Journal of Steroid Biochemistry Vol. 10, pp. 187 to 193

0022-4731/79/0201-0187$02.00/0

THE INDUCTION, PURIFICATION AND CHARACTERIZATION OF 17β-HYDROXY-C_{19}-STEROID DEHYDROGENASE OF THE FEMALE GUINEA PIG KIDNEY*

C. C. SHEN and CHARLES D. KOCHAKIAN†
Laboratory of Experimental Endocrinology, Medical Center,
University of Alabama in Birmingham, Birmingham, AL 35294, U.S.A.

(*Received* 19 *June* 1978)

SUMMARY

The female guinea pig kidney cytosol and microsomes contain a trace or no detectable $NADP^+$- or NAD^+-linked dehydrogenase activity for several hydroxy-C_{19}-steroids. The administration of testosterone induced a gradual increase in the 17β-C_{19}-steroid dehydrogenase activity of the cytosol, but not of the microsomes, to the level in the male after 50 days. The induced enzyme was purified by $(NH_4)_2SO_4$ precipitation, Sephadex G-75 filtration and DEAE-Sephadex A-50 and CM-Sephadex C-50 chromatography to give a protein which gave a molecular weight of 32,000 and a single protein and enzyme staining band on gel electrophoresis. The pure enzyme dissociated into three gel electrophoretic bands on removal of 2-mercaptoethanol from the solution and was restored by replacement of the mercaptoethanol. The enzyme in the cytosol, however, dissociated on storage at 4°C for 48 h or longer in the presence or absence of 2-mercaptoethanol. The K_M value, steroid specificity, gel electrophoretic pattern, isoelectric value, pH optimum and $NADP^+$ and NAD^+ requirement were practically identical with those of the purified major 17β-hydroxy-C_{19}-steroid dehydrogenase of the male guinea pig kidney. The several hydroxy-C_{19}-steroid dehydrogenase activities of the liver were not influenced by testosterone administration. The highest enzyme activity was exhibited with 5β-dihydrotestosterone as substrate.

INTRODUCTION

The male guinea pig kidney exhibits relatively high NAD^+- and $NADP^+$-linked 17β-hydroxy-C_{19}-steroid (testosterone) dehydrogenase activity [1, 2]. Castration produces a partial reduction in the enzyme activity which is restored to but not above normal by the daily injection of testosterone propionate [1]. The liver, on the other hand, contains an even higher concentration of the dehydrogenase activity which is not influenced either by castration or testosterone propionate injections. Since the activity of the kidney is partially dependent on the presence of testosterone, it seemed that the female guinea pig kidney should contain no or a trace of this activity.

This report demonstrates that the female guinea pig kidney contains only a trace of not only 17β-hydroxy-C_{19}-steroid dehydrogenase but also only a trace or no detectable enzyme activity for several other hydroxy-C_{19}-steroids. The administration of testosterone induced a progressive increase in the 17β-hydroxy-C_{19}-steroid dehydrogenase which was readily isolated, purified and characterized.

* This investigation was supported by grant AM 16729 from the National Institutes of Arthritis, Metabolic and Digestive Diseases.

† To whom reprint requests and correspondence should be sent.

MATERIALS AND METHODS

Guinea pigs. The female guinea pigs were purchased from Isaacs Lab. Stock, Litchfield, Il. and maintained as previously described [2]. Two cylindrical pellets of testosterone (15 mg each) were implanted subcutaneously over the shoulder [3] under Innovar-Vet anesthesia (0.4 ml/kg BW) at 400–500 g BW. Testosterone was absorbed from the pellets at 0.36 mg/d. The food was removed from the guinea pigs at 5 PM of the day preceding autopsy.

Materials. The chemicals and reagents were as previously described [2]. The purified male guinea pig kidney 17β-hydroxy-C_{19}-steroid dehydrogenase was from our previous study [2].

Procedures. The tissues were immediately homogenized in 2.5 vol. of 0.25 M sucrose/1 mM EDTA/7 mM 2-mercaptoethanol [4] The cytosol and microsomes were separated by differential centrifugation. The purification of the 17β-hydroxy-C_{19}-steroid dehydrogenase of the kidney and the analytical methods were as previously described [2].

RESULTS

C_{19}-Steroid dehydrogenase activity in normal kidney and liver

The kidney exhibited only traces or no 17β-hyd-

Table 1. Steroid dehydrogenase activity in female guinea pig kidney

Steroid	Cytosol		Microsomes	
	$NADP^+$	NAD^+	$NADP^+$	NAD^+
Testosterone	0.04	0.00	0.03	0.04
5α-Dihydrotestosterone	0.04	0.07	0.00	0.03
5β-Dihydrotestosterone	0.01	0.02	0.00	0.02
5α-Androsterone	0.00	0.00	0.04	0.14
5β-Androsterone	0.00	0.01	0.00	0.05
5α-Epiandrosterone	0.00	0.00	0.00	0.01
5β-Epiandrosterone	0.00	0.00	0.01	0.01
Dehydroepiandrosterone	0.00	0.00	0.00	0.00

The kidneys were immediately homogenized in cold (4°C) 0.25 M sucrose/1 mM EDTA/7 mM 2-mercaptoethanol, the cytosol and microsomes were separated by differential centrifugation [2] and enzyme activity was determined. The reaction mixture contained 0.3 M glycine/NaOH, pH 10.1, for $NADP^+$ (0.5 mM) or 0.2 M pyrophosphate, pH 9.6 for NAD^+ (3.8 mM), 0.2 mM steroid and 25 μl cytosol in a final volume of 1.0 ml. The absorbance at 340 nm was measured at 37°C in a Cary Model 14 spectrophotometer and the initial 3 min. of absorbance was used to calculate the U/mg kidney.

roxy- or 3α- and 3β-hydroxy-C_{19}-steroid dehydrogenase activity with the 5α- or 5β-steroids (Table 1). The liver, on the other hand, exhibited strong 17β-hydroxy-C_{19}-steroid dehydrogenase activity (Table 2). Testosterone and 5α-dihydrotestosterone (5α-DHT) were equally effective as substrate; of particular interest was the even greater activity of the cytosol with 5β-dihydrotestosterone (5β-DHT) as the substrate. The $NADP^+$-linked activities were greater than the NAD^+-linked activities in the cytosol and the reverse occurred in the microsomes. The 3-hydroxysteroids elicited no to low activity in both the cytosol and the microsomes in the presence of either NAD^+ or $NADP^+$.

Induction of 17β-hydroxy-C_{19}-steroid dehydrogenase activity

The administration of testosterone did not alter any of the enzyme activities of the liver after 5, 11 and 20 days. On the other hand, the $NADP^+$-17β-hydroxy-C_{19}-steroid dehydrogenase activity of the kidney cytosol (Fig. 1), but not microsomes, was progressively increased with duration of treatment so that after 50 days the enzyme activity was equal with that of the normal male. The NAD^+-linked activity exhibited a parallel increase. The substitution of 5α-DHT as substrate gave a response identical with that of testosterone but the enzyme activity was unchanged with 5β-DHT as substrate.

Gel electrophoresis of the cytosol

The immediate submission of the cytosol to gel electorophoresis exhibited a single enzyme band initially after 11 days of testosterone. The band progressively increased in intensity with further testosterone administration and two minor bands were also detected after 50 days (Fig. 2A). Storage of the cytosols at 4°C for 48 h with or without 2-mercaptoethanol yielded multiple bands. The absence of 2-mercaptoethanol (Fig. 2C) resulted in three enzyme bands, while the presence of mercaptoethanol (Fig. 2B) limited the number to two distinct and one barely

Table 2. Steroid dehydrogenase activity in female guinea pig liver

Steroid	Cytosol		Microsomes	
	$NADP^+$	NAD^+	$NADP^+$	NAD^+
Testosterone	1.00 ± 0.08	0.53 ± 0.06	0.14 ± 0.01	1.05 ± 0.09
5α-Dihydrotestosterone	1.01 ± 0.09	0.46 ± 0.04	0.11 ± 0.01	0.76 ± 0.09
5β-Dihydrotestosterone	1.60 ± 0.10	1.42 ± 0.07	0.18 ± 0.02	0.60 ± 0.05
5α-Androsterone	0.23 ± 0.02	0.05 ± 0.01	0.24 ± 0.04	0.07 ± 0.01
5β-Androsterone	0.22 ± 0.02	0.18 ± 0.03	0.01	0.12 ± 0.01
5α-Epiandrosterone	0.09 ± 0.01	0.09 ± 0.04	0.13 ± 0.01	0.07 ± 0.02
5β-Epiandrosterone	0.00	0.60 ± 0.14	0.00	0.20 ± 0.01
Dehydroepiandrosterone	0.01	0.00	0.03	0.01

See footnote to Table 1 for details. The values were lower than but parallel to those obtained with male guinea pig liver (e.g. testosterone as substrate. Cytosol: $NADP^+$ 1.42, NAD^+ 0.98; Microsomes: NAD^+ 0.17, NAD^+ 1.58 U/mg (unpublished)). The values are mean ± S.E. for 6 guinea pigs. The values of the treated were combined with those of the untreated guinea pigs since testosterone treatment had no effect.

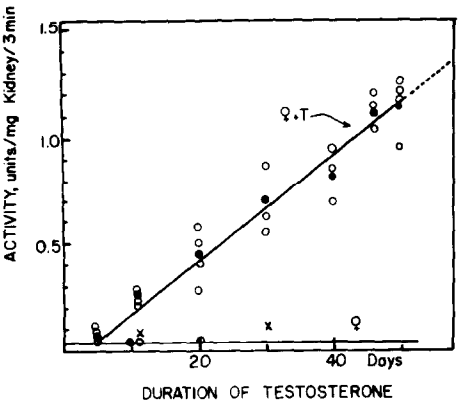

Fig. 1. The induction of 17β-hydroxy-C_{19}-steroid dehydrogenase activity in the female guinea pig kidney by testosterone. The treatment of the guinea pigs is described in the text. Dehydrogenase activity was determined with testosterone as substrate and $NADP^+$ as coenzyme (see Table 1) in a Cary Spectrophotometer Model 14. ○ = individual value and ● = mean value for the respective groups. Two of the control guinea pigs were at near term pregnancy (×) but the enzyme values were not significantly different from the non-pregnant animals. The value for 6 normal male guinea pigs was 1.3 U/mg and 0.8 U/mg for 6 castrated male guinea pigs. NAD^+-linked activity for testosterone and both NAD^+- and $NADP^+$-linked activity for 5α-DHT were determined at 5, 11 and 20 days of testosterone treatment and exhibited progressive increases parallel with the above.

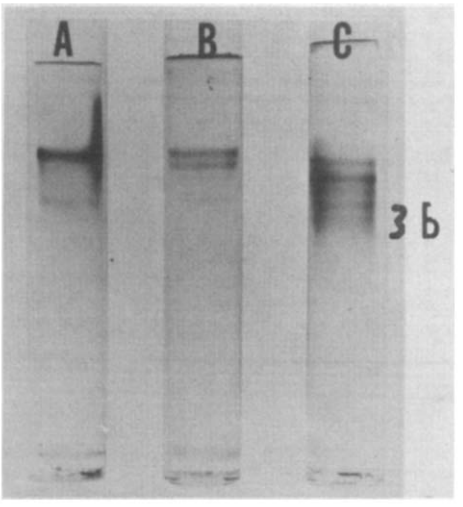

Fig. 2. Disc gel electrophoresis of the induced 17β-hydroxy-C_{19}-steroid dehydrogenase of female guinea pig kidney cytosol after 50 days of testosterone. The cytosols were prepared in 0.25 M sucrose/1 mM EDTA with (A and B) and without (C) 7 mM 2-mercaptoethanol. A: cytosol immediately analysed. B and C: cytosol stored at 4°C for 48 h. b = non-steroid dehydrogenase bands.

detectable bands. The fourth band in A and B and the fourth and fifth bands in C correspond with non-steroid dehydrogenase bands observed in control gels.

Isolation and purification

Trial experiments indicated that 80% of the enzyme activity was precipitated from the cytosol by $(NH_4)_2SO_4$ between 30 and 80% saturation. $(NH_4)_2SO_4$ at 40-75% saturation precipitated 64% of the enzyme activity (Table 3). The pooled enzyme fractions gave a symmetrical single peak on filtration through Sephadex G-75 (Fig. 3A) with a recovery of 43% of the cytosol activity (Table 3). Chromatography of the active fractions on DEAE-Sephadex (Fig. 3B) followed by chromatography on CM-Sephadex C-50 (Fig. 3C) gave single symmetrical peaks with a final yield of 19% (Table 3).

Purity of the isolated induced enzyme

Gel electrophoresis of the isolated enzyme in 7 mM 2-mercaptoethanol (Fig. 4) gave a single protein (Coomassie blue) strain (Fig. 4A) and enzyme (testosterone/$NADP^+$) (Fig. 4B) band. SDS-gel electrophoresis (Fig. 4C) also yielded a single band. Two distinct protein and enzyme bands, however, were apparent (Fig. 4D) after the mercaptoethanol was removed from the enzyme solution by dialysis against 0.25 M

Table 3. Summary of the purification of the induced 17β-hydroxy-C_{19}-steroid dehydrogenase of female guinea pig kidney

	Total protein (mg)	Total activity (U)	Specific activity (U)	Yield %
Cytosol	1,860	34,160	18	100
$(NH_4)_2SO_4$ ppt (40–75%)	632	22,070	35	64
Sephadex G-75	134	14,870	111	43
DEAE-Sephadex A-50	15	10,250	683	30
CM-Sephadex C-50	2	6,590	3,300	19

The kidneys were from the guinea pigs treated with testosterone for 40, 46 and 50 d (See Fig. 1). Homogenization was performed immediately after autopsy in 2.5 vol. of 0.25 M sucrose/1 mM EDTA/7 mM 2-mercaptoethanol. The cytosol was separated by differential centrifugation and the enzyme activity was precipitated with $(NH_4)_2SO_4$ 40–75% saturation. The precipitate was dissolved in 0.02 M Tris–HCl/0.25 M sucrose/7 mM 2-mercaptoethanol (pH 7.2) and stored at −20°C for further purification (Fig. 3). The kidneys from the animals treated for 11, 20 and 30 days were treated in the same manner and gave similar results with a final yield of 20%.

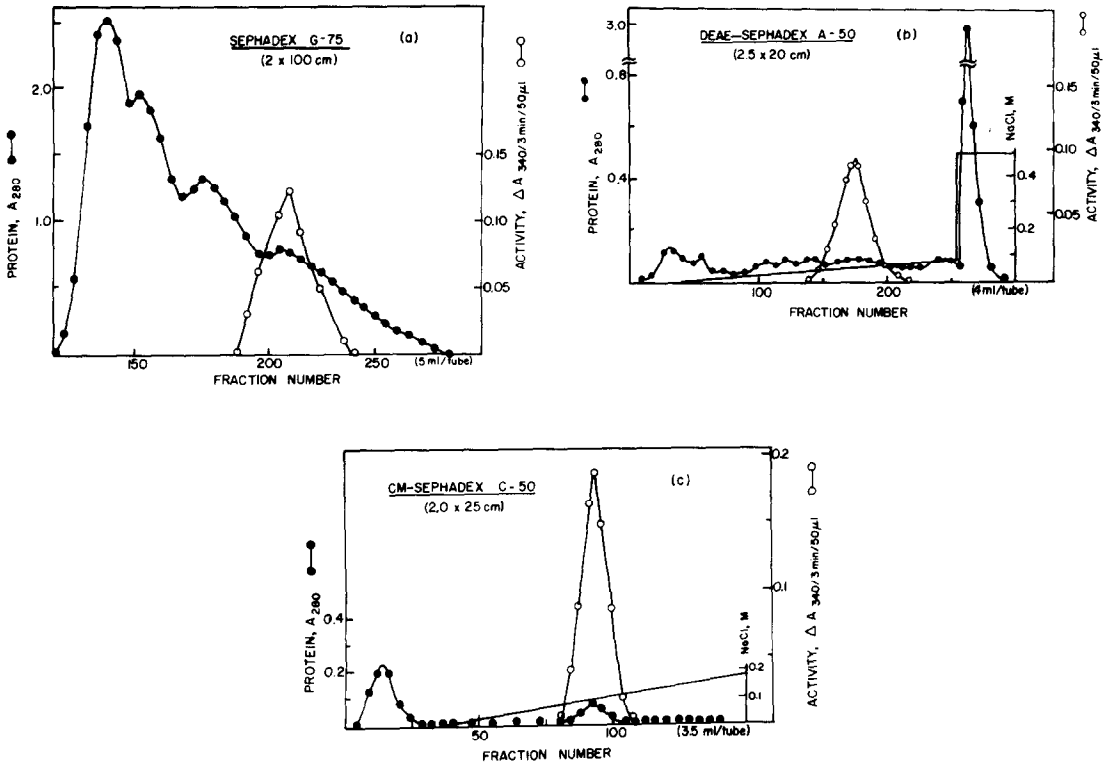

Fig. 3. Purification of induced 17β-hydroxy-C_{19}-steroid dehydrogenase of female guinea pig kidney. The $(NH_4)_2SO_4$ precipitate of the cytosol (Table 3) was dissolved in 15 ml of 0.02 M Tris·HCl/0.25 M sucrose/7 mM 2-mercaptoethanol (pH 7.2) and dialysed against the same solution to remove the $(NH_4)_2SO_4$. The dialysed solution was placed on a previously prepared column of Sephadex G-75 and the enzyme was eluted with the above buffer (Fig. 3A). The active fractions were pooled, condensed to 30 ml by vacuum ultrafiltration, dialysed against the above buffer and placed on the prepared DEAE-Sephadex A-50 column. The enzyme was eluted with the buffer containing the indicated NaCl linear gradient (Fig. 1B). The active fractions were pooled, condensed as above dialysed against 0.25 M sucrose/0.02 M phosphate/7 mM 2-mercaptoethanol, pH 7.2, and placed on a CM-Sephadex C-50 column. The enzyme was eluted with a linear gradient of NaCl in the buffer (Fig. 3C). The above procedures were carried out at 4°C.

sucrose/1 mM EDTA. A third faster moving band was barely detectable. The restoration of the mercaptoethanol to the solution reversed the multiple bands to a single band (Fig. 4E).

Molecular weight

A molecular weight of 34,000 was estimated for the enzyme from the SDS-gel electrophoresis and 32,000 after Sephadex G-100 filtration.

Isoelectric focusing

The enzyme gave a pI of 6.6 (Fig. 4F) which was identical with that of male guinea pig kidney enzyme I_1 (2) determined at the same time.

pH Dependence. The enzyme activity increased with the increase in pH to a maximum at 10.6 (Fig. 5). The simultaneous analysis of purified enzyme I_1 from male guinea pig kidney gave a parallel effect.

Substrate specificity

The purified enzyme exhibited the same or slightly higher activity with 5α-dihydrotestosterone than with testosterone (Table 4). 5β-Dihydrotestosterone, however, evoked only a fraction of the enzyme activity as did androsterone. The enzyme was unable to dehydrogenate epitestosterone, dehydroepiandrosterone, estradiol-17β and ethanol.

K_M values

Lineweaver–Burk plots of enzyme activity indicated (Fig. 6) that both the purified induced enzyme and the purified enzyme I_1 of the male guinea pig kidney had the same K_M of 2×10^{-4} M. The value was the same with NAD^+ and $NADP^+$ and testosterone and 5α-dihydrotestosterone (Fig. 6).

The effect of phosphate ions on enzyme activity

The substitution of pyrophosphate for glycine buffer did not alter the K_M for testosterone but increased the V_{max} from 400 to 2000 U/mg when NAD^+ was the coenzyme (Fig. 7). On the other hand, the K_M for NAD^+ was 9×10^{-3} M in the glycine buffer and 1.4×10^{-3} M in the pyrophosphate buffer and the V_{max} was 440 and 1100 U/mg respectively (Fig. 8). No such differences were detected when $NADP^+$ was the coenzyme.

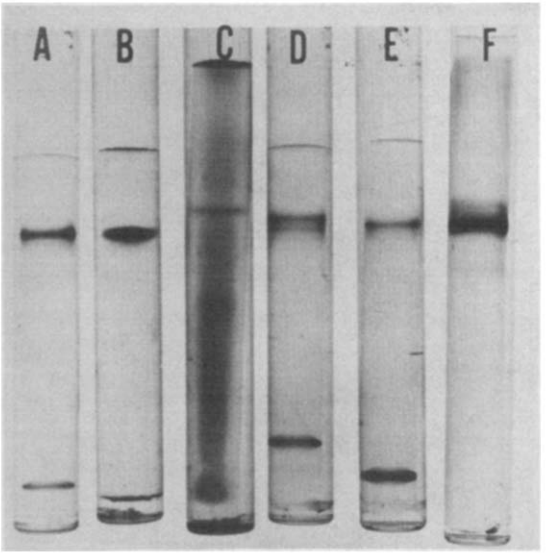

Fig. 4. Gel electrophoresis of the purified induced 17β-hydroxy-C_{19}-steroid dehydrogenase. The purified enzyme (50 μg) in 0.02 M phosphate/0.25 M sucrose/7 mM 2-mercaptoethanol pH 7.2 showed one band on enzyme staining (A), one band on protein staining (B) and one band on the SDS gel (C). The enzyme dissociated into two major and 1 minor bands when the mercaptoethanol was removed by dialysis (D). The enzyme reassociated to one band when the mercaptoethanol was added (E). Gel electrofocusing of the purified enzyme gave a single band (F).

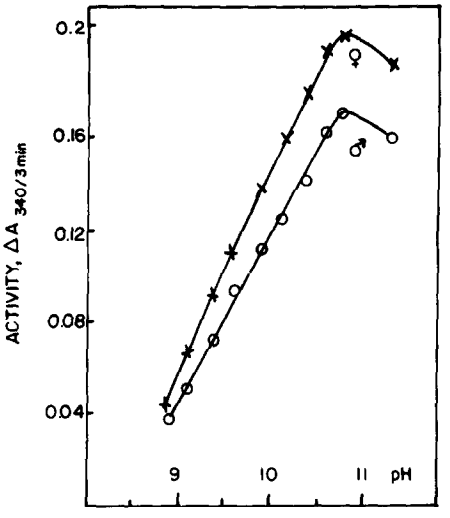

Fig. 5. Effect of pH on the activity of the purified induced enzyme on the female guinea pig. The assays were carried out in 0.3 M glycine/NaOH, 0.5 mM $NADP^+$, 0.2 mM testosterone. The purified male kidney enzyme (♂) of a previous preparation [2] was analysed simultaneously with the purified induced enzyme of the female guinea pig kidney (♀) (Table 3, Fig. 3).

DISCUSSION

The kidney of the female guinea pig in contrast to that of the male guinea pig contains at best only traces of 3- and 17-hydroxy-C_{19}-steroid dehydrogenases. The activity of these enzymes apparently is dependent upon the stimulation of the specific gene by a specific steroid(s). Testosterone administration gradually increased the 17β-hydroxy-C_{19}-steroid dehydrogenase concentration of the female guinea pig kidney from a trace to that of the male after 50 days. The stimulating effect of testosterone was specific for testosterone and 5α-dihydrotestosterone. The induction was not apparent for 5β-dihydrotestosterone indicating ring as well as hydroxyl specificity. The detection of a trace of enzyme activity in the cytosol with 5β-dihydrotestosterone suggests that the potential for such activity is present and possibly could be induced by the administration of 5β-dihydrotestosterone. In support of this possibility is the detection and purification of a dehydrogenase in guinea pig liver which is specific for 17β-hydroxy-5β-androstanes [5, 6]. The other hydroxy-androstane dehydrogenases also possibly may be induced by administration of the corresponding specific steroid. An enzyme has been isolated from male guinea pig kidney which has a preference for androsterone [2] as substrate.

The lack or slight trace of 17β-hydroxy-5α-androstane dehydrogenase activity in the female guinea pig kidney suggests that no or only a trace of testosterone is produced by the female guinea pig. The male guinea pig plasma contains traces of testosterone and

Table 4. Substrate specificity of the purified induced 17β-hydroxy-C_{19}-steroid dehydrogenase of the female guinea pig kidney

	Specific activity (U/mg)
Testosterone	3,050
5α-Dihydrotestosterone	3,200
5β-Dihydrotestosterone	400
Androsterone	350

The enzyme assay was done in 0.3 M glycine/NaOH, pH 10.1, 0.5 mM $NADP^+$, 0.2 mM steroid and 5.5 μg enzyme protein in a final volume of 1.0 ml. The change in absorbance at 340 nm was measured in a Gilford Model 240 spectrophotometer with a Model 242 recorder. Estradiol-17β, epitestosterone, dehydroepiandrosterone and ethanol were ineffective as substrates.

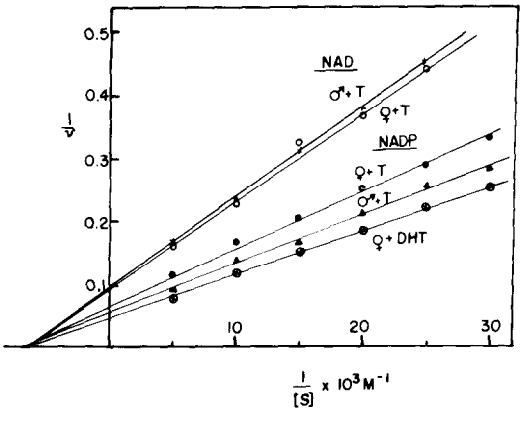

Fig. 6. Lineweaver–Burk plots of the purified 17β-hydroxy-C_{19}-steroid dehydrogenase of female and male guinea pig kidneys. The enzyme activity was determined in 0.3 M glycine/NaOH, pH 10.1, and 0.5 mM $NADP^+$ or in 0.18 M pyrophosphate, pH 9.6, and 4 mM NAD^+. Testosterone (T) and 5α-dihydrotestosterone (5α-DHT) at 0.2 mM were used as substrates. The purified male kidney enzyme (♂) was from a previous study [2] and the induced female (♀) enzyme was from Fig. 3 and Table 3.

androstenedione in the prenatal and early post-natal days which increase in parallel with the increase in testosterone production by the testis [7]. Castration [1] produced only a partial decrease in the 17β-hydroxy-C_{19}-steroid dehydrogenase activity of the male kidney with a restoration to normal on administration of testosterone. The partial loss of the enzyme activity after castration suggests that the enzyme activity in the male guinea pig is only partially regulated by the testis production of testosterone. Some other source of testosterone or other factors are involved in the production of this enzyme.

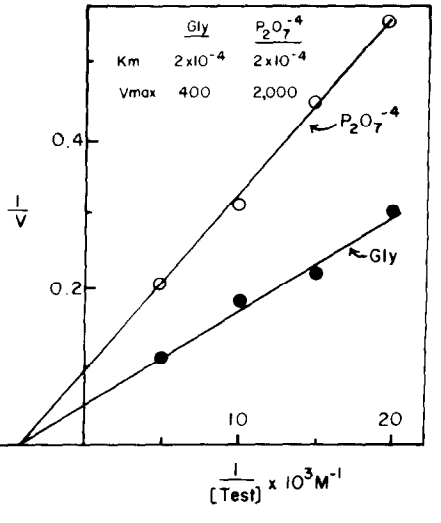

Fig. 7. Determination of K_M and V_{max} for NAD^+. The assays were carried out in 0.1 M pyrophosphate, pH 9.6 or 0.1 M glycine/NaOH pH 9.6, and 0.2 mM testosterone in 1 ml of reaction mixture at 37°C. The enzyme was added at 5 μg for the pyrophosphate and 50 μg for the glycine system.

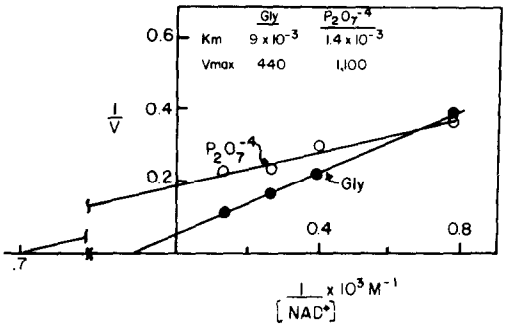

Fig. 8. Determination of K_M and V_{max} for testosterone. The assays were carried out in 0.1 M pyrophosphate, pH 9.6 or 0.1 M glycine/NaOH, pH 9.6 and 6 mM NAD^+ in 1 ml of the reaction mixutre at 37 C.

The adrenal cortex is a potential source of testosterone, especially after castration [8]. The existence of one or more other genes for the dehydrogenase is a possibility.

The identical properties of the purified induced enzyme with those of the main enzyme of the male guinea pig kidney indicate that the male and female kidneys contain an identical gene for the enzyme.

The slow gradual increase in the concentration of dehydrogenase activity in the kidney by testosterone is worthy of note. The process of induction apparently is not a simple all-or-none switch mechanism. The increase in enzyme concentration to the level in the male guinea pig kidney required 50 days. This suggests a progressive activation of the number of genes by an increase in the concentration of testosterone. A similar testosterone treatment of castrated guinea pigs, however, produced a maximum increase in weight and total RNA and DNA in the seminal vesicles and prostates within 30 days and a maximum concentration of the nucleic acids within two weeks [9]. The maximum rate of protein synthesis by homogenates of these tissues were attained even sooner –within one week [10]. A comparison of these tissues with the kidney may not be realistic. These parameters in the guinea pig kidney are not responsive to testosterone or castration. Other factors e.g. enzyme degradation should be considered.

The physiological significance of the dependence of the 17β-hydroxy-C_{19}-steroid dehydrogenase activity on testosterone is not completely clear. The enzyme could be present for the regulation of the level and metabolic action of testosterone in the kidney alone and/or the several other tissues of the body [1, 11, 12].

The microsomal NADH- and NADPH-linked 3α-hydroxy-steroid oxidoreductase activities of the rat kidney have been recently reported to be regulated by testicular hormones [8]. Castration reduced the high level of enzyme activities which was restored by the administration of testosterone, 5α-dihydrotestosterone or 5α-androstane-3α,17β-diol esters. On the other hand, the 17β-hydroxy-C_{19}-steroid dehydrogenase activity of the rat kidney cytosol was increased by castration or estradiol-17β-injections [13]. The

regulation of the enzyme activity was attributed to a multihormonal effect via the anterior pituitary. Apparently, the regulation of 17β-hydroxy-C_{19}-steroid dehydrogenase activity in the rat kidney cytosol is very different from that in the guinea pig kidney.

In contrast to the guinea pig kidney, the dehydrogenases of the liver did not respond to the administration of testosterone suggesting that the regulation of the liver enzymes in the female as in the male guinea pig are under regulatory mechanisms which are different from those in the kidney. It is noteworthy that the highest dehydrogenase activity in the liver was obtained with 5β-dihydrotestosterone. The 5β-androstanes have proven to be biologically inactive in all of the common tests. Neonatal administration of 5β-dihydrotestosterone, however, can induce irreversible changes in the mammary glands, pituitary, ovaries and genital tract of mice similar to those seen with biologically active steroid hormones including marked stimulation of spontaneous mammary tumorigenesis [14].

REFERENCES

1. Kochakian C. D. and Endahl B. R.: Testosterone regulation of 17β-hydroxysteroid dehydrogenases. *Proc. Soc. Exptl. Biol. Med.* **104** (1960) 720–722.
2. Shen C. C. and Kochakian C. D.: Purification and properties of 17β-hydroxy-C_{19}-steroid dehydrogenases of adult male guinea pig kidney. *Endocrinology.* **103** (1978) 2040–2052.
3. Kochakian C. D., Hill J. and Costa G.: Amino acid composition of the proteins of the muscles and organs of the normal, castrated and testosterone-treated guinea pigs. *Acta endocr. Copenh.* **45,** (1964) 613–622.
4. Liu D. K. and Kochakian C. D.: Partial purification and some properties of guinea pig kidney 17β-hydroxy-C_{19}-steroid dehydrogenase. *Steroids* **19** (1972) 701–719.
5. Kobayashi K. and Kochakian C. D.: 17β-Hydroxy-C_{19}-steroid dehydrogenases from male guinea pig liver. *J. biol. Chem.* **253** (1978) 3653–3642.
6. Kageura E. and Toki S.: Purification and properties of a new testosterone 17β-dehydrogenase ($NADP^+$) from guinea pig liver. *Biochem. J.* **163** (1977) 401–407.
7. Rigaudiere N., Pelardy G., Robert A. and Delost P.: Changes in the concentrations of testosterone and androstenedione in the plasma and testis of the guinea pig from birth to death. *J. Reprod. Fert.* **48** (1976) 291–300.
8. Verhoeven G. and De Moor P.: Androgenic control of the microsomal 3α-hydroxysteroid oxidoreductases in rat kidney. *J. steroid Biochem.* **8** (1977) 113–119.
9. Kochakian C. D., Hill J. and Harrison D. G.: Regulation of nucleic acids of muscles and accessory sex organs of guinea pigs by androgens. *Endocrinology* **74** (1964) 635–642.
10. Kochakian C. D.: Effect of castration and testosterone on protein biosynthesis in guinea pig tissue preparations. *Acta endocr. (Copenh.)* **46** (Supp 92) (1964) 3–16.
11. Kochakian C. D.: Regulation of kidney growth by androgen. In *Steroid Biochemistry and Pharmacology* (Edited by M. H. Briggs and G. A. Christie). Academic Press, London, New York, San Francisco, Vol. 6 (1978) p. 1.
12. Kochakian C. D.: Sites of tissue formation. In *Anabolic-Androgenic Steroids* (Edited by C. D. Kochakian). Springer-Verlag Berlin, Heidelberg, New York (1976) pp. 73–154.
13. Ghraf R., Lax E. R. and Schrieffers H.: Multihormonal regulation of sex-dependent 17β-hydroxysteroid dehydrogenase activity in rat kidney cytosol. *Z. Physiol. Chem.* **358** (1977) 165–168.
14. Yanai R., Mori T. and Nagasawa H.: Long term effects of prenatal and neonatal administration of 5β-dihydrotestosterone on normal and neoplastic mammary development in mice. *Cancer Res.* **37** (1977) 4456–4459.