[Chem. Pharm. Bull. 25(3) 420—426 (1977)]

UDC 615.631.011.4.076.9 : 577.158.01.02

Glucuronidation of Testosterone by Rat Liver Microsomes[1]

Mitsuteru Numazawa, Keiko Suzuki, and Toshio Nambara[2]

Pharmaceutical Institute, Tohoku University[3]

(Received May 19, 1976)

The properties of testosterone glucuronyltransferase of rat liver microsomes were studied. The conjugate which was formed from testosterone was identified as the 17β-glucuronide by thin-layer chromatography, hydrolysis with β-glucuronidase, and reverse isotope dilution analysis of the acetate-methyl ester derivative. The microsomal testosterone glucuronyltransferase of liver was shown to increase with age of the animals up to 15—16 weeks. Treatment of the isolated microsomes with Triton X-100 or with deoxycholate enhanced apparent glucuronyltransferase activity 5- and 2-fold at maximum, respectively. This increase of the enzyme activity was due to the elevation of the *V*max value. Pretreatment of the animals with phenobarbital, 3-methylcholanthrene, diphenylhydantoin, phenylbutazone, or with 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) increased the liver glucuronyltransferase activity by 130—230%. The microsomes obtained from 3-methylcholanthrene-treated rats showed the glucuronyltransferase activity with smaller *K*m value for testosterone. The kinetic studies also demonstrated that *p*-nitrophenol inhibited glucuronidation of testosterone competitively, whereas both bilirubin and estradiol inhibited the reaction noncompetitively.

Keywords——testosterone; 17β-hydroxysteroid glucuronyltransferase; rat liver; subcellular localization; development with age; kinetic properties

Testosterone 17β-glucuronide is a principal conjugate of male hormone in human urine[4] and is formed *in vitro* from testosterone by the liver preparation.[5] It is now well established that conjugation of an acceptor substrate with glucuronic acid is catalyzed by uridine diphosphate (UDP) glucuronate glucuronyltransferase (EC 2.4.1.17) resulting in formation of the β-glucuronide.[6] It has been suggested that multiple enzyme may probably be involved in glucuronidation of a large number of endogenous and exogenous substrates.[7,8] During recent years the formation of steroid glucuronides has been extensively studied and a variety of glucuronyltransferases have been shown to participate in the metabolism of steroid hormones. In particular, the properties of estrogen glucuronyltransferases and their role in the enterohepatic circulation of female hormones have been investigated.[9] As for the metabolism of

1) Part CXVII of "Studies on Steroids" by T. Nambara; Part CXVI: K. Arisue, Y. Katayama, Z. Ogawa, C. Hayashi, M. Miyata, K. Shimada, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **24**, 3093 (1976). In this paper the following trivial names were used: testosterone, 17β-hydroxyandrost-4-en-3-one; testosterone 17β-glucuronide, 3-oxoandrost-4-en-17β-yl-β-D-glucopyranosiduronic acid; testosterone 17β-glucuronide acetate-methyl ester, methyl (3-oxoandrost-4-en-17β-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate; estradiol, estra-1,3,5(10)-triene-3,17β-diol.

2) To whom any inquiries should be addressed.

3) Location: *Aobayama, Sendai.*

4) K. Schubert and K. Wehrberger, *Naturwissenschaften*, **47**, 281 (1960); A. Camacho and G.J. Migeon, *J. Clin. Endocrinol. Metab.*, **23**, 301 (1963); H.E. Hadd and R.K. Rhamy, *ibid.*, **25**, 876 (1965).

5) W.H. Fishman and H.G. Sie, *J. Biol. Chem.*, **218**, 335 (1956); H.H. Wotiz, H.G. Sie, and W.H. Fishman, *ibid.*, **232**, 723 (1958).

6) K.J. Isselbacher, "Recent Progress in Hormone Research," Vol. 12, ed. by G. Pincus, Academic Press, New York, 1956, pp. 134—151.

7) G.J. Dutton, "Glucuronic Acid," ed. by G.J. Dutton, Academic Press, New York, 1966, pp. 185—299.

8) *a*) E. Halac and A. Reff, *Biochim. Biophys. Acta*, **139**, 328 (1967); *b*) K.W. Bock, W. Fröhling, H. Remmer, and B. Rexer, *ibid.*, **327**, 46 (1973).

9) A.A. Sandberg, W.R. Slaunwhite, Jr., and R.Y. Kirdani, "Metabolic Conjugation and Metabolic Hydrolysis," Vol. II, ed. by W.H. Fishman, Academic Press, New York, 1970, pp. 123—152.

testosterone, glucuronidation also appears to be an important transformation in the living animal. The blood level of testosterone glucuronide is nearly equal to that of free testosterone,[10] and the 17β-glucuronide is further metabolized through preferential 5β-hydrogenation in man.[11] Therefore it seems to be an attractive problem to clarify the characteristics of testosterone glucuronyltransferase in connection with the physiological significance of conjugation. The present paper deals with the properties of testosterone glucuronyltransferase from the microsomal fraction of rat liver.

Experimental

Materials——Testosterone-7α-^{3}H (specific activity 7.6 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, England) and purified by thin-layer chromatography (TLC) using silica gel G or H (E. Merck AG, Darmstadt). Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia), beef-liver β-glucuronidase (Tokyo Zōkikagaku Co., Tokyo), bilirubin and UDP-glucuronic acid (Sigma Chemical Co., St. Louis) were purchased. All solvents used were of analytical grade.

Animals and Pretreatment with Drugs——Immature male Wistar rats (100—120 g) having free access to food and water were used except in the study on the effect of aging. The animals were maintained in the local animal facilities for 3—5 days prior to use. In one experiment, phenobarbital (40 mg/kg), diphenylhydantoin (40 mg/kg), or phenylbutazone (50 mg/kg) dissolved in isotonic saline was injected intraperitoneally in the animals twice daily for 4 days, respectively. Injections of 3-methylcholanthrene (20 mg/kg) and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) (25 mg/kg) were performed in the same fashion but corn oil was used as the solvent. The control animals received similarly intraperitoneal injections of the appropriate vehicles (0.2 ml). In all experiments the rats were starved for 24 hr prior to sacrifice.

Separation of Subcellular Fractions——The rats were sacrificed by decapitation and liver was immediately removed. When the animals were pretreated with drugs, the enzyme assay was performed 24 hr after the last injection. All subsequent steps were carried out at 0—4°. The tissue was weighed, minced with scissors, and then homogenized with 4 volumes of 0.25 M sucrose by a Potter-Elvejhem homogenizer (Teflon pestle). The homogenate was centrifuged at $600\times g$ for 10 min and the supernatant was in turn centrifuged at $8000\times g$ for 10 min. The sediment was washed twice with 0.25 M sucrose and used as a mitochondrial fraction. The $8000\times g$ supernatant was then centrifuged at $25000\times g$ for 10 min. The lysosomal sediment thus obtained was discarded and the supernatant was centrifuged at $105000\times g$ for 60 min. The sediment was washed once with 0.25 M sucrose and used as a microsomal fraction. The supernatant fraction was again centrifuged at $105000\times g$ for 90 min. In ordinary runs, however, the homogenate was centrifuged at $10000\times g$ for 30 min and the resulting supernatant was recentrifuged at $105000\times g$ for 60 min. Unless otherwise specified, liver microsomes from normal rats (no induction drug injection) were used. The microsomal pellet thus obtained was resuspended in 1.15% KCl in such a way that 1.0 ml should be equivalent to 200 mg wet weight of liver. Microsomal protein was determined by the method of Lowry, *et al.*[12]

Enzyme Assay——Each incubation medium contained testosterone-7α-^{3}H (200 nmoles, 0.5 μCi) in MeOH (0.1 ml), UDP-glucuronic acid (400 nmoles) and $MgCl_2$ (30 nmoles) in 0.05 M Tris–HCl buffer (pH 7.4) (0.1 ml), the microsomal preparation in 1.15% KCl (1 ml, equivalent to 2—3 mg of protein), and sufficient 0.05 M Tris–HCl buffer to make the total volume 2 ml. Incubations were performed at 37° for 10 min or 15 min with air as a gas phase in a test tube and terminated by the addition of ether (5 ml). The incubation mixture was diluted with water (3 ml) and then extracted with ether (5 ml×3). The preliminary experiments confirmed that by this procedure the glucuronide could be separated quantitatively from the unconjugated substrate. An aliquot of the glucuronide fraction was submitted to the radioactivity counting.

Separation and Characterization of Testosterone 17β-Glucuronide——An aqueous phase was layered gently onto a column packed with Amberlite XAD-2 resin (30 ml) and allowed to percolate through the column. After thorough washing with water for removal of polar substances, the glucuronide was eluted with MeOH. The effluent was evaporated *in vacuo* below 50°, and a portion of the residue was dissolved in 50% MeOH and chromatographed on the thin-layer plate (silica gel G) in $CHCl_3$–MeOH–formic acid (15: 5: 3, v/v) employing testosterone 17β-glucuronide as a carrier. Another portion of the glucuronide fraction was dissolved in 0.1 M acetate buffer (pH 4.5) and incubated with beef-liver β-glucuronidase (200 Fishman units) at 37° for 24 hr. The radioactive aglycone liberated was chromatographed on the thin-layer plate (silica gel G) in hexane–AcOEt (2: 3, v/v) employing testosterone as a carrier. The areas corresponding to testosterone (*Rf* 0.59) and its 17β-glucuronide (*Rf* 0.64) were eluted and submitted to the radioactivity counting, respectively. To a remaining portion of the glucuronide fraction was added an ethereal solution of CH_2N_2 and allowed to stand at room temperature for 30 min. After the addition of a drop of AcOH the resulting solution was evaporated

10) H.G. Burger, J.R. Kent, and A.E. Kellie, *J. Clin. Endocrinol. Metab.*, **24**, 432 (1964).
11) P. Robel, R. Emiliozzi, and E.-E. Baulieu, *J. Biol. Chem.*, **241**, 20 (1966).
12) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

with an aid of N_2 gas stream. The residue was dissolved in pyridine (0.2 ml)–Ac_2O (0.2 ml) and allowed to stand at room temperature for 12 hr. After usual work-up the crude product obtained was subjected to preparative TLC on silica gel H. On multiple run using hexane–AcOEt (2: 1, v/v) as a developing solvent the area corresponding to testosterone 17β-glucuronide acetate-methyl ester was scraped and eluted with AcOEt. The eluate was diluted with testosterone 17β-glucuronide acetate–methyl ester (26 mg) as a carrier and crystallized repeatedly up to a constant specific activity.

Radioactivity Counting——Counting was carried out on a Packard Tri-Carb Model 3380 liquid scintillation spectrometer using the Bray's scintillator.[13] The quenching was corrected by the external standard method.

Results

The conjugate formed by incubation with the tissue preparations showed the mobility identical with that of testosterone 17β-glucuronide on TLC. The aglycone liberated by hydrolysis with β-glucuronidase was also identified as testosterone by TLC. The complete structure of the glucuronide was unequivocally characterized by leading to the acetate-methyl ester in the usual manner, followed by the reverse isotope dilution analysis as listed in Table I.

Examinations were then made on the subcellular distribution of testosterone glucuronyltransferase. The acceptor substrate was incubated in the presence of UDP-glucuronic acid with the whole homogenate and three subcellular fractions, mitochondria, microsome, and supernatant, respectively. It is evident from the data in Table II that testosterone glucuronyltransferase in rat liver is localized mainly in the microsomal fraction.

Table I. Identification of Testosterone 17β-Glucuronide Acetate-Methyl Ester by Reverse Isotope Dilution Method

Crystallization[a]		Specific activity[b] (dpm/mg)
No.	from	
1	MeOH	8450
2	acetone–H_2O	8100
3	acetone–H_2O	8050

a) Nonradioactive testosterone 17β-glucuronide acetate-methyl ester (26 mg) was added as a carrier.
b) calculated: 8700 dpm/mg

Table II. Subcellular Distribution of Testosterone Glucuronyltransferase in Rat Liver[a]

Fraction	Activity (nmole/g wet tissue/min)	Specific activity (nmole/mg protein/min)
Whole homogenate	10.30	0.057
Mitochondrial (600—8000 × *g* ppt.)	0.69	0.007
Microsomal (25000—105000 × *g* ppt.)	6.47	0.570
Soluble (105000 × *g* sup.)	0.77	0.020

a) The figures represent the mean of three determinations.

Treatment of the rat liver microsomes with Triton X-100 and deoxycholate caused a marked increase of the glucuronyltransferase activity. As illustrated in Fig. 1, effects of the detergents were dependent upon their concentrations and maximum with *ca.* 0.075% Triton X-100 and 0.025% deoxycholate, which resulted in five- and two-fold increase of the enzyme activity, respectively. However, deoxycholate at the concentration over 0.075% exerted rather an

13) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).

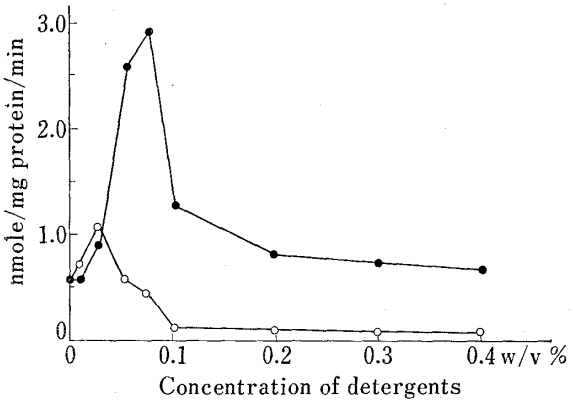

Fig. 1. Effects of Detergents on the Activities of Testosterone Glucuronyltransferase of Rat Liver Microsomes

Microsomal suspension (2.5 mg protein/ml) in 0.05M Tris-HCl buffer (pH 7.4) was preincubated with various concentrations of detergents at room temperature for 10 min.
●—●: Triton X-100, ○—○: deoxycholate
Figures on the *abscissa* indicate the final concentration.

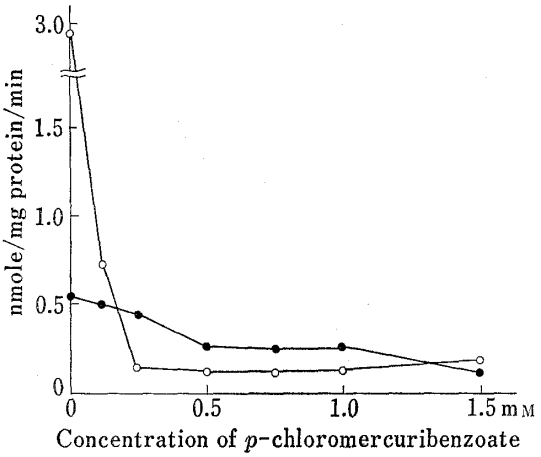

Fig. 2. Effect of *p*-Chloromercuribenzoate on the Activities of Testosterone Glucuronyltransferase of Native and Triton X-100-treated Rat Liver Microsomes

Microsomal suspension (2.5 mg protein/ml) in 0.05M Tris-HCl buffer (pH 7.4) was preincubated with various concentrations of *p*-chloromercuribenzoate at room temperature for 10 min. Triton X-100-treated microsomes exhibiting the maximum activity in Fig. 1. were used.
●—●: Native, ○—○: Triton X-100-treated
Figures on the *abscissa* indicate the final concentration.

inhibitory effect on testosterone glucuronyltransferase. On the other hand Triton X-100 did not show any inhibition even at higher concentrations.

The effect of *p*-chloromercuribenzoate on the activity of testosterone glucuronyltransferase was then explored. As can be seen in Fig. 2, the 0.075% Triton X-100-treated enzyme was much more sensitive for the sulfhydryl-blocking agent than the native one. The activity of the treated enzyme decreased rapidly with an increasing concentration of *p*-chloromercuribenzoate and only 4% of the initial activity remained at 1.5 mM of the sulfhydryl reagent.

The kinetic studies on the properties of testosterone glucuronyltransferase were then undertaken. A Lineweaver-Burk plot of the reaction velocity against the concentration of testosterone gave an apparent *K*m value of 59.9×10^{-6}M and a *V*max value of 0.77 nmole/mg protein/min for the control. Another double reciprocal plot of the reaction velocity against varying concentrations of UDP-glucuronic acid showed the apparent *K*m and *V*max values to be 5.5×10^{-6} M and 0.50 nmole/mg protein/min, respectively. The liver microsomal glucuronyltransferase of 3-methylcholanthrene–treated animals showed smaller *K*m value and slightly greater *V*max value. When the same enzyme of the control animals was treated with Triton X-100 *in vitro*, there was an increase of the *V*max value as it was observed above, but the apparent *K*m value was rather slightly decreased (Table III).

It has been reported that estradiol is conjugated with glucuronic acid *via* the 17β-hydroxyl group in rat liver, while *p*-nitrophenol and bilirubin undergo glucuronidation with different glucuronyltransferases.[7,14] Accordingly, the effects of these acceptors on conjugation of testosterone appeared to be of particular interest. A Lineweaver-Burk plot of the reaction velocity against the concentration of the substrate in the presence of *p*-nitrophenol, bilirubin, or estradiol was undertaken, separately. *p*-Nitrophenol showed competition with testosterone in glucuronidation, whereas both estradiol and bilirubin inhibited the reaction noncompetitively (Fig. 3).

The change of the liver glucuronyltransferase activity with the age of the animals was then

14) H.G. Sie and W.H. Fishman, *J. Biol. Chem.*, **225**, 453 (1975).

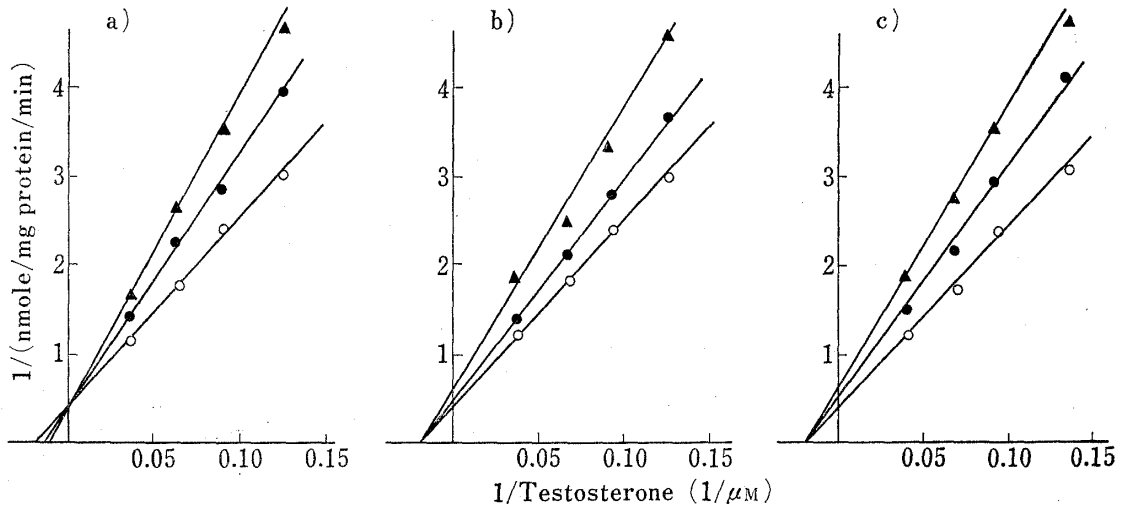

Fig. 3. Double Reciprocal Plots of Velocity against Varying Concentrations of Testosterone in the Presence of *p*-Nitrophenol, Bilirubin, or Estradiol

The incubations were carried out as described in the text.
a) *p*-nitrophenol: ○—○ none; ●—● 7.5 μM; ▲—▲ 15 μM
b) bilirubin: ○—○ none; ●—● 20 μM; ▲—▲ 40 μM
c) estradiol: ○—○ none; ●—● 10 μM; ▲—▲ 30 μM

TABLE III. Effects of 3-Methylcholanthrene (*in Vivo*) and Triton X-100 (*in Vitro*) on the Kinetic Constants of Testosterone Glucuronyltransferase in Rat Liver Microsomes

Kinetic constant	Control	Treated	
		3-Methylcholanthrene[a] (*in vivo*)	Triton X-100[b] (*in vitro*)
$Km \times 10^6$ (M)	59.9	38.5	50.0
Vmax (nmole/mg protein/min)	0.77	0.92	1.56

a) Rats were administered intraperitoneally with 3-methylcholanthrene as described in the text.
b) Microsomal suspension was preincubated with 0.075% Triton X-100 as described in the footnote of Fig. 1.

TABLE IV. Testosterone Glucuronyltransferase Activity of Liver Microsomes in Rats of Various Ages[a]

Age (weeks)	Body weight (g)	Activity (nmole/g wet tissue/min)	Specific activity (nmole/mg protein/min)
5	100—110	5.05±0.16	0.49±0.014
10—11	200—220	6.88±0.21	0.55±0.013
15—16	350—400	10.51±0.53	0.86±0.025

a) The figures represent the mean and standard error of five determinations ($p<0.01$).

investigated. Table IV shows that the enzyme activity increased gradually with age and the rats at 5 weeks of age had one-half the activity of those at 15 to 16 weeks of age.

Various glucuronyltransferases are inducible in liver by pretreatment of the animals with some drugs, polycyclic hydrocarbons, or insecticides.[7,14,15] These induction studies, however,

15) *a*) J.K. Inscoe and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **129**, 128 (1960); *b*) P. Zeidenberg, S. Orrenius, and L. Ernster, *J. Cell. Biol.*, **32**, 528 (1967); *c*) G.J. Mulder, *Biochem. J.*, **117**, 319 (1970); *d*) R.F. Potrepka and J.L. Spratt, *Biochem. Pharmacol.*, **20**, 2247 (1971); *e*) P.L.M. Jansen and P. Th. Henderson, *ibid.*, **21**, 2457 (1972).

Table V. Effects of Pretreatment of Rats with Some Drugs on Testosterone Glucuronyltransferase Activity of Liver Microsomes[a]

Drug	Control (nmole/mg protein/min)	Treated (nmole/mg protein/min)
Phenobarbital	0.44±0.03	0.66±0.04[b]
3-Methylcholanthrene	0.37±0.02	0.84±0.06[c]
Diphenylhydantoin	0.41±0.04	0.65±0.03[b]
Phenylbutazone	0.37±0.02	0.57±0.02[b]
DDT	0.39±0.02	0.51±0.02[c]

a) The figures represent the mean and standard error of five determinations.
b) $p<0.05$, *c*) $p<0.01$

have not fully dealt with conjugation of steroid hormones. Therefore, the effects of the representative inducers on glucuronidation of testosterone were examined. Intraperitoneal injections of 3-methylcholanthrene increased the glucuronyltransferase activity by 230%. In addition, the administration of phenobarbital, diphenylhydantoin, phenylbutazone, and DDT also stimulated the enzyme activity for conjugation of testosterone by 130—160% (Table V).

Discussion

Breuer and his coworker[16] reported the purification and substrate specificity of 17β-hydroxysteroid glucuronyltransferase in the 150000×g supernatant (termed as ground plasma) prepared from human intestine. The existence of this enzyme system in the human intestinal microsomes was also clarified.[17] In the present study the formation of testosterone 17β-glucuronide by rat liver has been unequivocally demonstrated. To the best of our knowledge this is the first report of characterizing 17β-hydroxysteroid glucuronyltransferase in the rat liver microsomes. It is also to be noted that the conjugate formed from testosterone by rat liver was the 17β-glucuronide but not the 3-enol glucuronide.[18] Testosterone glucuronyltransferase was principally localized in hepatic microsomes. The transfer activity observed in the soluble and mitochondrial fractions would probably be ascribable to contamination of the microsomal enzyme.

Testosterone glucuronyltransferase was markedly fortified by treatment with the detergents. It is known that the enzyme activity is affected to a different extent depending on the acceptor substrate when treated with Triton X-100 or deoxycholate.[19] The p-nitrophenol glucuronyltransferase activity is elevated by treatment with 0.1% Triton X-100 or 0.06% deoxycholate and is depressed with over 0.1% deoxycholate. The present result together with the previous finding implies the closely similar properties between testosterone and p-nitrophenol glucuronyltransferases. It is supposed that glucuronyltransferase bound with the microsomal membrane is supported by the phospholipid micelles.[20] Although the enzyme can be "solubilized" by treatment with the detergent and ultrasonic oscillation, inspection of the electron micrograph demonstrates the partly purified fraction being of the intact membranous structure.[8a,21] The experiments with p-chloromercuribenzoate indicate that the conformational change of the microsomal membrane with the detergent would result in exposure of the thiol groups around the active site.

16) K. Dahm and H. Breuer, *Biochim. Biophys. Acta*, **128**, 306 (1966).
17) K. Dahm, H. Breuer, and M. Lindlan, *Z. Physiol. Chem.*, **345**, 139 (1966).
18) M. Matsui, M. Kawase, and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **22**, 2530 (1974).
19) A.B. Graham and G.C. Wood, *Biochim. Biophys. Acta*, **276**, 392 (1972).
20) A.B. Graham and G.C. Wood, *Biochem. Biophys. Res. Commun.*, **37**, 567 (1969).
21) A.P. Mowat and I.M. Arias, *Biochim. Biophys. Acta*, **212**, 65 (1963); B.P.F. Adlard and G.H. Lathe, *Biochem. J.*, **119**, 437 (1970).

Bock, *et al.*[8b] disclosed that glucuronidation of *p*-nitrophenol is selectively stimulated by pretreatment with 3-methylcholanthrene, while conjugation of bilirubin is significantly increased with phenobarbital. Mulder[15c] also reported that pretreatment with phenobarbital does not exert any increase in glucuronidation of *p*-nitrophenol unless the activated enzyme preparation is employed. There can be seen a similarity in the mode of induction between *p*-nitrophenol and testosterone glucuronyltransferases. Considering preferential 5β-hydrogenation of testosterone 17β-glucuronide in human,[11] it seems to be important in the metabolism of male hormone that glucuronyltransferase may possibly be influenced by a variety of substances.

Determination of the kinetic constant with a heterogeneous system does not permit the precise analysis of the enzymatic reaction. The data obtained in this study, however, appear to follow the Michaelis-Menten kinetics and hence comparison of the kinetic constant between the pretreated and control animals is profitable. Treatments with 3-methylcholanthrene and Triton X-100 exerted influences on the kinetic constant of glucuronyltransferase in the different way. An alteration in the kinetic constant observed with the enzyme from the 3-methylcholantherene–treated animal suggests that the increased activity is mainly due to the enhanced affinity for the acceptor. The similar finding has already been reported on glucuronidation of *o*-aminophenol in the rat.[22] In sharp contrast, treatment with Triton X-100 increased the reaction velocity. A marked increase in the glucuronyltransferase activity would be ascribable to demasking of the active site but not to the rise in the affinity for the substrate. It has been suggested that the detergent may alter the structure of the microsomal membrane resulting in exposure of the active site on the enzyme which is normally inaccessible to the substrate due to its location in the membrane.[15c,23] In addition, Vessey, *et al.*[24] presented a speculation that the phospholipid membrane may constrain glucuronyltransferase in a rigid conformation of the reduced activity and treatment with the detergent may permit the enzyme to assume a fully active conformation.

The inhibition studies indicate that the different enzyme systems may be involved in glucuronidation of the 17β-hydroxyl group between androgen and estrogen. The substrate specificity of testosterone glucuronyltransferase appears to be analogous to that of *p*-nitrophenol glucuronyltransferase. It is of particular interest that the multiple enzyme is associated with glucuronidation of the hydroxyl group at the same position in a steroid molecule. Considering the physiological significance and biotransformation of these hormones in the living animal, it seems very likely that glucuronyltransferase may participate in the metabolic regulation of the hormonal level. The inhibitory effect of bilirubin on conjugation of male homone will be an attractive problem to be solved in connection with jaundice.

Further studies on the role of glucuronyltransferase in the action and metabolism of steroid hormones are being conducted in these laboratories.

Acknowledgement This work was supported in part by Grants-in-Aid from the Naito Foundation and the Ministry of Education, Science and Culture, which are gratefully acknowledged.

22) R.D. Howaland and A. Burkhalter, *Biochem. Pharmacol.*, **20**, 1463 (1971).
23) A. Winsnes, *Biochim. Biophys. Acta*, **191**, 279 (1969).
24) D.A. Vessey and D. Zakim, *J. Biol. Chem.*, **246**, 4649 (1971).