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EVALUATION OF THE USE OF CYPROTERONE ACETATE COMPETITION TO DISTINGUISH BETWEEN HIGH-AFFINITY BINDING OF [^{3}H]-DIHYDROTESTOSTERONE TO HUMAN PROSTATE CYTOSOL RECEPTORS AND TO SEX HORMONE-BINDING GLOBULIN

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SUMMARY

From preliminary experiments, it was concluded that cyproterone acetate (CA) was the most satisfactory of several anti-androgens investigated in distinguishing between high-affinity binding of [^{3}H]-5α-dihydrotestosterone to rat ventral prostate cytosol and to human serum, using a simple incubation technique followed by removal of free steroid by Dextran-coated charcoal. Rat prostate cytosol (androgen receptor) binding of [^{3}H]-DHT was almost eliminated by concentrations of CA which affected human serum binding to a relatively slight, though unfortunately variable, extent.

The total, high-affinity, and CA-inhibitable binding of [^{3}H]-DHT by prostatic cytosol of a number of patients with benign prostatic hypertrophy and prostatic carcinoma was investigated. In the untreated patients and in some patients treated by orchidectomy and/or estrogens, the total and CA-inhibitable high affinity binding of [^{3}H]-DHT were correlated with the endocrine status of the patient as determined by the serum testosterone level and the high affinity [^{3}H]-DHT binding capacity of the serum (equivalent to sex-hormone binding globulin (SHBG)).

It was concluded that the use of cyproterone acetate to distinguish between [^{3}H]-DHT binding to the androgen receptor and serum components in human prostate cytosol permits a semi-quantitative evaluation of the amounts of these two components.

INTRODUCTION

Investigation of the binding of androgen by human prostatic cytosol has been severely hampered by the presence of sex-hormone binding globulin (SHBG) in tissue preparations. This protein, which has high binding affinity for testosterone and 5α-dihydrotestosterone (DHT) [1], does not occur in the rat [2], which has been the animal most commonly used in studies of prostatic function. Much effort has been expended in devising techniques which would permit discrimination between the high affinity binding of androgens to receptor molecules from that to SHBG. Some authors have found sucrose density centrifugation satisfactory for this purpose [3, 4]; others have not [5]. Agar gel electrophoresis has also been used with varying success [5, 6]. Another method which has been used is Sephadex gel chromatography, either alone [7], or combined with other fractionation techniques [8, 9]. Geller *et al.*[9] combined gel filtration with competition with cyproterone acetate (CA), on the basis that this anti-androgen competes with [^{3}H]-DHT for high affinity binding sites on androgen receptors, but not for those on SHBG. We have also used an approach using anti-androgen competition in attempting to develop a technique simple enough for routine assay. We examined the effectiveness of a number of anti-androgens in inhibiting binding of [^{3}H]-DHT to components containing androgen receptor (rat prostate cytosol) and SHBG (human serum) in a simple assay in which cytosol/serum was incubated with [^{3}H]-DHT in the presence or absence of excess cold DHT or anti-androgen, followed by the removal of free steroid with Dextran-coated charcoal (DCC). We concluded that cyproterone acetate was the most effective of the anti-androgens investigated in discriminating between binding to the two components. Using this method we examined the total, high-affinity, and CA-inhibitable binding of [^{3}H]-DHT to prostatic cytosol from a series of patients with benign prostatic hyperplasia (BPH) and prostatic carcinoma. "Normal" prostatic tissue was also investigated. In some cases we related the results to the levels of bound and free testosterone in the patients' serum and to its SHBG content, as indicated by the capacity of the serum to bind [^{3}H]-DHT with high affinity.

MATERIALS AND METHODS

[1,2-^{3}H]-Dihydrotestosterone (44 Ci/mmol) was obtained from New England Nuclear Corp.: on arri-

val it was diluted to 10 μCi/ml in redistilled benzene–ethanol (9:1 v/v) and stored at 4°; an appropriate aliquot was prepared before each experiment by evaporating the solvent under nitrogen and redissolving the [^{3}H]-DHT in buffer. Cyproterone acetate (SH 714; 17α-acetoxy-6-chloro-1α-2α-methylene-pregna-4,6-diene-3,20-dione) and flutamide (SCH 13521; α,α,α-trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide) were supplied by Schering Berlin and Schering Corp. N.J., respectively, Compound 1 (2′,3′ α-tetrahydrofuran-2′-spiro-17(6,7α-difluoro-methylene-1,2α-methylene-4-androsten-3-one) by Merck, Sharp & Dohme, and DIMP (N-(3,5-dimethyl-4-isoxazolylmethyl) phthalimide) by Hoffman-La Roche. BOMT (6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one) was kindly provided by Dr. W. I. P. Mainwaring, Imperial Cancer Research Fund, London, England. Non-radioactive steroids and DNA standard were obtained from Sigma Chemical Co., MO: Dextran T 70 from Pharmacia, Montreal, and charcoal (Norit A) from Matheson, Coleman and Bell. The scintillator used was 5 g diphenyloxazole (PPO) and 0.1 g 1,4-bis[2-(5-phenyloxazolyl)] (POPOP) (Amersham–Searle), per liter of toluene.

For the serum testosterone assays, [1α,2α-^{3}H]-testosterone (55 Ci/mM) was purchased from New England Nuclear Corp. (Dorval, Quebec, Canada), checked for purity and counted as described by Bird *et al.*[10]. The testosterone antibody used in the testosterone radioimmunoassay was obtained from Dr. G. Abraham (Torrance, CA, U.S.A.). All other compounds used were of reagent grade. Solvents were redistilled twice prior to use.

Ventral prostate glands from mature male Wistar rats (Canadian Breeding Laboratories or High Oak Ranch) weighing approximately 280 gm were removed one day after castration. Human prostatic tissue, usually from transurethral resection, but occasionally from needle biopsy or open prostatectomy, was placed in a vial in an iced container immediately after removal and refrigerated until being brought to the laboratory, usually within an hour. Obviously damaged tissue was trimmed off and discarded. Rectus abdominis or pyramidalis muscle, used as control tissue, was treated in the same way. Such tissue was considered to be a valid control as, although androgen receptors have been demonstrated in skeletal muscle in the rat, the concentration was sixty times less than that in the prostate[11]. 'Normal' prostatic tissue was obtained from cadavers of subjects aged 19, 38 and 48 years, who had died within the previous 6–24 h of causes unrelated to prostatic disease. Samples of all human tissue were fixed for histological examination, to ensure that the tissue used for assay was similar to that described in the pathology reports. As could be expected from a series of unselected patients, the histology was varied. The majority of the cases with BPH were described as having nodular areas of fibromuscular and glandular hyperplasia. Some also had areas of inflammation. Two were described as having cystic glands. In the carcinoma cases, the proportion of malignant tissue present was variable, and ranged from well to poorly differentiated, even within the same gland. Mitotic figures were observed in a few cases. Squamous metaplasia was present in two estrogen-treated patients. Benign hyperplastic tissue was also present in some of the carcinomatous prostates. Two had mild inflammatory cell infiltration.

Ten ml of venous blood was taken from some patients at the time of operation. This was non-heparinized, allowed to clot, and the serum was spun off and stored in aliquots at −17°.

[^{3}H]-DHT *binding assay*

All procedures were carried out between 0 and 4°. Rat prostatic tissue was homogenized in a motor driven Potter–Elvejhem type homogenizer at 790 rev./min in Tris HCl buffer, pH 7.4, containing 1.5 mM EDTA and glycerol 10% v/v (TEG buffer). Homogenization was carried out in an ice-bath in 15 s bursts, with 45 s cooling intervals. An aliquot of the homogenate, whose final concentration was approximately 50 mg/ml, was removed for DNA determination by the method of Dische[12]. The remainder of the homogenate was centrifuged at 800 ***g*** for 10 min, and the supernatant from this spin was recentrifuged in a Beckman L5-50 centrifuge at 98,000 ***g*** for 1 h. The supernatant cytosol fraction was assayed for [^{3}H]-DHT binding activity. Replicate 1 ml. aliquots were diluted 1:1 with [^{3}H]-DHT in TEG buffer, so that the final concentration of [^{3}H]-DHT was 2.2×10^{-9} M, either with or without non-radioactive DHT at a concentration of 4.4×10^{-7} M. 2.2×10^{-9} M [^{3}H]-DHT was found to be a saturating concentration for the androgen receptor sites in the rat ventral prostate when assayed under these conditions. Blanks containing 1 ml of buffer instead of cytosol were run concurrently. Incubation was carried out at 0° for 2 h. Incubation for longer periods (up to 7 h) did not significantly alter the amount of binding observed. At the end of the incubation period, unbound hormone was removed by the addition of 1.0 ml of a suspension of 0.5% charcoal and 0.05% Dextran in Tris–HCl EDTA (TE) buffer. The tubes were shaken briefly, allowed to stand in an ice-bath for 15 min and centrifuged at 15,000 ***g*** for 10 min. The supernatant was removed and recentrifuged to remove the charcoal completely. Preliminary experiments established that the amount of radioactive steroid removed by the charcoal did not alter significantly during 5–20 min of DCC treatment. Bound DHT in the supernatant was extracted with methylene chloride which was evaporated to dryness in counting vials. Scintillator was added and radioactivity was measured in a Packard scintillation counter (Model 3375) at an efficiency of approximately 44%. Quenching was corrected for by using an external standard. High-affinity DHT-binding was calculated

by subtracting radioactivity in the presence of cold DHT (low-affinity binding) from total binding (in the absence of cold DHT). Binding was expressed as fmol [^{3}H]-DHT per mg wet weight of tissue and/or per μg DNA.

For the rat prostate cytosol/human serum experiments, aliquots of male human serum diluted 1:100 with TEG buffer were assayed for [^{3}H]-DHT binding in the same way as rat prostate cytosol. The binding capacity of a mixture of equal quantities of rat prostate cytosol and diluted human serum were also determined. For the competition studies, anti-androgens were added at concentrations ranging from $2.2 \times 10^{-9} - 2.2 \times 10^{-7}$ M, i.e. 1–100 times that of [^{3}H]-DHT. The percent reduction in high affinity binding of [^{3}H]-DHT was calculated, taking the reduction caused by the presence of non-radioactive DHT as 100%.

Human prostatic tissue was assayed for [^{3}H]-DHT binding capacity either immediately after being brought to the laboratory or after refrigeration in ice overnight. Tissue was rinsed briefly in TEG buffer and blotted dry before weighing. The assay was identical with that for the rat prostate except that the concentration of [^{3}H]-DHT used was reduced to 1.1×10^{-9} M because preliminary experiments indicated that the concentration of sites available for assay in human material was generally lower than that in the rat prostate. The lower concentration of [^{3}H]-DHT was therefore used to minimise low-affinity binding. Three sets of aliquots of human prostate cytosol were incubated as follows: (a) with [^{3}H]-DHT alone to determine total binding; (b) with [^{3}H]-DHT + 1.1×10^{-7} M "cold" DHT to determine low-affinity binding, and (c) with [^{3}H]-DHT + 1.1×10^{-7} M CA to determine CA-inhibitable [^{3}H]-DHT binding.

Initial assays of the [^{3}H]-DHT capacity of patients' serum were carried out with serum diluted 1:100 with TEG buffer. However, it became clear that the degree of reduction of high affinity binding of [^{3}H]-DHT by the addition of CA varied considerably with the binding capacity of the sera. In subsequent assays the serum of each patient was diluted to give a standard count of approximately 40,000 dpm bound per ml after incubation with [^{3}H]-DHT and treatment with Dextran-coated charcoal as described.

Determination of serum testosterone

Serum testosterone levels were determined by the radioimmunoassay procedure of Abraham *et al.*[13] with minor changes; these included using solvents that were not equilibrated with ethylene glycol for the Celite columns. Also 3 ml rather than 3.5 ml vol. of solvents were used for elution of the columns. Levels of binding of testosterone to plasma proteins were measured by a modification [14] of the equilibrium dialysis method of Forest *et al.*[15]. The testosterone free index (TFI) was calculated [16] by multiplying the total plasma testosterone level (ng/100 ml) by the unbound fraction [%] and is expressed as ng/100 ml.

RESULTS

Competition by anti-androgens for high affinity sites for [^{3}H]-DHT *in rat ventral prostate cytosol and human serum*

Curves showing the competition by CA for high-affinity [^{3}H]-DHT binding by rat ventral prostate cytosol, diluted human serum, and a mixture of the two are shown in Fig. 1. When CA was present in concentrations ten or more times that of [^{3}H]-DHT, the high-affinity binding of the latter by rat ventral prostate cytosol was reduced to 10–20% of that in the absence of CA, whereas binding to the serum used in this experiment was unaffected. The reduction of high-affinity binding of [^{3}H]-DHT in a 1:1 mixture of rat prostate cytosol and human serum was intermediate. Unfortunately, further experiments showed that the reduction of [^{3}H]-DHT binding by human serum brought about by CA varied with different sera.

The results of competition studies with cyproterone acetate, Compound 1, DIMP and flutamide in rat ventral prostate, human serum, and mixtures of the two are presented in Table 1. BOMT was tested with serum only. When used at a concentration 100× that of [^{3}H]-DHT, there was some competition by this anti-androgen for sites in human sera, varying from 0–30% in different sera. The mean reduction in high affinity binding of [^{3}H]-DHT to serum sites brought about by CA and Compound 1 was approximately 12.0% when used at concentrations 100× that of [^{3}H]-DHT, and there was considerable variation between sera with both anti-androgens. However, in 75% of these assays CA gave a reduction of less than 10%, and Compound 1 a reduction of less than 15%. In subsequent assays of serum binding, in which the serum of 34 patients was diluted to give a standard amount of [^{3}H]-DHT binding, the mean reduction of [^{3}H]-DHT binding brought about by 100 fold concentration of CA was 7.5% ± 7.4 S.D. DIMP and Flutamide resulted in some reduction of high-affinity binding of [^{3}H]-DHT to serum sites at concentrations which were not very effective in reducing binding to prostatic sites. Geller[9] also found that flutamide displaced bound [^{3}H]-DHT from plasma. The relative effectiveness of the anti-androgens used in competing for prostatic sites confirms results reported by other workers (e.g. 17, 18, 19). In particular, flutamide, which has been shown to have marked antiandrogenic effects *in vivo* [20, 21], was relatively ineffective in competing for [^{3}H]-DHT binding sites *in vitro* [17, 18, 22]. CA was the most effective anti-androgen investigated in competing for [^{3}H]-DHT binding prostatic sites.

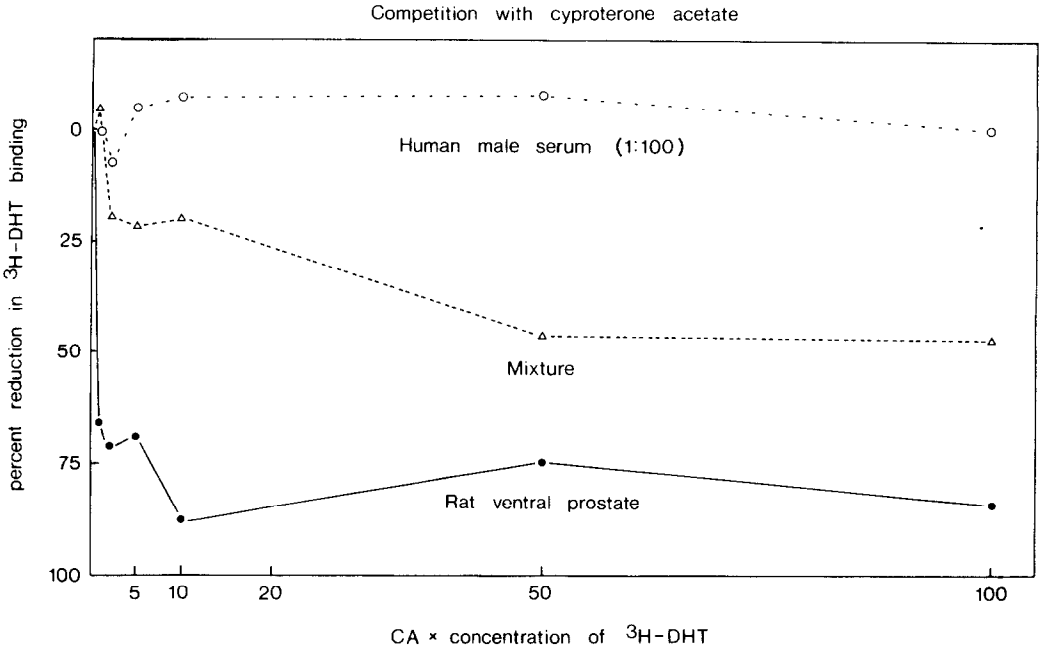

Fig. 1. Reduction in high-affinity binding of [^{3}H]-DHT by rat ventral prostate cytosol, male human serum diluted 1:100, and a 1:1 mixture by CA, present in the incubation at concentrations ranging from 0–100 times that of the concentration of [^{3}H]-DHT (2.2×10^{-9} M).

Assay of total high-affinity- and CA-inhibitable-binding of [^{3}H]-DHT *by human prostatic cytosol*

Total high-affinity- and CA-inhibitable-binding of [^{3}H]-DHT was assayed in prostatic cytosol from 14 patients with benign prostatic hyperplasia (BPH) and from 14 patients with prostatic carcinoma, 6 of whom were untreated. Four of the carcinoma patients had been treated with estrogens alone, 1 had been orchidectomized and 3 had been treated by both estrogen and orchidectomy. The tissue obtained from one estrogen-treated patient was iliac crest invaded by metastic prostatic carcinoma. The binding values were related to wet weight of tissue and the DNA content in all cases.

The binding values related to tissue weight are presented in Fig. 2. The total high affinity binding of [^{3}H]-DHT by prostatic tissue from all untreated subjects was less than 4.2 fmol per mg wet weight of tissue and less than 1.3 fmol per μg DNA. The CA-inhibitable binding was negligible in the cytosol of all untreated subjects except one, in which 32.3% (1.2 fmol/mg) [^{3}H]-DHT binding was inhibited by CA. The probable reasons for this will be discussed later. In 5/8 of the treated patients the total high-affinity [3H]-DHT binding was considerably higher than in the untreated subjects. This confirms our earlier work [23]. There were also higher values for CA-inhibitable binding, which ranged from 0.3 fmol/mg in the bone metastasis to 2.3 fmol/mg in prostatic tissue from two patients (one orchidectomized and one estrogen-treated). This represented a reduction by CA in [^{3}H]-DHT binding in cytosol from treated patients of up to 26.4%. In muscle from a female subject, used as a non-target control tissue,

Table 1. Percent reduction in high-affinity [^{3}H]-DHT binding caused by presence of anti-androgens

Anti-androgen	(a) Rat prostate cytosol	(c) Prostate: dil. serum 1:1	(b) Male human serum dil. 1:100
Cyproterone acetate	84.7 (78.3–91.5)* $n = 4$	47.3	12.6 (0–37.1) $n = 12$
Compound 1	71.8 (62.4–79.7) $n = 3$	32.2	11.9 (0–38.1) $n = 11$
DIMP	34.4	9.9	11.2
Flutamide	14.2	3.8	8.3

Reduction of high-affinity [^{3}H]-DHT binding to (a) rat ventral prostate cytosol, (b) human serum (dil. 1:100) and (c) 1:1 mixtures of (a) and (b), brought about by the addition of anti-androgens to incubated cytosol/serum. [^{3}H]-DHT was used at a concentration of 2.2×10^{-9} M, and anti-androgens at 100 times this concentration. N = Number of experiments, where this was >1. *Mean and range

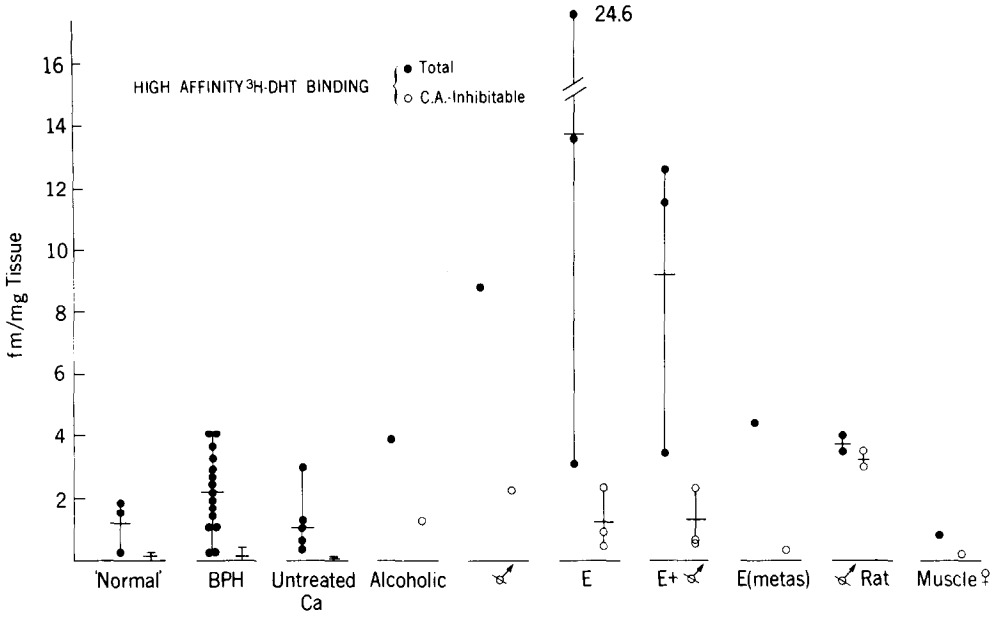

Fig. 2. High-affinity-(●) and CA-inhibitable-(○) binding of [^{3}H]-DHT in cytosol from prostatic tissue of "normal" subjects, and patients with BPH and prostatic carcinoma. [^{3}H]-DHT binding of cytosol from rat ventral prostate and a specimen of rectus abdominis muscle for a female patient measured under the same conditions are included for comparison. "Normal" tissue was from cadavers, as described in the text: BPH; benign prostatic hyperplasia; Ca; carcinoma; ♂; orchidectomized: E; estrogen treated: metas; metastasis: muscle ♀: muscle from a female patient. Due to overcrowding, individual values for CA-inhibitable binding have not been included for "normal", BPH, and untreated carcinoma patients, but means and ranges are shown.

total high affinity binding of [^{3}H]-DHT was 0.91 fmol/mg, and competition with CA was negligible. In Fig. 2 the results of an assay in prostate cytosol from one day castrated rats carried out under the same conditions are presented for comparison with the results in human prostatic cytosol. From previous experiments, we know that the concentration of [^{3}H]-DHT used does not saturate binding sites in the rat prostate cytosol. As expected in the rat prostate, the CA-inhibitable binding is almost equal to the total high affinity binding of [^{3}H]-DHT. The amount of CA-inhibitable binding is of the same order as that in the cytosol of some of the treated patients (approximately 4 fmol/mg). When related to DNA content, the pattern of results was similar and the CA-inhibitable [^{3}H]-DHT binding in 2 of the patients (one estrogen-treated and one estrogen-treated and orchidectomized) exceeded the value for high affinity [^{3}H]-DHT binding in the castrated rat prostate (2.1–1.0 fmol/μg DNA) respectively, as against 0.8 fmol/μg DNA in the rat, when assayed under the same conditions.

It is unlikely that the diethylstilbestrol (DES) used for estrogen therapy affected [^{3}H]-DHT binding as, in contrast to estradiol, DES has been found to be a poor competitor for [^{3}H]-DHT binding to both androgen receptor and SHBG [23].

[^{3}H]-DHT-*Binding capacity of human serum*

The capacity of the serum to bind [3H]-DHT with high affinity was measured in 11 patients with BPH, 4 with untreated carcinoma (including one suffering from alcoholism) and in 4 treated carcinoma patients. The results are shown in Table 2. Since SHBG is the only serum protein reported to bind DHT with high affinity, the capacity of serum to bind this steroid is taken as a measure of SHBG concentration. As the affinity of DHT for SHBG is higher than that of other endogenous steroids [1], the only possible interference in this assay is from endogenous DHT itself, which is present in male serum at a concentration of approximately 50–90 ng/100 ml [24, 25]. This may have resulted in a slight underestimation of [^{3}H]-DHT binding capacity in untreated patients with high levels of endogenous androgens. Rosner,

Table 2. High-affinity [^{3}H]-DHT binding capacity in sera of patients with BPH and prostatic carcinoma

Diagnosis and treatment (no. of patients)		Mean and range of high-affinity [^{3}H]-DHT binding by serum (μg/100 ml)
BHP	No treatment (11)	0.847 (0.362–1.478)
Ca	No treatment (3)	0.959 (0.577–1.675)
Ca	Alcoholic, no treatment (1)	2.375
Ca	Orchidectomized (2)	0.924 (0.440, 1.409)
Ca	Estrogen treated (2)	5.390 (2.140, 7.681)

Table 3. Values for total testosterone, testosterone free index (TFI) and high-affinity [^{3}H]-DHT binding capacity in sera from patients with BPH and prostatic carcinoma related to the high-affinity- and CA-inhibitable-[^{3}H]-DHT binding of cytosol from their prostatic tissue. N.D. = not done

Patient	Diagnosis and pre-op treatment	Serum			Prostate		
		Testosterone (ng/100 ml)		High-affinity [^{3}H]-DHT binding capacity	High-affinity [^{3}H]-DHT binding		CA-inhibitable [^{3}H]-DHT binding
		Total	TFI	μg/100 ml	fmol/mg	fmol/μg DNA	fmol/mg
H.H.	BPH, none	834	37.5	N.D.	2.5	0.4	0.25
P.S.	BPH, none	467	29.4	1.180	1.5	0.4	0.32
W.C.	BPH, none	712	23.5	1.380	3.7	0.6	0
H.J.	BPH, none	649	20.8	1.478	3.3	1.0	0.1
E.D.	Ca, DES	1187	N.D.	2.140	3.1	0.5	0.41
E.H.	Ca, none Alcoholic	341	7.8	2.375	3.8	0.4	1.24
A.A.	Ca, orchiex	82	2.2	1.409	8.9	1.1	2.28
A.N.	Ca, orchiex DES	114	1.1	3.117	12.7	5.5	2.29

who measured binding capacity after precipitation of SHBG with ammonium sulphate [26] reported no interference from endogenous steroids. The values we obtained for [^{3}H]-DHT binding capacity in the untreated, non-alcoholic patients agree well with those obtained by other authors for normal untreated men [14, 27]. Others have also observed elevated DHT-binding capacity in serum from estrogen-treated men [14] and in subjects with liver cirrhosis [26].

Free and bound testosterone levels in serum

Serum free and bound testosterone was assayed in 12 of the patients in this series, 10 of whom were untreated. In the untreated patients, the testosterone values were within the normal range for the methods used. The total testosterone level in the serum of the alcoholic patient was low in the normal range, as has been observed previously in men with liver cirrhosis [28], and, as expected from the high androgen binding capacity of his serum, the testosterone free index was low. The determinations on 8 patients are presented in Table 3 together with the [^{3}H]-DHT binding capacity of the serum and the data on [^{3}H]-DHT binding in the prostatic cytosol of the same patients. It is clear that the highest amounts of CA-inhibitable binding occurred in the prostatic cytosol of the patients whose endogenous testosterone levels were lowest, whether due to alcoholism, orchidectomy or orchidectomy combined with estrogen treatment.

DISCUSSION

As in all biochemical studies on the diseased human prostate gland, the interpretation of results is hampered by the scarcity of truly normal prostatic tissue for comparison, and by the histological variation between different glands, and even within the same gland. Glands diagnosed as malignant may have only a small proportion of malignant tissue present. Inflammatory infiltrate may raise the DNA content of the gland irrespective of the cellularity of the tumour. These difficulties will remain until satisfactory techniques are available for separating the gland components.

However, with these limitations in mind, some evaluation of the results obtained by this method is possible. The high values for high-affinity [^{3}H]-DHT binding in the prostatic cytosol of some treated patients reported in this paper and in our previous work [23] are likely to be due to two causes. Firstly, it is known that estrogen treatment (and possibly also orchidectomy) raises the levels of SHBG in the blood [14]. This is confirmed in our work by the high [^{3}H]-DHT binding capacity of the serum in the treated patients. Since prostatic tissue extracts contain SHBG [4–9, 23], these high serum levels are reflected in the high-affinity binding of [^{3}H]-DHT by prostatic cytosol. Secondly, since estrogen treatment and orchidectomy both lower endogenous androgen levels, the androgen receptors present will be largely unoccupied by endogenous androgen and thus will be available for measurement in our assay. It may also be that, in the prostate gland unstimulated by androgen, a high proportion of receptors are present in the cytosol, whereas in the untreated patient, the majority of receptors may be present in the nuclei. The results of competition experiments in rat prostate cytosol and human serum show that the CA-inhibitable fraction of [^{3}H]-DHT bound approximates the amount of androgen receptor present. This amount was negligible in untreated subjects and control tissue, but approximated 2.3 fmol/mg in some treated patients. Rosen *et al.*[4] combined sucrose density gradient centrifugation of [^{3}H]-DHT labelled human BPH cytosol with radioimmunoassay of endogenous DHT present in the corresponding 8 *S* fraction (representing androgen receptor) of unlabelled cytosol. They estimated that as many as 90% of the androgen receptor sites were occupied by endogenous androgens, that the total receptor capacity in BPH cytosol was

2.6 fmol/mg, and that the SHBG binding component corresponded to 4.0 fmol/mg of tissue. The values reported in this paper for the concentration of receptor sites "emptied" by orchidectomy and/or estrogen treatment, and for the concentration of high affinity [^{3}H]-DHT binding not inhibited by CA in cytosol from untreated patients, are in complete agreement with their results. However, in our experiments, the concentration of [^{3}H]-DHT used for the incubation may have resulted in an underestimation of the concentration of binding sites in some human specimens, especially those from treated patients, whose total [^{3}H]-DHT binding capacity was high. As previously mentioned, this concentration was chosen to minimise low-affinity binding in prostatic tissue from untreated patients, and in order to obtain results comparable throughout the patient series, this concentration was used throughout. Wagner, using agar gel electrophoresis, has also reported low assayable receptor concentrations in normal prostate and BPH cytosol. No values were given for receptor concentration in carcinoma tissue, but they were stated to be variable [6]. We also found variable CA-inhibitable [^{3}H]-DHT binding in the prostatic tissue of treated patients (Fig. 2) even when variations due to differences in cellularity were eliminated by relating binding values to DNA concentration. This could be due to the ineffectiveness of treatment in lowering serum testosterone levels as in patient E.D. (Table 3), or to variations in the mean receptor content per cell. This may be the basis for differences in androgen dependence among prostatic carcinomas.

The method we have described in this report is rapid and extremely simple. It can be carried out in any laboratory equipped with an ultracentrifuge and a scintillation counter. We do not consider that the measurement of CA-inhibitable [^{3}H]-DHT binding gives a completely quantitative value for androgen receptor levels in the human prostate. CA does compete to a certain extent for high-affinity binding sites in serum also and the amount of competition appears to vary between sera from different patients. To this extent the method reported here will give an overestimate of the concentration of receptor sites. Also CA does not completely compete out high-affinity [^{3}H]-DHT binding to androgen receptor sites, which will therefore be underestimated by approximately 10%. However, the method described does permit a semi-quantitative evaluation of androgen receptor sites which are unoccupied by endogenous androgens. The total and CA-inhibitable-[^{3}H]-DHT high-affinity binding values reported here related well to the endocrine status of those patients in whom the latter was established. We are currently investigating a fully quantitative method, which will be the subject of a further report.

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