Testosterone Metabolism *in vivo* by Human Prostatic Tissue

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Although 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) has been accepted as an active androgen in the rat for a number of years (Dorfman, 1956; Saunders, 1963), evidence has only recently been obtained to suggest that this compound, rather than testosterone, may well be the active hormone in the accessory sex organs of this species (Bruchovsky & Wilson, 1968*a,b*; Anderson & Liao, 1968; Mainwaring, 1970). The formation of 5 α -dihydrotestosterone from testosterone by preparations of human benign hyperplastic prostatic tissue *in vitro* has also been demonstrated (Farnsworth & Brown, 1963; Ofner, Smakula, Wotiz, Lemon & Mescon, 1965; Chamberlain, Jagarinec & Ofner, 1966), and the stimulatory effect this steroid has on cell division with the induction of epithelial hyperplasia in maintained explants of rat prostate (Baulieu, Lasnitzki & Robel, 1968*a,b*) is noteworthy in this respect.

More recently, Kowarski, Shalf & Migeon (1969) reported the presence of 5 α -dihydrotestosterone in high concentration in the prostates of dogs after the intravenous infusion of [^3H]testosterone. This communication now describes preliminary investigations to study the metabolites found in the prostate gland after infusion via the cephalic vein of [7α - ^3H]testosterone (10 μCi , specific radioactivity 1.5 Ci/mmol) into human subjects 20 min before a Millen retropubic prostatectomy was performed on men with benign prostatic hypertrophy. The enucleated tissue was transferred to the laboratory on ice, chopped into smaller pieces and homogenized in 5 vol. of acetone containing 500 μg each of the carrier non-radioactive steroids investigated. The filtrates and acetone washes of the mixtures were pooled and dried *in vacuo*. Steroids were fractionated as described by Fahmy, Griffiths, Turnbull & Symington (1968) and isolated on thin layers of silica gel HF_{254/366} and alumina HF_{254/366} and also on Woelm alumina. Table 1 also shows the derivatives formed and indicates the t.l.c. systems adopted for their isolation. The following solvent systems were used: solvent I, chloroform–acetone (37:3, v/v); solvent II, chloroform–acetone (150:1, v/v); solvent III, cyclohexane–ethyl acetate (7:3, v/v); solvent IV, cyclohexane–ethyl acetate (9:11, v/v); solvent V, cyclohexane–ethyl acetate (4:1, v/v);

solvent VI, ether–ethyl acetate (19:1, v/v); solvent VII, cyclohexane–ethyl acetate (1:1, v/v).

Portions of the neutral steroid fraction were applied as bands to thin layers of silica gel. After chromatography in solvent I, bands corresponding in mobility to 5 α -androstane-3,17-dione (R_F 0.75), androstenedione (androst-4-ene-3,17-dione) (R_F 0.60), androsterone (3 α -hydroxy-5 α -androstane-17-one) and 5 α -dihydrotestosterone (R_F 0.46), testosterone and epitestosterone (17 α -hydroxyandrost-4-en-3-one) (R_F 0.30), 5 α -androstane-3 α ,17 β -diol, 5 α -androstane-3 β ,17 α -diol and 5 α -androstane-3 β ,17 β -diol (R_F 0.22) and 5 α -androstane-3 α ,17 α -diol (R_F 0.14) were eluted. Androsterone and 5 α -dihydrotestosterone were acetylated and separated by re-running in solvent II (R_F 0.45 and 0.58 respectively). After hydrolysis the free steroids were oxidized to 5 α -androstane-3,17-dione and chromatographed in solvent III on silica gel (R_F 0.42).

Testosterone and epitestosterone were separated by running on Woelm alumina plates in solvent IV (R_F 0.50 and 0.39 respectively). Further purification was obtained by acetylation and re-running again on alumina in solvent III. The 5 α -androstane-3,17 β , 3 α ,17 β , 3 β ,17 α and 3 β ,17 β , were separated as described elsewhere (Harper, Pierrepoint, Fahmy & Griffiths, 1970*a*).

Derivatives were prepared as described previously (Griffiths, Grant & Whyte, 1963). Δ^4 -3-Oxo steroids were measured after elution by their selective absorption of light at 240 nm, and the remaining steroids and their derivatives were determined by g.l.c. [1% XE-60 on Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa., U.S.A.) at 200°C]. Radioactivity was measured by using a Nuclear–Chicago liquid-scintillation spectrometer. The specific radioactivities of the carrier steroids and their derivatives were used to calculate the total radioactivity associated with each steroid investigated. These results are also expressed as percentages of the total radioactivity isolated in the prostate.

Table 1 shows the evidence for the identification of the radioactive carrier steroids isolated from the human benign hypertrophic prostatic tissue and their contribution, as a percentage, to the total radioactivity extracted from the tissue. In Expts.

Table 1. *Evidence of the identification of testosterone metabolites isolated from human prostatic tissue*

The dashes indicate that the compounds were lost during processing. S, Silica gel; Al, alumina.

Steroid investigated and derivative formed	Solvents used for purifications	Sp. radioactivities (d.p.m./ μ mol)			% of total radioactivity in prostate tissue		
		Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Testosterone	I (S), I (Al)	Trace	2350	Trace	~1.0	4.6	Trace
Androstenedione	I (S), VII (S)		2110				
Testosterone acetate	III (Al), VII (S)						
5 α -Dihydrotestosterone	I (S), VII (S)	11150	13250	2300			
5 α -Androstane-3,17-dione	III (S), V (S), I (S)	11450	12490	2310	44.6	27.3	5.1
5 α -Dihydrotestosterone acetate	II (S), III (S)	11010	13990				
Epitestosterone	I (S), I (Al)			2310			
Epitestosterone acetate	I (S), VII (S)			2520			5.3
Androstenedione	III (Al), VII (S)			2380			
Androstenedione	I (S), VII (S)		8360				
Testosterone	I (S), VII (S)	—	8600	5160	—	17.2	11.9
Testosterone acetate	III (S)		7930	5410			
5 α -Androstane-3,17-dione	I (S), V (S)	1220	460	4030			
5 α -Androstane-3 β ,17 β -diol	IV (S)	1850		3640	4.9	0.9	9.4
5 α -Androstane-3 β ,17 β -diacetate	III (S)	1170	420				
5 α -Androstane-3 α ,17 β -diol	I (S), VI (S)						Trace
5 α -Androstane-3,17-dione	V (S), I (S)	0	2760		0	5.6	
5 α -Androstane-3 β ,17 β -diol	IV (S)		2850				
5 α -Androstane-3 β ,17 β -diol	I (S), VI (S), VII (Al)						
5 α -Androstane-3,17-dione	V (S), I (S)	2660	3240		10.8	6.8	Trace
5 α -Androstane-3 β ,17 β -diol	IV (S)	2800	3510				
5 α -Androstane-3 β ,17 α -diol	I (S), VI (S), VI (Al)						
5 α -Androstane-3,17-dione	V (S), I (S)	4500	0		18.3	0	Trace
5 α -Androstane-3 β ,17 β -diol	IV (S)	4800					

1, 2 and 3, 0.20, 0.38 and 0.35% of the administered radioactivity were isolated respectively in the prostatic tissue extracts. Extensive metabolism of the tritiated testosterone by the tissue would appear to occur with the formation of radioactive metabolites similar to those described from studies with this tissue *in vitro* (Farnsworth & Brown, 1963; Farnsworth, 1970). Their studies showed that the hypertrophic tissue converted testosterone into a number of products, the major one being 5 α -dihydrotestosterone, thus tending to support the recent concept (Bruchovsky & Wilson, 1968a,b) that this steroid is the active androgen in this target organ. Although it is often implied that the metabolic pattern of steroid formation from incubation studies is of little significance, it would seem that the investigations carried out *in vitro* by Farnsworth (1970) are closely related to the situation as it pertains *in vivo*, and demonstrated by the experiments now described. The amount of 5 α -dihydrotestosterone formed *in vivo* would suggest that it may have a significant role as the principal androgen in the human prostate. It is noteworthy that 5 α -dihydrotestosterone has been shown to stimulate DNA nucleotidyltransferase (EC 2.7.7.7) isolated from human benign hypertrophic prostatic tissue (Harper, Fahmy, Pierrepont & Griffiths, 1970). The formation from testosterone of compounds with a 17 α -hydroxyl group, epitestosterone (17 α -hydroxyandrost-4-en-3-one) and 5 α -androstane-3 α ,17 α -diol, may be important, possibly in relation to studies with the canine prostate (Harper, Pierrepont, Fahmy & Griffiths, 1970b) indicating that 5 α -androstane-3 α ,17 α -diol may have some role in regulating DNA polymerase activity in this tissue. The significance of the presence of these diols must await further study, perhaps along the lines of Baulieu *et al.* (1968a,b), who have described a differential action of the androstanediols and 5 α -dihydrotestosterone on rat prostate in culture. Such studies may indicate enzyme systems that are hyperactive in diseased prostatic tissue, thus causing a steroid imbalance and a hyperplastic condition.

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- Anderson, K. M. & Liao, S. (1968). *Nature, Lond.*, **219**, 277.
- Baulieu, E.-E., Lasnitzki, I. & Robel, P. (1968a). *Biochem. biophys. Res. Commun.* **32**, 575.
- Baulieu, E.-E., Lasnitzki, I. & Robel, P. (1968b). *Nature, Lond.*, **219**, 1155.
- Bruchovsky, N. & Wilson, J. D. (1968a). *J. biol. Chem.* **243**, 2012.
- Bruchovsky, N. & Wilson, J. D. (1968b). *J. biol. Chem.* **243**, 5953.
- Chamberlain, J., Jagarinec, N. & Ofner, P. (1966). *Biochem. J.* **99**, 610.
- Dorfman, R. I. (1956). In *Androgens*, p. 118. Ed. by Dorfman, R. I. & Shipley, R. A. New York: John Wiley and Sons Inc.
- Fahmy, D., Griffiths, K., Turnbull, A. C. & Symington, T. (1968). *J. Endocr.* **41**, 61.
- Farnsworth, W. E. (1970). In *Proc. 3rd Tenovus Workshop: Some Aspects of the Biochemistry and Aetiology of Prostatic Cancer*. Ed. by Griffiths, K. & Pierrepont, C. G. Caerphilly: Alpha Omega Alpha Publishing Co. (in the Press).
- Farnsworth, W. E. & Brown, J. R. (1963). *J. Am. med. Ass.* **183**, 436.
- Griffiths, K., Grant, J. K. & Whyte, W. G. (1963). *J. clin. Endocr.* **23**, 104.
- Harper, M. E., Fahmy, A. R., Pierrepont, C. G. & Griffiths, K. (1970). *Steroids*, **15**, 89.
- Harper, M. E., Pierrepont, C. G., Fahmy, A. R. & Griffiths, K. (1970a). *J. Endocr.* (in the Press).
- Harper, M. E., Pierrepont, C. G., Fahmy, A. R. & Griffiths, K. (1970b). *Biochem. J.* **119**, 785.
- Kowarski, A., Shalf, J. & Migeon, C. J. (1969). *J. biol. Chem.* **244**, 5269.
- Mainwaring, W. I. P. (1970). In *Proc. 3rd Tenovus Workshop: Some Aspects of the Biochemistry and Aetiology of Prostatic Cancer*. Ed. by Griffiths, K. & Pierrepont, C. G. Caerphilly: Alpha Omega Alpha Publishing Co. (in the Press).
- Ofner, P., Smakula, E. E., Wotiz, H. H., Lemon, H. M. & Mescon, H. (1965). *Biochim. biophys. Acta*, **100**, 247.
- Saunders, F. J. (1963). *Natn. Cancer Inst. Monog.* no. 12, p. 139.