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COMPARISON OF THE NUCLEAR 5α-REDUCTION OF TESTOSTERONE AND ANDROSTENEDIONE IN HUMAN PROSTATIC CARCINOMA AND BENIGN PROSTATIC HYPERPLASIA

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Summary—The nuclear conversion of testosterone (T) to dihydrotestosterone (DHT) and androstenedione (Δ^4A) to androstanedione (5α-Adione) was compared in the separated stromal and epithelial fractions of hyperplastic ($n = 6$) and malignant ($n = 3$) prostatic tissues. Assay conditions were linear with respect to time and protein concentration and were optimal for NADPH concentration. The apparent K_m values for the stromal enzymes were 0.2 and 0.02 μM for hyperplasia and carcinoma, respectively, using T as substrate. The apparent K_m values, using Δ^4A as substrate, were 0.03 and 0.02 μM, respectively. Apparent V_{max} values for the stromal formation of DHT were 16.5 ± 5.4 and 1.97 ± 0.45 pmol/mg protein/30 min incubation, respectively, for the hyperplastic and malignant tissues. The apparent V_{max} values for the formation of 5α-Adione were 2.8 ± 1.3 and 6.5 ± 1.2 pmol/mg/protein/30 min incubation.

The apparent K_m values for the epithelial enzyme, for hyperplastic and malignant tissue were 0.04 and 0.04 μM, for T, and 0.05 and 0.03 μM for Δ^4A. The respective apparent V_{max} values were 4.6 ± 0.93 and 0.65 ± 0.07 for DHT and 2.0 ± 0.86 and 6.4 ± 0.45 pmol/mg protein/30 min incubation for 5α-Adione. Δ^4A was a competitive inhibitor of T 5α-reduction.

These results provide further evidence that different rates of 5α-reduction at least partially explain the differences in androgen levels seen in the hyperplastic and the malignant prostate.

INTRODUCTION

Considerable evidence has accumulated demonstrating that the 5α-reduction of testosterone (T) to dihydrotestosterone (DHT) occurs more efficiently in the benign hyperplastic prostate than in normal or malignant prostatic tissues [1–11]. Furthermore, the *in vitro* rate of conversion of T to DHT in malignant tissue is substantially lower than that occurring in normal tissue at all substrate concentrations [3, 7, 9]. These different rates of enzymatic activity have been suggested to be at least a partial explanation of the differences in endogenous levels of T and DHT in the three types of prostatic tissues [3, 7, 9]. DHT levels are higher in hyperplastic tissue than in both the other two prostates [1, 2, 12, 13]. Malignant tissue contains higher concentrations of T than either of the other two tissues [2, 13].

Androst-4-ene-3,17-dione (androstenedione, Δ^4A) is also a possible substrate for the 5α-reductase, being converted to 5α-androstane-3,17-dione (androstanedione, 5α-Adione) [14]. The levels of Δ^4A may differ from normal in the neoplastic prostate [13, 15]. Habib *et al.*[15] have demonstrated that Δ^4A levels are higher than T levels in the hyperplastic prostate. However, these steroid concentrations are nearly equal in malignant prostatic tissue.

Because Δ^4A is a potential competitive inhibitor for the 5α-reduction of T, it was considered important to examine the kinetics of its conversion to 5α-Adione and to compare this conversion with that of T to DHT.

EXPERIMENTAL

Radioactive steroids and chemicals

The radioactive steroids used [^{3}H]T–85 Ci/mM, [^{3}H]DHT–51 Ci/mM, [^{3}H]Δ^4A–48.5 Ci/mM, [^{3}H]-5α-Adione–51 Ci/mM, [^{14}C]DHT–55 mCi/mM and [^{14}C]5α-Adione–55 mCi/mM) were obtained from the New England Nuclear Corp., Boston, Mass. Each steroid was purified by thin-layer chromatography (TLC) and the purity was checked by recrystallization with an authentic standard prior to use. Non-radioactive steroids, T, DHT, Δ^4A and 5α-Adione (Sigma Chemical Co., St Louis, Mo.) were purified further by TLC and recrystallization prior to use.

All other reagents obtained or prepared were as reported previously [7, 9, 16].

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Source of prostatic tissue

Hyperplastic and malignant prostatic tissues were obtained from men undergoing transurethral resection. Prostatic tissue was placed in ice-cold HEPES buffer (N_2-hydroxyethyl-piperazine-N^1-2-ethane sulfonic acid-pK 7.5) and immediately transferred to the laboratory. Aliquots of each specimen were sent to Pathology for histological assessment.

Separation of stromal and epithelial fractions

All procedures were performed at 4°C. The tissue (1–2 g) was minced with scalpel blades and the stromal and epithelial cells were separated by forcing the tissue through a stainless steel wire screen (30 mesh, 0.55 mm grid) with a teflon pestle, as described previously [9]. The epithelial fraction obtained was suspended in 0.1 M MES buffer, (2-[*N*-morpholino-ethane sulfonic acid]-pK 6.1), pH 6.5, 0.25 M sucrose, 1.0 mM NADPH, (3:1, v/w). The stroma was suspended in 0.1 M HEPES buffer, pH 7.4, 0.25 M sucrose, 1.0 mM NADPH (3:1, v/w) as described previously [9].

Preparation of subcellular fractions

Homogenization of the stromal and epithelial fractions and the preparation of the subcellular fractions were identical to methods reported previously [7, 9]. The nuclear fractions obtained from the epithelial cells were suspended in 0.1 M MES buffer to a protein concentration of 0.5–1.0 mg/ml. The nuclear fractions representing the stromal cells were suspended in 0.1 M HEPES buffer to a protein concentration of 0.5–1.0 mg/ml.

Incubation procedures

Standard assay conditions for the 5α-reduction of T were: 1.0 μM T plus 4–6 × 10^5 dpm [^{3}H]T, 1.0 mM NADPH, 2.0 mM EDTA and 0.5–1.0 mg nuclear protein in a total volume of 1.1 ml HEPES buffer, pH 7.4 (stroma) or MES buffer, pH 6.5 (epithelium). These conditions have been shown previously to be optimal for prostatic T 5α-reductase activity [7, 9].

The conversion of Δ^4A to 5α-Adione (1 μM Δ^4A plus 4–6 × 10^5 dpm [^{3}H]Δ^4A) was carried out under conditions identical to those for the 5α-reduction of T.

Incubations were performed in duplicate in a metabolic shaker for 30 min at 30°C in room air. Any deviations from standard conditions are noted in the Results section.

Control incubations were performed in the absence of added nuclei to determine the extent of non-enzymatic conversion of T to DHT or Δ^4A to 5α-Adione. The extent of this conversion varied from 0 to 0.1%. This figure was subtracted from sample values when calculating enzymatic conversion of substrate to product.

Product isolation and quantitation

Incubations were terminated by the addition of 3 ml of cyclohexane–ethyl acetate (1:1, v/v). Known amounts of [^{14}C]DHT or [^{14}C]5α-Adione were added to each incubation to permit correction for losses due to isolation. Steroids were extracted with the cyclohexane–ethyl acetate and the residues of these extracts, after evaporation, were chromatographed twice in the TLC system methylene chloride–methanol (99:1, v/v) for the separation of Δ^4A and 5α-Adione and in the TLC system toluene:ethyl acetate (3:2, v/v) for the separation of T and DHT.

The regions of the chromatogram corresponding to the DHT or 5α-Adione standards were scraped from the plates and extracted three times with 3 ml of ethyl acetate. The total amount of DHT or 5α-Adione formed by enzymatic activity was calculated in pmol/mg protein/30 min incubation, from the total amount of radioactive product isolated, following chromatography and correction for losses, and the specific activity of the substrate.

Protein determination

Protein was measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Mississauga, Ontario) with bovine gamma globulin as the standard.

Assessment of purity of stromal and epithelial fractions

The measurement of acid phosphatase, hydroxyproline and prolyl hydroxylase as markers for the epithelial and stromal fractions were as reported previously [9].

Determination of radioactivity

Radioactivity was determined in a Beckman LS 5801 Scintillation Analyzer as described previously [9, 16].

RESULTS

Purity of stromal and epithelial fractions

Previously reported studies [9], measuring acid phosphatase concentrations in the stromal and epithelial fractions have demonstrated that most of the acid phosphatase in hyperplastic and malignant prostatic tissue is associated with the epithelial fraction. A small amount of activity is associated with the stroma. This may represent epithelial contamination of the stroma or may represent endogenous activity in the stromal fraction.

Measurement of hydroxyproline and prolyl hydroxylase activity in the separated stromal and epithelial fractions, has revealed previously [9] that the epithelial fraction has no hydroxyproline or prolyl hydroxylase activity. These markers reside exclusively in the stromal fraction [9].

Conversion of Δ^4A to T or 5α-Adione to DHT

In initial experiments, the possible conversion of Δ^4A to T was examined by separately incubating

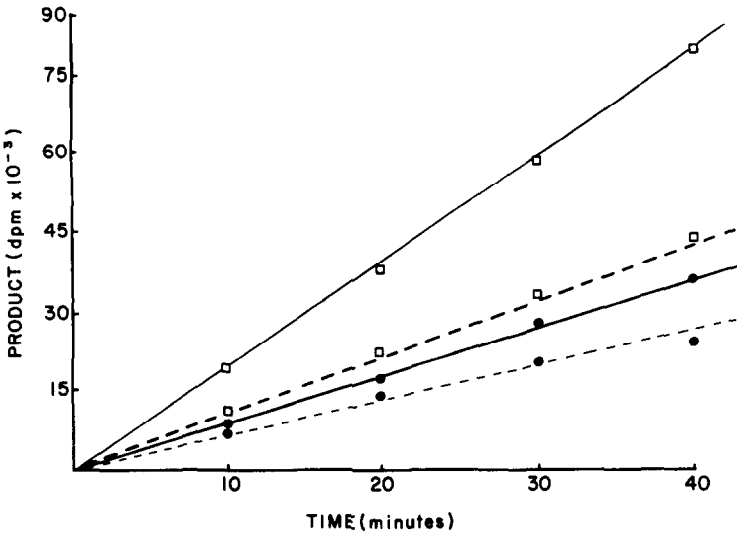

Fig. 1. The effect of time on the 5α-reduction of testosterone (T) (solid lines) and androstenedione (Δ^4A) (dotted lines) by the nuclei of stromal (□) and epithelial (●) fractions of hyperplastic human prostatic tissue. Each incubation mixture contained: [^{3}H]T or [^{3}H]Δ^4A, NADPH, and EDTA as in the standard assay and 0.15 mg nuclear protein. DHT and 5α-Adione production was assayed at 10, 20 and 30 min.

5×10^5 dpm Δ^4A with the nuclear fractions of the epithelial and stromal cells and extracting the region of the chromatograms corresponding to the T standard. No radioactivity associated with the region corresponding to the T standard was found.

Similarly, incubations of [^{3}H]5α-Adione with the nuclear fractions of both the epithelial and stromal cells revealed no radioactivity associated with the region of the chromatogram corresponding to the DHT standard.

Effect of time and protein concentration

Figure 1 illustrates the effect of time on the 5α-reduction of T and Δ^4A by the nuclei of the stromal and epithelial fractions by hyperplastic tissue. The rate of formation of DHT and 5α-Adione, by both fractions, was constant with time for at least 40 min and was proportional to a protein concentration of at least 1.5 mg/1.1 ml (data not shown). All subsequent experiments reported were performed under conditions of linearity with respect to time and protein concentration.

Effect of NADPH concentration

The effect of NADPH concentration on the 5α-reduction of Δ^4A is shown in Table 1. It can be seen that the formation of 5α-Adione increased to an NADPH concentration of 0.1 mM. Further increases in ANDPH concentration were not associated with any further 5α-Adione formation. Previous studies [7] have shown that the 5α-reduction of T follows a similar pattern. The standard assay utilizes on NADPH concentration of 1.0 mM. Therefore NADPH concentration was not rate limiting for either substrate.

Effect of substrate concentration

The effect of substrate concentration on 5α-reductase activity in the nuclei from hyperplastic tissue is shown in Fig. 2. The nuclei had been incubated with concentrations of T or Δ^4A varying from 0.002 to 10 μM. When the reciprocal of the velocity of the reaction was plotted against the reciprocal of substrate concentration, a straight line was obtained indicating that the enzymes in both cellular fractions and with both substrates, obey Michaelis–Menton kinetics. In separate experiments, apparent K_m values for the stromal enzymes were found to be 0.2 and 0.02 μM, respectively, for hyperplastic ($n = 6$) and malignant ($n = 3$) tissues using T as substrate (Table 2). The apparent K_m values for these tissues, using Δ^4A as a substrate, were 0.03 and 0.02 μM, respectively. The apparent V_{max} values for the formation of DHT in the stroma of the hyperplastic and malignant tissues were 16.5 ± 5.4 and 1.97 ± 0.45 pmol/mg protein/30 min, respectively. The apparent V_{max} values for the formation of

Table 1. The effect of NADPH concentration on the 5α-reduction of androstenedione by hyperplastic prostatic tissue

NADPH (mM)	Enzyme activity (dpm/mg protein) Stroma	Epithelium
0	0	0
0.001	12,100	5,940
0.005	19,250	7,210
0.01	26,240	8,100
0.05	36,120	10,125
0.1	43,290	12,420
0.5	46,525	15,100
1.0	44,620	14,380
2.0	42,050	15,945

The nuclei from hyperplastic tissue were incubated with 5×10^5 dpm [^{3}H]Δ^4A as described in the Experimental section.

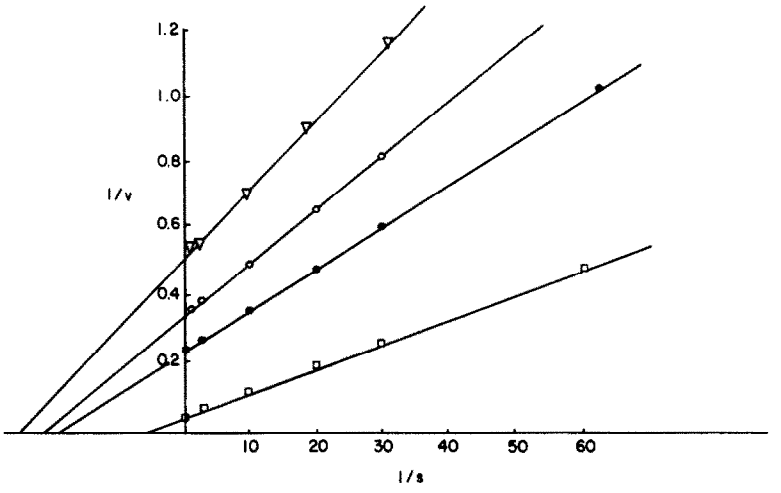

Fig. 2. The effect of T and Δ^4A concentration on 5α-reduction by the nuclei from stromal and epithelial fractions of hyperplastic prostatic tissues. The stromal nuclei were incubated with varying concentrations of T (□) or Δ^4A (○). The epithelial nuclei were incubated with varying concentrations of T (●) or Δ^4A (▽). Product formation is as described in text.

5α-Adione for the same tissues, were 2.8 ± 1.3 and 6.5 ± 1.2 pmol/mg protein/30 min incubation. The apparent K_m values for the epithelial enzyme, using T as substrate, for the hyperplastic and malignant tissues were 0.04 and 0.04 μM, respectively. The apparent K_m values using Δ^4A as substrate in these same tissues were 0.05 and 0.03 μM, respectively. The apparent V_{max} values for the formation of DHT in the epithelium of the hyperplastic and malignant tissues were 4.6 ± 0.93 and 0.65 ± 0.07 pmol/mg protein/30 mg incubation, respectively. The apparent V_{max} values, for these same tissues, using Δ^4A as substrate were 2.0 ± 0.86 and 6.4 ± 0.45 pmol/mg protein/30 min incubation, respectively.

Effect of Δ^4A on the 5α-reduction of T

Figure 3 shows the effect of 3.5 and 35 pmol of Δ^4A on the conversion of T to DHT by the nuclei of hyperplastic prostatic tissue. It can be seen from the $1/V$ vs 1/s plot that both concentrations of Δ^4A inhibited the formation of DHT. The pattern of inhibition is consistent with Δ^4A being a competitive inhibitor of the 5α-reduction of T.

Table 2. Apparent K_m and V_{max} values for the 5α-reduction of T and Δ^4A by the nuclei of hyperplatsic and malignant prostatic tissues

	T	Δ^4A
Hyperplasia ($n = 6$)		
Stroma—K_m	0.2	0.03
—V_{max}	16.5 ± 5.4	2.8 ± 1.3
Epithelium—K_m	0.04	0.05
—V_{max}	4.6 ± 0.93	2.0 ± 0.86
Malignant ($n = 3$)		
Stroma—K_m	0.02	0.02
—V_{max}	1.97 ± 0.45	6.5 ± 1.2
Epithelium—K_m	0.04	0.03
—V_{max}	0.65 ± 0.07	6.4 ± 0.45

K_m (μM); V_{max} (pmols/mg protein/30 min).

DISCUSSION

It has been demonstrated that concentrations of DHT in hyperplastic prostatic tissue exceed that in normal and malignant tissues [1, 2, 8, 11]. Additionally, T levels are higher in malignant prostate than in either of the other two tissues [2, 13]. Several investigators have shown that differences in the rates of 5α-reduction of T to DHT in the three prostatic tissues are at least a partial explanation for the differences in T and DHT concentrations [2–8, 11].

Habib *et al.* have shown that Δ^4A concentrations are higher than T concentrations in the hyperplastic prostate [13, 15]. In malignant tissue, however, both T and Δ^4A concentrations are more nearly equal [13, 15].

Although the intra-prostatic content of any steroid is due to several factors, including uptake from serum, formation from precursor(s) and metabolism to product(s), one possible explanation for the higher intra-prostatic concentration of Δ^4A than T in the hyperplastic prostate is a different rate of 5α-reduction of each steroid. The data from the current study has demonstrated that the rate of conversion of T to DHT in both the stromal and epithelial fractions of hyperplastic prostatic tissue exceeds the conversion of Δ^4A to 5α-Adione. The conversion of both substrates was carried out with the same tissues under conditions found to be optimal for the nuclear 5α-reduction of testosterone [7, 8, 16]. The conditions of the experiments were linear with respect to time and protein concentration for both substrates.

It is of interest that the apparent K_m for Δ^4A was an order lower than that for T in the stromal fraction of hyperplastic tissue. This indicates that the affinity of the enzyme for Δ^4A is greater than that for T. The velocity of the reaction, however, at least in hyperplastic tissue, is greater for T than for Δ^4A.

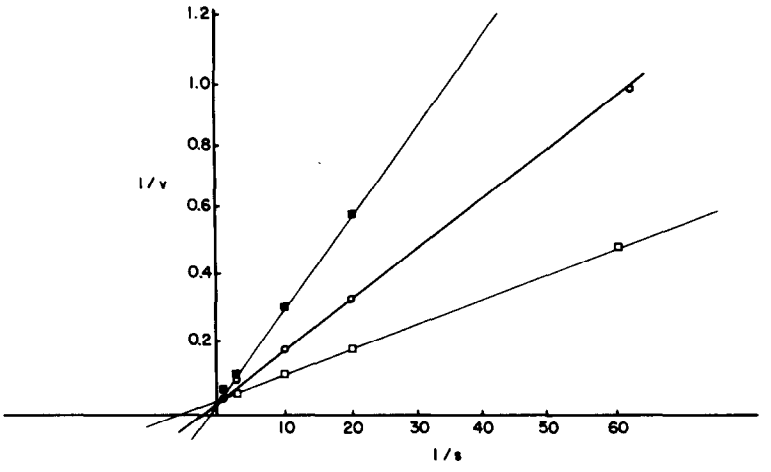

Fig. 3. The effect of Δ^4A on the 5α-reduction of T by the nuclei of the stromal fraction of hyperplastic prostatic tissue. The nuclei were incubated with varying concentrations of T in the absence (□) or presence of 3.5 pmols (○) or 35 pmols (●) Δ^4A.

The opposite rate of conversion was seen in the three malignant prostates. The apparent V_{max} for the conversion of Δ^4A to 5α-Adione was greater than the apparent V_{max} for T in both the stromal and epithelial fractions of the malignant tissues.

The reason for the different relative rates of 5α-reduction of T and Δ^4A in the two types of neoplasia is not known. The concentration of NADPH used was such that this cofactor's concentration was not rate limiting for either substrate. It has been shown that there may be at least two (relatively), substrate specific 5α-reductases in rat prostatic tissue [14]. One may be specific for Δ^4A, the other for T [14]. The effect of Δ^4A on the conversion of T to DHT demonstrated in the present study suggests that Δ^4A is a competitive inhibitor of the 5α-reduction of T in this tissue. Further, detailed studies on the potential for either substrate to compete with the other must be undertaken.

It is possible that the observed differences in the 5α-reduction of T and Δ^4A in this study could be due to differences in the further metabolism of DHT or 5α-Adione or in the interconversion of T and Δ^4A in the two types of prostate. Previous studies from this laboratory have demonstrated that DHT is not metabolized to 3α- or 3β-Androstanediol by the nuclear fraction of the human prostate [17]. Furthermore, the current study, and previously reported studies [16, 17] have shown no interconversion between T and Δ^4A or DHT and 5α-Adione by the nuclei of the human prostate. Therefore, it is unlikely that the differences in the 5α-reduction of T and Δ^4A demonstrated in the present study are due to other than differences in the activities of these enzymes in prostatic carcinoma and benign hyperplasia. These differences in enzyme activities help to explain the differences in the levels of T and Δ^4A in these tissues.

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REFERENCES

1. Farnsworth W. F. and Brown J. R.: Androgen of the human prostate. *Endocr. Res. Commun.* **3** (1976) 105–117.
2. Geller J., Albvert J., Lopez D., Geller S. and Niwayama G.: Comparison of androgen metabolites in benign prostatic hypertrophy (BPH) and normal prostate. *J. Clin. Endocr. Metab.* **43** (1976) 686–688.
3. Bruchovsky N. and Lieskovsky G.: Increased ratio of 5α-reductase: 3α(β)-hydroxysteroid dehydrogenase activities in the hyperplastic human prostate. *J. Endocr.* **80** (1979) 289–301.
4. Djoseland O., Tveter K. J., Attramadal A., Hannson V., Haughen H. N. and Mathisen W.: Metabolism of testosterone in the human prostate and seminal vesicles. *Scand. J. Urol. Nephrol.* **11** (1979) 1–6.
5. Wilkin R. P., Bruchovsky N., Shnitka T. K., Rennie P. S. and Comeau T.L.: Stromal 5α-reductase activity is elevated in benign prostatic hyperplasia. *Acta Endocr. (Copenh.)* **94** (1980) 284–288.
6. Bruchovsky N., McLoughlin M. G., Rennie P. S. and To M. P.: Partial characterization of stromal and epithelial forms of 5α-reductase in human prostate. *Prog. Clin. Urol. Res.* **75** (1981) 161–175.
7. Hudson R. W., Moffitt P. M. and Owens W. A.: Studies of the nuclear 5α-reductase of human prostatic tissue: comparison of enzyme activities in hyperplastic, malignant, and normal tissues. *Can. J. Biochem. Cell. Biol.* **61** (1983) 750–755.
8. Voigt K. D. and Bartsch W.: Intratissular androgens in benign prostatic hyperplasia and prostatic cancer. *J. Steroid Biochem.* **25** (1986) 749–757.
9. Hudson R. W.: Comparison of nuclear 5α-reductase activities in the stromal and epithelial fractions of

human prostatic tissue. *J. Steroid Biochem.* **26** (1987) 349–353.
10. Klein H., Bressel M., Kastendieck H. and Voigt K. D.: Quantitative assessment of endogenous testicular and adrenal sex steroids and of steroid metabolizing enzymes in untreated human prostatic cancerous tissue. *J. Steroid Biochem.* **30** (1988) 119–130.
11. Bruchovsky N., Rennie P. S., Batzold F. H., Goldenberg S. L., Fletcher T. and McLoughlin M. G.: Kinetic parameters of 5α-reductase activity in storm and epithelium of normal, hyperplastic and carcinomatous human prostate. *J. Clin. Endocr. Metab.* **67** (1988) 806–816.
12. Siiteri P. K. and Wilson J. D.: Dihydrotestosterone in prostatic hypertrophy. I. Formation and content of dihydrotestosterone in the hypertrophic prostate of men. *J. Clin. Invest.* **49** (1970) 1737–1745.
13. Habib F. K.: Zinc in the steroid endocrinology of the human prostate. *J. Steroid Biochem.* **9** (1978) 403–407.
14. Martini L., Zoppi S. and Motta M.: Studies on the possible existence of two 5α-reductases in the rat prostate. *J. Steroid Biochem.* **24** (1986) 177–182.
15. Habib F. K., Lee S. R., Stitch S. R. and Smith P. H.: Androgen levels in the plasma and prostatic tissues of patients with benign hypertrophy and carcinoma of the prostate. *J. Endocr.* **71** (1976) 99–107.
16. Hudson R. W., Studies of the nuclear 5α-reductase of human hyperplastic prostatic tissue. *J. Steroid Biochem.* **14** (1981) 579–584.
17. Hudson R. W.: Studies of the cytosol 3α-hydroxysteroid dehydrogenase of human prostatic tissue: comparison of enzyme activities in hyperplastic, malignant and normal tissues. *J. Steroid Biochem.* **16** (1982) 373–377.