Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 301–307. Pergamon Press. Printed in Great Britain

PROSTATE CONCENTRATIONS OF ENDOGENOUS ANDROGENS BY RADIOIMMUNOASSAY*

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(*Received* 6 *August* 1975)

SUMMARY

A method for simultaneously determining concentrations of major androgens in prostate has been developed. Extraction techniques used to isolate the androgens from minced tissue include homogenization with high-speed blades in Delsal's solvent mixture, adsorption to silica gel, followed by column and one thin-layer chromatography (t.l.c.). Radioimmunoassays (RIA) of small aliquots of t.l.c. eluates are used to quantitate picogram amounts of 5α-dihydrotestosterone (DHT) and 5α-androstanediols (Diol) and to estimate testosterone (T) and androstenedione (Ad). Contamination of blanks was reduced to RIA sensitivity limits primarily by treatment of glassware in a self-cleaning oven. The specificity of the method for each androgen was established by t.l.c. separations of known prostate metabolites, antisera specificities, and parallelism of sample aliquots to androgen RIA standards. The overall precision, in terms of coefficients of variation, was 21% for DHT and 24% for Diol. T and Ad could not be measured with acceptable precision because their very low concentrations in prostate (≤0·5 ng/g tissue) were less than RIA sensitivity limits. Accuracy studies indicated recoveries ranging from 96% for Diol to 121% for DHT. In human benign hypertrophic prostate tissue, DHT averaged 153 ng/g soluble protein (5·8 ng/g tissue) which was 17 times higher than values obtained in human spleen and kidney; Diol in prostate showed no consistent differences from values noted in kidney or spleen.

INTRODUCTION

Steroid hormone target organs such as prostate and uterus contain unique concentrations or unusual ratios of biologically active steroid metabolites compared to those in blood, urine and non-target tissues [1, 2, 3]. Measurements of steroids in the intracellular compartment may be important in identifying pathophysiologic states not detectable by extracellular compartment assays [4, 5]. Such an example is the elevated level of DHT in benign prostatic hypertrophy (BPH) as compared to normal prostate tissue, and its implication in the pathogenesis of BPH as hypothesized by Siiteri and Wilson [6]. Methodology for analysis of tissue steroids in general is needed. We have developed such methodology designed specifically for the assay of endogenous intracellular androgens in the human prostate.

EXPERIMENTAL

Solvents. Dried aliquots of all solvents were compared to blank tubes in the RIA. If significant interference was found, the solvents were redistilled in glass and retested. Distilled water was passed through a deionizer and stored in polyethylene carboys.

Cleaning of glass containers and pipets. Glassware (including glass vials reused for liquid scintillation counting) were soaked in mild detergent solution and rinsed with tap and deionized water. Traces of organic contaminants were then volatilized off the glass surfaces at 500°C for 6 h in a self-cleaning oven (Frigidaire Model RBE 94U). After each use, the t.l.c. tanks were rinsed with deionized water and "oven-cleaned." VirTis flasks and centrifuge tubes used to contain DHT in methanol were treated with a 1% Siliclad (Clay–Adams) solution after oven-cleaning.

Cleaning of metal equipment. All metal surfaces, including mincing scissors, forceps, homogenizing blades and shaft were cleaned with detergent solution and methanol, using ultrasonication when necessary. T.l.c. scraping razor blades were cleaned with ethyl acetate–acetone, 1:1 v/v.

Steroid nomenclature (name or trivial name acceptable by IUPAC-IUB; trivial name used here, if different; abbreviated):

5α-androstane-3α or 3β,17α or 17β-diol; androstanediol; Diol

5α-androstane-3,17-dione; androstanedione

5-androstene-3β,17β-diol; 5-androstenediol

4-androstene-3,17-dione; androstenedione; Ad

androsterone; A

dehydroepiandrosterone; DHA

5α-dihydrotestosterone; dihydrotestosterone; DHT

6,17-dimethyl-4,6-pregnadiene-3,20-dione; medrogestone; M

3-epiandrosterone; epi-A

17-epitestosterone; epi-T

testosterone; T.

* Presented at the 30th Annual Northwest Regional Meeting of the Americal Chemical Society, University of Hawaii, Honolulu, June 13, 1975. A preliminary report also appeared in *Clinical Research 23* (1975) 127A.

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Preparation and cleaning of columns and adsorbents. Disposable capillary pipets for collection of silica gel zones and 10-ml pipets for silica gel columns were plugged with glass wool, oven-cleaned, and treated with 1% Siliclad. Silica gel (100–200 mesh, ASTM D1319-61T, Grade 923) and aluminum oxide (neutral powder for chromatography, Brockmann Activity Grade 1) were each suspended in methanol to remove the fines by decanting. The adsorbents were dried in a vented oven at 60°C and then oven-cleaned. Silica gel G/MH, 0·25 mm on 20 × 20-cm. glass plates (Analtech Uniplates), was developed with methanol, air-dried, and stored in an air-tight chamber above Drierite.

Monitoring for adequacy of cleaning. Dried aliquots of solvent rinses of glassware and metal parts were compared to those of unused solvent by RIA.

Non-labelled steroid standards. Steroids (Steraloids, Pawling, NY, or Sigma Chemical Co., St. Louis, MO) were kept in a desiccator above Drierite and used without purification. Stock solutions of 10 mg/ml in absolute ethanol were stored below 5 C. Successive dilutions were made in methanol (or ethyl acetate for DHT) to prepare working standards of 1 ng/ml, which were stored at −15° C. All solutions were kept in glass tubes stoppered with Teflon-lined screw caps to minimize concentration changes. The most consistently reproducible and stable standards for RIA were found to be aliquots of 1 ng/ml solutions evaporated to dryness in assay tubes in the vacuum oven (NAPCO Model 15831) at 40°C and stored under Parafilm sheets at −15 C.

Radioactive androgens. [1,2-^{3}H]-5α-Androstan-3α,17β-diol (^{3}H-Diol), 44 Ci/mmol; [4-^{14}C]-testosterone (^{14}C-T), 0·0575 Ci/mmol; 5α-dihydrotestosterone-1,2-^{3}H (^{3}H-DHT), 44 Ci/mmol; [4-^{14}C]-5α-dihydrostestosterone (^{14}C-DHT), 0·0506 Ci/mmol; and [4-^{14}C]-4-androsten-3,17-dione (^{14}C-Ad), 0·0575 Ci/mmol, obtained from New England Nuclear (NEN), were diluted in benzene:ethanol, 9:1, v/v, to concentrations which were low enough to minimize corrections for pg of radioactive androgens in RIA aliquots and high enough to obtain adequate counting precision in aliquots for measurements of steroid recoveries. Only ^{3}H-labelled steroids of >40 Ci/mmol (NEN) were used for RIA. All solutions of labelled steroids were stored at 2–5°C in tightly-stoppered glass containers. Purities were monitored monthly by t.l.c., and no significant trends were noted over at least a 20-month period.

Solvent evaporation. Evaporation of "non-bumping" solvents (ethyl acetate, benzene, hexane, acetone, ethanol, chloroform, toluene and small amounts of methanol) were carried out in the vacuum oven at 40°C with water aspiration. Evaporations of solvents (ether and methylal) which tend to bump under vacuum, especially in tubes, and of solvents from silica gel were carried out in a 37°C water bath in a vented hood with aid of a gentle stream of dry nitrogen through a capillary pipet plugged with glass wool.

Specimens. Surgical prostate specimens were obtained by either transurethral resection (TURP) or suprapubic prostatectomy ("Open" tissue). Tissues from TURP were cleaned of excess blood and charred remnants, weighed and stored in Parafilm at −20 C until extracted. "Open" tissues were cleaned of connective and adipose tissue, minced, mixed to obtain homogeneous samples, and stored frozen as preweighed 2-g aliquots in Parafilm. Autopsy specimens were obtained within 38 h of death and frozen after cleaning and mincing as described for the "Open" tissues.

Homogenization. A 2-g sample of minced tissue, to which radioactive androgens had been added for determining steroid recoveries, was homogenized each of 3 times in 20 ml of Delsal's [7, 8] solvent (methylal–methanol, 4:1, v/v) at 23,000 rev./min with a VirTis Model 23 [6] for 5 min. Ice was added to the cooling cup surrounding the flask to keep the solvent from overheating and giving rise to higher blanks. The 3 supernatant extracts were combined and divided into replicates equivalent to 1-g extracts or portions thereof. The blank consisted of an identical volume of Delsal's solvent exposed to the same homogenizing conditions and apparatus.

For accuracy tests, both radioactive and non-radioactive androgens were added to combined supernatants of homogenates prior to dividing the supernatant into replicates. For determination of protein content, 100 mg of each tissue was homogenized in 1·0 ml of 0·05 M Tris buffer, pH 7·4, in ice water, using a Teflon pestle driven by a 2000 rev./min variable-speed drill and kept below the buffer surface to avoid foaming. The supernatant was analyzed for protein by a modification of the classical method of Lowry *et al.* [9], using bovine serum albumin as standard.

Silica gel adsorption of steroids [10] *and column chromatography* [8]. Silica gel (3–4 ml) was mixed into each sample and blank replicate. The solvent was very gradually evaporated at 37°C (to avoid bumping or blowing out the dry gel) with an extremely fine stream of nitrogen. When silica gel was dry enough to flow from the tip of the tube, about 3 ml of n-hexane was mixed with the gel-adsorbed sample and the solvent was evaporated to dryness.

A slurry of each silica gel-adsorbed sample in about 10 ml of hexane was transferred to a column and eluted successively with 15 ml more of hexane, which contained the bulk of non-steroidal lipids, and with 40 ml of ethyl acetate, which contained androgen metabolites. The 5% ether–benzene eluant used by Goldzieher, *et al.* [8] was omitted, since significant losses of labelled androgens occurred in our hands.

Thin-layer chromatography. 10 μg of medrogestone (Ayerst), non-reactive in our androgen RIA's, was added as an internal marker to each evaporated ethyl acetate eluate. Duplicate samples and their corresponding procedural blank were transferred in ethyl acetate onto a silica gel plate as 2-cm. streaks.

The plate was developed with benzene to remove some of the remaining nonpolar lipids [11]. Steroids were concentrated at a new origin by repeatedly placing the plate in an open trough of ether [12]. Separation of androgens was accomplished by developing with a fresh solvent system of chloroform:acetone, 92·5:7·5, v/v, at 2–5°C for 2 h 45 ± 15 min. This solvent system was also used by others [13–15] with apparently good separations of at least 5 androgens at room temperature. Our initial difficulty in obtaining consistently adequate separations was resolved when we maintained the temperature at 2–5°C.

The plate was sprayed with a methanol solution of 0·005% Rhodamine 6G, and markers were outlined in pencil under ultraviolet light. Androgens were located on the basis of previously determined R_F values (Ad = 0·88, 0·86; DHT = 0·67, 0·60; T = 0·50, 0·45; Diol = 0·34, 0·26) of the leading and trailing edges of the androgens relative to the leading and trailing edges of medrogestone, respectively.

Each androgen zone (and its parallel blank) was scraped from the plate and aspirated into the wide end of a disposable capillary pipet which had been plugged with glass wool and filled with 1–2 cm. of aluminum oxide [16] by aspiration. Androgens were eluted from the silica gel-alumina microcolumns into glass tubes with about 5 ml of ethyl acetate–acetone, 1:1, v/v. The eluates were evaporated to dryness and redissolved in 2·5 ml of ethyl acetate.

Recoveries and separations (or overlaps) of adjacent androgen isotopes on t.l.c. Forty per cent of each sample was counted in 8 ml of PPO scintillant (5 g of PPO/L of toluene + 1% Triton X-100, v/v), on both ^{3}H- and ^{14}C-channels to determine isotope recovery and androgen overlap. (Beckman LS counting efficiencies were ^{3}H = 55–58% for RIA, ^{3}H = 20–23% with cross-over of 3–5% of ^{14}C in the ^{3}H-channel, ^{14}C = 73–78% with cross-over of 0·5–3% of the latter ^{3}H-channel in the ^{14}C-channel.) An external standard, counted for every sample, indicated no quenching. The remainder of each sample was stored at −15°C until used for RIA.

Our ^{14}C- and ^{3}H-labelled androgens, found in alternating locations on t.l.c. (see relative R_F values), provided a highly sensitive means of determining the efficiency of separations, as well as the recoveries of the 4 androgens. Three pairs of radioactive androgens (^{3}H-Diol + ^{14}C-T, ^{14}C-T + ^{3}H-DHT, ^{3}H-DHT + ^{14}C-Ad) were chromatographed in separate lanes to determine the overlaps anticipated from the separation of all 4-radioactive androgens in a tissue extract.

Radioimmunoassays. Antisera for the DHT, Diol, and T assays were obtained from a goat inoculated with DHT-3-oxime-BSA conjugate (Endocrine Sciences). Antiserum for the Ad assay was purchased from Endocrine Sciences (No. AN 6-22). Antisera were diluted in 0·05 M borate, pH 8, to appropriate concentrations for RIA as needed. Included in the diluted working antisera were the corresponding ^{3}H-labelled androgens in appropriate concentrations, 0·1% bovine serum albumin (Schwarz–Mann No. 751) and 0·05% bovine gamma globulin (Schwarz–Mann No. 3004). Aliquots for each assay (at least 2 from each sample and blank) usually ranged from 25 µl (1/100) to 200 µl (1/12·5) depending on the androgen concentration anticipated from previous assay results. After removal of solvent, samples were redissolved in 250 µl of dilute antiserum and allowed to incubate for 16 to 22 h at room temperature.

Reagents and the ammonium sulfate separation technique were adapted from the method of Furuyama *et al.* [17]. Additional modifications included: preparing the 100%-free samples in PPO scintillant at the start of the incubation period to allow for the required equilibration time; omitting the 10-min incubation after adding sat. ammonium sulfate to assay tubes; directly pipetting 250 µl of unbound fractions from precipitates to counting vials, instead of first decanting the supernatants to other containers; vortex mixing the samples with 8 ml of the PPO scintillant in glass vials briefly, instead of mechanically shaking for 10 min; and counting to 2–5% S.D. (or 10 min).

Standard curves were constructed as the best fit of valid points, plotted as per cent free (unbound) radioactivity vs unlabelled androgen. Picograms of androgen in samples were read from their per cent-free intersections with the standard curve. A biostatistical evaluation of RIA data was made with the following quality control tests in order to verify each RIA: (1) Replicates greater than 2 S.D. (110 c.p.m.) apart were discarded. (2) If standards were within 2 S.D. of their respective inter-assay means (in % free), they were considered valid. The 0–100 pg ratio or "rise" was also monitored by cpm ratios of 100 to 0 pg (1·6–3·8 for DHT, 2·2–4·6 for T, 1·2–2·6 for Diol, and 1·3–4·7 for Ad). (3) If the mean pg readings of internal control pools (extracts, blanks, and differences) were within 2 S.D. of their respective interassay means, the RIA was considered valid. If these quality control limits for standards and controls were exceeded, the RIA was repeated. (4) Pg readings of blank extracts were examined to see if they were less than 2 S.D. above their interassay means for each aliquot size assayed. If these quality control limits were exceeded, the blanks were considered contaminated and the tissue extraction was repeated. These sensitivity limits (inter-assay blank means + 2 S.D.), according to Abraham [18], were established by assaying a number of different blank extracts at each aliquot size in a number of assays for each androgen.

Results were calculated using the following formulas:

$$\frac{\text{ng androgen}}{\text{g tissue}} = \frac{\text{mean}(X - B) - I}{R} \cdot \frac{2{\cdot}5\ \text{ml}}{\text{aliquot size (ml)}} \cdot \frac{1}{Y}$$

where

X = pg in sample aliquot
B = pg in blank aliquot (mean of all replicates used to minimize blank variability)
I = pg isotope in sample aliquot[a]
R = fraction isotope recovered
Y = g tissue

[a] Pg amounts of radioactive androgens in sample extracts were calculated from the cpm of large aliquots taken for recovery and overlap determinations times the specific activities. RIA measurements of specific activities of ^{14}C-T, -DHT, and -Ad confirmed the accuracy of our T, DHT, and Ad standards.

$$\frac{\text{ng androgen}}{\text{g protein}} = \frac{\text{ng}}{\text{g tissue}} \cdot \frac{1}{z}$$

where z = g protein/g tissue (from Lowry analysis)

RESULTS

T.l.c. Separation of radioactive androgens. The overlaps of androgen metabolite zones were tolerable, i.e., insignificant amounts of interfering steroids were present based upon their relative concentrations and antisera specificities. The most likely overlaps found (on the average, from many t.l.c. runs with alternating ^{3}H- and ^{14}C-androgens) were $\leq 2\%$ DHT in T or Ad zones, $\leq 2\%$ T in DHT or in Diol zones. Also, up to 3% of androstanedione may be found in the Ad zone.

Recovery. At each stage of the extraction it was found that recovery of the ^{3}H-DHT was consistently lower than other radioactive androgens. Most of the ^{3}H-DHT losses occurred during homogenization (mean = 30%), evaporation to dryness (mean = 12%), and silica gel chromatography ($\geq 10\%$). Overall recovery was better without tissue (63% vs 57%), suggesting adsorptive losses of ^{3}H-DHT on tissue particles in the Delsal's suspension. To obtain acceptable ^{3}H-DHT recoveries we found it necessary to use sharp homogenizing blades, silica gel to adsorb androgens during evaporation, Siliclad-treated glassware, redistilled methanol in the Delsal's solvent mixture, and ethyl acetate as solvent in other steps. Recent studies with ^{14}C-DHT have provided improved recoveries, suggesting loss of ^{3}H from ^{3}H-DHT.

Radioimmunoassay precision. Intra-assay coefficients of variation (C.V.'s) for androgen standards and pooled androgen extracts of prostate samples and blanks (internal quality controls) ranged from 5·7 to 10% over the 100-pg range used. Interassay C.V.'s ranged between 5 and 15% for standards and between 13 and 17% for controls. C.V.'s were higher in the low and high ranges of standard curves; for this reason, sample results were accepted only for those aliquots which gave pg readings in the middle of the standard curves (15–70 pg). Standard curves for the RIA of DHT and Diol using our goat antiserum are displayed in Figs. 1 and 2, respectively.

Sensitivity. Sensitivity limits (inter-assay blank means + 2 S.D.) for each of the 4 assays ranged from 3 to 44 pg for 1/250 to 1/25 aliquot, respectively, of the total blank extract. Most of the samples were measured in a range in which the sensitivity was 10 to 22 pg (1·0–1·1 ng androgen/g tissue).

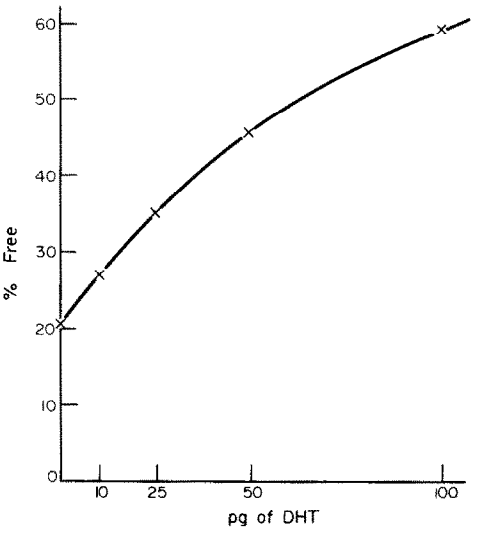

Fig. 1. RIA standard curve: % Free ^{3}H-DHT vs pg DHT.

Methodological precision. The intra- and inter-assay precision of the entire method for each of the 4 androgens were determined using 2 "Open" BPH tissues separately. The coefficients of variation ranged between 21% for DHT and 24% for Diol (Table 1). Unacceptably high C.V.'s (49–99%) were obtained for T and Ad.

Accuracy. The accuracy of the method in recovering 1 to 5 ng amounts (corrected for isotope recoveries) added to pooled supernatant replicates of BPH tissue homogenates appears adequate for DHT and Diol (Table 2). Recoveries of both T and Ad appeared better than expected on the basis of precision studies and may have been related to the higher concentrations measured.

Tissue storage. No statistically significant (>2 S.D.) changes, based on our inter-assay precision (Table 1), in androgen concentrations of a minced BPH tissue

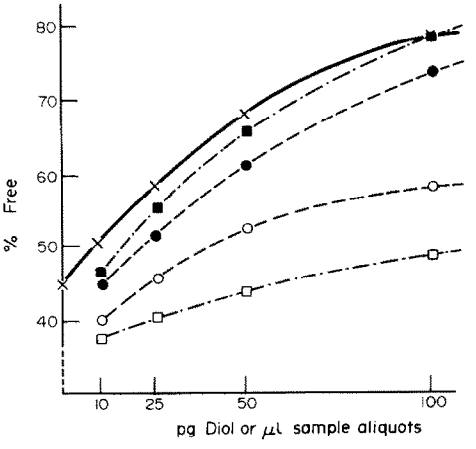

Fig. 2. Comparison of prostate (·····●), kidney (-·-■), and blank (○, □) extracts to Diol standards (——×).

Table 1. Precision of tissue extraction—RIA method

	Intra-assay N = 4		Inter-assay N = 3	
	DHT	Diol	DHT	Diol
C.V.	21	24	19	22
mean, ng/g tissue	9·0	2·9	6·3	1·8

Table 2. Recoveries of added androgens

	DHT		Diol		T		Ad	
ng Added	1·0	5·0	1·0	5·0	1·0	2·0	1·0	2·0
% Recovered	110	121	107	96	54	65	110	122
S.E.		10		24		10		2
N Extracts	1	3	1	2	1	3	1	2

occurred up to 12 h at room temperature plus 26 h of refrigeration. All sample tissues were therefore processed (for extraction or freezer storage) within these limits of time and temperature, since an additional refrigeration time caused significant decrease in DHT and a striking increase in Ad (Diol and T remained unchanged). Tissues not wrapped in Parafilm became desiccated while stored frozen, which resulted in significantly higher amounts of androgens per unit of tissue mass in comparison to values of non-desiccated tissues. However, we noted no significant differences from non-desiccated tissues when these same androgen concentrations were expressed in terms of tissue protein mass. This suggested that concentrations expressed in ng androgen/g protein would be more reliable for comparisons of tissues varying in water content (from dehydration to edema).

Specificity. The specificity of the method for identification of each of the androgens in prostate extracts was established by the following criteria: (1) constancy of DHT in 3 prostate extracts after t.l.c. in our solvent system (5·6 ± 1·1 S.D. in ng/g tissue) as compared to RIA of the same 3 eluates after t.l.c. in different solvent systems (5·1 ± 1·1 S.D.) developed by Farnsworth and Brown for the same androgens (toluene–methanol–methylal, 88·5:1·5:10, by vol., unsaturated, followed by toluene–methanol, 189:11, v/v, saturated; private communication); (2) the t.l.c. separation of androgen metabolites reported by others to be present in prostate extracts subjected to the same or similar solvent systems as used by us and the antisera specificities of our androgen assays (Table 3); and (3) parallelism between androgen standards and sample aliquots, and non-parallelism between androgen standards and aliquots of blank extracts (Fig. 2).

Tissue androgen concentrations. Androgen concentrations in 4 different types of human tissue were compared (Table 4). Average DHT levels in ng/g tissue were 6–10 times higher in prostates of patients with untreated BPH as compared to normal kidney, normal spleen, or to prostatic cancer tissue from patients with low circulating testosterone because of castration and/or estrogen therapy. When the data was expressed in terms of ng androgen/g protein, even greater-fold differences were noted (17 times more DHT in BPH than in kidney or spleen). Concentrations of Diol did not appear to be remarkable in prostates of patients with BPH as compared to these other tissues.

DISCUSSION

Despite the potential importance of intracellular steroid assays for both clinical and investigative purposes, very few tissue steroid analyses have been reported. To date, the method of Siiteri and Wilson appears to be the only reliable published technique for the measurement of the low amounts of endogenous androgens in prostate [6], but it does not appear to have been adopted by others.

Table 3. RIA specificities

Androgen RIA	Other possible metabolites present within or adjacent to androgen zones	% Cross-reactivity at same % free Found in the androgen t.l.c. zone	Found adjacent to the androgen t.l.c. zone
Diol	T		100
	3β,17β-Diol	88	
	5-androstenediol	42	
T	DHT		109
	3α,17β-Diol		30
	Androsterone(A)		12
	epi-T	5·8	
	epi-A	2·8	
DHT	T		65
	Ad		16
	A	11	
	DHA	3·4	
Ad	Androstanedione		35
	A		3·5
	DHT		0·5

Table 4. Human tissue androgen concentrations

Tissue:	Means ± S.E. (for N tissues) Open BPH (N = 6)	Prostate Cancer[a] (N = 4)	Kidney[b] (N = 1)	Spleen[b] (N = 1)
Androgen	A. ng Androgen/g tissue			
DHT	5·8 ± 1·1	0·88 ± 0·22	0·65[c]	0·56[c]
Diol	2·7 ± 0·3	3·5 ± 0·9	6·0	2·1
T[c]	0·15 ± 0·06	0·07 ± 0·04	0·29	0·35
Ad	0·64[c] ± 0·07	0·74[c] ± 0·14	1·5	6·1
	B. ng Androgen/g protein			
DHT	153 ± 39	28 ± 6.	8·6[c]	9·0[c]
Diol	75 ± 8·6	116 ± 18	79	34
T[c]	4·0 ± 1·6	2·5 ± 2·0	3·9	5·8
Ad	17[c] ± 1·1	27[c] ± 7	20	100

[a] castrated and/or estrogen-treated
[b] normal
[c] semi-quantitative values

We have attempted to develop a method for simultaneous assay of 4 androgens in human prostate tissue. The major advantage of our method over the modified double-isotope derivative assay of Siiteri and Wilson is its relative simplicity. Our tissue analysis of 4 steroids can be completed in 7 days, including 30% of the time used for steps involving evaporation of organic solvents. RIA incubation and sample counting. Except for the cleaning of glassware at 500°C and meticulous precautions to prevent contamination detected by sensitive radioimmunoassay, our method utilizes a few relatively basic techniques, including homogenization, solvent extraction, silica gel adsorption chromatography in a column and on one thin-layer plate.

Our method for DHT and Diol appears to have adequate precision, sensitivity, and accuracy in comparison to other published steroid methods [4, 18–20]. Although C.V.'s of our entire method for DHT (21%) and Diol (24%) were somewhat higher than those (10–12%) reported for T, DHT and Diol in testes extracts [21, 22], our method measured pg amounts by RIA whereas the others measured ng amounts by competitive protein binding analysis. In tissue steroid analyses reported with this latter technique [21–23] or with gas chromatography [24–26] sensitivity was limited to about 5 ng/g tissue, which is inadequate for measuring androgen concentrations in a gram or less of prostate. In the only other published pg-sensitive method for the assay of endogenous androgen metabolites in human prostate, Siiteri and Wilson did not report precision studies.

The very low concentrations of T and Ad (≤0·5 ng/g prostate) prevented their precise quantitation, and the values found can be considered approximations only. Because relatively large aliquots of extracts were needed for detection of measurable amounts of steroids, relatively high blanks were encountered. As a result, the net pg amounts of the T and Ad found in the prostate did not exceed our sensitivity limits as defined by Abraham [18].

Recoveries ranged from 96% for Diol to 121% for DHT (Table 2) in our accuracy tests of 1–5 ng added to tissue homogenates. Siiteri and Wilson listed a recovery of 86% for 23 ng DHT added to water [6].

The criteria we have relied upon for identification of the specific steroids in our immunoassay (Results: *Specificity*) have also been used by others for measuring these androgens in human plasma or tissue [4, 22, 27]. Additional steroid metabolites identified by others in human prostate by *in vivo* and *in vitro* pulse-labelling techniques [3, 13, 14, 15, 28, 29], and listed in Table 3, would not contribute significantly to the RIA of DHT or T, since they had little cross-reactivity with our antisera. We have not attempted to separate Diol isomers in order to determine the relative concentrations of each in tissues.

The very low levels of DHT found in other tissues examined and in prostates of castrated patients (Table 4) compared to BPH tissue, and the similarity of our results to those reported by Siiteri and Wilson [6] working with a method of established specificity, lend further support to the reliability of our method for the identification and quantitation of DHT.

The presence of contaminating androgens on glassware and other equipment in contact with tissue extracts has led to falsely higher values. In order to overcome these artifactual problems and to consistently obtain blanks below the RIA sensitivity limits, we cleaned all glassware in a self-cleaning oven, as first described by Kushinsky and Paul [30] for this purpose. In addition, other cleaning techniques were used and special precautions were taken to avoid cross-contamination of samples, blanks, RIA reagents, isotope solutions, solvents, evaporation apparatus, chromatographic material and equipment.

Low DHT recovery appeared to be a combination of adsorption to glass surfaces and tissue, instability, and susceptibility to degradation by solvent impurities. The DHT decomposition that we found after evaporation of methanol (unpublished data) confirmed the observations of Haltmeyer and

Eik-Nes [24]. Techniques previously mentioned helped to increase our recovery of DHT to over 30%, in agreement with DHT recoveries reported by Podesta and Rivarola [22] for tissue and by others [19, 20, 24] for plasma analyses.

Acknowledgements—We are deeply grateful for (1) financial support from the National Cancer Institute (NIH Grant No. 5 RO1 CA12448-04 and -03), from the Edna Dowdy Fund for cancer research at Mercy Hospital, and Mrs. Albert D. Lasker of New York City; (2) the medical research facilities and support personnel of Mercy Hospital; (3) the cooperation of urologists, pathologists and their staffs at Mercy Hospital and the Veterans Administration Hospital at San Diego who supplied tissue samples; (4) the consultation by Dr. Stanley Kushinsky on the oven-cleaning of glassware, and by Mr. Frank Ewing and Dr. Guy Abraham on RIA calculations and quality control; and (5) programming of the Olivetti Programma 101 by Mr. Thomas Cantor and assistance in the calculations by Miss Jamie Geller.

REFERENCES

1. Bruchovsky N. and Wilson J. D.: *J. biol. Chem.* **243** (1968) 2012–2021.
2. Braunsberg H., Irvine W. T. and James V. H. T.: *Br. J. Cancer* **21** (1967) 714–726.
3. Becker H., Kaufmann J., Klosterhalfen H. and Voigt K. D.: *Acta endocr. Copenh.* **71** (1972) 589–599.
4. Strickland A. L., Apland M. and Bruton J.: *Steroids* **21** (1973) 27–46.
5. Mahoudeau J. A., Delassalle A. and Bricaire H.: *Acta endocr. Copenh.* **77** (1974) 401–407.
6. Siiteri P. K. and Wilson J. D.: *J. clin. Invest.* **49** (1970) 1737–1745.
7. Delsal J. L.: *C.r. hebd. Séanc. Acad. Sci., Paris* **244** (1957) 2252–2255.
8. Goldzieher J. W., Baker R. A. and Riha E. C.: *J. clin. Endocr. Metab.* **21** (1961) 62–71.
9. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
10. Kushinsky S., Demetriou J. A., Nasutavicus W. and Wu J.: *Nature, Lond.* **182** (1958) 874–875.
11. Bullock L. P. and Bardin C. W.: *J. steroid Biochem.* **4** (1973) 139–151.
12. Leav I., Morfin R. F., Ofner P., Cavalos L. F. and Leeds E. B.: *Endocrinology* **89** (1971) 465–483.
13. Pike A., Peeling W. B., Harper M. E., Pierrepoint C. G. and Griffiths K.: *Biochem. J.* **120** (1970) 443–445.
14. Briggs M. H. and Briggs M.: *J. clin. Endocr. Metab.* **36** (1973) 600–604.
15. Harper M. E., Pike A., Peeling W. B. and Griffiths K.: *J. Endocr.* **60** (1974) 117–125.
16. Mayes D. and Nugent C. A.: *J. clin. Endocr. Metab.* **28** (1968) 1169–1176.
17. Furuyama S., Mayes D. and Nugent C. A.: *Steroids* **16** (1970) 415–428.
18. Abraham G. E.: *Acta endocr., Copenh. Supp. 183* **75** (1974) 7–42.
19. Barberia J. M. and Thorneycroft I. H.: *Steroids* **23** (1974) 757–766.
20. Coyotupa J., Parlow A. F. and Abraham G. E.: *Analyt. Let.* **5** (1972) 329–340.
21. Morse H. C., Horike N., Rowley M. J. and Heller C. G.: *J. clin. Endocr. Metab.* **37** (1973) 882–886.
22. Podesta E. J. and Rivarola M. A.: *Endrocrinology* **95** (1974) 455–461.
23. Nilsson I.: *Acta Obstet. Gynec. scand.* **51** (1972) 117–120.
24. Haltmeyer G. C. and Eik-Nes K. B.: *Analyt. Biochem.* **46** (1972) 45–61.
25. Ruokonen H., Laatikainen T., Laitinen E. A. and Vihko R.: *Biochemistry* **11** (1972) 1411–1416.
26. Karunakaran M. E., Pochi P. E., Strauss J. S., Valerio E. A., Wotiz H. H. and Clark S. J.: *J. Invest. Dermat.* **60** (1973) 121–125.
27. Brenner P. F., Guerrero R., Cekan Z. and Diczfalusy E.: *Steroids* **22** (1973) 775–794.
28. McMahon M. J. and Thomas G. H.: *J. Endocr.* **48** (1970) xx–xxi.
29. Hampl R., Rohling S., Petrik R. and Starka L.: *Endocr. Exp. (Bratisl)* **6** (1972) 147–156.
30. Kushinsky S. and Paul W.: *Analyt. Biochem.* **30** (1969) 465–467.