIMENT)

Bird no.	Direction and distance of implant center from midline (mm)	Location of implant center						Region[b]	Weight of two testes (mg)	Weight of two seminal sacs (mg)
		OCT	IN	INC	ME	IR	ALP			
1333	L 0.27		*						1.25	1.84
1339	L 0.15		*						1.89	3.41
1409	R 0.18		*						1.81	2.61
1140	L 0.15		*					C	2.08	3.30
1298	R 0.03		*						1.71	3.27
1166	L 0.20		*						1.30	0.77
1292	L 0.34	*						B	1.92	2.28
0398	R 0.07		+			+		D	2.10	3.56

INC = a central portion of the basal infundibular nucleus 0.07 to 0.16 mm above the roof of the infundibular recess of the third ventricle (see Fig. 4A). Other symbols and designations as in Tables 2 and 3.

[a]One bird that died is not included in this analysis.

[b]For comparisons among Tables 6, 7, and 9.

TABLE 7

LOCATIONS OF COCOA BUTTER IMPLANTS IN TREE SPARROWS[a]
(CYPROTERONE EXPERIMENT)

Bird no.	Direction and distance of implant center from midline (mm)	Location of implant center						Region	Weight of two testes (mg)	Weight of two seminal sacs (mg)
		OCT	IN	INC	ME	IR	ALP			
1367	L 0.13			*					2.58	3.00
1352	R 0.11			*				A	1.89	1.81
1168	L 0.27			*					2.49	3.82
1387	L 0.75		#						3.00[b]	3.77
1353	L 0.42		*						1.92	2.43
0400	L 0.14		*					C	3.82	3.19
1182	L 0.07		*						2.20	--
0389	0		*						1.70	2.45
1176	R 0.10				+		+	E	5.11	3.26
1328	R 0.02	+	+						4.26	2.83
1312	R 0.09	+	+						1.27	1.35

= implant near, but displaced from, structure specified. Other symbols and designations as in Table 6.

[a]One bird that died and one whose implant tube became dislodged are not included in this analysis.

[b]Weight of one testis.

TABLE 8

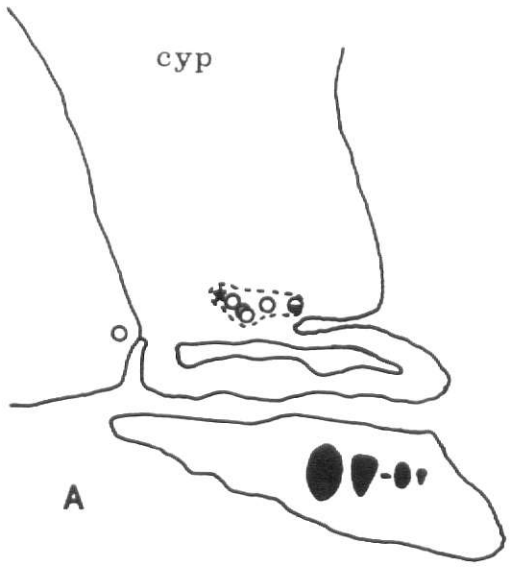
HISTOLOGICAL CONDITION OF SELECTED TESTES AND SEMINAL SACS (CYPROTERONE EXPERIMENT)[a]

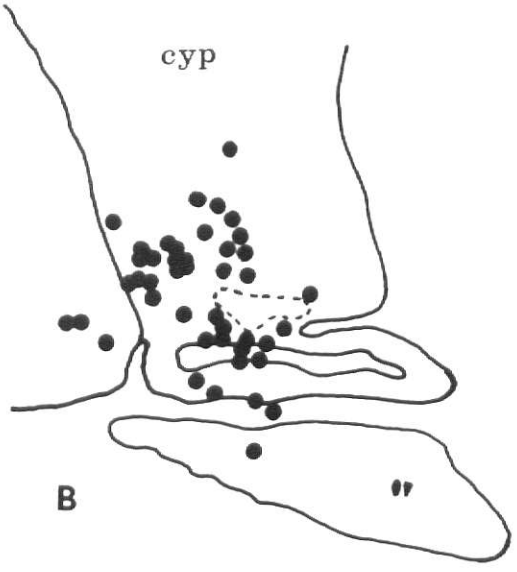
Bird no.	Combined testicular wt. (mg)	Histological condition of testes								Combined seminal sac wt. (mg)	Histological condition of seminal sacs			
		F1	F2	R1	R2	R3	R4	R5	R6		F	R1	R2	R3
CYP-CCB implants: effective (7 of 7)														
1336	599.8	4	16							152.7	6			
0384	130.5				9	11				11.00			6	
1175	162.6				8	12				11.04			6	
1137	95.7					15	5			6.12				6
1183	108.3					4	16			5.02				6
1384	82.7					2	18			4.37				6
1313	29.2						11	9		6.05				6
CYP-CCB implants: ineffective (6 of 43)														
1173	4.83							17	3	1.99				6
1184	2.87							9	11	3.71				6
1297	1.16							8	12	2.36				6
1203	1.46							7	13	2.43				6
1391	2.32							2	18	2.12				6
1300	2.50							2	18	4.15				6
CCB implants: ineffective (4 of 11)														
1353	1.92							14	6	2.43				6
1176	5.11							10	10	3.26				6
1312	1.27							7	13	1.35				6
1328	4.26							2	18	2.83				6

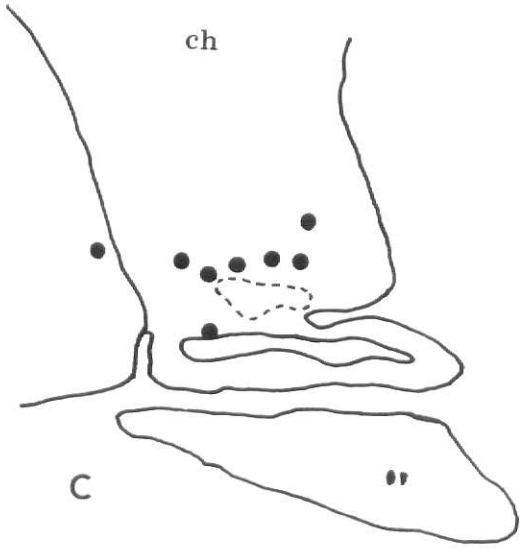
CH-CCB implants: ineffective (4 of 8)					
1292	1.92	7	13	2.28	6
0398	2.10	7	13	3.56	6
1333	1.25	3	17	1.84	6
1140	2.08	6	14	3.30	6
Initial controls (6 of 16)					
1219	1.98		20	--	
0397	1.60		20	--	
1130	1.23		20	--	
1317	1.41		20	--	
1204	1.52		20	--	
1216	1.24		20	--	

[a]Designations and methods of analysis as in Table 4.

Fig. 4. Schematic parasagittal sections through the hypothalamo-pituitary region showing locations of steroid and cocoa butter implants and their effects on spontaneous testicular regression (stars = prevention; open circles = severe inhibition; half-open circles = moderate inhibition; closed circles = no effect) in Tree Sparrows after 17 weeks on 20-hour daily photoperiods. Size of ellipses and cones reflects relative size and functional status of testes and seminal sacs, respectively, as evidenced histologically. (A) Cyproterone implants: complete to moderate inhibition of spontaneous testicular regression (7 birds: combined testicular weights, 29.2-599.8 mg; combined seminal sac weights, 4.37-152.7 mg). (B) Cyproterone implants: no effect on spontaneous testicular regression (43 birds: combined testicular weights, 1.16-4.83 mg; combined seminal sac weights, 1.34-5.44 mg). (C) Cholesterol implants: no effect on spontaneous testicular regression (8 birds: combined testicular weights, 1.25-2.10 mg; combined seminal sac weights, 0.77-3.56 mg). (D) Cocoa butter implants: no effect on spontaneous testicular regression (11 birds: combined testicular weights, 1.27-5.11 mg; combined seminal sac weights, 1.35-3.82 mg).


cyp
A


cyp
B


ch
C

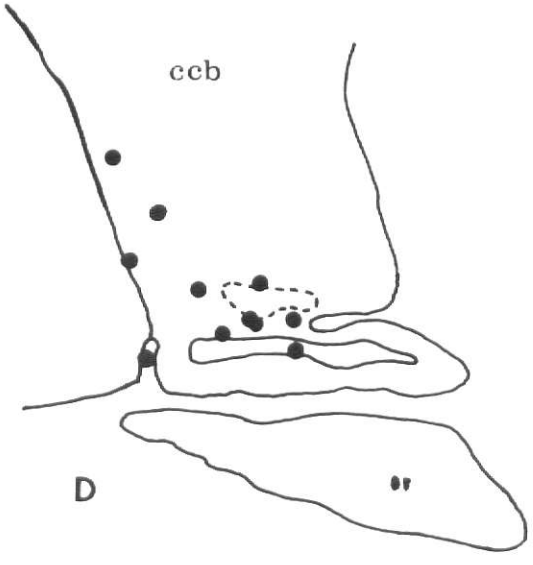

ccb
D

Fig. 5. Schematic frontal section through the hypothalamo-pituitary region showing locations of cyproterone implants that prevented or retarded spontaneous testicular regression in Tree Sparrows held 17 weeks on 20-hour daily photoperiods. IN = infundibular nucleus; ME = median eminence; ALP = anterior lobe of pituitary; CS = cavernous sinus; ST = sella turcica; ICA = internal carotid artery; OT = optic tract. Symbols as in Fig. 4A.

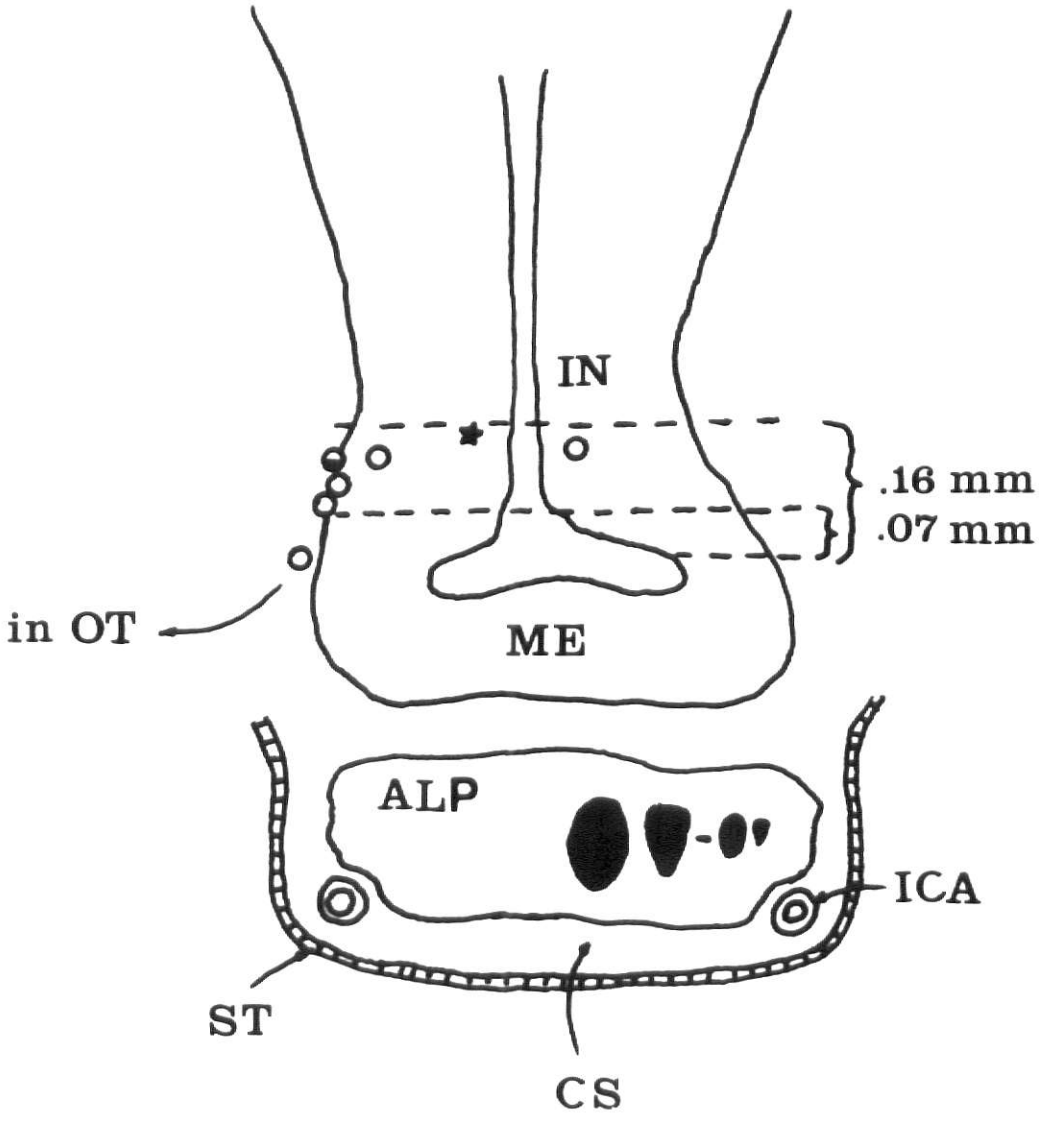
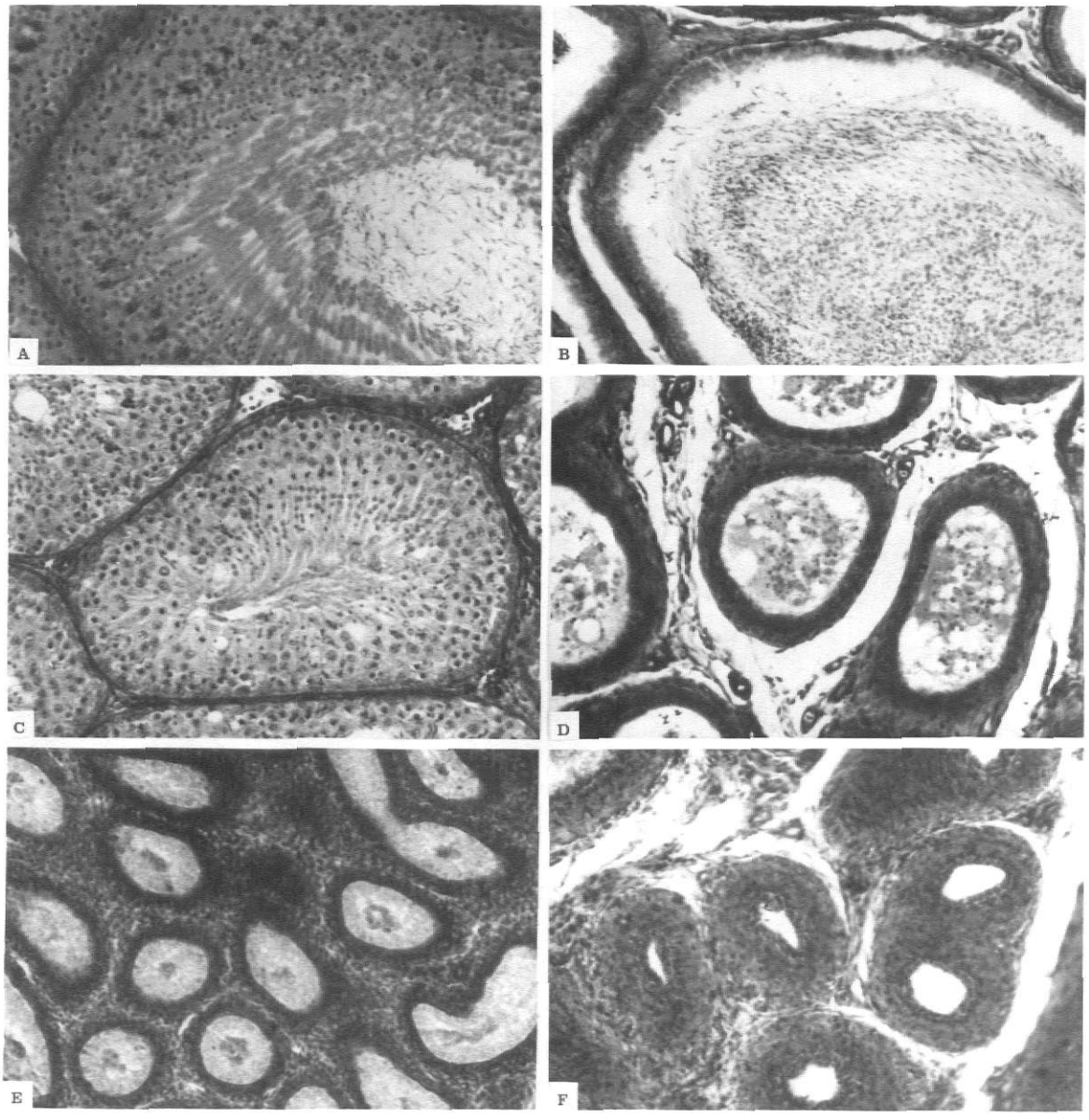

IN
.16 mm
.07 mm
in OT
ME
ALP
ICA
ST
CS

Fig. 6. Transverse sections of testes (left column) and seminal sacs (right column) of birds implanted with cyproterone and held 17 weeks on 20-hour daily photoperiods. (A,B) 1336; combined testicular weight, 599.8 mg; combined seminal sac weight, 152.7 mg. See Fig. 7 for implantation site. (C,D) 0384; combined testicular weight, 130.5 mg; combined seminal sac weight, 11.00 mg. (E,F) 1184; combined testicular weight, 2.87 mg; combined seminal sac weight, 3.71 mg. (See also Tables 6 and 7.) AFA. Periodic acid Schiff's and hematoxylin. X172.


A
B
C
D
E
F

zone, the least effective was the most posterior and made least contact with hypothalamic tissue. The most effective was the most anterodorsal and was in complete contact with hypothalamic tissue (Figs. 4, 5, and 7; Table 8). One CYP implant that terminated posteroventrally and laterally in the left optic tract markedly inhibited regression (Figs. 4 and 5). Histological analysis revealed that a thin layer of optic fibers and some loose connective tissue separated it from the cavernous sinus surrounding the pars distalis (Vitums et al., 1965, 1966).

All CYP implants in the infundibular nucleus that terminated above, below, anterior, or posterior to the effective zone (Fig. 4; Table 9) were ineffective and did not retard spontaneous testicular regression (Table 8). Implants in the median eminence, in the pars distalis, or at their common borders (Fig. 4; Table 9) were also ineffective, as were 3 of 4 implants in the optic chiasm or tract. Several ineffective CYP implants at various levels of the hypothalamus made contact with the third ventricle or its infundibular recess.

TABLE 9

LOCATIONS OF CYPROTERONE IMPLANTS IN TREE SPARROWS[a]

Bird no.	Direction and distance of implant center from midline (mm)	Location of implant center						Region	Weight of two testes (mg)	Weight of two seminal sacs (mg)
		OCT	IN	INC	ME	IR	ALP			
1336	L 0.07			*					599.8	152.7
0384	R 0.07			*					130.5	11.00
1175[b]	L 0.33			*				A	162.6	11.04
1183	L 0.18			*					108.3	5.02
1384	L 0.20			*					82.7	4.37
1313[b]	L 0.28			*					29.2	6.05
1137	L 0.29	*							95.7	6.12
1194	L 0.44	*						B	2.17	2.56
1192	L 0.13	*							1.49	2.13
1267	L 0.20	*							1.83	3.60
1201	R 0.22		*						2.13	2.09
1281	L 0.09		*						1.97	1.67
1217	R 0.03		*						3.00	2.08
1139[b]	L 0.10		*						3.04	3.46
1149	L 0.14		*						2.58	3.43
1379	R 0.15		*						2.13	2.40
1305	L 0.03		*						2.21	2.47
1307	0		*						2.10	3.10
1258	L 0.08		*						1.40	1.40
1148	L 0.35		*						2.10	2.78
1286	R 0.05		*						2.91	1.83
1391	R 0.06		*						2.32	2.12
1407	L 0.07		*						1.88	1.81
1138	L 0.08		*						3.55	3.22
1300	R 0.10		*						2.50	4.15
1173	L 0.03		*						4.83	1.99

1297	L 0.02	*				1.16	2.36
1271	L 0.31	*			C	2.53	3.09
1153	R 0.04	*				2.41	3.45
1327	L 0.22	*				1.98	2.18
1150	L 0.24	*				4.01	5.44
1370	L 0.11	*				1.51	2.99
1315	L 0.31	*				1.92	4.41
1208	L 0.30	*				2.48	3.90
1404	L 0.07	*				3.31	2.77
1202	L 0.03	*				3.61	2.23
1196	L 0.19	*				0.92[c]	1.34
1318	L 0.06	*				1.42	2.29
1184	R 0.06	*				2.87	3.71
1262	--	*				2.61	2.09
1212	R 0.04	+		+	D	3.90	1.84
1359	R 0.02		+	+		1.56	2.73
1316	L 0.03		+	+	E	2.36	3.18
1210	L 0.08		+	+		2.29	3.24
1135	L 0.27		*			2.36	4.21
1190	L 0.18		*		F	1.78	2.30
1178	L 0.03		*			2.81	4.00
1158	L 0.20		+	+	G	1.97	1.58
1203	L 0.02			*	H	1.46	2.43
1186	--					1.59	1.43

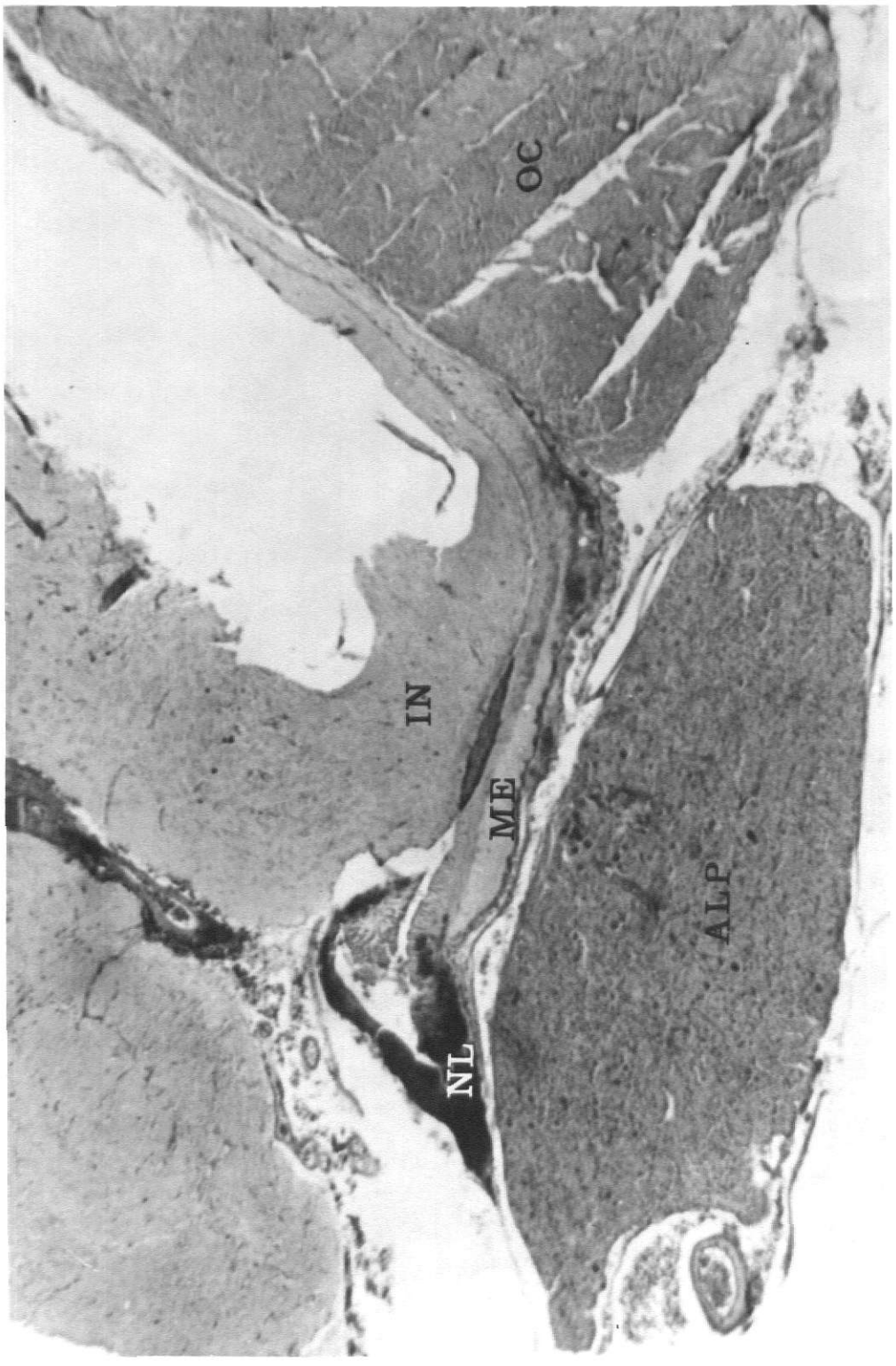
Symbols and designations as in Table 6.

[a]Ten birds that died and 2 whose implant tubes became dislodged are not included in this analysis.

[b]Tube contents in contact with pars tuberalis cells and loose connective tissue surrounding ventral hypothalamus.

[c]Weight of one testis.

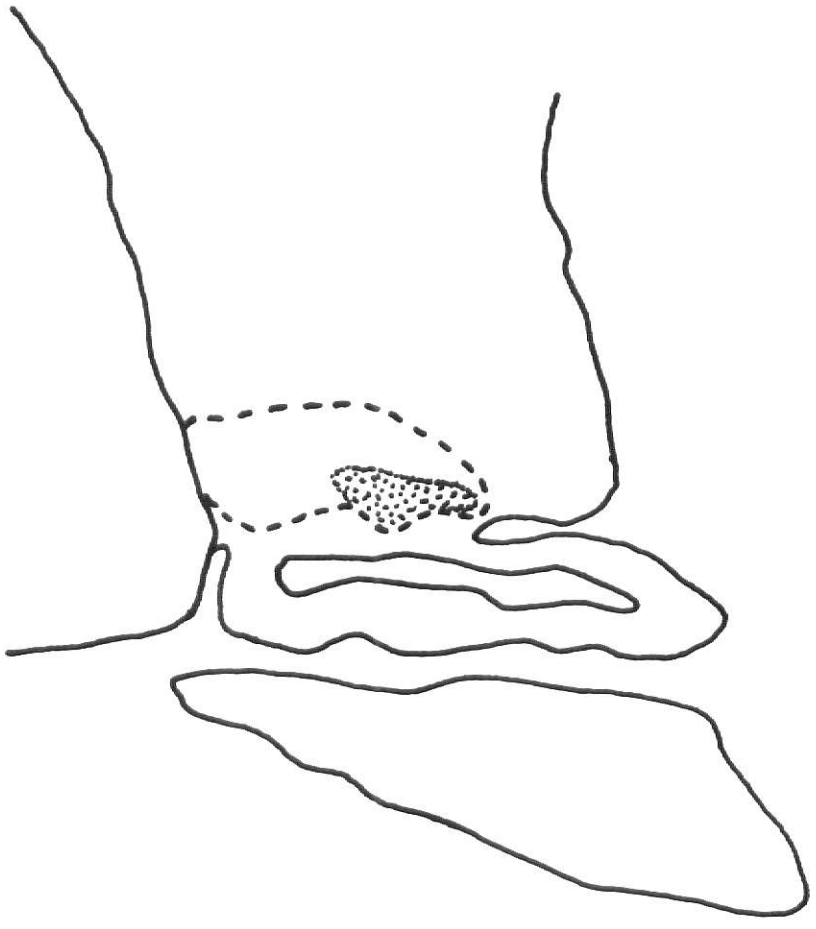
Fig. 7. Implantation site of a 27-gage tube containing cyproterone. Steroid-tissue contact in basal infundibular nucleus (IN). 1336: Weight of testes and seminal sacs after 17 weeks of photostimulation: 599.8 and 152.7 mg, respectively. See also Fig. 5. (ALP) anterior pituitary; (ME) median eminence; (NL) neural lobe; (OC) optic chiasm. Bouin. Parasagittal section (0.07 mm left of midline). Paraldehyde-fuchsin. X100.


OC
IN
ME
NL
ALP

SUMMARY OF RESULTS

TP implants in the basal infundibular nucleus of the hypothalamus induced moderate to severe gonadotropic insufficiency in photostimulated Tree Sparrows. Spontaneous testicular regression was retarded or prevented in photostimulated Tree Sparrows when the antiandrogen CYP was chronically implanted in a rather specific region of the testosterone-sensitive basal hypothalamus (Fig. 8).

Fig. 8. Schematic parasagittal section through the hypothalamo-pituitary region of the Tree Sparrow showing the relationship between regions sensitive to testosterone propionate and cyproterone. Area circumscribed by dashed line represents region sensitive to testosterone propionate. Stippled area represents region sensitive to cyproterone.



DISCUSSION

All TP implants in the basal infundibular nucleus of the hypothalamus caused some degree of gonadosuppression in photostimulated Tree Sparrows (as evidenced by weight or spermatogenic state of the testes and weight or histological condition of the seminal sacs), but CH implants in or near that region did not. Therefore, the possibility is slight that the gonadosuppressive effects of TP implants stem from tissue elimination or damage or from a nonspecific effect of steroid. Since subcutaneous TP implants did not induce regression of testes or seminal sacs, effects of TP in effectively implanted birds were presumably exerted locally rather than systemically. TP implants dorsal to the basal infundibular region (in der erster dorsaler Abteilung of Oehmke, 1968) or in the optic chiasm, median eminence, or pars distalis had no apparent effect on testicular growth or spermatogenesis, but implants in the latter 3 regions did affect the endocrine function of the testes as noted by decreases in weight of the androgen-dependent seminal sacs (see Bailey, 1953). These observations may indicate a greater sensitivity to TP by CNS components involved in the control of LH-like release than by those involved in the control of FSH-like secretion, at least in 3- to 7-week photostimulated birds. Alternatively, differing degrees of gonadosuppression caused by TP implants in the basal infundibular region may be due to variability in TP sensitivity in photostimulated birds. At any rate, these data suggest that the CNS site of inhibitory feedback of androgen is less clearly defined for LH- than for FSH-like secretion since TP implants in the optic chiasm, median eminence, and pars distalis, as well as those in the basal infundibular nuclear region, inhibited androgen secretion (as evidenced by weight and histological condition of seminal sacs).

According to Wilson (1970), although TP implants in (or sometimes near) the median eminence partially blocked the photoperiodic testicular response of Tree Sparrows, TP implants in the anterobasal infundibular nucleus dorsal to the median eminence were far more effective. Data of the present study are generally consistent with those of Wilson (1970) and indicate that when TP is implanted into the infundibular nucleus, including its anterobasal portion, testicular regression is induced. Though one implant in and one rostral to the median eminance did not induce regressive changes in the testes, they did cause regression, but not loss of function, of the seminal sacs. Gogan (1968) observed in the Pekin duck that TP implants in the median eminence did not produce gonadosuppression, whereas those in the dorsomedian-ventromedian nuclear complex or in the mediobasal region of the middle hypothalamus did. The primary focus of TP sensitivity in Gogan's study was the ventromedial nucleus which is somewhat dorsal to the TP-sensitive region in the Tree Sparrow as observed in this study and that of Wilson (1970).

The free alcohol of cyproterone has been instrumental in elucidating androgenic functions related to control of male reproductive and accessory organs in several mammalian species. CYP is believed to be a competitive inhibitor of androgen-sensitive receptors while having no progestational or estrogenic activities and little or no androgenic activity. When injected systemically, it prevents androgen-induced differentiation of the male reproductive system in fetuses or neonates and depresses accessory glands (and testes to some extent) in adults. Implantation of CYP in the median eminence causes marked gains in testicular, prostatic, and seminal vesicle weights in prepuberal rats, but has less marked effects in adults.

CYP has thus been fairly extensively used in the study of androgenic functions of the mammalian male reproductive system (for reviews, see Davidson and Bloch, 1969; Neumann et al., 1970). It has also been demonstrated that CYP antagonizes testosterone-stimulated comb growth in cockerels (Boris et al., 1970).

The present study shows that CYP, when implanted into a relatively specific portion of the basal infundibular nucleus of the hypothalamus, can retard, if not prevent, spontaneous testicular regression in the Tree Sparrow. The possibility that this effect is due to tissue elimination or damage or to a nonspecific effect of steroid or CCB is also unlikely since CH or CCB implants in or near the basal infundibular nucleus had no effect. Six of 7 CYP implants that inhibited testicular regression were in contact with hypothalamic cells at least 0.07 to 0.16 mm above the roof of the infundibular recess of the third ventricle in a central portion of the basal infundibular nucleus. Implants ventral to the effective zone, yet in the basal infundibular nucleus, along with implants in the median eminence or along its dorsal border, and in or dorsal to the pars distalis were ineffective. Thus, structures sensitive to CYP and, therefore, probably to androgen are not likely eminential or adenohypophysial. Retardation or prevention of spontaneous testicular regression can, therefore, best be explained as specific effects of CYP on androgen-sensitive structures in a fairly discrete portion of the basal infundibular nucleus. One effective CYP implant in the left lateral aspect of the optic tract most likely produced its effect at a distant site. As noted before, only a thin layer of optic fibers and loose connective tissue separated the implant from the cavernous (venous) sinus surrounding the anterior pituitary, but the fate of the steroid or its site of action cannot be determined from the data available.

Wilson (1970) also reported that a CYP implant at the rostral tip of the cephalic lobe of the anterior pituitary prevented testicular regression in a Tree Sparrow held 15 weeks on 20-hour daily photoperiods. That implant was in contact with a vascular channel whose destination could not be determined, and so the site of action of CYP in that bird is also dubious.

Halász et al. (1962) hypothesized on the basis of pituitary transplant studies in rats that substances necessary for normal pituitary function are synthesized by the parvicellular nuclei of the mediobasal hypothalamus and carried by fibers of the tubero-infundibular tract to the eminential zone in contact with the portal circulation (see also Halász et al., 1965). TP implants in the arcuate (= infundibular) nucleus-median eminence region of dogs (Davidson and Sawyer, 1961) and rats (Lisk, 1962) cause testicular atrophy and reduce pituitary FSH. On the other hand, CYP implants in the median eminence region of prepuberal rats stimulate testicular and accessory organ growth and increase pituitary FSH (see Davidson and Bloch, 1969). These observations suggest that the mediobasal hypothalamus (including median eminence) may respond to circulating levels of androgen by altering the rate of release of gonadotropin-releasing factors into the hypophysial portal system. Data of the present study and those of Wilson (1970) and of Gogan (1968), while not inconsistent with those on mammals, point toward the basal infundibular nucleus rather than the median eminence as a primary site of androgen feedback.

As noted before, the region of CYP sensitivity in the Tree Sparrow is located centrally within the basal infundibular nucleus and lies within the region shown in this study and in that of Wilson (1970) to be sensitive to TP. CYP is less soluble than TP (Bloch and Davidson, 1967), a fact that may account for the difference in size of the CYP- and TP-sensitive

regions. Locations of effective CYP implants may, therefore, indicate more precisely the region of androgen sensitivity in the hypothalamus of the Tree Sparrow. The relative insolubility of CYP as compared to TP plus the observation that CYP implants in the infundibular nucleus ventral to the sensitive region or in or near the median eminence were ineffective make an eminential site of androgen feedback even more unlikely. Ineffective implants in or dorsal to the pars distalis also tend to discount this structure as a site sensitive to feedback of androgen.

The importance of the infundibular nucleus and median eminence in the mechanism of photoinduced testicular growth has been demonstrated in the White-crowned Sparrow by Wilson (1967) and Stetson (1969). Electrocoagulation of the entire infundibular nucleus or its median basal portion (Wilson, 1967) prevents testicular growth as does destruction of the entire median eminence (Wilson, 1967; Stetson, 1969) or its posterior division (Stetson, 1969). Gonadotropic function is also impaired when lesions destroy the basal tuberal region in male domestic fowl (Lepkovsky and Yasuda, 1966; Graber et al., 1967; Snapir et al., 1969). The present study, as well as those of Wilson (1970) and Gogan (1968), shows that the basal infundibular region sensitive to androgen roughly corresponds to regions shown by ablation to block pituitary gonadotropin secretion. Recent studies by Sharp and Follett (1969) on Japanese Quail show that destruction of not only the median eminence and basal infundibular nucleus but of the nucleus hypothalamicus posterior medialis (posterodorsal to the basal infundibular region) as well prevents testicular recrudescence. This observation raises the possibility that lesions in the basal infundibular nucleus may sever connecting neurons between the median eminence and some higher region of the hypothalamus. Although insufficient data make it uncertain whether posterodorsal portions

of the hypothalamus are sensitive to TP in Tree Sparrows, CYP implants in or near the nucleus hypothalamicus posterior medialis, as described by Sharp and Follett (1969), did not retard spontaneous testicular regression. While it is possible that a higher region may be involved in the synthesis of neurohormones controlling pituitary gonadotropic secretion, indications are that the rate of neurohormone release is modulated by components of the basal infundibular nucleus.

Data from this and Wilson's (1970) study indicate that androgen has the potential for inhibitory feedback control of pituitary gonadotropin secretion and strongly suggest that feedback of androgen onto the basal infundibular nucleus may be an essential part of the mechanism triggering spontaneous testicular regression in the Tree Sparrow. When TP is appropriately distributed to androgen-sensitive receptors, photoperiodic testicular growth can be blocked or regression can be induced. As a corollary to this, when CYP is appropriately distributed (both temporally and spatially) to possibly the same androgen-sensitive receptors, testicular regression can be prevented and full reproductive activity prolonged.

Testicular function in birds like the Tree Sparrow is terminated naturally by regression which signals the onset of photorefractoriness. Photorefractoriness was first described in 1936 by Riley, and although mechanisms underlying its control remain enigmatic, inhibition of gonadotropin secretion by androgen feedback onto the hypothalamus has been suspected, though little supported, for some time (see Farner, 1961, 1964, 1967, 1970; Farner and Follett, 1966; Farner et al., 1967; Hamner, 1968; Kobayashi and Farner, 1966; Wilson, 1970). Effects of systemically administered TP on testicular function are inconsistent and confusing, though seemingly determined by the phase of the testicular cycle during which it is administered,

and probably pharmacologic (see Lofts, 1962; Hamner, 1968). On the other hand, if, as some authors contend (see Threadgold, 1960), refractoriness need not necessarily follow spontaneous regression, then mechanisms underlying their control might be separate.

Although the possibility for inhibitory feedback of androgen on gonadotropin secretion is confirmed by our data, it is not known whether birds bearing TP implants that induced complete testicular regression were photorefractory as after spontaneous testicular regression. However, as noted before, chronic CYP implants in a specific portion of the basal infundibular nucleus (which presumably reduce the local concentration of androgen) can retard or even prevent testicular regression. Clearly, the one CYP-implanted bird in full breeding condition (see also Wilson, 1970) after 17 weeks on long days was still photosensitive. In light of this, it seems likely that androgen plays an important, if not key, role in the mechanism of spontaneous testicular regression and, possibly, of photorefractoriness. That a different mechanism initiates photorefractoriness cannot be unequivocally refuted by the data available; however, the prevention of spontaneous testicular regression and of photorefractoriness that naturally follows by certain CYP implants in this and Wilson's (1970) study suggest that the two phenomena may be closely related mechanistically.

REFERENCES

BAILEY, R. E. (1953). Accessory reproductive organs of male fringillid birds. Anat. Rec. 115, 1-19.

BLOCH, G. J., and DAVIDSON, J. M. (1967). Antiandrogen implanted in brain stimulates male reproductive system. Science 155, 593-595.

BLOCH, G. J., and DAVIDSON, J. M. (1971). Behavioral and somatic responses to the antiandrogen cyproterone. Horm. Behav. 2, 11-25.

BORIS, A., COX, D. C., and HURLEY, J. E. (1970). Comparison of the effects of six antiandrogens on chick comb stimulation by testosterone. Proc. Soc. Exptl. Biol. Med. 134, 985-987.

DAVIDSON, J. M. (1969a). Role of CNS in control of reproductive mechanisms by gonadal steroids. In "Metabolic Effects of Gonadal Hormones and Contraceptive Steroids," (H. H. Salhanick, D. M. Kipnis, and R. L. Vandewiele, eds.) pp. 722-728. Plenum Press, New York.

DAVIDSON, J. M. (1969b). Feedback control of gonadotropin secretion. In "Frontiers in Neuroendocrinology," (W. F. Ganong and L. Martini, eds.) pp. 343-388. Oxford University Press, New York.

DAVIDSON, J. M., and BLOCH, G. J. (1969). Neuroendocrine aspects of male reproduction. Biol. Reprod. Suppl. 1, 67-92.

DAVIDSON, J. M., and SAWYER, C. H. (1961). Evidence for a hypothalamic focus of inhibition on gonadotropin by androgen in the male. Proc. Soc. Exptl. Biol. Med. 107, 4-7.

DONOVAN, B. T. (1958). Restraint and anesthesia of caged birds. Vet. Med. 53, 541-543.

FARNER, D. S. (1961). Comparative physiology: photoperiodicity. Ann. Rev. Physiol. 23, 71-96.

FARNER, D. S. (1964). Photoperiodic control of reproductive cycles in birds. Am. Scient. 52, 137-156.

FARNER, D. S. (1967). The control of avian reproductive cycles. In "Proceedings of the XIV International Ornithological Congress," (D. W. Snow, ed.) pp. 107-133. Blackwell Scientific Publications Ltd., Oxford.

FARNER, D. S. (1970). Some glimpses of comparative avian physiology. Fed. Proc. 29, 1649-1663.

FARNER, D. S., and FOLLETT, B. K. (1966). Light and other environmental factors affecting avian reproduction. J. Anim. Sci. Suppl. 25, 90-118.

FARNER, D. S., WILSON, F. E., and OKSCHE, A. (1967). Neuroendocrine mechanisms in birds. In "Neuroendocrinology," Vol. II (L. Martini and W. F. Ganong, eds.) pp. 529-582. Academic Press, New York.

GOGAN, F. (1968). Sensibilité hypothalamique à la testostérone chez le Canard. Gen. Comp. Endocrinol. 11, 316-327.

GOGAN, F., and KORDON, C. (1964). Influence du feed-back par la testostérone sur la gonadostimulation induite par la lumière chez le Canard. J. Physiol. (Paris) 56, 364-365.

GOSLAR, H. G., MEHRING, M., and NEUMANN, F. (1970). Einfluss der Antiandrogene Cyproteron und Cyproteronacetat auf das Enzymmuster im Hoden juveniler und erwochsener Ratten. Histochemie 23, 51-58.

GRABER, J. W., FRANKEL, A. I., and NALBANDOV, A. V. (1967). Hypothalamic center influencing the release of LH in the cockerel. Gen. Comp. Endocrinol. 9, 187-192.

HALÁSZ, B., PUPP, L., and UHLARIK, S. (1962). Hypophysiotrophic area in the hypothalamus. J. Endocr. 25, 147-154.

HALÁSZ, B., PUPP, L., UHLARIK, S., and TIMA, L. (1965). Further studies on the hormone secretion of the anterior pituitary transplanted into the hypophysiotrophic area of the rat hypothalamus. Endocrinology 77, 343-355.

HAMNER, W. H. (1968). The photorefractory period of the House Finch. Ecology 49, 211-227.

KARFSMEIER, K. H., and DAVIDOFF, M. (1970). Autoradiographische Untersuchungen zur Mitosiaktivitat im Hypophysenvorderlappen mannlicher Ratten nach Behandlung mit Antiandrogen und nach Kastration. Z. Zellforsch. 104, 275-281.

KING, J. R., FOLLETT, B. K., FARNER, D. S., and MORTON, M. L. (1966). Annual gonadal cycles and pituitary gonadotropins in Zonotrichia leucophrys gambelii. Condor 68, 476-487.

KOBAYASHI, H., and FARNER, D. S. (1966). Evidence of a negative feedback on photoperiodically induced gonadal development in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Gen. Comp. Endocrinol. 6, 443-452.

KOIKE, T., and LEPKOVSKY, S. (1967). Hypothalamic lesions producing polyuria in chickens. Gen. Comp. Endocrinol. 8, 397-402.

KORDON, C., and GOGAN, F. (1964). Localisation par une technique de microimplantation de structures hypothalamiques responsables du feed-back par la testostérone chez le Canard. C. R. Soc. Biol. (Paris) 158, 1795-1798.

LEPKOVSKY, S., and YASUDA, M. (1966). Hypothalamic lesions, growth and body composition of male chickens. Poultry Sci. 45, 582-588.

LISK, R. D. (1962). Testosterone-sensitive centers in the hypothalamus of the rat. Acta. Endocr. (Kbh.) 41, 195-204.

LOFTS, B. (1962). The effects of exogenous androgen on the testicular cycle of the Weaver-Finch, (Quelea quelea). Gen. Comp. Endocrinol. 2, 394-406.

LOFTS, B., FOLLETT, B. K., and MURTON, R. K. (1970). Temporal changes in the pituitary-gonadal axis. Mem. Soc. Endocrinol. 18, 545-572.

NEUMANN, F., VON BERSWORDT-WALLRABE, R., ELGER, W., STEINBECK, H., HAHN, J. D., and KRAMER, M. (1970). Aspects of androgen dependent events as studied by antiandrogens. Recent Prog. Hormone Res. 26, 337-405.

OEHMKE, H.-J. (1968). Regionale Strukturunterschiede im Nucleus infundibularis der Vögel (Passeriformes). Z. Zellforsch. 92, 406-421.

OKSCHE, A., LAWS, D. F., KAMEMOTO, F. I., and FARNER, D. S. (1959). The hypothalamo-hypophysial neurosecretory system of the White-crowned Sparrow (Zonotrichia leucophrys gambelii). Z. Zellforsch. 51, 1-42.

RILEY, G. (1936). Light regulation of sexual activity in the male sparrow (Passer domesticus). Proc. Soc. Exptl. Biol. Med. 34, 331-332.

SHARP, P. J., and FOLLETT, B. K. (1969). The effects of hypothalamic lesions on gonadotropin release in Japanese Quail (Coturnix coturnix japonica). Neuroendocrinology 5, 205-218.

SNAPIR, N., NIR, I., FURUTA, F. and LEPKOVSKY, S. (1969). Effect of administered testosterone propionate on cocks functionally castrated by hypothalamic lesions. Endocrinology 84, 611-618.

STERN, J. W., and EISENFELD, A. J. (1969). Androgen accumulation and binding to macromolecules in seminal vesicles: inhibition by cyproterone. Science 166, 233-235.

STETSON, M. H. (1969). The role of the median eminence in control of photoperiodically induced testicular growth in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Z. Zellforsch. 93, 369-394.

THREADGOLD, L. T. (1960). Testicular response of the House Sparrow, Passer domesticus, to short photoperiods and low intensities. Physiol. Zool. 33, 190-205.

VITUMS, A., MIKAMI, S., and FARNER, D. S. (1965). Arterial blood supply to the brain of the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Anat. Anz. 116, 309-326.

VITUMS, A., ONO, K., OKSCHE, A., FARNER, D. S., and KING, J. R. (1966). The development of the hypophysial portal system in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Z. Zellforsch. 73, 335-366.

VON BERSWORDT-WALLRABE, R., and NEUMANN, F. (1967). Influence of a testosterone antagonist (cyproterone) on pituitary and serum FSH content in juvenile male rats. Neuroendocrinology 2, 107-112.

VON BERSWORDT-WALLRABE, R., and NEUMANN, F. (1968). Influence of a testosterone antagonist (cyproterone) on pituitary and serum ICSH content in juvenile male rats. Neuroendocrinology 3, 332-336.

WILSON, F. E. (1967). The tubero-infundibular neuron system: a component of the photoperiodic control mechanism of the White-crowned Sparrow, Zonotrichia leucophrys gambelii, Z. Zellforsch. 82, 1-24.

WILSON, F. E. (1970). The tubero-infundibular region of the hypothalamus: a focus of testosterone sensitivity in male Tree Sparrows (Spizella arborea). In "Aspects of Neuroendocrinology," (W. Bargmann and B. Scharrer, eds.) pp. 274-286. Springer-Verlag, Berlin.

WILSON, F. E., and FARNER, D. S. (1965). Effects of hypothalamic lesions on testicular growth. Fed. Proc. 24, 129.

WILSON, F. E., and HANDS, G. R. (1968). Hypothalamic neurosecretion and photoinduced testicular growth in the Tree Sparrow, Spizella arborea. Z. Zellforsch. 89, 303-319.

ACKNOWLEDGMENTS

Several individuals that aided in the completion of this project deserve mention.

First, I would like to express my appreciation to Dr. Fred E. Wilson whose laboratory direction, assistance in constructing the experimental design, and critical review of the manuscript greatly enhanced the completion of the project. Doctors C. L. Chen, M. F. Hansen, and J. S. Weis have also critically reviewed the manuscript.

I would like to thank D. J. Pihlaja for assistance in photomicroscopy, H. M. Schuler for suggestions in staining techniques and Dr. H. T. Gier and associated graduate students for technical assistance at various times throughout the course of the project.

J. V. Morrison deserves mention for aid in collecting Tree Sparrows and assistance in performing laparotomies.

I appreciate the suggestions of D. A. Rehovsky and R. S. Wampler in selection of materials used in surgical procedures.

I am grateful to French's Pet Bird Laboratory, Rochester, New York, who provided part of the parakeet foods, and Berlin Laboratories, Inc., New York, New York, for the cyproterone.

Finally, I wish to thank my wife, Janice, for her support throughout the project, for assisting in the care of experimental animals, and for typing the manuscript.

EFFECTS OF INTRAHYPOTHALAMIC IMPLANTATION OF TESTOSTERONE PROPIONATE AND OF CYPROTERONE (AN ANTIANDROGEN) ON THE TESTICULAR CYCLE OF THE TREE SPARROW (SPIZELLA ARBOREA)

by

EDWARD KENT CUSICK

B. A., University of Northern Colorado, 1968

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

BIOLOGY

Division of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

The precise mechanism by which spontaneous testicular regression is induced in photoperiodic species of birds is unknown. Previous investigations using intrahypothalamic implantation techniques suggest that androgen has an inhibitory feedback effect on gonadotropin secretion, and some investigators believe that feedback of androgen may play an important role in the initiation of seasonal testicular regression. The site at which androgen exerts its influence on gonadotropic function has not been elucidated with absolute certainty, but recent studies point to the tuberoinfundibular region of the hypothalamus as an important focus of androgen sensitivity.

To determine the effect of androgen on gonadotropin secretion during photoinduced testicular growth, photostimulated Tree Sparrows (Spizella arborea) were implanted stereotaxically with testosterone propionate 3 weeks after transfer from 8- to 20-hour daily photoperiods. At sacrifice, after 4 additional weeks on long days, all birds with testosterone propionate implants in the basal infundibular nucleus showed histological signs of regression of both testes and seminal sacs; magnitude of regression ranged from moderate to complete. Testes and seminal sacs of birds with cholesterol implants were fully active. These data confirm the potential for inhibitory feedback control of gonadotropin secretion by androgen during photoinduced testicular growth in the Tree Sparrow.

To assess whether androgen is involved in the onset of spontaneous testicular regression, 27-gage stainless steel tubes containing the free alcohol of cyproterone, an androgen antagonist, were implanted into the hypothalamo-pituitary axes of photosensitive, nonphotostimulated males. On the day following implantation, birds were transferred from 8- to 20-hour daily photoperiods. At sacrifice, 17 weeks thereafter, 1 bird was in full

breeding condition and testes of 6 others were partially regressed; regression of seminal sacs in the latter ranged from partial to complete. Cyproterone implants in 6 of the 7 (including the bird in full breeding condition) were located centrally in the basal infundibular nucleus and within that region of the hypothalamus shown sensitive to testosterone propionate. Birds with control implants or cyproterone implants outside the effective zone had fully regressed testes and seminal sacs. These results support the hypothesis that androgen plays a role in the onset of spontaneous testicular regression and suggest that androgen sensitivity is a property of a relatively discrete region of the basal infundibular nucleus of the hypothalamus.