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Adrenocortical Function of the Domestic Fowl: Effects of Orchiectomy and Androgen Replacement[1] (42539)

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Abstract. The effect of orchiectomy and androgen replacement on cockerel adrenocortical function was investigated. Orchiectomized cockerels (2 weeks old) were implanted with Silastic tubing containing various amounts of one of the following steroids: cholesterol, testosterone (T), androstenedione (A_4), and 5α-dihydrotestosterone (DHT). Birds were administered additional implants, containing doses of steroids equivalent to those of the initial implants, at 4 and 8 weeks of treatment (i.e., 6 and 10 weeks of age). Sham-operated cockerels administered empty implants served as intact controls for comparison of data. Animals were killed after 10 weeks of treatment (12 weeks old). Trunk plasma corticosterone (B) and plasma T, and B production by collagenase-isolated adrenocortical cells incubated briefly (2 hr) with or without steroidogenic agents were measured by radioimmunoassay. Orchiectomy with implantation of the inert sterol, cholesterol (hereafter referred to as orchiectomy), did not alter plasma B concentrations and did not affect basal cellular B production or cellular B production induced by a maximal steroidogenic concentration of ACTH or that maximally supported by 25-hydroxycholesterol. However, orchiectomy did lower maximal 8-bromo-cyclic AMP-induced B production by 30%. Low-implant doses of A_4 (1-cm implant) and T (0.3-cm implant), that maintained comb growth, lowered plasma B concentrations by 24–42%, whereas a high-implant dose of T (3-cm implant) and all implant doses of DHT had no effect on plasma B concentrations. Thus, androgen replacement had different effects on plasma B depending on the type of androgen and the implant dose. In contrast, androgen replacement consistently suppressed basal and maximal ACTH-induced cellular B production regardless of the type of androgen. Furthermore, the degree of suppression was dose-dependent. These results suggest that the differential effect of androgen replacement on plasma B concentrations was due to differences in the clearance of circulating B and/or differences in blood volume. In addition, the present study suggests that in the absence of the testes, androgens are suppressants of adrenocortical cell function in the domestic fowl.

Studies with the orchiectomized rat suggest that testicular androgens play a role in the regulation of mammalian adrenocortical function. For example, studies *in vitro* have shown that orchiectomy results in an increase in adrenal pregnenolone synthesis, whereas testosterone (T) replacement restores pregnenolone synthesis to normal values (1). In addition, other work *in vivo* and *in vitro* has shown that orchiectomy reduces adrenal corticosterone (B) secretion rates (2, 3) and B production by adrenal slices and homogenates (1, 4–6), whereas these effects of orchiectomy are reversed with T replacement (1, 3). Furthermore, studies have demonstrated that the effects of orchiectomy and T on adrenal function are mediated by their opposite actions on adrenal 5α-reductase, an enzyme that degrades B to 5α-reduced metabolites (1, 7).

In contrast to these reports of mammalian adrenocortical function, little information is available on the role of the testis and androgens in the regulation of avian adrenocortical function. Earlier work *in vivo* has shown that orchiectomy can either increase (cortical hypertrophy) or decrease relative adrenal weight depending on domestic fowl strain, age of orchiectomy, and duration of the orchiectomized state. In addition, in the case of cortical hypertrophy after orchiectomy, histological examination suggested an increase in cortical

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function (8). In support of the earlier work, a more recent study has shown that orchiectomy increases the stress-induced release of B in the adrenal vein effluent. Furthermore, T replacement prevented this effect of orchiectomy and *in vitro* suppressed B production by adrenal tissue from intact cockerels (9).

Although previous studies suggest that orchiectomy increases and T suppresses adrenocortical function in the domestic fowl, there have been no reports on the role of the testis and androgens in the regulation of fowl adrenocortical cell function per se. Accordingly, in the present study we evaluate the influence of orchiectomy and androgen replacement on domestic fowl adrenocortical function at the cellular level by measuring the B response of isolated domestic fowl adrenocortical cells incubated briefly with steroidogenic agents.

Materials and Methods. *Animals.* White Leghorn cockerels (2 weeks old) were orchiectomized or sham operated according to procedures described elsewhere (10, 11). Orchiectomized cockerels were implanted with Silastic tubing (1.57 mm i.d., 3.80 mm o.d.; Dow Corning) (sc, flank region) of various lengths (0.3, 1, and 3 cm) containing various amounts of one of the following crystalline steroids: cholesterol, testosterone, 5α-dihydrotestosterone (DHT), and androstenedione (A_4). Implants were packed manually with crystalline steroids and the ends were plugged with silicone. The amounts of steroid contained in the implants were as follows (means $\pm$ SEM of 20 randomly chosen implants, 5 for each steroid): 2.6 ± 0.2 mg (0.3 cm), 8.9 ± 0.4 mg (1.0 cm), and 29.2 ± 0.7 mg (3.0 cm). Cockerels received additional implants, containing doses of steroids equivalent to those of the initial implants, at 4 and 8 weeks of treatment (6 weeks and 10 weeks of age) to ensure adequate androgen replacement with increasing growth. Sham-operated cockerels receiving empty implants served as intact controls for comparisons of data. Orchiectomized cockerels receiving implants containing the inert sterol, cholesterol (hereafter referred to as orchiectomy), served as controls for steroid replacement. Birds were housed randomly in Petersime brood units under a 12-hr light:12-hr dark photocycle in a temperature and humidity controlled room and had free access to food and water. At 12 weeks of age (10 weeks of treatment), birds from the different treatment groups were collected together in plastic carrying crates, transported to a surgery room, and then quickly killed at random by decapitation and exsanguination. Trunk blood was collected in chilled heparinized plastic culture tubes and quickly centrifuged (1000*g*, 15 min, 4°C) to prepare plasma. Adrenal glands and livers were quickly removed, trimmed free of adhering connective tissue, and weighed. Adrenal glands were then immediately immersed in cold Krebs–Ringer Hepes (*N*-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (24.2 m*M* Hepes, 118.5 m*M* NaCl, 4.75 m*M* KCl, 2.54 m*M* $CaCl_2$, 1.20 m*M* KH_2PO_4, 1.20 m*M* $MgSO_4$, pH 7.5) containing 11.1 m*M* glucose (KRHG) until processing for the isolation of adrenocortical cells. KRHG was the basic medium for cell isolation and incubation.

Adrenocortical cell isolation and incubation. Adrenal glands were diced into small pieces (about 2 mm^3) for cell isolation. In each experiment eight cockerels from each treatment group were used to provide sufficient adrenal tissue for the desired cell concentration. The yield of cells per adrenal was not different among the groups (approximately 630,000 cells/adrenal).

Enriched adrenocortical cell populations were prepared essentially as described in detail previously (12, 13) using collagenase (0.2%) (Type I, Sigma Chemical Co.) and mechanical agitation followed by Percoll (Pharmacia Fine Chemicals) continuous density gradient centrifugation in order to separate adrenocortical cells from cell debris and other adrenal cell types. For each treatment group, light microscopy was used to establish the paucity of medullary cells and that 85–93% of the cell populations were comprised of adrenocortical cells, as determined by the presence of lipid droplets. Recent work (14) using electron microscopy has confirmed the light microscopic identification of adrenocortical cells. Adrenocortical cells were suspended in KRHG containing 0.25% bovine serum albumin and diluted to a final cell concentration of 1×10^5 cells/ml. Aliquots of cell suspensions were incubated with various concentrations of ACTH [ACTH-(1-24), Cortrosyn; Organon, Inc.], 8-bromo-cyclic AMP (8-Br-cAMP) (Sigma Chemical Co.) and 25-hydroxycholesterol (Steraloids, Inc.) at 39.5–41.0°C for 2 hr. The

incubation volumes were 200–500 μl (90% of the incubation volume was cell suspension; 10% of the incubation volume was a solution containing a steroidogenic agent). Prior to the addition of these agents to cell suspensions, ACTH was dissolved in a solution of 0.9% NaCl, pH adjusted to 3.0 with 1.0 *N* HCl, containing 0.1% bovine serum albumin, whereas 8-Br-cAMP and 25-hydroxycholesterol were made up in the incubation medium. Initial dilutions of 25-hydroxycholesterol were carried out in absolute ethanol. However, the final maximal concentration of ethanol (1%) did not affect corticosteroidogenesis. In each experiment at least 92% of the cells were viable as indicated by trypan blue dye exclusion (15).

Radioimmunoassay for B and T. Aliquots of plasma (200 μl) were extracted with dichloromethane (1 vol plasma:10 vol dichloromethane) and then the vacuum-dried residue from the extract was processed for radioimmunoassay for B. Recoveries were routinely 82%. Cell suspensions were assayed directly for B. B, the major glucocorticoid secreted by the domestic fowl adrenal gland (16), was measured by a modification of the radioimmunoassay procedure of Roy *et al.* (17) using specific antibody (Miles Research Products). As little as 0.1 ng B/ml could be detected. Radioimmunoassay of aliquots of the same pooled plasma samples or cell suspensions (performed with each radioimmunoassay) showed intraassay and interassay coefficients of variation of 6.3 and 10.1%, respectively. Plasma T was measured directly using a highly specific radioimmunoassay kit (antibody-coated tubes, ^{125}I-T) (Diagnostic Products Inc.). As little as 15 pg T/ml could be detected.

Analysis of data. The data of the responses of isolated adrenocortical cells to steroidogenic agents were analyzed using a four-parameter logistic equation model that is available as a computer program (ALLFIT, Biomedical Computing Technology Information Center, Vanderbilt Medical Center, Nashville, TN). This method allowed the simultaneous testing of a family of dose–response curves (18). Values for half-maximal steroidogenic concentrations (ED_{50}) and maximal production values were calculated and statistically analyzed using this program.

Other data (adrenal gland weights, plasma B values, etc.) were statistically analyzed by analysis of variance (19). Data are expressed as means ± SEM; means were deemed significantly different at $P \leq 0.05$.

Results. *Effect of orchiectomy and androgen replacement on adrenal growth.* In the work reported here, data of the various orchiectomized treatment groups are compared to the data of sham-operated (intact) cockerels receiving empty implants. In addition, orchiectomized cockerels receiving implants containing the inert sterol, cholesterol, are referred to as orchiectomy.

Table I shows the effect of orchiectomy and a fixed level of androgen replacement (1-cm implants) on body weight, adrenal and liver weights, and relative adrenal and liver weights (weights expressed as mg/100 g body wt and g/100 g body wt, respectively). Although orchiectomy did not affect body and liver weights and relative liver weight, it slightly decreased adrenal weight and relative adrenal weight by 11.0 and 8.7%, respectively. T replacement, sufficient to maintain comb growth almost to the value of intact cockerels (Table II), maintained adrenal weight and relative adrenal weight in orchiectomized birds. However, T replacement did not affect body weight, liver weight, and relative liver weight. In contrast to the effects of T replacement, DHT replacement, which induced a 23% greater comb growth than that of the sham-operated, intact group (Table II), decreased adrenal weight by 18.5% which was 8.4% lower than the decrease resulting from orchiectomy. However, DHT replacement decreased body weight by 19.6%; thus, relative adrenal weight was maintained by DHT. In contrast, DHT did decrease liver weight and relative liver weight by 27.2 and 10.3%, respectively.

Effects of orchiectomy and androgen replacement on plasma B concentrations. Orchiectomy had no affect on plasma B concentrations (Table II). T replacement, which partially maintained plasma T concentrations and partially maintained comb growth, decreased plasma B values by 42.4%. However DHT replacement, which had a greater androgenic effect than T replacement in terms of comb growth, had not effect on plasma B concentrations when compared to the sham-operated group; however, it did raise plasma B to a level that was significantly greater than that due to orchiectomy.

TABLE I. EFFECTS OF ORCHIECTOMY AND ANDROGEN REPLACEMENT ON BODY WEIGHT, ADRENAL WEIGHT, RELATIVE ADRENAL WEIGHT, LIVER WEIGHT, AND RELATIVE LIVER WEIGHT OF DOMESTIC FOWL

Treatment	N[a]	Body weight (g)[b]	Adrenal weight (mg)[b]	Relative adrenal weight (mg/100 g body wt)[b]	Liver weight (g)[b]	Relative liver weight (g/100 g body wt)[b]
Sham-operated, empty implants	30	1030.8 ± 18.7	110.5 ± 3.1[d]	10.73 ± 0.26[d]	21.1 ± 0.5	2.04 ± 0.03
Orchiectomized, implanted						
Cholesterol	22	1004.9 ± 21.4	98.3 ± 3.2[c]	9.79 ± 0.26[c]	20.5 ± 0.4	2.05 ± 0.04
Testosterone	26	1003.9 ± 21.3	106.0 ± 3.4	10.56 ± 0.27	21.0 ± 0.6	2.09 ± 0.04
5α-Dihydro-testosterone	18	828.6 ± 30.5[c,d]	90.0 ± 3.5[c,d]	11.02 ± 0.45	15.3 ± 1.0[c,d]	1.83 ± 0.08[c,d]

Note. Cockerels (2 weeks old) were implanted with Silastic tubing (1 cm) containing nothing (sham operated) or crystalline steroids (orchiectomized). Cockerels were administered additional 1-cm implants at 4 and 8 weeks of treatment and then were killed at 12 weeks of age (total treatment period of 10 weeks).

[a] Values of N for treatments of orchiectomized birds were different due to the exclusion of operated birds having testis remnants.

[b] Values are the means ± SEM.

[c] Values significantly different from values for sham-operated birds administered empty implants ($P \leq 0.05$).

[d] Values significantly different from values for orchiectomized birds implanted with cholesterol ($P \leq 0.05$).

Effect of orchiectomy and androgen replacement on the response of isolated adrenocortical cells to steroidogenic agents. The preceding results suggest that some aspects of orchiectomy and androgen replacement affected adrenocortical function. To learn if adrenocortical function per se was affected, we measured the influence of the treatments *in vivo* on B production by isolated adrenocortical cells. Regardless of treatments *in vivo,* steroidogenic agents stimulated cellular B production in a dose-dependent manner over approximately two log orders of concentration (Fig. 1). However, the data of Fig. 1 indicate that the responses of cells isolated from the various treatment groups were different. In order to quantitate the effects of the various treatments on cell function, the data of Fig. 1 were sta-

TABLE II. EFFECTS OF ORCHIECTOMY AND ANDROGEN REPLACEMENT ON COMB GROWTH AND PLASMA CONCENTRATIONS OF TESTOSTERONE AND CORTICOSTERONE OF DOMESTIC FOWL

Treatment	N[a]	Approximate comb area[b] (cm^2)[c]	Plasma testosterone (ng/ml)[c]	Plasma corticosterone (ng/ml)[c]
Sham-operated, empty implants	30	29.5 ± 1.6[e]	0.86 ± 0.13[e]	2.73 ± 0.33
Orchiectomized, implanted				
Cholesterol	22	3.2 ± 0.2[d]	0.13 ± 0.04[d]	2.28 ± 0.38
Testosterone	26	24.4 ± 0.9[d,e]	0.39 ± 0.04[d,e]	1.58 ± 0.18[d,e]
5α-Dihydrotestosterone	18	36.4 ± 1.3[d,e]	0.09 ± 0.03[d]	3.37 ± 0.42[e]

Note. Cockerels (2 weeks old) were implanted with Silastic tubing (1 cm) containing nothing (sham operated) or crystalline steroids (orchiectomized). Cockerels were administered additional 1-cm implants at 4 and 8 weeks of treatment and then were killed at 12 weeks of age (total treatment period of 10 weeks).

[a] Values of N for treatments of orchiectomized birds were different due to the exclusion of operated birds having testis remnants.

[b] One-half (base) × maximal height of the planar projection of the comb.

[c] Values are the means ± SEM.

[d] Values significantly different from values for sham-operated birds administered empty implants ($P \leq 0.05$).

[e] Values significantly different from values for orchiectomized birds implanted with cholesterol ($P \leq 0.05$).

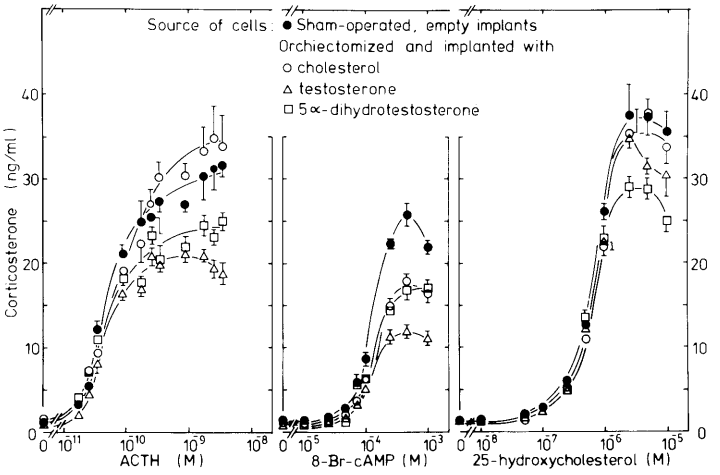

FIG. 1. Effect of orchiectomy and a fixed dose (1-cm implants) of androgen replacement on corticosterone production by isolated domestic fowl adrenocortical cells. Cells (1×10^5 cells/ml) were incubated with various concentrations of ACTH, 8-Br-cAMP, and 25-hydroxycholesterol for 2 hr. Each symbol represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments).

tistically analyzed by computer (see Materials and Methods), and maximal B production induced by each agent (Table III) and the ED_{50} for each agent (Table IV) were calculated.

Orchiectomy did not affect basal cellular B production. However, both T and DHT replacement decreased basal cellular B production by 22.8 and 25.2%, respectively (Table III). Similarly, orchiectomy did not affect maximal B production induced by ACTH and that supported by 25-hydroxycholesterol, whereas orchiectomy did decrease maximal 8-Br-cAMP-induced B production by 29.6%. T replacement decreased maximal 8-Br-cAMP-induced B production to a greater extent than orchiectomy (cholesterol implanted) (decrease of 50.9%), whereas DHT replacement did not alter the suppressive effect of orchiectomy. However, both T and DHT replacement decreased maximal B production induced by

TABLE III. EFFECTS OF ORCHIECTOMY AND ANDROGEN REPLACEMENT ON BASAL AND MAXIMAL CORTICOSTERONE PRODUCTION BY ISOLATED ADRENOCORTICAL CELLS FROM DOMESTIC FOWL

Source of cells	Basal	ACTH	8-Br-cAMP	25-Hydroxycholesterol
Sham-operated, empty implants	1.83 ± 0.04	30.04 ± 2.65	23.26 ± 1.28[b]	36.44 ± 2.15
Orchiectomized, implanted				
Cholesterol	1.85 ± 0.04	32.91 ± 3.05	16.37 ± 1.01[a]	35.45 ± 2.62
Testosterone	1.41 ± 0.07[a,b]	20.01 ± 0.70[a,b]	11.42 ± 0.35[a,b]	30.44 ± 1.72[a,b]
5α-Dihydrotestosterone	1.37 ± 0.04[a,b]	23.07 ± 1.08[a,b]	16.01 ± 0.72[a]	28.77 ± 1.30[a,b]

Note. Adrenocortical cells were isolated from the adrenal glands of sham-operated cockerels administered empty implants (1 cm) and orchiectomized cockerels administered a fixed dose of steroid replacement (1-cm implants). Cells (1×10^5 cells/ml) were incubated with various concentrations of ACTH, 8-Br-cAMP, and 25-hydroxycholesterol for 2 hr at 39.5–41.0°C. Corticosterone response data (Fig. 1) were statistically analyzed using the ALLFIT computer program. Values (ng/ml) are the means ± SEM from three experiments.

[a] Values significantly different from values for cells from sham-operated birds administered empty implants ($P \leq 0.05$).

[b] Values significantly different from values for cells from orchiectomized birds implanted with cholesterol ($P \leq 0.05$).

TABLE IV. EFFECTS OF ORCHIECTOMY AND ANDROGEN REPLACEMENT ON THE HALF-MAXIMAL STEROIDOGENIC CONCENTRATION (ED_{50}) OF STEROIDOGENIC AGENT WITH ISOLATED ADRENOCORTICAL CELLS FROM DOMESTIC FOWL

Source of cells	ACTH (10^{-10} *M*)	8-Br-cAMP (10^{-4} *M*)	25-Hydroxycholesterol (10^{-7} *M*)
Sham-operated, empty implants	4.28 ± 1.30	1.32 ± 0.13	7.32 ± 0.93
Orchiectomized, implanted			
Cholesterol	5.90 ± 1.26	1.43 ± 0.13	7.03 ± 0.97
Testosterone	4.38 ± 0.87	1.21 ± 0.19	8.13 ± 0.93
5α-Dihydrotestosterone	3.97 ± 0.92	1.24 ± 0.14	6.44 ± 0.87

Note. Adrenocortical cells were isolated from the adrenal glands of sham-operated cockerels administered empty implants (1 cm) and orchiectomized cockerels administered a fixed dose of steroid replacement (1-cm implants). Cells (1×10^5 cells/ml) were incubated with various concentrations of ACTH, 8-Br-cAMP, and 25-hydroxycholesterol for 2 hr at 39.5–41.0°C. Corticosterone response data (Fig. 1) were statistically analyzed using the ALLFIT computer program. Values are the means ± SEM from three experiments. Corresponding values between the treatment groups were not significantly different.

ACTH (decreases of 33.3 and 23.2%, respectively) and that supported by 25-hydroxycholesterol (decreases of 16.5 and 21.1%, respectively).

In contrast to the effects of treatments *in vivo* on cellular B production, there were no effects of treatments *in vivo* on steroidogenic agent ED_{50} values for cells isolated from the various fowl groups (Table IV). Since ED_{50} values are a measure of cellular sensitivity [the lower the agent ED_{50} value the greater the sensitivity of the cellular locus at which the agent acted (20)], the data suggest that orchiectomy and androgen replacement did not affect the binding or interaction of each agent with its cellular receptor or binding site (e.g., ACTH receptors, protein kinase, steroidogenic enzymes).

Dose dependency of the effect of androgen replacement on adrenocortical function. In the absence of the testis, a fixed dose of androgen replacement (1-cm implants) suppressed domestic fowl adrenocortical cell function. To determine the dose dependency of the suppressive effect by androgens, we implanted orchiectomized cockerels with various lengths of androgen-filled Silastic tubing (0.3, 1.0, and 3.0 cm), and after 10 weeks of treatment, trunk plasma B and T concentrations were measured (Table V) and the responses of isolated adrenocortical cells to ACTH and 25-hydroxycholesterol were evaluated (Fig. 2). Here again, sham-operated cockerels receiving empty implants served as controls for all comparisons of data.

Low-replacement doses of T (0.3- and 1.0-cm implants) decreased plasma B concentrations by 23.4 and 39.8%, respectively. Similarly, a fixed replacement dose of A_4 (1.0-cm implant) decreased plasma B values by 24.6%. However, a high dose of T (3.0-cm implant) did not affect plasma B concentrations. In contrast to the bimodal effect of T replacement doses, all levels of DHT replacement did not affect plasma B values. These differences in the effects of androgen replacement did not appear to be due to androgen availability to tissues, since plasma T values were either partially or completely maintained. In addition, comb growth, an index of tissue-accessible androgens, was completely maintained regardless of type of androgen or androgen replacement dose (Table V).

In contrast to the equivocal actions of the androgen replacement doses on plasma B concentrations, all androgen replacement doses consistently suppressed cellular basal and maximal ACTH-induced B production, and greater replacement doses (1.0- and 3.0-cm implants) of T and DHT suppressed maximal B production supported by 25-hydroxycholesterol (Fig. 2). In addition, the degree of the suppressive effect of androgen replacement on basal and maximal ACTH-induced B production by isolated adrenocortical cells was dependent on the dose of androgen replacement *in vivo*. However, the suppressive effect was quantitatively different depending on the agent added to the incubations. The maximal dose of androgen replacement (T and DHT,

TABLE V. EFFECTS OF ORCHIECTOMY AND ANDROGEN REPLACEMENT ON COMB GROWTH AND PLASMA CONCENTRATIONS OF TESTOSTERONE AND CORTICOSTERONE OF DOMESTIC FOWL

Treatment	N^a	Approximate comb area[b] (cm^2)[c]	Plasma testosterone (ng/ml)[c]	Plasma corticosterone (ng/ml)[c]
Sham-operated, empty implants	15	29.3 ± 1.2^e	0.83 ± 0.10^e	2.64 ± 0.18
Orchiectomized, implanted				
Cholesterol (1.0 cm)	15	3.9 ± 0.2^d	0.23 ± 0.04^d	2.95 ± 0.25
Androstenedione (1.0 cm)	14	26.5 ± 1.9^e	0.73 ± 0.12^e	$2.13 \pm 0.14^{d,e}$
Testosterone				
(0.3 cm)	14	28.6 ± 2.5^e	$0.37 \pm 0.04^{d,e}$	$2.03 \pm 0.08^{d,e}$
(1.0 cm)	14	$33.5 \pm 1.3^{d,e}$	$0.60 \pm 0.09^{d,e}$	$1.73 \pm 0.13^{d,e}$
(3.0 cm)	14	$37.2 \pm 1.8^{d,e}$	$1.92 \pm 0.18^{d,e}$	2.58 ± 0.21
5α-Dihydrotestosterone				
(0.3 cm)	14	32.0 ± 1.6^d	0.26 ± 0.04^d	2.96 ± 0.19
(1.0 cm)	14	$40.2 \pm 1.6^{d,e}$	0.19 ± 0.09^d	3.01 ± 0.31
(3.0 cm)	9	$41.9 \pm 2.5^{d,e}$	0.15 ± 0.05^d	2.89 ± 0.29

Note. Cockerels (2 weeks old) were implanted with Silastic tubing (1 cm) containing nothing (sham operated) or crystalline steroids (orchiectomized). Cockerels were administered additional 1-cm implants at 4 and 8 weeks of treatment and then were killed at 12 weeks of age (total treatment period of 10 weeks).

[a] Values of N for treatments of orchiectomized birds were different due to the exclusion of operated birds having testis remnants.

[b] One-half (base) × maximal height of the planar projection of the comb.

[c] Values are the means ± SEM.

[d] Values significantly different from values for sham-operated birds administered empty implants ($P \leq 0.05$).

[e] Values significantly different from values for orchiectomized birds implanted with cholesterol ($P \leq 0.05$).

3.0 cm) suppressed basal and maximal ACTH-induced B production by 55.1 and 63.9%, respectively, whereas it suppressed maximal 25-hydroxycholesterol-supported B production by 31.1%.

Discussion. The present study indicates that in the absence of the testes, androgen replacement suppressed domestic fowl adrenocortical cell function. T replacement suppressed basal and maximal cellular B production stimulated by ACTH, 8-Br-cAMP, and that supported by 25-hydroxycholesterol (Fig. 1 and Table III), and the suppression was dependent on the replacement dose (Fig. 2). A similar suppressive effect on adrenocortical cell function occurred with DHT replacement except that DHT replacement did not suppress maximal 8-Br-cAMP-induced B production to any greater extent than did orchiectomy. Our results with isolated cells are in agreement with the results of earlier work *in vivo* which demonstrated that T replacement lowered the concentration of B in adrenal vein effluent of stressed orchiectomized cockerels (9). In addition, our results extend the previous work in that they show a dose dependency of androgen replacement-induced suppression of domestic fowl adrenocortical function. Moreover, our results demonstrate that androgen replacement suppressed andrenocortical cell function per se. However, in contrast to the earlier work *in vivo* suggesting an enhancement of adrenocortical function by orchiectomy (9), our results suggest that orchiectomy had no effect on cellular function with the exception of an inexplicable suppression of maximal 8-Br-cAMP-induced B production (Fig. 1 and Table III). This discrepancy between our results and results of the earlier work may be due to the fact that the earlier work involved studies *in vivo,* whereas we conducted studies on isolated cells at the same cell concentration for each treatment group.

Some aspects of how androgen replacement suppressed domestic fowl adrenocortical cell function are suggested by the data. Androgen replacement suppressed basal and maximal B production (Figs. 1 and 2 and Table III) but did not affect steroidogenic agent ED_{50} values (Table IV). The data suggest that androgen replacement decreased the amount of factors (receptors, binding sites, etc.) operating at the steroidogenic pathway steps at which each steroidogenic agent acted but did not affect the

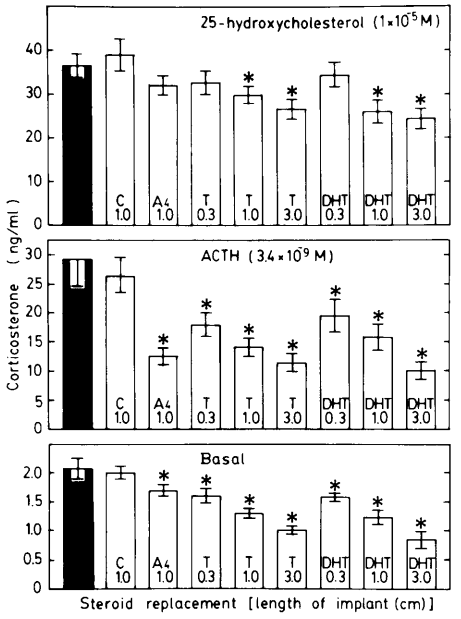

FIG. 2. Effect of orchiectomy and various doses of androgen replacement on basal and maximal corticosterone production by isolated domestic fowl adrenocortical cells. Cells (1×10^5 cells/ml) were incubated with or without maximal steroidogenic concentrations of ACTH (3.4×10^{-9} *M*) and 25-hydroxycholesterol (1×10^{-5} *M*) for 2 hr. Each column represents the mean of corticosterone values from nine cell incubations (three cell incubations from each of three experiments). Treatments: solid columns, sham-operated cockerels administered empty implants (1 cm); open columns, orchiectomized birds administered various length implants filled with crystalline steroids. SEM are represented by bars. (Values for cells from sham-operated cockerels administered empty implants and for cells from orchiectomized cockerels administered cholesterol implants were not significantly different. The * indicates values that are significantly different from values for cells from sham-operated cockerels administered empty implants and from orchiectomized cockerels administered cholesterol implants; $P \leq 0.05$).

interaction of the steroidogenic agents with these factors. In support of this notion, other work *in vitro* has demonstrated a direct suppression of adrenal protein synthesis by testosterone (21). However, a noncompetitive mechanism of suppression of steroidogenic steps is equally plausible since it would produce results similar to those presented here, that is, reduced B production without a change in steroidogenic agent ED_{50} values. In addition, our data (Table III and Fig. 2) suggest that steps earlier in the steroidogenic pathway were affected more than later steps since maximal ACTH- and 8-Br-cAMP-induced B production were suppressed to a greater degree than maximal B production supported by the steroidogenic precursor, 25-hydroxycholesterol.

Plasma B concentrations did not always reflect the function of isolated adrenocortical cells. On the one hand, orchiectomy, which did not alter cellular B production (with the exception of that maximally stimulated by 8-Br-cAMP) (Figs. 1 and 2, Tables III and IV), did not alter plasma B values (Tables II and V). In addition, T replacement doses (0.3 and 1 cm), which suppressed cellular B production (Figs. 1 and 2, Table III), decreased plasma B values (Tables II and V). On the other hand, a high-replacement dose of T (3 cm) and all replacement doses of DHT, which suppressed cellular B production (Fig. 2), had no effect on plasma B values (Tables II and V). These inconsistencies between plasma B and cellular B production values may have been due to the decreased clearance of circulating B caused by the high-replacement dose of T and all replacement doses of DHT. Indeed, there is evidence for a decreased clearance of B by administration of T to rats (22, 23). However, T does not appear to affect plasma B clearance in the domestic fowl (9). Another plausible explanation is that the high-replacement doses of T and DHT caused a sufficient decrease in body weight and organ weight resulting in a decreased blood volume and, thus, a subsequent increase in plasma B.

In addition to the different metabolic activities of the various androgens used in this study (24), it is possible that the different rates of androgens released into the plasma played a role in the differential effects of the androgens on plasma B and cellular B production. Other work with domestic fowl and similarly constructed implants (25) has demonstrated that T is released at a rate which is 1.4 times that of DHT. In the present study only plasma T was measured, and the replacement plasma concentrations were well within the physiologic range of domestic fowl (11, 24). Since plasma DHT concentrations are 20–50% of those of plasma T (24, 26), and since DHT is released more slowly from Silastic implants than T (25), presumably replacement plasma DHT concentrations were also within the

physiologic range. Thus, replacement plasma concentrations of T and presumably DHT, the principal physiologic androgens of the domestic fowl (24), were not pharmacologic concentrations.

Our results with orchiectomized cockerels can be compared with the results of other work with orchiectomized rats. In contrast to our work and the work of others (9) showing no change or enhanced adrenocortical B production with orchiectomy of the domestic fowl, other work *in vivo* and *in vitro* with orchiectomized rats indicates that adrenocortical B production is suppressed (1–7). In addition, the present study and earlier work (9) indicate that androgen replacement suppresses adrenal B production in the orchiectomized domestic fowl, whereas it maintains and restores adrenal B production in the orchiectomized rat (1, 3, 5, 7). Thus, the role of testicular androgens in the regulation of adrenocortical function may be considerably different between avian and mammalian species.

Somewhat puzzling is the fact that orchiectomy is required to demonstrate the different effects of androgen replacement on adrenocortical function in both rats (5) and domestic fowl (9). Equally puzzling are our results showing suppression of B production of cells from orchiectomized birds by replacement doses of T that maintained plasma T values at levels that were close to the physiologic levels of intact cockerels (11). We propose that the domestic fowl testis regulates adrenal B secretion by secreting factors that affect adrenocortical cell function: One of these factors, T, suppresses cellular function, whereas another factor(s) counteracts T action. Thus, in the intact bird, the opposing actions of these testicular substances bring about a fine regulation of adrenal B secretion. With orchiectomy, all testicular substances are removed and the adrenal is affected little. However, with orchiectomy and T replacement, the suppressive effect of T is unopposed resulting in a decrease in adrenal B production. Although this notion requires further testing, it is in keeping with the hypothesis that factors other than pituitary ACTH may be important regulators of adrenocortical function in the domestic fowl (13, 27, 28). Possibly a direct action of T and other testicular factors on the domestic fowl adrenocortical cell and an indirect action of T through the modulation of ACTH secretion, as evinced by studies with the orchiectomized rat (29), bring about a fine regulation of corticosteroidogenesis in the domestic fowl.

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