Androgen Dynamics *in vitro* in the Human Prostate Gland

EFFECT OF OESTRADIOL-17 β

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Normal, hyperplastic and adenocarcinomatous human prostatic tissue was perfused *in vitro* with radioactively labelled androstenedione, testosterone and 5 α -dihydrotestosterone with and without added oestradiol-17 β . Various parameters of tissue-steroid relationship were measured at the steady state. When oestradiol (0.11 or 0.22 μ mol/l) was added to the perfusing medium, the entry of the steroids into the tissue and their metabolism was increased in the majority of the glands studied. The 'uptake' of all the steroids varied, in response to the addition of oestradiol, in both normal and adenocarcinomatous glands in a way differing from the response of hyperplastic glands. As a consequence, the tissue clearance of the steroids, particularly of androstenedione and testosterone, increased in normal and adenocarcinomatous glands in the presence of oestradiol, and decreased in the hyperplastic tissues. At a concentration 0.33 μ mol/l, oestradiol decreased the entry of the steroids in all the tissues studied, while the clearance of steroids tended to decrease. The significance of these findings in terms of the regulation of androgen dynamics *in vivo* in the normal and diseased human prostate, with particular regard to the response to oestrogen treatment, is discussed.

The relationship between entry, 'uptake' and removal of androstenedione (androst-4-ene-3,17-dione), testosterone (17 β -hydroxyandrost-4-en-3-one) and 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androst-3-one) *in vitro* by human normal and hyperplastic prostatic tissue has been described in a previous paper (Giorgi *et al.*, 1971). The parameters referred to above are defined as follows: 'entry' is the fraction of perfused steroid which penetrates from the medium into the tissue and reflects the permeability of the tissue to the steroid; 'uptake' is the ratio of the concentration of the steroid in the tissue and in the perfusing medium, and expresses the ability of the tissue to retain the steroid; 'removal' is the sum of the fraction of the perfused steroid which is transferred unchanged back into the medium from the tissue ('release') and the fraction of the perfused steroid which is irreversibly converted by the tissue into metabolites ('irreversible metabolism'). Different proportions of the three androgens may enter the tissue. The 'uptake' of 5 α -dihydrotestosterone is much greater than that of the other two steroids. Conversion of testosterone into 5 α -dihydrotestosterone is more extensive than the interconversion of testosterone and androstenedione; the extent of the irreversible metabolism of the three androgens may vary in the same tissue. Only 5 α -dihydrotestosterone is released unchanged from the tissue in measurable amounts. When the steroid concentration in the perfusing medium is raised from 0.11 to

1.1 μ mol/l, normal glands increase the 'uptake' and metabolism proportionally to the rise in steroid concentration. The hyperplastic glands, on the other hand, increase the 'uptake' more than the metabolism, although the entry of the steroids decreases. These findings suggest that the diminished flexibility of hyperplastic tissue in regulating retention of androgens could be accounted for by either a deficiency in androgen-metabolizing enzymes or by an excess of non-specific binding sites in the tissue. The hypothesis has been put forward by Wurtman (1968) that there are in target tissues 'storage' receptors, i.e. macromolecules that store hormones in an inactive form, but that can release the bound hormones to the 'specific' receptors. These 'storage' receptors would have a much greater capacity for the hormones than the 'specific' receptors. Thus, at the steroid concentrations used in our experiments, binding to these hypothetical receptors would completely mask 'specific' binding. Proteins that are present in target tissues in greater amounts than the 'specific' receptors and that bind steroids with low affinity (Mainwaring, 1969; Mester *et al.*, 1970) might be 'storage' receptors. Further, less specialized components of human prostate, such as stromal and muscular tissue, might act as 'storage' of steroids, and hyperplasia of these tissues may be important in determining the difference observed in the regulation of androgen retention in hyperplastic glands as opposed to normal glands.

The present paper reports the results of further investigations of androgen dynamics in human prostatic tissue *in vitro*, with particular reference to the effect of oestradiol-17 β at different concentrations.

Materials and Methods

Tissues studied

Details of patients and the prostatic tissues investigated are summarized in Table 1. Prostates used in Expts. 3, 8 and 9 were removed during a total cystectomy for carcinoma of the bladder. They were of normal size, and appeared histologically normal

or showed only mild hyperplasia. Except in the case of patient H.R., other prostates were removed by retropubic operation for acute urinary retention or symptoms related to chronic enlargement of the prostate. These prostates showed marked nodular hyperplasia of both glandular and fibromuscular tissue, with considerable cystic change in some instances. Adenocarcinoma was diagnosed by prostate biopsy in patient H.R. He was treated with stilboestrol, 90mg daily, for 2 weeks before transurethral resection. The prostate from patient W.D. showed areas infiltrated by poorly differentiated carcinoma of prostatic origin.

Table 1. *Details of patients*

Expt. no.	Patient	Age (years)	Prostatic tissue removed at operation Wt. (g)	Histology
1	Jo.T.	73	50	Benign hyperplasia especially of the glandular tissue
2	C.McG.	78	60	Nodular hyperplasia of the glandular and fibromuscular tissue
3	D.F.	60	14	Normal (carcinoma of the bladder)
4	D.C.	61	65	Simple hyperplasia of the glandular and muscular tissue
5	A.K.	61	40	Benign hyperplasia of the glandular and muscular tissue; well-marked cystic change
6	Jo.C.	62	33	Nodular hyperplasia of the glandular and fibromuscular tissue; well-marked cystic change
7	Jo.H.	73	125	Nodular hyperplasia of the glandular and fibromuscular tissue; well-marked cystic change
8	A.S.	59	16	Normal (carcinoma of the bladder)
9	Th.B.	66	20	Mild adenomatous and fibromuscular hyperplasia (carcinoma of the bladder)
10	W.D.	70	25	Nodular hyperplasia of the glandular and muscular tissue with areas of squamous metaplasia; one area infiltrated by poorly differentiated squamous carcinoma
11	D.S.	88	17	Benign hyperplasia of the glandular and fibromuscular tissue
12	I.T.	91	75	Benign hyperplasia of the glandular and fibromuscular tissue
13	Jo.M.	70	130	Benign hyperplasia of the glandular and fibromuscular tissue; considerable cystic change
14	H.R.	83	—*	Fragments of tissue of prostate adjacent to the bladder neck; some of the fragments are infiltrated by poorly differentiated carcinoma of the clear cell type
15	A.B.	64	75	Benign nodular hyperplasia of the glandular and muscular tissue; moderate cystic change
16	P.D.	72	56	Benign nodular hyperplasia of the glandular and fibromuscular tissue
17	J.L.	83	38	Benign hyperplasia especially of the glandular tissue

* The prostatic tissue was obtained by transurethral resection.

Radioactive steroids

[4- ^{14}C]Testosterone (specific radioactivity 58.2 mCi/mmol), [1,2- ^3H]testosterone (specific radioactivity 44.1 Ci/mmol), [4- ^{14}C]androstenedione (specific radioactivity 56.6 mCi/mmol), [7 α - ^3H]androstenedione (specific radioactivity 3.23 mCi/mmol), [4- ^{14}C]oestradiol-17 β (specific radioactivity 51.4 mCi/mmol) and 5 α -dihydro[1,2- ^3H]testosterone (specific radioactivity 33.1 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Carrier steroids were purchased from Steraloids Ltd., Croydon, Surrey, U.K.

The radioactive steroids were purified before use, and the purity of the carrier steroids was checked as described by Giorgi *et al.* (1971).

Experimental design

The experimental design was essentially that proposed by Gurpide & Welch (1969). The models on which the experiments were based are illustrated in Figs. 1 and 2. A solution containing [^{14}C]testosterone, 5 α -dihydro[^3H]testosterone and/or [^3H]androstenedione is perfused through tissue slices at a constant rate of 0.33 ml/min. The perfusion is continued for

90 min, at which point the steady state is achieved in all experiments, as checked by the procedure described by Giorgi *et al.* (1971). The concentrations of the radioactive steroids are then measured in the perfusates, in the tissue and in the perfusing medium, after isolation and chemical purification. During perfusion, as indicated in Fig. 1, some of the [^{14}C]testosterone is converted into 5 α -dihydro[^{14}C]testosterone (ρ_{TDHT}) so that the 5 α -dihydrotestosterone in the tissue and that released back into the medium (β_{DHT}) have both the original ^3H label and the ^{14}C label derived from testosterone. It is thus possible to distinguish between the portion of 5 α -dihydrotestosterone which bypasses the tissue ($1 - \alpha_{\text{DHT}}$) and that which is released from the tissue. This, in turn, permits the measurement of the fraction entering the tissue (α_{DHT}). Since reduction of the steroid 17-oxo group in human prostate slices occurs only to a small extent (Giorgi *et al.*, 1971), it was expected that conversion of androstenedione into 5 α -dihydrotestosterone would be negligible. Therefore, the use of 5 α -dihydro[^3H]testosterone and [^3H]androstenedione in the same perfusion should not introduce any error in the measurement of the parameters of 5 α -dihydrotestosterone. In the model with the three

Fig. 1. Experimental design

The symbols used in this figure and in the text have the following meaning: C indicates concentration: the superscripts indicate the isotope, the subscripts DHT and T indicate 5 α -dihydrotestosterone and testosterone respectively; the subscript p indicates that the compound is measured in the perfusate; α , fraction of perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid (c.p.m./ml) in the tissue and in the perfusing medium; β , fraction of the perfused steroid released from the tissue back into the medium; im , fraction of the perfused steroid irreversibly metabolized; ρ_{TDHT} , conversion factor of testosterone into 5 α -dihydrotestosterone.

Fig. 2. Experimental design with [3H]androstenedione, [^{14}C]testosterone and 5α-dihydro[3H]testosterone present together in the perfusing medium

The symbols are the same as in Fig. 1; in addition, the subscript A indicates androstenedione, ρ_{AT} the conversion factor of androstenedione into testosterone and ρ_{TA} the conversion factor of testosterone into androstenedione.

radioactive androgens (Fig. 2), any testosterone released from the tissue would be labelled with ^{14}C and 3H , since it would arise in part from [3H]androstenedione; similarly, any androstenedione released would be labelled with ^{14}C and 3H . Thus this model permits the measurement of the parameters of the three steroids simultaneously, and in particular allows the measurement of the release of testosterone (β_T), which is not possible with the model in Fig. 1. It will be noted that the ρ factors (ρ_{TDHT} , conversion of testosterone into 5α-dihydrotestosterone; ρ_{AT} , conversion of androstenedione into testosterone, and ρ_{TA} , conversion of testosterone into androstenedione) indicate the portion of the steroid entering the tissue which is transformed into a specific metabolite. The irreversible metabolism (im in Figs. 1 and 2) indicates the fraction of perfused steroid that 'disappears' on conversion into the sum of its metabolites; as such, im is given by the difference between the concentration of radioactive steroid (c.p.m./ml) in the perfusing medium and in the perfusate, divided by the concentration in the perfusing medium (e.g. $im_T = [C_T^{14C} - (C_T^{14C})_p] / C_T^{14C}$). The 'uptake' is given by the ratio of the concentration of radioactive steroid (c.p.m./ml) in the tissue and in the perfusing

medium [e.g. $uptake_T = (C_T^{14C})_t / C_T^{14C}$]. This expresses the ability of the tissue to retain the perfused steroid. The equations defining the other parameters in Figs. 1 and 2 are given in the previous paper (Giorgi *et al.*, 1971).

Gurpide *et al.* (1971) have introduced, for experiments *in vitro*, the concepts of production rate and of intracellular clearance. By analogy with experiments with tracers *in vivo*, they define production rate as the amount of compound appearing in the tissue *de novo* per unit time. Therefore it is given by the sum of the rate of entry and of the rate of intracellular formation. For example, the production rate (PR) of 5α-dihydrotestosterone (ng/mg) is:

$$PR_{DHT} = v_{DHT} + v_{TDHT} = \phi C_{DHT} \cdot \alpha_{DHT} + \phi C_T \cdot \alpha_T \cdot \rho_{TDHT} \quad (1)$$

where v_{DHT} = rate of entry, v_{TDHT} = rate of intracellular formation from testosterone, ϕ = flow (ml/min), C is the concentration (ng/ml) in the perfusing medium and the other symbols have the meaning given in the legend to Fig. 1.

The intracellular clearance is defined as the weight of tissue irreversibly cleared of the steroid per unit time and is obtained from the production rate of the

steroid per g of tissue (PR/W) divided by its intracellular concentration in ng/ml. For example, the intracellular clearance (IC) of 5α-dihydrotestosterone (g/min) is:

IC_{DHT} = PR_{DHT} / W * 1 / (C_{DHT})_t = v_{DHT} + v_{TDHT} / W * C_{DHT})_t (2)

where the concentration of steroid in the tissue (C_{DHT})_t can be calculated from the isotopic data and the specific radioactivity of the labelled steroids in the medium, since at the steady state the endogenous compounds are completely replaced by the perfused steroids.

Details of the perfusion technique are given by Giorgi *et al.* (1971). Krebs-Ringer bicarbonate containing glucose and equilibrated with O₂+CO₂ (95:5) was used as perfusion medium. The concentration of ¹⁴C in this was 5000-10000 c.p.m./ml and that of ³H was 12000-25000 c.p.m./ml. Suitable amounts of unlabelled steroids were added to achieve the required concentration. Tissue slices were rinsed with Krebs-Ringer bicarbonate solution before and after perfusion. The apparatus was kept in a thermostatically controlled bath at 38°C during the perfusion. The perfusates were collected in tubes standing in crushed ice.

Isolation and purification of radioactive steroids in the samples

The methods already described (Giorgi *et al.*, 1971) were used: the samples were extracted with ethyl acetate containing 200μg of each steroid to be identified; the steroids were separated and purified on

two successive silica gel t.l.c. plates with chloroform-acetone (7:1, v/v) and ethyl acetate-hexane (3:2, v/v). In the experiments with both [³H]androstenedione and 5α-dihydro[³H]testosterone, after the first t.l.c. the androstenedione fractions were treated overnight at room temperature with acetic anhydride and pyridine and run on thin layers of silica gel with benzene-ethyl acetate (9:1, v/v). [¹⁴C]Testosterone and [¹⁴C]oestradiol were separated by t.l.c. with cyclohexane-ethyl acetate (1:1, v/v). The amounts of testosterone, androstenedione and oestradiol recovered were measured by u.v. spectrophotometry, and that of 5α-dihydrotestosterone by g.l.c. After these measurements, 3-5mg of carrier steroid was added to the remainder of the steroid solution and the compounds were crystallized repeatedly until the ³H/¹⁴C ratio and the specific radioactivity were constant. The concentration of the radioactive steroids in c.p.m./ml was derived from the data of crystallization, after correction for losses during the extraction procedure.

Results

It has been shown that the models in Figs. 1 and 2 can be applied to experiments of perfusion of human prostate slices with the pairs of steroids 5α-dihydro[³H]testosterone and [¹⁴C]testosterone and [³H]androstenedione and [¹⁴C]testosterone (Giorgi *et al.*, 1971). The use of two tritiated compounds simultaneously, as in the model in Fig. 2, would, however, only be justified if conversion of androstenedione into 5α-dihydrotestosterone were so small

Table 2. Verification of the experimental design of perfusion experiments of human prostate slices with [³H]androstenedione, [¹⁴C]testosterone and 5α-dihydro[³H]testosterone (0.11 μmol/l)

The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.522±0.030 g. α, fraction of the perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β, fraction of the perfused steroid released unchanged from the tissue; im, fraction of the perfused steroid irreversibly metabolized; ρ_{ADHT}, conversion factor of androstenedione into 5α-dihydrotestosterone.

Expt. no.	Perfusion with:	ρ _{ADHT}	5α-Dihydro[³ H]testosterone			
			α _{DHT}	'Uptake' _{DHT}	β _{DHT}	im _{DHT}
1 (Hyperplastic)	[¹⁴ C]Androstenedione+[³ H]dihydrotestosterone	0.0083				
	[¹⁴ C]Testosterone+[³ H]dihydrotestosterone		0.312	3.535	0.052	0.260
	[³ H]Androstenedione+[¹⁴ C]testosterone+[³ H]dihydrotestosterone		0.335	3.435	0.061	0.275
2 (Hyperplastic)	[¹⁴ C]Androstenedione+[³ H]dihydrotestosterone	0.032				
	[¹⁴ C]Testosterone+[³ H]dihydrotestosterone		0.104	2.951	0.036	0.068
	[³ H]Androstenedione+[¹⁴ C]testosterone+[³ H]dihydrotestosterone		0.101	2.095	0.029	0.072

as not to alter the parameters for the latter. This was checked by perfusing three samples of the same gland, one with [^{14}C]androstenedione and 5α -dihydro[^3H]-testosterone, another with [^{14}C]testosterone and 5α -dihydro[^3H]testosterone and a third with all three steroids. The results of two such experiments with hyperplastic prostate tissue are reported in Table 2. The fractions of androstenedione converted into 5α -dihydrotestosterone were 0.008 and 0.032 respectively. The parameters for 5α -dihydrotestosterone were essentially the same in the presence or absence of [^3H]androstenedione in both experiments.

Effect of addition of oestradiol on the entry, uptake, release and metabolism of androstenedione, testosterone and 5α -dihydrotestosterone by human prostate slices

In each experiment, two samples of slices from the same gland were perfused separately with medium containing the three androgens ($0.11\ \mu\text{mol}$ of each/l). To one medium oestradiol ($0.11\ \mu\text{mol/l}$) was added. The results of five experiments (Expts. 3–7) are reported in Table 3. In the presence of oestradiol, an increase in the entry and 'uptake' of the androgens was observed in three of the four hyperplastic glands. In the normal gland (Expt. 3), α_{T} and α_{DHT} were raised, but the 'uptake' remained unchanged. The release of androstenedione and testosterone was not affected by the addition of oestradiol, but this parameter for 5α -dihydrotestosterone was elevated in two hyperplastic glands (Expts. 6 and 7). Thus in at least two hyperplastic glands oestradiol not only modified the entry of androgens from the medium into the tissue, but also favoured the passage from the tissue back into the medium. A greater fraction of the perfused steroids was irreversibly metabolized in the presence of the oestrogen in both the normal and the hyperplastic glands. In one hyperplastic gland (Expt. 5), the changes observed in the presence of oestradiol were the reverse of those found in the other cases. It may be important to note that, in this experiment, on perfusing androgens alone, entry and metabolism were greater than usual.

In a second group of seven experiments (Expts. 8–14), three simultaneous perfusions were performed with slices from the same gland. In one perfusion, the medium contained $0.11\ \mu\text{mol}$ of each of the three androgens/l. In the other two, this concentration was raised to $1.10\ \mu\text{mol/l}$. One of these two contained, in addition, oestradiol ($0.11\ \mu\text{mol/l}$). The results are reported in Table 4.

In one normal gland (Expt. 8), the addition of oestradiol had no effect on the entry of steroids, but decreased significantly the 'uptake' of androstenedione and testosterone. In the other normal gland (Expt. 9), a large increase of steroid entry occurred, whereas the 'uptake' of the three steroids was not

Table 3. *Effect of oestradiol ($0.11\ \mu\text{mol/l}$) on the entry, 'uptake', release and irreversible metabolism of [^3H]androstenedione, [^{14}C]testosterone and 5α -dihydro[^3H]testosterone at concentrations of $0.11\ \mu\text{mol/l}$ by human prostate slices in vitro*

Expt. no.	Oestradiol	[^3H]Androstenedione				[^{14}C]Testosterone				5α -Dihydro[^3H]testosterone			
		α_{A}	'Uptake' A	β_{A}	im_{A}	α_{T}	'Uptake' T	β_{T}	im_{T}	α_{DHT}	'Uptake' DHT	β_{DHT}	im_{DHT}
3 (Normal)	—					0.132	0.137	0*	0.132	0.203	2.188	0.063	0.140
4 (Hyperplastic)	+	0.182	0.210	0	0.182	0.175	0.169	0*	0.175	0.264	2.106	0.047	0.217
5 (Hyperplastic)	+	0.337	0.339	0	0.337	0.149	0.221	0	0.149				
6 (Hyperplastic)	+					0.290	0.443	0	0.290	0.371	3.068	0.113	0.259
7 (Hyperplastic)	+	0.080	0.324	0	0.080	0.340	0.380	0*	0.340	0.154	0.836	0.029	0.125
	—	0.116	0.675	0	0.116	0.115	0.222	0*	0.115	0.121	2.008	0.029	0.093
	+	0.048	0.242	0	0.048	0.144	0.381	0	0.144	0.236	4.703	0.083	0.154
	—	0.135	0.720	0	0.135	0.149	0.982	0	0.149	0.241	1.384	0.022	0.219
	+					0.065	0.701	0	0.065	0.320	4.543	0.086	0.234
	—					0.123	1.090	0	0.123				

* In this experiment it was assumed that $\beta_{\text{T}} = 0$.

The flow was $0.33\ \text{ml/min}$ and perfusion lasted 90 min. The weight of the tissues was $0.510 \pm 0.037\ \text{g}$. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without oestradiol. α , fraction of the perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β , fraction of the perfused steroid released unchanged from the tissue; im , fraction of the perfused steroid irreversibly metabolized. The subscripts A, T and DHT indicate androstenedione, testosterone and 5α -dihydrotestosterone respectively.

Table 4. Effect of increase of androgen concentration and of oestradiol (0.11 $\mu\text{mol/l}$) on the entry, 'uptake', release and irreversible metabolism of $[\text{^3H}]$ androstenedione, $[\text{^14C}]$ testosterone and 5α -dihydro $[\text{^3H}]$ testosterone by human prostate slices *in vitro*

The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.508 ± 0.040 g, excluding Expt. 14 in which the weights were 0.415, 0.421 and 0.436 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without oestradiol addition. α , fraction of the perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β , fraction of the perfused steroid released unchanged from the tissue; *im*, fraction of the perfused steroid irreversibly metabolized. The subscripts A, T and DHT indicate androstenedione, testosterone and 5α -dihydrotestosterone respectively.

Expt. no.	Concn. of androgen ($\mu\text{mol/l}$)	Oestradiol	$[\text{^3H}]$ Androstenedione				$[\text{^14C}]$ Testosterone				5α -Dihydro $[\text{^3H}]$ testosterone			
			α_A	'Uptake' $_A$	β_A	<i>im</i> $_A$	α_T	'Uptake' $_T$	β_T	<i>im</i> $_T$	α_{DHT}	'Uptake' $_{\text{DHT}}$	β_{DHT}	<i>im</i> $_{\text{DHT}}$
8 (Normal)	0.11	—					0.267	0.700	0*	0.267	0.230	3.837	0.088	0.141
	1.10	—	0.304	3.114	0.162	0.142	0.196	1.764	0*	0.196	0.232	3.498	0.082	0.150
	1.10	+	0.288	0.616	0.088	0.200	0.206	1.176	0*	0.206	0.269	3.758	0.100	0.165
9 (Normal)	0.11	—	0.193	0.325	0	0.193	0.122	0.368	0	0.122	0.252	2.510	0.056	0.196
	1.10	—	0.151	0.426	0	0.151	0.146	0.461	0	0.146	0.161	2.436	0.033	0.129
	1.10	+	0.434	0.368	0	0.434	0.438	0.575	0	0.438	0.437	1.890	0.035	0.415
10 (Hyperplastic squamous carcinoma)	0.11	—					0.137	0.310	0*	0.137	0.209	1.436	0.043	0.166
	1.10	—					0.104	0.361	0*	0.104	0.110	1.349	0.032	0.078
	1.10	+					0.128	0.312	0*	0.128	0.196	1.807	0.035	0.165
11 (Hyperplastic)	0.11	—	0.049	0.524	0	0.049	0.139	0.844	0	0.139	0.139	3.942	0.064	0.071
	1.10	—	0.067	1.720	0	0.067	0.091	2.378	0	0.091	0.060	3.814	<0.010	0.060
	1.10	+	0.066	1.694	0	0.066	0.087	2.218	0	0.087	0.150	4.948	0.065	0.085
12 (Hyperplastic)	0.11	—	0.277	0.234	0	0.277	0.266	0.330	0	0.266	0.222	4.342	0.156	0.066
	1.10	—	0.274	0.434	0	0.274	0.245	0.874	0	0.245	0.265	4.404	0.146	0.120
	1.10	+	0.178	0.439	0	0.178	0.159	0.773	0	0.159	0.285	3.859	0.114	0.175
13 (Hyperplastic)	0.11	—	0.306	0.154	0	0.306	0.098	0.248	0	0.098	0.132	2.918	0.066	0.076
	1.10	—	0.217	0.288	0	0.217	0.081	0.827	0	0.081	0.086	4.552	0.029	0.058
	1.10	+	0.202	0.684	0	0.202	0.143	1.214	0	0.143	0.200	3.791	0.035	0.152
14 (Hyperplastic adenocarcinoma)	0.11	—	0.056	2.119	0	0.056	0.131	2.486	0	0.131	0.215	5.476	0.047	0.168
	1.10	—	0.106	6.377	0	0.106	0.080	3.538	0	0.080	0.035	5.525	<0.010	0.035
	1.10	+	0.124	2.335	0	0.124	0.109	2.548	0	0.109	0.081	4.045	0.010	0.081

* In this experiment it was assumed that $\beta_T = 0$.

significantly changed. Androgen metabolism was also substantially raised in this gland.

In the majority of the hyperplastic glands, the fraction of androgens entering the tissue decreased at the higher steroid concentration in the medium. In the presence of oestradiol, this decrease either did not occur or was less marked. The 'uptake' of testosterone and androstenedione was more than doubled at the higher steroid concentration in the hyperplastic glands, with the exception of the two glands containing adenocarcinomatous tissue (Expts. 10 and 14). By contrast, 'uptake' of 5 α -dihydrotestosterone remained the same in the majority of the cases. Oestradiol did not affect the 'uptake' of the three steroids, with the exception of Expts. 13 and 14. In the latter, as already mentioned, the slices contained mainly adenocarcinomatous tissue.

The fraction of steroid released from the tissue remained unchanged in the normal glands and in one hyperplastic gland (Expt. 10), whereas it decreased in some other hyperplastic glands at the higher concentration. Oestradiol did not prevent this decrease. The fraction of the perfused steroids metabolized by the tissue tended to decrease in the hyperplastic glands when the concentration became tenfold. There was no decrease in the two normal glands (Expts. 8 and 9). However, oestradiol raised the androgen metabolism in the normal as well as in the hyperplastic glands.

The tissue used in Expt. 12 behaved differently from the other specimens of hyperplastic glands. Entry and metabolism of the steroids did not diminish

with elevation of the concentration, and α_A and α_T decreased in the presence of oestradiol.

Effect of oestradiol on the interconversion of testosterone and androstenedione and on the formation of 5 α -dihydrotestosterone from testosterone

Tables 5 and 6 report the conversion factors ρ_{AT} , ρ_{TA} and ρ_{TDHT} measured at the lower and higher androgen concentration respectively. The major metabolite of testosterone was 5 α -dihydrotestosterone. Androstenedione and testosterone were interconverted only to a small extent. However, in Expt. 14 (Table 6), with a tissue infiltrated by adenocarcinoma, the factor ρ_{TDHT} was unusually low. Addition of oestradiol tended to decrease the interconversion of testosterone and androstenedione in hyperplastic glands perfused with the low steroid concentration (Table 5), whereas there was no definite trend in the changes observed in the experiments at the higher steroid concentration (Table 6). The conversion of testosterone into 5 α -dihydrotestosterone was significantly increased by the addition of oestradiol in three hyperplastic glands (Expts. 6, 11 and 13). In a gland containing adenocarcinomatous tissue (Expt. 14), on the contrary, the formation of 5 α -dihydrotestosterone from testosterone was decreased in the presence of oestradiol.

If, however, one considers the rate of intracellular formation, v_{TDHT} in eqn. (1), it is evident that this can vary with α independently of changes in the ρ

Table 5. *Effect of oestradiol (0.11 μ mol/l) on the interconversion in vitro by human prostate slices of [14 C]testosterone and [3 H]androstenedione, and on the formation of 5 α -dihydro[3 H]testosterone from [14 C]testosterone at an androgen concentration in the medium of 0.11 μ mol/l*

The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.510 ± 0.037 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without oestradiol addition. ρ_{AT} , conversion factor of androstenedione into testosterone; ρ_{TA} , conversion factor of testosterone into androstenedione; ρ_{TDHT} , conversion factor of testosterone into 5 α -dihydrotestosterone; v_{TDHT} , rate of formation of 5 α -dihydrotestosterone from testosterone (eqn. 1).

Expt. no.	Oestradiol	Conversion factors			v_{TDHT} (ng/min)
		ρ_{AT}	ρ_{TA}	ρ_{TDHT}	
3	—			0.970	1.41
(Normal)	+			0.920	1.77
4	—	0.090	0.093		
(Hyperplastic)	+	0.034	0.013		
5	—			0.845	3.16
(Hyperplastic)	+			0.945	1.19
6	—	0.041	0.153	0.506	0.80
(Hyperplastic)	+	0.015	0.083	0.810	1.33
7	—	0	0.073	0.923	0.66
(Hyperplastic)	+	0	0.038	0.722	0.98

Table 6. *Effect of oestradiol (0.11 $\mu\text{mol/l}$) on the interconversion in vitro by human prostate slices of [^{14}C]testosterone and [^3H]androstenedione and on the formation of 5 α -dihydro[^3H]testosterone from [^{14}C]testosterone at an androgen concentration in the medium of 1.1 $\mu\text{mol/l}$*

The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.507 ± 0.040 g, excluding Expt. 14, in which the weights were 0.421 and 0.436 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without oestradiol addition. ρ_{AT} , conversion factor of androstenedione into testosterone; ρ_{AT} , conversion factor of testosterone into androstenedione; ρ_{TDHT} , conversion factor of testosterone into 5 α -dihydrotestosterone; v_{TDHT} , rate of formation of 5 α -dihydrotestosterone from testosterone (eqn. 1).

Expt. no.	Oestradiol	Conversion factors			v_{TDHT} (ng/min)
		ρ_{AT}	ρ_{TA}	ρ_{TDHT}	
8	—	0.060	0.248	0.421	9.07
(Normal)	+	0.085	<i>0.153</i>	0.387	8.77
9	—	0.042	0.078	0.552	8.85
(Normal)	+	<i>0.017</i>	<i>0.050</i>	0.567	<i>24.28</i>
10	—			0.625	7.20
(Hyperplastic squamous carcinoma)	+			0.755	<i>10.63</i>
11	—	0.017	0.055	0.148	1.47
(Hyperplastic)	+	0.018	0.042	<i>0.600</i>	<i>5.74</i>
12	—	0	0.079	0.627	16.90
(Hyperplastic)	+	<i>0.120</i>	0.067	0.691	<i>11.75</i>
13	—	0.014	0.202	0.691	6.51
(Hyperplastic)	+	<i>0.031</i>	<i>0.052</i>	<i>0.843</i>	<i>13.25</i>
14	—	0.043	0.097	0.060	0.53
(Hyperplastic adenocarcinoma)	+	0.061	0.133	<i>0.028</i>	<i>0.33</i>

factors. This is shown for the rate of formation of 5 α -dihydrotestosterone from testosterone (v_{TDHT}) in the last column of Tables 5 and 6. In Expts. 3, 7 (Table 5), 9, 10 and 13 (Table 6), in which α_{T} was much higher in the presence of oestradiol, the rate of formation of 5 α -dihydrotestosterone was also much greater. Conversely, v_{TDHT} decreased in the two experiments (Expt. 5, Table 5, and Expt. 12, Table 6) in which the entry of testosterone diminished in the presence of the oestrogen.

Effect of change of concentration and addition of oestradiol on the intracellular clearance of androstenedione, testosterone and 5 α -dihydrotestosterone by human prostate slices

The overall effect of the variations of the parameters described above can best be seen in the changes in the intracellular clearance (IC) of the steroids. This parameter expresses the weight of tissue cleared of steroid per unit time, eqn. (2), and establishes a quantitative relationship between entry, production, retention and removal. Fig. 3 shows the values of the intracellular clearance of the three androgens at 0.11 $\mu\text{mol/l}$ with and without addition of oestradiol

at the same concentration. It will be noted that the values varied from one gland to the other, but IC_{A} and IC_{T} were consistently higher than IC_{DHT} . As indicated in eqn. (2), this difference depends on the higher tissue concentration of 5 α -dihydrotestosterone. In the hyperplastic glands, IC_{A} and IC_{T} were either unchanged or decreased by the addition of oestradiol; IC_{DHT} was increased in two out of three glands. No significant variation of the clearances was observed in the normal gland (Expt. 3).

Fig. 4 shows the intracellular clearances in the experiments performed at the two androgen concentrations and at the higher concentration with the addition of oestradiol (0.11 $\mu\text{mol/l}$). In the hyperplastic glands (Expts. 10–14), the tenfold increase in androgen concentration in the medium caused a dramatic decrease in IC_{A} and IC_{T} , and a smaller decrease in IC_{DHT} . Addition of oestradiol either did not change or decreased IC_{A} and IC_{T} , but elevated IC_{DHT} . In the normal glands (Expts. 8 and 9), the clearances were lowered at the tenfold concentration in the medium, but became significantly greater in the presence of oestradiol. Variations of the clearances, similar to those observed in the normal glands, occurred in the two glands containing adenocarcinomatous tissue (Expts. 10 and 14). Clearances of the

Fig. 3. Intracellular clearance (g/min) of (a) [^3H]-androstenedione, (b) [^{14}C]-testosterone and (c) 5 α -dihydro[^3H]-testosterone at a concentration of 0.11 $\mu\text{mol/l}$, with and without addition of equimolar concentration of oestradiol, in human prostate slices

□, Intracellular clearances of androstenedione (IC_A), testosterone (IC_T) and 5 α -dihydrotestosterone

steroids in the presence of oestradiol were also decreased in the two hyperplastic glands in which oestradiol inhibited entry (Expts. 5 and 12).

In conclusion, although both normal and hyperplastic glands were unable to maintain the same clearance of the androgens when the concentration was elevated, some differences were evident between the two types of glands. In the presence of oestradiol, the normal glands could increase the clearances at the high androgen concentration by diminishing the retention and by increasing the metabolism. In the hyperplastic glands, whatever the extent of increase in the rate of entry and intracellular formation, there was a proportionately higher retention and the clearances became lower. Oestradiol did not reverse this change with respect to testosterone and androstenedione. However, IC_{DHT} was increased by oestradiol at both androgen concentrations; this might be related to the fact that in the presence of oestradiol more 5 α -dihydrotestosterone is released unchanged from the tissue (e.g. Expts. 6 and 7, Table 3).

Effect of increasing concentrations of oestradiol on the entry, uptake, release and metabolism of androstenedione, testosterone and 5 α -dihydrotestosterone by human prostate slices

Three simultaneous perfusions were performed with slices from the same gland. The perfusing media contained 0.11 μmol of each of the three androgens/l, and two of them contained also oestradiol at concentrations of 0.22 and 0.33 $\mu\text{mol/l}$ respectively. The results of experiments with three hyperplastic glands are reported in Table 7.

Oestradiol (0.22 $\mu\text{mol/l}$) caused an increase in the entry and metabolism of the steroids in two hyperplastic glands (Expts. 15 and 16), without changing the 'uptake' significantly. In the third gland, the same concentration of the oestrogen decreased entry, 'uptake' of the three steroids and the release of 5 α -dihydrotestosterone. When oestradiol concentration was increased to 0.33 $\mu\text{mol/l}$, the entry and metabolism of the steroids diminished, with the exception of α_{DHT} and im_{DHT} in Expt. 15; at the same time, androgen uptake tended to become greater.

It appears that the effect of oestradiol on the permeability of prostate slices to androgens is strictly concentration-dependent. A very small change,

(IC_{DHT}) in the absence of oestradiol; ▨, intracellular clearances in the presence of oestradiol. The number of each experiment is shown at the foot of the figure. NOR, normal tissue; HYPER, hyperplastic tissue.

Fig. 4. Intracellular clearance (g/min) of (a) [^3H]androstenedione, (b) [^{14}C]testosterone and (c) 5 α -dihydro[^3H]testosterone at concentrations of 0.11 and 1.1 $\mu\text{mol/l}$, with and without addition of oestradiol (0.11 $\mu\text{mol/l}$), in human prostate slices

□, Intracellular clearances of androstenedione (IC_A), testosterone (IC_T) and 5 α -dihydrotestosterone (IC_DHT) at a concentration of 0.11 $\mu\text{mol/l}$; ▨, intracellular clearances at a concentration of 1.1 $\mu\text{mol/l}$; ▩, intracellular clearances at a concentration of 1.1 $\mu\text{mol/l}$ with the addition of oestradiol (0.11 $\mu\text{mol/l}$). The number of each experiment is shown at the foot of the figure. NOR, normal tissue; HYPER, hyperplastic tissue; CA, squamous or adenomatous carcinoma.

namely from 0.22 to 0.33 $\mu\text{mol/l}$, can produce opposite effects. The intracellular clearances of testosterone and 5 α -dihydrotestosterone in these three experi-

ments are illustrated in Fig. 5. The clearance of testosterone was decreased in all the experiments at an oestradiol concentration of 0.33 $\mu\text{mol/l}$. Clearance

Table 7. Effect of increasing concentrations of oestradiol on the entry, 'uptake', release and irreversible metabolism of [^3H]androstenedione, [^{14}C]testosterone and 5 α -dihydro[^3H]testosterone by human prostate slices *in vitro*

Expt. no.	Concn. of oestradiol ($\mu\text{mol/l}$)	[^3H]Androstenedione				[^{14}C]Testosterone				5 α -Dihydro[^3H]testosterone			
		α_A	'Uptake' $_A$	β_A	im_A	α_T	'Uptake' $_T$	β_T	im_T	α_{DHT}	'Uptake' $_{DHT}$	β_{DHT}	im_{DHT}
15	0					0.106	0.237	0*	0.106	0.137	2.362	0.056	0.081
(Hyperplastic)	0.22					0.143	0.257	0*	0.143	0.232	2.303	0.080	0.152
	0.33					0.083	0.329	0*	0.083	0.213	3.589	0.048	0.165
16	0					0.121	0.458	0*	0.121	0.210	2.367	0.031	0.179
(Hyperplastic)	0.22					0.243	0.446	0*	0.243	0.244	3.236	0.085	0.158
	0.33					0.099	0.459	0*	0.099	0.085	2.745	0.030	0.055
17	0	0.231	0.504	0	0.231	0.260	0.704	0	0.260	0.301	4.673	0.087	0.213
(Hyperplastic)	0.22	0.273	0.247	0	0.273	0.060	0.509	0	0.060	0.067	2.709	0.040	0.026
	0.33	0.109	0.466	0	0.109	0.113	1.196	0	0.113	0.221	4.739	0.122	0.129

* In this experiment it was assumed that $\beta_T = 0$.

The concentration of the androgens was 0.11 $\mu\text{mol/l}$ in all the experiments. The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.466 ± 0.038 g. Values in *italics* indicate significant differences ($P < 0.0027$) between results with and without oestradiol addition. α , fraction of the perfused steroid entering the tissue; 'uptake', ratio of the concentration of the perfused steroid in the tissue and in the perfusing medium; β , fraction of the perfused steroid released unchanged from the tissue; *im*, fraction of the perfused steroid irreversibly metabolized. The subscripts A, T and DHT indicate androstenedione, testosterone and 5 α -dihydrotestosterone, respectively.

Fig. 5. Intracellular clearance (g/min) of (a) $[^{14}\text{C}]$ -testosterone (IC_T) and (b) 5 α -dihydro[^3H]testosterone (IC_{DHT}) at concentrations of 0.11 $\mu\text{mol/l}$, with and without addition of oestradiol, in human prostatic tissue slices

□, IC_T and IC_{DHT} ; ▨, clearances in the presence of 0.22 μmol of oestradiol/l; ▤, clearances in the presence of 0.11 μmol of oestradiol/l. HYPER, hyperplastic tissue.

of 5 α -dihydrotestosterone was decreased in one experiment.

Uptake and metabolism of oestradiol by human prostate slices

In order to measure the uptake and metabolism of oestradiol by human prostate, in nine experiments $[^{14}\text{C}]$ oestradiol-17 β (0.11 $\mu\text{mol/l}$) was added to the perfusing medium in place of unlabelled compound, together with the labelled androgens. As shown in Table 8, some of the tissues concentrated the oestrogen up to 6 times the concentration in the medium. Metabolism of a large fraction of the perfused oestradiol (im_{E_2}) was observed in one normal (Expt. 9) and in two hyperplastic glands (Expts. 6 and 11), whereas in the other six hyperplastic glands im_{E_2} was well below 0.100, that is much lower than the fraction of androgens metabolized (Tables 3 and 4). In Expt. 17, both the uptake and the metabolism of oestradiol increased when its concentration in the medium was raised from 0.22 to 0.33 $\mu\text{mol/l}$. No difference was apparent between oestradiol uptake in perfusions with the low (Expts. 5, 6, 7 and 17) and high (Expts. 9, 11, 12, 13 and 14) androgen con-

centration. Further, in Expt. 17 the uptake of the androgens and of the oestrogen rose simultaneously. These observations seem to exclude competition between the two types of steroids for binding sites inside the tissue at the concentrations used in our experiments.

Discussion

The effect of oestradiol on the metabolism and 'uptake' of testosterone by the prostate *in vitro* has been studied by different groups of workers. Concentrations of from 2 to 17 μmol oestradiol/l decrease the metabolism of testosterone in human and canine tissue. No significant change is found, however, at 0.4 μmol of oestradiol/l (Farnsworth, 1969; Groom *et al.*, 1971). Jonsson (1969) has not observed any competition between oestrogen and androgens for tissue 'uptake' on incubation with human hyperplastic prostate.

We find that oestradiol at a concentration of 0.11 $\mu\text{mol/l}$ can increase the entry of androgens into human prostatic tissue *in vitro*, whereas at a concentration of 0.33 $\mu\text{mol/l}$ oestradiol decreases the entry. Riggs (1964) has described a similar concentration-dependent effect of oestradiol on the transport of amino acids into the rat uterus *in vitro*. The mechanism of entry of steroids into cells is not yet understood. This mechanism, however, appears to have some specificity. Thus the permeability of a sample of tissue may differ for the three androgens investigated here (Tables 3 and 4). Moreover, oestradiol affects the entry of testosterone and 5 α -dihydrotestosterone more than that of androstenedione.

The effect of oestradiol is independent of the androgen concentration and takes place in both normal and diseased prostates. The increased entry of androgens results, in hyperplastic glands, in a greater 'uptake' under certain circumstances (Table 3) and in lower clearances of testosterone and androstenedione in most cases. The same changes are observed when larger amounts of androgens enter the tissue after a tenfold rise in their concentration in the medium (Table 4). The experiments with oestradiol have also shown that activity of androgen-metabolizing enzymes does not limit the removal of the steroids from the tissue, since the fraction of steroids metabolized is increased in parallel with their entry. Therefore the greater 'uptake' and lower clearances observed in the hyperplastic glands, when entry is increased, must depend on accumulation of the steroids in the tissue through binding to 'storage' receptors. Normal and adenocarcinomatous glands do not show such a tendency to accumulate steroids in excess, and steroid clearances can be elevated by addition of oestradiol. As mentioned earlier, this difference between normal or adenocarcinomatous

Table 8. *Uptake and metabolism of [¹⁴C]oestradiol-17 β by human prostate slices in vitro in the presence of [³H]androstenedione, [¹⁴C]testosterone and 5 α -dihydro[³H]testosterone*

The flow was 0.33 ml/min and perfusion lasted 90 min. The weight of the tissues was 0.500 ± 0.020 g, except in Expt. 14, where the weight was 0.436 g. 'Uptake_{E₂}', ratio of the concentration of oestradiol in the tissue and in the perfusing medium; *im*_{E₂}, fraction of the perfused oestradiol irreversibly metabolized.

Expt. no.	Concn. of steroids (μ mol/l)		[¹⁴ C]Oestradiol	
	[¹⁴ C]Oestradiol	Labelled androgens	'Uptake' _{E₂}	<i>im</i> _{E₂}
5 (Hyperplastic)	0.11	0.11	0.787	0.056
6 (Hyperplastic)	0.11	0.11	3.675	0.186
7 (Hyperplastic)	0.11	0.11	6.619	0.029
9 (Normal)	0.11	1.10	1.942	0.415
11 (Hyperplastic squamous carcinoma)	0.11	1.10	4.539	0.381
12 (Hyperplastic)	0.11	1.10	2.771	0.054
13 (Hyperplastic)	0.11	1.10	4.845	0.065
14 (Hyperplastic adenocarcinoma)	0.11	1.10	4.254	0.010
17 (Hyperplastic)	0.22	0.11	5.092	0.054
	0.33	0.11	8.180	0.129

glands and hyperplastic glands may depend on the presence in hyperplastic glands of a greater proportion of stromal and muscular tissue.

The significance of these findings in terms of the situation *in vivo* may be illustrated as follows. Tissues containing a large number of binding macromolecules will prevent the intracellular concentration of the steroids from falling below a certain concentration when the amount of steroid available is low (Tait & Burstein, 1964). Further, as the intracellular clearance is inversely proportional to the half-life of the compound in the tissue (Gurpide *et al.*, 1971), decreased clearances result in prolonged presence of the steroids in the tissue. This might change the hormonal stimulation from acute to chronic and be a cause of hyperplasia.

The limitations of experiments *in vitro* do not need to be stressed. But it is important to point out that the general pattern of metabolism and uptake of androgens by the prostate appears to be the same *in vivo* and *in vitro*. In both conditions, the major metabolite of testosterone is 5 α -dihydrotestosterone. This steroid is accumulated more than testosterone and androstenedione and is retained for a longer time, although it is in part released unchanged (Ofner, 1968; Fang *et al.*, 1969; Eik-Nes, 1971). Further, it has been reported that hyperplastic glands contain more dihydrotestosterone than normal glands (Siiteri *et al.*, 1970) and that they concentrate circulating androgens even when their content is low (Farnsworth, 1971). These findings support the hypothesis that greater cellular retention of androgens occurs in hyperplasia.

With regard to the action of oestradiol on the prostate *in vivo*, it is well established that hyperplastic glands do not respond to doses of oestradiol, or of synthetic oestrogens, that induce regression of prostatic carcinoma (Chisholm, 1970). It is generally agreed (Arduino *et al.*, 1967) that this regression is related to decrease in circulating androgens caused by the action of oestradiol on the hypothalamic-hypophyseal system. This does not explain why a decrease in circulating androgens is not effective in hyperplasia. The different changes brought about by oestradiol in hyperplastic and adenocarcinomatous glands that have been shown in our experiments may help to clarify this point.

Finally, another observation pertinent to carcinomatous tissue is noteworthy. The conversion of testosterone into 5 α -dihydrotestosterone was exceptionally small in one of these glands (Expt. 14), although testosterone was metabolized to the usual extent. The patient had been treated with diethylstilboestrol. However, also in two other cases of adenocarcinomatous tissues from untreated patients (Giorgi *et al.*, 1971; I. M. Shirley, unpublished work), the conversion of testosterone into 5 α -dihydrotestosterone was very small.

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