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Urological Research

Study of the Effect of an Anti-Androgen (Oxendolone) on Experimentally Induced Canine Prostatic Hyperplasia

I. Morphological Analysis

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Summary. To investigate the effect of anti-androgens on BPH, Oxendolone (OXD), a pure anti-androgen, was tested in experimentally induced BPH in 17 beagle dogs, alone or in combination with medroxyprogesterone acetate (MPA) which displays both anti-androgenic and anti-estrogenic acitivity. The relatively early stage of canine BPH was induced by administration of 3α-androstanediol (3α-A) plus estradiol (E_2) for 6 months and followed by testosterone propionate (TP) plus E_2 for another 6 months during the anti-androgenic treatment. By the manipulation with T, a decrease in volume of glandular component associated with a relative increase in stromal tissue was achieved, which mimics human BPH histology. The prostate substituted with T and E_2, however, gradually decreased in size. Therefore the effect of OXD or OXD + MPA was not significant agianst the untreated controls (T-E control). The weight of the prostate in these OXD ± MPA groups was however significantly reduced as compared to that of BPH controls which received 3α-A and E_2 throughout the experimental period. On histological examination, atrophic changes were observed in the hormone-treated groups compared to the T-E control. The finding was the most striking in the OXD + MPA group with small non-involuted acini scattered in the abundant stromal tissue. This was almost identical to the appearances of castrated control groups. Atrophy may be due not only to the anti-androgenic but also to the anti-estrogenic property of MPA. A report on the hormonal background of this experiment will appear in the second article.

Key words: Canine BPH – Prostate size – Histology – Anti-androgen – Anti-estrogen – Oxendolone – Medroxyprogesterone acetate

Introduction

Since a definitive role of androgens became evident in the development of human prostatic hyperplasia (BPH), several anti-androgens have been used in the medical mangement of this pathologic condition in Japan. We have previously demonstrated the significant clinical benefits of 2 different types of anti-androgens against early-stage BPH in a double blind study [17, 18]. Since the rationale of this therapy, however, has not yet been clarified, the present study was undertaken to investigate the effect of an anti-androgen, alone or in combination with an anti-estrogenic compound, on experimentally induced mild to moderate BPH in dogs.

Evaluated were: the size and histological findings of the prostate, androgen and estrogen content in blood, and androgen content as well as quantification of the receptors of both hormones in the tissue.

Part I deals with the morphological evaluation.

Materials and Methods

As shown in Table 1, the experiments took one year and consisted of 2 major parts: the induction of BPH during the first 6 months and treatment of BPH with hormones during the latter 6 months.

Induction of BPH in Dogs

The induction of BPH was made in 18 eleven-month-old beagle dogs by means of Walsh and Wilson's method [16]. Twenty-four hours after castration of all the animals, 75 mg/week of 3α-androstanediol[1]

[1] Structural names of hormones used and their origins:
3α-androstanediol = 5α-androstan-3α,17β-diol
estradiol = 1,3,5(10,–estratriene-3,17β-diol)
Oxendolone = 16β-ethyl-17β-hydroxy-estr-4-en-3-one
medroxyprogesterone acetate = 6α-methyl-17α-acetoxyprogesterone

The above hormones except for Oxendolone were purchased from Sigma Chemical Co., St Souis, Mo., and Oxendolone was supplied from the products for clinical use manufactured by Takeda Chemical Industries Ltd., Osaka, Japan. The origin of other chemical compounds used in the experiments will be summarized in fhe following report

Table 1. Experimental scheme

Induction of BPH	
3α-androstanediol (3α-A)	75 mg/week
estradiol (E_2)	0.75 mg/week
Hormone manipulation	
0: Castrated control (sesame oil 1 ml/w + aq. vehicle 2 ml/w)	
1: BPH control (3α-A 25 mg/w + E_2 0.25 mg/w)	
2: T-E control (TP 10 mg/w + E_2 5 μg/w)	
3: T-E + anti-androgen (Oxendolone 200 mg/w)	
4: T-E + OXD + anti-estrogen (MPA 30 mg/w)	

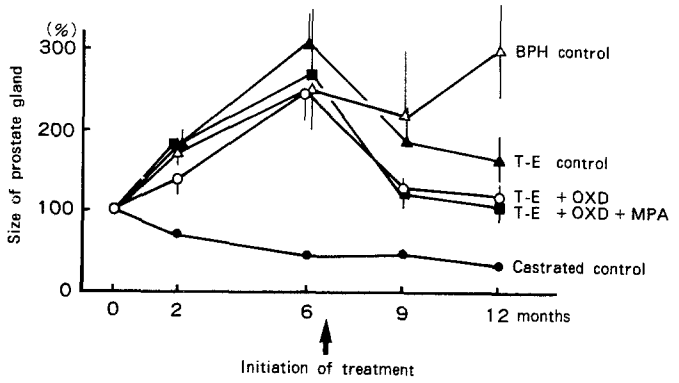


Fig. 1. Sequential change of the size of canine prostate in percent of the initial values as estimated by percutaneous measurement in 2 dimensions and expressed by mean ± SEM

(3α-A) disolved in 3 ml of a hydrophilic vehicle and 0.75 mg/week of estradiol (E_2) in 1.5 ml of sesame oil were given. These ammounts were divided into 3 weekly doses, and were injected intramuscularly into 16 dogs for 6 months. The remaining 2 dogs received the vehicles only and served as castrate controls.

Every 3rd month all of the animals were anesthesized to measure the body weight and size of the prostate gland. The development of BPH was assured by the growth curve of the prostate gland and through a biopsy done after six months. The status of the prostate was assumed to be a mild stage of BPH with size and corresponding histological findings in the biopsy specimen adequate for the succeeding stage of the experiment.

Treatment of BPH with Hormone(s)

After the BPH development had been assured, the experimental hormonal treatment was commenced. The anti-androgen chosen was Oxendolone (OXD) which has been characterized as a potent competitive inhibitor at the level of the androgen receptor [10, 12], and has been in clinical use for BPH in Japan during recent years. As an anti-estrogenic agent medroxyprogesterone acetate (MPA), a potent progestational agent was selected, which acts as an anti-androgen at high dosage [11].

One dog died during the induction of BPH; the remaining 17 were divided into 5 groups, the 2 castrate controls, Group 0, remained untreated (Table 1).

The 3 dogs in Group 1, receiving another half-year administration of 3α-A and E_2 but at a dose reduced to one third, served as BPH controls. The remaining 12 dogs in Group 2 to 4, 4 dogs in each, received 10 mg/week of testosterone propionate (TP) and 5 μg/week of E_2 by i.m. injection instead of 3α-A + E_2. Both drugs were disolved in 1.5 ml of sesame oil. The substitution of 3α-A with TP had two major purposes: 1) to prevent advanced BPH and 2) to try to achieve tissue characteristics similar to those in the human. The 4 dogs without hormonal treatment, called T-E control, was designated as the third control group. Group 3 and 4, 4 dogs in each, which were respectively treated with OXD and OXD + MPA. OXD was given biweekly at a dose of 200 mg/week in a 2 ml aqueous suspension and 30 mg/week of MPA in the same manner in a 2 ml solution as for clinical use. These hormones were injected i.m. for 6 months up to the end of the experiment (Table 1).

Measurement of the Size and Histological Examination of the Prostate

Before and every 3rd month after the initiation of the experiment, all of the dogs were anesthesized intravenously with sodium pentobarbital. The size of the prostate was represented as a maximum area of the gland by length x width measured with a flexible rule percutaneously on the abdomen. The body weight was checked each time.

At 6th month, when needle biopsy of the prostate was undertaken in all of the animals except for Group 0, the acomplishment of BPH was also confirmed histologically. At the end of the experiment all of the dogs were agian anesthesized, weighed and sacrificed by bleeding. The removed prostate was weighed and cut in half perpenqicularly to the urethra. Half of the prostate was sliced and fixed in standard formalin fixative for histological examination. The remaining tissue was frozen and stocked for further endocrinological and biochemical study.

Results

The results are summarized in three categories: measurements of prostate size, hostology, and hormones and their receptor content in the tissue. This part deals only with prostatic size and histology.

Size of the Prostate

The size of the prostate was monitored before, during, and after the experiment (Table 1). Sequential changes in size were expressed in percentages of that at the beginning (Fig. 1).

Up-to 6 months the size of the prostate increased continuously in all of the animals except in the castrated control. Subsequently, however, a constant reduction in size was noticed in Group 2 (T-E controls) and in the corresponding hormone treated groups. On the contrary, an increase in prostate size was noticed in Group 1 (BPH controls) in spite of the dose reduction of 3α-A and E_2 to one third. T scariely maintained the hyperplastic condition after induction by 3α-A.

Consequently, the difference in the final weight of the prostate measured at autopsy was minimal among the groups except for the BPH and the castrate control group, but a decrease in size was evident in the T-E, T-E + OXD and T-E + OXD + MPA groups, when expressed per kg body weight

Table 2. Comparison of prostate gland weights (actual weight anq gm/kg body weight) by experimental groups (mean ± SEM) at autopsy. Except for Group 0 in which prostates were extremely small, a significant reduction in prostate weight, both in actual weight and per kg body weight, was noticed in Group 3 and 4 against Group 1 at $P < 0.05$ and 0.01, respectively. The difference, however, was not significant between 1 and 2, 2 and 3 or 4, and 3 and 4

Treated group	Dog No.	Body weight (kg)	Prostate Weight (gm)	
			actual wt.	per kg BW
0: Castrated	B 682	14.0	1.7	0.12
	U 182	15.0	3.1	0.21
		14.5 ± 0.5	2.4 ± 0.7	0.17 ± 0.50
1: BPH control	H 282	15.5	48.2	3.11
	H 482	12.0	48.0	4.00
	I 282	12.0	69.6	5.80
		13.2 ± 1.2	55.3 ± 7.2	4.30 ± 1.37
2: T-E control	A 182	13.6	14.2	1.04
	A 282	10.5	13.3	1.27
	H 182	16.0	30.0	1.88
	U 282	13.0	48.2	3.71
		13.3 ± 1.1	26.4 ± 8.2	1.98 1.21
3: T-E + OXD	A 482	12.0	18.4	1.53
	B 282	13.0	17.7	1.36
	B 582	13.5	23.3	1.73
	W 182	14.0	38.0	2.71
		13.1 ± 0.4	24.4 ± 4.7	1.83 ± 0.60
4: T-E + OXD + MPA	A 582	12.0	22.4	1.87
	B 182	14.0	14.6	1.04
	B 482	16.0	15.6	0.98
	H 585	15.5	20.0	1.29
		14.4 ± 0.9	18.2 ± 1.8	1.30 ± 0.41

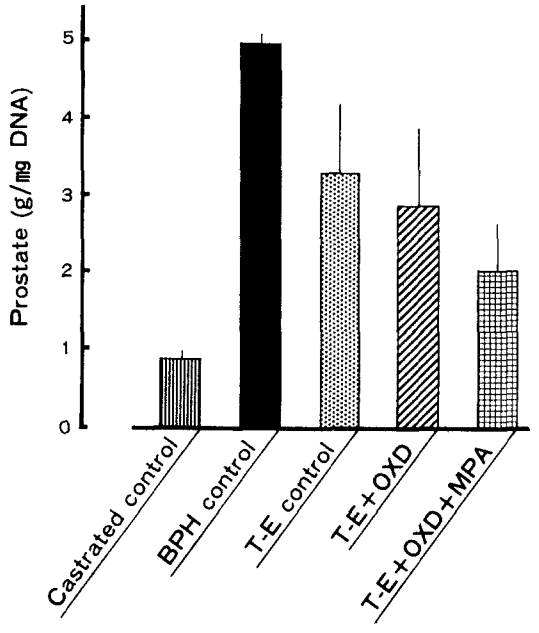


Fig. 2. Comparison of prostate gland weights by experimental gorups, expressed by gm per mg tissue DNA content (mean ± SEM). As mentioned in the explanation of Table 2, a significant decrease in the prostate weights in Group 3 (T-E + OXD) and 4 (T-E + OXD + MPA) was noticed agianst Group 1 (BPH controls) at $P < 0.05$ and 0.01, respectively, but not against the T-E controls. The difference of the weight between the BPH controls and T-E controls was not significant either

(Table 2). These findings were confirmed when the prostate weight was expressed per mg DNA per prostate, which indicates the wieght per unit of cellular mass in the tissue (Fig. 2). (The method for determining DNA in the prostate will be described in Part II of this report.)

Estimation of th size of the prostate during the experiment was evaluated at autopsy whether or not it represented the actual size. The measurements before autopsy were compared to those obtained at autopsy. Although some discrepancies exist between two methods, the percutaneous measurement seems acceptable when each mean value was expressed as the ratio to that of Group 0 in each group (Table 3).

Histologcial Examination

The prostate of the BPH controls, Group 1, showed purely glandular hyperplasia with a scarce stromal component, as previously reported by several investigators (Fig. 3) [1, 16]. Neither Cystic hyperplasia nor squamous metaplasia of epithelia were seen as reported by Tunn et al. and which Funke et al. had documented [2, 14, 15]. In contrast, such glandular proliferation was greatly diminished in the prostates of Group 2, which was designated as the control of

Table 3. Comparison of the prostate size expressed as maximum area (width x length), percutaneously measured before autopsy, with that actually measured after removal of the gland (mean ± SEM)

Group	No. dogs	Maximum area of prostate (cm^2)			
		percutan. measure	ratio	actual measure	ratio
0	2	2.4 ± 0.2	1	2.8 ± 0.4	1
1	3	19.5 ± 0.7	8.1	23.7 ± 0.7	8.5
2	4	9.3 ± 0.5	3.9	15.2 ± 0.7	5.4
3	4	7.9 ± 0.3	3.3	14.1 ± 0.4	5.1
4	4	8.4 ± 0.4	3.5	10.7 ± 0.4	3.8

the hormone treated groups. There was also relatively abundant stromal tissue surrounding the acinar component, similar to that seen in human BPH (Fig. 4).

The increased ratio of stroma to acini must have resulted from the replacement of 3α-A by T leading to less 3α-A and DHT stimulation. This becomes more evident in the following hormone treated groups. The histological picture of Group 3, T-E + OXD, showed a more pronounced decrease of the acinar and an increase of the fibro-muscular component. On the whole, the greater glandular atrophy compared with the T-E controls was associated with diminished acinar branching and a diminished heigh of the epithelial cells (Fig. 5). Such findings were most prominent in Group 4 treated with OXD and MPA. The glands were severely atrophic, being alomost comptaible with that of castrate control (Figs. 6, 7).

Discussion

Although histology is not quite the same as in humans, the dog prostate has often been used as an experimental model for human benign prostatic hyperplasia (BPH). Although evidence for the etiology of BPH and its relation to androgen metabolism has largely been accumulated from studies investigating spontaneous or experimentally induced canine BPH, little experience has been obtained on the effect of hormonal treatment of this disease. A series of the reports from Senge's laboratory deal with the prostate which is in the process of developing BPH and the prostate in castrated and in intact dogs [2, 14].

The reasons for the lack of such experiments may be both considerations of cost and the histological dissimilarity between dog and human BPH. The typical histological finding is pure glandular hyperplasia in the dog, independent of whether the condition occurs spontaneously or is experimentally induced [1, 6, 16].

In this experimental study we hoped to obtain answers to the following questions: First, what is the effect of an anti-andorgenic agent on BPH? Second, if estrogen has an influence on the occurrence of BPH, is it of therapeutic value to combine an anti-estrogen with an anti-androgen? And finally, though it may be most difficult, would it be possible to change experimentally the histological pattern of dog BPH in such a way that it resembles more closely human BPH?

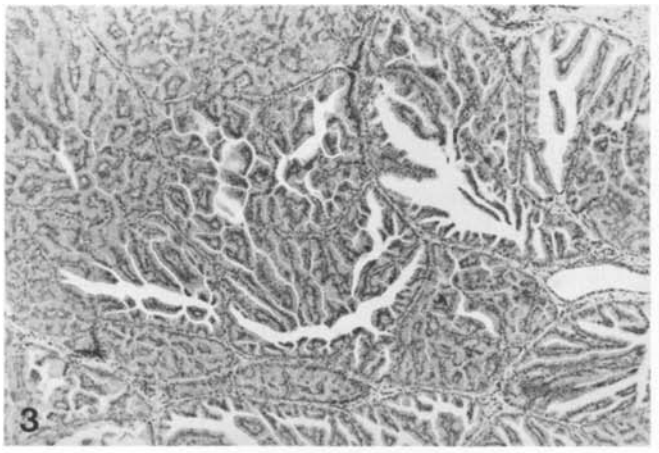
It has been generally recognized that hormonal manipulation is only effective in early BPH. Therefore, it was also desirable that the experiments should not be conducted with 'mature' BPH. As Jacobi et al. had previously demonstrated, the effect of 3α-androstanediol (3α-A) reaches a maximum at 12-weeks of administration [7]. On th other hand, Walsh and Wilson continued hormone administration for 2 years and induced mature glandular hyperplasia [16]. We therefore shortened the administration period of 3α-A and estradiol (E_2) to 6 months.

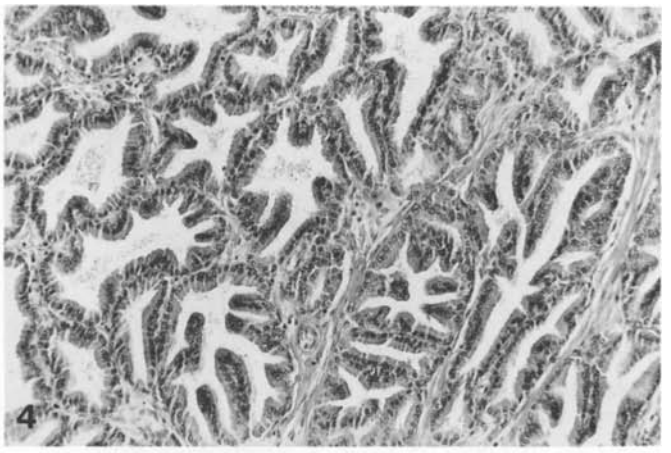
Among other anti-androgens, we chose Oxendolone (OXD) in this study. The compound was synthesized from dehydroepiandrosterone by demethylation at the C_{19} position [4]. OXD exerts its anti-androgenic activity by competing with androgen at the receptor site without exhibiting any other hormonal activity [8, 10, 12]. This compound, therefore, seemed to be a reasonable choice in evaluating the effect of an anti-androgen on the prostate gland.

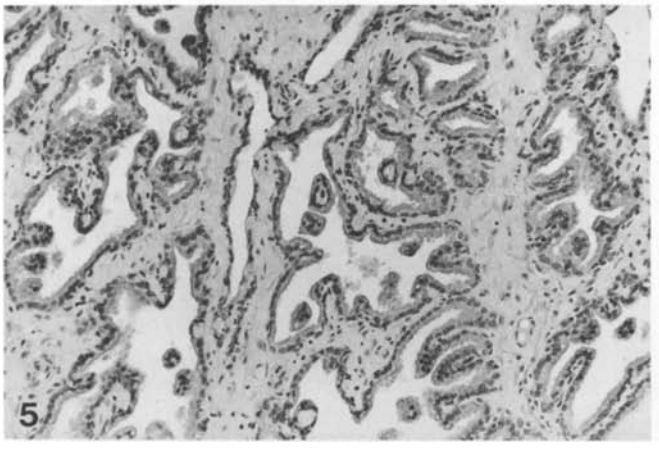
On the other hand, the selection of an anti-estrogen was extremely difficult. We did not use Tamoxifen, a familiar anti-estrogen and easily available, because its biological action differs according to animal spieces, and in dogs it is known to be estrogenic [3]. Finally medroxyprogesterone acetate (MPA) was selected; this anti-estrogen is also generally accepted as a potent anti-androgen if administrated in large doses, especially in male subjects [11]. According to reported data, MPA directly inhibits growth of the cells of an estrogen-dependent cell line [11], and modulates the estrogen stimulation of the uterine endometrium by enhancing the enzyme activity of the estradiol converting enzyme to a far less estrogenic hormone, estrone [13].

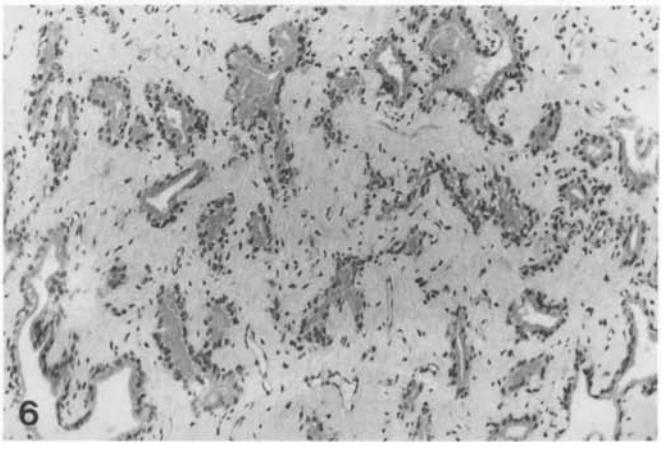
However, we have not ignored the possibiity of anti-androgenic influence of MPA in combination with OXD, because the similar histological finding in the dog prostate treated with cyproterone acatate (CA) was also demonstrated by Tunn et al. [14, 15]. They treated castrated dogs with a large amount of CA (600 mg/week), with 3α-A ± E_2 together, and found severly atrophic acinar component associated with pronounced stromal proliferation. Although th experimental procedure markedly differs from ours and the dosage of CA used was higher than that of MPA, both agents have a similar hormonal properties as gestagens. However, it should be emphasized that the atrophy of the prostate seen histologically after the combined administration of OXD and MPA was more pronounced that we had expected. Limitation in the number of dogs prevented us from setting up another group treated with MPA alone.

As documented in the reports by Walsh et al. [16], and others [1, 7, 9], and confirmed in the present experiments, a contuinuous 3α-A + E_2 administration resulted in purely glandular hyperplasia in spite of a reduction in dosage of hormonal manipulation to 1/3. On the other









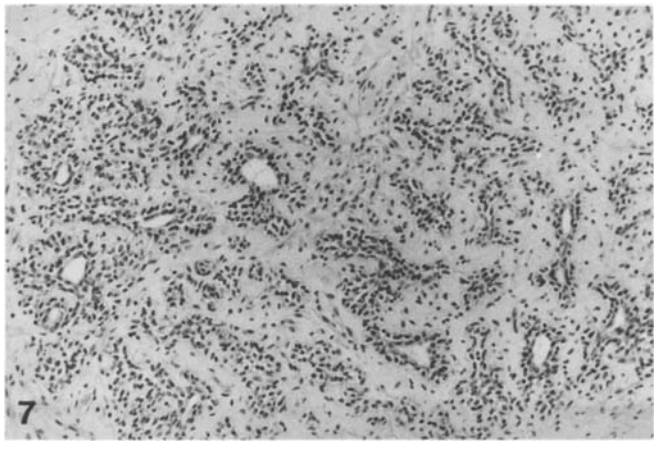


Fig. 3. Histology of a Group 1 prostate after 1-year administration of androstanediol (3α-A) + estradiol (E_2). Marked, purely glandular hyperplasia is seen with scanty stromal tissue (H-E stain, magnified 145 times)

Fig. 4. Histology of a Group 2 prostate from a T-E control, in which testosterone propionate (TP) + E_2 was substituted during the 6 months following induction of BPH with a 6-month manipulation with 3α-A + E_2. The glandular components are less dominant than those of Group 1 and more abudnant stromal tissue can be seen. The appearnace is more similar to human BPH (H-E stain, magnified 172 times)

Fig. 5. Histology of a Group 3 prostate from an Oxendolone (OXD)-treated T-E dog. The findings which are recodnized in Fig. 5 are more pronounced (H-E stain, magnified 145 times)

Fig. 6. Histology of a Group 4 prostate treated with OXD + medroxyprogesterone acetate (MPA). The glands have become completely atrophic, characterized by a loss of acinar folds, diminished epithelial height and abundant stromal tissues. Secreted fluid, however, can be seen in the acinar lumen (H-E stain, magnified 145 times)

Fig. 7. Histology of the prostate gland of a castrated dog. The severe atrophy corresponds to the long-term androgen depletion. A similar atrophy was seen in the prostates of the OXD + MPA-treated dogs with the difference of no secretion in the acinar lumen (H-E stain, magnified 72 times)

hand, it is known that estrogen stimulates stromal growth in the canine prostate [2, 5, 14, 15]. E_2 stimulation, therefore, under mild androgen replacement would possibly maintain the 3α-A-induced BPH in association with increased stromal tissue. In order to carry out our study by the use of a BPH model closely resembling human BPH, we discontinued 3α-A + E_2 which we substituted with testosterone (T) + E_2. This regimen was applied in the control gorup (Group 2, T-E controls) and the hormone-treated groups (Gourp 3 and 4). The histological findings in the T-E controls were more similar to those in human BPH than in the BPH controls which continously received 3α-A

and E_2. However, the T-E manipulation could not adequately support prostatic growth, even after BPH had been initiated. Consequently, the size of the prostate among the hormone-treated groups did not differ markedly, though the histology did.

These findings will further be documented in the following endocrinological report.

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