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A DYNAMIC STUDY OF ANDROGEN BINDING TO MACROMOLECULES IN THE HUMAN PROSTATE

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SUMMARY

A new experimental design has been used to study steroid-tissue relationships in the human prostate. The usefulness of results obtained in attempting to understand the interactions between steroids and intracellular macromolecules is discussed.

IT HAS been said that our lack of understanding of how hormones, including steroids, bring about their effects is one of the great outstanding problems of modern biology. In attempting to grapple with this problem, we may choose to study the response of a target organ to steroids. We soon find that the response depends on a variety of factors including the permeability of cell membranes to steroids, and the interaction, within the cells, between steroids and macromolecules. While it is known that the entry of *amino acids* into uterine cells is an 'active' process[1], influenced by the concentration of oestradiol available[2], we know virtually nothing about the mechanism of entry of steroids into cells. The macromolecules within cells with which steroids react may be the enzymes which catalyse the metabolic transformation of steroid hormones, if such changes occur, or they may be one or other of the 'receptors', the definition of which has been discussed by Wurtman and Jensen[3]. Some choose to isolate and study the properties of these 'receptors' and by so doing lay themselves open to the criticism that they are obtaining interesting results but are nevertheless studying artifacts. We prefer to find out what we can about steroid 'receptor' interactions by an indirect approach. 'In our current state of profound ignorance as to the molecular basis of sex hormone' actions[4], we may perhaps be forgiven for our presumption.

The human prostate is a convenient androgensensitive tissue to study. It is frequently surgically removed in amounts and in a fresh state suitable for biochemical investigation. Such tissue most commonly exhibits benign hyperplasia or may contain regions of adenocarcinoma. For control purposes, apparently normal tissue is removed during the surgical treatment of carcinoma of the bladder. Tissue investigated is invariably examined by an experienced histopathologist.

Prostatic tissue concentrates androgens, a phenomenon referred to as 'uptake' of these steroids[5–8]. It metabolises these steroids[9], and rodent prostate contains macromolecules which bind testosterone and dihydrotestosterone (17β-hydroxy-5α-androstan-3-one, DHT)[10–14]. The presence of such macromolecules in human prostate is inferred but requires confirmation. Dynamic studies of androgen behaviour in prostatic tissue offer the prospect of investigating such hypothetical 'receptors' in an indirect manner. For this purpose continuous-flow incubation or 'superfusion' has many advantages over 'batch' incubations in closed flasks[15–17]. Thus, products, which may influence reaction rates or be further metabolised, do not accumulate. The cells of tissue slices are not broken

by the usual shaking of incubation flasks. As a result, the metabolic responses of the tissue as a whole are not interfered with by those of 'homogenates' produced unintentionally. Moreover, accurately timed collections are possible. The technique is thus sufficiently different from conventional incubations to provide qualitatively and quantitatively different information.

Apart from trying to overcome disadvantages of *in vitro* techniques, it is important to try to obtain a better measure of the extent to which events actually occur in the tissue during incubation. Experiments are thus designed to measure parameters characterising processes occurring during superfusion. The experimental design proposed by Gurpide and Welch[18] is convenient for this purpose: medium containing metabolically related substances, each labelled with a different radioactive isotope, flows continuously at constant rate over tissue slices in an all-glass apparatus (Fig. 1) maintained at constant temperature. Measurements of concentrations and ratios of isotopes at the steady state permit the calculation of parameters of behaviour of the substances in the tissue. We have shown[19] that this experimental design can be applied to the study of the 'uptake' and metabolism of testosterone, androstenedione (4-androstene-3,17-dione) and DHT by prostatic tissue from human subjects.

Details of the experimental procedure have been published[19]. The following is a brief outline. The tissue was placed in crushed ice on removal from the patient. Particular care was taken to cut uniform tissue slices (0·5 mm thick) using a Stadie–Riggs wet tissue microtome. Batches of slices of 500 mg wet weight were employed in most experiments. The dry weight was determined on one batch of slices. The perfusion medium was Krebs–Ringer bicarbonate glucose solution equilibrated with $O_2 + CO_2$ (95:5). It contained 0·11 and 1·1 μmol of each radioactive steroid per litre. The radioactivities were 6 to 10×10^3 c.p.m. ^{14}C and 11 to 20×10^3 c.p.m. ^3H per ml. Tissue slices (about 500 mg) were perfused at 38°C at a constant rate of 20 ml per h for 90 or 120 min. Perfusates were collected for 3 or

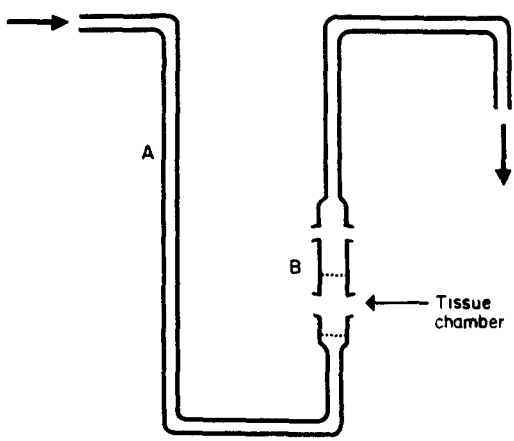
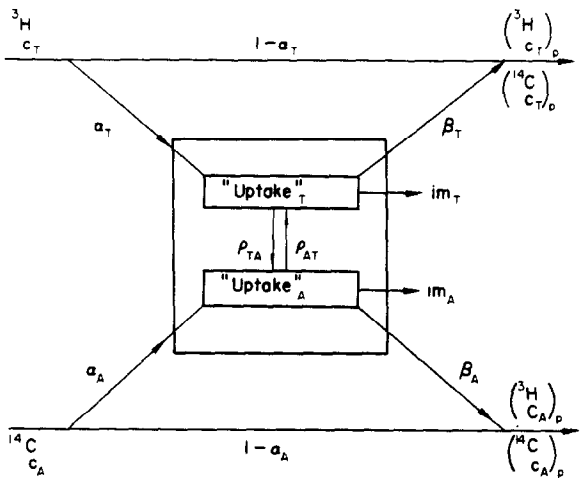


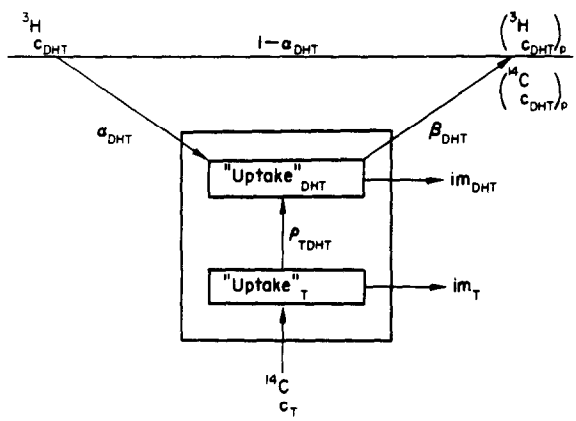
Fig. 1. Superfusion apparatus. The parts of the apparatus, which are of glass, have ground joints and are held in position by metal clips. The length of the vertical branches of the apparatus is 26 cm. The internal diameter of the tubing is 0·3 cm. The chamber into which the tissue slices are introduced is formed by the space between two sintered glass discs in A and B (indicated by the broken line). The volume of the chamber is about 2 ml.

4 periods of 30 min. Observations made on the last collections were used in the calculation of parameters. Up to three superfusion units were set up at one time so that random batches of slices from the one prostate could be superfused simultaneously under different conditions of androgen concentrations or with added substances.

Radioactive steroids were extracted from perfusates collected, with ethyl acetate containing 200 μg of each steroid investigated. The steroids were separated



MODEL 1



MODEL 2

Fig. 2. Models of superfusion experiments. The tissue is represented by the larger rectangles; the perfusion media by horizontal lines at top and bottom. The fraction of steroids entering the tissue (α) is disposed of as follows: part is retained (tissue 'uptake', i.e. the ratio of concentration in tissue to that outside the tissue c_i/c_o), part is converted to other steroids (metabolism *im*) and part is released back into the medium (β). Since [^{14}C]androstenedione is converted into [^{14}C]testosterone in the tissue, testosterone released from the tissue will, in Model I, bear both ^{3}H and ^{14}C labels. Similarly, androstenedione released will bear both labels. In Model II, [^{14}C]testosterone is converted into [^{14}C]DHT, but the reverse reaction cannot occur. Thus, DHT released from the tissue will bear both labels but the fraction of testosterone released, in this case, cannot be determined.

and purified by t.l.c. in two different solvent systems. Carbon and tritium radioactivity in part of the eluates from the second t.l.c. plate was determined by liquid scintillation counting. The mass of steroids recovered was measured in the remaining part by U.V. spectrophotometry or g.l.c. After these measurements of recovery, 3–5 mg carrier steroid was added and the compounds recrystallized to constant $^3H/^{14}C$ ratio. Steroids in tissue slices were measured similarly after homogenizing the slices in 0.9% NaCl.

The following equations, derived by Gurpide and Welch[18], were used in the calculations of parameters of androgen behaviour in the tissue. They are written here for [^{3}H]testosterone and [^{14}C]androstenedione as the metabolically related substances (see Fig. 2, Model I). The symbols used in the calculations and in the models (Fig. 2) have the following meanings. Concentrations (c.p.m./ml) are represented by c. Superscripts ^3H or ^{14}C indicate the nature of the isotope with which the steroids shown in the subscripts are labelled. T, A and DHT stand for testosterone, androstenedione and dihydrotestosterone. Additional subscript p indicates that the steroid is measured in the perfusate, and t that it is measured in the tissue.

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

$$c^{3H}_{T} \text{ or } \left(c^{3H}_{T}\right)_p = \frac{^3\text{H (c.p.m.) in counting vial} \cdot 10}{\text{volume extracted} \cdot \text{recovery \%}}. \tag{1}$$

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

$$\left(c^{3H}_{T}\right)_t = \frac{^3\text{H (c.p.m.)/mg} \cdot \text{mg carrier T}}{\text{wet wt tissue (g)} \cdot \text{recovery \%}}(1+2/10) \tag{2}$$

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

The following parameters of steroid behaviour in the prostate tissue may then be calculated. The fraction of perfused steroid entering the tissue:

$$\alpha_T = 1 - \frac{\left(c^{3H}_{T}\right)_p - [(^3H/^{14}C)_T]_t \cdot \left(c^{14C}_{T}\right)_p}{c^{3H}_{T}}. \tag{3}$$

The fraction of perfused steroid leaving the tissue:

$$\beta_T = \frac{[(^3H/^{14}C)_T]_t \cdot \left(c^{14C}_{T}\right)_p}{c^{3H}_{T}}. \tag{4}$$

The 'uptake' of steroid by the tissue:

$$(c_i/c_0)_T = \frac{\left(c\,{}^{3}_{T}\mathrm{H}\right)_t}{c\,{}^{3}_{T}\mathrm{H}} \tag{5}$$

where c_i is the concentration inside the tissue and c_0 the concentration outside. The irreversible metabolism:

$$im_T = \alpha_T - \beta_T = \frac{c\,{}^{3}_{T}\mathrm{H} - \left(c\,{}^{3}_{T}\mathrm{H}\right)_p}{c\,{}^{3}_{T}\mathrm{H}}. \tag{6}$$

The fraction of testostesterone entering the tissue and undergoing conversion to androstenedione:

$$\rho_{TA} = \frac{\alpha_A}{\alpha_T} \cdot \frac{[(^3\mathrm{H}/^{14}\mathrm{C})_A]_t}{c\,{}^{3}_{T}\mathrm{H} \Big/ c\,{}^{14}_{A}\mathrm{C}}. \tag{7}$$

By substituting appropriate symbols, these equations apply similarly to calculations of parameters for [^{14}C]-androstenedione, 5α-dihydro[^{3}H]-testosterone and [^{14}C]testosterone in models I and II (Fig. 2).

Recently, Gurpide[20] has introduced the concepts of *Production Rate* and *Intracellular Clearance* for *in vitro* experiments, by analogy with *in vivo* experiments with tracers. The *in vitro Production Rate* of a compound is defined as the amount of the compound entering the tissue *de novo* in unit time. It is thus the sum of the rate of entry and the rate of *Intracellular Formation*. The *Production Rate* of DHT (ng/min) might thus be written

$$\mathrm{PR_{DHT}} = \mathrm{V_{DHT}} + \mathrm{V_{TDHT}} = \phi c_{\mathrm{DHT}} \cdot \alpha_{\mathrm{DHT}} + \phi c_T \cdot \alpha_T \nabla \rho_{\mathrm{TDHT}} \tag{8}$$

where $\mathrm{V_{DHT}}$ = rate of entry of DHT

$\mathrm{V_{TDHT}}$ = rate of intracellular formation of DHT from testosterone

ϕ = rate of perfusion (ml/min)

c = concentration of steroid in the perfusing medium (ng/min).

The *Intracellular Clearance* is defined as the weight of tissue irreversibly cleared of the steroid per unit time. It is obtained from the *Production Rate* of the steroid per g of tissue (PR/W) divided by the intracellular concentration of the steroid (ng/ml). Thus, *Intracellular Clearance* of DHT (g/min) is:

$$\mathrm{IC_{DHT}} = \frac{\mathrm{PR_{DHT}}}{\mathrm{W}} \cdot \frac{1}{(c_{\mathrm{DHT}})_t} = \frac{\mathrm{V_{DHT}} + \mathrm{V_{TDHT}}}{\mathrm{W} \cdot (c_{\mathrm{DHT}})_t}. \tag{9}$$

The *Intracellular Clearance* thus establishes a useful quantitative relationship between entry, production, retention and removal of a steroid.

The overall percentage error of the measurements for equations (1) and (2) and

for the measurement of isotope ratio was calculated from the standard deviation from their means of the results of 40 duplicate determinations (21). These were

$$c\,{}^{3}_{T}\mathrm{H} \text{ or } \left(c\,{}^{3}_{T}\mathrm{H}\right)_{p} 3{\cdot}26 \text{ per cent, } \left(c\,{}^{3}_{T}\mathrm{H}\right)_{t} 10{\cdot}85 \text{ per cent, and}$$

$^{3}H/^{14}C$ in the range 0·01–100 5·83 per cent. 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 those in which the probability of agreement was < 0·0027 [22].

In order that these calculations may be applied to the models shown in Fig. 2. the radioactive steroid concentration and the steroid isotope ratio must be measured in the tissue and in the perfusate at a steady state. In other words, at the time of making the measurements, the concentration of radioactive steroids in tissue and perfusate must be constant. We investigated this in a variety of ways. In preliminary experiments, we used an apparatus [19] which permitted removal of portions of slices from the perfusion chamber at intervals during the perfusion and compared their radioactive steroid concentration with that in the perfusates. In these experiments, steroid concentration and isotope ratio were constant between 60 and 90 min after the start of the perfusion. The achievement of steady state was checked by comparing the radioactive steroid concentration (c.p.m./ml) in the perfusate collected not less than 60 min from start of perfusion, and used in the calculations with the concentration in the perfusate collected during the preceding

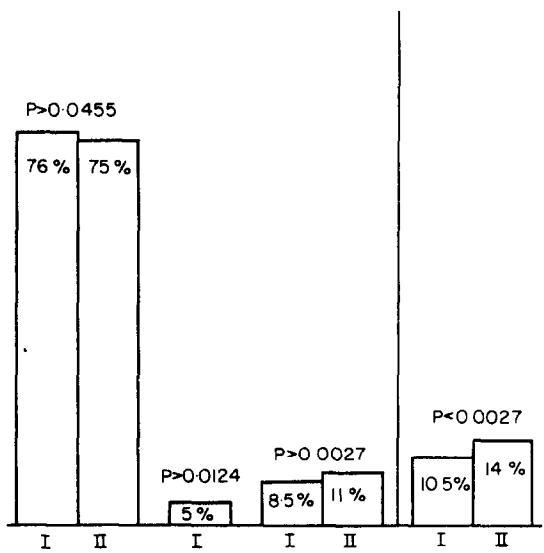


Fig. 3. Statistical analysis of the difference between steroid concentrations (c.p.m./ml) in consecutive perfusates. The results of the analysis are expressed as percentage of the total number of experiments in which the *P* value (the probability of agreement) lay within the ranges > 0·0455, > 0·0124, > 0·0027 and < 0·0027. Measurements were made at two sets of time intervals; I – in which perfusions lasted 90 min and perfusates collected from 30 to 60 and from 60 to 90 min were compared in 16 experiments, and II – in perfusions lasting 120 min with the 60–90 and 90–120 min collections compared in 14 experiments. [^{3}H]Testosterone, [^{14}C]androstenedione and [^{3}H]DHT at 0·11 and 1·1 μmol/l were used.

30 min interval. The results of this comparison are shown in Fig. 3. There was no significant difference between steroid concentrations in consecutive perfusates collected after 60 min and up to 90 and 120 min. The assumption that the isotope ratio in steroids is the same in tissue and perfusate while observations were being made was checked by eluting or 'washing-out' of the steroids from the tissue by changing from a medium with labelled steroids to one with unlabelled steroids. A second check involved determination of the isotope ratio of a metabolite in the tissue and perfusate, which is derived exclusively via one of the two perfused radioactive steroids. This latter method has advantages when the perfused steroids are not released from the tissue in significant amounts, i.e. they cannot be recovered by 'wash-out' experiments. This is the case for testosterone and androstenedione for which $\beta = 0$. Some results are shown in Table 1. A further check of the validity of the experimental procedure was to show that perfusates contained no soluble enzymes which might metabolise steroids. Such enzymes might be released from the tissue slices. None was found. In a typical experiment, tissue slices converted 19 per cent of added [^{3}H]-testosterone to [^{3}H]-DHT; conversion by perfusates was negligible.

Measurements of parameters for all three steroids indicated that their uptake by the tissue (c_i/c_0) varied considerably from one prostate to another. Less variation appeared to occur in fractions of steroids entering the tissue (α) or being metabolised (im). These parameters were of similar magnitude for all three steroids. In most experiments, however, a significantly different fraction of each of the two steroids perfused entered the tissue (particularly at the higher concentration) (Fig. 4). This suggests that the permeability of the tissue is not necessarily equal for all steroids.

Prostatic tissue appears to be able to concentrate DHT from the perfusing medium to a much greater extent than in the case of either testosterone or androstenedione (Fig. 5).

On increasing the steroid concentration in the medium tenfold, a marked difference in behaviour of normal and hyperplastic tissue was observed. Tissue from normal glands increased the 'uptake' and metabolisms proportionally to the increase in the steroid concentration in the medium. Tissue from hyperplastic glands, however, showed a disproportionately high 'uptake' (c_i/c_0), whereas

Table 1. Comparison of the steroid isotope ratios in tissue (t) and in perfusate (p) for DHT and in a metabolite (5α-androstane-3α,17β-diol – A'diol) derived exclusively from DHT

Steroids perfused	Time*	^{3}H/^{14}C DHT		^{3}H/^{14}C A'diol	
		t	p	t	p
[^{3}H]DHT + [^{14}C]T to 60 min followed by unlabelled DHT + T to 120 min (0·11 μmol/l)	90–120	2·91	4·17†	7·12	3·56†
^{3}H DHT + ^{14}C T (1·10 μmol/l)	60–90	2·83	—	2·83	2·72

*min from start of experiment.
†significant difference ($P < 0{\cdot}0027$)

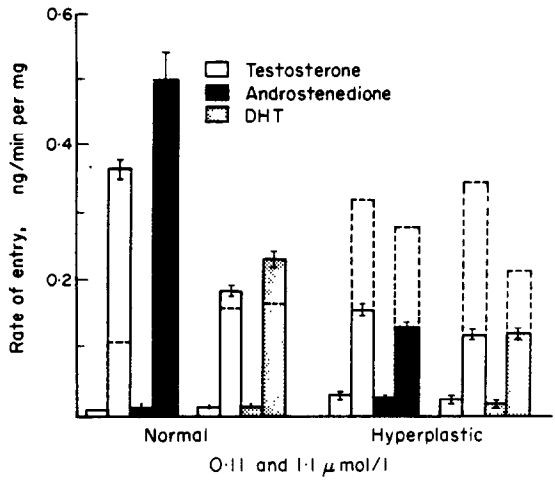


Fig. 4. Rates of entry of testosterone (T), androstenedione (A) and DHT into slices of prostatic tissue. The first column for each steroid indicates the rate of entry ($\alpha \times$ ng/min/mg tissue) at the lower steroid concentration or flow; the second, the rate of entry at the higher concentration. Broken lines in the second column for each steroid indicate the rate of entry which would be proportional to the increase in steroid concentration. Bars indicate the limits of the theoretical error of the measurements.

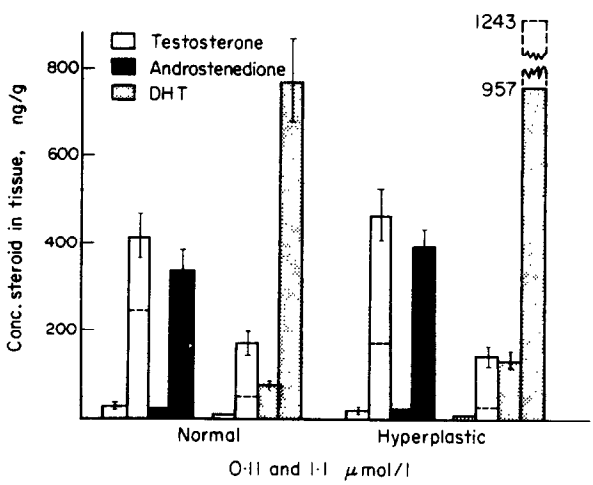


Fig. 5. Concentration of radioactive testosterone (T), androstenedione (A) and DHT in prostatic tissue at the steady state (c_i ng/g wet weight). The first column for each steroid indicates the concentration of the steroid in the tissue at the lower concentration in the perfusing medium (lower rate of flow); the second column gives the concentration at the higher rate of flow. Dotted lines in the second column represent the concentration inside the tissue, which would be proportional to the increase in steroid concentration in the medium which is tenfold. Bars indicate the limits of the theoretical error of the measurements.

entry (α) and metabolism (im) (Fig. 6) could not keep pace with the increased supply of steroids. In the case of DHT, the impaired entry was accompanied by a relative increase in the fraction of DHT leaving the tissue unchanged (β_{DHT}).

From the point of view of physiological roles of steroid 'uptake' (c_i/c_0) and

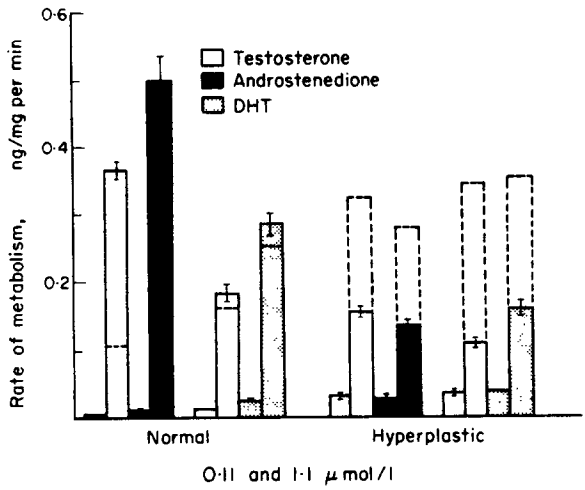


Fig. 6. Rate of irreversible metabolism of testosterone (T) androstenedione (A) and DHT by prostatic tissue at the steady state. The first column for each steroid indicates the rate of metabolism at the lower concentration of steroid in the perfusing medium (lower rate of flow); the second column gives the rate of metabolism at the higher rate of flow. Dotted lines in the second column represent the rate of metabolism, which would be proportional to the increase in steroid concentration in the medium which is tenfold. The bars at the top of the columns indicate the limits of the theoretical error of the measurements.

least in some species, there are other proteins which bind androgens[13]. These are present in amounts larger than the 'true receptors' and have a lower specificity of binding. They may be the 'storage receptors' of Wurtman and Jensen[3] which are considered to bind the steroid unchanged preventing its metabolism. A steroid hormone bound in this way would be inactive but would be in equilibrium with free steroid which can react with the 'true receptor'. In this sense, 'storage receptors' with bound steroid might play an important part in bringing about the chronic effect of the hormone on the prostate. Cells containing larger amounts of 'storage receptors', or perhaps those in which more steroid is bound to such receptors because of decreased metabolism, might retain a pool of free ('active') steroid hormone for longer than cells containing less 'storage receptors' or less 'stored' steroid.

In our observations on prostatic tissue, we have found no evidence of saturation of binding—the characteristic of 'true receptors'. It would thus seem likely that the 'uptake' of androgens which we have measured was an indication of non-specific binding to 'storage-receptors'. Indeed, Mainwaring[13] has reported predominantly non-specific binding of androgens in rat prostate *in vitro*.

The physiological significance of the reduction of testosterone to DHT remains a matter for discussion. We have, however, in common with others[23, 24], noted that DHT is the major metabolite of testosterone in the human prostate, and it has been reported [25] that the concentration of DHT in hyperplastic prostatic tissue is significantly greater than in normal glands. Moreover, the concentration is particularly high in the periurethral region, where hyperplasia of the prostate usually commences. It may thus be particularly interesting that we find DHT to be present in a diffusible form which can be released from cells. Thus, β_{DHT} ranges from 0·043 to 0·115 when DHT is perfused at a concentration of 0·11 μmol/l.

concentrated by prostatic tissue (Table 2). In one case, the 'uptake' (c_i/c_0) was as high as 6·619. No significant difference was observed in oestrogen uptake when the androgen concentration in the medium was increased tenfold. These observations Under similar circumstances, β_A and β_T are zero. This may reflect the ease with which DHT, bound to 'storage receptors', may contribute to the pool of free, 'active' DHT. It might also mean that a source of DHT other than blood[26] is made available to prostatic cells which may be unable to make DHT. Information is at present lacking regarding prostatic cells which make DHT and those influenced by this steroid. There is evidence, however[27], that different cell populations in the human prostate may be dependent on each other.

Oestradiol has an interesting effect on the entry (α) of androgens into prostatic tissue slices (Fig. 7). There is a significant increase in entry of testosterone and DHT, but not androstenedione. Androgen entry is apparently strictly oestrogen concentration dependent, since a small increase in concentration of oestrogen reverses the effect on androgen entry into the prostatic cells. Oestradiol is itself

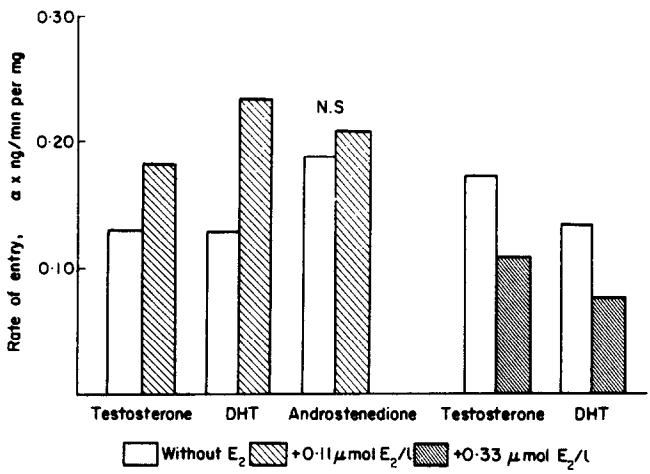


Fig. 7. The effect of oestradiol-17β (E_2) on the rate of entry of testosterone (T), androstenedione (A) and DHT. The column on the left for each steroid indicates the rate without E_2. The column on the right indicates the rate in the presence of E_2. The concentration of the androgens was 0·11 μmol/l. For the three experiments on the left-hand side of the figure the concentration of E_2 was 0·22 μmol/l and for the two experiments on the right hand side 0·33 μmol/l. NS = not significant.

Table 2. The uptake of [^{14}C]oestradiol-17β, [^{14}C]-testosterone and [^{3}H]DHT by human prostatic tissue slices

Steroid concentration (μmol/l)		'Uptake' (c_i/c_0)		
oestradiol	androgen	E_2	T	DHT
0·11	0·11	3·675	0·982	4·703
0·11	0·11	6·619	1·090	4·543
0·11	1·10	4·539	2·218	4·948
0·11	1·10	4·845	1·214	3·791
0·22	0·11	5·092	0·509	2·709

metabolism (*im*) by the prostate, the most important aspect of 'uptake' is the interaction with 'true receptors.[3]. This is perhaps the first step in the action of steroids at the cellular level. In addition to 'true receptors' in prostatic cells, at suggest that oestrogens and androgens have different binding sites in the prostatic cells.

As already mentioned, hyperplastic prostatic tissue increases 'uptake' (c_i/c_0) more than metabolism (*im*) as androgen concentration increases, even although the entry (α) may be somewhat impaired at higher androgen concentrations. There is also a decreased Intracellular Clearance Rate (Fig. 8). This diminished flexibility in the regulation of androgen dynamics in the prostate might be explained by impaired androgen metabolising enzymes or by an excess of non-specific steroid binding sites ('storage' receptors) in the tissue. Experiments with oestradiol in the perfusing medium have shown that the activity of androgen metabolizing enzymes is not limiting, since the fraction of steroids metabolized (*im*) is increased in parallel with their entry into the tissue (α) (Table 3). One might, therefore, conclude that the increased uptake and decreased clearance observed in the hyperplastic tissue on exposure to increased androgen concentration must depend on the accumulation of steroids in the tissue through binding to 'storage' receptors.

We do not suggest that our observations can be extrapolated directly to the situation *in vivo*. The limitations of *in vitro* techniques do not need to be stressed,

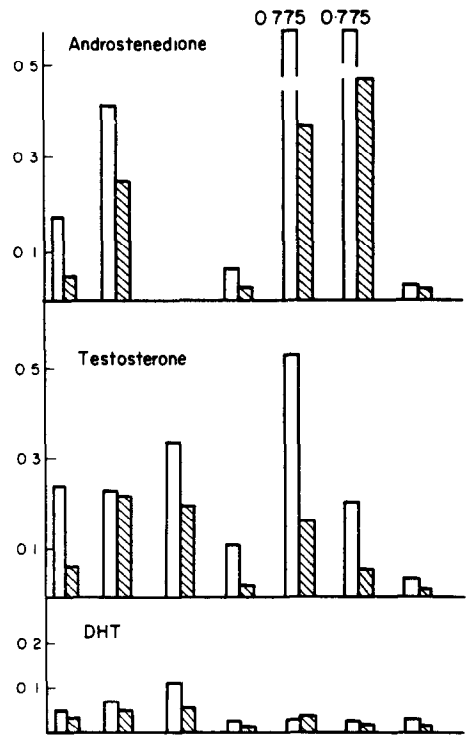


Fig. 8. The effect of increasing the concentration of androgen in the perfusing medium on the intracellular clearance rates of androstenedione (A), testosterone (T) and DHT. The columns □ indicate clearance rate at an androgen concentration of 0·11 μmol/l and ▨ at a concentration of 1·10 μmol/l in the perfusing medium.

Table 3. The effect of adding oestradiol-17β to the perfusing medium on the fraction (α) of androstenedione (A), testosterone (T) and dihydrotestosterone (DHT) entering hyperplastic prostatic tissue and on the fraction of androgen metabolised (*im*)

Oestrogen concentration (μmol/l)	Androstenedione*		Testosterone*		Dihydrotestosterone	
	α_A	im_{α_T}	($0{\cdot}11$ μmol/l)	im_T	α_{DHT}	im_{DHT}
0	0·182	0·182	0·149	0·149	—	—
0·11	0·337	0·337	0·290	0·290	—	—
			($1{\cdot}10$ μmol/l)			
0	0·151	0·151	0·146	0·146	0·161	0·129
0·11	0·434	0·434	0·438	0·438	0·437	0·415
0	0·321	0·231	0·260	0·260	0·301	0·210
0·22	0·273	0·273	0·060	0·060	0·067	0·026
0·33	0·109	0·109	0·113	0·113	0·221	0·129

*$im = \alpha - \beta$

In separate experiments no significant amounts of androstendione or testosterone were found to be released from the tissue; i.e. β_A and $\beta_T = 0$. Hence $im = \alpha$.

and we recognise that we have used high concentrations of steroids. It is worth noting, however, that the general pattern of metabolism and uptake of androgens by the prostate appear to be similar *in vivo* and *in vitro*. In both conditions, the major metabolite of testosterone is DHT, and this steroid is accumulated more than testosterone or androstenedione. The relationships between oestrogens and the prostate *in vivo* are less clear. It is well established that hyperplastic glands do not respond to oestradiol or other oestrogens which produce regression of prostatic carcinoma.

We conclude that the decreased flexibility in the regulation of uptake and metabolism observed in hyperplastic prostatic tissue must reflect a basic difference, pre-existing *in vivo* from the normal gland. This may be related to increased amounts of non-specific 'storage' receptors in a particular type of cell or to variations in the cellular composition of hyperplastic tissue. We hope to separate the different cell types for future investigations, and believe that our particular approach to this problem will provide us with useful information on relationships between steroids and macromolecules.

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DISCUSSION

Collins: Dr. Grant, I would like to ask what factors determined the concentrations of steroids in the perfusing fluid, and whether you have tried adding various proteins to the perfusing medium?

Grant: We are concerned that we are not working with physiological concentrations of androgens. One problem is that you cannot get carbon-labelled androgens with sufficiently high specific activity. We picked this rather curious 0·11 μM concentration because it happens to give 40 ng/ml in the superfusion medium. To overcome the problem of the androgen concentration, we've recently been using androgens with a 17α tritium, which you can remove at will by oxidation. We now hope to do superfusions with both steroids tritium-labelled, one from which you can remove the tritium at will, and with the high specific activities available we should be able to work at concentrations of androgens nearer the physiological level. We have done experiments using plasma but it presents special problems.

Morfin: When you found 5α-androstanediol being released from the slices, could you say whether it was a 3α- or a 3β-epimer which was released or both of them?

Grant: It was the 3α,17β-diol. It is released quite well in circumstances when testosterone and androstenedione are not released.

Morfin: Have you found any traces of the 3β-epimer?

Grant: We haven't looked for it.

Morfin: Since it is a superfusion technique, you renew constantly the medium, but what would happen to the native cofactors which should be present in the tissue?

Grant: We are aware that we are not superfusing plasma and we don't have the factors present such as protein or peptide hormones. I'm assuming that you're not thinking of cofactors like pyridine nucleotides, because I believe that you do not need to add pyridine nucleotide to intact cells. I would say that again we wanted to establish this technique as Gurpide had established it for the uterus, and having done this we are considering using whole plasma, which as I say raises enormous problems, but this is on the go.

Morfin: Yes, but don't you think that in 120 min the relative amounts of the native pyridine nucleotides, like $NADP^+$ or NAD^+, could be modified?

Grant: We of course use Krebs–Ringer bicarbonate with glucose, and we did measure the total $NADH^+$ concentration. In that one particular instance there was no difference. We also monitored the oxygen content before and after.

There's a drop of oxygen across the apparatus but we only use up about 25 per cent of the oxygen, and we think these cells, after not 120 min, but 60 to 90 min, are in pretty good shape.

Jensen: Your comparison of the normal and hyperplastic tissues is especially interesting, but I'm not sure I have it straight. You're saying that at low amounts of steroid, there isn't much difference between the two, but if you increase the amounts, then the hyperplastic tissue becomes saturated at a much lower concentration than the normal tissue, indicating that hyperplastic has fewer total receptors in than normal?

Grant: No, Dr. Jensen, we don't think it becomes saturated. I'm sorry I didn't make this clearer. We think that there is a defect in the hyperplastic tissue in that it fails to metabolize the androgen so well. The rate of entry is defective, the rate of entry may go up, the rate of metabolism will possibly go down, and the concentration in the case of a steroid like dihydrotestosterone can go up to a high level, but we have no evidence of saturation. We've only got three points admittedly; I've only shown you two points, but we can go up to a 10-fold concentration of androgen presented to the tissue without evidence of saturation. We realize of course that there must be binding to specific receptors, and we're only claiming that what we are measuring is binding to storage receptors; we're not pretending that the specific receptor binding is not there.

Jensen: So the abnormality is in the metabolic potential rather than in receptor content. Is that what you're saying?

Grant: No. The abnormality is not entirely in the metabolic behaviour. Hyperplastic tissue increases the "uptake" of androgens more than the irreversible metabolism as the concentration of androgens increases. One might explain this diminished flexibility in handling androgens by impairment of androgen metabolising enzymes in the hyperplastic tissue or by an excess of "storage" receptors in this tissue. When oestradiol is added to the perfusing medium, androgen metabolism is seen to be not limiting since the fraction of steroids metabolised increases in parallel with their entry into the cells. One might thus attribute the increased "uptake" in the hyperplastic tissue to accumulation of the androgens in the tissue due to binding to "storage" receptors.

Munck: Do you have any problem with the sintered glass filters plugging up in this superfusion? Do you have to superfuse very slowly?

Grant: The superfusion rate is very slow at 20 ml/h. We use a coarse sintered glass filter and wash the tissue slices rather carefully before they go into the apparatus. So we don't have any little fragments and the filter doesn't plug up.

Munck: What is the ratio between the amount of steroid in the tissue and the amount of steroid in the medium? Are the weights you gave wet weights or dry weights?

Grant: They are wet weight of tissue. The uptake—concentration inside over the concentration outside—can go up to quite high levels: with dihydrotestosterone it can concentrate up to 6 times the amount in the medium. Interestingly enough, the estrogen does remarkably well: it will concentrate better than dihydrotestosterone. We've only tried the three androgens and estradiol, and estradiol concentrates better than any of them.