Androgen Dynamics *in vitro* in the Normal and Hyperplastic Human Prostate Gland

By ELEONORA P. GIORGI, JOAN C. STEWART, J. K. GRANT AND R. SCOTT

*University Department of Steroid Biochemistry and
Department of Urology, Royal Infirmary, Glasgow C.4, U.K.*

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The dynamics of uptake and metabolism *in vitro* of androgens by normal and hyperplastic human prostate glands was studied by means of a new experimental design proposed by Gurpide & Welch (1969). Prostate slices were perfused with a medium containing [³H]testosterone and [¹⁴C]androstenedione, or 5 α -dihydro-[³H]testosterone and [¹⁴C]testosterone. The entry into the slices, the irreversible metabolism, the conversion between the compounds and the tissue retention or 'uptake' of the steroids were measured at the steady state. A similar portion of the three androgens entered the tissue and was irreversibly metabolized. Conversion of testosterone into 5 α -dihydrotestosterone was much greater than the interconversion of testosterone and androstenedione. The prostate slices retained 5 α -dihydrotestosterone at a concentration three times that in the medium, whereas testosterone and androstenedione were retained to a smaller extent. At a steroid concentration of 0.11 μ mol/l in the medium, the various parameters did not differ significantly in experiments performed with slices from normal and hyperplastic glands. When the steroid concentration in the medium was increased tenfold, however, a difference between normal and hyperplastic glands was evident. The normal glands increased the uptake and metabolism proportionally to the elevation of the steroid concentration in the medium. In the hyperplastic glands the entry and metabolism lagged behind the increase in steroid supply, whereas the tissue uptake became disproportionately high. The possible causes of this finding are discussed.

The concept that the trophic action of androgenic steroids on the prostate gland involves some interplay between gland and hormone has been the subject of much study (Vollmer, 1963). The human prostate can metabolize androgens (for review see Ofner, 1968), and it is known that at least one of the metabolites, 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one), possesses considerable androgenic activity in certain tests (Dorfman & Shipley, 1956; Huggins & Mainzer, 1957). Prostate glands of various species can concentrate androgenic steroids, a phenomenon referred to as steroid 'uptake' (Pearlman & Pearlman, 1961; Harding & Samuels, 1962; Tveter & Attramandal, 1968; Kowarski, Shalf & Migeon, 1969). Proteins that bind testosterone (17 β -hydroxyandrost-4-en-3-one) and 5 α -dihydrotestosterone have been demonstrated in the prostate glands of rodents (Anderson & Liao, 1968; Bruchowsky & Wilson, 1968; Mainwaring, 1969*a,b*; Unhjem, Tveter & Aakvaag, 1969) but not definitely in man.

Little attention, however, seems to have been paid to the need to study androgen uptake and metabolism in this tissue in a dynamic fashion. The experimental design proposed by Gurpide & Welch

(1969) may be used for this purpose. These workers perfused tissue slices with a continuous flow of medium containing two metabolically related compounds each labelled with a different isotope. Measurements of isotopic concentrations and ratios at the steady state permitted the calculation of various parameters of steroid behaviour in the tissue. The advantages of such continuous-flow perfusion, or 'superfusion', over 'batch' incubations have been discussed by various authors (Orti, Baker, Lanman & Branch, 1965; Tait, Tait, Okamoto & Flood, 1967; Matthews & Saffran, 1967).

In the investigation now reported we have used the experimental design of Gurpide & Welch (1969) to study the dynamics of uptake and metabolism of androgens in human, normal and hyperplastic prostatic tissue *in vitro*.

MATERIALS AND METHODS

Tissue studied. The details of the patients and prostatic tissue are summarized in Table 1. In the case of four patients, the prostate gland was removed during a total cystectomy for carcinoma of the bladder, and was

Table 1. *Details of patients*

Cy indicates that the operation was cystectomy. In these cases the whole prostate gland was received and the weight is that of the whole gland. RPP indicates retropubic prostatectomy. TUR indicates transurethral resection.

Patient	Age (years)	Expt. no.	Wt. of prostatic tissue removed at operation (g)	Histology of prostate
J.C.	42	Cy1	15	Normal (carcinoma of the bladder)
D.F.	60	Cy2	14	Normal (carcinoma of the bladder)
J.K.	62	Cy3	14	Normal (carcinoma of the bladder)
A.S.	59	Cy4	16	Mild adenomatous and fibromuscular hyperplasia (carcinoma of the bladder)
T.B.	71	RPP1	24	Hyperplasia of the glandular and muscular tissue
Ro.K.	75	RPP1a	75	Hyperplasia of the glandular and muscular tissue
C.B.	73	RPP2	25	Simple nodular hyperplasia of the glandular and muscular tissue; foci of inflammation
G.K.	70	RPP2a	20	Benign hyperplasia of the glandular and muscular tissue
A.C.	69	RPP3	25	Simple nodular hyperplasia of the glandular and muscular tissue; foci of inflammation
Jo.H.	60	RPP4	40	Benign hyperplasia of the glandular and muscular tissue
Th.K.	81	RPP5	64	Hyperplasia of the glandular and muscular tissue; foci of acute and chronic inflammation
L.A.	67	RPP6	77	Simple hyperplasia of the glandular and muscular tissue; slight inflammatory activity
Ja.P.	68	RPP7	33	Nodular hyperplasia of the glandular and muscular tissue
J.H.	84	RPP8	35	Benign hyperplasia of the glandular and muscular tissue
Th.C.	73	RPP9a	45	Benign nodular hyperplasia of the glandular and muscular tissue; slight inflammatory activity
D.McD.	66	RPP10	25	Benign nodular hyperplasia of the glandular and muscular tissue
D.C.	61	RPP11	65	Simple hyperplasia of the glandular and muscular tissue
D.McA.	66	RPP12a	40	Hyperplasia of the glandular and muscular tissue; areas of adenocarcinoma
W.D.	70	RPP13	25	Nodular hyperplasia of the glandular and muscular tissue with areas of squamous metaplasia; one area infiltrated by poorly differentiated squamous carcinoma
D.McL.	66	TUR1	—	Fibromuscular tissue containing ducts lined by transitional epithelium showing areas of squamous metaplasia, with much acute and chronic inflammation
Ja.C.	58	TUR2	—	Extensive permeation of the prostate stroma by poorly differentiated small acinar carcinoma
A.McN.	54	TUR3	—	Fibromuscular tissue containing a few dilated glands

regarded as normal on the basis of weight and histological appearance. Cy before the experiment number in these cases indicates that the operation was cystectomy. All the other patients were operated upon either because of acute urinary retention or because of symptoms related to chronic enlargement of the prostate gland. When the operation was retropubic prostatectomy, this is indicated by RPP. TUR indicates transurethral resection of the prostate.

Radioactive steroids. [4-¹⁴C]Testosterone (specific radioactivity 58.2 mCi/mmol), [6,7-³H]testosterone (specific radioactivity 41.8 Ci/mmol), [4-¹⁴C]androstenedione

([4-¹⁴C]androst-4-ene-3,17-dione) (specific radioactivity 56.6 mCi/mmol) and [7 α -³H]androstenedione (specific radioactivity 3.23 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. 5 α -Dihydro[1,2-³H]testosterone (specific radioactivity 66.1 Ci/mmol) was prepared from 17 β -acetoxy-5 α -androst-1-en-3-one by The Radiochemical Centre. These steroids were purified by paper chromatography in hexane-methanol-water (5:4:1, by vol.). They were located by means of a radiochromatogram scanner (model 2700; Packard Instrument Co. Ltd., Wembley, Middlesex, U.K.) and eluted with methanol. A sample of the eluate was

Fig. 1. Perfusion apparatus. The various sections of the apparatus, which are all glass, have ground joints and are held in position by means of metal clips. The length of the vertical branches of the apparatus is 26 cm; the inside diameter is 0.3 cm. The perfusion chamber in which the tissue is inserted is formed by the space between the two sintered-glass filters in *A* and *B* (indicated by the broken line); its volume is 2 ml. In *C'*, which is used connected to *A*, the tissue is put above the sintered-glass filter, so that samples of tissue can be taken without interrupting the perfusion.

crystallized with added carrier steroid. Constant specific radioactivity of both crystals and mother liquors in successive crystallizations was taken as proof of radiochemical homogeneity. Carrier steroids were purchased from Steraloids Ltd., Croydon, Surrey, U.K. Their purity was checked by chromatography on silica-gel t.l.c. plates developed with chloroform–acetone (5:3, v/v) and ethyl acetate–hexane (3:2, v/v). 5 α -Androstanediol (5 α -androstane-3 α ,17 β -diol) was also run in benzene–ether (9:1, v/v).

Perfusion technique. Tissue was carried to the laboratory in crushed ice, and was immediately sliced by hand with a Stadie–Riggs microtome. Two samples (about 0.5 g each) were prepared for use in paired perfusions at two different concentrations of steroids in the medium. Only 0.25 g of slices for each perfusion could be obtained from curettings taken at the transurethral operation. In each experiment a small sample of sliced tissue was weighed and used for the determination of dry weight. From the area and the weight of single slices taken at random, the mean thickness was calculated to be 0.54 mm. After being weighed, the slices were rinsed with 0.9% NaCl and inserted into the perfusion chambers (Fig. 1 ABC), which were then immersed in a thermostat at 38°C. Each chamber was connected to a 100 ml syringe fitted to a pump (Multisyringe Attachment, F135; C. F. Palmer Ltd., London S.W.2, U.K.) which delivered the medium at a constant rate of 20 ml/h. Less than 40 min intervened between removal of the tissue and the start of perfusion.

Perfusions lasted 90 or 120 min, during which time samples of perfusates were collected in tubes standing in crushed ice for three or four periods of 30 min.

Observations made on the last collection were used in the calculations of the parameters. At the end of the perfusion the slices were removed from the apparatus, rinsed with ice-cold Krebs–Ringer bicarbonate solution (Cohen, 1957) and suspended in 3 ml of acetone. Medium from the syringe was collected for analysis. All the samples were kept at –5°C until processed.

The perfusion medium was prepared by adding the steroids, in 1 ml of ethanol, to 70 ml of the Krebs–Ringer bicarbonate solution containing glucose (5.5 mmol/l) and equilibrated with O₂ + CO₂ (95:5). The concentration of ¹⁴C was 6000–10000 c.p.m./ml and that of ³H was 11000–20000 c.p.m./ml in all the experiments. Suitable amounts of unlabelled steroids were added to achieve steroid concentrations of 0.11 and 1.1 μ mol/l used in the experiments. In some experiments the oxygen concentration of the medium, before and after perfusion, was tested by using a pO₂ electrode (Radiometer, Copenhagen, Denmark). The mean values were 515 and 345 mmHg respectively.

Isolation and purification of radioactive steroids in the samples. A measured volume of perfusing medium, and of each perfusate collected, was extracted with 10 ml of ethyl acetate containing 200 μ g of each steroid to be identified. Extracted steroids were separated and purified on two t.l.c. chromatograms with chloroform–acetone (7:1, v/v) and ethyl acetate–hexane (3:2, v/v). The eluted steroids were dissolved in ethanol, and one-tenth of the solution was transferred to counting vials and evaporated to dryness. ¹⁴C and ³H radioactivities present were counted, after the addition of toluene-based phosphor, by using a two-channel Tri-Carb liquid-scintillation spectrometer (Packard Instrument Co.). Counts (2000) were accumulated in each channel to give a S.E.M. < 5%.

The amount of testosterone and androstenedione recovered was measured by u.v. spectrophotometry on one-tenth of the eluted steroid solution. Recovery of 5 α -dihydrotestosterone was measured on one-tenth of the steroid solution by g.l.c. with a 1% OV-1 packed column in a model 402 high-efficiency chromatograph (F & M Scientific Division of Hewlett–Packard, Slough, Bucks., U.K.). Pregnenolone acetate was used as internal standard.

After these measurements, 3–5 mg of carrier steroid was added to the remainder of the steroid solution and the compounds were recrystallized from acetone–hexane until the ³H/¹⁴C ratio was constant. In many cases the crystalline steroids were acetylated at room temperature overnight with acetic anhydride and pyridine, and the product was chromatographed by t.l.c. on silica gel with benzene–ethyl acetate (9:1, v/v) and recrystallized to constant isotope ratio.

The tissue slices were removed from the acetone, minced, suspended in 0.9% NaCl and homogenized in a Potter–Elvehjem all-glass homogenizer. The homogenate and the acetone were extracted together and processed as described above for the perfusates. However, because of the presence of substantial amounts of metabolites in the tissue, it was thought necessary to determine the concentration of the steroids in tissue from the constant specific radioactivity of the crystals.

Calculations. The symbols used in the equations below

Scheme 1. Models of the perfusion experiments. The tissue is represented by the square box; the perfusing medium by the horizontal lines at the top and bottom of the models. The compounds entering the tissue (α) are partly retained in the tissue (c_1/c_0), partly converted into other compounds (im) and partly released back into the medium (β). Since [^{14}C]androstenedione is converted in the tissue into [^{14}C]testosterone, testosterone released from the tissue will bear both the 3H and the ^{14}C label. Conversely, androstenedione released from the tissue will have both labels (model 1). In model 2 there is conversion of [^{14}C]testosterone into 5 α -dihydro[^{14}C]testosterone, but not vice versa. Thus 5 α -dihydrotestosterone released from the tissue will bear the label of testosterone, but release of testosterone cannot be determined. For the meanings of the other symbols in the scheme, see the text.

and in Scheme 1 have the following meanings. The superscript indicates the isotope; the subscripts T, A and 2HT indicate testosterone, androstenedione and 5 α -dihydrotestosterone respectively; the additional subscript p indicates that the compound is measured in the perfusate, and t that it is measured in the tissue. Eqns. (1)–(9) are written for [3H]testosterone in experiments with this labelled steroid and [^{14}C]androstenedione (Scheme 1, model 1). For their derivation see Gurpide & Welch (1969).

The radioactive steroid concentration (c.p.m./ml) in the perfusing medium or the perfusate

$$c_T^{3H} \text{ or } (c_T^{3H})_p = \frac{{}^3H \text{ (c.p.m.) in vial} \cdot 10}{\text{volume extracted} \cdot \text{recovery (\%)}} \quad (1)$$

The radioactive steroid concentration in the tissue (c.p.m./ml, assuming a tissue specific gravity of 1), is calculated from the specific radioactivity of the crystals (c.p.m./mg) as follows:

$$(c_T^{3H})_t = \frac{{}^3H \text{ (c.p.m.)/mg} \cdot \text{carrier (mg)}}{\text{wet wt. of tissue (g)} \cdot \text{recovery (\%)}} \cdot (1 + 2/10) \quad (2)$$

where 2/10 is the portion taken for determination of recovery and counting.

The following parameters were calculated.

The fraction of perfused steroid entering the tissue:

$$\alpha_T = 1 - \frac{(c_T^{3H})_p - [(^3H/^{14}C)_T]_t \cdot (c_T^{14C})_p}{c_T^{3H}} \quad (3)$$

The fraction of perfused steroid leaving the tissue:

$$\beta_T = \frac{[(^3H/^{14}C)_T]_t \cdot (c_T^{14C})_p}{c_T^{3H}} \quad (4)$$

The uptake of steroid by the tissue:

$$(c_1/c_0)_T = \frac{(c_T^{3H})_t}{c_T^{3H}} \quad (5)$$

where c_1 is the concentration inside the tissue and c_0 the concentration outside.

The irreversible metabolism:

$$im_T = \alpha_T - \beta_T = \frac{c_T^{3H} - (c_T^{3H})_p}{c_T^{3H}} \quad (6)$$

and for

$$\beta_T = 0 \quad \text{and} \quad im_T = \alpha_T \quad (7)$$

the fraction of testosterone entering the tissue that is converted into androstenedione:

$$\alpha_{TA} = \frac{\alpha_A}{\alpha_T} \cdot \frac{[{}^3\text{H}/{}^{14}\text{C}]_A}{c_T/c_A} \quad (8)$$

according to Gurpide, MacDonald, VandeWiele & Lieberman (1963).

The fraction of testosterone released into the medium as androstenedione:

$$\gamma_{TA} = \frac{(c_A^{{}^3\text{H}})_P}{c_T^{{}^3\text{H}}} \quad (9)$$

By substituting appropriate symbols, eqns. (3)–(9) apply similarly to the calculations of the parameters for [${}^{14}\text{C}$]androstenedione, 5α -dihydro[${}^3\text{H}$]testosterone and [${}^{14}\text{C}$]testosterone in models 1 and 2 (Fig. 2), provided the isotope ratio is expressed as (${}^3\text{H}/{}^{14}\text{C}$) in the equations for the ${}^{14}\text{C}$ -labelled steroids. In model 2 (Scheme 1) α_T is calculated from eqn. (7), which would introduce an error if testosterone were released from the tissue. Whether testosterone is released from the tissue under the experimental conditions can, however, be verified from the experiments according to model 1 (Scheme 1). It will also be noted that in eqns. (3) and (4) the isotope ratio in the tissue is used to calculate the concentration of [${}^3\text{H}$]testosterone released from the tissue. This requires the assumption that the isotope ratio is similar in tissue and in the perfusate, which must be validated for each steroid in models 1 and 2.

The overall percentage error of the measurements in eqns. (1) and (2) and in the determination of the isotope ratio was calculated from the standard deviation of results of 40 determinations in duplicate from their means (Snedecor, 1952). This error was 3.26% for the perfusate and perfusing medium (1), 10.85% for the tissue (2), and 5.83% for the isotope ratio (${}^3\text{H}/{}^{14}\text{C}$) in the range from 0.01 to 100.

On the basis of the percentage error of all the measurements involved, the overall theoretical error of each parameter was calculated according to the laws for the combination of additive and multiplicative errors. Significant differences between results were considered to be those in which the probability of agreement was <0.0027 (Dahlberg, 1948).

All the calculations were performed on a desk computer (Programma 101; British Olivetti, London W.1, U.K.), by means of programs especially prepared by us.

RESULTS

Verification of the models. A condition necessary for the application of the analysis, reported above, to the models in Scheme 1 is that the radioactive steroid concentration and the steroid isotope ratio in tissue and perfusate must be measured at the steady state. This may be defined as the constancy of concentration of the radioactive steroids in the tissue and consequently in the perfusates. To determine at what time during perfusion steady

state was attained, the following preliminary experiments were performed. Slices from hyperplastic prostate glands were perfused in the apparatus shown in Fig. 1 (AC') with Krebs–Ringer bicarbonate solution containing glucose and [${}^3\text{H}$]testosterone and [${}^{14}\text{C}$]androstenedione at concentrations of both steroids of 0.11 and 1.1 $\mu\text{mol/l}$ (Expts. RPP 1 and RPP 1a). In two further experiments, 5α -dihydro[${}^3\text{H}$]testosterone and [${}^{14}\text{C}$]testosterone were perfused at the two concentrations (Expts. RPP 2 and RPP 2a). Portions of the tissue were taken from the chamber at intervals of 15 or 30 min. They were washed with ice-cold 0.9% sodium chloride solution, blotted on filter paper, weighed and put into acetone. Steroid concentrations in the tissues were measured as described in the Materials and Methods section. In these experiments, both steroid concentration and isotope ratio approached constancy between 60 and 90 min. Thus the parameters defined by eqns. (3)–(9) were calculated from

Fig. 2. Statistical analysis of the difference between steroid concentrations (c.p.m./ml) in consecutive perfusates of the experiments reported in Tables 5–8. The findings of the analysis are expressed as the percentage of the total number of experiments in which the P value (probability of agreement) lay within the ranges >0.0455 , >0.0124 , >0.0027 , <0.0027 . The experiments were carried out at two sets of time-intervals; in those designated (I) perfusion lasted 90 min and perfusates were compared at 30 and 90 min (16 experiments); in those designated (II) perfusions lasted 120 min and perfusates were compared at 60 and 120 min (14 experiments). The experiments were carried out with [${}^3\text{H}$]testosterone, [${}^{14}\text{C}$]androstenedione and [${}^{14}\text{C}$]testosterone at concentrations of 0.11 and 1.1 $\mu\text{mol/l}$ of medium.

Table 2. Comparison of the isotope ratio of 5 α -dihydrotestosterone and 5 α -androstenediol in tissue and perfusate

($^3\text{H}/^{14}\text{C}$)_t is isotope ratio in tissue; ($^3\text{H}/^{14}\text{C}$)_p is isotope ratio in perfusate. The values of $\alpha_{2\text{HT}}$ and $\beta_{2\text{HT}}$ shown were obtained by using each isotope ratio in eqns. (3) and (4), while keeping the other experimental values in the equations unchanged.

Expt. no. RPP 3	Time from start of perfusion (min)	5 α -Dihydrotestosterone		5 α -Androstenediol	
		($^3\text{H}/^{14}\text{C}$) _t	($^3\text{H}/^{14}\text{C}$) _p	($^3\text{H}/^{14}\text{C}$) _t	($^3\text{H}/^{14}\text{C}$) _p
Perfusion with 5 α -dihydro[^3H]testosterone and [^{14}C]testosterone (at 0.11 $\mu\text{mol/l}$)	90	3.0563	3.8031	2.9982	3.8682
			($\alpha_{2\text{HT}} = 0.599$) ($\beta_{2\text{HT}} = 0.029$)		($\alpha_{2\text{HT}} = 0.591$) ($\beta_{2\text{HT}} = 0.037$)
Perfusion with 5 α -dihydro[^3H]testosterone and [^{14}C]testosterone (at 0.11 $\mu\text{mol/l}$)	120	2.9071	4.1738*	7.1210	3.2570*
Perfusion with 5 α -dihydro[^3H]testosterone and [^{14}C]testosterone (at 1.1 $\mu\text{mol/l}$)	90	2.0594	($\alpha_{2\text{HT}} = 0.203$) ($\beta_{2\text{HT}} = 0.063$)	2.0173	3.5726*
					($\alpha_{2\text{HT}} = 0.197$) ($\beta_{2\text{HT}} = 0.108$)†
	90	2.8269	($\alpha_{2\text{HT}} = 0.233$) ($\beta_{2\text{HT}} = 0.044$)	2.8034	2.7162
					($\alpha_{2\text{HT}} = 0.233$) ($\beta_{2\text{HT}} = 0.042$)

* Significant difference between the isotope ratio in tissue and perfusate ($P < 0.0027$).

† Significant difference between $\beta_{2\text{HT}}$ in fourth and ninth column ($P < 0.0027$).

data from perfusates collected after not less than 60min from the start of perfusion. The values of these parameters are reported in Tables 5–8. Achievement of steady state in all these experiments was checked by comparing the steroid concentration (c.p.m./ml) in the perfusate used in the calculations with the steroid concentration in the perfusate collected during the preceding 30min interval. These concentrations are not reported here for the sake of brevity. The results of this comparison are given in Fig. 2. The *P* values (probability of agreement) were greater than 0.0027 in 90% of cases (left-hand side of Fig. 2). That is to say, there was no significant difference between the steroid concentration in the two consecutive perfusates, irrespective of whether perfusion lasted 90 or 120min. However, steady state did not appear to exist at the time of measurements in Expt. Cy1 for either testosterone or androstenedione at 1.1 μ mol/l. Nor was there steady state for androstenedione at either 0.11 or 1.1 μ mol/l in Expt. TUR1.

It has also been pointed out in the calculations that the assumption that the isotope ratio of the compounds is the same in tissue and in perfusate must be validated if eqns. (3) and (4) are to be used for the calculation of the rate of entry and release of steroids from the tissue. This can be tested in two ways. One is to elute the steroids retained in the tissue with unlabelled steroids, by changing the buffer at one point of the perfusion ('washing-out' experiment). This enables a direct determination of the actual isotope ratio in the compound leaving the tissue to be made. The second way is to determine the isotope ratio in the perfusate of a metabolite that is derived exclusively via one of the two perfused steroids. Such a compound is 5 α -androstanediol, since 5 α -dihydrotestosterone is an obligatory intermediary between testosterone and this diol. In Expt. RPP3 perfusion with [14 C]testosterone and 5 α -dihydro[3 H]testosterone was stopped after 60min, the tissue was removed from the chamber, rinsed with buffer and one portion was set aside, and the remainder was transferred to another chamber. Perfusion with unlabelled steroids, at the same concentration as with labelled steroids, was continued for a further hour. Other portions of the tissue were treated similarly at 90

and 120min. Steroids were isolated from both tissue and perfusates, and crystallized to constant isotope ratio. Results are given in Table 2.

Table 2 shows that in Expt. RPP3 the $^3\text{H}/^{14}\text{C}$ ratios of 5 α -dihydrotestosterone and 5 α -androstanediol in tissue and perfusate were very similar at 90min but significantly different at 120min. In Expt. Cy2 with 1.1 μ mol of steroids/l of medium, the $^3\text{H}/^{14}\text{C}$ ratios of 5 α -dihydrotestosterone in the tissue and of 5 α -androstanediol in the perfusate were similar. The values of $\alpha_{2\text{HT}}$ and $\beta_{2\text{HT}}$ calculated from eqns. (3) and (4) by using the isotope ratio in tissue and perfusate are also reported in Table 2. Only in one case was there a significant difference in the value of $\beta_{2\text{HT}}$. These results therefore validate eqns. (3) and (4) for 5 α -dihydrotestosterone. An important corollary to the 'washing-out' experiment was that binding to human prostate tissue of testosterone, 5 α -dihydrotestosterone and 5 α -androstanediol appeared to be reversible, as the radioactive steroids could be displaced by the unlabelled compounds.

'Washing-out' experiments with testosterone and androstenedione proved to be unnecessary, since, as seen from Tables 5 and 7, these two androgens are not generally released from the tissue (i.e. $\beta = 0$). This observation also justifies the use of eqn. (7) for the calculation of α_T in model 2 (Scheme 1).

An experiment was performed to confirm that there were in the perfusates no soluble enzymes that might metabolize the steroids. For this 0.5g slices of tissue were perfused with Krebs–Ringer solution containing no steroids. The perfusates were collected at 30min intervals, transferred to flasks containing 53000 c.p.m. of [14 C]testosterone and 5000 c.p.m. of 5 α -dihydro[3 H]testosterone and incubated at 37°C with shaking in an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5). The incubation was stopped with 20ml of acetone after 30min. 5 α -Dihydrotestosterone was isolated and crystallized with carrier steroid until the isotope ratio was constant. Another similar portion of slices from the same prostate gland was incubated under the same conditions with 53000 c.p.m. of [14 C]testosterone. Labelled 5 α -dihydrotestosterone was isolated and crystallized repeatedly to constant specific radioactivity after addition of carrier. The results of the

Table 3. Expt. RPP4: conversion of [14 C]testosterone on incubation with prostatic tissue and perfusates

Perfusates Time of collection (min)	5 α -Dihydrotestosterone $^3\text{H}/^{14}\text{C}$ ratio	Conversion of testosterone into 5 α -dihydrotestosterone (%)
0–30	100.27	0.01
31–60	135.52	0.007
61–90	99.61	0.01
Tissue	—	18.65

Table 4. *Results of perfusion experiments in duplicate*

Expt. no.	Steroid	α	β	c_1/c_0	im	$\alpha\tau_{2HT}$ or $\alpha\tau_A$
RPP 6	5 α -Dihydrotestosterone	0.250	0.083	3.894	0.167	
Perfusion with		0.279	0.083	2.364	0.196	
5 α -dihydro[³ H]testosterone						
and [¹⁴ C]testosterone	Testosterone	0.250	0	0.952	0.250	0.428
(at 0.11 μ mol/l)		0.278	0	0.538*	0.278	0.495
RPP 7	Testosterone	0.200	0	0.298	0.200	0.023
Perfusion with		0.140*	0	0.239	0.140*	0.029
[³ H]testosterone and	Androstenedione	0.099	0	0.430	0.099	0
[¹⁴ C]androstenedione		0.096	0	0.347	0.096	0
(at 0.11 μ mol/l)						

* $P < 0.0027$

experiment are reported in Table 3. It was evident that, although the tissue converted 18.65% of the testosterone present into 5 α -dihydrotestosterone, conversion by perfusates alone was negligible.

Finally, the reproducibility of results with two specimens of tissue slices from the same prostate was tested by performing four experiments in duplicate. The results of two such experiments are reported in Table 4. A significant difference between duplicate values was observed only for α_T and im_T in Expt. RPP 7 and for $(c_1/c_0)_T$ in Expt. RPP 6, and in one of the two other experiments not reported in Table 4.

Experimental results. The results of perfusions of prostate slices with testosterone and androstenedione are reported in Table 5, and of perfusions with 5 α -dihydrotestosterone and testosterone in Table 6. The concentration of the steroids in the medium was 0.11 μ mol/l.

Considering first the parameters measured for all three steroids, it is evident that the uptake (c_1/c_0) varied greatly from one gland to the other. The fraction entering (α) and that being metabolized (im) varied less and were of a similar order of magnitude for all three steroids studied. However, in most experiments a significantly different fraction of each of the two perfused steroids entered the tissue, indicating that the permeability of the tissue is not necessarily equal for all steroids.

With regard to steroid uptake, it appeared that prostatic tissue was able to concentrate 5 α -dihydrotestosterone up to three times the concentration in the perfusing medium. Neither testosterone nor androstenedione was concentrated by the tissue ($c_1/c_0 < 1$). In fact, prostate slices retained 5 α -dihydrotestosterone formed from perfused testosterone more than testosterone itself (last column of Table 5). It is also noteworthy that in the experiments with curettings of prostate gland (TUR 1, 2 and 3) the tissue content of the three androgens was very high.

Release of testosterone and androstenedione into

the perfusate was observed only in Expt. TUR 1. Some testosterone was, however, released back into the medium as 5 α -dihydrotestosterone (last column of Table 6). 5 α -Dihydrotestosterone, when perfused, was released from the tissue (β_{2HT}) as a fraction of approx. 0.10 in six out of eight experiments. Since release back into the medium indicates that a portion of the steroid in the tissue is diffusible, these observations demonstrated that 5 α -dihydrotestosterone bound intracellularly was in equilibrium with some unbound compound, and that testosterone and androstenedione not so released were, on the contrary, prevalently in a bound form.

The conversion of testosterone into 5 α -dihydrotestosterone ($\alpha\tau_{2HT}$) was much greater than the interconversion of testosterone and androstenedione. The extent of the latter varied greatly between different glands.

The values of the various parameters for the four glands, which were regarded as normal, did not differ significantly from the values obtained with the hyperplastic glands.

The results of the experiments with perfusions at a steroid concentration of 1.1 μ mol/l are reported in Tables 7 and 8. As in the experiments reported in Tables 5 and 6, the values of the parameters measured at the steady state varied greatly from one gland to the other. The uptake of 5 α -dihydrotestosterone was, in general, higher than that of the other two androgens, and the conversion of testosterone into 5 α -dihydrotestosterone was much greater than the interconversion of testosterone and androstenedione. However, at the higher steroid concentration in each experiment the values of some of the parameters varied from those observed with the same gland at the lower concentration. The values showing these variations are printed in *italics* in Tables 7 and 8. The variations were as follows.

The fraction of the steroid entering the tissue (α) in the normal glands was not significantly changed, with the exception of Expt. Cy 3. By contrast, in

Table 5. *Perfusion of prostate slices with [³H]testosterone and [¹⁴C]androstenedione at 0.11 μmol/l*

Expt. no.	Flow rate of testosterone and androstenedione (ng/min per mg)	[³ H]Testosterone					[¹⁴ C]Androstenedione				
		α _T	β _T	(c ₁ /c ₀) _T	im _T	α _{PTA}	α _A	β _A	(c ₁ /c ₀) _A	im _A	$\frac{(c_1)_{2HT}}{(c_0)_T}$
Cy1	0.11	0.103	0	0.722	0.103	0.164	0.126	0	0.267	0.126	1.391
Cy3	0.11	0.150	0	0.204	0.150	0.049	0.108	0	0.206	0.108	0.035
RPP5	0.15	0.120*	0	0.501*	0.120*	0.058*	0.119*	0	0.390*	0.119*	0.930*
RPP7	0.13	0.170*	0	0.269*	0.170*	0.026*	0.098*	0	0.389*	0.098*	0
RPP10†	0.10	0.173	0	0.125	0.173	0.092	0.166	0	0.062	0.166	0.019
RPP11	0.13	0.149	0	0.221	0.149	0.093	0.182	0	0.210	0.182	0.090
RPP12a†	0.13	0.257	0	0.583	0.219	0.048	0.219	0	0.553	0.219	0.111
TUR1	0.29	0.716	0.636	1.334	0.080	0.032	0.266	0.063	0.781	0.203	0.052

* Mean of duplicate determinations.
† In this experiment [¹⁴C]testosterone and [³H]androstenedione were used.

Table 6. *Perfusions of prostate slices with 5α-dihydro[³H]testosterone and [¹⁴C]testosterone at 0.11 μmol/l*

Expt. no.	Flow rate of 5 α -dihydrotestosterone and testosterone (ng/min per mg)	5 α -Dihydro[³ H]testosterone					[¹⁴ C]Testosterone				
		α_{2HT}	β_{2HT}	$(c_1/c_0)_{2HT}$	im_{2HT}	α_T	$(c_1/c_0)_T$	im_T	α_{PT2HT}	γ_{T2HT}	
Cy2	0.12	0.203	0.063	2.188	0.140	0.132	0.137	0.132	0.970	0.069	
Cy4	0.14	0.230	0.088	3.837	0.141	0.267	0.700	0.267	0.442	0.045	
RPP6	0.17	0.264*	0.083*	3.129*	0.182*	0.264*	0.745*	0.264*	0.496*	0.041*	
RPP8	0.14	0.306	0.068	2.650	0.238	0.163	0.069	0.163	0.941	0.064	
RPP9a	0.13	0.286	0.115	3.768	0.170	0.277	0.073	0.277	1.025	0.115	
RPP13	0.17	0.209	0.043	1.436	0.166	0.137	0.310	0.137	0.755	0.051	
TUR2	0.26	0.129*	0	8.293*	0.129*	0.066*	3.966*	0.066*	0.161*	0	
TUR3	0.14	0.215	0	7.511	0.215	0.149	3.916	0.149	0.317	0	

* Mean of duplicate determinations.

Table 7. *Perfusions of prostate slices with [³H]testosterone and [¹⁴C]androstenedione at 1.1 μ mol/l*Values in *italics* indicate significant differences with paired perfusion at steroid concentration 0.11 μ mol/l ($P < 0.0027$).

Expt. no.	Flow rate of testosterone and androstenedione (ng/min per mg)	[³ H]Testosterone					[¹⁴ C]Androstenedione				
		α_T	β_T	(c_1/c_0) _T	im_T	α_{PTA}	α_A	β_A	(c_1/c_0) _A	im_A	$\frac{(c_1)_{2HT}}{(c_0)_T}$
Cy1	1.70	0.220	0	1.244	0.220	1.177	0.295	0	1.006	0.295	1.032
Cy3	1.16	0.114	0	0.982	0.114	0.036	0.075	0	0.572	0.075	0.998
RPP5	1.20	0.048	0	1.398	0.048	0.422	0.066	0	1.161	0.066	0.469
RPP10*	1.12	0.096	0	0.290	0.096	0.054	0.101	0	0.334	0.101	2.572
RPP11	1.38	0.136	0	0.486	0.136	0.023	0.130	0	0.467	0.130	1.353
RPP12a*	1.27	0.123	0	0.601	0.123	0.030	0.105	0	0.478	0.105	0.753
TUR1	3.06	0.498	0.321	2.736	0.177	0.012	0.281	0.034	2.018	0.247	0.255

* In this experiment [¹⁴C]testosterone and [³H]androstenedione were used.Table 8. *Perfusions of prostate slices with 5 α -dihydro[³H]testosterone and [¹⁴C]testosterone at 1.1 μ mol/l*Values in *italics* indicate significant differences with paired perfusion at steroid concentration 0.11 μ mol/l ($P < 0.0027$).

Expt. no.	Flow rate of 5 α -dihydrotestosterone and testosterone (ng/min per mg)	5 α -Dihydro[³ H]testosterone					[¹⁴ C]Testosterone				
		α_{2HT}	β_{2HT}	$(c_1/c_0)_{2HT}$	im_{2HT}	α_T	$(c_1/c_0)_T$	im_T	α_{PT}	γ_{T2HT}	
Cy2	1.24	0.233	0.044	2.319	0.189	0.151	0.504	0.151	0.714	0.035	
Cy4	1.22	0.232	0.082	3.498	0.150	0.196	1.768	0.196	0.421	0.029	
RPP8	1.45	0.099	0.040	3.829	0.059	0.103	0.689	0.103	0.630	0.025	
RPP9a	1.25	0.135	0.039	2.899	0.096	0.096	0.424	0.096	1.055	0.030	
RPP13	1.63	0.110	0.032	1.343	0.078	0.104	0.361	0.104	0.629	0.034	
TUR3	1.48	0.195	0.053	7.862	0.141	0.147	3.578	0.147	0.254	0.014

Fig. 3. Rates of entry of testosterone ($\square$), androstenedione ($\boxtimes$) and 5α -dihydrotestosterone ($\boxplus$) into slices of prostatic tissue. The first column for each steroid indicates the rate of entry ($\alpha \times \text{ng/min per mg}$) at the lower steroid flow; the second column indicates the rate of entry at the higher steroid flow. These rates were calculated from the values of α and the flow rates of steroids shown in Tables 5–8. The broken lines in the second column of each steroid indicate the rate of entry that would be proportional to the increase of the steroid flow. This rate was calculated from the values of α shown in Tables 5 and 6 multiplied by the higher flow rates shown in Tables 7 and 8. The bars indicate the limits of the theoretical error of the measurements. The values of these limits are given in parentheses for Expt. TUR1.

the hyperplastic glands the value of α was generally diminished. There was a significant increase in the release of 5α -dihydrotestosterone in Expt. TUR3; in two hyperplastic glands there was a relative increase in the value of $\beta_{2\text{HT}}$ with respect to $\alpha_{2\text{HT}}$, so that proportionally more of the steroid entering the tissue was released unchanged. On the other hand, in the normal glands $\beta_{2\text{HT}}$ decreased relatively to $\alpha_{2\text{HT}}$, and less of the steroid entering the tissue was released unchanged. As a consequence the fraction metabolized ($im_{2\text{HT}}$) was raised in the normal gland and diminished in the hyperplastic glands. Also, the fraction of testosterone and androstenedione metabolized was generally smaller in the hyperplastic glands, but was not significantly changed in the normal glands. In the hyperplastic glands conversion of testosterone into androstenedione (αp_{TA}) tended to be diminished and the reverse reaction (αp_{AT}) tended to increase. The conversion of testosterone into 5α -dihydrotestosterone (αp_{T2HT}) remained the same, but, because of the lowered entry of testosterone, less 5α -dihydrotestosterone was formed [see $(c_1)_{2\text{HT}}/(c_0)_\text{T}$ in the last column of Table 7]. The uptake (c_1/c_0) of testosterone and

androstenedione by the normal glands was raised, but not as much as in some hyperplastic glands. The uptake of 5α -dihydrotestosterone did not change in the normal glands nor in the hyperplastic glands, with the exception of RPP8.

The variations in α , im and uptake at the higher steroid concentration are further illustrated in Figs. 3, 4 and 5. In Figs. 3 and 4 the results from Tables 5–8 have been expressed as rates of entry and metabolism. Thus, for example, the rate of entry equals α times the flow rate of steroid and is expressed in ng/min per mg dry wt. of tissue. In Fig. 5 c_1 is the concentration of radioactive steroids inside the tissue, expressed in ng/ml or ng/g wet wt. of tissue. Fig. 3 shows that the rate of entry of the three androgens into the normal glands increased proportionally to the steroid concentration in the medium. Normal glands also raised metabolic activity and tissue steroid content proportionally to the flow rate of the steroids (Fig. 4). Hyperplastic glands, on the contrary, either could not increase the rate of metabolism according to the availability of steroids, or retained a disproportionate concentration of steroids in the tissue. Thus in Expts.

Fig. 4. Rates of metabolism of testosterone (□), androstenedione (▨) and 5α-dihydrotestosterone (▩) by slices of prostatic tissue. The first column of each steroid indicates the rate of metabolism at the lower steroid flow (rate of metabolism = $im \times ng/min$ per mg in Tables 5 and 6); the second column indicates the rate of metabolism at the higher steroid flow in the medium (rate of metabolism = $im \times ng/min$ per mg in Tables 7 and 8). The broken lines in the second column of each steroid indicate the rate of metabolism that would be proportional to the increase of the steroid flow. This rate was calculated from the values of im shown in Tables 5 and 6 multiplied by the higher flow rates shown in Tables 7 and 8. The bars indicate the limits of the theoretical error of the measurements.

RPP5 and RPP9a the tenfold increase in steroid concentration in the medium resulted in a 100-fold increase in $(c_1)_T$ and a 50-fold increase in $(c_1)_A$; in Expt. RPP8 the concentration in the tissue of 5α-dihydrotestosterone became about 15 times that observed at the lower steroid flow rate (Fig. 5).

Finally, it was observed that the three tissue specimens obtained by transurethral resection (TUR1, TUR2 and TUR3) gave results different from those of the other specimens. They appeared to have a high permeability to the steroids (Expt. TUR1), high uptake and low conversion of testosterone into 5α-dihydrotestosterone at both steroid concentrations. It will be noted from Table 1 that such specimens contained little or no acinar epithelium. On the other hand, in the two hyperplastic glands which presented some adenocarcinomatous degeneration (RPP12a and RPP13), the tissue uptake of steroids was not disproportionately increased at the higher steroid concentration in the medium, in contrast with the finding in the other hyperplastic glands.

DISCUSSION

The results reported in Tables 2 and 3 demonstrated that the experimental design and the analysis of the results at the steady state proposed by Gurpide & Welch (1969) can be applied to the study of uptake and metabolism of androgen by prostatic tissue from human subjects. The use of two different steroid concentrations in perfusion of tissue from the same prostate gland allowed us to study variations in the values of the parameters. This provided an insight into mechanisms determining the uptake and metabolism of androgens by the normal and hyperplastic human prostate *in vitro*.

The physiological role of uptake and metabolism of androgens by the prostate gland requires some comment. The most important aspect of uptake of hormones by target tissues is the interaction with the 'true receptors', which might be the first step of the mechanism of action of the steroids at the cellular level (Wurtman & Jensen, 1968).

Fig. 5. Concentration of radioactive testosterone ($\square$), androstenedione (▨) and 5α -dihydrotestosterone (▩) inside the tissue at the steady state (c_i) in ng/g wet wt. of tissue. The first column of each steroid indicates the concentration of steroid in the tissue at the lower steroid flow; the second column gives the concentration at the higher steroid flow. The dotted lines in the second column represent the concentration inside the tissue that would be proportional to the increase in steroid concentration in the medium, which is tenfold. The bars indicate the limits of the theoretical error of the measurements. The values of these limits are given in parentheses in Expts. Cy4, RPP8, RPP9a and TUR3.

Another aspect of uptake of steroids by prostatic tissue has to be considered. There are in prostatic tissue, besides 'true receptors', other proteins binding androgens (Mainwaring, 1969b). The latter are present in larger quantities than the 'true receptors' and have a lower binding specificity. They might represent 'storage receptors', which, according to Wurtman & Jensen (1968), are substances that bind the hormone unchanged, preventing its metabolism. The hormone thus bound is thought to be inactive as such, but it is in equilibrium with a pool of free hormone that can react with the 'true receptors'. Thus 'storage receptors' would have an essential function in bringing about the chronic effects of the hormone, as follows. Cells containing more intracellular binding substances, or in which more hormone is bound to these substances because of decreased metabolism, would retain a pool of free active hormone for a longer time than cells containing less 'storage receptors' or less 'stored' hormone.

It is probable that the uptake of androgens by prostate slices, measured in our experiments, reflected predominantly this non-specific 'storage' binding, since no evidence of saturation, typical of

binding to 'true receptors', was observed. In fact, binding of testosterone and 5α -dihydrotestosterone to sites of low specificity predominates in the rat prostate *in vitro* (Mainwaring, 1969b).

Metabolism of steroids by target tissues may generally be regarded as a means of maintaining their intracellular concentration as constant as possible. However, in the case of testosterone (and androstenedione) in the prostate, metabolic reduction mainly to 5α -dihydrotestosterone may also have the important function of producing a more potent androgen. Proof that 5α -dihydrotestosterone is the really active androgen in the prostate gland in man is, however, still lacking (Gloyna & Wilson, 1969). It is noteworthy that in our experiments 5α -dihydrotestosterone was found to be the major metabolite of testosterone, and that its uptake by the tissue was much greater than that of either testosterone or androstenedione. Similar observations in experiments *in vitro* with human prostatic tissue have already been reported (Farnsworth & Brown, 1963; Chamberlain, Jagarinec & Ofner, 1966; Gloyna & Wilson, 1969). It was also apparent from our experiments that some of the 5α -dihydrotestosterone present in the tissue was in

a diffusible form. Therefore a source other than blood would be made available to prostatic cells unable to form this compound.

From our perfusion experiments with two steroid concentrations in the medium it appeared that hyperplastic tissue cannot respond to a tenfold rise in the concentration of perfused androgens in the same manner as normal prostatic tissue. The normal tissue increased both the rate of metabolism and the uptake proportionally to the elevation in the steroid concentration in the medium. In the hyperplastic glands the rate of metabolism lagged behind the increase in steroid supply, whereas the uptake became disproportionately high. As mentioned above, such increased uptake or increased 'storage' of unchanged hormone would, if happening *in vivo*, augment the chronic effect of the hormone at the cellular level. Further, in some hyperplastic glands the entry of the steroids into the tissue was somewhat impaired at the higher concentration. In the case of 5α -dihydrotestosterone this was accompanied by a relative increase in the steroid leaving the tissue unchanged. This finding suggested that in the hyperplastic glands there might be a decreased ability of the enzyme systems to metabolize the steroid.

The observation that the entry of the steroids into the tissue was independent of the steroid concentration in the medium in the normal and in some of the hyperplastic glands seems to exclude the existence of mechanisms of active transport, which may be saturated, and rather suggests a mechanism of simple diffusion. Simple diffusion has also been postulated for the entry of oestrogens and androgens into human endometrium *in vitro* (Gurpide & Welch, 1969), and of oestradiol into rat uterus *in vivo* (Jensen, De Sombre & Jungblut, 1967).

We do not suggest that our findings can be extrapolated to the situation *in vivo*. The concentrations of steroids used in our experiments were, for example, five and 50 times the concentration of testosterone in male plasma (Mayes & Nugent, 1968), 25 and 250 times that of androstenedione (Rosenfield, 1969) and 60 and 600 times that of 5α -dihydrotestosterone (A. Vermeulen, personal communication). However, we suggest that the decreased flexibility in the regulation of uptake and metabolism observed in the hyperplastic glands must reflect a basic difference, pre-existing *in vivo*, from the normal gland. This might be related to a deficiency in the enzyme systems, as suggested above, to increased amounts of non-specific binding proteins in a particular type of cell or to the different cellular composition of the hyperplastic tissue, which contains more stromal and muscular cells than normal tissue. These cells might accumulate steroids without metabolizing them, as indicated by the observation (Expts. TUR1, 2 and 3) that

specimens with only a small amount of acinar epithelium exhibited a very high uptake of androgens even at the lower steroid concentration. Thus the prevalence in the tissue of non-acinar cells might bring about an increase in the uptake and a diminution in the rate of metabolism per unit weight of tissue. Experiments with homogeneous cell types could help to clarify this point.

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