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# Localization of Type 5 17β-Hydroxysteroid Dehydrogenase, 3β-Hydroxysteroid Dehydrogenase, and Androgen Receptor in the Human Prostate by *in Situ* Hybridization and Immunocytochemistry

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**ABSTRACT**

An important source of androgens in the human prostate are those synthesized locally from the inactive adrenal precursor dehydroepiandrosterone (DHEA) and its sulfated derivative DHEA-S. Three β-HSD (hydroxysteroid dehydrogenase) converts DHEA into androstenedione (4-dione), whereas type 5 17β-HSD catalyzes the reduction of 4-dione into testosterone in the human prostate and other peripheral intracrine tissues. In the present study, we have used two complementary approaches, namely *in situ* hybridization and immunocytochemistry, to identify the cells that contain the type 5 17β-HSD messenger RNA and enzyme in human benign prostatic hyperplasia (BPH). Localization of 3β-HSD and of the androgen receptor (AR) was also investigated by immunostaining in the same tissue. To find out whether there are any differences between BPH and normal prostate tissue, the localization of type 5 17β-HSD was reexamined by immunocytochemistry in the normal human prostate samples and also in normal prostate epithelial cell line (PrEC). The *in situ* hybridization results obtained with a tritiated uridine triphosphate ($^3$H-UTP)-labeled type 5 17β-HSD riboprobe are in agreement with the immunostaining data obtained with a specific antibody to the enzyme. The immunostaining results obtained from normal prostate tissue and BPH were found to be similar. Thus, in the glandular epithelium, basal cells highly express the messenger RNA and the enzyme, whereas luminal cells show a much lower and variable level of expression. In the stroma and walls of blood vessels, fibroblasts and the endothelial cells lining the blood vessels show positive staining. Similar results are observed when the cellular distribution of 3β-HSD is investigated. AR immunoreactivity, however, shows a different distribution because, in the epithelium, most of the nuclei of basal cells are negative, whereas the majority of nuclei of the luminal cells show positive staining. A strong reaction for AR is also found in most stromal cell nuclei and in the nuclei of most endothelial cells, as well as in some other cells of the walls of blood vessels. In conclusion, human type 5 17β-HSD, as well as 3β-HSD, are highly expressed, not only in the basal epithelial cells and stromal fibroblasts but also in the endothelial cells and fibroblasts of the blood vessels. AR, on the other hand, is highly expressed in the luminal cells. The present data suggest that DHEA is transformed in the basal cells of the glandular epithelium into 4-dione by 3β-HSD and then into testosterone by type 5 17β-HSD, whereas dihydrotestosterone is synthesized in the luminal cells after diffusion of testosterone from the underlying layer of basal cells. The potential role of androgen formation and action in blood vessels is unknown and opens new avenues of investigation for a better understanding of the multiple roles of androgens. (*Endocrinology* **140:** 1481–1491, 1999)

ANDROGENS PLAY AN essential role in prostate development, growth, and function. On the other hand, it is well recognized that androgens in men originate from two sources of comparable importance, namely the endocrine testis and the local intracrine synthesis from the adrenal precursors dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) (1). The importance of the androgens of adrenal origin is illustrated by the finding that 30–50% of dihydrotestosterone (DHT) remains in the prostate after elimination of all testicular androgens by surgical or medical castration (2, 3). The human prostate thus synthesizes its own androgens, namely testosterone and DHT from circulating DHEA and DHEA-S. In fact, all the enzymes required for the transformation of the DHEA and DHEA-S precursors into DHT have recently been shown to be expressed in the human prostate. The structure of the complementary DNAs (cDNAs) and/or genes encoding the following human steroidogenic enzymes has been elucidated: 3β-hydroxysteroid dehydrogenase (3β-HSD) types 1 (4–7) and 2 (8, 9); 17β-HSD type 1 (10, 11), type 2 (12, 13), type 3 (14), type 4 (15) and type 5 (16), as well as 5α-reductase type 1 (17, 18) and type 2 (19, 20).

The enzyme 3β-HSD is essential for the biosynthesis of all classes of hormonal steroids, namely progesterone, glucocorticoids, mineralocorticoids, androgens, and estrogens. The 3β-HSD enzyme is present in the adrenal, testis, ovary, and placenta, as well as in a long series of peripheral intracrine tissues, including the prostate, breast, liver, and skin (21, 22). The 3β-HSD converts DHEA into androstenedione (4-dione), whereas the formation of testosterone from 4-dione and DHT from 5α-androstane-3, 17-dione (A-dione) and their respective backward reactions are catalyzed by 17β-HSDs. The five human 17β-HSDs characterized so far show only approximately 20% homology in their amino acid sequences. Moreover, the various 17β-HSDs differ markedly in their tissue distribution and substrate specificity (23–25). In fact, we have recently shown, using intact transfected cells in culture, that

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the activity catalyzed by each type of 17β-HSD is unidirectional. The recently cloned 17β-HSD type 5 (16) selectively catalyzes the reduction of 4-dione into testosterone and 4-dione into DHT. In peripheral intracrine tissues, the various levels of expression of types 1, 2, 4, and 5 17β-HSD activities play a critical role in regulating the formation (types 1 and 5) or inactivation (types 2 and 4) of active estrogens and androgens.

To obtain precise information on the cellular distribution of type 5 17β-HSD and gain a better knowledge of the role of this enzyme in the human prostate, we performed *in situ* hybridization and immunocytochemical localization studies in human hyperplastic prostatic tissue [benign prostatic hyperplasia (BPH)]. Normal human prostate tissue and the epithelial prostate cell line (PrEC) were also investigated by immunostaining. In the same series of experiments, the immunocytochemical localization of 3β-HSD was examined to compare the distribution of the two enzymes that are both involved in the biosynthesis of androgens from DHEA. To determine the site(s) of action of the locally produced androgens, we have also identified the immunocytochemical localization of the androgen receptor (AR).

**TABLE 1.** Percentage of immunostained cells of BPH and normal human prostate tissues

| | | Type 5 17β-HSD | Androgen receptor |
|---|---|---|---|
| | | (%) | (%) |
| Epithelium | Basal cells | 100 | 37 |
| | Luminal cells | 22[a] | 94 |
| Fibro-muscular stromal cells | Fibrocytes | 100 | 66[b] |
| | Smooth muscle cells | 0 | |
| Blood vessels | Endothelial cells of tunica intima | 100 | 75 |
| | Smooth muscle cells of tunica media | 35[c] | 82 |
| | Fibrocytes of tunica adventitia | 100 | 56 |

[a] This percentage represents the number of stained luminal cells only in about 5% of the alveoli, as shown in Figs. 3c and 4b. The vast majority of alveoli (about 85%) did not show stained luminal cells, whereas all luminal cells were stained in about 10% of them (Fig. 3b).

[b] The number represents the percentage of stained nuclei of fibrocytes and smooth muscle cells together.

[c] The staining intensity is low in the stained smooth muscle cells of tunica media (as seen in Fig. 4f), as compared with other stained cells.

## Materials and Methods

### *Tissue preparation*

Adult prostatic tissue was obtained from 12 patients, with symptomatic BPH, undergoing transurethral prostatectomy. The specimens were fixed by immersion in 2% glutaraldehyde, 4% formaldehyde, and 3% dextran in 0.05 M phosphate buffer (pH 7.4). After 4 h, the specimens were processed and embedded in paraffin or frozen at −70 C. Four paraffin blocks of normal human prostate, fixed in 4% formaldehyde (age of patients, between 37 and 73 yr) were kindly provided by Dr. Bernard Tetu, Department of Pathology, Hotel-Dieu de Québec.

### *Cultured cells*

Normal prostate epithelial cells PrEC 5500–1 were cultured in PrEGM medium (Clonetics, Walkersville, MD) and harvested, after the third passage, using a rubber policeman. The cells were then fixed in 2% glutaraldehyde, 4% formaldehyde, and 3% dextran in 0.05 M phosphate buffer for 20 min and centrifuged at 700 rpm for 5 min. After removing the supernatant, 2% agarose in 0.05 M phosphate buffer was added to the pellet at 55 C (the volume of agarose was twice the volume of the pellet). After mixing the cells with agarose, the pellet was solidified at 4 C and immersed in the same fixative for 2 h, then washed, processed, and paraffin embedded.

### *In situ hybridization*

Two different procedures were used for *in situ* hybridization of BPH tissue. In the first one, 10-μm sections were cut from frozen tissue with a cryostat and processed as previously described (26). The second procedure will be described in detail elsewhere (El-Alfy *et al.*, unpublished data). In brief, thick paraffin sections (20-μm) were cut, and the unmounted sections were deparaffinized in toluene. The sections were subsequently rehydrated; postfixed in 2% glutaraldehyde, 4% formaldehyde, and 3% dextran in 0.05 M phosphate buffer; and washed in the same buffer containing 7.5% glycine. Hybridization of the floating sections was performed overnight at 40 C with a $^{3}$H-UTP riboprobe, as previously described (27). After hybridization, they were postfixed in osmium tetroxide, flat-embedded in Epon, and cut at 0.7 μm with an ultramicrotome. Both frozen (10-μm) and semithin (0.7-μm) sections were coated with liquid photographic emulsion (Kodak NTB-2, Eastman Kodak Co., Rochester, NY) and processed after 14 days (semithin sections) or 28 days (frozen sections) of exposure.

Sense and antisense riboprobes were generated by *in vitro* transcription from the p-Bluescript phagemid containing a cDNA insert of 35 nucleotides of the human type 5 17β-HSD. [$^{35}$S]- and [$^{3}$H]-UTP riboprobes were used for hybridization with the frozen and floating deparaffinized sections, respectively.

**TABLE 2.** Intensity of the *in situ* hybridization and immunostaining reactions in the different cell types of BPH and normal human prostatic tissue

| | | *In situ* hybridization Type 5 17β-HSD | Immunostaining | | |
|---|---|---|---|---|---|
| | | | 3β-HSD | Type 5 17β-HSD | Androgen receptor |
| Epithelium | Basal cells | +++ | +++ | +++ | +/− |
| | Luminal cells | + | +/− | +/− | +++/− |
| Fibro-muscular stromal cells | Fibrocytes | +++ | +++ | +++ | +++/− |
| | Smooth muscle cells | − | − | − | +++/− |
| Blood vessels | Endothelial cells of tunica intima | +++ | +++ | +++ | +++/− |
| | Smooth muscle cells of tunica media | +/− | +/− | +/− | +++/− |
| | Fibrocytes of tunica adventitia | +/− | ++ | ++ | +++/− |

The presence of silver grains or positive immunostaining reaction is indicated by (+), graded from 1 to 3. The number of (+)s thus corresponds to the intensity of the reaction and takes into account the percentage of labeled cells.

The absence of reaction is indicated by (−).

The possibility of being positively or negatively labeled is indicated by (+/−).

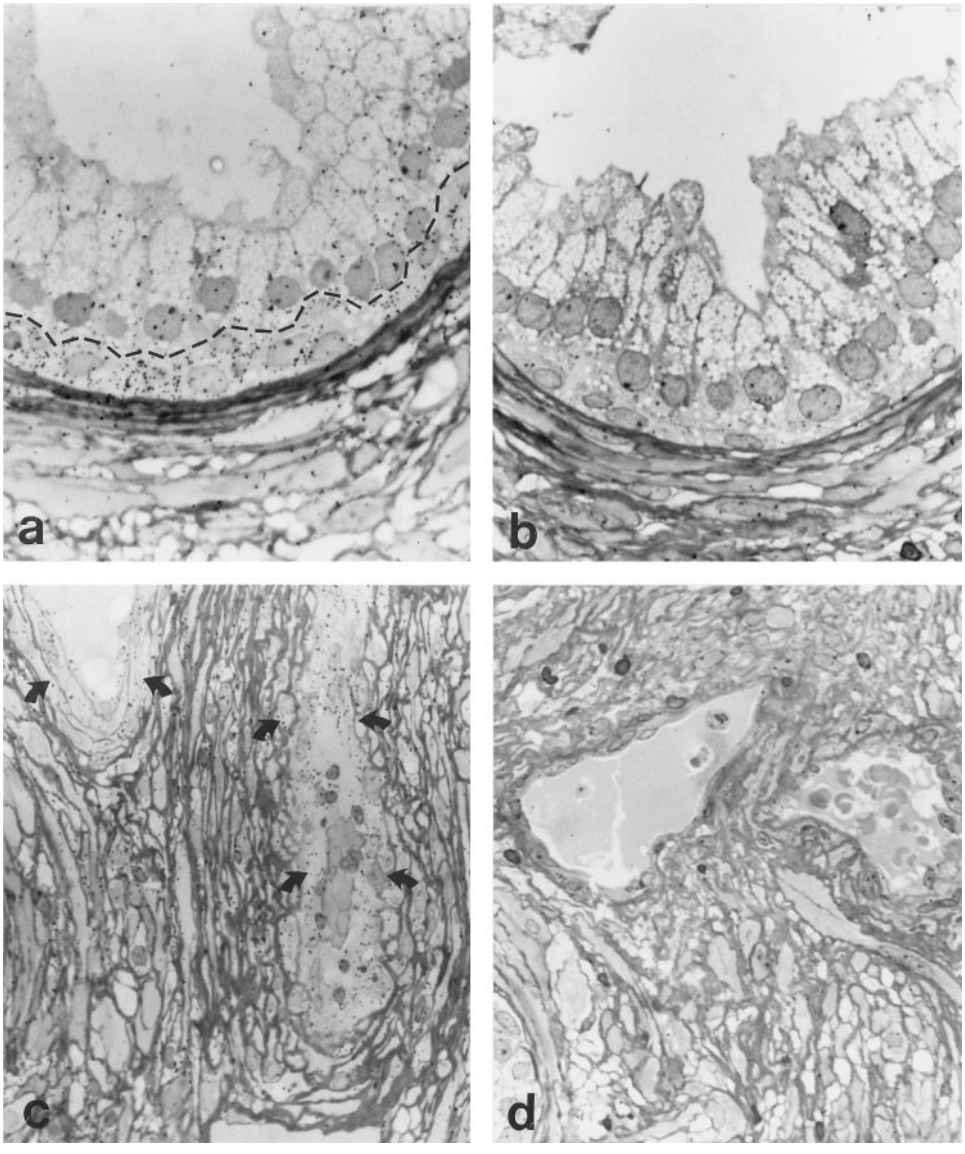


FIG. 1. Autoradiographs of $^3$H-labeled type 5 17β-HSD antisense and sense riboprobes (35 bp), hybridized *in situ* to human BPH tissue. a, Semithin Epon section (0.7-μm thick) hybridized with the antisense probe. Epithelial cells lining the tube-alveoli, as well as some of the surrounding stromal cells, are labeled. In the epithelium, the *dashed line* indicates the approximate boundary between the basal and the luminal cells. The basal cells are intensively labeled, in comparison with the luminal cells, where only a few grains are seen (× 1000). b, Similar area, from the same prostate, hybridized with the sense probe as a control. Only scattered silver grains can be detected (× 1000). c, The antisense probe generates strong radioautographic signals in the wall of blood vessels (*arrows*) (× 600). d, Similar blood vessels, hybridized with the sense probe as a control. No significant labeling could be seen (× 600).

### *Immunocytochemistry*

Twelve paraffin-embedded BPH samples, four normal prostate specimens, and PrEC cells in paraffin blocks were serially cut at 4 μm. Sections were incubated overnight at 4 C, with the human type 5 17β-HSD antiserum diluted at 1:1000 in Tris-saline, pH 7.6. The sections were then washed and incubated at room temperature for 4 h with peroxidase-labeled goat antirabbit γ-globulin (Hyclone Laboratories, Inc., Logon, UT), diluted 1:500, as previously described (28). Endogenous peroxidase activity was eliminated by preincubation with 3% $H_2O_2$ for 30 min, and peroxidase was then revealed during incubation with 10 mg 3,3-diaminobenzidine in 100 ml Tris-saline buffer containing 0.03% $H_2O_2$. The intensity of staining was controlled under the microscope. The sections were then counterstained with hematoxylin. On other sections, immunostaining was performed using a commercial kit (Vectastain ABC Kit; Vector Laboratories, Inc., Burlingame, CA), and diaminobenzidine was used as the chromogen to visualize the biotin streptavidin-peroxidase complex. A microwave retrieval technique was applied for the AR staining (29). Control experiments were performed on adjacent sections by substituting nonimmunized rabbit serum (1:1000). In the case of type 5 17β-HSD antiserum (diluted 1:1000), immunoabsorption with an excess ($10^{-6}$ M) of the synthetic peptide used to raise the antibodies was also performed. The number of stained cells (type 5 17β-HSD) and nuclei (ARs) were counted from colored photographs and their number presented in Table 1. The intensity of staining was compared and evaluated between the different stained cell types of the prostate on the same section. Similarly, the density of silver grains was compared between the labeled cells on the same section. The intensity of immunostaining and *in situ* hybridization reaction was presented in Table 2. Paraffin sections of cultured cells were immunostained, using type 5 17β-HSD antiserum as mentioned above, and the number of immunostained cells presented as a percentage of stained cells.

### *Antibody preparation*

*Type 5 17β-HSD.* The type 5 17β-HSD peptide sequence N-GLDRNLHYFNSDSFASHPNYPYS, located at amino acid position 297–320 of the human type 5 17β-HSD (16), was synthesized by Le Service de Séquence de Peptides de l'Est du Québec (CHUL Research Center) and purified by HPLC. New Zealand rabbits (2.5 kg) received an sc injection of 100 μg of the peptide solubilized in 1 ml PBS containing 50% complete Freund's adjuvant. The animals received, at 1-month intervals, two successive booster injections with 50 μg of the peptide in 1 ml incomplete Freund's adjuvant. Two weeks after the last injection, the rabbits were killed and the blood collected. The antiserum was obtained by decan-

tation and separation by centrifugation, then affinity-purified and stored at −80 C.

Specificity of the antiserum was examined by immunoblot analysis. In brief, human embryonal kidney cells (293) were transfected with CMV-neo vectors expressing human type 5 17β-HSD, types 1 and 3 3α-HSD and types 1 and 2 5α-reductase, respectively. Stable transfectants were selected by their resistance to $10^7$ M G-418. Positive clones were confirmed by their ability to efficiently transform the appropriate substrate (16). Forty micrograms of protein of the homogenate of each cell line were electrophoresed on a 5–15% SDS-polyacrylamide gel, as described (30), before being transferred to the nitrocellulose filter using a Bio-Rad apparatus for 4 h at 60 V. The blot was treated 3 times with 5% fat-free milk in PBS containing 0.1% Nonidet P-40 for 30 min. The antiserum developed against the type 5 17β-HSD peptide was diluted to 1:1000, and the blot was then incubated at 4 C for 18 h in the diluted antiserum. The blot was then washed three times with PBS containing 5% fat-free milk and 0.1% Nonidet P-40. After incubation with horseradish peroxidase-conjugated antirabbit IgG in solution for 2 h, the membrane was washed, and bound antibodies were detected with ECL detection reagents (Amersham, Oakville, Ontario, Canada), and finally, the membrane was exposed to Hyperfilm.

*3β-HSD.* The antiserum used for immunocytochemical studies was raised by immunizing rabbits with purified human placental 3β-HSD (4). This antiserum has been widely used to localize the enzyme in tissues of several species, including the human (31).

*AR.* AR rabbit antiserum was generated against a synthetic peptide corresponding to the first 20 amino acid residues of the N-terminal domain of the human and rat AR. The antiserum was purified by immunoprecipitation and did not show any cross-reactivity with estrogen or progesterone receptors (32). This antiserum was kindly provided by Dr. Théo H. van der Kwast, Department of Pathology, Erasmus University Rotterdam, The Netherlands.

## Results

### *Type 5 17β-HSD*

*In situ hybridization.* In the prostate specimens hybridized with the [$^3$H]-labeled type 5 17β-HSD probe, all basal cells are intensively labeled, whereas the luminal secretory cells are poorly labeled (Fig. 1a). In the fibromuscular stroma, none (or only few) silver grains are located over smooth muscle cells, whereas the fibroblasts dispersed throughout the stroma or in association with the wall of blood vessels are well labeled (Fig. 1c). Interestingly, the endothelial cells lining blood vessels are strongly labeled. The smooth muscle cells and fibroblasts of the tunica media and adventitia show variable labeling intensity. When the hybridization was performed using $^3$H-labeled sense riboprobe as control, only a few scattered silver grains were detected over the epithelium (Fig. 1b) and blood vessels (Fig. 1d). The results obtained using [$^{35}$S]-UTP riboprobes (not shown) were found to be similar to the above mentioned results.

### *Immunostaining*

*Type 5 17β-HSD distribution.* As illustrated in Fig. 2, immunoblot analysis indicates that the antiserum specifically reacts with type 5 17β-HSD. In fact, no cross-reactivity was detected, either with types 1 or 3 3α-HSD (which share 84% and 86% identity with type 5 17β-HSD, respectively) or with types 1 and 2 5α-reductase, two enzymes which are abundant in prostatic tissue (17, 33).

When immunostained paraffin sections of BPH specimens and normal prostatic tissues were examined, similar results were found (Figs. 3, a–c; and 4, a and b). Some variation in the distribution and intensity of immunostaining was observed between the twelve BPH specimens examined. A similar degree of variation was found between the normal prostate specimens, and the overall pattern was similar between normal and BPH prostatic tissues. The variation in staining of the epithelium lining the tube-alveoli was observed, not only between the different specimens but also between the tube-alveoli of the same specimens. A constant finding was the positive reaction detected in the stromal fibroblasts, whereas the smooth muscle cells were not stained (Figs. 3, a–c; and 4, a, b, and d). Strong staining was consistently found in the basal cells of the epithelium (Figs. 3a, 4a, and 5a). In contrast, the luminal secretory cells exhibited highly variable and usually low immunoreactivity. In fact, in most alveoli (about 85%), no luminal cells were labeled (Figs. 3a and 4a), whereas about 10% of alveoli contained a low detectable (but positive) reaction of all luminal cells (Fig. 3b), the basal cells being always strongly labeled. In some alveoli (about 5%), labeling of few luminal cells and all basal cells (Table 1) was found (Figs. 3c and 4b).

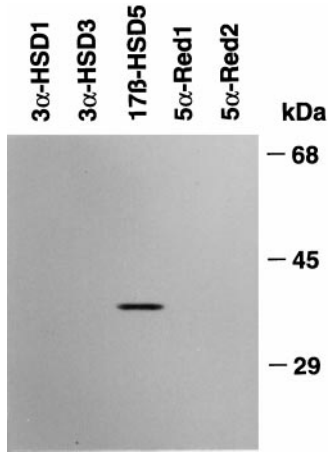


FIG. 2. Specificity of the antiserum used in the immunostaining of type 5 17β-HSD. Transfected human kidney cells (293) with human type 5 17β-HSD, types 1 and 3 3α-HSD, and types 1 and 2 5α-reductase (5α-Red1 and -Red2) were used for immunoblot analysis. The results indicate that the antiserum specifically reacts with type 5 17β-HSD. Types 1 and 3 3α-HSD, which share 84 and 86% amino acid identity with type 5 17β-HSD, respectively, and 5α-Red1 and 5α-Red2, two other androgen-synthesizing enzymes present at high concentration in prostatic tissue, were used as controls.

When the antiserum was immunoabsorbed with the antigen or when nonimmunized rabbit serum was used, no staining could be detected (Fig. 4c). The endothelial cells of small (Fig. 4e) and large (Fig. 4, e–g) blood vessels were strongly immunoreactive. The staining reaction was variable in the smooth muscle cells of the tunica media, whereas fibroblasts of the tunica adventitia were intensively stained. Veins appeared strongly labeled because of the large number of fibroblasts in their walls (Fig. 4e). In arteries, the tunica media was lightly stained (Fig. 4, f and g), whereas the tunica adventitia was well stained (Fig. 4f).

When the paraffin sections of cultured epithelial cells were examined after immunostaining, using type 5 17β-HSD antibody, 58% of these cells were found to be positively stained (Fig. 3d).

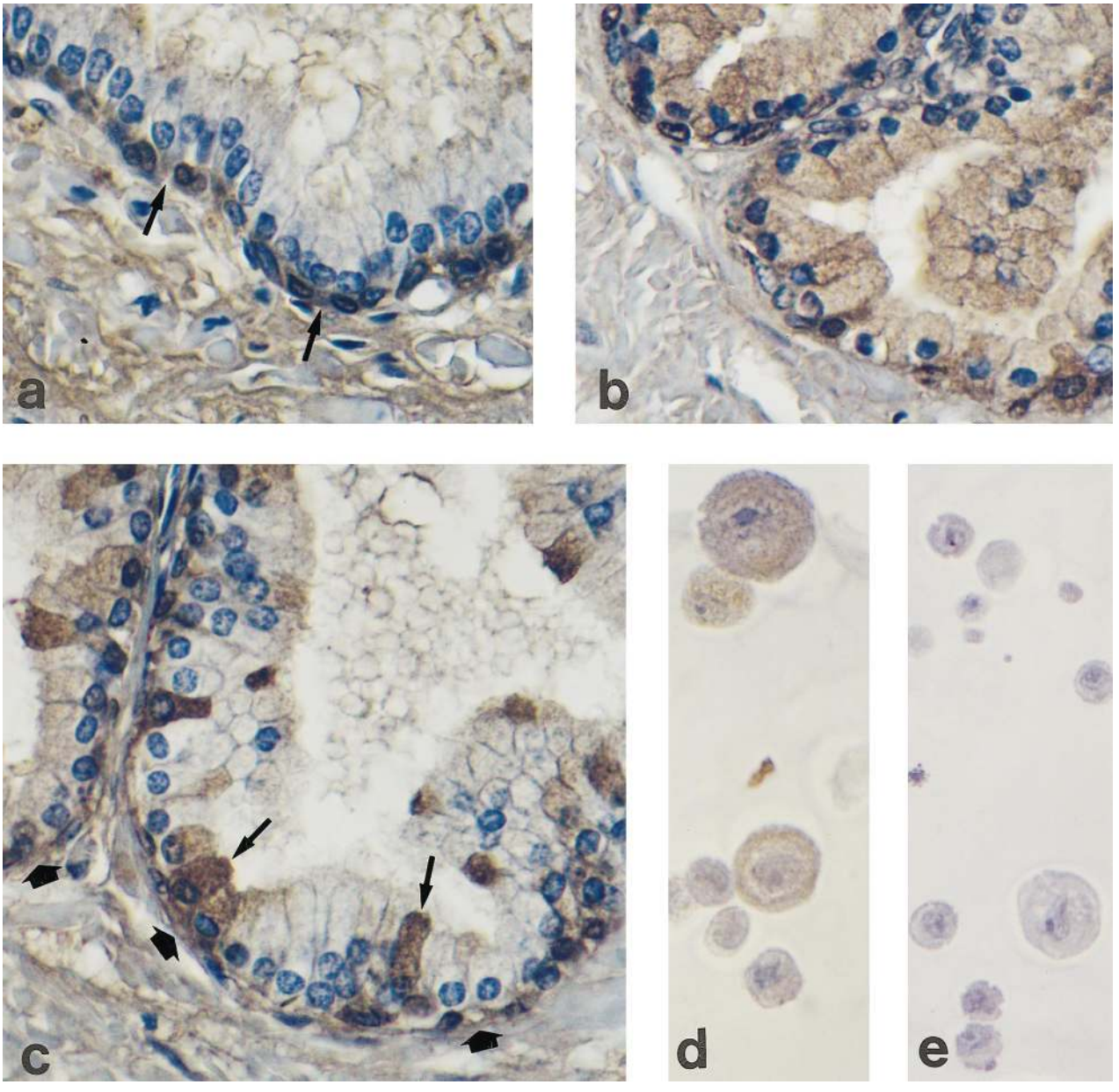


FIG. 3. Paraffin sections of normal human prostate and cultured epithelial cells (PrEC 5500–1), immunostained with antibody to type 5 17β-HSD (× 800). a, The staining reaction is observed in basal cells (*arrows*) and in most of the stromal cells situated below. The luminal cells of the epithelium (above the basal cells) are not reactive. b, All epithelial cells are immunoreactive. c, In this section, the basal cells are not very well seen (*thick arrows*), but they are labeled, as well as some luminal cells (*thin arrows*) of the tube-alveoli, d, Immunostained paraffin section of normal human prostate epithelial cells (PrEC 5500–1). The staining reaction can be seen in the cytoplasm of most of the cells. e, The same cultured cells as in d, but the antiserum was incubated with an excess of antigen. No staining reaction can be seen.

*3β-HSD Distribution.* The results obtained, after immunostaining with antibody to 3β-HSD, were found to be very similar to those generated with the type 5 17β-HSD antiserum (Fig. 5, a and b). Although the staining reaction was generally weaker for 3β-HSD, the cellular distribution of the enzyme corresponds very well to that described above for type 5 17β-HSD. In the glandular epithelium of the prostate, all the basal cells were generally labeled; whereas in the luminal cells, the staining was variable, being intense in some cells and weak or absent in most others. In the stroma, the staining was restricted to the cytoplasm of fibroblasts. As observed for type 5 17β-HSD, specific immunolabeling was found in the endothelial cells and fibroblasts of blood vessel walls, including arteries, veins, and capillaries. In all the 3β-HSD-containing cells, the staining was restricted to the cytoplasm, no significant nuclear staining being detected.

*AR distribution.* The AR appears exclusively localized in the nuclei of prostate cells in all the specimens examined. In the epithelium, immunostaining is detected in almost all the nuclei of the luminal cells, whereas most of the basal cell nuclei do not exhibit positive staining (Fig. 5c and Table 1). In the stroma, the majority of nuclei of the fibromuscular cells are labeled, but unstained nuclei of smooth muscle cells are

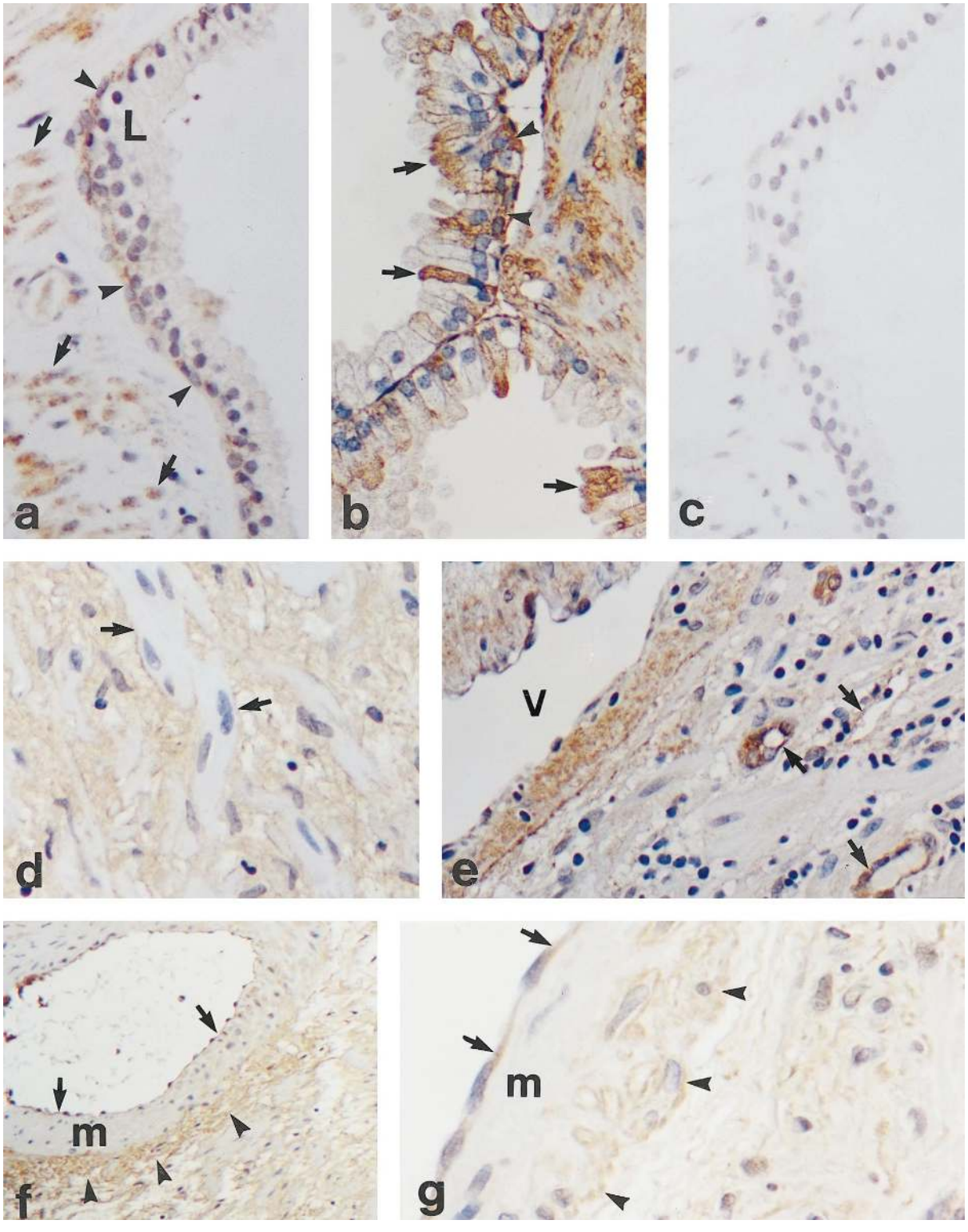


FIG. 4. Paraffin sections of BPH tissue, immunostained with antibody to type 5 17β-HSD. a, All of the basal cells show a strong positive staining reaction (*arrowheads*). Note the absence of reaction in the luminal cells (L), whereas the fibroblasts of the stroma are stained (*arrows*) (× 500). b, Some of the luminal cells are immunoreactive (*arrows*), as well as basal cells (*arrowheads*) (× 500). c, Section consecutive to that shown in Fig. 4a. Immunoabsorption of the antiserum, with an excess of antigen, completely prevents immunostaining (× 500). d, In the stroma, smooth muscle cells (*arrows*) are not labeled, whereas the surrounding fibroblasts are stained (× 800). e, Photograph, showing the positive staining in the wall of a large vein (V). The endothelial cells of small blood vessels are well-labeled (*arrows*) (× 800). f, Low magnification of an artery, showing the labeling of endothelial cells (*arrows*) and of fibroblasts of the tunica adventitia (*arrowheads*), whereas smooth muscle cells of tunica media (m) are weakly labeled (× 200). g, High magnification of an artery, which clearly demonstrates the labeling in the cytoplasm of endothelial cells (*arrows*), as well as of fibroblasts (*arrowheads*), whereas smooth muscle cells of the tunica media (m) are not labeled (× 800).

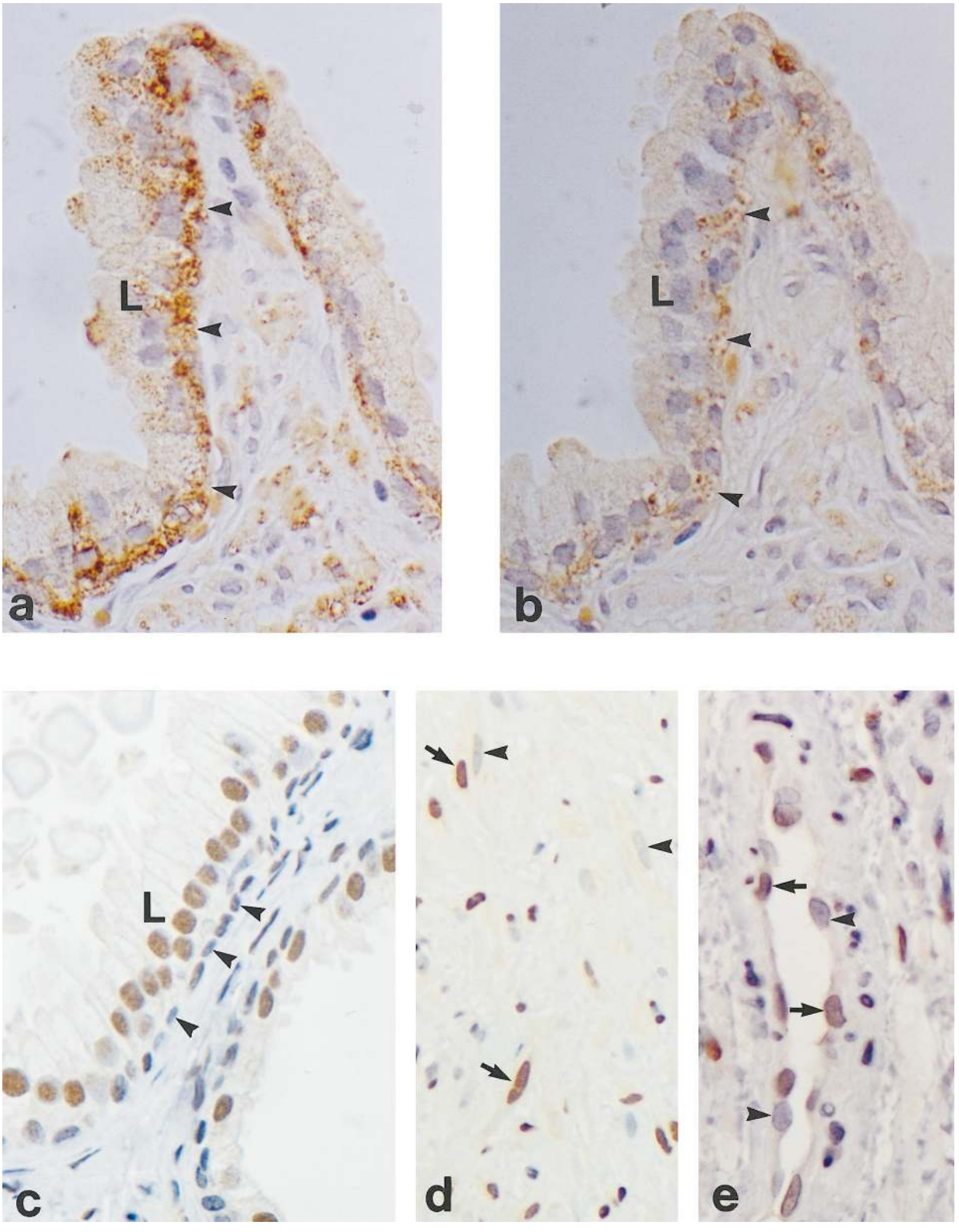


FIG. 5. Paraffin sections of BPH tissue, immunostained with antibodies for type 5 17β-HSD (a), 3β-HSD (b), and AR (c, d, and e) (× 800). a and b, Consecutive sections, immunostained for type 5 17β-HSD (5a) and 3β-HSD (5b). Although the reaction for 3β-HSD is somewhat weaker than that obtained for type 5 17β-HSD, the distribution of the two enzymes in basal (*arrowheads*) and luminal cells (L) is similar. c, AR immunoreactivity was found to be exclusively localized in the nuclei. In the epithelium, the reaction can be seen in the majority of the luminal cells (L) nuclei but not in the nuclei of most basal cells (*arrowheads*). d, In the fibromuscular stroma, most of the nuclei are labeled. Some smooth muscle cell nuclei are labeled (*arrows*), whereas others show nondetectable reaction (*arrowheads*). e, The wall of the blood vessels shows labeled and unlabeled nuclei. Some of the nuclei of the endothelial cells lining the lumen of an arteriole are labeled (*arrows*), whereas others show no staining (*arrowheads*).

also observed (Fig. 5d and Table 1). In the blood vessels, several nuclei of the endothelial cells lining the lumen are positive, but some display no reaction (Fig. 5e). In the tunica media of the arteries, most of the nuclei of the smooth muscle cells are stained, whereas some remain negative (not shown). Comparable results were obtained for the nuclei of fibrocytes of the tunica adventitia.

## Discussion

Different types of 17β-HSDs, from types 1–5 in the human, type 6 in the rat (34), from types 1–5 (35) and recently type 7 (36) in the mouse, have been identified so far. Each of these isoenzymes is thought to be expressed in a tissue- and even a cell-specific manner. However, little is known about the detailed cellular distribution of these important enzymes. Type 1 17β-HSD is known to exclusively catalyze the conversion of estrone (E1) to estradiol (E2), primarily in the ovary and placenta (37, 38). The type 2 enzyme is preferentially expressed in the placenta and catalyzes the interconversion of E1 and E2, 4-dione and testosterone, as well as androstanedione and DHT (38). This enzyme is also said to be highly expressed in the liver and secretory endometrium (37). Type 3 17β-HSD is expressed mainly in the testes (14), where it catalyzes the conversion of 4-dione to testosterone (38). Type 4 17β-HSD, on the other hand, transforms E2 into E1 and 5-diol into DHEA; this enzyme is widely expressed in peripheral tissues, with the highest expression being found in the liver, kidney, ovary, and testes (39). The type 5 isoenzyme has a reductive preference and has been shown to transform 4-dione into testosterone (38). Castagnetta *et al.* (40) have examined the expression and activities of the types 1–4 17β-HSDs in human prostate cancer cells. These authors have concluded that distinct 17β-HSDs may be differently regulated in cells with different sensitivities to sex steroids, leading to a differential accumulation of biologically active hormones.

In the present study, we have used two complementary approaches, namely *in situ* hybridization (using BPH specimens) and immunocytochemistry (using BPH, normal prostate tissues, and cultured epithelial cells) to identify the cells that express type 5 17β-HSD in the human prostate. This enzyme was found mainly in the basal cells of the tube-alveoli, the fibroblasts of the stroma and blood vessels, and in the endothelial cells of the blood vessels (Tables 1 and 2). This double approach permits us to identify not only type 5 17β-HSD messenger RNA (mRNA) but also the enzyme itself. The present data are in agreement with results from this laboratory, which indicated the presence of androgenic 17β-HSD activity in human and rhesus monkey prostates (38).

The stratified epithelium lining the tube-alveoli is divided into two layers, namely the basal layer made of low cuboidal cells and a layer of columnar secretory cells (luminal cells). It is generally believed that prostatic stem cells are located in the basal cell layer (see Ref. 41). As revealed by both *in situ* hybridization and immunocytochemistry, the basal cells are expressing type 5 17β-HSD at a much higher level than the luminal cells. In fact, whereas many luminal cells exhibited some detectable hybridization signal, they have shown a high degree of variation and usually low level of immunostaining (Figs. 3c and 4b). On the other hand, the majority of alveoli contained only strongly labeled basal cells (Figs. 3a and 4a). However, in a few alveoli, staining was detectable either in some luminal cells (Figs. 3c and 4b) or in all of them (Fig. 3b). This variable staining in luminal cells might be explained by variations in the biosynthetic activity among alveoli or among different luminal cells in the same alveolus. It is quite possible that the low level of the protein in an unknown proportion of luminal cells cannot be detected by immunocytochemistry. It is noteworthy to mention that very similar results were obtained with the antibody against 3β-HSD.

The cultured epithelial cells PrEC 5500–1 have shown approximately the same pattern of expression of type 5 17β-HSD as the epithelial cells of BPH and normal prostate tissues. We assumed that the cultured epithelial cells are a mixture of basal and luminal cells. Therefore, it is not surprising to find that only 58% of these cells expressed the enzyme.

It has been reported that types 1 and 2 5α-reductases are produced by both epithelial and stromal cells in the prostate (33, 42–44). Using immunocytochemistry, Eicheler *et al.* (45) have shown that staining for type 2 5α-reductase could be seen in both basal and luminal epithelial cells. On the basis of studies performed with human prostatic *in vitro* models, it has been suggested that the basal cells exert a stem cell role (46–48). On the other hand, *in vivo* studies performed in the rat prostate during maturation have established that both basal and secretory luminal cells are self-replicating cell types (49). The presence of type 5 17β-HSD, 3β-HSD, and 5α-reductase isoenzymes in the basal cells suggests that this cell type is actively involved in androgen production and cannot be considered as being only a precursor of the luminal secretory cells.

Using cells transfected with the cDNAs of different types of 17β-HSD, Luu-The *et al.* (50) have shown that types 1 and 3 17β-HSD catalyze the reduction of E1 to E2 and 4-dione to testosterone, respectively. They have also shown that these enzymes are substrate- and orientation selective. In fact, type 3 and 5 17β-HSDs have the same selective function, but type 3 was detected only in the testis (14) and was not found in the human prostate (51). Therefore, in the prostate, the reduction of 4-dione to testosterone is probably caused by type 5 17β-HSD. Beause type 5 17β-HSD and 3β-HSD are both highly expressed in basal cells, whereas the AR is mainly present in luminal cells (Fig. 5c and Table 1), it is tempting to suggest that testosterone synthesized in the basal cells reaches the luminal cells in a paracrine fashion, to be ultimately transformed into DHT in the luminal cells, where the androgenic action is exerted and AR is highly expressed. DHT, made in the luminal cells by the action of 5α-reductase, would then exert its action in the luminal cells themselves, thus meeting the definition of intracrine activity (1, 52). The involvement of two cell types in the biosynthesis of steroids has already been shown to occur in the ovary. In fact, in the ovary, C19 steroids (4-dione and testosterone), synthesized by theca interna cells, are transferred to granulosa cells, where they are aromatized into estrogens (53). The present data suggest the possibility of a similar two-cell mechanism of androgen formation in the human prostate: testosterone is

first synthesized in the basal cells before diffusing into the luminal cells, where transformation into DHT occurs.

In the present study, the fibroblasts present in the stroma, as well as those associated with blood vessels, are shown to contain type 5 17β-HSD mRNA, as well as the immunoreactive type 5 17β-HSD and 3β-HSD enzymes. The two types of 5α-reductase have also been detected in this cell type by various techniques (33, 45, 54, 55). The role of the steroidogenic enzymes in fibroblasts remains to be established, but because ARs are present in the nuclei of most stromal cells (Table 1), it is likely that DHT could act in the fibroblasts themselves (intracrine action) to modulate the activity of these cells.

The previous study by Leav *et al.* (56) has shown that, in normal prostate, basal cells contained the mRNA for AR but lacked an immunodetectable receptor, whereas in the luminal cells, both mRNA and immunodetectable receptor were present. These authors have also stated that AR localization in BPH was identical to that observed in normal prostate. Similarly, Loda *et al.* (57) found that the nuclei of the luminal and the majority of stromal cells were positive to AR antibody in hyperplastic, as well as normal prostatic glands. They have also found that primary (as well as metastatic) prostate carcinomas show nuclear staining for AR. Ruizeveld De Winter *et al.* (58), using AR antibody, found that the proportion and the intensity of immunostained human prostate tumor cells decreased in the more aggressive tumors. Bonkhoff and Remberger (59) found that the basal cells also express nuclear AR in normal and hyperplastic tissue. However, the receptor was most frequently expressed at lower levels in the basal cells, compared with the staining intensity detected in secretory luminal cells. Iwamura *et al.* (60) found that AR immunostaining was localized to the nuclei of luminal cells but was weak or absent in basal cells and of variable intensity in the stromal cells. In the present study, whereas 94% of luminal cells expressed nuclear AR, only 37% of basal cells were stained (Table 1), and their staining intensity was lower than that of luminal cells (Table 2). The majority (66%) of fibromuscular stromal cells also expressed AR. The findings of the present study are thus in agreement with previous studies performed in human, rat, and mouse tissue (61–63). Because the stroma/epithelium cell ratio is higher in the hyperplastic prostate (64), it can be hypothesized that androgens synthesized intracellularly by fibroblasts can influence the production of collagen and elastic fibers in the stroma.

An unexpected finding was the localization of type 5 17β-HSD and 3β-HSD in blood vessel walls, including the endothelial cells. This observation, however, correlates well with recent findings from this laboratory indicating the presence of types 1 and 2 5α-reductase mRNA in blood vessel walls in human prostate and skin (33). Recently, we have also observed that immunoreactive type 5 17β-HSD is present in the blood vessel walls in other tissues, such as skin, breast, uterus, and ovary (Pelletier *et al.*, unpublished data). The role of the steroidogenic intracrine enzymes in these vascular structures is unknown.

Previously, it has been shown that ARs were present in vascular smooth muscle and endothelial cells of human skin (65, 66). Bergh and Damber (67) found that nuclear ARs were present in the muscular layer of almost all arteries within the rat testis. These authors have suggested that testicular blood vessels could be a target organ for androgens and may mediate some of the effects of androgens on testicular microcirculation. Furthermore, in the developing human prostate, Aumuller *et al.* (68) found that AR was positive in the nuclei of vascular smooth muscle and endothelial cells. Because ARs are present in the endothelial cells, smooth muscle cells, and fibroblasts of blood vessels (Table 1), it may be speculated that locally biosynthesized androgens are exerting a paracrine and/or intracrine action in blood vessels. It is also possible that these androgens are, up to an unknown extent, released into the blood circulation, to reach some target tissues, although their global impact is likely to be minimal. Interestingly, Franck-Lissbrant *et al.* (69) have shown that, in the rat prostate, testosterone could induce a rapid response of the vasculature that precedes growth of the glandular epithelium. It might well be that cells of the blood vessels are stimulated by locally made androgens, to produce paracrine growth factors, which could promote the growth of the secretory epithelium. Further studies are required to elucidate the role of the steroids synthesized by cells of the blood vessel walls. The present data clearly indicate new mechanisms of androgen formation, which may play an important role, not only in normal human prostate physiology but also in the pathogenesis of BPH and possibly prostate cancer.

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## Erratum

In the article "Insulin-like growth factor I suppresses parathyroid hormone (PTH)/PTH-related protein receptor expression via a mitogen-activated protein kinase pathway in UMR-106 osteoblast-like cells" by Kawane and Horiuchi (*Endocrinology* **140:**871–879), 1999, please note the following correction. On pages 871 (in the footnotes) and 876 (in Fig. 9 legend), the correct GenBank accession no. should be AB012944. *The authors regret this error.*