Studies on a Testosterone Glucuronyltransferase from the Cytosol Fraction of Human Liver

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An enzyme that conjugates the 17 β -hydroxyl group of testosterone was found in the cytosol fraction of human liver. The same enzyme preparation also conjugates the 16 α -hydroxyl group of oestril. The enzymic activity could not be sedimented by centrifuging the cytosol fraction at 158000g_{av} for 120 min. The testosterone-conjugating as well as the oestril-conjugating activities were found in the precipitate obtained after 30% saturation of the cytosol fraction with ammonium sulphate. Filtration of the precipitate through Sephadex G-200 enriched the testosterone-conjugating enzyme 50-fold and the oestril-conjugating enzyme 100-fold. No separation of the two activities was achieved. With labelled testosterone the product of the reaction, testosterone 17 β -glucuronide, was identified by paper chromatography and by crystallization to constant specific radioactivity. Testosterone 17 β -glucuronyltransferase was active between pH 7.0 and 8.6 in tris-HCl and tris-maleate buffers. The apparent K_m values for testosterone and UDP-glucuronic acid were 6.4 and 25 μ M respectively. The enzyme was active between 37 and 45°C; the activation energy was calculated to be 5 kcal/mol. Oestril did not influence the glucuronidation of testosterone. Controlled heating as well as alternate freezing and thawing of the purified enzyme preparation led to an inactivation of both testosterone-conjugating and oestril-conjugating activities at similar rates. Testosterone and oestril, when incubated together, gave a reaction rate that was approximately equal to the sum of the rates when the two substrates were incubated separately. The present findings suggest that testosterone and oestril are conjugated by two separate enzymes.

In a previous paper (Rao, Rao & Breuer, 1970) the occurrence of an enzyme conjugating the 16 α -hydroxyl group of oestril with glucuronic acid in the cytosol fraction of human liver was reported. In the present investigation testosterone was tested as a possible substrate for conjugation with glucuronic acid. The enzyme preparation described in this study conjugated testosterone as well as oestril. The testosterone-conjugating and oestril-conjugating activities were partially purified; characteristics of the testosterone-conjugating system were studied by kinetic experiments. An effort was also made to distinguish between the two enzymic activities occurring in the purified preparation.

MATERIALS

Buffer solutions were prepared as described by Rao *et al.* (1970).

[4-¹⁴C]Testosterone (17 β -hydroxy-4-androsten-3-one; specific radioactivity 51.4 mCi/mmol) and [4-¹⁴C]oestril [1,3,5(10)-oestratriene-3,16 α ,17 β -triol; specific radio-

activity 51.4 mCi/mmol] were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Testosterone 17 β -monoglucuronide was obtained from Ikapharm, Ramat-Gan, Israel.

UDP-glucuronic acid disodium salt was purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. DEAE-cellulose and CM-cellulose were obtained from Serva, Heidelberg, Germany. Sephadex G-200, DEAE-Sephadex and CM-Sephadex were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Hydroxyapatite was obtained from Schuchhardt, Munich, Germany.

EXPERIMENTAL

Liver was obtained from the surgery unit during kidney transplantation. The subject was a 17-year-old male who had been involved in a traffic accident. The liver was macroscopically normal.

Preparation of the tissue and the cytosol fraction was carried out as described by Rao *et al.* (1970).

Determination of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Protein concentrations were expressed in mg/ml

from a standard curve, obtained with dry bovine serum albumin (Serva Entwicklungslabor, Heidelberg, Germany).

Assay of glucuronyltransferase activity. The assay method was basically similar to that described by Rao *et al.* (1970), except that the steroid substrate used in the present investigation was testosterone. [$4\text{-}^{14}\text{C}$]Testosterone was tested for purity in two different paper-chromatographic systems before use. The radioactive testosterone was diluted with recrystallized unlabelled testosterone to give 116000 c.p.m. equivalent to 30 nmol in 0.1 ml of an ethanolic solution. Then 0.1 ml of this solution was pipetted into a tube, propylene glycol (2 drops) was added and the ethanol was evaporated under a gentle stream of N_2 in a water bath at 40°C . The incubation mixture contained 1.0 ml of 50 mM-tris-HCl buffer, pH 8.0, $1.0\text{ }\mu\text{mol}$ of UDP-glucuronic acid, $20\text{ }\mu\text{mol}$ of MgCl_2 and the enzyme preparation, containing 100–200 μg of protein. Incubation was carried out at 37°C for 60 min. In each experiment a control incubation was carried out with a mixture containing all the above ingredients except UDP-glucuronic acid.

Extraction, measurement of radioactivity and calculation. These procedures were carried out exactly as described by Rao *et al.* (1970). The activity of the enzyme was calculated from the following relationship:

$$\frac{(a-b) \times 30}{116000}$$

where a is c.p.m. in the butan-1-ol fraction of the test incubation mixture and b the c.p.m. in the butan-1-ol fraction of the control incubation mixture; 30 denotes the nmol of substrate and 116000 (c.p.m.) the amount of radioactivity incubated.

Unit of enzyme activity. A unit of enzyme activity was defined as the amount of enzyme necessary to catalyse the conjugation of 1 nmol of testosterone/h under the standard assay conditions. Specific activity was defined as units/mg of protein.

RESULTS

Purification of the enzyme

Ammonium sulphate precipitation. Fractionation of the cytosol fraction with ammonium sulphate revealed that the enzymic activity was confined to the sediment obtained after 0–30% saturation with ammonium sulphate.

Gel filtration on Sephadex G-200. Washing of the

gel and packing of the gel into a column was done as described previously (Rao *et al.* 1970). The sediment obtained after 30% saturation of the cytosol fraction with ammonium sulphate was dissolved in a minimum amount of 2.5 mM-tris-hydrochloric acid buffer, pH 8.0, and applied to the Sephadex column; 5 ml fractions were collected. The void volume contained the highest specific activity and was used as a source for characterization studies of the enzyme. Details of purification are presented in Table 1.

Identification of testosterone 17β -glucuronide. The butan-1-ol extracts from several incubations, containing the radioactive glucuronide, were collected and evaporated under nitrogen in a water bath at 45°C . The residue was taken up in dry butan-1-ol and the solution centrifuged to remove inorganic salts; the clear butan-1-ol layer was removed and evaporated to dryness. The residue was chromatographed on paper in the solvent system ethyl acetate-toluene-*n*-hexane-2-methylpropan-2-ol-acetic acid-water (12:15:8:5:12:28, by vol.) (Schneider & Lewbart, 1959) after authentic testosterone 17β -glucuronide had been added as carrier. Scanning of the chromatogram in a Packard radiochromatogram scanner showed exact coincidence between the peak of radioactivity and the u.v.-absorbing zone of testosterone 17β -glucuronide. The radioactive zone was eluted from the paper, mixed with 4.0 mg of non-radioactive testosterone 17β -glucuronide and passed through a column of Sephadex G-25. The glucuronide was eluted with water. The fractions containing the glucuronide were combined; the water was evaporated under nitrogen in a water bath at 45°C and the residue crystallized to constant specific radioactivity (Table 2).

Properties and kinetic studies of the purified enzyme. During the purification of the testosterone 17β -glucuronyltransferase, simultaneous purification of the oestriol 16α -glucuronyltransferase (Rao *et al.* 1970) was also obtained; at all stages of purification the activity of the latter enzyme was higher than that of the testosterone 17β -glucuronyltransferase. Both activities were found in the same

Table 1. Purification of the testosterone glucuronyltransferase from the cytosol fraction of human liver

The enzyme preparations were incubated with [$4\text{-}^{14}\text{C}$]testosterone, as described in the Experimental section. Further details about the purification of the enzyme are given in the text.

Fraction	Total protein (mg)	Total activity (units)	Yield (%)	Specific activity (unit/mg of protein)	Purification
158000g supernatant	400	4.0	100	0.01	—
$(\text{NH}_4)_2\text{SO}_4$ (0–30% satn.) precipitate	32.6	5.8	146	0.18	18
Sephadex G-200 gel filtrate	7.8	4.2	105	0.54	54

Table 2. Recrystallization to constant specific radioactivity of [4-¹⁴C]testosterone 17 β -glucuronide formed during incubation of [4-¹⁴C]testosterone with the purified testosterone 17 β -glucuronyltransferase from the cytosol fraction of human liver

The solvent mixture used was methanol-water-acetone.

Specific radioactivity (c.p.m./mg)				
	Initial	First crystallization	Second recrystallization	Third recrystallization
694	Crystals	630	625	624
	Mother liquor	770	631	653

Fig. 1. Elution pattern of the testosterone and the oestriol 16 α -glucuronyltransferases from the cytosol fraction of human liver during filtration through a column (2.5 cm $\times$ 90 cm) of Sephadex G-200. The 0–30% saturation-(NH₄)₂SO₄ precipitate was applied in a volume of 2 ml and eluted with 2.5 mM-tris-HCl buffer, pH 8.0. The volume of each fraction was 5.6 ml. Enzyme activities with [4-¹⁴C]testosterone and [4-¹⁴C]-oestriol were measured as described in the text. ○, Protein (E_{280}); ●, testosterone glucuronyltransferase activity; ■, oestriol 16 α -glucuronyltransferase activity.

fractions of Sephadex G-200 gel filtration (Fig. 1). The ratio of the two glucuronyltransferase activities ranged from 7.0 to 12.0 in fractions 15–20. After Sephadex G-200 filtration, the purification factors for the testosterone 17 β -glucuronyltransferase and for the oestriol 16 α -glucuronyltransferase were 50 and 100 respectively. This finding led to the assumption that two different enzyme systems might be present in the Sephadex G-200 fraction; accordingly, efforts were directed towards the separation of the two activities. The following methods were tried: column chromatography with DEAE-cellulose, DEAE-Sephadex, CM-Sephadex and hydroxyapatite; with none of these procedures was a separation of the two enzymic activities achieved. At this stage it was thought that kinetic studies might possibly help to obtain more informa-

tion on the nature of the two glucuronyltransferase activities.

Effect of pH on the activity of the testosterone 17 β -glucuronyltransferase. The activity was tested in four different 50 mM buffers (Fig. 2). The activities in tris-maleate buffer, tris-hydrochloric acid buffer and phosphate buffer do not exhibit a sharp optimum. The activity in glycine-sodium hydroxide buffer is somewhat higher than in the above mentioned buffers. For routine incubations tris-hydrochloric acid buffer, pH 8.0, was used.

Determination of K_m for testosterone and UDP-glucuronic acid. The K_m for testosterone was determined in the presence of saturating concentration of UDP-glucuronic acid and that for UDP-glucuronic acid in the presence of saturating concentration of testosterone. The results obtained

Fig. 2. Effect of pH on the testosterone glucuronyltransferase; enzyme activity was measured as described in the text. ■, Phosphate buffer; ▲, tris-maleate buffer; ●, tris-HCl buffer; ○, glycine-NaOH buffer. All buffers were 50mM.

were plotted in a double-reciprocal manner by the procedure of Lineweaver & Burk (1934) (Figs. 3a and 3b). The K_m values were calculated to be $6.4\mu\text{M}$ for testosterone and $25\mu\text{M}$ for UDP-glucuronic acid.

Temperature optimum of the testosterone 17 β -glucuronyltransferase. The enzyme shows highest activity in the range 37–45°C; a well-defined temperature optimum is apparently lacking. For the purpose of comparison, the optimum temperature for the oestriol 16 α -glucuronyltransferase was simultaneously determined (Fig. 4). The activation energy for the testosterone glucuronyltransferase, calculated for two temperatures (White, Handler & Smith, 1964) 32 and 40°C, was 5 kcal/mol.

Effect of Mg^{2+} and cysteine on the testosterone 17 β -glucuronyltransferase. Mg^{2+} , in concentrations between 5 and $25\mu\text{M}$, stimulates enzymic glucuronidation of testosterone two- to four-fold. Cysteine exhibited some unexpected properties when incorporated in the incubation mixture. The addition of $10\mu\text{mol}$ of cysteine apparently increased sixfold the yield of radioactivity in the butan-1-ol fraction (glucuronide fraction). A further addition of cysteine led to a further remarkable increase in the radioactivity extractable by butan-1-ol. However, this increase appeared rather suspicious, since amounts of cysteine that would normally denature the enzyme gave elevated radioactivities in the butan-1-ol fraction. Under these circumstances a glucuronidation of testosterone could not be detected by paper chromatography. Attempts to identify the new compound by paper chromato-

graphy were unsuccessful. Although cysteine stimulated the enzyme at lower concentrations, it was not included in the incubation mixtures because of the variability of the results.

Effect of oestriol on the testosterone 17 β -glucuronyltransferase. The effect of oestriol on the glucuronidation of testosterone was studied by adding oestriol (5 and 20nmol) to the incubation mixtures with various concentrations of testosterone. The concentration of UDP-glucuronic acid (0.5mM) was kept constant. The velocities and substrate concentrations were plotted in a double-reciprocal manner by the procedure of Lineweaver & Burk (1934) (Fig. 5). Apparently oestriol, in the two concentrations used, does not affect the enzyme conjugating testosterone.

Rate of thermal inactivation. This was done as described by Linn & Lehman (1965). Portions of the purified enzyme preparation were kept for various times at 50°C as indicated in Fig. 6. The samples were cooled in ice-water and incubated as usual at 37°C for 60min with oestriol and testosterone separately. Fig. 6 shows that the two glucuronyltransferase activities are inactivated at similar rates.

Effect of freezing and thawing. During the purification it was observed that freezing and thawing of the purified enzyme preparation (Sephadex G-200 filtrate) resulted in a somewhat lower conjugation rate for oestriol, whereas that for testosterone did not show any significant decrease. Hence it was decided to investigate further this phenomenon by extending the number of times of freezing and thawing of the enzyme and testing the activities towards testosterone and oestriol. The enzyme preparation was frozen at -20°C and thawed at room temperature six times. As is shown in Fig. 7, the decrease in the two glucuronyltransferase activities is not significantly different from each other and both activities are inactivated at similar rates.

Rate of reaction for the two substrates separately and together. The 'mixed-substrate' method as described by Dixon & Webb (1964) is one of several criteria used to determine whether two enzymic reactions are in fact due to the same enzyme or to two different enzymes. In this method the total rate of reaction will be less than the sum of the rates of the reactions measured separately (at saturating concentrations of the substrates) when the two substrates are attacked by the same enzyme. However, when the rates are additive it is probable that two independent enzymes are involved. Table 3 shows the results of an experiment in which testosterone and oestriol were incubated separately as well as together. The concentrations of testosterone and oestriol used in both incubations lie in the range of saturation.

Fig. 3. (a) Double-reciprocal plot of initial velocity against variable testosterone concentrations at saturating concentration of UDP-glucuronic acid; (b) double-reciprocal plot of initial velocity against variable UDP-glucuronic acid concentrations at saturating concentration of testosterone. The incubation contained in addition to the enzyme 20 μ mol of $MgCl_2$. Further details are given in the text; v , μM -testosterone glucuronide formed. The insets show a direct plot of initial velocity against substrate concentration.

When both substrates were incubated separately the sum of the rates of conjugation was 0.73 μM , and when incubated together it was 0.646 μM .

DISCUSSION

It is obvious from the results that the cytosol fraction from the human liver contains an enzyme catalysing the formation of testosterone 17 β -glucuronide in the presence of UDP-glucuronic acid. The same enzyme preparation obtained from the cytosol fraction also conjugates oestriol in the presence of UDP-glucuronic acid (Rao *et al.* 1970).

When two different enzymic reactions are catalysed by an enzyme preparation, the question arises whether this is due to one enzyme only or to two different enzymes. As pointed out by Dixon & Webb (1964), a number of criteria for the identity of enzymes can be formulated; none of them is absolutely conclusive by itself, but they provide strong evidence when taken together. The experiments described in the present paper were carried out to study the extent to which the properties of the two different glucuronyltransferase activities are congruent with each other.

The fact that testosterone and oestriol do not

show competition for the enzyme or for one active site facilitated the study of the possible existence of two different enzymes. The conjugation with oestriol as substrate was, on average, 8 times that with testosterone (Fig. 1), the ratio varying between 7.0 and 12.0. The testosterone glucuronyltrans-

ferase could be purified 50-fold and the oestriol 16 α -glucuronyltransferase 100-fold.

Inactivation by frequent freezing and thawing is a common feature of several enzymes. Alternate freezing and thawing of the enzyme preparation

Fig. 4. Effect of temperature on the testosterone glucuronyltransferase and the oestriol 16 α -glucuronyltransferase from the cytosol fraction of human liver. Incubation mixtures contained [4- 14 C]testosterone or [4- 14 C]oestriol, UDP-glucuronic acid, MgCl₂ and the purified enzyme. The tubes were incubated for 60min; details of the measurement of the activities are described in the Experimental section. ●, Glucuronidation of testosterone; ▲, 16 α -glucuronidation of oestriol.

Fig. 6. Rates of heat inactivation of the testosterone glucuronyltransferase and of the oestriol 16 α -glucuronyltransferase activities. A fresh enzyme preparation (Sephadex G-200 filtrate) was incubated in the absence of substrates (testosterone, oestriol, UDP-glucuronic acid) and MgCl₂ at 50°C. At the times indicated, portions of the enzyme preparation were removed and added to previously prepared reaction mixtures and incubated at 37°C for 60min. Activity measurements of the glucuronyltransferases were made as described in the text. ■, Formation of testosterone 17 β -glucuronide; ●, formation of oestriol 16 α -monoglucuronide.

Fig. 5. Double-reciprocal plot of initial velocity against variable testosterone concentrations at saturating concentration of UDP-glucuronic acid; in the absence of oestriol (●), in the presence of 2.5 μ M-oestriol (▲) and in the presence of 10 μ M-oestriol (■). Details of the measurement of the activity are described in the Experimental section. v , μ M-Testosterone 17 β -glucuronide formed; the three lines were fitted according to the method of least squares.

revealed that both activities were inactivated at similar rates.

Another method commonly used for inactivation is controlled heating. Maintenance of a constant ratio between the two activities as the enzyme undergoes inactivation is an indication of the presence of one enzyme (Dixon & Webb, 1964). Linn & Lehman (1965) used the procedure of thermal inactivation as one of the criteria to show that a single enzyme catalyses the hydrolysis of DNA and RNA. Thermal inactivation of the two enzymic activities conjugating testosterone and oestriol indicated that both were inactivated at the same rate.

A consideration of the properties of the testosterone-conjugating enzyme system and of the oestriol-conjugating system reveals more dissimilarities than similarities. As has already been mentioned, both activities appear in the same ammonium sulphate fraction (0–30% saturation) and in the same protein fractions after filtration of

the ammonium sulphate precipitate through Sephadex G-200. The ratios of the specific activities for the oestriol-conjugating system to the testosterone-conjugating system in fractions 15–20 were 7.9, 11.4, 10.9, 12.0, 8.5 and 7.0. Similarly, differences in the rates of specific activities were also observed after chromatography on CM-Sephadex, where the protein was not adsorbed.

Differences in the behaviour of the enzymic activities in different buffers become evident from Fig. 2. Testosterone 17 β -glucuronyltransferase activity does not show a well-defined optimum in phosphate buffer, tris-hydrochloric acid buffer or tris-maleate buffer. The oestriol 16 α -glucuronyltransferase has a pH optimum of 8.0 in tris-hydrochloric acid buffer (Rao *et al.* 1970), and a broad activity region in the range pH 7–8.6 in tris-maleate buffer. Although the differences may be considered to be minor, it is obvious that the two activities show distinct characteristics.

Testosterone 17 β -glucuronyltransferase activity does not exhibit a well-defined temperature optimum, but appears to be the same in the temperature range between 32 and 45°C. In contrast, the activity of the enzyme conjugating oestriol shows a well-defined temperature optimum of 37°C. The activation energy for the glucuronidation of testosterone was calculated to be 5 kcal/mol, whereas that for oestriol was 11.1 kcal/mol (Rao *et al.* 1970). This difference in the activation energies for the two reactions may be attributed to two different enzymes. In fact, as mentioned by Dixon & Webb (1964), when two or more enzymes catalyse the same reaction (in the present investigation the same type of reaction), a different activation energy is obtained with each enzyme; however, if only one enzyme acts on several substrates, the same activation energy is often obtained. They further mention that the activation energy appears to be more characteristic of the enzyme than of the substrate.

The mixed-substrate method (Dixon & Webb, 1964) gives information on whether two reactions

Fig. 7. Rates of inactivation of the testosterone glucuronyltransferase and the oestriol 16 α -glucuronyltransferase. A fresh enzyme preparation (Sephadex G-200 filtrate) was frozen at -20°C and thawed at $5-8^{\circ}\text{C}$ under running tap water. Portions from the thawed preparation were added to previously prepared reaction mixtures and incubated at 37°C for 60 min. Measurements of the glucuronyltransferase activities were made as described in the text. ■, Formation of testosterone 17 β -glucuronide; ●, formation of oestriol 16 α -monoglucuronide.

Table 3. Rates of glucuronidation of testosterone and oestriol when incubated separately and together

The rates of conjugation of testosterone and oestriol were measured as described in the Experimental section. The rate of conjugation of testosterone in the presence of oestriol and vice versa was determined first by measuring the conjugation of testosterone and secondly by measuring the conjugation of oestriol.

Substrate	Concentration (μM)	Rate of conjugation (μM)		
		Testosterone	Oestriol	Sum
Testosterone	10	0.04		
	20	0.05		
Oestriol	10		0.44	0.48
	20		0.68	0.73
Testosterone + oestriol (each)	10	0.046	0.43	0.476
Testosterone + oestriol (each)	20	0.046	0.60	0.646

are catalysed by one enzyme. During a study of the requirements for the side-chain cleavage of cholesterol and cholesterol sulphate by enzymes from bovine adrenal mitochondria, Young & Hall (1969) concluded that the two substrates are cleaved by separate enzymes, since the sum of the cleavage of the two substrates incubated separately equalled approximately the total cleavage of both substrates incubated together. The results in Table 3 indicate that the rate of glucuronidation of the two different steroid substrates incubated together is approximately equal to the sum of the rate of the conjugation when the steroids are incubated separately. This is true at the two different concentrations of the two substrates used (10 and 20 μM). From these results it is probable that the purified enzyme system contains two separate enzymes for the glucuronidation of testosterone and of oestriol.

The apparent K_m value for testosterone was found to be 6.4 μM and that for oestriol 11.4 μM ; approximately the same value (13.3 μM) was found for the oestriol 16 α -glucuronyltransferase described previously (Rao *et al.* 1970). The K_m value for UDP-glucuronic acid with respect to oestriol as the substrate was 100 μM , which is the same as that found in the previous investigation (Rao *et al.* 1970). With testosterone as substrate, the K_m for UDP-glucuronic acid was found to be 25 μM .

From these observations four possible models of enzymes may be postulated: (a) one enzyme with one site for UDP-glucuronic acid and one common site for testosterone as well as for oestriol; (b) one enzyme with one common site for UDP-glucuronic acid and two different sites for oestriol and testosterone respectively; (c) one enzyme with two sites for UDP-glucuronic acid and two different sites for oestriol and testosterone respectively; (d) two different independent enzymes, one conjugating testosterone and the other oestriol.

Model (a). If only one enzyme were present, a competition between testosterone and oestriol should have taken place. From the results it may be seen that this is not the case.

Model (b). If there were two binding sites, one for testosterone and oestriol respectively and one for UDP-glucuronic acid, then the latter should be equally available to both testosterone and oestriol resulting in a similar K_m for UDP-glucuronic acid at saturating concentration of both steroid substrates. However, the K_m values are significantly different, depending on whether testosterone or oestriol is the substrate.

Model (c). Since the K_m values for UDP-glucuronic acid are different, two binding sites on the same enzyme may be proposed; in this case the double-reciprocal plot of UDP-glucuronic acid with respect to oestriol as substrate should have

been non-linear (Webb, 1963). It also appears that the site for UDP-glucuronic acid, when testosterone is used as the other substrate, is saturated much earlier because of its greater affinity. As already mentioned, the K_m value for oestriol is higher than that for testosterone. If one of the two substrates had changed the K_m for UDP-glucuronic acid, an allosteric effect should have been observed. Since the double-reciprocal plots of UDP-glucuronic acid were linear with respect to testosterone and oestriol as substrates, this model also appears to be improbable.

Model (d). This proposes the existence of two separate enzymes. Taking into consideration the differences in the kinetic behaviour of the two enzymic activities this model appears to be the more probable one. The similar rates of inactivation caused by controlled heating and by freezing and thawing seem to indicate that both enzymes possess similar physical properties. Separation and further purification of the two activities should answer several questions as to the origin, specificity and kinetic properties of the testosterone 17 β -glucuronyltransferase.

Finally, it may be mentioned that, although the purified enzyme preparation described here conjugated testosterone and oestriol, the enzyme preparation described by Rao *et al.* (1970) conjugated only oestriol. This phenomenon is particularly surprising since the same cell fraction was used in both instances. The reason for this difference in conjugating steroids by two different livers is not clear.

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