ANDROGEN LEVELS IN THE PLASMA AND PROSTATIC TISSUES OF PATIENTS WITH BENIGN HYPERTROPHY AND CARCINOMA OF THE PROSTATE

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SUMMARY

Specific radioimmunoassays for testosterone, dihydrotestosterone (DHT) and androstenedione were carried out to measure the concentrations of the three hormones in the plasma and prostatic tissue of ten patients with benign prostatic hypertrophy (BPH) and ten patients with carcinoma of the prostate.

The results indicate that there are no significant differences between the peripheral plasma concentrations of testosterone, DHT and androstenedione in BPH [19.7 ± 2.6, 2.6 ± 0.9 and 5.5 ± 1.7 (s.e.m.) nmol/l respectively] and in carcinoma [16.9 ± 2.8, 2.4 ± 0.5, 4.4 ± 1.1 nmol/l respectively], (in all cases P > 0.1). In contrast, the prostate tissue ratios DHT : testosterone (3.59 ± 0.55 for BPH and 0.66 ± 0.09 for carcinoma) and androstenedione : testosterone (2.83 ± 0.38 for BPH and 1.07 ± 0.16 for carcinoma) are significantly less in carcinoma than in benign hypertrophy (in all cases P < 0.01).

The accumulation of testosterone in the carcinoma, relative to values found in BPH tissue is, therefore, not associated with changes in the concentrations of androgens in the plasma pool but may be related to local factors and metabolic changes within the prostate.

INTRODUCTION

A major difference between the prostate and other accessory reproductive glands is its susceptibility to hypertrophy and neoplasia in ageing men. The need to understand the biochemical factors in these diseases has stimulated a wide interest in the field of prostatic research and it has been suggested by a number of investigators that an endocrine imbalance in the ageing male might be causal in this process (Huggins, 1947; Ofner, 1968). Although recent reports are unanimous in showing that the plasma testosterone is significantly lower in men over the age of 60 than in men between 18 and 40 years of age (Vermeulen, Rubens & Verdonck, 1972; Pirke & Doerr, 1975), data on the concentration of 17β-hydroxy-5α-androstane-3-one (5α-dihydrotestosterone, DHT) in blood remain confusing (Mahoudeau, Delasalle & Bricaire, 1974; Horton, Hsieh, Barberia, Pages & Cosgrove, 1975; Pirke & Doerr, 1975). The human prostate requires the presence of androgens for its normal development and maturation (Huggins & Clark, 1940) and hypertrophy of this gland does not occur in subjects castrated in later life (Huggins & Clark, 1940; Balogh & Szendro, 1968), suggesting that androgens are directly or indirectly involved in the hypertrophic process. However, there is no evidence that benign prostatic hypertrophy is brought about by any

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change in the total hormonal status of the patient (Becker, Kaufmann, Klozterhalfen & Voigt, 1972; Steins, Krieg, Hollmann & Voigt, 1974) but local factors within the prostate may be involved. There is no difference between the normal prostate and the hypertrophied gland in the total content of testosterone and 4-androstene-3,17-dione (androstenedione) but there is a fivefold increase in the concentration of DHT in benign hypertrophy (Siiteri & Wilson, 1970).

The rate of conversion of testosterone to DHT is the same in hypertrophic prostatic tissue as in the normal prostate, and the raised concentrations of DHT can be related to abnormal accumulation of the metabolite (Siiteri & Wilson, 1970). Since DHT is a potent stimulant of hyperplasia in canine prostates (Gloyna, Siiteri & Wilson, 1970) its accumulation in the human gland suggests that it may be associated with the equivalent condition in man.

The relationship of androgens to the development of prostatic neoplasia is unclear, and the present study was designed to explore the relationship between testosterone, DHT and androstenedione and carcinoma of the prostate in man. We have therefore measured the concentrations of the three androgens in prostatic tissue and plasma samples from patients with benign hypertrophy and carcinoma of the prostate.

**MATERIALS AND METHODS**

**Radiochemicals and steroids**

\[1,2,6,7-^3\text{H}]\text{Androstenedione} (sp. act. 83 kCi/mol), \[4-^{14}\text{C}]\text{Androstenedione} (sp. act. 60 kCi/mol), \[1,2,6,7-^3\text{H}]\text{DHT} (sp. act. 47 kCi/mol), \[1,2,6,7-^{13}\text{H}]\text{Testosterone} (sp. act. 91 kCi/mol), \[4-^{14}\text{C}]\text{Testosterone} (sp. act. 59 kCi/mol) were obtained from the Radiochemical Centre, Amersham. Non-radioactive steroids were obtained from Organon Laboratories, Lanarkshire, Scotland. Purity of radioactive steroids was checked every 2 months by paper chromatography on a Bush A system [petroleum ether (b.p. 80-100°C): methanol: water (10:8:2, by vol.)] at 37°C (Bush, 1952) followed by scanning in a Nuclear Chicago Actigraph III radiochromatogram scanner. The purified radioactive steroids were eluted with methanol.

**Other reagents**

All reagents were of A.R. grade except for ethylene and propylene glycol (Chromapak Vickers Ltd, Wharfedale Labs, Burley-in-Wharfedale, England), 2,5-diphenyloxazole (PPO) and 1,4-dimethyl-(5-phenylenazoyl)-benzene (dimethyl POPOP) (Hopkins and Williams Ltd, Freshwater Road, Chadwell Heath, Essex) and the Triton X-100, charcoal and dextran (Sigma Chemical Company, Kingston-upon-Thames, Surrey, England).

The charcoal-dextran mixture (10:1, w/w) was prepared in Tris-HCl buffer, pH 8·5, at a concentration of 12·1 g/l. Before use the charcoal-dextran solution was mixed for at least 20 min. Celite 545 (Johns-Maneville Ltd, Parkridge House, The Little Green, Richmond, Surrey) was purified with 6 m-HCl according to the method described by Korenman, Perrin & McCallum (1969).

**Prostatic tissue and plasma samples**

Prostatic tissue was obtained at the time of transurethral resection from ten patients between 60 and 87 years of age (age mean = 72) with BPH and ten patients between 64 and 91 years (age mean = 73) with carcinoma of the prostate. The resection chips were split lengthways; one of each pair was used for histological examination and the other for hormone analysis.

Blood was drawn from each patient within 2 min of the induction of halothane anaesthesia between 11·00 and 12·00 h and placed into heparinized tubes. Plasma was separated.
and along with the tissue was stored at $-25^\circ C$ for 1–4 months before determinations were made.

None of the patients included in this study had previously undergone any major surgery, none was receiving any form of hormonal therapy and all were Caucasians.

**Androgen extraction and chromatography**

Celite columns were employed, as described by Barberia & Thorneycroft (1974) to separate testosterone, DHT and androstenedione from other androgens which are present in significant concentrations in blood and prostatic tissue.

Finely minced prostatic tissue (20 mg dry wt) was placed in glass tubes and 24 000 d.p.m. each of tritiated testosterone, DHT and androstenedione in Tris–HCl buffer, pH 8.4 (0.1 ml) were added to monitor manipulative losses. After standing for 45 min at room temperature the sample was homogenized in two consecutive aliquots of 0.5 ml Tris–HCl buffer, pH 8.4, with the strokes of a tightly fitting glass piston. Steroids were extracted by mixing three times with 2 ml anhydrous ethyl ether and after freezing the extract in a solid CO$_2$-methanol bath, the pooled organic phase was dried under vacuum for subsequent chromatography.

The blood extraction method was identical to that of the tissue except that 0.3 ml of the plasma was used as starting material.

The dried ether extracts were redissolved in iso-octane and separated on chromatography columns (1 × 5 cm) packed with the purified Celite. A 1:1 mixture of propylene glycol:ethylene glycol was used as stationary phase and the columns were washed with 2 × 3.5 ml of iso-octane followed sequentially by 3.5 ml portions of 5% benzene in iso-octane and 40% benzene in iso-octane. Eluates were collected as 0.5 ml portions. Fractions 4–10, 12–19 and 24–28 contained the androstenedione, DHT and testosterone respectively and were combined. Duplicate samples from each of the combined eluates (0.1, 0.0 and 0.1 ml for the plasma and 1.0, 1.5 and 1.0 ml for the tissue) were transferred into 75 × 9.5 mm assay tubes and dried under reduced pressure before radioimmunoassay (RIA). Two ml of the remainder of each fraction were added to scintillation vials, dried down and subsequently counted for individual recovery estimations.

**Radioimmunoassay**

The testosterone was determined by RIA using a testosterone antiserum, raised in a rabbit against testosterone-3-([O-carboxymethyl]-oxime–bovine serum albumin conjugate and kindly given to us by Miss S. S. Eccles of the Division of Steroid Endocrinology. Dextran-coated charcoal was used for removing the free steroid and the bound fraction was counted in a liquid scintillation counter. The assay for DHT and androstenedione is, in principle, the same as that for testosterone, save only that standards and traces for testosterone were replaced by those for DHT and androstenedione. The testosterone antiserum showed a 52% cross-reaction with DHT and was therefore used for both assays. The dilution of the antibody was 1:10 000 for testosterone and 1:7000 for DHT. The antiserum for the androstenedione was kindly supplied by Dr M. Johnson of Queen Charlotte's Hospital for Women, London and was used at a dilution of 1:400. In all assays standard curves were constructed between 0 and 200 pg for each of the three steroids. All samples from the same subject were measured in the same assay and Student's $t$ test was used for evaluation of significant differences.

**Specificity**

The specificity of the method is dependent on the efficacy of the purification procedure and on the specificity of the antiserum. It has been shown that the only steroid to occur in significant amounts in plasma and prostatic tissue and to cross-react with the testosterone antiserum is DHT. Similarly, using the same method, androstenedione is separated from...
5α-androstane-3β,17β-diol, 5α-androstane-3α,17β-diol, 3α-hydroxy-5β-androstan-17-one and 3α-hydroxy-5α-androstan-17-one, which all show a high cross-reactivity with the androstenedione antiserum.

**Sensitivity**
The reagent blanks, obtained by extracting 1 ml of distilled water and following the extraction through the entire procedure, were not significantly different from the zero value of the standard curve, and the sensitivity of the final RIA was 10 pg/tube for the testosterone and DHT determinations and 12 pg/tube for the androstenedione.

**Accuracy**
Accuracy of the method was determined by adding 2000 pg testosterone, 300 pg androstenedione and 150 pg DHT to 1 ml of water. The percentage recoveries (mean ± s.d.) were 106 ± 3 % for testosterone, 92 ± 6 % for DHT and 96 ± 4 % for androstenedione.

**Precision**
The intra-assay precision was studied by measuring the steroid concentrations in all plasma pools. The coefficient of variation was 5-8 % for testosterone, 14-2 % for DHT and 10-6 % for androstenedione.

The inter-assay precision was determined on the same plasma pool but on different days, showing a coefficient of variation of 8-2 % for testosterone, 19-5 % for DHT and 12-1 % for androstenedione.

![Graph](image_url)

**Fig. 1.** Concentrations of testosterone (T), dihydrotestosterone (DHT) and androstenedione (And.) in benign prostatic hypertrophy (open bars) and carcinomatous (hatched bars) tissues. Vertical lines indicate ± S.E.M.
RESULTS

Androgen levels in tissue

Fig. 1 shows the difference in androgen levels between hypertrophic and carcinomatous tissues. The mean values (pmol/g dry tissue) for androstenedione [42·0 ± 7·9 (S.E.M.)] and testosterone (39·6 ± 6·2) in the neoplastic tissue were significantly higher than those in the hyperplastic tissue (testosterone = 14·1 ± 2·4; androstenedione = 30·0 ± 7·6).

In contrast to the higher concentrations of androstenedione and testosterone in the malignant tissue, the levels of DHT (22·4 ± 2·4) were lower than those in hyperplastic tissue (45·5 ± 5·8). The differences in the hormonal values of both types of tissues are highly significant (in all cases \( P < 0·01 \)).

![Graph showing levels of testosterone (T), dihydrotestosterone (DHT), and androstenedione (And.) in plasma from patients with benign prostatic hypertrophy (open bars) and carcinomatous tissue (hatched bars). Vertical lines indicate ±S.E.M.](image)

Fig. 2. Levels of testosterone (T), dihydrotestosterone (DHT), and androstenedione (And.) in plasma from patients with benign prostatic hypertrophy (open bars) and carcinomatous tissue (hatched bars). Vertical lines indicate ±S.E.M.

Analysis of the individual testosterone, DHT and androstenedione values in BPH by linear regression resulted in a direct correlation between testosterone and DHT (\( r = 0·65, \) \( P < 0·002 \)), androstenedione and DHT (\( r = 0·68, \) \( P < 0·01 \)), and testosterone and androstenedione (\( r = 0·49, \) \( P < 0·05 \)).
A similar evaluation of the carcinoma tissue showed no correlation between testosterone and DHT \((r = 0.3, P > 0.1)\), nor between androstenedione and DHT \((r = 0.1, P < 0.1)\). A significant correlation was, however, shown to exist between testosterone and androstenedione \((r = 0.82, P < 0.001)\).

Fig. 3. The relation between testosterone, dihydrotestosterone (DHT) and androstenedione concentrations in the plasma of ten patients with benign prostatic hypertrophy (○) and ten patients with carcinoma of the prostate (●) and age.
Androgen levels in plasma

The concentrations of the three androgens in peripheral plasma of patients with BPH and carcinoma are shown in Fig. 2; there are no significant differences between the hormonal concentrations in each group (in all cases $P < 0.1$). A significant correlation, however, does exist between testosterone and DHT in both groups ($r = 0.68$, $P < 0.05$ for BPH; $r = 0.69$, $P < 0.05$ for carcinoma) and a second correlation was found between the values of androstenedione and DHT in the BPH group ($r = 0.89$, $P < 0.001$).

Plasma hormone levels and age

The concentrations of testosterone, DHT and androstenedione are plotted against age in Fig. 3. The present study does not show any discernible trends and the values for testosterone remain within the normal range.

DISCUSSION

Before the measurement of androgens in plasma and tissue, chromatographic separation and purification of the steroid hormones was necessary since antibodies raised to the steroid hormone–protein conjugates are not specific and will react with other steroids present. The method presented in this paper is more sensitive than any other published procedure and therefore required less sample for assay, thus allowing the measurement, for the first time, of the concentrations of testosterone, DHT and androstenedione in the malignant parts of the prostate.

In view of the reported variation in the levels of androgen with age and the rarity of normal prostates after the age of 60, we assumed that all prostates in the age-range under investigation belonged to either the benign prostatic hyperplasia or carcinoma categories. Normal control tissues from this age-range are, therefore, not available in the present study.

The data reported in this paper clearly indicate that the appearance of malignancy in the prostate is not related to levels of circulating androgens since no great differences were found in the plasma hormonal levels of patients with either type of prostatic disease ($P > 0.05$). The values for plasma testosterone for the two groups are of the same order as those described in recent publications (Mahoudeau et al. 1974; Pazzagli, Forti, Cappellini & Serio, 1975) with the mean value for men above the age of 60 slightly below the mean values from normal men aged between 18 and 40 years but none the less within the normal range.

The present study also showed that the total plasma DHT concentration for patients with hypertrophied (2·6 ± 0·9 nmol/l) and neoplastic (2·4 ± 0·5 nmol/l) prostates was not significantly different. Although our results for DHT levels are considerably lower than those found by Pirke & Doerr (1975) in normal men, they are in agreement with Horton et al. (1975) who demonstrated raised concentrations in elderly men with BPH. Since blood samples were taken, in the present study, immediately after the induction of anaesthesia, whilst the patients were under stress, the levels of luteinizing hormone and testosterone are expected to be lower (Garstensen, Amer, Wide & Amer, 1973). This in turn may account for a slight reduction in the levels of DHT compared with the data of Horton et al. (1975).

It is, however, evident from these results that the testes of patients with either type of pathological condition maintain similar testosterone levels in blood to those found by other workers (Vermeulen et al. 1972; Pirke & Doerr, 1975) in normal men. The increases in the concentrations of DHT in hyperplastic tissues when compared with normal tissues (Siiteri & Wilson, 1970; Habib, Hammond, Stitch & Dawson, 1975) might account for the increases.
in plasma DHT detected in our studies. However, in view of the significant correlation found between testosterone and DHT, suggesting that testosterone is the most important precursor for DHT production, the contribution of the prostate gland to the DHT plasma pool remains minimal.

The present study clearly demonstrates that the mean value for peripheral plasma androstenedione in BPH (5.5 ± 1.7 nmol/l) and carcinoma (4.4 ± 1.1 nmol/l) are of the same order of magnitude as those obtained by Sciarra, Sorcini, Di Silverio & Gagliardi (1971) for patients with prostatic adenocarcinoma. These authors have also shown that the androstenedione levels in patients with carcinoma were slightly higher than those found in normal males, and attributed this increase to an extra-gonadal origin.

The concentration of testosterone and DHT in the two types of tissue differed considerably. Levels of testosterone were significantly higher in the carcinomatous tissue, with the DHT:testosterone ratio greater in hyperplastic prostates. However, no significant differences were detected in the concentration of androstenedione. To our knowledge this is the first demonstration of the accumulation of testosterone in prostatic carcinomatous tissue. This accumulation may reflect a metabolic pattern different from that of the BPH or normal tissue in which the 5α-reduced metabolites are predominant.

Although our experiments suggest that the accumulation of testosterone is related to the development of carcinoma of the prostate, the mechanism responsible for inducing the tumour remains totally unexplained. We have, however, shown that tumours in the prostate are not associated with changes in plasma levels of the three androgens investigated but might be initiated by factors within the gland. A decrease in the 5α-reductase activity might be causal in the accumulation of testosterone and may be attributed to the low zinc content found in malignant tissue (Grant, Minguell, Taylor & Weiss, 1971) or to the absence of a co-factor. Alternatively, as suggested by Yamaguchi, Kasai, Minesita, Kotoh & Matsumoto (1974), who considered the 5α-reductase activity in an androgen-dependent mouse mammary tumour, the androgen dependency may be more closely related to a high degree of testosterone binding in the cytosol and nucleus than to a relatively high activity of 5α-reductase.

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REFERENCES


