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The Effects of Castration on the Ventral Prostate of the Outbred Syrian Hamster (39754)

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Introduction. Androgens are required for the maintenance of a variety of target tissues including the accessory sexual glands. However, postcastrational characteristics of prostatic epithelial cells vary with species, genetic strain, and age (1), also with the specific lobe of the prostate and possibly with adrenal influences (2). In most species the prostate and seminal vesicles atrophy after castration and subsequent testosterone administration restores their RNA, protein, and DNA synthesis (3, 4). Compared to rats, atrophy of hamster target tissues after castration is slower and less pronounced (5). The studies herein reported describe some histological and biochemical changes observed in ventral prostate glands of outbred Syrian hamsters after castration and adrenalectomy.

Materials and methods. Outbred [LaK: LVG (SYR)] male hamsters (*Mesocricetus auratus*) (Lakeview Hamster Colony, Newfield, N. J.) averaged 90 to 120 g in body weight. All were provided commercial rodent chow and tap water, *ad libitum*. They were housed in groups of five or six and exposed to 11 hr of light and 13 hr of darkness daily. All castrations were performed via the scrotal route under ether anesthesia. Since adrenalectomized male hamsters (Lakeview Hamster Colony, 90–120 g) cannot be maintained with saline (6), our animals were given 5 mg of cortisone acetate (Research Plus Steroid Laboratory, Danville, N. J.) sc in 0.2 ml of corn oil daily. Cortisone acetate was used rather than desoxycorticosterone because of the reported progestational action of the latter in female hamsters (7). Animals given cyproterone acetate (Table II) were killed after receiving sc injections (100 mg/day) of the antiandrogen for 10 days beginning on the fifth day after castration.

All animals were killed by decapitation. Tissues for histology were fixed in Bouin's solution and sections were stained with hematoxylin and eosin. At necropsy precautions were followed to retain the secretions in the ventral prostate and combined seminal vesicles and coagulating glands. The glands and their secretions were weighed and stored at −30°c. The tissue extraction was basically that of Glazier and Weber (9). RNA was determined spectrophotometrically with baker's yeast RNA as the standard (10). DNA was determined according to Schneider (11) as modified by Burton (12) using salmon sperm DNA as the standard. Protein was assayed according to Lowry *et al.* (13) with bovine serum albumin as the standard.

Tartrate-sensitive prostatic acid phosphatase was assayed with *p*-nitrophenyl phosphate as the substrate (Sigma Kit 104-ATL, Sigma Chemical Company, St. Louis, Mo.) (14). The enzyme assays were performed on whole ventral prostates homogenized in 3 ml of deionized water. Other chemicals included 5α-dihydrotestosterone (5α-androstan-17β-ol-3-one), obtained from the Sigma Chemical Co., and cyproterone acetate (6α-chloro-17α-acetoxy-1α,2α-methylene-4,6-pregnadiene-3,20-dione), donated by Dr. Stanley Gould, Berlin Laboratories, New York, N. Y.

Results and discussion. Prolonged castration or combined castration and adrenalectomy had little or no effect on the histology of the ventral prostate gland of hamsters (Fig. 1). Thus, epithelial cell height was similar to that of normal hamsters at 22 days after adrenalectomy with castration (Figs. 1a and b). Prostatic acini were distended with secretions and the epithelium still ap-

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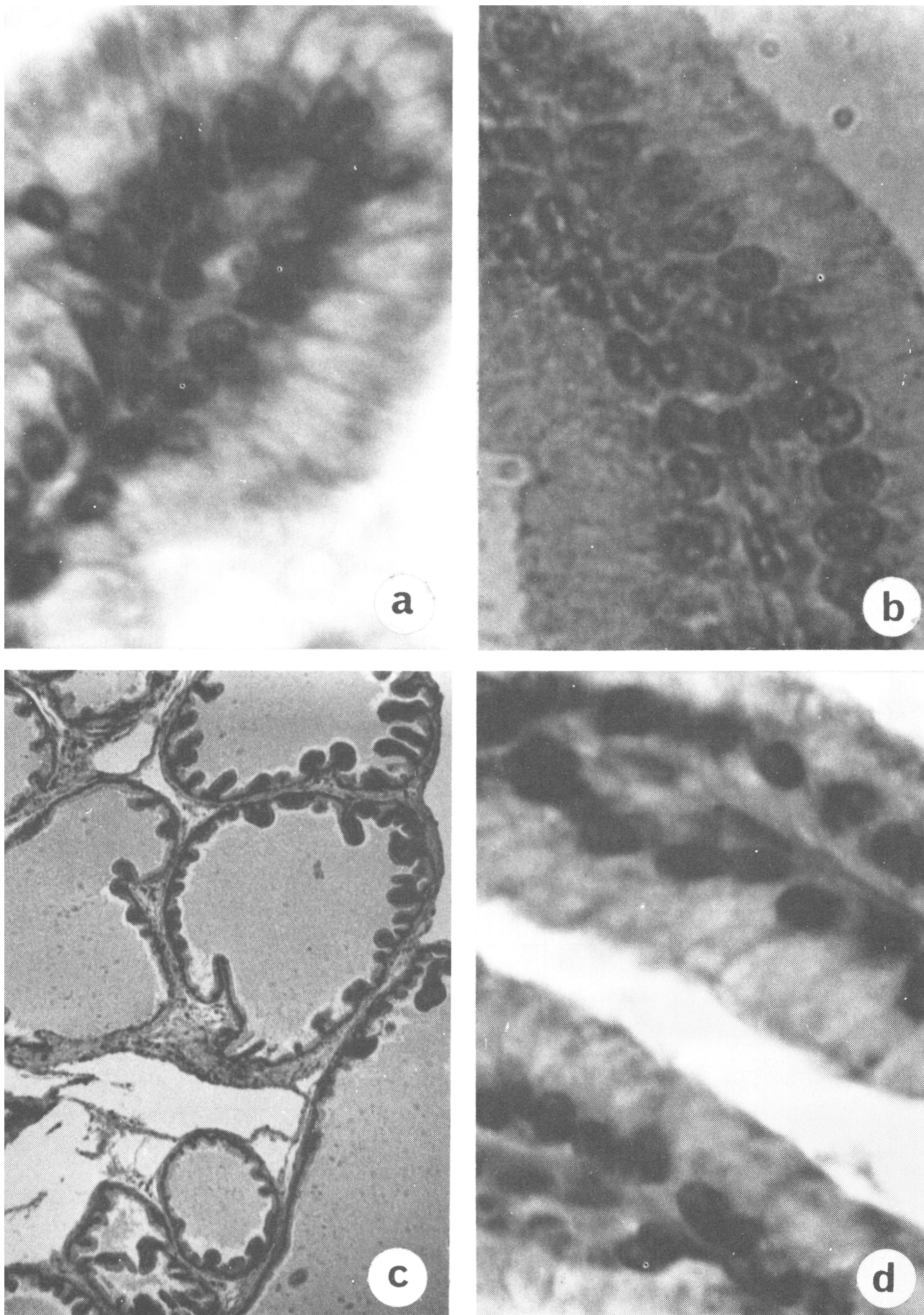

FIG. 1. (a) Ventral prostate gland of intact hamster. H&E 1520×. (b) Ventral prostate gland of castrated-adrenalectomized hamster (22 days). 1520×. (c) Ventral prostate gland of hamster castrated for 364 days. 60×. (d) Ventral prostate gland of hamster castrated for 364 days. 1520×.

peared normal after 364 days of castration (Figs. 1c and d). Normal maintenance of epithelial cell height and secretions was also observed in castrated hamsters given cyproterone acetate subcutaneously (5 mg/day) for 3 weeks. There were no apparent differences in the distribution of fibromuscular tissue between treatment groups.

The results suggest that continuous secretion of gonadal androgens was not required for the maintenance of prostatic epithelium and its secretions in adult hamsters. Ortiz *et al.* (1), who first reported that the accessory sexual glands of hamsters regress slowly after castration, speculated that the adrenal cortex of hamsters increases its androgen production after orchiectomy. However, our results show that the prostatic epithelium was maintained after castration combined with adrenalectomy, making it unlikely that adrenal androgens were involved. This conclusion agrees with the demonstration that hamster adrenals are not required for sexual activity after castration (15) even though adrenals of other species are known to produce significant amounts of androgens. Delost (2) reported that the ventral prostate of the vole increased its secretory activity 1 month after castration, and that it involuted completely following adrenalectomy. Although it is possible that cortisone acetate administered to the adrenalectomized hamster in the present study exerted androgenic effects, it is unlikely that it stimulated the ventral prostate while having no effect on the other accessory sexual glands. The histological studies showed that the other accessory glands atrophied at the same rate in castrated hamsters as in castrated plus adrenalectomized animals given cortisone acetate. Frenkel and Havenhill (8) reported that a similar dosage of cortisone acetate reduced the liver, spleen, thymus, and body weight of intact male hamsters, but they did not include a description of changes in accessory sexual glands.

Some species show changes in the androgen-producing *X* zone of the adrenals after castration. Although rats do not show these changes, there is development of a secondary *X* zone in castrated adult mice (16). Howard noted that 100 days after castration the secondary zone in mice had disappeared and the seminal vesicles showed uniform degeneration (16). An *X* zone in the adrenals of immature or mature hamsters, whether intact or castrated, has not been found (17). Likewise, accessory adrenal tissue has not been detected in males of this species (15). Thus it seems unlikely that adrenal tissue was supplying androgens sufficient to explain the lack of postcastrational secretory epithelial atrophy or loss of wet weight of the ventral prostate in the present studies.

The wet weights of the prostate glands of adult (120 g) and young adult (70 g) hamsters increased immediately following castration and remained elevated for approximately 20–25 days in the adult animals (Fig. 2a). The dramatic decline in acid phosphatase activity in these enlarged glands immediately following castration (Fig. 2b) reflects the androgen dependency of this enzyme, which is also characteristic of the rat prostatic enzyme (18). In contrast, Butler *et al.* (19) reported that acid phosphatase activity in the ventral prostates of adult inbred (LSH/Ss LaK) hamsters increased following castration, despite their decreased wet weight. However, histological changes accompanying these changes in weight were not described.

Table I summarizes the changes in wet weights, protein, RNA, and DNA content of the accessory sexual glands 2 weeks after castration. Although prostatic wet weight and total protein increased significantly, total DNA was significantly decreased ($P < 0.05$). Despite the apparent reduction in cell number, the decrease in total RNA was not significant. The protein/DNA ratio again reflected accumulation of proteinaceous secretions. In contrast, Giegel *et al.* (20) reported significant decreases in wet weight, protein, RNA, and DNA of hamster prostate glands after castration for 2 weeks. However, these workers did not specify the hamster strain, the prostatic lobe assayed, or whether the weights included glandular secretions. The significant decreases in wet weight, protein, and RNA of the Cowper's glands and combined seminal vesicles and coagulating glands which we observed reflect their androgen dependency. The decreased RNA/DNA and protein/DNA ratios indicate that the remaining cells in these glands contained less RNA and that proteinaceous secretions were greatly reduced.

The ability of cyproterone acetate (10 mg/day) to block the stimulation by dihydrotestosterone (DHT) administered to castrated adult hamsters is summarized in Table II. Castration appeared to increase the wet weight of the ventral prostate but not

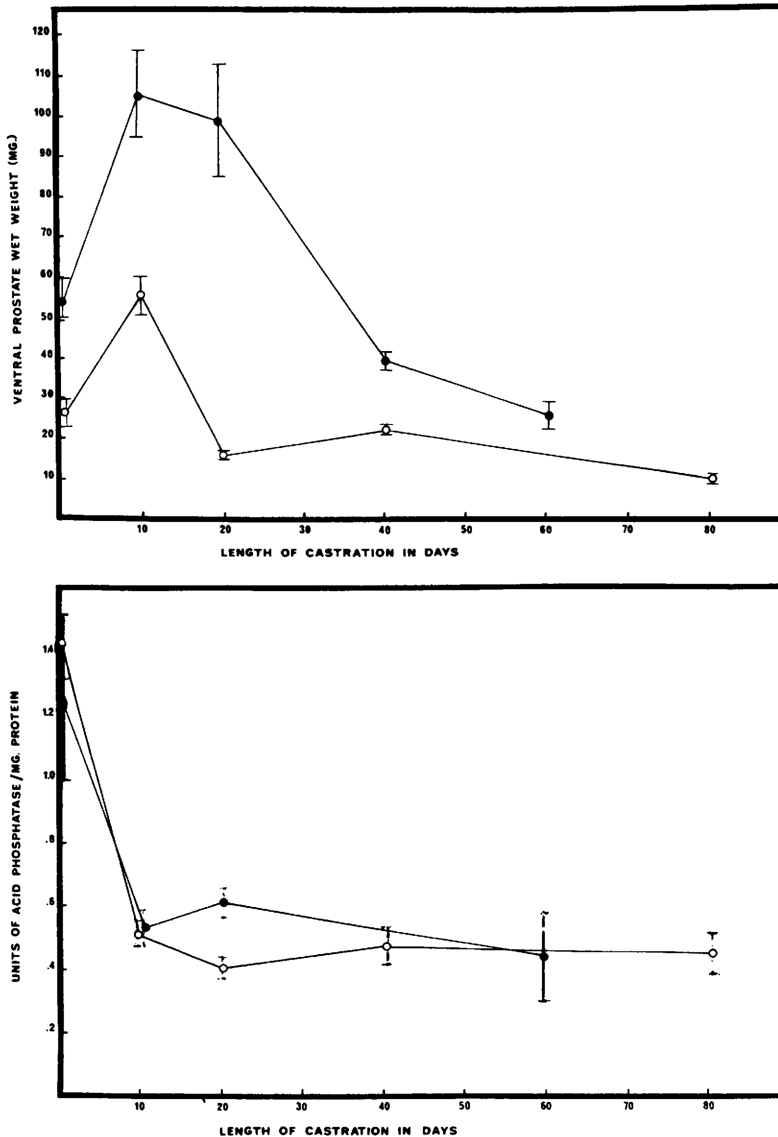

FIG. 2a. The effects of castration on the wet weight of the ventral prostate glands of adult (——) and young adult (O——O) outbred hamsters. Mean ± SEM for four experimental hamsters.

FIG. 2b. The effects of castration on the prostatic acid phosphatase concentration in the ventral prostate glands of adult (——) and young adult (O——O) outbred hamsters. One Sigma unit of phosphatase liberates 1 μmole of *p*-nitrophenyl/hr at 37°. Mean ± SEM for four experimental hamsters.

significantly in this experiment. Castration did cause statistically significant reduction in the wet weights of the other accessory sexual glands. Administration of DHT to castrates also restored the wet weights of the other glands to values which did not differ significantly from those of intact controls. Butler *et al.* (19) reported that castration of adult inbred LSH (strain 2.4) hamsters did not influence the weights of seminal vesicles and ventral prostates, although they were significantly increased by testosterone (0.2 mg/day). Although cyproterone acetate significantly reduced the effect of DHT on the wet weight of the Cowper's glands in the present study, its effect on the combined

TABLE I. WET WEIGHT AND NUCLEIC ACID AND PROTEIN CONTENT OF ACCESSORY SEXUAL GLANDS OF THE OUTBRED MALE SYRIAN HAMSTER 2 WEEKS AFTER CASTRATION.

10 10	Ventral prostate		Cowper's glands		Seminal vesicles and coagulating glands	
10	Intact	Castrated	Intact	Castrated	Intact	Castrated
Total weight (mg)	76±10 (9)[a]	106±8 (12)*	35±2 (10)	11±1 (12)*	1417±29 (10)	302±44 (12)*
Total DNA (μg)	503±19 (9)	420±11 (8)*	107±8 (10)	102±6 (8)	1391±74 (10)	1234±45 (10)
Total RNA (μg)	191±20 (8)	158±13 (11)	42±5 (9)	21±1 (11)*	897±57 (10)	597±42 (10)*
Total protein (μg)	3450±327 (8)	4370±286 (11)*	945±94 (10)	473±29 (10)*	80668±4085 (10)	12444±1444 (10)*
RNA/DNA	0.37	0.37	0.39	0.20	0.64	0.48
Protein/DNA	6.9	10.4	8.9	4.6	58.0	10.1

[a] Mean ± SEM Number of hamsters per group is given in parentheses.
* Difference between intact and castrated group is significant using Student T test ($P < 0.05$).

TABLE II. THE *In Vivo* EFFECT OF CYPROTERONE ACETATE ON THE WET WEIGHTS OF ACCESSORY SEXUAL GLANDS OF OUTBRED MALE SYRIAN HAMSTERS.[a]

Treatment	Daily dosage (mg) (5α-DHT + cyproterone acetate)[b]	Ventral prostate	Seminal vesicles and coagulating glands	Cowper's glands	Kidney
Intact controls	0.0 + 0.0	58.7 ± 10.0	1285.4 ± 86.8[c,d]	32.1 ± 1.2[c,d]	841.3 ± 18.3
Castrated controls	0.0 + 0.0	76.9 ± 18.6	152.9 ± 6.6[c,e,f]	7.9 ± .4[c,e,f]	901.2 ± 34.3
Castrated + 5α-DHT	0.5 + 0.0	75.5 ± 14.4	1061.1 ± 118.3[e]	33.0 ± 3.6[e,g]	950.7 ± 53.6
Castrated + 5α-DHT + cyproterone acetate	0.5 + 10.0	60.1 ± 3.1	765.3 ± 125.3[d,f]	21.0 ± 1.1[d,f,g]	1004.9 ± 18.5

[a]All values represent mean ± S.E.M. (four animals/treatment). All values for a given tissue with common superscripts (*c*–*g*) differ significantly ($P < 0.05$) when analyzed by Neuman Keuls pairwise contrasts procedure. All wet weights are expressed as mg/100 g body weight.

[b]All animals were castrated for 5 days prior to DHT + cyproterone acetate treatment for 10 days. First value is 5α-DHT dosage, the second is cyproterone acetate dosage.

weight of the seminal vesicles and coagulating glands was not significant. Antiandrogenic effects of cyproterone acetate on the ventral prostates may have been masked by the increase (not statistically significant) in wet weight which occurred after castration. It is possible, however, that higher doses of antiandrogen would decrease prostate weight. From these results it appears that the wet weight of the ventral prostate of the outbred hamster may not be a reliable endpoint for determining antiandrogenic potency.

The mechanism responsible for the postcastrational increase in the protein content of the hamster ventral prostate gland is unclear. Although the effects of testosterone on the prostate are potentiated by prolactin and growth hormone (21, 22), it is unlikely that either of these hormones exerted a significant stimulatory effect on the ventral prostates of our castrated hamsters. Despite the stimulatory effects of *in vivo* administration of prolactin in castrates (23, 24), most reports indicate that prolactin levels decline rapidly following castration (25), perhaps because of increased synthesis and/or release of prolactin release–inhibiting hormone by the hypothalamus. Aragona and Friesen (26) also observed that castration of male rats causes a decrease in binding of prolactin to membrane receptors in the ventral prostate. In contrast, FSH concentrations in other species increase following castration probably because of removal of the postulated inhibitory protein "inhibin," which is thought to be synthesized in the cytoplasm of developing spermatids (27). However, although FSH stimulates the production of androgen-binding protein by Sertoli cells (28), data showing that this gonadotrophin can directly stimulate the ventral prostate without affecting other accessory sexual glands are lacking.

Summary. Prolonged castration or castration combined with adrenalectomy had virtually no effect on epithelial cell height in the ventral prostate glands of outbred Syr-

ian hamsters. Although the wet weight of the prostate glands of adult and young adult hamsters increased following castration, the acid phosphatase activity in these glands decreased dramatically. The increase in wet weight was apparently due to the increased accumulation of proteinaceous secretions rather than to prostatic hyperplasia, since the DNA content of the glands decreased significantly after castration for 2 weeks. When administered *in vivo,* the antiandrogen cyproterone acetate had no detectable effect on prostatic epithelial cell height or overall wet weight. The data suggest that although the ventral prostate of the outbred hamster is an androgen-responsive tissue, neither its wet weight nor its secretory epithelium show typical postcastrational atrophy.

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