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QUANTIFICATION OF CYTOSOLIC STEROID RECEPTORS IN SECRETORY AND NON-SECRETORY EPITHELIAL CELLS OF THE CANINE PROSTATE

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Summary—Radiolabelled methyltrienolone, dihydrotestosterone and estradiol were used as ligands to identify and quantify androgen and estrogen receptors in freshly dispersed cells from the canine prostate. Soluble extracts (cytosols) were obtained from secretory and non-secretory epithelial cells separated on the basis of their density in Percoll gradients. For both cell types, as well as for the whole prostate, Scatchard plot analyses were linear and showed a single class of high affinity binding sites: K_d values of $3.6 \pm 2.2 \times 10^{-9}$ M and $3.0 \pm 1.2 \times 10^{-10}$ M were measured for the androgen and estrogen receptors, respectively. The number of binding sites for the cytosolic androgen receptor, expressed per mg of protein or per mg of DNA, was 2.4- to 6.7-fold higher in the non-secretory cells compared to the secretory cells. However, these two cell types contained a similar number of specific sites for the estrogens. The specificities of the androgen and estrogen receptors were shown to be identical for the two cell types: the binding of [^{3}H]R1881 was strongly inhibited by unlabelled R1881, 5α-androstane-3α,17β-diol and dihydrotestosterone, while 5α-androstane-3β,17β-diol, estradiol and estrone did not displace bound R1881. The addition of triamcinolone acetonide did not alter the binding of R1881 in extracts of either cell type or in the whole prostate. The binding of [^{3}H]estradiol to the estrogen receptor was highly specific since a strong displacement was only observed with estradiol (83%).

INTRODUCTION

The etiology and pathogenesis of benign prostatic hyperplasia (BPH) remain uncertain, although it is widely accepted that hormonal factors are involved, namely androgens and estrogens. Indeed, BPH is the major age-related neoplastic disease [1, 2] in man and dog and its development requires the presence of functioning testes [2]. It has been suggested that the accumulation of dihydrotestosterone (DHT) may be responsible for the development of both human and canine BPH [3–7] and the nuclear androgen receptor content is also elevated in the hyperplastic canine prostate [8, 9]. However, it has been recently reported that, in age-matched dogs and in humans, the DHT content is similar in normal and hyperplastic glands [10, 11].

Androgen administration can activate basal and secretory cells of the canine prostatic epithelium, while it has also been shown that estrogens inhibit the activity of secretory cells, produce squamous metaplasia in the acini and increase the fibromuscular stroma [12–14]. Estrogen treatment potentiates the BPH-inducing effect of androgens [15] and increases the number of androgen receptors in the cytosol and nucleus [7–9]. A loss of estrogen-induced squamous metaplasia [12, 15] and a histological pattern of squamous metaplasia with stromal metaplasia [16] have both been reported in castrated dogs treated with estradiol and androstanediol.

In most studies, androgen and estrogen receptors have been measured and characterized in the cytosolic and nuclear fractions derived from the whole prostate, which would include all cell types as well as the extracellular compartment [8,9,17–23]. However, the proportion and function of these different cell types can be altered in pathological conditions as well as via endocrine manipulation. Moreover, the different cell types may be under separate control mechanisms [24]. Consequently, the measurement of the receptor content for the sex steroids in the individual cell types may add to the understanding of the regulation of the different cellular functions.

In this study, a model which has been characterized in our laboratory [25] was used to identify and measure androgen and estrogen receptors in the cytosol of secretory (S) and non-secretory (NS) epithelial cells of the canine prostate.

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Abbreviations used: DHT: 5α-androstan-17β-ol-3one (dihydrotestosterone); T: 4-androsten-17β-ol-3one (testosterone); 3α,17β-diol: 5α-androstane-3α,17β-diol; 3β,17β-diol: 5α-androstane-3β,17β-diol; Androstenedione: 4-androstene-3,17-dione; E_2: 1,3,5(10)-estratriene-3,17β-diol (estradiol); E_1: 1,3,5(10)-estratrien-3β-ol-17-one (estrone); epiT: 4-androsten-17α-ol-3-one (epitestosterone); epiDHT: 5α-androstan-17α-ol-3-one (epidihydrotestosterone); P: 4-pregnene-3,20-dione (progesterone); TA: Triamcinolone acetonide; R1881: 4,9,11-estratrien-17β-ol-17α-methyl-3-one (methyltrienolone).

EXPERIMENTAL

Reagents

Hank's balanced salt solution without calcium and magnesium (HBSS), tissue culture medium (minimum essential medium, MEM, Eagle) and collagenase were obtained from Gibco, Canada. Percoll was purchased from Pharmacia.

[17α-methyl-^{3}H]R1881, (87 Ci/mmol), [1,2,4,5,6,7-^{3}H]DHT (123 Ci/mmol) and [2,4,6,7-^{3}H]E_2 (102 Ci/mmol) as well as "Ready-Solv" scintillation fluid, were purchased from New England Nuclear, Boston, MA. Radioactive steroids were purified as already described [26]. Radioinert steroids (New England Nuclear or Steraloids, Wilton, NH) were crystallized from methanol–water, dissolved in absolute ethanol and stored at 4°C.

Isolation of prostatic cells

Prostates were obtained from normal mature mongrel dogs. The glands, cooled at 4°C in HBSS, were dissected and the external envelope, urethra and the vasa differentia were discarded. The remaining tissue was minced with scissors into small explants (3 mm^3). Prostatic cells were obtained after successive incubations with collagenase as described [25]. Thereafter, the cells were separated by centrifugation at 25,300 ***g*** for 30 min in a Sorvall RC-2B (rotor SS-34) using a preparative Percoll gradient (40 ml polycarbonate tubes). Epithelial S cells (density: 1.02–1.03 g/ml) and NS cells (density: 1.05–1.06 g/ml) recovered from the gradient were diluted with HBSS, sedimented for 10 min at 1000 rpm and resuspended in MEM at a concentration of 10×10^6 cells/ml until homogenization. Cell concentration was determined with a hemacytometer and cellular viability was assessed by Trypan blue dye exclusion (> 95%). One g of canine prostatic tissue yielded $0.76 \pm 0.29 \times 10^8$ cells (mean of 29 glands).

For homogenization, each cell preparation was resuspended in TEDG (50 mM Tris–HCl, 2 mM EDTA, 1 mM DTT, 10% glycerol, pH 7.4) at a concentration of $50–100 \times 10^6$ cells per ml. Homogenization was performed with a Teflon homogenizer at 4°C and cell disruption was confirmed by light microscopy. Cytosolic extracts were obtained by centrifugation of the homogenate (105,000 ***g***, 60 min) in a Beckman L5-65 ultracentrifuge. The supernatants contained 1–10 mg of protein per ml. For purposes of comparison, a portion of the whole prostate was suspended in 2 vol of TEDG, homogenized with a Polytron PCW-2-110 (Brinkman Instr., Rexdale, Ontario) and centrifuged at 105,000 ***g***, 60 min, to obtain the cytosol.

Binding studies

Saturation analyses were performed by serial incubations in the presence of radio-labelled R1881, DHT or E_2 at concentrations ranging from 1 to 15 nM for the androgens and from 1 to 5 nM for E_2. Non-specific binding was evaluated by the addition of 150–1,000-fold excess of radioinert ligand to parallel incubations in duplicate. Since preliminary experiments demonstrated that the maximum specific binding in the two cell types was achieved after 16–20 h at 4°C, incubations were performed under these conditions after addition of each cytosolic fraction. Unbound steroids were removed by the addition of 1 ml of ice-cold Dextran-coated charcoal suspension (0.5% Dextran, 0.5% charcoal in TEDG) for 10 min before centrifugation. The supernatant was transferred to scintillation vials and 10 ml of "Ready-Solv" scintillation fluid was added. The radioactivity was determined in an Intertechnique liquid scintillation counter employing an external standard for quench correction.

Protein and DNA determination

Protein concentrations were determined by the method of Bradford[27] with bovine serum albumin as a standard. For the whole prostate, as well as for the separated S and NS cells, DNA was extracted from the 105,000 ***g*** pellets according to the method of Schneider[28] and assayed by the diphenylamine reaction using calf thymus DNA as standard [29].

Statistical analyses

Scatchard plots [30] were calculated by simple linear regression lines. Mean values were compared by the paired t-test.

RESULTS

Androgen receptors

Saturation analysis showed that the binding of R1881 to the cytosolic fraction was maximal with 10–15 nM of radiolabelled R1881 in the two cell types as well as in the whole prostate (Fig. 1). Non-specific binding, expressed as a percentage of the total binding, was similar in the whole prostate (mean of $47 \pm 5\%$) and in the NS cells ($43 \pm 5\%$) but much higher in the S cells (mean of $86 \pm 4\%$). From the Scatchard plots, illustrated in Fig. 1, one class of high affinity binding sites for R1881 was detected and no significant difference in equilibrium dissociation constants was noted between the cytosol of the NS cells, S cells and the whole prostate. K_d values were 3.2, 4.3 and 3.3 nM for NS cells, S cells and the whole prostate, respectively. However, the number of binding sites, expressed per mg of protein, was different in the two cell types: 57 fmol for the NS cells and 14 fmol for the S cells. The number of binding sites in the cytosol of whole prostate was 78 fmol per mg of protein. When DHT was used as ligand, similar K_d values and number of binding sites for both cell preparations were obtained (results not shown).

Since the cytosolic concentration of protein per cell could differ in the two cell types, it was of interest to compare the number of binding sites as expressed per mg of DNA. Values obtained for NS and S cells originating from the same prostate are shown in Table 1-A (4 different preparations). When expressed

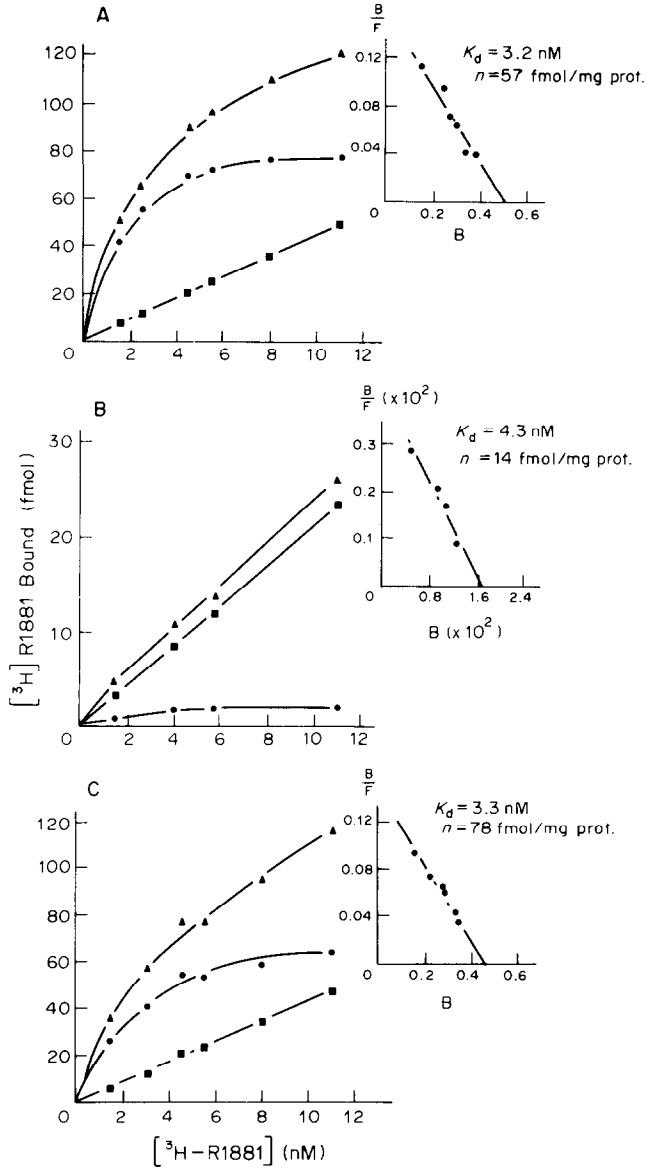

Fig. 1. Binding of [³H]R1881, specific (●), non-specific (■), total (▲) and Scatchard plots for: Non-secretory cells (cytosol: 9 mg prot./ml) (A). Secretory cells (cytosol: 1.2 mg prot./ml) (B). Whole prostate (cytosol: 6.0 mg prot./ml) (C).

Table 1. Comparison of the number of androgen and estrogen binding sites per mg of protein and per mg of DNA in cytosols of NS and S cells

Prostate weight (g)	Androgen binding sites (fmol/mg prot.) NS	S	NS/S	(fmol/mg DNA) NS	S	NS/S
6	116	45	2.6	1125	465	2.4
14	65	25	2.6	254	95	2.7
16	98	27	3.6	1470	220	6.7
25	31	13	2.4	243	64	3.8
Mean	77.5 ± 37.5	27.5 ± 13.2	2.8 ± 0.5	773 ± 622	211 ± 182	3.9 ± 2.0

Prostate weight (g)	Estrogen binding sites (fmol/mg prot.) NS	S	NS/S	(fmol/mg DNA) NS	S	NS/S
17	82	97	0.8	539	573	0.9
19	87	57	1.5	313	308	1.0
29	47	24	2.0	315	259	1.2
Mean	72 ± 22	59 ± 37	1.4 ± 0.6	389 ± 130	380 ± 169	1.0 ± 0.2

per mg of protein, mean values of 77.5 ± 37.5 and 27.5 ± 13.2 fmol/mg prot. were obtained for NS and S cells, respectively (paired t-test, $P < 0.005$), resulting in a ratio NS/S of 2.8 ± 0.5. However, when expressed per mg of DNA, the difference between the number of binding sites in the two cell types became greater and the NS cell cytosol contains a 4-fold higher number of androgen receptors than the S cells (paired t-test, $P < 0.05$). As can be seen in this table, there is no relationship between the number of binding sites and the prostatic weight. Moreover, it was verified that the half-life of the androgen receptor complex was also identical in the two cell preparations.

The binding specificity was studied by incubating a fixed concentration (10 nM) of [^{3}H]R1881 with a 10-fold excess of various unlabelled steroids (Table 2). The potency of these competitors to displace [^{3}H]R1881 was R1881 > 3α,17β-diol, DHT > T. The steroids, 3β,17β-diol, androstenedione, epiDHT, epiT, E_1, E_2 and P showed no significant competition at the concentration used. It was also found that the specificity of the R1881 binding to S and NS cell preparations was similar.

Since R1881 binds with high affinity to both androgen and progesterone receptors [31], a 500-fold excess (5 μM) of the anti-progestin Triamcinolone acetonide (TA), was added to the cytosolic preparation in order to determine the contribution of the progesterone receptor to the binding of R1881. The results (expressed in % of control binding without TA) demonstrate that the binding of R1881 was not significantly modified by the addition of TA to the two cell preparations (NS cells: 85% of the control value; S cells: 99% of the control value) as well as in the whole prostate (90% of the control value).

Estrogen receptors

High affinity binding of radiolabelled E_2 was demonstrated in the two epithelial cell types with saturation at 2 nM of estradiol (Fig. 2). No significant difference in the apparent equilibrium dissociation constants or in the number of binding sites was noted in the cytosols of the two cell types; K_d values of 0.35 and 0.25 nM were obtained in NS cells and S cells with a mean number of binding sites per mg of protein of 82 and 97 fmol, respectively.

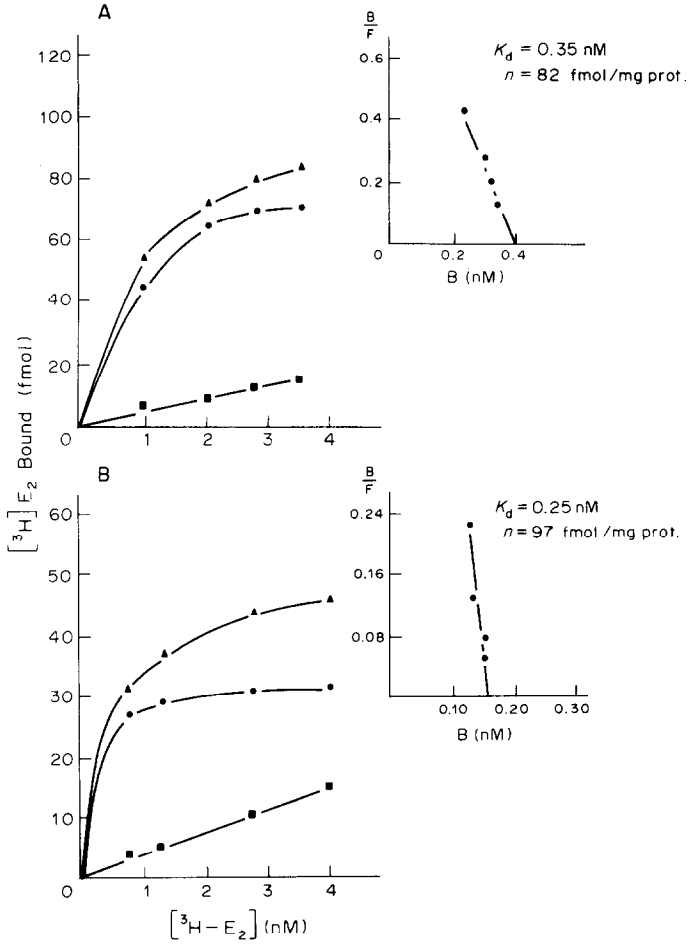

Fig. 2. Binding of ^{3}H-E_2, specific (●), non-specific (■), total (▲) and Scatchard plots for: Non-secretory cells (cytosol: 1.8 mg prot./ml) (A). Secretory cells (cytosol: 4.8 mg prot./ml) (B).

Table 2. Specificity of the androgen receptor in cytosols of NS and S cells

Steroids*	Competitive activity (%)**	
	NS cells	S cells
R-1881	83	95
3α,17β-Diol	67	57
DHT	61	56
T	52	—
3β,17β-Diol	11	16
Androstenedione	12	—
epiDHT	14	—
epiT	5	—
E_1	11	3
E_2	13	16
P	18	—

*Incubations contained 10 nM [^{3}H]R1881 and 100 nM unlabeled steroid.

**Measured by comparison with the control value (100% displacement): Control = [Binding with 10 nM ^{3}H-R1881] − [Binding with 10 nM ^{3}H-R1881 + 1.25 μM R1881].

Table 3. Specificity of the estrogen receptor in the cytosol of NS and S cells

Steroids*	Competitive activity (%)**	
	NS cells	S cells
E_2	83	84
E_1	31	35
Androstenedione	< 1	10
T	< 1	< 1
DHT	< 1	< 1
3α, 17β-diol	7	1
3β, 17β-diol	10	5
epiT	3	2
epiDHT	2	2
P	18	8

*Incubations contained 4.2 nM ^{3}H-E_2 and 25 nM unlabeled steroid.

**Measured by comparison with the control value (100% displacement): Control value = [Binding with 4.2 nM ^{3}H-E_2] − [Binding with 4.2 nM ^{3}H-E_2 + 1 μM E_2]

The number of binding sites expressed per mg of protein or DNA was not different in the two cell types (Table 1-B, $P > 0.5$). The NS cell cytosol contained 389 ± 130 fmol per mg of DNA while the S cells contained 380 ± 169 fmol per mg of DNA (mean of 3 different preparations) for a final ratio NS/S of 1.0 ± 0.2.

The specificity of the receptor was demonstrated by competition studies with the addition of a 5-fold excess of various steroids (Table 3). [^{3}H]E_2 binding in the two cell types was strongly inhibited by unlabelled E_2, moderately inhibited by E_1, and not significantly altered by the other steroids investigated.

DISCUSSION

The data reported herein demonstrate the presence of an androgen binding component with those properties which are characteristic of a receptor (high affinity, limited capacity, steroid specificity) in the cytosolic fraction of the two epithelial cell types of the canine prostate. The apparent K_d's obtained with R1881 as ligand are slightly higher than those reported by others [8, 17–19, 22, 23]. This difference may be attributed to the use of different cytosol preparations (isolated cells vs whole tissue) or other experimental procedures such as temperature which is known to influence the stability of the receptor or alter binding kinetics. Thus, Shain and Boesel [19] have shown that the K_d of the cytosolic androgen receptor measured at 15°C was 17-fold higher than that measured at 2°C. In the present study, the binding was measured at 4°C.

The number of cytosolic androgen binding sites in the canine prostate varies from 40 fmol [17, 19] to 83 fmol/mg protein [18] when measured with R1881 as ligand. The latter value is similar to that obtained in our experiments with cytosol obtained from whole prostatic tissue (78 fmol/mg protein). However, a significant difference in the androgen receptor content was observed in cytosols of two epithelial cell types originating from the same prostate. Even though variations were observed for different prostates, the androgen binding was always higher in the NS cells than in the corresponding S cells. This difference was further emphasized when the results were expressed in fmol/mg of DNA. Since the proportion of these cell types can vary from one prostate to another [25], this could represent an important variable in the measurement of androgen binding sites in whole prostatic tissue.

It is unlikely that this difference in the number of androgen binding sites between the two cell types could be explained by a preferential effect of cell dispersion and separation on Percoll gradients on one individual cell type. Since the stabilizing effect of sodium molybdate on androgen receptors has been demonstrated [32], it would be interesting to verify this effect in the two epithelial cell types. Certain proteases, located for the most part in the glandular portion of the prostate [33] can also affect one cell type in particular. Nuclear translocation, as well as the nuclear concentrations of the hormone–receptor complex should also be studied in the two cell types in order to evaluate more thoroughly the significance of this difference in cytosolic receptor content.

However, we have verified that there is no relationship between the prostatic weight and the number of androgen receptors in NS and S cells, expressed either per mg of protein or mg of DNA.

Interestingly, it has been shown [34] that the NS cells in culture have the capacity to divide and undergo a maturation process which transforms them into S cells. While active differentiation of the latter cells has been demonstrated [34, 35], they possess no or minimal mitotic activity. Therefore, the population of NS cells, with the higher cytosolic receptor content, is more susceptible to undergo hyperplastic changes in the prostate of the aging dog. Indeed, a lower secretory activity has been reported to occur in BPH [24].

The specificity of the R1881 binding in both cell types was also demonstrated and similar percentages of displacement were obtained with the different

competitors in both NS and S cells. With the exception of 3α,17β-diol, our competition studies provided results which are similar to those reported with the human, rat and dog prostate [17–19, 22, 36–38]; R1881 and DHT were strong inhibitors while the displacement with unlabelled testosterone was moderate. All other radioinert steroids that were tested were poor competitors. The strong displacement of [^{3}H]R1881 observed in both cell types with an excess of 3α,17β-diol could be explained by the conversion of 3α,17β-diol to DHT, which is known to occur at 0°C in the rat prostate [39] and at 4°C in our epithelial cells (unpublished results). Even though progesterone receptors have been found in the canine prostate [21], their contribution to the binding of [^{3}H]R1881 in our cellular preparations was limited as indicated by the fact that an excess of anti-progestin did not result in any significant displacement of the R1881. These results confirm the existence of very low concentrations of progesterone receptors in the prostate of intact mature dogs [21].

The estrogen sensitivity of the secretory and basal cells is also well documented [40, 41]. Indeed, in the present study, the presence of an estrogen binding component with receptor-like properties has been shown in the cytosolic fraction of both cell types. The K_d values, as determined by Scatchard analysis, were similar for both cell types and to those published for the cytosols of whole prostates [8, 20–23]. The number of binding sites in each cell type was not statistically different and these values are in accord with those reported for the cytosol of the whole prostate [20, 22, 42].

Competition studies for the estrogen receptor demonstrate its high specificity in both cell types. An important displacement was observed with E_2 and an intermediate displacement was found with E_1. All other steroids used (androstenedione, T, DHT, 3α,17β-diol, 3β,17β-diol, epiT, epiDHT and P) had no effect. With the exception of estradiol displacement by 3β,17β-diol reported by Dubé *et al.*[21], our results are in accord with those published [8, 20–23]. The present data also confirm the presence of a specific estrogen binding in the epithelial cells of the canine prostate.

In contrast to the androgen receptors, the quantification of estrogen receptors provided similar values in the NS and S cells when expressed per mg of DNA (mean of 389 and 380 fmol per mg of DNA, respectively). These concentrations are lower than those reported by Trachtenberg *et al.*[8], i.e. 2830 fmol per mg of DNA in the cytosol of whole prostate. However, the higher concentration of estrogen receptor found in canine stromal tissue [42] could explain, at least in part, the lower concentration measured in separated epithelial cells when compared to those found in whole prostate.

This model could provide more pertinent information with regard to the intra tissue distribution of androgen and estrogen receptors particularly in view of the fact that normal, as well as the hyperplastic canine prostate, consist mainly of epithelial cells.

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