A Study of the Effects of a New Anti-Androgen on the Hyperplastic Dog Prostate (37384)

J. R. BROOKS, R. D. BUSCH, D. J. PATANELLI, AND S. L. STEELMAN
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

Man and the dog commonly experience the naturally occurring pathological condition known as benign prostatic hyperplasia or BPH (1, 2). In man, BPH is characterized by a nodular enlargement localized in the inner group of prostatic tubules whereas that of the dog is of a diffuse cystic nature (3). Although a voluminous literature relating to BPH is available, its etiology remains obscure. Prostatic enlargement is generally accepted to be a result of gonadal hormone stimulation or imbalance (4, 5). However, it is not known whether androgens, or estrogens, or a combination of the two are of primary importance in the onset of the disease.

Franks (6) made a strong case for the premise that human BPH begins with estrogenic stimulation of the "inner gland," an estrogen-sensitive area immediately surrounding the urethra. Other investigators maintain that androgens are mainly responsible for prostatic hyperplasia in the dog (7, 8) and man (9).

Medical nonsurgical therapy has been largely empirical and a variety of hormones have been given for relief of BPH. Estrogen administration caused a diminution of prostate size in both the dog (10) and man (11, 12). However, it has been pointed out (13) that estrogen therapy in the male is tempered by such unfortunate side effects as impotence, sterility and gynecomastia. Treatment with androgens was ineffective in at least one series of patients (14). Also, androgen administration is probably inappropriate if for no other reason than it may stimulate proliferation of a latent, androgen-dependent prostatic tumor (15). The use of progestational agents has usually yielded either negative (16) or inconclusive results (17). A few years ago a strongly anti-androgenic, highly progestational substance, cyproterone acetate (6-chloro-Δ^6-1,2α-methylene 17α-hydroxy progesterone acetate, SH-714) became available (18, 19), and studies in dogs (20) and man (21, 22) were conducted with encouraging results. However, cyproterone acetate possesses considerable potency as a gonadotrophin inhibitor (23) and would be expected to cause a decline in libido and spermatogenesis with continued administration.

In our laboratories a potent anti-androgen having virtually no other endocrine properties has recently been developed (24). It inhibits the uptake of labeled testosterone by the rat prostate and largely prevents the increase in ventral prostate weight of immature rats treated with testosterone. The compound, 6α,7α-difluoromethylene-4′,5′-dihydro-1α,2α-methylene-(17R)-spiro-[androst-4-ene-17,2′-(3′H)-furan]-3-one (Compound I) was tested in aged beagles in order to determine its effects on prostatic size and histology. This report will discuss the results of those dog studies and their possible import to human BPH.

Materials and Methods. Experiment 1. Seven beagles at least six and up to eleven years of age and weighing 10–16 kg were used. They were laparotomized after being anesthetized with Nembutal; length, width, and depth of the prostate were measured and it was needle biopsied. The dogs were allowed to recover for about 10 days prior to treatment. Three then received a daily subcutaneous injection of sesame oil vehicle, two were given 0.33 mg/kg/day of Compound I and two received 1.0 mg/kg/day. Compound I was administered subcutaneously in sesame oil. They were treated for thirty-five days and sacrificed the day following the final dose. At sacrifice, each prostate

TABLE I. Effect of Compound I Administered Subcutaneously for 35 Days on the Prostate Size of Intact Dogs.

Dog	Age, yr	Dose mg/kg/Day	Prostate dimensions (mm)							
			Pretreatment				After 35 days treatment			
			Length	Width	Depth	Vol. (cm³)	Length	Width	Depth	Vol. (cm³)
2172	8	Control	28	22	18	11.09	30	22	15	9.90
2173	8	Control	31	27	20	16.74	30	27	20	16.20
3929	8	Control	23	23	18	9.52	26	22	19	10.87
		Avg.	27	24	19	12.45	29	24	18	12.32
2177	8	0.33	34	27	22	20.20	33	21	15	10.40
2168	8	0.33	32	24	23	17.66	29	17	14	6.90
2317	6	1.0	33	29	24	22.97	26	23	18	10.76
2021	11	1.0	40	30	26	31.20	39	23	15	13.46
		Avg.	35	28	24	23.01	32	21	16	10.38

was measured *in situ* after which it was removed and a cross section taken for histology. A section of testis was also removed for histological examination.

Experiment 2. Four 10 to 12-year-old beagles were used. They were laparotomized and the length, width, and depth of the prostate were measured. The day after laparotomy a 48-day treatment period began. During that time two dogs received a daily oral dose of Compound I at a level of 2 mg/kg of body weight while two were given a placebo. Each dose of test compound was placed in a gelatin capsule which was then filled with lactose. Placebo capsules contained lactose alone. The capsules were hidden in a bolus of ground beef which was fed and eagerly accepted.

A second laparotomy was performed the day following the end of treatment and a third was carried out 60 days later. At each operation prostate measurements were taken. The dogs were sacrificed 120 days after the treatment period ended and *in situ* prostate dimensions were determined.

In both Expt 1 and Expt 2 the control dogs were selected as those having the smallest prostates at the time of the pretreatment laparotomy. Dogs with larger prostates received Compound I. Selections were made because a very limited amount of Compound I was available and it was thought best to use it in a situation where its effect, if any, would be most noticeable. Also, in previous work involving repeated measurements of the dog prostate over a span of several months, we had determined that prostate dimensions of untreated animals underwent little change within that period.

Results. Experiment 1. Thirty-five days of treatment produced a marked regression of prostate size which was unrelated to dose level. Length, width, and depth of the gland were less after the dosing period than before in each dog which received the compound. Average measurements of length, width, and depth for treated dogs were 35, 28, and 24 mm before treatment and 32, 21, and 16 mm afterwards, respectively. By contrast, prostate dimensions of the three control animals changed very little over the course of

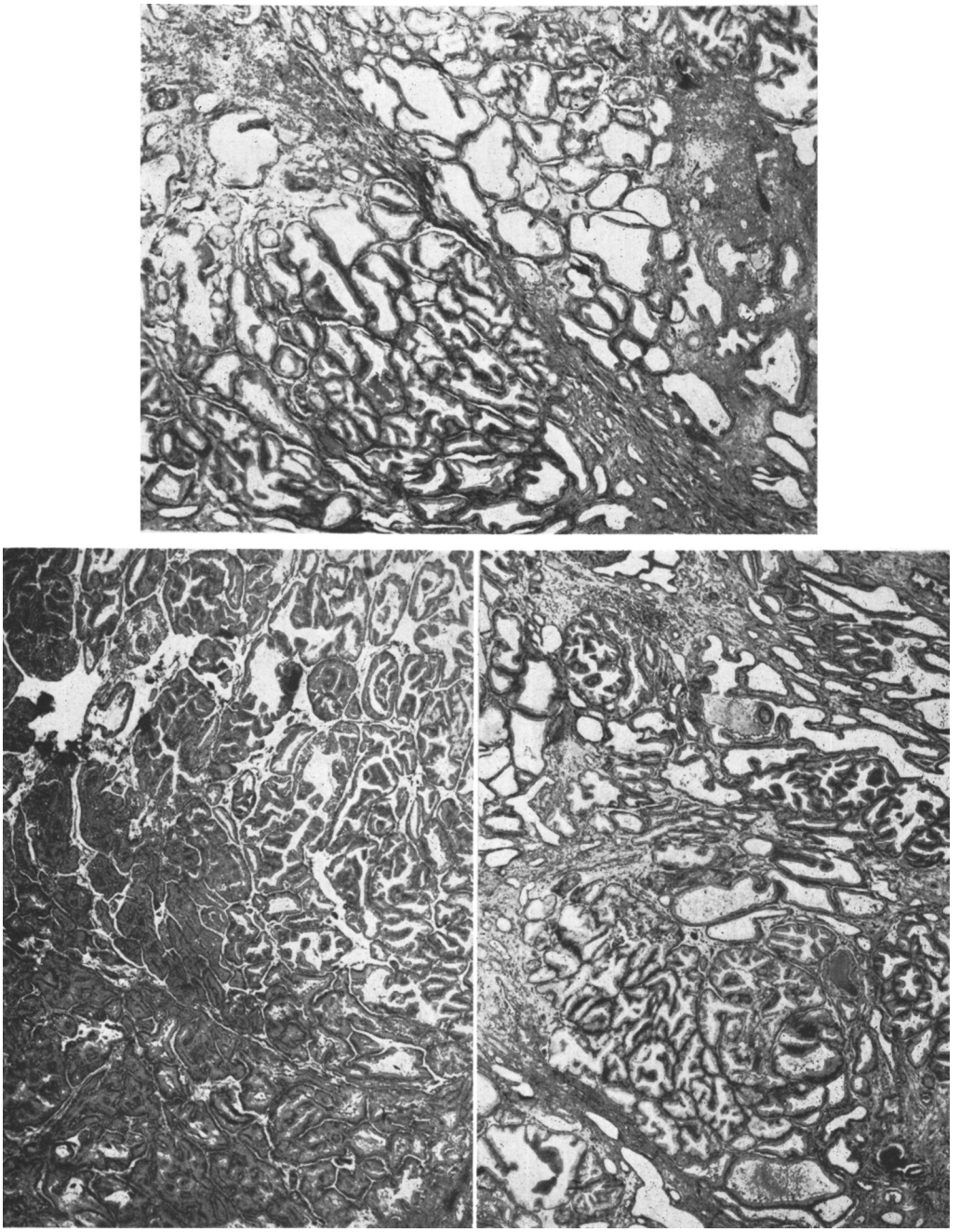

FIG. 1. Cross-sectional areas of prostates taken from control dogs which had received no Compound I. × 63. (Top) Dog 2172; (Bottom left) Dog 2173; (Bottom right) Dog 3929. Columnar epithelium predominates in each animal.

the experiment. In those dogs, the mean figures for length, width, and depth were 27, 24, and 19 mm at the pretreatment laparotomy and 29, 24, and 18 mm at the time of sacrifice (Table I).

Before treatment, the average volume for

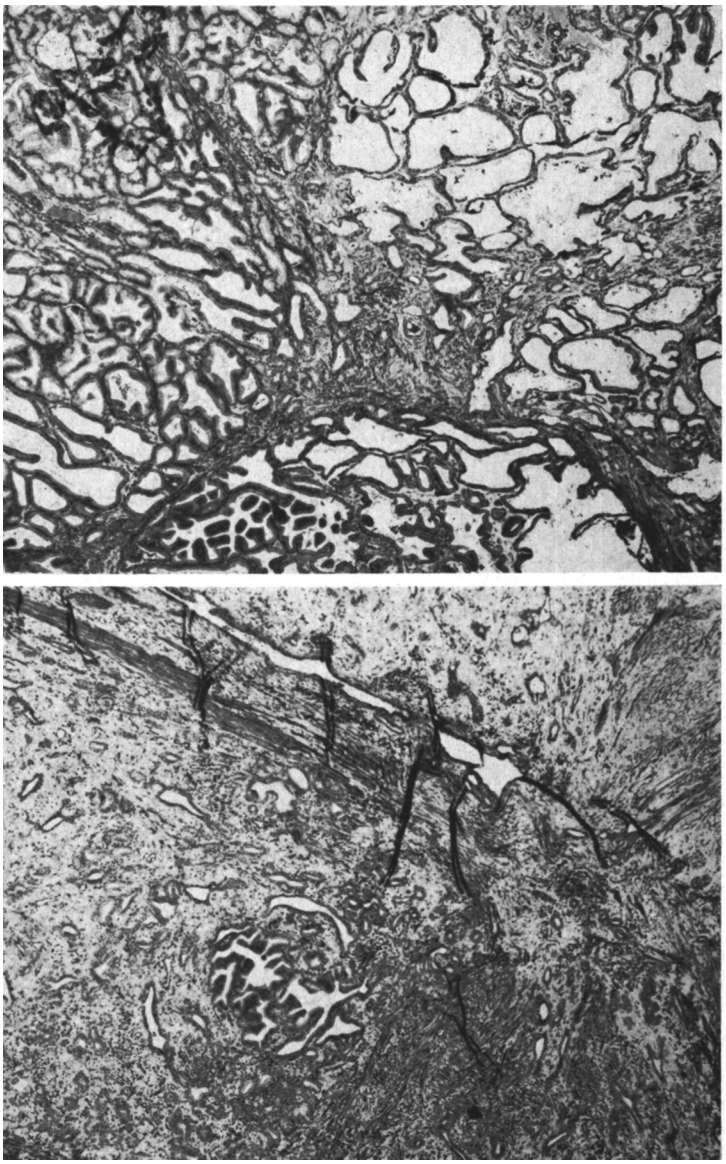

FIG. 2. Cross-sectional areas from the prostates of dogs treated with 0.33 mg/kg/day of Compound I for 35 days. × 63. (Top) Dog 2177; (Bottom) Dog 2168. Note atrophic acini present in both sections.

the four dogs receiving Compound I was 23.01 cm^3. At completion of the dosing period the mean volume was 10.38 cm^3. Comparable figures for the three control dogs were 12.45 cm^3 before and 12.32 cm^3 after treatment.

Microscopic examination revealed the presence of prominent focal areas of atrophic acini containing flattened epithelium in the prostates of all treated dogs. Considerable interalveolar fibrosis was also noted. Although these treatment effects were much in evidence, normal appearing acini with simple columnar epithelium and basally arranged nuclei were found in each treated animal. In control dogs, few areas of thinned out acini were seen. The predominant cell type consisted of columnar epithelium with

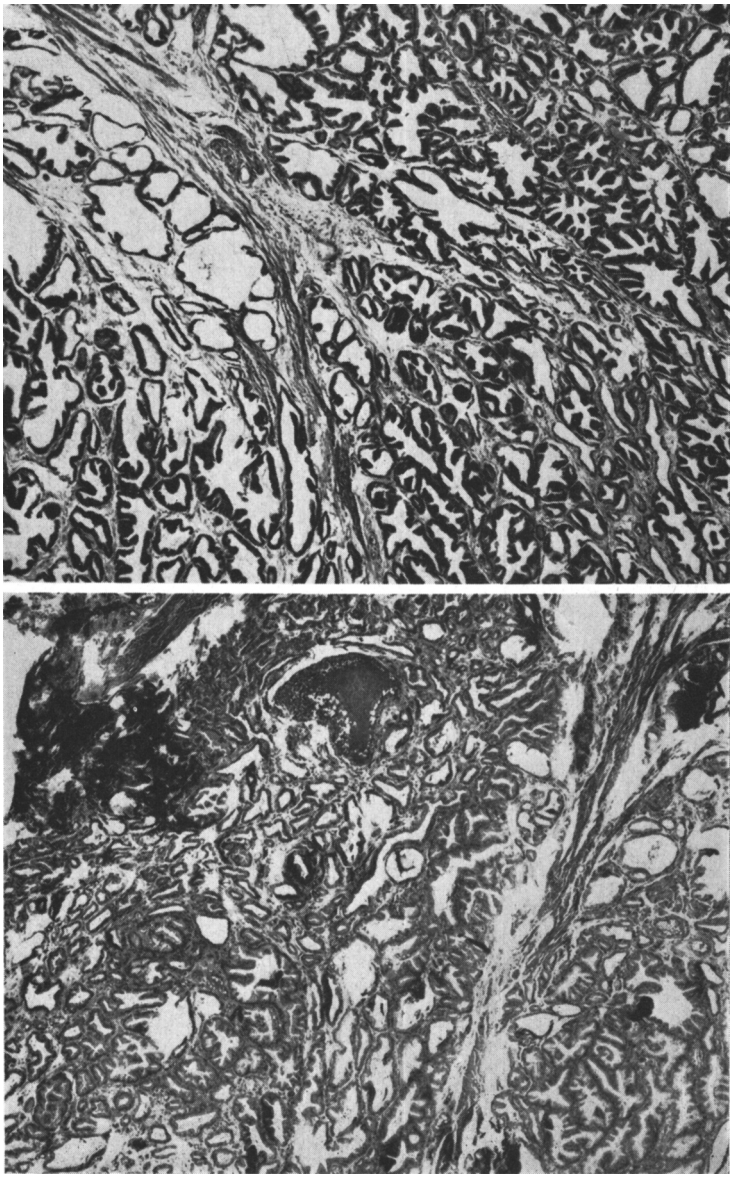

FIG. 3. Cross-sectional areas of prostates from dogs given 1.0 mg/kg/day of Compound I for 35 days. × 63. (Top) Dog 2177; (Bottom) Dog 2168. Areas of thinned-out acini in both sections.

basally arranged nuclei. Epithelial infolding was noted and abundant epithelial secretion was present in all instances, indicating good glandular activity (Figs. 1–3). There was no apparent effect of treatment on spermatogenesis nor on Leydig cells (Fig. 4).

Experiment 2. Average pretreatment dimensions of the prostate in the two dogs given Compound I were 38, 37, and 33 mm for length, width, and depth, respectively. Mean pretreatment prostate volume was 46.4 cm^3.

After 48 days of treatment with orally administered Compound I, the respective mean measurements for prostate length, width, and depth were 29, 24 and 20 mm.

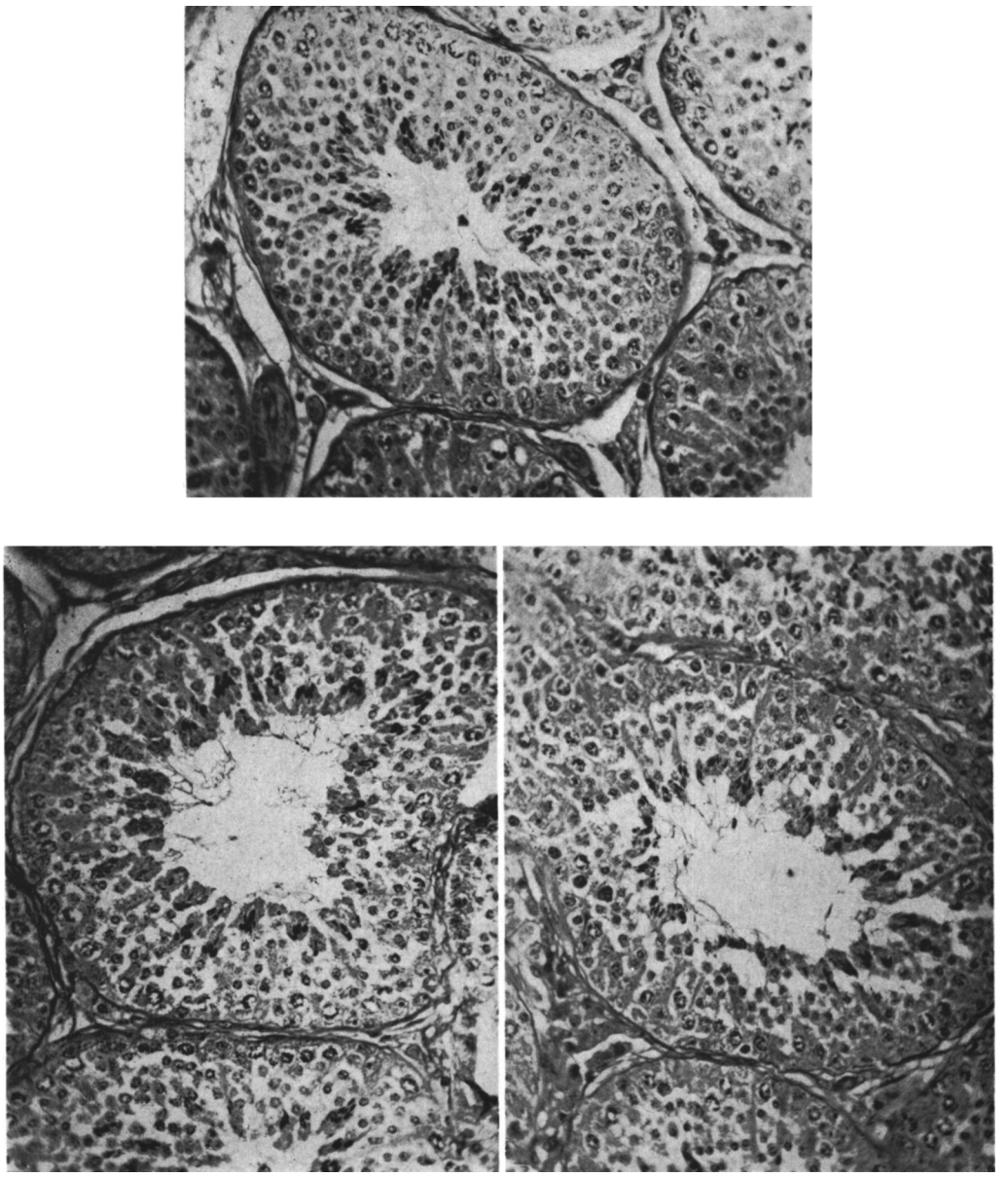

FIG. 4. Seminiferous tubules from testes of dogs 2172, 2168 and 2317, respectively. × 313. (Top) Dog 2172 which had received no Compound I. (Bottom left) Dog 2168 which had been given 0.33 mg/kg/day for 35 days. (Bottom right) Dog 2317 which had received 1.0 mg/kg/day for 35 days. Note the normal spermatogenesis occurring in all three animals.

Average prostate volume was 13.9 cm^3.

In contrast to the marked reduction in prostate size associated with Compound I administration, mean prostate dimensions of the two control dogs changed very little. At the time of the pretreatment laparotomy their average prostate dimensions were 30, 28, and 26 mm for length, width, and depth of gland, respectively. Mean prostate volume was 21.8 cm^3. After receiving a placebo capsule daily for 48 days the respective means for length, width, and depth were

31, 28, and 26 mm. The average volume was 22.6 cm^3.

When the dogs were sacrificed, 120 days after the 48-day treatment period ended, the prostates of those which had received Compound I were much smaller than they were before treatment began. The prostates of control dogs were approximately the same size as they were before the treatment period (Table II).

Discussion. The results of this study indicate that Compound I is capable of causing a very pronounced reduction in the size of the hyperplastic dog prostate. In view of the fact that the compound possesses minimal endocrinological properties other than anti-androgenicity (24), this finding is of considerable significance. It would appear that Compound I is a very attractive candidate for the treatment of BPH. To date, nonsurgical therapy for BPH has centered on drugs having estrogenic, progestational or androgenic activity. All such treatment has been associated with undesirable side effects, notably impotence, gynecomastia and sterility. Recently, cyproterone acetate has been the compound of choice (21, 22) even though it is known to be strongly progestational (19) and to inhibit gonadotrophins (23). Compound I, with its very narrow endocrinological spectrum and little or no estrogenic, progestational or androgenic activity, would seem to possess a theoretical advantage over all compounds currently being used against BPH.

One hundred and twenty days after the cessation of treatment with Compound I, the prostate dimensions of two dogs had not materially changed from measurements recorded immediately after the treatment period ended. This indicated prostatic regression induced by the compound was maintained without further treatment over a relatively long term. It would further suggest that the prostate was not responding to endogenous androgens by an increase in size. The reason for this unresponsiveness is not understood, especially since a long-lasting effect of the compound has not been established. Even so, the maintenance of prostatic regression could be of considerable clinical importance. An intermittent dosing regi-

TABLE II. Effect of Compound I Administered Orally for 48 Days on the Prostate Size of Intact Dogs.

Dog	Dose mg/kg/day	Prostate measurements (mm)															
		Pretreatment				After 48 days treatment				60 days after end of treatment				120 days after end of treatment			
		Length	Width	Depth	Vol. (cm^3)	Length	Width	Depth	Vol. (cm^3)	Length	Width	Depth	Vol. (cm^3)	Length	Width	Depth	Vol. (cm^3)
654	—	30	28	25	21.0	31	27	23	19.3	32	30	23	22.1	36	32	24	27.6
646	—	29	28	26	21.1	30	29	28	24.4	29	28	22	17.9	31	30	19	17.7
	Avg.	30	28	26	21.8	31	28	26	22.6	31	29	23	21.5	34	31	22	23.2
2175	2.0	43	43	32	59.2	27	22	16	9.5	30	25	19	14.3	33	28	21	19.4
652	2.0	33	31	33	33.8	30	25	23	17.3	29	24	22	15.3	28	24	21	14.1
	Avg.	38	37	33	46.4	29	24	20	13.9	30	25	21	15.8	31	26	21	16.9

men might be expected to keep prostate size reduced and continuous therapy would not be required.

Prostatic histology of treated dogs suggested that a reduction of glandular secretory activity had occurred. This was indicated by numerous areas in which the acini were characterized by low epithelium and relative lack of secretion present in the lumen. Such effects would be expected from an anti-androgen since it has been shown that castration or estrogen treatment abolished prostatic secretion in dogs (10).

Areas of normal or near normal epithelium were noted. This result is not surprising since some columnar epithelium persists in men for several months after castration (4) and in castrate dogs treated with a combination of estrogens and androgens (10). It is possible that had the treatment period been longer or the dosages higher in the present study, more of the epithelium would have reverted to the low cuboidal type.

Treated dogs showed no evidence of gonadotrophin inhibition as judged by the fact that Leydig cells were unaffected and spermatogenesis was normal at the time of sacrifice. These observations are of particular interest since both spermatogenesis and libido are gonadotrophin-dependent phenomena, albeit the latter is mediated through androgens.

Several investigators have reported decreased libido in men following the administration of progestational agents (22, 25, 26), estrogens (13) or a combination of the two (16, 26). This reduction in sexual drive is presumably due to gonadotrophin inhibition with a resultant decline in androgen production. In that context, the present results are encouraging. Although a selective anti-androgenic effect was obtained on the prostate, neither Leydig cells nor spermatogenesis was affected, indicating there was no central action of the compound on the hypothalamus or pituitary.

Summary. Aged beagles with enlarged prostates were given daily subcutaneous injections of the anti-androgen, 6α,7α-difluoromethylene-4′,5′-dihydro-1α,2α-methylene-(17 R)-spiro-[androst-4-ene-17,2′-(3′H)-furan]-3-one, Compound I, at levels of 0.33 or 1.0 mg/kg body weight for a period of thirty-five days. Similar dogs were given a daily oral dose of 2 mg/kg body weight for forty-eight days. A comparison of prostate measurements taken before and after treatment revealed that all treatments caused a very marked reduction in size of the gland. The prostate regression persisted over a four-month post-treatment follow-up period in the two dogs which had been treated for forty-eight days.

Histologically, treatment was associated with decreased epithelial cell height, less glandular secretion and increased fibrosis of the prostate.

Neither Leydig cells nor spermatogenesis was affected by treatment, indicating that gonadotrophins were not inhibited and also suggesting that Compound I does not act at the hypothalamic or pituitary level.

The aid of Dr. A. Segaloff in the interpretation of histological material is gratefully acknowledged.

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Received Jan. 5, 1973. P.S.E.B.M., 1973, Vol. 143.