Studies on the Human Epididymis: Partial Characterization of 3α - and 3β -Hydroxysteroid Dehydrogenase, Regional Distribution of 5α -Reductase and Inhibitory Effect of $4\text{-}^3\text{-Oxosteroids}$ on 5α -Reductase

YUHZO KINOSHITA

Department of Urology, Yokohama City University, School of Medicine
3-46 Urafune-cho, Minami-ku, Yokohama 232

Abstract

When $[4\text{-}^{14}\text{C}]\text{-}5\alpha\text{-dihydrotestosterone}$ was incubated with the homogenate of human epididymis, $5\alpha\text{-androstane-}3\alpha, 17\beta\text{-diol}$ and $5\alpha\text{-androstane-}3\beta, 17\beta\text{-diol}$ were identified as major metabolites. The ratio of 3α - to 3β -epimer in androstanediol formation was approximately 2.4. $5\alpha\text{-Androstane-}3, 17\text{-dione}$ was also identified as a minor metabolite. Among the subcellular fractions, both the human epididymal 3α - and 3β -hydroxysteroid dehydrogenases were localized almost exclusively in the cytosol fraction ($105,000\times$ supernatant). Both enzymes had optimum pH at 7.5 and optimum temperature at 46°C . NADPH was a more preferable cofactor than NADH for both dehydrogenases. The Michaelis constants (K_m) of 3α - and 3β -hydroxysteroid dehydrogenase for $5\alpha\text{-dihydrotestosterone}$ were similar and estimated as $8\times 10^{-5}\text{M}$, but the enzymes were unsaturable with the substrate under the conditions investigated, indicating low affinity and high capacity of both dehydrogenases for $5\alpha\text{-dihydrotestosterone}$.

The human epididymal 5α -reductase revealed a regional difference in activity. The 5α -reductase activity in the most proximal part of the head (ductuli efferentes) was one seventh to one tenth the activity in the remaining part of the epididymis which was constructed of ductus epididymis. Except for this finding, the activity of 5α -reductase was highest in the head, then declined along the course to the tail portion. The 5α -reductase for testosterone was competitively inhibited by $4\text{-}^3\text{-oxosteroids}$ such as progesterone, $20\alpha\text{-dihydroprogesterone}$, $17\alpha\text{-hydroxyprogesterone}$, 4-androstenedione, 11-deoxycorticosterone, corticosterone and 11-deoxycortisol, which had inhibition constants (K_i) of $3.3\times 10^{-9}\text{M}$, $2.2\times 10^{-9}\text{M}$, $1.8\times 10^{-8}\text{M}$, $1.3\times 10^{-8}\text{M}$, $8.3\times 10^{-9}\text{M}$, $1.5\times 10^{-7}\text{M}$ and $8.7\times 10^{-8}\text{M}$, respectively, suggesting the possibility that the 5α -reduction of testosterone is regulated by other $4\text{-}^3\text{-oxosteroids}$.

Received June 24, 1981.

The following abbreviations and trivial names are used in the text: progesterone, 4-pregnene-3, 20-dione; $20\alpha\text{-dihydroprogesterone}$, $20\alpha\text{-hydroxy-}4\text{-pregnen-}3\text{-one}$; $17\alpha\text{-hydroxyprogesterone}$, $17\alpha\text{-hydroxy-}4\text{-pregnene-}3, 20\text{-dione}$; pregnenolone, $3\beta\text{-hydroxy-}5\text{-pregnen-}20\text{-one}$; $17\alpha\text{-hydroxypregnenolone}$, $3\beta, 17\alpha\text{-dihydroxy-}5\text{-pregnen-}20\text{-one}$; corticosterone, $11\beta, 21\text{-dihydroxy-}4\text{-pregnene-}3, 20\text{-dione}$; 11-deoxycorticosterone, $21\text{-hydroxy-}4\text{-pregnene-}3, 20\text{-dione}$; cortisol, $11\beta, 17\alpha, 21\text{-trihydroxy-}4\text{-pregnene-}3, 20\text{-dione}$; 11-deoxycortisol, $17\alpha, 21\text{-dihydroxy-}4\text{-preg-$

$\text{nene-}3, 20\text{-dione}$; dehydroepiandrosterone, $3\beta\text{-hydroxy-}5\text{-androstene-}17\text{-one}$; 4-androstenedione, 4-androstene-3, 17-dione; testosterone, $17\beta\text{-hydroxy-}4\text{-androstene-}3\text{-one}$; $5\alpha\text{-dihydrotestosterone}$, $17\beta\text{-hydroxy-}5\alpha\text{-androstane-}3\text{-one}$; androstanediol, $5\alpha\text{-androstane-}3\alpha(\beta), 17\beta\text{-diol}$; androsterone, $3\alpha\text{-hydroxy-}5\alpha\text{-androstane-}17\text{-one}$; estradiol, 1, 3, 5(10)-estratriene-3, $17\beta\text{-diol}$; estriol, 1, 3, 5(10)-estratriene-3, $16\alpha, 17\beta\text{-triol}$; estrone, 3-hydroxy-1, 3, 5(10)-estratriene-17-one; POPOP, 1, 4-bis-2 (5-phenyloxazolyl)-benzene; DPO, 2, 5-diphenyloxazole.

Studies on a variety of species have proved that spermatozoa, which are still immature in the testis, undergo a functional change to enable fertilization of the ova during passage through the epididymis (Young, 1931; Blandau and Rumery, 1964; Bedford, 1966; Horan and Bedford, 1972; Dyson and Orgebin-Crist, 1973). The role of the epididymis in supporting sperm maturation is also suggested in man (Hinrichsen and Blaquier, 1980). These epididymal functions or the maturation processes of spermatozoa are regulated by androgens (Blaquier *et al.*, 1972; Dyson and Orgebin-Crist, 1973; Lubicz-Nawrocki, 1973a), and 5 α -reduced androgens are more effective than testosterone in restoring the fertilizing ability of epididymal spermatozoa (Lubicz-Nawrocki, 1973b). There is extensive evidence showing that the epididymis actively converts testosterone to 5 α -reduced metabolites (Inano *et al.*, 1969; Gloyna and Wilson, 1969; Sowell and Eik-Nes, 1972; Djøseland *et al.*, 1974; Kinoshita *et al.*, 1980; Scheer and Robaire, 1980), and that the concentration of 5 α -dihydrotestosterone in this organ is remarkably high (Vreeburg and Aafjes, 1971; Purvis *et al.*, 1978; Leinonen *et al.*, 1980). On the other hand, androgen receptors are present in the nuclei and cytosol of the epididymis (Blaquier, 1971; Tindall *et al.*, 1972; Blaquier and Calandra, 1973; Hansson *et al.*, 1975; Danzo and Eller, 1978; Lea *et al.*, 1979) and androgen binding proteins (ABP) probably facilitate the intratubular transport of androgens from the testis to the epididymis (Hansson *et al.*, 1974). Thus, the metabolism of androgens in the epididymis appears to be of extreme importance in the maintenance of its functions.

The present study was conducted to investigate the metabolism of androgens in the human epididymis. This paper focuses especially on the properties of the enzymes catalyzing the reduction of 5 α -dihydrotestosterone, regional differences in activity of

5 α -reductase, and the effects of steroid hormones on 5 α -reductase activity.

Materials and Methods

Chemicals

[4-¹⁴C]-Testosterone (58 mCi/mmol), [4-¹⁴C]-5 α -dihydrotestosterone (58 mCi/mmol), [1, 2-³H]-testosterone (59 Ci/mmol), and [1, 2-³H]-5 α -androstane-3 α , 17 β -diol (52 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, Ma.) and [1, 2-³H]-5 α -androstane-3 β , 17 β -diol (50 Ci/mmol) from Radiochemical Center (Amersham, England). Radiochemical purity of these steroids was confirmed by thin-layer chromatography before use. Non-radioactive steroid preparations and cofactors were obtained from Sigma Chemical Co. (St. Louis, Mo.). All reagents were of analytical grade. Silica gel G and GF₂₅₄ were purchased from E. Merck (Darmstadt, Germany).

Tissue preparation and incubation

Men aged 66 to 78 underwent bilateral orchiectomy for the treatment of prostatic carcinoma established by histological examination of biopsied specimens. None had received previous endocrine therapy. Immediately after castration, epididymides were separated from the testes and surrounding periepididymal fat and connective tissue was dissected from the organ. The epididymal tissue was minced with fine scissors and then homogenized in an ice cold 0.25 M sucrose solution with a loose fitting teflon-glass homogenizer. The homogenate was filtered through nylon net (100 mesh) to remove the few remaining unbroken strips of connective tissue. The filtrate was centrifuged at 800 $\times$ g for 20 min. The 800 $\times$ g precipitate was resuspended in a 0.88 M sucrose solution containing 1.5 mM CaCl₂, and the suspension was gently layered over the sucrose gradient which consisted of the 2.2 M sucrose and overlying 1.8 M sucrose solutions, both containing 0.5 mM CaCl₂. The nuclear fraction was prepared by centrifuging the discontinuous sucrose gradients at 33,000 $\times$ g for 90 min in a swinging bucket rotor as described by Suzuki and Tamaoki (1974). The 800 $\times$ g supernatant fluids were further fractionated by conventional differential centrifugation to obtain the mitochondrial fraction (800-10,000 $\times$ g precipitate), the microsomal fraction (10,000-105,000 $\times$ g precipitate) and the cytosol fraction (supernatant fluid at 105,000 $\times$ g) as described in a previous report (Kinoshita *et al.*, 1980). All procedures were performed at 0-4°C. A radioactive steroid of known specific activity was diluted with a corresponding authentic non-radioactive steroid to obtain appropriate substrate concentrations. The incubation

flask contained one of the enzyme preparations, a radioactive steroid substrate, 0.2 mM NADPH, 0.25 M sucrose, 17 mM tris (pH 7.4) and 1.7 mM MgCl_2 , unless otherwise specified. The sequence for preparing the incubation medium was the steroid substrate dissolved in two drops of propylene glycol, the cofactor dissolved in tris buffer (pH 7.4), and then the enzyme preparation. The final volume was adjusted to 3 ml. Incubations were performed at 37°C for 30 min in an atmosphere of 95% O_2 and 5% CO_2 with continuous shaking, unless otherwise indicated. Other conditions for incubation are described in each experimental section. Enzyme reactions were terminated by adding 10 ml of dichloromethane.

Extraction and isolation of products

After adding non-radioactive carrier steroids, the steroid metabolites were extracted from the incubation medium with 10 ml of dichloromethane. The extraction was repeated two more times. The combined extracts were dried over anhydrous sodium sulfate, then evaporated to dryness under reduced pressure. An aliquot of each extract was spotted on a thin-layer plate precoated with silica gel G and GF₂₅₄ (4:1, w/w). Steroid metabolites from testosterone were separated by ascending thin-layer chromatography using a solvent system of benzene: acetone (4:1, v/v). 5 α -Androstane-3 α , 17 β -diol and 5 α -androstane-3 β , 17 β -diol were distinctly separated by the thin-layer chromatography developed twice in the same direction with a solvent system of hexane: benzene: acetone (7:1:4, v/v). Spots of carrier steroids on the chromatogram were detected under ultraviolet light ($\lambda=254$ nm) or by exposing the plate to iodine vapor. The radioactive metabolites from ^{14}C -labeled substrates were detected by autoradiography obtained by exposing an X-ray film to the thin-layer plate for several days. The spots thus detected on the chromatogram were scraped off and then the steroids were exhaustively eluted from the silica gel with diethylether.

Quantitation of steroids and protein

The radioactivity of each steroid fraction was measured with a liquid scintillation spectrometer (Packard Model 3390-544) in 10 ml of dioxane containing POPOP (0.025%), DPO (1%) and naphthalene (10%). The counting efficiency was approximately 55% for ^{14}C and 30% for ^3H . The protein content in each enzyme preparation was measured by the method of Lowry *et al.* (1951). Steroid 5 α -reductase activity was expressed by the amount of 5 α -dihydrotestosterone produced from testosterone during 30 min incubations as previously described (Kinoshita *et al.*, 1980). The formation of 5 α -androstane-3 α (β), 17 β -diol from testosterone was negligible under the conditions employed.

Identification of metabolites

Identification of the radioactive steroid metabolite was based on identical mobility with the authentic steroid preparation on the thin-layer chromatogram, using different solvent systems, and finally confirmed by constant specific radioactivity through repeated recrystallization with the authentic non-radioactive steroid.

Results

Metabolism of 5 α -dihydrotestosterone in the human epididymis

When the whole homogenate of a human epididymis equivalent to approximately 90 mg wet tissue was incubated with [4- ^{14}C]-5 α -dihydrotestosterone (5.8×10^5 dpm, 74 nmol) in the presence of NADPH, 79% of the 5 α -dihydrotestosterone substrate was recovered unchanged, 7.1% of the radioactivity was recovered as ^{14}C -5 α -androstane-3 α , 17 β -diol, 2.8% as ^{14}C -5 α -androstane-3 β , 17 β -diol and 0.2% as ^{14}C -5 α -androstane-3, 17-dione (Fig. 1). Identification and radiochemical purity of these radioactive metabolites are demonstrated in Table 1. Other than these major metabolites from 5 α -dihydrotestosterone, only trace quantities of some polar compounds were observed. The cytosol fraction (6.3 mg protein) and [4- ^{14}C]-5 α -dihydrotestosterone (1.0×10^5 dpm, 0.8 nmol) were incubated with either [1, 2- ^3H]-5 α -androstane-3 α , 17 β -diol (9.9×10^5 dpm, 8.6 pmol) or [1, 2- ^3H]-5 α -androstane-3 β , 17 β -diol (9.6×10^5 dpm, 8.7 pmol) in the presence of NADP and NADPH. Under these conditions, 72% of the ^{14}C -labeled 5 α -dihydrotestosterone substrate was converted to 5 α -androstane-3 α , 17 β -diol and 5 α -androstane-3 β , 17 β -diol in three hours of incubation, during which time a minimal but distinct amount of ^3H -labeled 5 α -dihydrotestosterone was also formed from the ^3H -labeled 5 α -androstane-3 α , 17 β -diol or 5 α -androstane-3 β , 17 β -diol substrate, indicating reversible reactions catalyzed by 3 α - and 3 β -hydroxysteroid dehydrogenases. The activity of 3 α - and

Fig. 1. Autoradiogram of the metabolites from 5 α -dihydrotestosterone in the human epididymis. Radioactive metabolites from [4- 14 C] - 5 α - dihydrotestosterone were separated on a thin-layer chromatogram developed twice in the same direction by a solvent system of hexane: benzene: acetone (7:1:4, v/v).

Table 1. Identification of the metabolites produced from 5 α -dihydrotestosterone in the human epididymis.

Recrys- tallization	Solvents	Specific radioactivity (dpm/mg)		Solvents	5 α -androstane- 3,17-dione
		5 α -androstane- 3 α , 17 β -diol	5 α -androstane- 3 β , 17 β -diol		
1st	Methanol	1301	736	Benzene/hexane	229
2nd	Ethanol	1169	773	Acetone/hexane	206
3rd	Methanol/water	1283	733	Ethylacetate/hexane	198
4th	Ethanol/water	1291	730	Dioxane/hexane	211
Calculated specific radioactivity		1392	812		207

The radioactive metabolites tentatively identified on the chromatogram were recrystallized with authentic non-radioactive steroid preparations.

3β -hydroxysteroid dehydrogenase was compared with subcellular fractions of the human epididymal homogenate. Each subcellular fraction was incubated with $[4-^{14}\text{C}]-5\alpha$ -dihydrotestosterone (5×10^5 dpm, 73.5 nmol) in the presence of 0.2 mM NADPH. Both dehydrogenases revealed the highest specific activity in the cytosol fraction, as shown in Table 2.

Table 2. Intracellular distribution of $3\alpha(\beta)$ -hydroxysteroid dehydrogenase in the human epididymis.

Subcellular fractions	3α -hydroxysteroid dehydrogenase (nmol/mg protein/30 min)	3β -hydroxysteroid dehydrogenase (nmol/mg protein/30 min)
Whole homogenate	0.93	0.34
Nuclear fraction	0.21	0.11
Mitochondrial fraction	0.17	0.16
Microsomal fraction	0.25	0.17
Cytosol fraction	1.63	0.57

5α -Dihydrotestosterone (5×10^5 dpm, 73.5 nmol) was incubated with either the whole homogenate (4.6 mg protein), nuclear fraction (8.3 mg protein), mitochondrial fraction (1.2 mg protein), microsomal fraction (3.1 mg protein), or cytosol fraction (4.1 mg protein).

Expression of 3α - and 3β -hydroxysteroid dehydrogenase activity

Because no significant metabolites other than 5α -androstane- 3α , 17β -diol were detectable under the conditions employed in the present study, the amount of 5α -androstane- 3α , 17β -diol produced from the 5α -dihydrotestosterone substrate was defined as the activity of 3α -hydroxysteroid dehydrogenase. Similarly, the amount of 5α -androstane- 3β , 17β -diol formation from 5α -dihydrotestosterone could be defined as 3β -hydroxysteroid dehydrogenase activity. A time study revealed a linear increase in 5α -androstane- $3\alpha(\beta)$, 17β -diol with an incubation time of up to 60 min; a linear correlation was also confirmed between the amount of

these metabolites and enzyme concentrations.

Effect of pH and temperature on 3α - and 3β -hydroxysteroid dehydrogenase activity

Both 3α - and 3β -hydroxysteroid dehydrogenase had optimum pH around 7.5 (Fig. 2) and optimum temperature at 46°C (Fig. 3). The ratio of the 3α - to 3β -hydroxysteroid dehydrogenase activity remained nearly constant between 2.1 and 2.7, even when either the pH of the incubation medium or the incubation temperature was changed.

Cofactor requirement of 3α - and 3β -hydroxysteroid dehydrogenase

The formation of 5α -androstane- 3α , 17β -diol and 5α -androstane- 3β , 17β -diol from

Fig. 2. Effect of pH on the activity of 3α - and 3β -hydroxysteroid dehydrogenase in the human epididymis. The cytosol fraction (3.9 mg protein) and $[4-^{14}\text{C}]-5\alpha$ -dihydrotestosterone (7.5×10^5 dpm, 75 nmol) were incubated at the indicated pH. The buffers used were 0.1 M Na_2HPO_4 -0.2 M citrate for pH 6.0-6.4 and 0.2 M tris for pH 6.9-9.2. 3α -HSD (—○—): 3α -hydroxysteroid dehydrogenase and 3β -HSD (—△—): 3β -hydroxysteroid dehydrogenase.

Fig. 3. Effect of temperature on the activity of 3α - and 3β -hydroxysteroid dehydrogenase in the human epididymis. The cytosol fraction (2.3 mg protein) and $[4\text{-}^{14}\text{C}]\text{-}5\alpha$ -dihydrotestosterone (1.0×10^6 dpm, 77 nmol) were incubated at the indicated temperatures. See legend to Fig. 2 for abbreviations and symbols.

5α -dihydrotestosterone was enhanced by adding either NADPH or NADH. The activity of 3α - and 3β -hydroxysteroid dehydrogenase as a function of the concentrations of coenzymes is represented in Fig. 4, indicating that both dehydrogenases preferred NADPH to NADH as a hydrogen donor. Both dehydrogenases were adequately saturated with 0.2 mM NADPH which was currently used for the present study.

Michaelis constants of 3α - and 3β -hydroxysteroid dehydrogenase

The rate of 5α -androstane- $3\alpha(\beta)$, 17β -diol formation was determined at various concentrations of 5α -dihydrotestosterone substrate. As shown in Fig. 5, 5α -dihydrotestosterone even at a concentration of 30 μM was still insufficient to saturate the 3α - and 3β -hydroxysteroid dehydrogenase. Although the enzymes were not sufficiently saturated, the apparent Michaelis constants (K_m) of both dehydrogenases for 5α -dihy-

Fig. 4. Effect of pyridine nucleotide concentration on the activity of 3α - and 3β -hydroxysteroid dehydrogenase in the human epididymis. The cytosol fraction containing 2.6 mg of protein was incubated with $[4\text{-}^{14}\text{C}]\text{-}5\alpha$ -dihydrotestosterone (6.4×10^5 dpm, 74 nmol) in the presence of NADPH or NADH at the concentrations indicated. The activity of 3α -hydroxysteroid dehydrogenase (3α -HSD) in the presence of NADPH (—○—) or NADH (—●—) and of 3β -hydroxysteroid dehydrogenase (3β -HSD) in the presence of NADPH (—△—) or NADH (—▲—) is indicated as a function of the coenzyme concentrations.

drotestosterone were estimated approximately as 8×10^{-5} M by a graphic method of Lineweaver and Burk (1934).

Regional distribution of 5 α -reductase in the human epididymis

Human epididymides were divided into four segments, as shown in Fig. 6. The proximal part of the head, segment I, which showed dark brown pigmentation, was separated from the remainder of the epididymis. The junctional portion between segments I and II was slightly trimmed off and discarded. Landmarks of the divisions between segments II, III and IV were rather arbitrary. Histological examination indicated that most of the tubular structures observed in segment I were ductuli efferentes and that segments II, III and IV were composed of ductus epididymis. [4-¹⁴C]-Testosterone (1×10^5 dpm, 1.5 nmol) was incubated with the whole homogenate of each segment in the presence of 0.2 mM

NADPH. The mean value ($\pm$ SD) of the 5 α -reductase activity in segments I, II, III and IV were 23 ± 11 , 231 ± 50 , 209 ± 68 and 150 ± 47 pmol/100 mg tissue/30 min ($N=5$) respectively (Fig. 6). Activity of 5 α -reductase in ductuli efferentes (segment I) was 7–10 times lower than that in the other part of the epididymis (segment II, III and IV). The 5 α -reductase of ductus epididymis showed significantly higher activity in the head (segment II) than in the tail (segment IV).

Effect of steroids on the activity of 5 α -reductase in the human epididymis

As shown in Table 3, 5 α -reduction of testosterone by the microsomal 5 α -reductase was inhibited by several steroids. The order of inhibitory effects was progesterone, 20 α -dihydroprogesterone > 11-deoxycorticosterone, 17 α -hydroxyprogesterone, 4-androstenedione > corticosterone, 11-deoxycortisol, and inhibition of the enzyme by these

Fig. 5. Kinetic studies on 3 α - and 3 β -hydroxysteroid dehydrogenase for 5 α -dihydrotestosterone in the human epididymis. The cytosol fraction containing 3.1 mg of protein was incubated with [4-¹⁴C]-5 α -dihydrotestosterone (5.8×10^5 dpm, 11–91 nmol) at the concentrations indicated. A. Substrate vs product. B. Lineweaver-Burk plots. See legend to Fig. 2 for abbreviations and symbols.

Fig. 6. Regional distribution of 5 α -reductase in the human epididymis. [4-¹⁴C]-Testosterone (1×10^5 dpm, 1.5 nmol) was incubated with the whole homogenate (equivalent to 50mg of tissue) of each segment of the epididymis. Each column and vertical line represent the mean value $\pm$ SD in five elderly men. Segment I corresponds to ductuli efferentes. Segments II, III and IV, which are composed of ductus epididymis, represent the head, body and tail, respectively. The activity in I is significantly lower than in II, III and IV ($P < 0.01$) and the difference between II and IV is also significant ($P < 0.05$).

Table 3. Effect of various steroids on the activity of 5 α -reductase for testosterone in the human epididymis.

Added steroids	5 α -Reductase for testosterone (% activity)	
	10 nM	100 nM
None	100%	100%
Pregesterone	34	5
20 α -Dihydroprogesterone	31	5
17 α -Hydroxyprogesterone	69	27
4-Androstenedione	75	25
11-Deoxycorticosterone	65	19
Corticosterone	94	74
11-Deoxycortisol	86	67
Cortisol	93	97
Pregnenolone	100	97
17 α -Hydroxypregnenolone	100	103
Dehydroepiandrosterone	104	93
5 α -Dihydrotestosterone	96	91
5 α -Androstane-3 α , 17 β -diol	111	92
5 α -Androstane-3 β , 17 β -diol	103	98
Androsterone	99	102
Estradiol	98	98
Estriol	93	96
Estrone	96	97

[1, 2-³H]-Testosterone (5×10^5 dpm, 12 nM final concentration) was incubated with the microsomal fraction (0.05 mg protein) in the presence and absence of various non-radioactive steroids (10 nM and 100 nM final concentrations).

steroids was dose responsive. In contrast, none of Δ^5 -3 β -hydroxysteroids, 5 α -reduced steroids or estrogens inhibited 5 α -reductase activity for testosterone under the conditions employed in the present experiment. The mode of inhibition by some of Δ^4 -3-oxosteroids was analyzed by the graphic method of Lineweaver-Burk (Fig. 7). They revealed competitive inhibition to the enzyme with inhibition constants (K_i) tabulated in Table 4.

Fig. 7. Inhibition of 5 α -reductase for testosterone by various Δ^4 -3-oxosteroids. The microsomal fraction (0.05 mg protein) of the human epididymis was incubated with [1, 2- 3 H]-testosterone (6.1×10^5 dpm, 11-92 pmol) in the absence (None, —●—) or presence of a non-radioactive steroid additive: 5 nM progesterone (P₄, —△—), 5 nM 20 α -dihydroprogesterone (20-P₄, —x—), 20 nM 17 α -hydroxyprogesterone (17-P₄, —○—), 30 nM 4-androstenedione (Δ^4 , —□—), 150 nM corticosterone (B, —•—), 10 nM 11-deoxycorticosterone (DOC, —■—) or 50 nM 11-deoxycortisol (S, —▲—).

Table 4. K_i values of various steroids for 5 α -reductase in the human epididymis.

Steroids	Apparent K _i values
Progesterone	3.3×10^{-9} M
20 α -Dihydroprogesterone	2.2×10^{-9}
17 α -Hydroxyprogesterone	1.8×10^{-8}
4-Androstenedione	1.3×10^{-8}
11-Deoxycorticosterone	8.3×10^{-9}
Corticosterone	1.5×10^{-7}
11-Deoxycortisol	8.7×10^{-8}

K_i values were derived from the Lineweaver-Burk plots illustrated in Fig. 7. The K_m value of 5 α -reductase for testosterone was 1.0×10^{-8} M.

Discussion

5 α -Dihydrotestosterone, one of the most biologically active androgen metabolites from testosterone, is converted to 5 α -androstane-3 α (β), 17 β -diol by the reduction of 3-oxo-group in various androgen target organs such as the prostate (Chamberlain *et al.*, 1966; Shimazaki *et al.*, 1972; Shida *et al.*, 1975), epididymis (Inano *et al.*, 1969; Hastings and Djøseland, 1979; Kinoshita *et al.*, 1980), seminal vesicle (Suzuki and Tamaoki, 1974), and vas deference (Dupuy *et al.*, 1979). In the human epididymis, the major metabolites of 5 α -dihydrotesto-

sterone in the presence of either NADPH or NADH are 5α -androstane- 3α , 17β -diol and 5α -androstane- 3β , 17β -diol. The formation of 5α -androstane- $3,17$ -dione from 5α -dihydrotestosterone is also detectable, but the amount of the metabolite is minimal, indicating minor activity of 17β -hydroxysteroid dehydrogenase for the 5α -dihydrotestosterone substrate in the human epididymis. While many enzymes have been demonstrated to be relevant to steroid biosynthesis in the epididymis (Hamilton and Fawcett, 1970; Frankel and Eik-Nes, 1970), the main products from testosterone in rat epididymides are 5α -dihydrotestosterone and 5α -androstane- 3α , 17β -diol (Inano *et al.*, 1969; Hastings and Djøseland, 1979). The latter compound, 5α -androstane- 3α , 17β -diol, is almost a sole product from 5α -dihydrotestosterone in rat prostates (Shimazaki *et al.*, 1972; Taurog *et al.*, 1975; Hastings *et al.*, 1980); rat epididymides (Hastings and Djøseland, 1979) and seminal vesicles (Suzuki and Tamaoki, 1974), whereas its 3β -epimer, 5α -androstane- 3β , 17β -diol, is produced in considerable quantities in mouse, guinea-pig, rabbit and cat prostates (Jacobi and Wilson, 1979), and also in rabbit epididymides (Booth and Jones, 1979). In the human epididymis, 5α -androstane- 3β , 17β -diol occupies about 25–30% of total 5α -androstanediol formed, which is similar to the previous findings on the human prostate (Jacobi and Wilson, 1977) and human vas deferens (Dupuy *et al.*, 1979).

Both 3α - and 3β -hydroxysteroid dehydrogenase in the human epididymis localize mainly in the cytosol fraction. 3α -Hydroxysteroid dehydrogenase has been reported to localize in the cytosol fraction of most of androgen target organs such as rat prostates (Shimazaki *et al.*, 1972), rat epididymides (Hastings and Hansson, 1979), rat seminal vesicles (Suzuki and Tamaoki, 1974), and immature rat testes (Oshima *et al.*, 1970), whereas the enzyme has been de-

monstrated largely in microsomal fractions of canine prostatic tissue (Jacobi and Wilson, 1976) and the rat kidney (Verhoeven *et al.*, 1976). Although 3α -hydroxysteroid dehydrogenase can utilize both NADPH and NADH, NADPH is preferred to NADH as the hydrogen donor in both rat (Shimazaki *et al.*, 1972; Taurog *et al.*, 1975) and human prostates (Jacobi and Wilson, 1977) as observed in the present study. Hastings *et al.* (1980a) has reported that 3α -hydroxysteroid dehydrogenases in the prostatic, epididymal and testicular cytosol can be separated into fractions containing NADPH- and NADH-linked enzymes. Epididymal 3β -hydroxysteroid dehydrogenase is completely different from testicular Δ^5 - 3β -hydroxysteroid dehydrogenase. The latter prefers NADH to NADPH, localizes at testicular microsomal fractions and is linked with Δ^5 - Δ^4 isomerase (Fan and Troen, 1975; Oshima *et al.*, 1975).

The 3α - and 3β -hydroxysteroid dehydrogenase in the human epididymal cytosol have a similar optimum pH of 7.5 and also a similar optimum temperature around 46°C . The results coincide with properties of the 3α -hydroxysteroid dehydrogenase partially purified from rat prostatic (Taurog *et al.*, 1975; Inano *et al.*, 1977), epididymal (Hastings and Hansson, 1979) and testicular cytosol (Hastings *et al.*, 1980b). The K_m values of the human epididymal 3α - and 3β -hydroxysteroid dehydrogenase for 5α -dihydrotestosterone are 8×10^{-5} M, while the human epididymal 5α -reductase has a K_m value of 1×10^{-8} M for testosterone (Kinoshita *et al.*, 1980). The androgen metabolism by a high affinity enzyme from testosterone to 5α -dihydrotestosterone, and subsequently by a low affinity enzyme into 5α -androstane- $3\alpha(\beta)$, 17β -diol, seems to provide a mechanism for maintaining a high concentration of 5α -dihydrotestosterone in the human epididymis. Leinonen *et al.* (1980) has reported that the concentration of 5α -dihydrotestosterone in the human

epididymis is 3–4 times higher than that of 5 α -androstane-3 α , 17 β -diol and androstosterone.

5 α -Androstane-3 α , 17 β -diol is a biologically potent androgen in restoring the weight of the prostates of castrated rats (Verjans and Eik-Nes, 1976) and in decreasing the plasma LH levels through negative feedback (Verjans and Eik-Nes, 1976). The androgenic activity of 5 α -androstane-3 α , 17 β -diol has been assumed to be mediated by 5 α -dihydrotestosterone which is formed by the oxidation of the former compound in the tissue (Bruchovsky, 1971; Krieg *et al.*, 1975; Hastings and Djøseland, 1977). Orgebin-Crist *et al.* (1976) has reported that 5 α -androstane-3 α , 17 β -diol has less androgenic activity than 5 α -dihydrotestosterone on cultured epididymides of rabbit. Since in the rat epididymis and other androgen target organs (Djøseland *et al.*, 1976; Hastings and Djøseland, 1977; Verhoeven *et al.*, 1977) 5 α -androstane-3 α , 17 β -diol is readily converted to 5 α -dihydrotestosterone, the biological activity of 5 α -androstane-3 α , 17 β -diol can be due to its conversion to 5 α -dihydrotestosterone. The present study also confirmed the interconvertibility between 5 α -dihydrotestosterone and 5 α -androstane-3 α , 17 β -diol in the human epididymis. In contrast, some reports support the direct and specific effects of 5 α -androstane-3 α , 17 β -diol itself on the target organs. The report by Walsh and Wilson (1976) states that canine prostatic hypertrophy can be induced by the administration of 5 α -androstane-3 α , 17 β -diol more efficiently than 5 α -dihydrotestosterone. Further, 5 α -androstane-3 α , 17 β -diol administered *in vivo* is reported to exert a greater promoting effect on sperm maturation in the hamster epididymis than 5 α -dihydrotestosterone (Lubicz-Nawrocki, 1973b). On the other hand, 5 α -androstane-3 β , 17 β -diol is less physiologically active or rather inactive in inducing prostatic growth (Verjans and Eik-Nes, 1976) and in developing

sperm maturation in the epididymis (Lubicz-Nawrocki, 1973b; Orgebin-Crist *et al.*, 1976). Ofner *et al.* (1979) and Morfin *et al.* (1977) have shown that 5 α -androstane-3 β , 17 β -diol is a substrate for 6 β - or 7 α -hydroxylation in canine and human prostates, suggesting the possibility that the 3 β -hydroxysteroid dehydrogenase-hydroxylase system may be operating as a mechanism by which androgen target organs dispose of intracellular androgens. *In vitro* and *in vivo* studies, however, have indicated that 5 α -dihydrotestosterone is the major metabolite of testosterone in the epididymis of the human (Kinoshita *et al.*, 1980; Leinonen *et al.*, 1980) and several other animals (Vreeburg and Aafjes, 1971; Calandra *et al.*, 1974; Larminat and Blaquier, 1978). Therefore, 5 α -dihydrotestosterone appears to be the major 5 α -reduced androgen which mediates the maturation processes of spermatozoa in the human epididymis, although the above findings do not exclude the possibility that 5 α -androstane-3 α (β), 17 β -diol has its own direct action on the epididymis.

In the present study, it is demonstrated that the head of the human epididymis contains abundant 5 α -reductase activity with a gradual decline toward its tail portion. It is of interest that the proximal part of the head, which is histologically identified as the ductuli efferentes, has strikingly low 5 α -reductase activity, about 7–10 times lower than the other part of the epididymis composed of ductus epididymis. However, Purvis *et al.* (1978) and Leinonen *et al.* (1980) could not find any concentration gradients of testosterone, 5 α -dihydrotestosterone or 5 α -androstane-3 α , 17 β -diol in the human epididymis. In the rat, the head of the epididymis also has higher 5 α -reductase activity than the tail (Djøseland *et al.*, 1974; Saksena *et al.*, 1976; Robaire *et al.*, 1977; Pujol and Bayard, 1978; Kumari *et al.*, 1980) and, in addition, the concentration of 5 α -dihydrotestosterone in

epididymal tissues of the rat, bull and boar is also highest in the head portion (Aafjes and Vreeburg, 1972; Vreeburg, 1975). Similarly, it has been shown that the binding capacity of androgen binding protein (ABP) in the rat epididymis is highest in the head and gradually decreases toward the distal part of the epididymis with the decrease in 5α -reductase activity and 5α -dihydrotestosterone concentration (Hansson *et al.*, 1975). Since not only 5α -dihydrotestosterone concentration and ABP, but also 5α -reductase activity in the rat epididymis markedly declines after the ligation of the efferent ductules or gonadectomy (Aafjes and Vreeburg, 1972; French and Ritzén, 1973; Vreeburg, 1975; Larminat *et al.*, 1978; Pujol and Bayard, 1978), it has been assumed that testicular androgens supplied as precursor steroids to the epididymis by intratubular transport are involved in the maintenance of high 5α -reductase activity, especially in its proximal portion (Pujol and Bayard, 1978).

5α -Reductase for testosterone in the human epididymis is competitively inhibited by Δ^4 -3-oxosteroids including progesterone, 20α -dihydroprogesterone, 17α -hydroxyprogesterone, 4-androstenedione, 11-deoxycorticosterone and 11-deoxycortisol. It is verified that rat epididymal 5α -reductase reduces C_{19} - and C_{21} - Δ^4 -3-oxosteroids such as progesterone, 17α -hydroxyprogesterone, 4-androstenedione and testosterone (Inano *et al.*, 1969). Similarly, rat prostatic 5α -reductase reveals a higher affinity for progesterone, 17α -hydroxyprogesterone and 20α -dihydroprogesterone than testosterone itself, and also has a broad affinity for Δ^4 -3-oxosteroids, resulting in competitive inhibition of 5α -reductase activity for testosterone by these compounds (Frederiksen and Wilson, 1971; Nozu and Tamaoki, 1974). In the human epididymis, the K_i values of 5α -reductase for various steroids suggest that progesterone and 20α -dihydroprogesterone have higher affinity for the reductase than testosterone,

and that 17α -hydroxyprogesterone, 4-androstenedione and 11-deoxycorticosterone also reveal high affinity comparable to that for the testosterone substrate. These results are generally consistent with the properties of 5α -reductase in the rat prostate (Frederiksen and Wilson, 1971) and human skin (Voigt *et al.*, 1970; Voigt and Hsia, 1973). In the human epididymis, average concentrations of progesterone, 17α -hydroxyprogesterone, 4-androstenedione and testosterone have been reported as 2.1 ng/g, 14.5 ng/g, 7.4 ng/g and 37.3 ng/g, respectively (Leinonen *et al.*, 1980), while the former three compounds and other Δ^4 -3-oxosteroids are present only in considerably smaller quantities than testosterone in the human testis (Ruokonen *et al.*, 1972; Hosaka *et al.*, 1978). It therefore appears likely that 5α -reduction of testosterone to 5α -dihydrotestosterone in the human epididymis can be negatively regulated to some extent by other Δ^4 -3-oxosteroids.

Acknowledgements

The author is grateful to Prof. S. Takai for his directions in this study and to Dr. M. Hosaka for his continuous and helpful support. Kind encouragement and advice by Dr. R. Nishimura were also instrumental in the completion of this study. Thanks are also due to Dr. H. Oshima, Department of Urology, Faculty of Medicine, Tokyo Medical and Dental University, for valuable advice to this work.

References

- Aafjes, J. H. and J. T. M. Vreeburg (1972). Distribution of 5α -dihydrotestosterone in the epididymis of bull and boar, and its concentration in rat epididymis after ligation of efferent testicular ducts, castration and unilateral gonadectomy. *J. Endocrinol.* 53, 85-93.
- Bedford, J. M. (1966). Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. *J. Exp. Zool.* 163, 319-330.
- Blandau, R. J. and R. E. Rumery (1964). The relationship of swimming movements of epididymal spermatozoa to their fertilizing capacity. *Fertil.*

- Steril.* 15, 571-579.
- Blaquier, J. A. (1971). Selective uptake and metabolism of androgens by rat epididymis. The presence of a cytoplasmic receptor. *Biochem. Biophys. Res. Commun.* 45, 1076-1082.
- Blaquier, J. A., M. S. Cameo and M. H. Burgos (1972). The role of androgens in the maturation of epididymal spermatozoa in the guinea pig. *Endocrinology* 90, 839-842.
- Blaquier, J. A. and R. S. Calandra (1973). Intracellular receptor for androgens in rat epididymis. *Endocrinology* 93, 51-60.
- Booth, W. D. and R. Jones (1979). Metabolism of testosterone and 5 α -reduced androgens by the rabbit prostate and epididymis: Studies *in vitro* and *in vivo*. *J. Endocrinol.* 82, 207-214.
- Bruchovsky, N. (1971). Comparison of the metabolites formed in rat prostate following the *in vivo* administration of seven natural androgens. *Endocrinology* 89, 1212-1222.
- Calandra, R. S., E. J. Podesta, M. A. Rivarola and J. A. Blaquier (1974). Tissue androgens and androphilic proteins in rat epididymis during sexual development. *Steroids* 24, 507-518.
- Chamberlain, J., N. Jagarinec and P. Ofner (1966). Catabolism of [4-¹⁴C]-testosterone by subcellular fractions of human prostate. *Biochem. J.* 99, 610-616.
- Danzo, B. J. and B. C. Eller (1978). Androgen metabolism by and binding to rabbit epididymal tissue: Studies on nuclei. *J. Steroid Biochem.* 9, 477-483.
- Djoeseland, O., V. Hansson and H. N. Haugen (1974). Androgen metabolism by rat epididymis. 2. Metabolic conversion of ³H-testosterone *in vitro*. *Steroids* 23, 397-410.
- Djoeseland, O., C. D. Hastings and V. Hansson (1976). Androgen metabolism by rat epididymis. Metabolic conversion of ³H-5 α -androsterane-3 α , 17 β -diol, *in vivo*. *Steroids* 28, 585-596.
- Dupuy, G. M., P. Boulanger, K. D. Roberts, G. Bleau and A. Chapdelaine (1979). Metabolism of sex steroids in the human and canine vas deferens. *Endocrinology* 104, 1553-1558.
- Dyson, A. L. M. B. and M.-C. Orgebin-Crist (1973). Effect of hypophysectomy, castration and androgen replacement upon the fertilizing ability of rat epididymal spermatozoa. *Endocrinology* 93, 391-402.
- Fan, D.-F. and P. Troen (1975). Studies of the human testis. VII. Conversion of pregnenolone to progesterone. *J. Clin. Endocrinol. Metab.* 41, 563-574.
- Frankel, A. I. and K. B. Eik-Nes (1970). Metabolism of steroids in the rabbit epididymis. *Endocrinology* 87, 646-652.
- Frederiksen, D. W. and J. D. Wilson (1971). Partial characterization of the nuclear reduced nicotinamide adenine dinucleotide phosphate: Δ^4 -3-ketosteroid 5 α -oxidoreductase of rat prostate. *J. Biol. Chem.* 246, 2584-2593.
- French, F. S. and E. M. Ritzén (1973). A high-affinity androgen-binding protein (ABP) in rat testis: Evidence for secretion into efferent duct fluid and absorption by epididymis. *Endocrinology* 93, 88-95.
- Gloyna, R. E. and J. D. Wilson (1969). A comparative study of the conversion of testosterone to 17 β -hydroxy-5 α -androsterane-3-one (dihydrotestosterone) by prostate and epididymis. *J. Clin. Endocrinol. Metab.* 29, 970-977.
- Hamilton, D. W. and D. W. Fawcett (1970). In vitro synthesis of cholesterol and testosterone from acetate by rat epididymis and vas deferens. *Proc. Soc. Exp. Biol. Med.* 133, 693-695.
- Hansson, V., O. Trygstad, F. S. French, W. S. McLean, A. A. Smith, D. J. Tindall, S. C. Weddington, P. Petrusz, S. N. Nayfeh and E. M. Ritzén (1974). Androgen transport and receptor mechanisms in testis and epididymis. *Nature* 250, 387-391.
- Hansson, V., E. M. Ritzén, F. S. French and S. N. Nayfeh (1975). Androgen transport and receptor mechanisms in testis and epididymis. In: *Handbook of Physiology, Section 7, Volume V* (D. W. Hamilton, R. O. Greep and E. B. Astwood ed.), American Physiological Society, Washington D. C. pp. 173-201.
- Hastings, C. D. and O. Djoeseland (1977). Androgen metabolism by rat epididymis. Metabolic conversion of ³H-5 α -androsterane-3 α , 17 β -diol *in vitro*. *Steroids* 30, 531-539.
- Hastings, C. D. and O. Djoeseland (1979). Androgen metabolism by rat epididymis: Metabolic conversion of ³H-dihydrotestosterone *in vivo* and *in vitro*. *Arch. Androl.* 2, 215-222.
- Hastings, C. D. and V. Hansson (1979). Physicochemical characterization of the NADPH dependent soluble 3 α -hydroxysteroid oxidoreductase in the rat epididymis. *Int. J. Androl.* 2, 263-274.
- Hastings, C. D., I. Brekke, K. Purvis, A. Attramadal and V. Hansson (1980a). Cofactor dependency of soluble 3 α -hydroxysteroid oxidoreductases in rat testis, prostate, and epididymis. *Endocrinology* 107, 1762-1766.
- Hastings, C. D., I. Brekke, A. Attramadal and V. Hansson (1980b). Physico-chemical characterization of the soluble 3 α -hydroxysteroid oxidoreductase in the rat testis and prostate. *Int. J. Androl.* 3, 72-81.
- Hinrichsen, M. J. and J. A. Blaquier (1980). Evidence supporting the existence of sperm maturation in the human epididymis. *J. Reprod. Fertil.* 60, 291-294.
- Horan, A. H. and J. M. Bedford (1972). Development of the fertilizing ability of spermatozoa in the epididymis of the Syrian hamster. *J. Reprod. Fertil.* 30, 417-425.
- Hosaka, M., H. Oshima, H. Nankin and P. Troen

- (1978). Studies of the human testis. XI. Leydig cell clusters and levels of intratesticular testosterone and 20 α -dihydroprogesterone. *J. Clin. Endocrinol. Metab.* 47, 1164-1167.
- Inano, H., A. Machino and B. Tamaoki (1969). *In vitro* metabolism of steroid hormones by cell-free homogenates of epididymides of adult rats. *Endocrinology* 84, 997-1003.
- Inano, H., S. Hayashi and B. Tamaoki (1977). Prostate 3 α -hydroxysteroid dehydrogenase: Its partial purification and properties. *J. Steroid Biochem.* 8, 41-46.
- Jacobi, G. H. and J. D. Wilson (1976). The formation of 5 α -androstane-3 α , 17 β -diol by dog prostate. *Endocrinology* 99, 602-610.
- Jacobi, G. H. and J. D. Wilson (1977). Formation of 5 α -androstane-3 β , 17 β -diol by normal and hypertrophic human prostate. *J. Clin. Endocrinol. Metab.* 44, 107-115.
- Jacobi, G. H. and J. D. Wilson (1979). 3-Alpha-androstenediol and prostatic growth: Comparison of 3-alpha-androstenediol formation in prostates from 8 species including man and dog. *J. Urol.* 121, 612-614.
- Kinoshita, Y., M. Hosaka, R. Nishimura and S. Takai (1980). Partial characterization of 5 α -reductase in the human epididymis. *Endocrinol. Jpn.* 27, 277-284.
- Krieg, M., H.-J. Horst and M.-L. Sterba (1975). Binding and metabolism of 5 α -androstane-3 α , 17 β -diol and of 5 α -androstane-3 β , 17 β -diol in the prostate, seminal vesicle and plasma of male rats: Studies *in vivo* and *in vitro*. *J. Endocrinol.* 64, 529-538.
- Kumari, G. L., I. S. Allag, R. P. Das and J. K. Datta (1980). Regional differences in steroidogenesis and hormone levels in the epididymis and vas deferens of adult rats. *Int. J. Androl.* 3, 267-281.
- Larminat, M. A. and J. A. Blaquier (1978). Androgen concentration and partial characterization of 5 α -reductase in the epididymis of the rhesus monkey. *Steroids* 31, 129-138.
- Larminat, M. A., A. Monsalve, E. H. Charreau, R. S. Calandra and J. A. Blaquier (1978). Hormonal regulation of 5 α -reductase activity in rat epididymis. *J. Endocrinol.* 79, 157-165.
- Lea, O. A., E. M. Wilson and F. S. French (1979). Characterization of different forms of the androgen receptor. *Endocrinology* 105, 1350-1360.
- Leinonen, P., G. L. Hammond and R. Vihko (1980). Testosterone and some of its precursors and metabolites in the human epididymis. *J. Clin. Endocrinol. Metab.* 51, 423-428.
- Lineweaver, H. and D. Burk (1934). The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.* 56, 658-666.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Lubicz-Nawrocki, C. M. (1973a). Effect of testosterone metabolites on sperm maturation in the epididymis of castrated hamsters. *J. Reprod. Fertil.* 35, 588-589.
- Lubicz-Nawrocki, C. M. (1973b). The effect of metabolites of testosterone on the viability of hamster epididymal spermatozoa. *J. Endocrinol.* 58, 193-198.
- Morfin, R. F., S. Di Stéfano, J.-F. Charles and H. H. Floch (1977). Precursors for 6 β - and 7 α -hydroxylations of 5 α -androstane-3 β , 17 β -diol by human normal and hyperplastic prostates. *Biochimie* 59, 637-644.
- Nozu, K. and B. Tamaoki (1974). Characteristics of the nuclear and microsomal steroid 4 α -5 α -hydrogenase of the rat prostate. *Acta Endocrinol. (Kbh)* 76, 608-624.
- Ofner, P., R. L. Vena, I. Leav and D. W. Hamilton (1979). Metabolism of C₁₉-radiosteroids by explants of canine prostate and epididymis with disposition as hydroxylated products: A possible mechanism for androgen inactivation. *J. Steroid Biochem.* 11, 1367-1379.
- Orgebin-Crist, M.-C., N. Jahad and L. H. Hoffman (1976). The effect of testosterone, 5 α -dihydrotestosterone, 3 α -androstenediol, and 3 β -androstenediol on the maturation of rabbit epididymal spermatozoa in organ culture. *Cell Tissue Res.* 167, 515-525.
- Oshima, H., T. Sarada, K. Ochiai and B. Tamaoki (1970). 4 α -5 α -Hydrogenase in immature rat testes: Its intracellular distribution, enzyme kinetics, and influence of administered gonadotrophin and testosterone propionate. *Endocrinology* 86, 1215-1224.
- Oshima, H., D.-F. Fan and P. Troen (1975). Studies of the human testis. V. Properties of 4 α -3 β - and 17 β -hydroxysteroid dehydrogenases in the biosynthesis of testosterone from dehydroepiandrosterone. *J. Clin. Endocrinol. Metab.* 40, 573-581.
- Pujol, A. and F. Bayard (1978). 5 α -Reductase and 3 α -hydroxysteroid oxidoreductase enzyme activities in epididymis and their control by androgen and the rete testis fluid. *Steroids* 31, 485-493.
- Purvis, K., R. Calandra, S. Sander and V. Hansson (1978). Androgen binding proteins and androgen levels in the human testis and epididymis. *Int. J. Androl.* 1, 531-548.
- Robaire, B., L. L. Ewing, B. R. Zirkin and D. C. Irby (1977). Steroid 4 α -5 α -reductase and 3 α -hydroxysteroid dehydrogenase in the rat epididymis. *Endocrinology* 101, 1379-1390.
- Ruokonen, A., T. Laatikainen, E. A. Laitinen and R. Vihko (1972). Free and sulfate-conjugated neutral steroids in human testis tissue. *Biochemistry* 11, 1411-1416.
- Saksena, S. K., I. F. Lau and M. C. Chang (1976).

- The inhibition of the conversion of testosterone into 5 α -dihydrotestosterone in the reproductive organs of the male rat. *Steroids* 27, 751-757.
- Scheer, H. and B. Robaire (1980). Steroid 4 α -reductase and 3 α -hydroxysteroid dehydrogenase in the rat epididymis during development. *Endocrinology* 107, 948-953.
- Shida, K., J. Shimazaki, Y. Ito, H. Yamanaka and H. Nagai-Yuasa (1975). 3 α -Reduction of dihydrotestosterone in human normal and hypertrophic prostatic tissues. *Invest. Urol.* 13, 241-245.
- Shimazaki, J., N. Kato, H. Nagai, H. Yamanaka and K. Shida (1972). 3 α -Reduction of 5 α -dihydrotestosterone by rat ventral prostate. *Endocrinol. Jpn.* 19, 97-106.
- Sowell, J. G. and K. B. Eik-Nes (1972). Formation *in vivo* of 5 α -dihydrotestosterone by the canine epididymis. *Proc. Soc. Exp. Biol. Med.* 141, 827-829.
- Suzuki, K. and B. Tamaoki (1974). *In vitro* metabolism of testosterone in seminal vesicles of rats. *J. Steroid Biochem.* 5, 249-256.
- Taurog, J. D., R. J. Moore and J. D. Wilson (1975). Partial characterization of the cytosol 3 α -hydroxysteroid: NAD(P)⁺ oxidoreductase of rat ventral prostate. *Biochemistry* 14, 810-817.
- Tindall D. J., F. S. French and S. N. Nayfeh (1972). Androgen uptake and binding in rat epididymal nuclei, *in vivo*. *Biochem. Biophys. Res. Commun.* 49, 1391-1397.
- Verhoeven, G., W. Heyns and P. De Moor (1976). Interconversion between 17 β -hydroxy-5 α -androstan-3-one (5 α -dihydrotestosterone) and 5 α -androstane-3 α , 17 β -diol in rat kidney: Heterogeneity of 3 α -hydroxysteroid oxidoreductases. *Eur. J. Biochem.* 65, 565-576.
- Verhoeven, G., W. Heyns and P. De Moor (1977). Interconversion between 17 β -hydroxy-5 α -androstan-3-one (5 α -dihydrotestosterone) and 5 α -androstane-3 α , 17 β -diol: Tissue specificity and role of the microsomal NAD: 3 α -hydroxysteroid oxidoreductase. *J. Steroid Biochem.* 8, 731-733.
- Verjans, H. L. and K. B. Eik-Nes (1976). Effects of androstenes, 5 α -androstanes, 5 β -androstanes, oestrenes and oestratriens on serum gonadotrophin levels and ventral prostate weights in gonadectomized adult male rats. *Acta Endocrinol. (Kbh)* 83, 201-210.
- Voigt, W., E. P. Fernandez and S. L. Hsia (1970). Transformation of testosterone into 17 β -hydroxy-5 α -androstan-3-one by microsomal preparations of human skin. *J. Biol. Chem.* 245, 5594-5599.
- Voigt, W. and S. L. Hsia (1973). Further studies on testosterone 5 α -reductase of human skin, structural features of steroid inhibitors. *J. Biol. Chem.* 248, 4280-4285.
- Vreeburg, J. T. M. and J. H. Aafjes (1971). Dihydrotestosterone (5 α -androstan-17 β -ol-3-one) in the epididymis of rats. In: *Current Problems in Fertility* (A. Ingelman-Sundberg and N. O. Lunell ed.), Plenum Press, New York. pp. 203-206.
- Vreeburg, J. T. M. (1975). Distribution of testosterone and 5 α -dihydrotestosterone in rat epididymis and their concentrations in efferent duct fluid. *J. Endocrinol.* 67, 203-210.
- Walsh, P. C. and J. D. Wilson (1976). The induction of prostatic hypertrophy in the dog with androstenediol. *J. Clin. Invest.* 57, 1093-1097.
- Young, W. C. (1931). A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea-pig. *J. Exp. Biol.* 8, 151-162.