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ANDROGEN METABOLISM IN TISSUE RECOMBINANTS COMPOSED OF ADULT URINARY BLADDER EPITHELIUM AND UROGENITAL SINUS MESENCHYME

BLAKE LEE NEUBAUER*, NEIL G. ANDERSON‡, GERALD R. CUNHA†, JOHN F. TOWELL§ and LELAND W. K. CHUNG‡[a]

*Lilly Research Laboratories, Eli Lilly and Company. Indianapolis, IN 46285,
†Anatomy Department, School of Medicine, University of California, San Francisco, CA 94143,
‡Pharmacology/School of Pharmacy, University of Colorado, Boulder, CO 80309,
Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262 and
§Drug Treatment Center, VA Hospital, Wood, WI 53193, U.S.A.

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Summary—Epithelium of the adult mouse urinary bladder (BLE) was experimentally combined with mesenchyme of the urogenital sinus (UGM) and grown in intact male hosts to produce prostate-like glandular structures. To determine the extent to which the BLE is altered in a functional sense by inductive influences from UGM, investigations into the *in vitro* metabolism of tritiated testosterone (T) were undertaken.

An isocratic high performance liquid chromatographic (HPLC) method was developed in order to separate the metabolites of T in mouse bladder, prostate and UGM + BLE tissue recombinants. Using a C-18 reversed phase column and a tetrahydrofuran (20): methanol (40): H_2O (40) mobile phase, efficient and rapid separation of T, dihydrotestosterone, 3α-androstanediol, androstenedione, androstanedione and androsterone was achieved. The identities of the radiolabeled T metabolites were confirmed by recrystallization to constant specific activity.

The results of the present study revealed that tissue recombinants expressed testosterone metabolic profiles only partially toward that of the adult prostate. For example, percentage formation of 5α-androstanedione, 3α-androstanediol and unknown polar metabolites in the UGM + BLE resembled the prostate and differed significantly from the urinary bladder. Conversely, formation of the 3β-androstanediol and androsterone from testosterone resembled the urinary bladder and differed from the formation of these metabolites in the prostate. These results suggest that in contrast to histomorphology, androgen-induced DNA synthesis, androgen receptor binding activity and total tissue two-dimensional gel electrophoretic protein profiles, androgen metabolic profiles in the tissue recombinants showed only partial transformation into prostatic phenotypes. Analysis of steroid-metabolic profiles, therefore, may represent an exquisite and sensitive method to assess gene expression in various hormone-responsive target tissues.

INTRODUCTION

Prostate-like glandular structures develop when epithelium of the adult mouse urinary bladder (BLE) is experimentally combined with the embryonic mesenchyme of the urogenital sinus (UGM) and grown in intact male hosts [1]. The UGM + BLE recombinants are also androgen-sensitive with regard to protein and DNA synthetic activity [2]. To determine the extent to which the BLE may be reprogrammed with regard to its androgen metabolic activity by the UGM, investigations of the *in vitro* metabolism of [^{3}H]testosterone (T) were undertaken. An isocratic high performance liquid chromatographic (HPLC) method was developed to separate and compare the labeled metabolites of testosterone formed in rodent bladder, prostate and tissue recombinants.

Results of the present study suggest incomplete phenotypic reprogramming of adult BLE by the embryonic inductor, the UGM. The significance of these results is discussed in the context of the developmental competence of the UGM for the heterotypic induction of the prostate gland.

EXPERIMENTAL

Steroids

All non-radioactive steroids were purchased from Sigma. Tritiated testosterone was purchased from New England Nuclear and had a sp. act. of 135 Ci/mmol. This radioactive material was repurified 24 h prior to use using Sephadex LH-20 (Pharmacia) chromatography [3]. Radiolabeled 3α- and 3β-androstanediols were obtained from Amersham; androstenedione and androsterone were obtained from New England Nuclear. These steroids had specific activities of 40 Ci/mmol. Tritiated androstanedione was prepared from unlabeled an-

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[a]To whom correspondence should be addressed.

drostenedione by New England Nuclear. Fourteen mg of precursor was dissolved in 2 ml of dry dioxane; to this was added 14 mg of 5% Pd/C catalyst and one atmosphere of tritium gas. The reaction mixture was stirred for 1.5 h at room temperature. Labile tritium was removed, *in vacuo*, using methylene chloride–methanol (1:1, v/v) as solvent. After filtration from the catalyst, the product was again taken to dryness, *in vacuo*, and then reconstituted in benzene–ethanol (9:1, v/v). This material was repurified using the tetrahydrofuran–methanol–H_2O–HPLC system prior to use in the extraction recovery studies.

Animals

Epithelium from urinary bladders (BLE) of adult C57/6J BL mice were experimentally combined with mesenchyme from the urogenital sinus (UGM) of 16-day old embryonic mice and grown under the kidney capsules of intact male hosts for 24–28 days using method as described previously [4]. Following this growth period, male hosts under ether anesthesia were orchiectomized *via* the scrotal route and sacrificed by cervical dislocation 24 h later.

Mince assays

Tissues were rapidly excised, finely minced with scissors and placed in ice-cold (4°C) Kreb's Ringer phosphate buffer (pH 7.4). Minces of host bladder, UGM + BLE recombinants of dorsolateral and ventral prostatic tissues containing between 20 and 35 μg DNA were incubated in 0.5 ml Kreb's Ringer phosphate buffer containing [1, 2, 6, 7, 16, 17-^{3}H]T at a final concentration of 0.5 μM and a final sp. act. of 0.8 Ci/mmol.

The use of tissue minces instead of tissue homogenates for the testosterone metabolic studies was designed to preserve cellular compartmentalization and to efficiently utilize the endogenous replenishable co-factors during incubation [5]. The advantages of using tissue minces instead of tissue homogenates for androgen metabolic studies have been previously discussed by Isaacs and Coffey[6].

The incubations were carried out in glass culture tubes sealed with polypropylene caps in a Labquake shaker (Labindustries) at 37°C. At various times, the reactions were terminated with the addition of 2.5 ml freshly open anesthesia-grade diethyl ether (Mallinckrodt). Successive extractions of aqueous phase were performed with an additional 2.5 ml ether and 2.5 ml redistilled spectrophotometric grade ethyl acetate–*n*-hexane (1:9, v/v) [Fisher]. These organic extracts were pooled, taken to dryness under vacuum at 50°C and subsequently redissolved in 140 μl of the following reference steroids: androstenedione (10^{-5} M), T (10^{-5} M), androstanedione (10^{-2} M), dihydrotestosterone [DHT] (10^{-2} M) and androsterone (10^{-2} M) in HPLC grade methanol (Fisher). The 1-h incubation period for testosterone metabolism was chosen because of linearity with respect to substrate depletion and maximum formation of metabolites (see Results).

Extraction recoveries

Recoveries of steroids were examined in minced tissues. To determine the distribution of total radioactivity in aqueous and organic phases, tissue extracts prepared according to the procedures described in the previous section were extracted twice with 2.5 ml diethyl ether and once with 2.5 ml ethyl acetate–*n*-hexane (1:9, v/v). The organic extracts were combined, and both organic and aqueous extracts were dried in a vacuum oven at 50°C. To determine the recovery of individual radiolabeled steroids by the organic extraction procedure outlined above, tissue minces pretreated at 100°C for 10 min were added to 0.5 ml Kreb's Ringer phosphate buffer containing 10,000 dpm of each appropriate steroid. Samples were dried under vacuum at 50°C, 5.0 ml of MAXI-fluor scintillation cocktail (Baker) was added and radioactivity was counted on a Beckman LS-3133P scintillation counter (SmithKline Beckman). The counting efficiency for [^{3}H] was 60%.

High Performance Liquid Chromatography (HPLC)

An ALTEX high performance liquid chromatographic (HPLC) system utilizing a Model 110A solvent metering pump, Model 210 sample injection valve, C-18 ultrasphere ODS (25.0 cm) reversed-phase column and Model 153 ultraviolet analytical detector ($\lambda = 254$ nM) was employed to separate [^{3}H]T and its radiolabeled metabolites. An isocratic mobile phase of filtered (45 μ) tetrahydrofuran–methanol–H_2O (20:40:40, by vol) was pumped at 1.5 ml/min. Fractions corresponding to the steroid standard peaks or, in the case of the two androstanediols [5α-androstane-3 (α or β), 17β-diol)], intervals corresponding to the appropriate retention times determined for radiolabeled standards were collected into 7.0 ml scintillation vials using a fractomette 200 automatic fraction collector (Buchler Instruments). The samples were dried under vacuum and counted as described above.

Identification of testosterone and its metabolites

Replicate radioactive samples for recrystallization studies were separated by HPLC and added to 50 mg of unlabeled carrier steroid, reconstituted in benzene–ethanol (9:1, v/v), and the mixtures were crystallized from various solvent systems (cf. Table 1). Recrystallized material used for the determinations of specific activity exceeded 3.0 mg and the counting error with less than 5% in each case. The identity of each steroid was established by successive crystallizations to constant specific activity according to a procedure described previously [7].

DNA Content

Tissue DNA was extracted and determined by the

Table 1. Crystallization to constant specific activity of radioactive metabolites isolated by HPLC from two incubations of mouse prostate/bladder with [^{3}H] testosterone

Area number	Steroid	Sequence solvent pairs	Specific activity (dpm/mg) after recrystallization							Percent of initial activity present in last crystals
				First		*Second*		*Third*		
			Start	C_1	M_1	C_2	M_2	C_3	M_3	
2	Androstenedione	a,b,c	1530	1330	2720	1390	1700	1400	1390	92
3	Testosterone	a,b,c	21,000	19,700	19,600	20,400	20,300	20,600	20,400	98
4	5α-Androstane-3β, 17β-Diol* (3β-diol)	a,d,c	55	47	22	48	47	48	90	88
5	5α-Androstanedione*	a,b,c	142	105	368	52	77	60	67	42
6	Dihydrotestosterone	a,d,e	10,900	10,300	9890	10,400	9200	10,600	9430	97
7	5α-Androstane-3α, 17β-Diol (3α-diol)	a,d,e	1200	1180	963	1040	920	957	905	80
8	Androsterone*	a,b,c	70	55	170	35	47	48	42	69

Solvents: (a) acetone–H_2O. (b) acetone–*n*-hexane. (c) ethyl acetate–*n*-hexane. (d) chloroform–*n*-hexane. (e) ethyl acetate–cyclohexane.
*Isolated from bladder. C: crystals. M: mother liquor.

fluorometric method of Hinegardner [8] with calf thymus DNA (Sigma) as the reference standard.

Statistical analysis

Values presented for the tissue metabolic studies are expressed as the mean ± SEM of at least 6 independent observations. Data were analyzed using one-way analysis of variance for groups of unequal size and Dunnett's multiple comparison method [9].

RESULTS

The chromatogram of authentic standards of testosterone and six of the major metabolites known to be formed in male accessory sex organs is shown in Fig. 1. The rapid (< 17 min) and discrete separation of androstenedione, T, 3β-androstanediol, androstanedione, DHT, 3α-androstanediol and androsterone is readily apparent.

The identity of each steroid when eluted in a given peak was confirmed by crystallization to constant specific activity in three solvent pairs (Table 1). Although epiandrosterone eluted with the same retention time (9.8 min) as androstanedione, crystallization of the radiometabolite fraction formed from prostatic tissues with unlabeled epiandrosterone failed to confirm the identity of the material (not shown).

Under the defined reaction conditions, there was no apparent difference in the distribution of total radioactivity in aqueous and organic phases following extraction of various specimens containing viable or heat-treated tissues (Table 2).

Although there were small differences observed in the present recoveries of individual radiolabeled steroids which were added to these prostatic or bladder tissue minces prior to extraction (Table 3), these

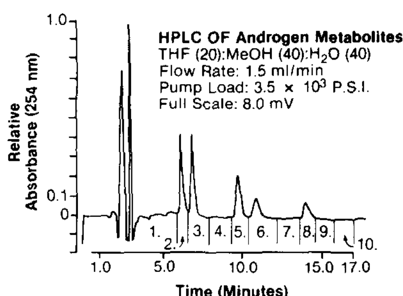

Figure 1
HPLC Profile of Androgen Standards Measured by U.V. Absorption (254 nm) and Fractions Collected for Radioactivity Detection

Steroid (Concentration)	Fraction Number (Duration)
Unknown polar metabolites	1. (< 5.9 min.)
Androstenedione (10^{-5}M)	2. (5.9- 6.5 min.)
Testosterone (10^{-5}M)	3. (6.5- 8.0 min.)
3β-androstanediol*	4. (8.0- 9.4 min.)
Androstanedione (10^{-2}M)	5. (9.4-10.4 min.)
Dihydrotestosterone (10^{-2}M)	6. (10.4-12.2 min.)
3α-androstanediol*	7. (12.2-13.7 min.)
Androsterone (10^{-2}M)	8. (13.7-14.6 min.)
Unknown**	9. (14.6-15.8 min.)
Unknown**	10. (15.8-17.0 min.)

*Determined by use of radiolabelled steroid
**Comprises <1 percent of total radioactivity

Fig. 1. HPLC profile of androgenic standards measured by u.v. spectrophotometric and radioactivity detection methods.

Table 2. Percent distribution of total radioactivity in aqueous and organic phases following extractions of incubation media with ether and ethyl acetate–*n*-hexane*

	Organic phase	Aqueous phase
No tissue	97	3
Boiled prostate*	95	5
Bladder	97	3
UGM + BLE	98	2
Prostate	97	3

*For details, see the Experimental section.

Table 3. Percent recovery of individual radiolabelled steroids in the organic phase following extractions of boiled bladder and prostate in incubation media with ether and ethyl acetate–*n*-hexane*

	Bladder	Prostate
Androstenedione	100 ± 2	100 ± 4
Testosterone	100 ± 8	100 ± 1
3β-Androstanediol	84 ± 3	88 ± 4
DHT	82 ± 3	88 ± 4
Androstanedione	91 ± 1	90 ± 1
3α-Androstanediol	95 ± 4	99 ± 1
Androsterone	82 ± 6	95 ± 6

*For details, see the Experimental section. Data represent the mean ± SEM of three separate experiments.

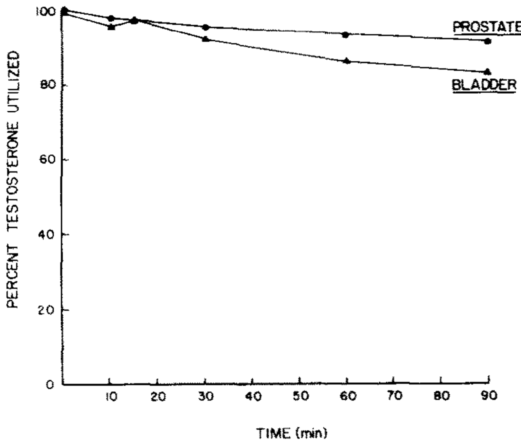

Fig. 2. Depletion of testosterone substrate as a function of incubation time in mouse prostate and bladder. (For experimental details, see the Experimental section.)

differences are not sufficient to account for major differences of androgen metabolic profiles in tissues studied.

The optimal assay conditions for testosterone metabolism by adult urinary bladder and prostatic tissues were determined. Figure 2 shows that testosterone was depleted as a function of time for at least 90 min. Urinary bladder metabolized testosterone to a greater extent than the prostate gland. Analysis of total testosterone metabolites formed by urinary bladder and prostate tissue minces showed that the maximal amount of major testosterone metabolites formed, dihydrotestosterone and androstenedione, peaked at 60 min (Figs 3a and b). Consequently, a 1-h incubation period was selected for the comparative study of testosterone metabolism by host prostate, host urinary bladder and UGM + BLE tissue recombinants.

Results of the metabolism of T by host prostate, bladder and UGM + BLE tissue recombinants under *in vitro* incubation conditions suggest that the amount of total metabolites formed by the tissue recombinants was lower than in prostate which in turn was less than in bladder (Table 4). In addition, the UGM + BLE recombinants produced percentages of metabolite formation comparable to the host prostate with regard to the generation of unknown polar metabolites, 5α-androstanedione, and 3α-androstanediol. The UGM + BLE recombinant exhibited a bladder-type phenotypic response with regard to the formation of 3β-androstanediol and androsterone from T while exhibiting a lower percentage of androstenedione formation than either host tissue. Formation of DHT was comparable in the three tissues examined (Table 4).

DISCUSSION

Previous studies from our laboratories have established the inductive potential of embryonic urogenital sinus mesenchyme in reprogramming the mor-

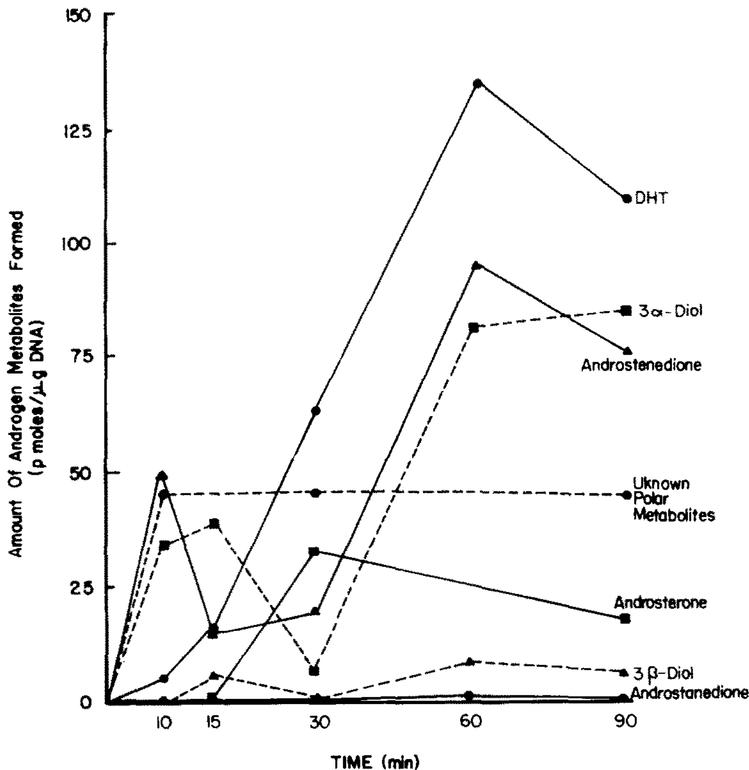

Fig. 3. (a) Formation of androgenic metabolites as a function of incubation time in mouse prostate. (For experimental details, see the Experimental section.)

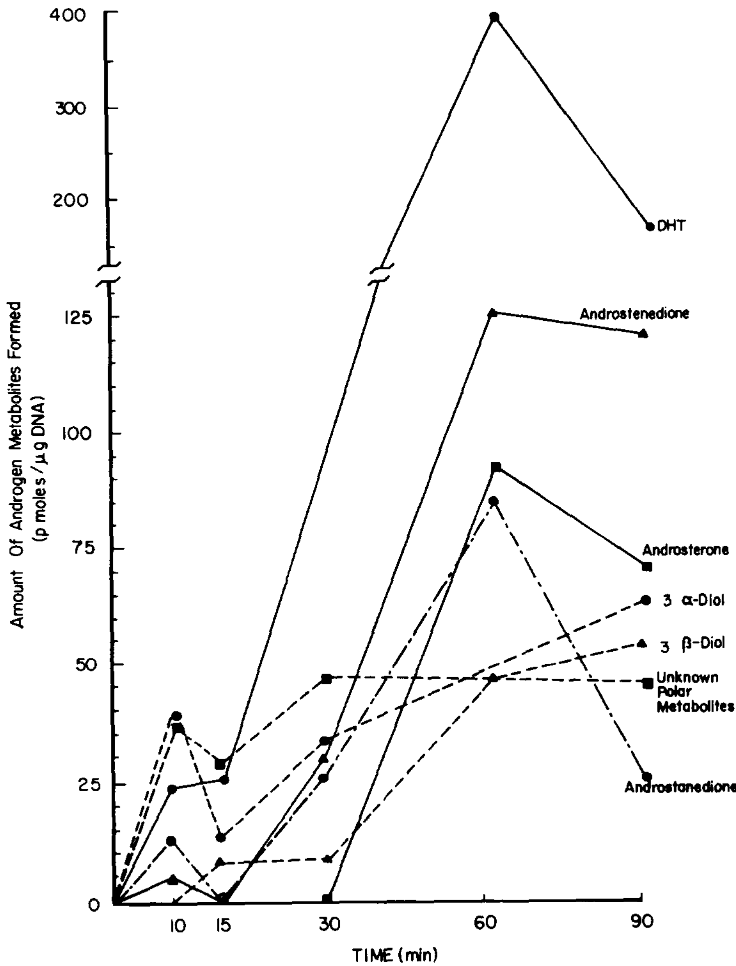

Fig. 3. (b) Formation of androgenic metabolites as a function of incubation time in mouse bladder. (For experimental details, see the Experimental section.)

Table 4. Radioactive steroids obtained after *in vitro* incubation of bladder, UGM + BLE tissue recombinant, and prostatic tissue minces with [^{3}H]testosterone

Area number	Steroid	Percentage of testosterone metabolites		
		UGM + BLE	Bladder	Prostate
1	Unknown polar metabolites	19.8 ± 4.8[a]*	6.8 ± 2.1[a]	17.6 ± 4.1[a]
		(47.1 ± 11.4)‡	(35.1 ± 10.8)‡	(61.9 ± 14.4)‡
2	Androstenedione	2.6 ± 1.9	18.3 ± 4.1	29.4 ± 5.2
		(6.2 ± 4.5)	(94.5 ± 21.1)	(103.4 ± 18.3)
3	Testosterone[b]	90.3 ± 2.2	78.7 ± 4.1	85.5 ± 8.9
		(2.2 × 10^3 ± 53.9)	(1.9 × 10^3 ± 99.1)	(2.1 × 10^{-3} ± 215.8)
4	3β-Androstanediol	13.4 ± 3.1†	10.0 ± 3.2	2.2 ± 1.5
		(31.8 ± 7.4)	(51.6 ± 16.2)	(7.7 ± 5.3)
5	5α-Androstanedione	3.6 ± 1.8*	10.7 ± 2.6	1.0 ± 0.5
		(8.6 ± 4.3)	(55.3 ± 31.4)	(3.5 ± 1.8)
6	Dihydrostestosterone	26.6 ± 4.3	18.8 ± 2.4	26.6 ± 3.8
		(63.2 ± 10.2)	(97.1 ± 12.4)	(93.5 ± 13.4)
7	3α-Androstanediol	16.6 ± 2.1*	9.0 ± 2.9	22.3 ± 4.2
		(39.4 ± 5.0)	(46.5 ± 15.0)	(78.4 ± 14.8)
8	Androsterone	17.6 ± 3.3†	23.0 ± 4.1	7.8 ± 2.5
		(41.9 ± 7.8)	(118.8 ± 21.2)	(27.4 ± 8.8)
Total metabolites formed		(238.1 ± 17.2)	(498.9 ± 78.8)	(375.8 ± 41.2)

[a]Values represent percentage mean ± SEM of six or more observations. [b]Percent of unutilized substrate. *Significantly different from bladder ($P < 0.05$). †Significantly different from prostate ($P < 0.05$). ‡Data in parentheses are expressed as pmol/µg DNA.

phogenesis and functional activities of the epithelium isolated from urinary bladder of adult hosts [1–2]. In these studies, it was observed that several biochemical functions of these recombinants (UGM + BLE) including androgen receptor activity, DNA synthetic activity and total two-dimensional electrophoretic gel tissue protein profiles resembled those of the host prostate and differed distinctly from the host urinary bladder. These results, supported by morphogenetic data, led to the suggestion that adult bladder epithelium was reprogrammed completely by UGM to form prostate. The present study was designed to test the extent to which the androgen metabolic activities of mouse urinary bladder epithelium may be reprogrammed by UGM to express prostatic phenotypes. To achieve this objective, we developed a simple and reproducible steroid extraction and chromatographic method for the analysis of androgen metabolism in male accessory sex organs in order to determine the alteration (if any) of the androgen metabolic phenotypes in UGM + BLE recombinants from host bladder or prostatic tissues. The relative simplicity of the present method and the clarity of the results suggest that this isocratic HPLC system is superior to existing systems for the separation and quantitation of testosterone as well as its principal metabolites and should, hopefully, facilitate future investigations. The efficient separation of these androgens has also been confirmed using simultaneous ultraviolet and in-line flow radioactivity detection [10]. Few studies have been performed on testosterone metabolism in mouse accessory sex tissues [11, 12], and these studies were limited in scope by the thin layer chromatographic techniques employed. This HPLC method has wide versatility and, in addition to androgens, can be used to quickly and efficiently separate estrone, estriol, as well as the 17α- and 17β-isomers of estradiol (unpublished).

The observation that murine bladder possesses a complement of androgen metabolic enzyme activities: 5α-reductase, 3α-hydroxysteroid oxidoreductase (3α-HSOR; EC 1.1.1.50) and 3β-hydroxysteroid oxidoreductase (3β-HSOR; EC 1.1.1.51) as the host prostate is somewhat surprising because the bladders of castrated male mice and rats do not respond to androgen stimulation with increases in protein and DNA synthesis [2]. These results suggest that in mouse urinary bladder, the relative androgen insensitivity is attributable to the absence of functional cytosolic androgen receptor activities rather than its ability to form biologically-active androgen metabolites [2]. The similarities of these androgen reductive oxidative enzyme activities between host bladder and host prostate of the murine species is in direct contrast to the dog, in which the canine exhibits a general pattern of reductive androgen metabolism in the prostate and oxidative metabolism in the bladder [13].

HPLC analysis of androgen metabolic profiles in the UGM + BLE recombinants revealed that both prostate (predominant) and bladder-like phenotypes were present in the tissue. These results are consistent with the observation that there are quantitatively smaller protein and DNA synthetic responses to androgens in the tissue recombinant when compared to the host prostate [2]. One interpretation of these observations is the UGM + BLE tissue recombinants developed all components of the prostatic complex (viz. the glandular acini, small amounts of urethra and urethral glands) which invariably differed morphologically and biochemically from host prostate [14]. Therefore, these other cellular phenotypes might be expressed, accounting for a diminution of the general prostate-like biochemical responses in the tissue recombinant [2]. The alternative interpretation of these data is that the urothelium is not completely induced by the UGM to form prostate. Consequently, androgen metabolism may be ideal in characterizing tissue-specific gene expressions in which the difference between tissue recombinants and host prostate or host bladder cannot be assessed by the aforementioned methods (e.g. androgen receptor, DNA synthetic and protein synthetic activities).

The concept that underlying mechanisms of tissue interactions may bear importance to growth disorders such as human benign prostatic hyperplasia has been proposed [15, 16, 17]. These studies suggest epithelial–stromal interactions resulting from an acquisition of embryonic inductive potential in adult prostatic stroma may be important to the expression of abnormal prostatic growth. The present study demonstrated that analysis of steroid metabolic profiles may provide a convenient and sensitive tool to distinguish tissue-specific expression of differentiative capability of androgen-sensitive tissues in their developing, normal and neoplastic states.

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REFERENCES

1. Cunha G. R., Lung G. and Reese B. A.: Glandular epithelial induction by embryonic mesenchyme in adult bladder epithelium of BALB/c mice. *Invest. Urol.* **17** (1980) 302–304.
2. Neubauer B. L., Chung L. W. K., McCormick K. A., Thompson T. C. and Cunha G. R.: Epithelial–mesenchymal interactions in prostatic development II. Biochemical observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult urinary bladder. *J. Cell Biol.* **96** (1983) 1671–1676.
3. Murphy B. E. P.: 'Sephadex' column chromatography as an adjunct to competitive protein binding assays of steroids. *Nature* **2321** (1971) 21–24.
4. Cunha G. R.: Tissue interactions between epithelium and mesenchyme of urogenital and integumental origin. *Anat. Rec.* **172** (1972) 179–187.
5. Isaacs J. T. and Coffey D. S.: Androgen control of prostatic growth: Regulation of steroid levels in prostate cancer. A series of workshops on the biology of human cancer. (Edited by D. S. Coffey and J. T. Isaacs).

International Union Against Cancer, Geneva, Vol. 48 (1979) pp. 112–125.
6. Isaacs J. T. and Coffey D. S.: Changes in dihydrotestosterone metabolism associated with the development of canine benign prostatic hyperplasia. *Endocrinology* **108** (1981) 445–453.
7. Chung L. W. K. and Coffey D. S.: Androgen glucuronides I. Direct formation in rat accessory sex organs. *Steroids* **30** (1977) 223–243.
8. Hinegardner R. T.: An improved fluorometric assay for DNA. *Analyt. Biochem.* **29** (1971) 197–201.
9. Chew V.: *Comparisons Among Treatment Means in an Analysis of Variance* (Edited by J. McGuire) Agricultural Research Service U.S.D.A., Washington, D.C. (1977) pp. 24–25.
10. Hanlin M. L. and Neubauer B. L.: In-line flow radioactivity detection of rat ventral prostatic [^{3}H]-testosterone metabolites separated by HPLC. *Pharmacologist* **24** (1982) 215.
11. Thomas J. A., Lloyd J. W., Smith M. T., Mawhinney M. G. and Smith C. G.: Effect of Dieldrin on the accumulation and biotransformation of radioactive testosterone by the mouse prostate gland. *Toxicol. Appl. Pharmac.* **26** (1973) 523–531.
12. Lloyd J. W., Thomas J. A. and Mawhinney M. G.: 2,4,5, T and the metabolism of testosterone-1,2-[$^{3}H_{2}$] by mouse prostate glands. *Archs environ. Hlth* **26** (1973) 217–220.
13. Morfin R. F., Aliapoulios M. A., Chamberlain J. and Ofner P.: Metabolism of testosterone-4-[^{14}C] by the prostate and urinary bladder *in vivo*. *Endocrinology* **87** (1970) 394–405.
14. Cunha G. R., Shannon J. M., Neubauer B. L., Sawyer L. M., Fujii H., Taguchi O. and Chung L. W. K.: Mesenchymal–epithelial interactions in sex differentiation. *Hum. Genet.* **58** (1981) 68–77.
15. Deming C. L. and Wolf J. S.: The anatomical origin of benign prostatic enlargement. *J. Urol.* **42** (1939) 566–580.
16. LeDuc I. E.: The anatomy of the prostate and the pathology of early benign hypertrophy. *J. Urol.* **42** (1939) 1217–1241.
17. McNeal J. E.: Origin and evolution of benign prostatic enlargement. *Invest. Urol.* **15** (1978) 340–345.