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# HYDROXYLATION OF [$^3$H]5α-ANDROSTANE-3β,17β-DIOL BY WHOLE TISSUE, EPITHELIAL CELLS AND FIBROBLASTS FROM THE SAME HYPERPLASTIC HUMAN PROSTATE

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**Summary**—Hydroxylations of 3β-hydroxy 5α-dihydro $C_{19}$-steroids are terminal reactions by which male accessory sex organs dispose of intracellular androgens. Cellular androgen egress is of particular interest in benign prostatic hyperplasia (BPH) where the elevated nuclear 5α-dihydrotestosterone-receptor content may be implicated in the etiology of the disease. We here report substitution of hydroxyl groups at C-6α, C-7β and predominantly at C-7α of [$^3$H]5α-androstane-3β,17β-diol on incubation of 3 and 8.5 nM substrate concentrations with minced and explanted human BPH tissue. Fibroblasts isolated from the same prostatectomy specimen hydroxylated 3 nM radiosubstrate mainly at C-6α, with extensive metabolism to 17-oxosteroids. Epithelial cells from the same tissue source substituted to the same extent at the three positions. Competing 3β-hydroxysteroid dehydrogenase exceeded hydroxylase activity only in epithelial-cell cultures. Our findings support previous evidence that prostatic epithelial and stromal cells make different contributions to androgen disposition by the 3β-hydroxysteroid pathway.

## INTRODUCTION

Prostatic radiosteroid disposition following *in vivo* administration of $^3$H-labelled natural androgens or exposure of organ cultures to the labelled hormones involves metabolic activation, with high-affinity nuclear binding of the agonist 5α-DHT, and egress from explanted tissue as hydroxylated 3β-hydroxysteroid radiometabolites [1–5]. While the experimental basis for the finding of a supranormal 5α-DHT content in canine and human BPH tissue has recently been called into question [6–9], the nuclear 5α-DHT-receptor content in BPH of both species has been reported as abnormally high [10, 11]. Thus this intracellularly-active androgen remains implicated in the etiology of the disease. Since the enzymes of the 5α-3-oxo$C_{19}$-steroid reductase/3β-hydroxy$C_{19}$-steroid hydroxylase system, the 3β-hydroxysteroid pathway, are a principal means of disposing of prostatic intracellular 5α-DHT, the capacity of human prostate adenoma to transform 3β-diol into egress metabolites is of considerable interest. We characterized the metabolites of [$^{14}$C]3β-diol by NADPH-supplemented minced BPH tissue as 6ξ- and, predominantly, 7ξ-triols [12]. The major radiometabolite was subsequently identified as 5α-androstane-3β,7α,17β-triol by Morfin *et al.* [13].

In the present study, we used the same prostatectomy specimen to identify the products of the hydroxylations of [$^3$H]3β-diol by minced and explanted tissue preparations and to compare the extent and patterns of metabolism of the radiosubstrate by primary cultures of epithelial cells and fibroblasts. We also determined the extent of competing dehydrogenation of the radiosubstrate to 3-oxo and 17-oxo steroids. With whole tissue preparations hydroxylation substituted mainly at C-7α, and to a considerable extent also at the C-7β and C-6α positions. Primary cultures of both epithelial cells and fibroblasts actively hydroxylated [$^3$H]3β-diol, but differed considerably in the extent of oxygenation at individual C-6 and C-7 positions. Fibroblast cultures metabolised the radiosubstrate extensively to 17-oxo$C_{19}$-steroids. We therefore surmise that the two cell populations make interactive contributions to the metabolite patterns generated by whole-tissue preparations.

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*The following abbreviations are used in this paper:* 5α-DHT, 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one; androstanedione, 5α-androstane-3,17-dione; iso-androsterone, 3β-hydroxy-5α-androstan-17-one; 3β-diol, 3β-androstanediol, 5α-androstane-3β,17β-diol; 3α-diol, 5α-androstane-3α,17β-diol; 6α-triol, 5α-androstane-3β,6α,17β-triol; 7α-triol, 5α-androstane-3β,7α,17β-triol; 7β-triol, 5α-androstane-3β,7β,17β-triol; 17-one 6α-diol, 3β,6α-dihydroxy-5α-androstan-17-one; 17-one 7α-diol, 3β,7α-dihydroxy-5α-androstan-17-one; BPH, benign prostatic hyperplasia.

## EXPERIMENTAL

### *Source of steroids*

[1α,2α-$^3$H]5α-Androstane-3β,17β-diol (40 Ci/mmol) was purchased from the Amersham-Searle Corp. and purified by HPLC to 98% purity. The following $C_{19}O_3$-steroids with the 3β-hydroxy 5α-androstane configuration were synthesized and crystallized in carrier quantities as previously described [5]: 6α-triol, 6β-triol, 7α-triol, 7β-triol and 17-one 6α-diol. Our supply of carrier quantity of 17-one 7α-diol was exhausted and only reference quantities for HPLC analysis remained. 3β-Hydroxy-5α-androstane-7,17-dione (NSC-37849) was donated by the Experimental Biology Projects Section, National Cancer Institute; it served as starting materal for the preparation of carrier quantity of 5α-androstane-3,7,17-trione as reported [12].

### *Human prostate tissue*

The BPH surgical specimen (95 g) was obtained by suprapubic prostatectomy from a 70-year old patient. Nodules were dissected and used as the tissue source for the present study.

### *Minced tissue incubation*

The nodular tissue was scissor-minced and incubated for 30 min with 3 nM [$^3$H]3β-diol in 5 ml M/15 phosphate buffer (pH 7.4) supplemented with 0.5 mM NADPH, 5 mM glucose-6-phosphate and 0.5 U glucose-6-phosphate dehydrogenase. The reaction was terminated by shaking the contents of the incubation flask with 8 ml methylene chloride–acetone (4:1, v/v). Extraction and chromatographic determination of radiosteroid patterns were as previously described [12].

### *Organ-culture incubation*

Ice-cold oxygenated Trowell's T8 medium (GIBCO, Grand Island, NY) containing 100 U/ml penicillin G (Squibb, Princeton, NJ) and 100 μg/ml streptomycin (Pfizer, New York, NY) was used as wash and transport medium. The organ-culture procedure was as we described it for canine prostate culture [4]. Briefly, nodular tissue was sectioned from hand-cut strips into 2 mm long, 2 to 3 mm wide, 0.7 mm thick explant-size slices with a McIlwain Tissue Chopper (Brinkman Instruments, Westbury, NY) and placed on rafts of lens paper on stainless steel grids in 35 × 10 mm culture dishes. All explants were incubated at 37°C for 21 h in serum- and organic-solvent-free T8 medium (2.5 ml) containing 8.5 nM [$^3$H]3β-diol. They were positioned between the aqueous and gaseous (95% $O_2$–5% $CO_2$) phases. After culture, the explants were rinsed with sterile water, blotted dry, and then weighed. The combined medium and rinse were extracted with methylene chloride–acetone (4:1, v/v). The explants were homogenized and extracted with the same solvent mixture.

### *Primary epithelial-cell and fibroblast cultures*

Human prostatic epithelial cells and fibroblasts were isolated using modifications of the procedure previously developed for isolating purified populations of canine prostatic epithelial cells and fibroblasts [14, 15]. The cell-isolation procedure was initiated within 30 min of prostatectomy. The human tissue was transported to the cell-culture laboratory in Moscona's saline containing 100 U pencillin G and 100 μg streptomycin/ml and 1.25 μg/ml gentamicin (Garamycin, Schering, Kenilworth, NJ). All extraneous capsular and nonparenchymal material was dissected and discarded. The remaining prostatic parenchyma was finely sliced to yield 3–5 mm$^3$ strips and then minced to yield 1–2 mm$^3$ fragments. The tissue fragments were washed 3 times with the tissue culture medium RPMI 1640 containing antibiotics and 10% fetal bovine serum. The washed fragments were incubated at 37°C in 15 ml of the same medium containing antibiotics, 1% fetal bovine serum, and 0.5% type I collagenase (Worthington, Freehold, NJ) for 30 min while gently shaking in a water bath.

After 30 min, the flask of cells was removed from the water bath and the tissue fragments were briskly triturated with a 20 ml syringe and 14 gauge cannula. The tissue fragments were allowed to settle and the supernatant, containing cellular aggregates dislodged by the trituration, was removed and transferred to a 50 ml conical centrifuge tube. Fifteen ml of 0.2% collagenase was added to the undigested tissue fragments and the flask was incubated in the shaking water bath for an additional 30 min. The supernatant from each digestion was diluted to a final volume of 50 ml and centrifuged at 50 **g** for 5 min. The resulting supernatant containing viable, single cells was transferred to a 50 ml tube and centrifuged at 150 ***g*** for 5 min. The supernatant was discarded and both pellets of cells were resuspended in culture medium and transferred to separate sets of 100 mm tissue culture dishes. The procedure of collagenase digestion and cell separation by differential centrifugation was repeated until the tissue fragments were totally dissociated (6 repeat cycles). Throughout this procedure, the sample was handled aseptically with sterile solutions and supplies.

The 100 mm tissue-culture dishes of human prostatic cellular aggregates and single, isolated cells were incubated overnight in a 5% $CO_2$ tissue-culture incubator, during which time they attached to the culture dish. The cellular aggregates spread out to form discrete patches of polygonal, epithelial-like cells while the single cells formed a disperse population of attenuated, fibroblast-like cells. Because the human prostatic epithelial-like cells had attached after 10 h, a differential-attachment procedure was not employed to segregate epithelial and stromal elements as in the procedure for isolating canine prostatic cells [14, 15]. However, on the basis of morphological observations, stromal contamination of the prostatic epithelial cells did not exceed 10% of the total cells

Table 1. Metabolism of [$^3$H]5α-androstane-3β,17β-diol by whole tissue preparations of benign hyperplastic human prostate

| Tissue preparation | Mince | Explants |
|---|---|---|
| Weight (mg) | 400 | 28 |
| % Substrate recovered | 69 | 34<br>42 |
| % Metabolites | | |
| 5α-Dihydrotestosterone | 2 | 9<br>2 |
| $C_{19}O_3$-Steroids | 21 | 26<br>49 |
| Total 17β-OH steroids | 25 | 36<br>35 |
| Total 17-oxo steroids | 3 | 28<br>31 |
| 17-Oxo/17β-OH Steroids | 0.12 | 0.78<br>0.89 |

Minced tissue was incubated as quadruplicates for 30 min at 37°C with 3 nM labelled substrate in the presence of 0.5 mM NADPH, 5 mM glucose-6-phosphate and 0.5 U glucose-6-phosphate dehydrogenase.

3 × 10 Explants were exposed for 21 h at 37°C to serum-free Trowell T8 medium containing 8.5 nM $^3$H-substrate. Solvent extracts of tissues and media of 3 organ cultures were separately combined. The upper and lower figures are results for tissue and medium, respectively.

present and epithelial cells comprised less than 5% of the single-cell-derived cultures. Both sets of cell cultures were maintained in RPMI 1640 with antibiotics and 10% horse serum which had been screened for selective growth stimulation of either prostatic fibroblasts or epithelial cells [16].

*Cell-culture incubations*

The primary cultures of prostatic epithelial cells and fibroblasts were grown to semiconfluency in 100 mm culture dishes. After rinsing the cells 3 times with Moscona's saline, cultures were constituted in previously-equilibriated serum-free F12K medium (7.7 ml) to which [$^3$H]3β-diol was added in ethanolic-F12K medium (0.3 ml); the final ethanol concentration of the incubate (0.125%) proved to be non-toxic to the cells. 3 nM Radiosubstrate was then incubated for 24 h at 37°C in an atmosphere of 5% $CO_2$ (v/v) in air. The incubations were terminated by shaking the medium and scraped cells with 8 ml each of methylene chloride–acetone (4:1, v/v); the medium was then extracted with another 8 ml of the solvent mixture. Radioactivity measurements of portions of the separate solvent extracts were made with a counting efficiency for tritium which averaged 24%. Sufficient counts were allowed to accumulate so that the error did not exceed ±2%.

*Characterization and identification of radiometabolites*

The [$^3$H]steroid extracts were resolved by TLC and HPLC as previously described [5, 12, 15]. TLC system 1 used for the preliminary separation was benzene–95% ethanol (9:1, v/v)2x on Merk Silica Gel G or $HF_{254+366\,nm}$. The 2-dimensional TLC system 2 used for resolving the $CrO_3$-oxidation products of an unidentified 5α-$C_{19}O_3$-radiometabolite was chloroform–ethyl ether (9:1, v/v)2x/chloroform–ethyl ether (7:3, v/v)1x on Silica Gel G or $HF_{254+366\,nm}$. Combined TLC eluates expected to contain 5α-dihydrotestosterone, androsterone and isoandrosterone and another zonal eluate expected to contain the 3β-diol substrate and its 3α-diol epimer were resolved by HPLC with the system methanol–water (65:35, v/v) on μBondapak C18 at a flow rate of 1 ml/min. The 5α-$C_{19}O_3$-radiometabolites were separated on the same HPLC column and at the same flow rate with the system methanol–water (45:55, v/v). Crystallization of radiometabolites in HPLC peaks with authentic radioinert peak-marker steroids (Fig. 1) to constant SA, or with complete loss of radioactivity, was by the procedure of Morfin *et al.* [17]. The following solvent systems were used for these crystallizations: acetone (A); methanol–water (M/W); acetone–water (A/W); acetone–*n*-hexane (A/H).

## RESULTS

*Metabolism of [$^3$H]5α-androstane-3β,17β-diol by minced human BPH tissue and its disposition by BPH explants*

Radiosubstrate recoveries and metabolite patterns are listed in Table 1. Explant uptake of 8.5 nM [$^3$H]3β-diol-derived radiosteroids in the 21 h serum-free organ cultures based on triplicate determinations was 3.8 pmol/100 mg tissue and accounted for 5% of the radioactivity added to the organ-culture medium prior to incubation. Recoveries of radioactivity in incubations with minced tissue and explants in organ culture exceeded 92%. In the absence of tissue, control radiosubstrate recoveries were greater than 93%. Total 17β-hydroxysteroid metabolites comprise 5α-DHT and 5α-dihydro 17β-hydroxy $C_{19}O_3$-steroids, the latter resolved by HPLC (Fig. 1) and identified (Tables 3 and 4) as 7β-triol (Peak I), 6α-triol (Peak II) and 7α-triol (Peak III); total 17-oxosteroids include androstanedione, androsterone, isoandrosterone and 5α-dihydro 17-oxo$C_{19}O_3$-steroids, the latter chromatographically resolved and identified as 17-one 7β-diol (HPLC zone A), 17-one 6α-diol (Peak IV) and 17-one 7α-diol (Peak VI).

Table 2. Metabolism of [$^3$H]5α-androstane-3β,17β-diol by primary epithelial-cell and fibroblast cultures of benign hyperplastic human prostate

| Cell Type | Epithelial | Fibroblast |
|---|---|---|
| Protein (mg) | 0.30 | 0.57 |
| % Substrate recovered | 8 | 8 |
| % Metabolites | | |
| 5α-Dihydrotestosterone | 15 | 6 |
| Androstanedione | 28 | 7 |
| Isoandrosterone | 12 | 37 |
| Androsterone | 12 | 16 |
| $C_{19}O_3$-Steroids | 24 | 28 |
| Total 17β-OH Steroids | 25 | 10 |
| Total 17-Oxo Steroids | 62 | 78 |
| 17-Oxo/17β-OH Steroids | 2.5 | 7.8 |

The cells derived from the surgical specimen used for the whole-tissue incubations (Table 1) were incubated for 24 h at 37°C with 3 nM labelled substrate in 8 ml serum-free F12K medium (single determination). Substrate recoveries and metabolite patterns are from cell-culture media. Uptake into epithelial cells and fibroblasts accounted for 1.1 and 1.2% of incubated radioactivity.

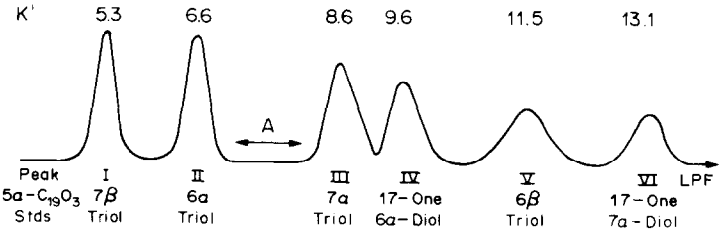


Fig. 1. Separation of six authentic 5α-dihydro$C_{19}O_3$-steroids which serve as markers in the HPLC resolution of the prostatic [$^3$H]5α-androstane-3β,17β-diol metabolites. A, zone containing radioactivity (see Table 3) with no available $C_{19}O_3$-marker (see Results). System μBondapak C18, methanol–water (45:55, v/v); flow rate: 1 ml/min.

The predominant contribution to [$^3$H]3β-diol metabolism was hydroxylation. Dehydrogenation to 3-oxosteroids (5α-DHT + androstanedione) was slight, but became evident in the accumulated [$^3$H]steroids in incubated explants, where the ratio of 3β-hydroxysteroid dehydrogenase/hydroxylase-generated radiosteroids was 0.56. Compared with the 30 min submerged minced-tissue incubations, 17-oxosteroid formation was greatly increased in the 21 h grid-supported explant cultures (refer to section on Results for data given in Table 3 based on characterization and identification of $C_{19}O_3$-radiometabolites).

*Metabolism of [$^3$H]5α-androstane-3β,17β-diol by primary epithelial-cell and fibroblast cultures of benign hyperplastic human prostate*

Radiosubstrate recoveries from the media and corresponding metabolite patterns are shown in Table 2. Uptake of 3 nM [$^3$H]3β-diol-derived radiosteroids by the epithelial cells and fibroblasts in 24 h serum-free cell cultures was 880 and 505 fmol/mg cell protein and accounted for 1.1 and 1.2%, respectively, of the initially-added radioactivity. Recovery of radioactivity from the incubated epithelial-cell culture was 94%, from the fibroblast culture 96%. In

Table 3. Human prostatic $C_{19}O_3$-metabolites of [$^3$H]5α-androstane-3β,17β-diol

| Peak | Percent of $C_{19}O_3$-Radiometabolites in HPLC Peaks and LPF | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | A | III | IV | V | VI | LPF |
| Incubation | | | | | | | | |
| Minced tissue* | 14 | 24‡ | 5 | 50 | 2 | 1 | 1 | 2 |
| Organ culture* | 11‡ | 5 | 9 | 39‡ | 17 | 1 | 7 | 6 |
| Epithelial cells† | 4 | 10 | 19 | 12 | 12 | 7 | 12 | 13 |
| Fibroblasts† | 2 | 4 | 13 | 4 | 41‡ | 2 | 17 | 11 |

For incubation conditions, see *Table 1 (organ-culture medium only analyzed) and †Table 2. Designation for Peaks I–VI as in Fig. 1; A, radioactive eluate with the indicated range of K'-values containing a major metabolite characterized as [$^3$H]3β,7β-dihydroxy-5α-androstan-17-one (see Results). LPF, less polar fraction eluted with 65 ml methanol–water (45:55, v/v) and 100 ml of methanol.

‡Based on crystallization to constant SA.

Table 4. Reverse-isotope-dilution analysis of HPLC fractions containing human prostatic $C_{19}O_3$—metabolites of [$^3$H]5α-androstane-3β,17β-diol

| Origin of fraction | Crystallization | | Specific activity* | | |
|---|---|---|---|---|---|
| | No. | System† | Starting | CR‡ | MLQ§ |
| | | | *Carrier: 5α-Androstane-3β,6α,17β-triol* | | |
| | 1 | *M/W* | 2075 | 1898 | 2250 |
| Minced—PII | 2 | A | | 1895 | 1904 |
| | 3 | A/W | | 1896 | 1896 |
| | | | *Carrier: 5α-Androstane-3β,7β, 17β-triol* | | |
| | 1 | M/W | 1610 | 1594 | 1623 |
| Organ culture—PI | 2 | A/W | | 1591 | 1597 |
| | 3 | A/H | | 1590 | 1590 |
| | | | *Carrier: 5α-Androstane-3α,7α,17β-triol* | | |
| Organ culture—PIII | 1 | M/W | 2804 | 2799 | 2810 |
| | 2 | A/W | | 2796 | 2798 |
| | | | *Carrier: 5α-Androstane-3β,6α-diol-17-one* | | |
| Fibroblast—PIV | 1 | M/W | 3281 | 3272 | 3300 |
| | 2 | A/W | | 3269 | 3274 |
| | | | *Carrier: 5α-Androstane-3,7,17-trione* | | |
| Epithelial + Fibroblast—PA¶ | 1 | A/W | 1435 | 1424 | 1440 |
| | 2 | M/W | | 1424 | 1423 |

P, HPLC peak no. (Table 3). *dpm/μmol. †cf. the Experimental Section. ‡Crystals. §Redistributed SA (17) of mother liquor. ¶$CrO_3$-oxidation product of PA.

the absence of cells, control radiosubstrate recovery was 95%.

The substrate was almost completely converted into $C_{19}O_2$-radiometabolites (5α-DHT, androstanedione, androsterone and isoandrosterone, see Table 2) and $C_{19}O_3$-radiometabolites (Fig. 1, Tables 3 and 4) at the cell-protein concentrations used. In contrast to the radiometabolite patterns generated by the whole-tissue preparations, 3β-hydroxysteroid dehydrogenase competed actively with hydroxylase for substrate. The ratio of 3β-hydroxysteroid dehydrogenase/hydroxylase-generated radiosteroids was 1.8 for the epithelial cells and 0.46 for fibroblasts. Discussion of Table 3 deals with the ratio of 17-oxo$C_{19}O_3$/17β-hydroxy$C_{19}O_3$-radiosteroids. The ratio of the total 17-oxosteroid/total 17β-hydroxysteroid radiometabolites was considerably higher in cell culture than in organ culture, and in fibroblast than epithelial-cell culture.

*HPLC characterization and identification of [$^3$H]5α-androstane-3β,17β-diol hydroxylation products generated by incubation with whole tissue and isolated cells of benign hyperplastic human prostate*

All polar radiometabolites which migrated on TLC with system 1 to various locations on the chromatogram from the origin to the position of 3β-diol were jointly eluted with ethyl acetate–methanol (1:1, v/v). The extracts were fractionated by HPLC in the presence of 6 authentic marker 5α-$C_{19}O_3$-steroids described under Experimental and shown in Fig. 1. They were resolved into radioactive components contained within the solvent volumes of peaks I–VI; the effluent volume extending over the K′-range marked A in Fig. 1 for which no authentic marker was available; and in the Less Polar Fraction, LPF (Table 3). Peak I–VI volumes, the volume comprising the A-range, and LPF accounted for 89–99% of the radioactivity applied to the columns.

We carried out reverse-isotope-dilution analysis of [$^3$H]$C_{19}O_3$-metabolites in those selected HPLC peak volumes which contained high levels of radioactivity as shown in Table 3. Radiometabolites listed in Table 4 were shown to be identical with the authentic marker steroids which had generated the differential-refractometry tracings of the peaks. They constituted 92–100% of the radioactivity in the peak volumes. Insufficient carrier was available for reverse-isotope-dilution analysis of Peak VI radioactivities. We assume that the preponderant radiometabolite is the peak tracer 17-one 7α-diol based on our previously-reported attainment of constant SA on carrier crystallization with Peak VI radioactivity derived from radiotestosterone metabolism by organ cultures of normal canine prostate [5].

Combined epithelial and fibroblast $C_{19}O_3$-radiometabolites present in the A-range effluent were oxidized with $CrO_3$ in acetone. The principal oxidation product isolated by TLC in the 2-dimensional system 2 was shown to be [$^3$H]5α-androstane-3,7,17-trione by crystallization with authentic carrier to constant SA (Table 4) and accounted for 76% of the HPLC fraction. We therefore conclude that the principal constituent in the A-range effluent is 3β,7β-dihydroxy-5α-androstan-17-one.

When crystallized twice with authentic 6β-triol, all of the radioactivity of Peak V radiometabolites generated by epithelial-cell cultures appeared in the mother liquor. Thus we could find no evidence for the transformation of [$^3$H]3β-diol to the 6β-hydroxylation product.

The reliability of the reverse-isotope-dilution analyses was checked by crystallizations of portions of Peak III radioactivity, isolated after organ-culture incubation and identified as 7α-triol, with its 7β-triol (Peak I marker) epimer and the Peak IV marker, 17-one 6α-diol. In both analyses, the crystalline carriers were devoid of radioactivity after the second crystallization.

In whole tissue [$^3$H]3β-diol incubations 7α-hydroxylation (Peaks III + VI; mince 51%, organ culture 46%) predominated over 6α- (Peaks II + IV, 26 and 22%) and 7β-hydroxylation (Peaks I + A, 19 and 20%), whereas in fibroblast culture 6α-hydroxylation (45%) exceeded 7α-(21%) and 7β-hydroxylation (15%). In epithelial cell cultures 7α-, 6α- and 7β-hydroxylation contributed equally (24, 22 and 23%). The data in Table 3 also show that 17β-hydroxy$C_{19}O_3$-metabolites (Peaks I, II, III) predominated over the 17-oxo derivatives (Peaks A, IV, VI) in whole-tissue incubations, whereas in the epithelial-cell and fibroblast cultures 17-oxo$C_{19}O_3$-metabolites were preponderant. Thus the relative patterns of 17β-hydroxy- and 17-oxosteroids at the $C_{19}O_2$- and $C_{19}O_3$-levels were similar (Tables 1–3). Radiometabolites in the LPF fractions remain unidentified.

## DISCUSSION

Prostatic metabolism of 3β-diol involves transformation of the substrate by hydroxylases and by the dehydrogenase activities of 3β-hydroxy 5α-$C_{19}$-steroid oxidoreductase and 17β-hydroxy$C_{19}$-steroid oxidoreductase. While 17β-hydroxy$C_{19}$-steroid dehydrogenase generates isoandrosterone which is also readily hydroxylated, 3β-hydroxy 5α-$C_{19}$-steroid dehydrogenase competes with the hydroxylases for the 3β-hydroxy 5α-androstane substrates. This report has described our identification of the major 3β-diol hydroxylases of human BPH tissue and the contributions of the three enzymes to the metabolic disposition patterns generated by whole-tissue preparations, and by the epithelial and fibroblast components isolated from the same prostatectomy specimen, in the presence of 3 and 8.5 nM concentrations of the radiosubstrate. A caveat in comparing the metabolic profiles needs to be emphasized. We selected different media for the serum-free organ and cell culture incubations as they had proved optimal for maintenance of morphology and function. Use of

the differing radiosubstrate concentrations permits internal comparison with data from previously-published work with rat ventral and canine prostate [4, 5, 15].

To our knowledge only Millington *et al.* [18] have determined the BPH tissue concentration of 3β-diol. Their mass-spectrometric determination of the 3α- and 3β-diol contents by high-resolution selected-ion monitoring gave $0.53 \pm 0.12$ ng/g wet weight ($n = 7$) equivalent to 1.8 nM for 3β-diol, and $1.06 \pm 0.23$ ng/g ($n = 8$) or 3.6 nM for 3α-diol. The 3α-diol content determination is in agreement with 3α-diol RIA data of Hammond [19] who found $1.40 \pm 0.12$ ng/g ($n = 10$) with an antibody which cross-reacted $<0.1\%$ with 3β-diol.

In addition to confirming 7α-hydroxylation of [$^3$H]3β-diol by whole BPH tissue [13], we have now demonstrated significant hydroxylation at C-7β and C-6α. This finding complements our recent identification of the same hydroxylated 3β-hydroxy 5α-dihydro radiosteroids in the organ-culture medium of human BPH tissue which had been incubated with [$^3$H]testosterone for 21 h [5]. To date both 7α- and 7β-hydroxylation by a male accessory reproductive organ has only been reported with the unsaturated 3β-hydroxysteroid substrate dehydroepiandrosterone, on incubation with minced normal human epididymis [20]. We could find no evidence for 6β-hydroxylation, in agreement with precipitous loss of radioactivity from carrier reported by Morfin *et al.* [13] on repeated crystallization with minced BPH-tissue $C_{19}O_3$-metabolites of [$^{14}$C]3β-diol.

There appears to be no species difference in [$^3$H]3β-diol hydroxylation by canine and human prostate tissue. Previous studies of the metabolism of 1α,2α-$^3$H- and 7β-$^2$H-labelled 3β-diol with canine prostate preparations had shown that hydroxylation occurs predominantly at C-7α [21, 22], and to a much smaller extent at C-6α [21] and C-7β (our unpublished data). In contrast, rat ventral prostate hydroxylates 3β-diol mainly at C-6α, and much less at C-7α [23].

Whereas 7α-hydroxylation was clearly predominant with minced and explanted human prostate adenoma preparations, the human fibroblasts substituted mainly at C-6α and the epithelial cells to the same extent at all three positions. Thus, with the proviso that our findings derive from comparative organ and cell culture studies with a single prostatectomy specimen, both BPH epithelial and stromal cells can contribute to explant production of the terminal 5α-$C_{19}O_3$-metabolites by the 3β-hydroxysteroid pathway. In this regard they have the metabolic capacity of their rat ventral and canine prostate counterparts [5, 15]. To date only the fibroblasts have been studied, with the prostatic smooth muscle cells remaining to be isolated and incubated with [$^3$H]3β-diol in cell culture.

Predominance of 3β-hydroxy 5α-$C_{19}$-steroid hydroxylase over dehydrogenase activities is a prerequisite if metabolic disposition and egress of intracellular androgens is to be accomplished by the 3β-hydroxysteroid pathway. The organ-culture [$^3$H]3β-diol metabolite pattern meets this requirement and supports evidence gained from our previous study of [$^3$H]testosterone disposition by BPH explants [5]. However, following dissociation, 3β-hydroxysteroid dehydrogenase (formation of 5α-DHT and 5α-androstane-3,17-dione) of the BPH epithelial cells exceeded their hydroxylase activity. In contrast, the fibroblast component of the human BPH stroma contributed considerable hydroxylase and little 3β-hydroxysteroid dehydrogenase activity and should thereby play an important role in the terminal disposition of intracellular androgens. However only a relative assessment of the activities of the competing enzymes can be made from the data, as 3β-diol transformation in the 24 h incubations was almost complete at the cell-protein concentrations used.

The level of dehydrogenase activity of prostatic 17β-hydroxysteroid oxidoreductase determines the structure of the [$^3$H]3β-diol $C_{19}O_3$-metabolites. The activity was highest in the BPH fibroblasts. Reporting on a study of [$^3$H]testosterone metabolism by human fibroblasts in monolayer culture, Schweikert *et al.* [24] also found a high content of the enzyme in BPH fibroblasts. Hepatic and peripheral enzyme activity probably contributed additionally to the *in vivo* metabolism of radiolabelled 3β-diol in human males to the sulfate and glucuronide of 3β,7α-dihydroxy-5-androstan-17-one which was isolated from its urinary conjugates in 20-fold greater yield than the 7α-triol [25]. These steroids may serve as an index of *in vivo* testosterone metabolism by the 3β-hydroxysteroid pathway in 5α-reductase-containing male target tissues.

The three triol metabolites of 3β-diol are devoid of androgenic activity in the ventral-prostate-growth bioassay [23, 26]. Apart from facilitating cellular androgen egress, hydroxylation may reduce or abolish the ability of 3β-diol to compete for prostatic estrogen receptor [27, 28], as demonstrated for the 5-androstene-3β,7α,17β-triol metabolite of 5-androstene-3β,17β-diol with respect to binding to the estrogen receptor in uterine cytosol [29]. Thus by modulating the levels of 5α-DHT and 3β-diol available for receptor interaction, the prostatic epithelial and stromal hydroxylases can exert regulatory control of the biological activities of these intracellular androgens. Since our work with rat ventral and canine prostate epithelial cells and fibroblasts had suggested that the 5α-3-oxo$C_{19}$-steroid reductase is the limiting enzyme of the 3β-hydroxysteroid pathway [15], a similar study of 5α-DHT metabolism with cells derived from human prostate adenoma is required to determine their capacities to generate 3β-diol substrate for the hydroxylation reactions described in this paper.

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