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A MINI-COLUMN METHOD FOR ROUTINE MEASUREMENT OF HUMAN PROSTATIC ANDROGEN RECEPTORS

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Summary—The complex heterogeneous nature of the human prostate gland is such that it is advisable to know the histological characteristics of each sample used for androgen receptor (AR) measurement. Adequate size of sample for AR determination is thus a problem if specimens provided during routine transurethral prostatectomy are to be used for both estimation of AR and histological examination. We present a simple method suitable for these small specimens in which [^{3}H]R1881 bound to AR is separated from free steroid on mini-columns of controlled-pore glass beads. Data obtained indicate a single class of binding sites of high affinity and low capacity with steroid specificity typical of an androgen receptor. The assay is suitable for samples as small as 20 mg wet weight and is linear using 25–125 μl cytosol (correlation coefficient 0.995). Intra-assay variation is 6.8% and interassay variation 25.8% ($n = 22$) over 4 months. A single saturating concentration of steroid measures 97% of AR calculated by Scatchard analysis. Inclusion of high salt (0.4 M KNO_3) and 10 mM dithiothreitol in incubation buffer at pH 8.4 are essential; inclusion of 10 mM sodium molybdate in the homogenisation buffer improves measurement. A comparison of AR measured in histologically similar samples obtained by a transurethral resectoscope (TUR) and a cold punch resectoscope (CPR) taken in juxtaposition demonstrated no difference in receptor content. Although carcinomatous samples contained significantly higher receptors levels than benign samples, no differences were observed between TUR and CPR specimens.

INTRODUCTION

Experience suggests that three-quarters of all patients with cancer of the prostate gland will respond to endocrine therapy for varying periods of time. Growth of the prostate is dependent upon androgens presumably acting upon androgen receptors (AR), therefore measurement of AR should define those patients more likely to respond. However progress with regard to successful prediction of response has been slow; this may be because of methodological difficulties inherent in prostatic AR measurement which do not occur to the same extent in other target organs or with other steroid hormone receptors. Problems include the presence of high levels of enzymic activity, the existence of other high affinity steroid binding components and, perhaps most importantly, the complex heterogeneous nature of the tissue.

Before conducting large-scale trials to determine the value of AR measurement it is important to have a reliable and reproducible assay suitable for routine use and also suitable sampling procedures for obtaining tissue. The sample used for AR measurement must be representative of the carcinoma and not of adjacent normal or benign hyperplastic tissue. It is therefore advisable to know the histological characteristics of each specimen. Open operations are now rarely performed. Part of each sample obtained during routine transurethral resection must be used for histological examination, thus the tissue remaining available for AR measurement will be small. Any assay chosen must be able to use these small specimens.

We describe a method, modified from that of Randall and Mainwaring [1] which fulfils these requirements. The principle of the method is as follows. AR, bound to the synthetic androgen [^{3}H]R1881 (methyltrienolone), is selectively retained by a mini-column of controlled-pore glass (CPG) beads. All other cytosol contaminants (which may interfere with the assay) and unbound [^{3}H]R1881 are washed through the column. Measurement of [^{3}H]R1881 eluted from the beads will give a direct measure of AR.

EXPERIMENTAL

Steroids

[^{3}H]Methyltrienolone (R1881, sp. act. 87 Ci/mmol), and radioinert R1881 and promegestone (R5020) were obtained from New England Nuclear Corporation (Southampton, U.K.). On arrival [^{3}H]R1881 was diluted to 400 nM with benzene–ethanol (9:1, v/v) and stored at 4°C. Milbolerone (a synthetic androgen, 7,17-dimethyl-19-nortestosterone) and Organon 2058 (a synthetic progestogen, 16-ethyl-21-hydroxy-19-norpregn-

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4-ene-3,20-dione) were obtained from Amersham International plc (Bucks, U.K.). Other unlabelled steroids were obtained from Sigma Chemicals (Dorset, U.K.).

Chromatographic materials

Glass balls (3.5–4.5 mm) were obtained from British Drug House (Middlesex, U.K.) and manually selected for size (3.5 mm) and uniform roundness. CPG beads (PG 500–200, mesh size 120–200) were purchased from Sigma Chemicals. Precision glass Pasteur pipettes were obtained from Bilbate Ltd (Coventry, U.K.).

Buffers and solutions

The following buffers were used. Homogenisation buffer (H): pH 7.4, 10 mM Tris, 1 mM EDTA, 1 mM dithiothreitol (DTT), 15% glycerol; Soaking buffer (A): pH 7.4, 10 mM Tris; Washing buffer (B): pH 8.4, 10 mM Tris, 0.4 M KNO_3; Incubation buffer (B+): pH 8.4, 10 mM Tris, 0.4 M KNO_3, 10 mM DTT; Incubation buffer (B++): pH 8.4, 10 mM Tris, 0.8 M KNO_3, 20 mM DTT. All water used was double distilled. DTT was added to solutions immediately prior to use. All pH values were determined at 4°C.

Measurement of radioactivity

Radioactive samples in aqueous–ethanol solution were mixed with 3.5 ml Beckman EP aqueous scintillation cocktail (Beckman, Bucks, U.K.) and allowed to equilibrate for 1 h prior to counting for 5 min in a Betatrac Liquid Scintillation Counter (Denley Ltd, Sussex, U.K.). Counting efficiency was 25–30% and was unusually low because of high ethanol quenching.

Tissue handling and storage

Samples were obtained during transurethral resection (TUR) surgery from patients presenting with prostatic obstruction and subsequently diagnosed as having either benign hyperplastic or malignant prostates. Specimens from the posterior peripheral region of the prostate (presumed peripheral zone) were obtained using the Trucut biopsy needle inserted perineally and guided by a finger in the rectum. The needle was palpated and guided to any suspect area when these were present. A second needle biopsy taken in juxtaposition (as located by ultrasound in some instances) was used for histological classification. Resected specimens were obtained using a Storz resectoscope with either the 24 French or 27 French sheaths, samples being taken both from the periurethal region and, towards the conclusion of the resection, from the presumptive capsule of the hyperplastic region and peripheral zone. TUR samples were carefully selected for assay; only large undamaged chips were used, and a portion of each specimen was taken for histological classification.

To investigate whether the TUR technique of obtaining samples caused erroneously low or falsely negative AR results as has been suggested by some groups [2, 3, 4] comparisons were made between this technique and samples obtained using a cold punch resectoscope which, as the name implies, generates no heat and does not cause tissue charring. The cold punch specimens were sampled entirely from the periurethral area towards the beginning of the resection using a Storz Mauermayer lithotrite with specially sharpened blades to obtain tissue specimens. Tissue was taken in juxtaposition by the two methods and the histological characteristics of each noted; only those pairs with similar features were used in comparisons.

All samples used for AR measurement were immediately (whilst still in the operating room) frozen in liquid nitrogen and stored frozen until required. All tissues were weighed before use, whilst still frozen. All subsequent procedures were carried out at 4°C unless specifically stated otherwise.

Cytosol preparation

The frozen tissue was pulverised using a microdismembrator (Braun, Melsungen, Germany) for 30 s at full speed. When the tissue weighed below 40 mg a 50 μl aliquot of buffer H was frozen in the Teflon vial with the tissue prior to pulverisation (the tungsten ball was frozen separately in this instance). The powder was carefully resuspended in 5 vol (w/v) buffer H. The homogenate was centrifuged at 105,000 g using a swing-out rotor for 30 min to separate the cytosol which was used immediately.

A quality control (QC) cytosol was included in each assay. A large volume of cytosol was prepared from BPH tissue obtained during retropubic prostatectomy and 1 ml aliquots were stored in liquid nitrogen until required. If the QC was AR negative, or Scatchard plots could not be drawn, or the AR value was outside two standard deviations from the mean, the results from other cytosols in the same assay were regarded as invalid.

To determine optimum assay conditions, for reproducibility, assay validation and steroid specificity studies, cytosol was prepared and divided into aliquots as for the preparation of QC.

Protein content of the cytosol was determined according to the method of Lowry [5] using bovine serum albumin as standard.

Modifications to androgen receptor assay procedure to determine optimum conditions

Preliminary work established the optimum assay conditions in an attempt to improve the suitability of the method for routine use. Some of the details are shown below.

Column preparation. A 50% suspension of CPG beads in buffer A was deaerated by vacuum pump for 1 h on the day of use with occasional very gentle swirling. Two-hundred μl settled volume of beads (settling time 15 min) in a 1 ml disposable syringe was

transferred to a Pasteur pipette blocked by a single 3.5 mm glass bead. The CPG suspension was immediately washed in with buffer A, allowed to settle under gravity and covered by a circle of glass fibre filter paper (Whatman grade GF/C). The use of a single glass ball to block the columns was found to give better reproducibility than the use of glass wool as recommended by Randall and Mainwaring [1]. Columns were used within 1 h of preparation; they were equilibrated with 2.5 ml buffer B and checked for signs of leakage, immediately before use.

Separation of bound and free [^{3}H]R1881. Solvent was evaporated from the [^{3}H]R1881 under nitrogen and the solid immediately redissolved in the appropriate incubation buffer (as described later) to prevent the generation of impurities. To determine total binding (TB) of steroid, cytosol was incubated with [^{3}H]R1881; to determine non-specific binding (NSB) of steroid a duplicate aliquot of cytosol was incubated with [^{3}H]R1881 in the presence of 100-fold excess unlabelled dihydrotestosterone. A 500-fold excess (relative to the radiolabelled material) of triamcinolone acetonide was also included in each incubate to prevent binding of [^{3}H]R1881 to progesterone receptors [6].

For Scatchard Analysis [7] a range of [^{3}H]R1881 concentrations (0.6–10 nM final concentration) was used. The concentration of AR was calculated from a Scatchard plot and expressed as fmol/g wet weight of tissue, the binding affinity (K_d) was calculated as moles/litre incubation mixture. Preliminary experiments used a single saturating concentration (SSC) of [^{3}H]R1881 (10 nM final concentration) and were performed in triplicate. Optimum conditions for AR labelling were investigated.

Labelling of AR on the column, according to Randall and Mainwaring [1], was carried out as follows. A 50 μl sample of cytosol was added to the column and allowed to run in. The column was washed with 8 ml buffer B to remove cytosol contaminants, with AR being retained by the column. Four-hundred μl of the appropriate [^{3}H]R1881 solution in buffer B+ was allowed to run in followed immediately by 200 μl and then by a further 400 μl of the same solution 30 min later to label the AR retained by the column. After varying periods of time (1–24 h) the column was washed with two 1 ml portions and then 20 ml buffer B.

Labelling of AR "in tube" was performed as follows. Fifty μl cytosol was added to 50 μl appropriate [^{3}H]R1881 solution (final concentration 10 nM) prepared in buffer B+ + (final salt and DTT concentrations of the incubate were the same as for the *in situ* column labelling method) and incubated for varying periods of time (1–24 h). The whole incubate was then transferred to the column and washed in with two 1 ml portions followed by 20 ml of buffer B.

The above procedures separated the free [^{3}H]R1881 from that bound to AR, with the free steroid being washed through the column and AR bound to either [^{3}H]R1881 (TB) or to DHT (NSB) being retained by the CPG bead columns. After this separation the [^{3}H]R1881 retained was allowed to dissociate from the receptor by leaving the columns for 1 h at room temperature. The steroid was then eluted with 1 ml absolute ethanol, the radioactivity counted and the AR concentration calculated.

Using either of these methods, specific binding (SB) of [^{3}H]R1881 was maximal at 16 h and decreased by 24 h thus 16 h incubations were adopted. Labelling of cytosol "in tube" was used in all further work as SB was higher, and NSB and background levels of radioactivity were lower when compared with labelling on the column (Table 1), thus giving greater sensitivity.

Binding of [^{3}H]R1881 was also measured in male serum diluted 1:10 with buffer H to give a similar protein concentration to cytosol. No SB was detected when labelled by either method, however, NSB was lower when labelled prior to separation on the column.

Optimum wash volume. Using SSC the effects of different washing procedures on TB, NSB and blanks (R1881 only) were investigated. The labelled cytosol or blank was applied to the columns and washed with varying volumes of buffer B from 0.5 to 20 ml, using 0.5 ml aliquots up to 5 and 1 ml aliquots thereafter.

The wash volume chosen for subsequent assays was 3 ml because with this volume blanks were consistently low and SB was constant. The smaller volume also has the major advantage that the assay is carried out more quickly (20 min compared to 140 min) thus making the assay more suitable for large numbers of samples and therefore suitable for routine use (12 Scatchards can easily be processed in one assay).

Table 1. Different labelling techniques of cytosol and serum

	Total binding	Non-specific binding	Specific binding	Blank
Cytosol				
Column labelling	1610 ± 177	784 ± 47	826 ± 107	275 ± 52
Tube labelling	1364 ± 57	130 ± 21	1234 ± 33	98 ± 16
Serum				
Column labelling	702 ± 84	707 ± 111	—	275 ± 52
Tube labelling	225 ± 44	243 ± 55	—	98 ± 16

Values are mean ± SE dpm eluted from column calculated from 3 replicates. Serum was diluted 1:10 with buffer H. In blanks buffer H replaced cytosol or serum. See text for further details of methods.

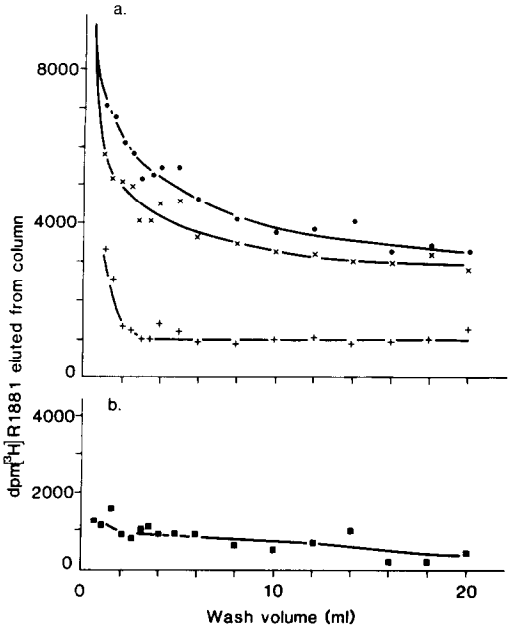

Fig. 1. Determination of optimum wash volumes. The effects of different washing procedures upon (a) total (●---●), non-specific (× --- ×) and blanks (+ --- +), and (b) specific binding (---). One-hundred μl aliquots of cytosol were labelled with the appropriate 10 nM [^{3}H]R1881 solutions (in triplicate) for 16 h at 4°C, applied to columns and washed with increasing volumes of buffer B from 0.5 ml to 20 ml, using 0.5 ml aliquots up to 5 ml and 1 ml aliquots thereafter. The mean ± SE dpm [^{3}H]R1881 eluted from each column are shown.

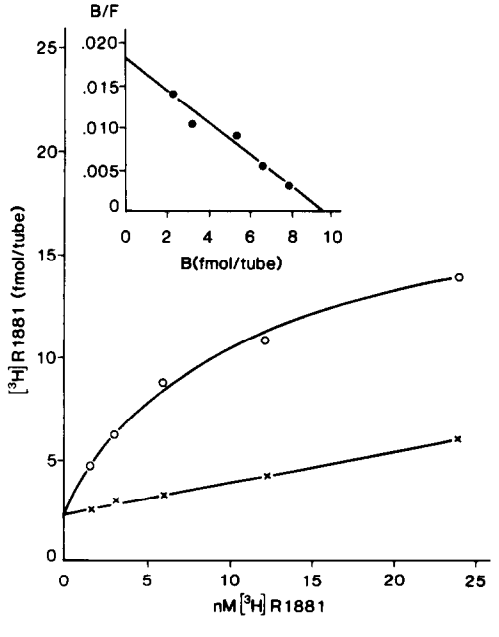

Fig. 2. Total (○---○) and non-specific (× --- ×) binding of [^{3}H]R1881 in human prostatic cytosol containing 893 fmol AR/g wet wt tissue, with binding affinity of 2.6×10^{-9} mol/l. For details of method see text. These data are also expressed in the form of a Scatchard plot (inset), B represents bound and F represents free [^{3}H]R1881.

Figure 1 is an example of the effects of increasing wash volumes on [^{3}H]R1881 binding in a cytosol containing a low concentration of AR. Similar results with regard to optimum wash volume have been obtained in further less detailed experiments using cytosols of varying AR concentrations. Although NSB was high in this particular cytosol other cytosols have shown NSB could be almost negligible relative to TB and not significantly different from blanks. A total volume of 3 ml discontinuous washing as described is preferable to the 20 ml continuous washing according to Randall and Mainwaring [1]. With larger volumes TB but not NSB was decreased resulting in lower SB, presumably due to dissociation of [^{3}H]R1881 from AR during the prolonged state of non-equilibrium.

Final modified method. An equal volume of cytosol was incubated with an equal volume of the appropriate [^{3}H]R1881 solution in buffer B + + and incubated overnight. The incubate (usually 100–200 μl) was transferred to the CPG bead column and washed in with 6 × 500 μl aliquots of buffer B, allowing each to run in before the next was added. After standing for 1 h at room temperature [^{3}H]R1881 retained by the column was eluted with 1 ml ethanol and the radioactivity in the eluate counted.

A representative binding curve with Scatchard plot is shown in Fig. 2.

Validation of method

The modified method was validated with regard to linearity, sensitivity and reproducibility. To demonstrate that binding occurred of [^{3}H]R1881 to AR only and not other cytosol contaminants, heat lability and steroid specificity were investigated.

RESULTS

Linearity and sensitivity of the assay

To determine the linearity of the assay procedures aliquots of cytosol (25–125 μl) were incubated with an equal volume of [^{3}H]R1881 (SSC) and applied to the CPG columns. SB of [^{3}H]R1881 was determined in each. The assay was found to be linear over this range of cytosol volumes with a correlation coefficient of 0.995 for SB (Fig. 3). High affinity binding was measurable in as little as 25 μl cytosol. Experience showed that values lower than 100 fmol/g wet weight tissue should be taken as negative.

Reproducibility

Eight replicate frozen cytosols were thawed and AR measured in the same assay using SSC [^{3}H]R1881. The mean TB, NSB and SB (calculated from the respective pairs) are presented in Table 2. The coefficients of variation (C.V.) were 4.0, 10.7 and 6.8% respectively, revealing an intra-assay variation of 6.8%.

Inter-assay variation was determined as follows. Aliquots of the same cytosol were included in each of

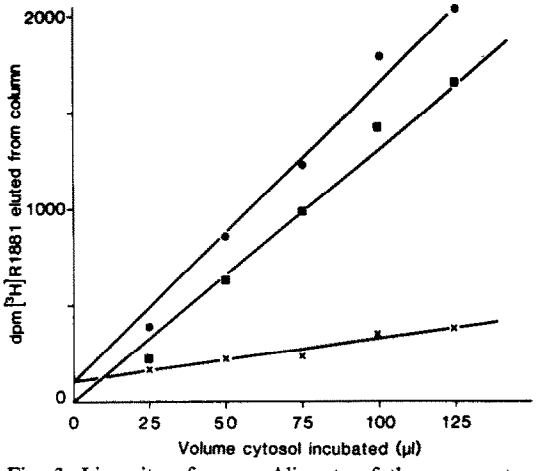

Fig. 3. Linearity of assay. Aliquots of the same cytosol (25–125 µl) were incubated for 16 h at 4°C with an equal volume of the appropriate [³H]R1881 solution to measure total (●——●) and non-specific binding (× --- ×) and applied to CPG columns. Specific binding (---) was expressed as dpm [³H]R1881 eluted.

22 assays over a period of 4 months. The cytosol contained 134.6 ± 7.2 fmol AR/ml of binding affinity $5.3 \pm 0.3 \times 10^{-9}$ mol/l (protein concentration 7.2 mg/ml). The inter-assay C.V. was 25.8%. There was no difference in AR measured in the first 11 assays (130.5 ± 11.1, C.V. 29.5%) compared with the last 11 assays (138.8 ± 9.2, C.V. 22.9%), although there was a slight improvement in reproducibility as shown by the lowering of the C.V. These results also demonstrate that cytosol stored in liquid nitrogen does not deteriorate over 4 months with regard to receptor content.

Single point determination compared with Scatchard analysis

A comparison of the saturating binding capacity of the same cytosol determined by Scatchard analysis with that determined by single point analysis revealed that both methods yield similar results. 14.5 ± 0.7 fmol/mg protein (mean ± SE, $n = 6$) were measured by a single saturating concentration, compared to 14.9 ± 1.6 fmol/mg protein ($n = 6$) by Scatchard analysis with a dissociation constant of $6.6 \pm 0.6 \times 10^{-9}$ mol/l. AR was measured alternately by each method over 12 consecutive assays.

Effects of dithiothreitol and molybdate on AR measurement

BPH tissue obtained from open prostatectomy was pulverised and the pooled frozen powder immediately freeze-dried. Lyophilisation has been shown not to produce qualitative or quantitative differences in recovery of prostatic AR [8]. Aliquots of powder were resuspended in the appropriate homogenisation buffer; the concentrations of DTT and sodium molybdate (Mo) buffers and B++ were varied as described in Fig. 4. AR were measured by Scatchard analysis and the results expressed in terms of fmol/g wet weight original tissue.

Figure 4a shows that the inclusion of DTT in buffer H had little effect on the concentration of AR measured, however, 1 mM DTT was routinely included as this gave a better Scatchard plot. In contrast, receptor levels increased with increasing concentrations of DTT up to a final concentration of 10 mM in buffer B++; in addition the quality of the Scatchard plot improved as the DTT concentration increased and at low DTT concentrations the K_d values were abnormally high. In a second experiment (not shown) AR could not be measured in either the absence of DTT or at low concentrations in the incubation mixture.

Increasing the concentration of Mo in buffer H (Fig. 4b) caused a continuous increase in measurable AR. This effect was only seen when Mo was present when the freeze-dried powder was resuspended; addi-

Table 2. Intra-assay variation

	Total binding	Non-specific binding	Specific binding
[³H]R1881 (dpm/column)	1188.5 ± 15.9	458 ± 16.2	718 ± 15.9
Coefficient of variation	4.05%	10.7%	6.77%

Values are mean ± SE and represent fmol [³H]R1881 retained by each column calculated from 8 replicates. For details of methods see text.

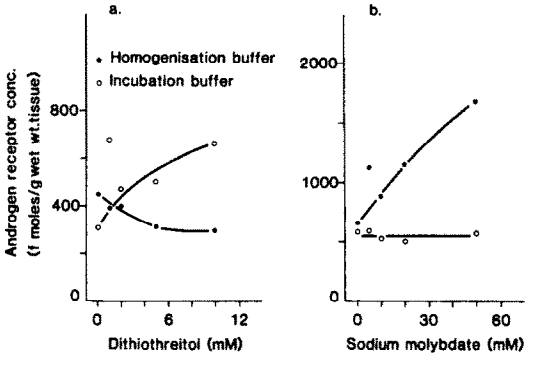

Fig. 4. Effects of dithiothreitol and sodium molybdate on measurement of androgen receptors. The effects of various concentrations of DTT (0–10 mM final concentration) and molybdate (0–50 mM final concentrations) added to either the homogenisation buffer (H) or to the incubation buffer (B++) were determined as follows. BPH tissue (7.7 g) obtained from open prostatectomy was pulverised and the pooled frozen powder immediately freeze dried and stored frozen in liquid nitrogen until required (within 2 weeks). 60 mg aliquots of powder were resuspended in 1.6 ml appropriate homogenisation buffer making allowances for loss of water to obtain the usual tissue:buffer ratio of 1:5 (wet wt:volume). The concentrations of DTT and Mo in buffer H were varied as indicated; in the presence of Mo 1 mM DTT was used. When DTT or Mo was varied in the buffer B++, "normal" buffer H was used to resuspend the powder. 10 mM DTT was included in buffer B++ at all times except when DTT was varied as indicated. Values represent fmol AR/g wet weight tissue and were determined from Scatchard analysis as described in text. (●——●) represents addition of DTT (a) or Mo (b) at homogenisation stage; (○---○) represents addition of DTT (a) or Mo (b) to incubation mixture.

Table 3. Steroid specificity of [^{3}H]R1881 binding in prostate cytosol

Competitor	10 nM (1-fold excess)	100 nM (10-fold excess)	1 mM (100-fold excess)	10 mM (1000-fold excess)
DHT	84.5	53.9	1.0	1.5
R1881	65.4	9.4	-0.9	-8.8
Mibolerone	94.1	59.9	20.5	12.7
Oestradiol	ND	83.5	76.2	20.0
Diethylstilboestrol	ND	100.4	96.2	90.9
Cortisol	ND	78.2	110.1	88.7
Triamcinolone acetonide	ND	79.0	71.3	69.8
R5020	ND	74.3	52.0	ND
Organon 2058	ND	66.3	54.9	61.5
Pregnenolone	ND	93.9	74.3	114.9
Progesterone	ND	ND	ND	49.8

Competition of binding of [^{3}H]R1881 (10 nM) to prostatic cytosol by various steroids. Non-specific binding (estimated in the presence of 1 mM DHT) was subtracted in all instances and the values shown are a percentage of the control (100%) and represent the mean of duplicate or triplicate determinations. ND indicates value not determined. For experimental details see text.

tion of Mo to the incubation mixture had no effect, with 94 ± 3% of receptors being measured compared to the control.

Steroid specificity

The steroid binding specificity of [^{3}H]R1881 was checked by incubating 100 μl cytosol with 100 μl [^{3}H]R1881 (final concentration 10 nM) in the presence and absence of 1, 10, 100 or 1,000-fold excess unlabelled steroid. The unlabelled steroid was dissolved in ethanol and a volume of 5 μl was added to each tube prior to the [^{3}H]R1881 and cytosol to give final concentrations of 10 nM, 100 nM, 1 mM and 10 mM. Control tubes contained 5 μl ethanol without unlabelled steroid. Each tube was treated following the same procedure as for "modified method" to measure AR. NSB, represented by the amount of binding not displaced by the 100-fold excess DHT, was subtracted in all instances to give SB. The displacement of binding by the competing steroid was expressed as a percentage of the control SB. The specificity of binding was typical of binding to an AR (Table 3). DHT, R1881, and mibolerone were found to be good competitors whereas oestradiol, Organon 2058, R5020 and triamcinolone acetonide were inefficient competitors. Oestradiol was however a good competitor at 1000-fold excess only. Pregnenolone, cortisol and diethylstilboestrol showed no competition.

Heat sensitivity

To demonstrate the heat lability of AR binding, cytosol was divided into two portions, one was heated at 45°C for 30 min and AR were measured using SCC method in aliquots from each portion. NSB was similar in both cytosols, however, TB was decreased in the heat-treated cytosol so that only 8% of SB remained compared with the control.

Investigation of methods for obtaining specimens

Samples were obtained as described in the Experimental section using a cold punch resectoscope (CPR) or a conventional transurethral resectoscope (TUR) from both benign and malignant prostates. Pairs of samples were taken in juxtaposition using the two procedures to minimise problems inherent in prostatic heterogeneity. Thus two samples obtained by different techniques were more likely to have similar histological and biochemical properties.

Thirty pairs demonstrated similar predominant histological characteristics, 10 pairs were dissimilar. Fifteen of the histologically matched pairs agreed on receptor status, in 7 both specimens were AR positive and in 8 both were AR negative. The receptor status differed in 15 pairs, CPR specimens only were AR positive in 8 cases whereas TUR specimens only were AR positive in 7 cases. Of the 10 histologically dissimilar pairs only three had the same receptor status and were all negative. Four CPR and three TUR samples were AR positive of those pairs which differed. Using these two sampling techniques, McNemara's test for significance shows there was no difference in receptor status of the two histologically similar groups.

Fifty percent of all CPR specimens and 47% of all TUR specimens contained measurable AR levels. In addition to AR status, the receptor concentrations in samples obtained by the two methods were compared (Table 4). AR levels were higher, although not significantly, in CPR specimens (778 ± 318 fmol/g tissue, mean ± SE, $n = 15$) than in TUR specimens (318 ± 67 fmol/g tissue, $n = 14$). It was evident that the highest AR concentrations were found in samples obtained from carcinomatous prostates. Carcinoma specimens contained 1,000 ± 384 fmol AR/g tissue ($n = 12$) whereas BPH specimens contained significantly lower levels of AR ($P < 0.02$) at 242 ± 29 fmol/g tissue ($n = 17$), irrespective of whether the specimens were obtained by the cold punch or transurethral resectoscope. When results were also classified as to both state of malignancy and method of sampling, AR levels of CPR and TUR specimens were not different in benign samples (240 ± 40 and 244 ± 46 fmol/g tissue, $n = 9$ and 8, respectively) and although levels were higher in carcinomatous CPR specimens (1585 ± 699 fmol/g

Table 4. Comparison of CPR and TUR sampling of carcinomatous and benign hyperplastic prostate

AR concentration (fmol/g tissue)	All samples ($n = 30$)		Malignant ($n = 11$)		Non-malignant ($n = 19$)		All samples	
	CPR	TUR	CPR	TUR	CPR	TUR	Maligant	Non-malignant
Mean ± SE	778 ± 318	318 ± 67	1585 ± 699	416 ± 140	240 ± 40	244 ± 46	1000 ± 383	242 ± 29
Median	226	230	934	306	190	202	461	212
Range	157–4746	104–1088	185–4746	166–1088	157–523	104–480	166–4746	104–523
% AR positive	50	47	55	55	47	42	54	45
Wet weight (mg)	107 ± 15	130 ± 16	114 ± 28	116 ± 18	108 ± 15	145 ± 21		

Androgen receptors were measured in pairs of specimens matched for histology and taken in juxtaposition from periurethral prostate during transurethral prostatectomy using a cold punch resectoscope (CPR) and a transurethral resectoscope (TUR). Eleven pairs were of carcinomatous tissue, 19 pairs were benign hyperplastic (BPH) tissue, 2 of these pairs were benign but from prostates in which carcinoma was present in further specimens taken from elsewhere in the gland.

tissue, $n = 6$) than in carcinomatous TUR specimens (416 ± 140 fmol/g tissue, $n = 6$), this difference was not significant.

Weights of specimens provided for AR measurement were similar for both methods of tissue sampling (Table 4). AR were measurable in as little as 24 mg tissue for CPR and 36 mg for TUR specimens.

DISCUSSION

We have described a simple, reproducible method suitable for measuring human prostatic AR in small specimens of known histological characteristics obtained during routine prostatectomy for obstruction in benign hyperplastic and carcinomatous prostates. Open prostatectomy is now very rarely performed. Samples from routine TUR prostatectomy are small and after some is sent for histology, the tissue remaining for receptor measurement is limited and a microassay is therefore necessary. The method is also suitable for specimens of needle biopsy size.

Androgen receptors were measured by exclusion chromatography in which porous glass granules (CPG beads) are used as molecular sieves. The AR are thought to be in a particular conformation so that they can be separated from other components by the column. Very large molecules which are excluded from the glass pores, will pass through the interstitial spaces whilst smaller molecules will be distributed between the solvent inside and outside the molecular sieve and will then pass through the column at slower rates. Free R1881 moves quickly through the column as demonstrated by the constantly low wash volume of blanks at 2 ml. AR with R1881 bound is of a size and conformation that will be retained by the column. It cannot be extracted from the column in its native state (Mainwaring, personal communication).

We have demonstrated that labelling of cytosol prior to separation on the column is preferable to labelling of cytosol components that have been already separated by the column. Using the former technique, assay sensitivity is greater; levels of radioactivity in the blanks and NSB of both cytosol and serum were lower. Although [^{3}H]R1881 does not bind specifically to serum components [9, 10] NSB to serum does occur. Thus NSB to cytosol may be due, at least in part, to serum contamination. Experiments with [^{3}H]DHT (not presented) demonstrated that SB to sex hormone binding globulin is not measured by this method, in agreement with Randall and Mainwaring [1]. NSB was, however, much lower when using [^{3}H]R1881 and this ligand does not undergo metabolism (unlike DHT) and exhibits some stabilising effect on AR [9].

We noted that reproducibility of the assay is better when new [^{3}H]R1881 is used and indeed, as the [^{3}H]R1881 ages, impurities increase and background levels of radioactivity increase. In spite of the well documented resistance of [^{3}H]R1881 to metabolism, the tritiated steroid demonstrates a remarkable in-

stability as a solid. If [^{3}H]R1881 was left as a solid for even a very short length of time during preparation of radioactive solutions, background levels of radioactivity were greatly increased and could be used to monitor the purity of the [^{3}H]R1881. Jensen and Hospelhorn (personal communication) have in fact used CPG beads to purify steroids. When impurities of [^{3}H]R1881 increase, poor quality Scatchards are produced and Smith *et al.*[11] have demonstrated that mishandling of [^{3}H]R1881 can result in a significant reduction in the number of AR sites measured.

The heat lability and steroid specificity studies indicate that only AR are measured by [^{3}H]R1881 in the presence of excess triamcinolone acetonide with NSB determined by competition with excess DHT. Using these conditions Scatchard analysis over an extended range of ligand gives a straight line plot with no evidence of curvature. The binding affinity of the receptor was sufficiently constant and the reproducibility of the assay such that a single point assay could be utilized for estimation of binding sites where tissues obtained were too small for Scatchard analysis. Even using the small volumes of cytosol suitable for this technique some needle biopsies are too small if protein concentrations are to be maintained at adequate levels (>2 mg/ml). Dilution of cytosol preparations to low protein concentrations leads to a high incidence of false negative results [12]. We have demonstrated that 97% of receptors measured by Scatchard plot can be measured by single saturating concentrations. However, if tissues are of adequate size we recommend that Scatchard analysis should be performed.

Conditions of high salt (0.4 M HNO_3) and alkaline pH 8.4 which favour the 4S form of receptor [13, 14, 15] are essential for measurement. The high concentration of DTT in the incubation buffer may be involved in maintaining the activated state of the AR as well as the transformed state, as suggested for glucocorticoid receptors [16] and progesterone receptors [17]. The present work indicates that the effects of Mo and DTT on AR can be additive if Mo is included in the buffer H but not if included only in the buffer B++. Inclusion of 10 mM Mo in buffer H is therefore recommended. We have similarly demonstrated that Mo influences measurement of progesterone receptors in cytosols prepared from malignant breast tissue only when included at the homogenisation stage and has no effect if added later [18]. Although progesterone receptors have recently been demonstrated in human prostatic tissue [19, 20] it is not thought that progesterone receptors in this assay contribute to the increased binding measured when Mo is included in buffer H; any binding of [^{3}H]R1881 to progesterone receptors is eliminated by triamcinolone acetonide [6]. Dahmer *et al.*[21] have suggested that molybdate–sulphydryl interactions might account for all of the Mo effects on steroid receptors. Further, they suggested that Mo does not interact with phosphate moieties of glucocorticoid receptors, acting elsewhere to prevent conformational changes that lead to irreversible inactivation after oxidation of the sulphydryl moiety required for steroid binding.

Using the Dunning R3327 prostatic tumour Rowley *et al.*[14] demonstrated that Mo acts to inhibit the salt-induced transformation of AR to the DNA-binding state, and also preserves the aggregated 8.5–9.0S form under high salt conditions where it would normally disaggregate to the smaller 4.4S form. Similarly salt-mediated transformation of progesterone [22], oestrogen [23] and glucocorticoid [24] receptors or temperature-mediated transformation of oestrogen [23], glucocorticoid [24] and androgen [25] receptors have been shown to be inhibited by Mo. Thus it is paradoxical that we have demonstrated that even though high salt conditions are essential for this method, Mo still appears to have a stabilising effect on AR if added prior to the salt. Smith *et al.*[11] and Sirett and Grant[26] have also demonstrated a stabilising effect of Mo upon prostatic AR.

Since open prostatectomy is now rarely performed, if AR are to be measured at routine operation, tissues obtained by TUR must be shown to be suitable for assay. There is, however, controversy regarding the use of TUR material for biochemical investigations. Some investigators have reported satisfactory results using such tissues whilst others consider results unsatisfactory, and suggest that erroneously low or negative AR results are obtained when compared to samples from open prostatectomy. It is possible that any differences observed may be due to differences in sample location and histological composition caused by the heterogeneous nature of the prostate gland rather than the effect of surgical procedures on the tissue. We have investigated these limitations. Firstly, the method described above permits the measurement of AR in samples of known histology (part of each specimen is used for histological characterisation). Secondly, we have taken tissue in juxtaposition to the TUR specimen using a cold punch resectoscope (CPR) which generates no heat, so that both samples are likely to have similar histological and thus biochemical features, and both are of periurethral tissue. In spite of these precautions 22% of paired specimens were histologically dissimilar. Of the remainder, we found no significant difference in AR status. Thus, if care is taken to select only those TUR specimens of adequate size showing little signs of heat damage, as in this study, satisfactory results can be obtained using TUR material. In agreement with our findings the groups of Ghanadian[3] and Habib[27] have also demonstrated that as long as special care is taken when selecting TUR chips, transurethral resection does not significantly damage the receptor proteins.

Pertschuk *et al.*[2] have shown in a histochemical study that severely burnt TUR specimens showed autofluorescence at the periphery of each sample; whilst beneath was a variable zone up to 200 μm wide where non-specific uptake of ligand conjugate

occurred. They excluded from their studies these damaged samples which were also receptor negative. Kyprianou *et al.*[4] compared nuclear AR in TUR and open prostatectomy specimens but found no correlation between the two methods, however, in their study only 30% of TUR specimens contained AR; these were the largest specimens and were free from charring. They also found no correlation between TUR and CPR specimens, but specimens were obtained from different areas of the prostate gland. They did find a significant correlation between CPR and open resection specimens.

We have also classified specimens into those from benign and malignant prostates. There was no significant difference in AR levels of CPR and TUR specimens from either benign or malignant prostates. However, specimens from malignant prostates contained significantly higher levels than those from non-malignant prostates, irrespective of whether the specimens were obtained using the transurethral or cold punch resectoscopes. This finding emphasises the need to know the histological characteristics of specimens investigated and a more detailed study of influence of histological characteristics upon AR in the human prostate is now being undertaken.

Mini-columns of controlled-pore glass beads thus seem to provide a sensitive and reproducible method for the separation of receptor-bound label from the free and non-specifically bound label in small amounts of cytosol. The method allows the routine measurement of AR in small TUR specimens of known histology and in needle biopsies. Progress with regard to successful prediction of response in cancer of the prostate may now be made more quickly.

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