Partial Purification and Kinetic Properties of a Soluble Estrogen Glucuronyltransferase from Pig Intestine*

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SUMMARY

A soluble enzyme from the small intestine of the pig, capable of conjugating uridine diphosphate glucuronic acid with 17β-estradiol, has been purified 5- to 10-fold. The glucuronide formed was identified as 17β-estradiol 3-monogluconuride by paper chromatography and microchemical reactions. This estrogen glucuronyltransferase is present in the 150,000 x g supernatant and shows an absolute requirement for UDP-glucuronic acid.

The glucuronidation reaction is optimal in Tris-HCl buffer between pH 8.0 and 8.6. Magnesium ions and cysteine increase the activity of the enzyme; p-chloromercuribenzoate and iodoacetamide are inhibitors of the enzyme. Km values were found to be 0.74 x 10⁻⁶ m for 17β-estradiol and 0.84 x 10⁻⁶ m for UDP-glucuronic acid. The glucuronyltransferase is stable for at least 3 months at -20°C, while it loses 50% of its initial activity within 20 days when kept at +5°C. The enzyme shows a temperature optimum of 42°C. The reaction has an activation energy of 15 kcal per mole within the range of 22°C to 42°C. ATP competes with UDP-glucuronic acid for the enzyme, whereas UDP inhibits the enzyme noncompetitively.

The following steroidal and nonsteroidal substrates are not attacked to any significant extent by the estrogen glucuronyltransferase: estriol, 17α-estradiol, dehydroepiandrosterone, testosterone, bilirubin, and p-nitrophenol. However, estrone is conjugated by the enzyme and also acts as a competitive inhibitor of the glucuronidation of 17β-estradiol; the inhibition constant, Ki, for estrone was calculated to be 0.79 x 10⁻⁶ m. The similarity of the Km value for estrone and the Km value for 17β-estradiol makes it likely that only one enzyme catalyzes the glucuronidation of both 17β-estradiol and estrone.

Uridine diphosphate glucuronate glucuronyltransferases (EC 2.4.1.17) catalyze the transfer of glucuronic acid from uridine 5'-pyrophosphate and glucopyranosiduronic acid to a large number of acceptors, thereby forming water-soluble conjugates. It has been shown that the glucuronyltransferases are localized in the endoplasmic reticulum (1). So far, attempts to solubilize the microsomal glucuronyltransferases and to obtain stable enzyme preparations have not been very successful (1).

During recent years, the formation of steroid glucuronides has been extensively studied. Thus, various glucuronyltransferases have been shown to conjugate C-19 as well as C-18 steroids (2-4). A soluble glucuronyltransferase was first described in 1966 (5); this enzyme is present in the 150,000 x g supernatant (ground plasma) of the human intestine and forms estriol 3-monogluconurone, estriol 16α-monogluconurone, and estriol 17β-monogluconuride, the last conjugate being the main product (6).

In the present paper, we wish to report the partial purification of a highly specific, soluble estrogen glucuronyltransferase from pig intestine. With this stable enzyme preparation, kinetic studies were carried out to investigate further and characterize the properties of the estrogen glucuronyltransferase.

EXPERIMENTAL PROCEDURE AND RESULTS

Materials—The following radioactive steroids were purchased from The Radiochemical Centre, Amersham, England: estrone-4-14C (specific activity 51.0 mC per mmole), 17β-estradiol-4-14C (specific activity 51.0 mC per mmole), estriol-4-14C (specific activity 27.5 mC per mmole), and testosterone-1,2-3H (specific activity 100 mC per mmole). 17α-Estradiol-6,7-3H was made available by Dr. R. Knuppen, Bonn, Germany. All radioactive steroids were tested for impurities by paper chromatography. Subsequently, those that needed to be purified were chromatographed on paper in two different systems before use. 17β-Estradiol 17β-monogluconuride and 17β-estradiol 3-monogluconuride were gifts from Professor A. E. Kellie, London, England. Bilirubin and 4-nitrophenol were purchased from E. Merck, Darmstadt, Germany. Uridine 5'-diphosphate glucuronic acid disodium salt, uridine 5'-diphosphate dipotassium salt, uridine 5'-diphosphate N-acetylgalactosamine disodium salt, adenosine 3',5'-monophosphate, adenosine triphosphate, nicotinamide adenine dinucleotide phosphate, and reduced nicotinamide adenine dinucleotide phosphate were purchased from Biochemica Boehringer, Mannheim, Germany. Sephadex G-100 and G-200 were purchased from Pharmacia, Uppsala, Sweden.

Enzyme Assay—The radioactive substrate, 17β-estradiol-4-14C, was diluted with recrystallized cold 17β-estradiol to give 100,000 cpm equivalent to 33 mmole in 0.1 ml of ethanol. To 0.1 ml of the solution, 2 drops of propylene glycol were added, and the alcohol was evaporated under nitrogen in a water bath maintained at 40°C. To this was added 1.0 ml of 0.05 M Tris-HCl buffer.
buffer, pH 8.0; 1.26 μmoles of UDP glucuronic acid disodium salt dissolved in 0.1 ml of the same buffer; 5 μmoles of MgCl₂; 5 μmoles of a freshly prepared and neutralized solution of cysteine hydrochloride; and the enzyme preparation containing 300 to 600 μg of protein. Incubation was carried out at 37°C for 60 min. The final concentration of the steroid was 2.2 × 10⁻⁸ M, and that of UDP-glucuronic acid 0.84 × 10⁻⁴ M.

After the incubation, the tubes were cooled in ice-water, and the contents were extracted 3 times with 5 ml of water-saturated ethyl acetate. Several experiments showed that this procedure quantitatively removed the unconjugated substrate. To the water phase, containing the glucuronides, NaCl was added until a slight excess remained undissolved. The glucuronide was extracted with 2 ml of n-butyl alcohol saturated with water and allowed to stand for 30 min. An aliquot of 0.5 ml of the n-butyl alcohol extract was transferred to a counting vial; 15 ml of scintillator and 1 ml of methanol were added. The radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer, model 3003. The settings used for 14C gave an efficiency of 82%. The test vials never showed any significant quench. The composition of the scintillator was 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis(2-(4-methyl-5-phenyloxazolyl))benzene in 1 liter of toluene. A control incubation was done simultaneously in the same manner, except that it did not contain UDP-glucuronic acid (Table I). A unit of enzyme activity was defined as the amount necessary to catalyze the conjugation of 1 pmole of substrate and 100,000 the amount of radioactivity in the final step for obtaining the enzyme preparation. It was transported in ice, and all procedures thereafter were carried out at 4°C in a cold room. The adhering fat was removed, and the intestine was cut up into pieces 5 to 6 cm long. The pieces were cut open and vigorously shaken in 200 ml of 0.25 M sucrose, with the sucrose solution being changed frequently. About 450 to 500 g of tissue pieces were combined and repeatedly washed with sucrose, until the sucrose solution was fairly clear. After passing the tissue through a meat grinder, the ground tissue was homogenized with 3 volumes of 0.25 M sucrose in a Waring Blender intermittently for 1 min. The homogenate was centrifuged in a cooled centrifuge at 12,000 × g for 30 min. The supernatant was then centrifuged at 150,000 × g for 60 min. The clear supernatant thus obtained was transferred to fresh tubes and centrifuged at 150,000 × g for another 60 min. The supernatant was decanted and stored in portions of 20 to 25 ml at -20°C until used. Protein was determined by the procedure of Lowry et al. (9), with bovine serum albumin as standard.

Purification of Enzyme—To the 150,000 × g supernatant, finely powdered ammonium sulfate was added to a saturation of 45%. After standing for 30 min, the mixture was centrifuged at 12,000 × g for 15 min. The supernatant was discarded, and the tubes were drained for 5 to 10 min. The precipitate was then dissolved in a small volume of 0.0025 M Tris-HCl buffer, pH 8.0. The protein solution was pipetted onto a column of Sephadex G-100 (1.8 × 90 cm) and eluted with the same buffer. Sephadex G-100 had been previously allowed to swell for 3 days in distilled water and washed thoroughly again with distilled water; this was followed by extensive washing with the buffer used for elution of the column. The column was equilibrated overnight in the cold room before the ammonium sulfate fraction was applied. Elution of protein was monitored by an LKB Uvicord II. Fractions of 2 ml were collected. Most of the activity was found to be present in a few fractions of the protein peak; Fractions 25 to 29 were pooled and used for kinetic studies. At this stage a 5- to 10-fold purification as compared with the activity of 150,000 × g supernatant was usually obtained (Table II). Further purification of the estrogen glucuronyltransferase presented several problems. The Sephadex G-100 fraction could only be partly adsorbed on a column of DEAE-SN cellulose, because the column invariably became blocked. Adsorption did not occur on carboxymethyl cellulose nor did the specific activity of the unadsorbed fraction increase. Preliminary experiments with calcium phosphate gel indicated that with each addition of gel, the enzyme was also adsorbed. The adsorbed enzyme could not be eluted from the gel-protein complex with 0.05 M Tris-HCl buffer, pH 8.0, containing 20% ammonium sulfate. Filtration of the Sephadex G-100 fraction through a column of Sephadex G-200 did not give any increase in specific activity. Since filtration through Sephadex G-100 lasted only about 4 to 5 hours and gave a specific activity which could not be improved by other methods of purification, it was chosen as the final step for obtaining the enzyme preparation. It was occasionally observed that the initial activity of the 150,000 ×

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**Table I**

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Unconjugated substrate</th>
<th>Conjugated substrate</th>
<th>Total recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm x 10⁻⁴</td>
<td>%</td>
<td>cpm x 10⁻⁴</td>
</tr>
<tr>
<td>Test</td>
<td>1.65</td>
<td>90</td>
<td>912</td>
</tr>
<tr>
<td>Control</td>
<td>1.82</td>
<td>99</td>
<td>2.3</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>150,000 × g supernatant</td>
<td>270</td>
<td>108</td>
<td>0.38</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ (0-45%)</td>
<td>70</td>
<td>116</td>
<td>1.66</td>
<td>107</td>
</tr>
<tr>
<td>Sephadex G-100 filtration</td>
<td>30</td>
<td>114</td>
<td>3.80</td>
<td>105</td>
</tr>
</tbody>
</table>
g supernatant rose 3- to 4-fold from 0.1 to 0.4 within 10 to 20 days, when stored at −20°. A corresponding increase in activity of the Sephadex G 100 fraction was also obtained. A single column of Sephadex G-100 could be used two or three times after washing thoroughly with elution buffer before each filtration.

The enzyme could be frozen at −20° and reused without any loss of activity; subsequent freezing and thawing, however, led to losses of activity. At +5° the enzyme lost 50% of its initial activity within 20 days.

Identification of 17β Estradiol 3-Monoglucuronide—For identification purposes, 1.04 μC of 17β-estradiol-4,13C corresponding to 5.3 μg of 17β-estradiol, 800 μg of UDP-glucuronic acid, 5 μmoles of MgCl₂, and 5 μmoles of cysteine hydrochloride were incubated with 450 μg of the purified enzyme preparation in 1.5 ml of 0.05 M Tris-Cl buffer, pH 8.0, at 37° for 60 min. Removal of the unconjugated substrate and extraction of the glucuronide fraction were done as described under “Enzyme Assay.” Aliquots from the ethyl acetate extract (free steroid faction) and from the n-butyl alcohol extract (glucuronide fraction) were counted. For identification of the glucuronide fraction, the n-butyl alcohol extract was evaporated under nitrogen; and the residue was dissolved in 3 drops of a mixture of n-butyl alcohol and 70% aqueous methanol, 1:3, and chromatographed on paper in ethyl acetate-toluene-n-hexane-t-butyl alcohol-acetic acid-water, 60:75:40:25:140 (10), with 17β-estradiol-17β-monoglucuronide and 17β-estradiol 3-monoglucuronide as carrier and parallel standards. After 3 hours of chromatography, the paper was dried for 20 min at 60° and scanned in a Packard radiograph scanner, model 7200. A single peak of radioactivity was obtained. The standard, 17β-estradiol-17β-monoglucuronide, was visualized as a blue spot by Folin-Ciocalteu reagent and the radioactive compound was cut lengthwise into two parts. One of them was stained with Folin-Ciocalteu reagent and the other with aninmyl trichloride in chloroform. The peak of radioactivity had the same mobility as authentic 17β-estradiol 3-monoglucuronide. The chromatogram with the radioactive compound was cut lengthwise into two parts. One of them was stained with Folin-Ciocalteu reagent and the other with aninmyl trichloride in chloroform. The peak of radioactivity corresponded exactly with the light yellow spot with antimony trichloride in chloroform. The peak of radioactivity corresponded exactly with the light yellow spot with antimony trichloride in chloroform.

Effect of pH—The activity of the estrogen glucuronyltransferase at different pH values is shown in Fig. 1. The optimum pH in Tris-HCl and glycine-NaOH buffers was found to be between 8.0 and 8.6. The activity of the enzyme was somewhat lower in Tris-maleate and phosphate buffers. Most probably,

### Table III

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity Specific activity (μmol/hr/mg protein)</th>
<th>Activity Specific activity (μmol/hr/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>30.3</td>
<td>0.60</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>1.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Microsomal</td>
<td>28.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Soluble (150,000 X g supernatant)</td>
<td>0.2</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**Subcellular Distribution of Estrogen Glucuronyltransferase**—Pieces of intestine were cleaned, passed through a meat grinder, and homogenized in 0.25 M sucrose. The homogenate was centrifuged at 600 X g for 10 min, and the supernatant was then centrifuged at 8,000 X g for 10 min; the sediment was used as the mitochondrial fraction. This fraction was washed and re-centrifuged twice in 0.25 M sucrose at 16,000 X g for 20 min. The 8,000 X g supernatant was subjected to centrifugation at 25,000 X g for 10 min. The lysosomal sediment thus obtained was discarded and the supernatant was centrifuged at 105,000 X g for 60 min. The sediment, consisting of the microsomal fraction, was washed and centrifuged twice in 0.25 M sucrose at 105,000 X g. The supernatant fraction was centrifuged twice at 150,000 X g for 60 min. The four fractions (whole homogenate, mitochondrial, microsomal, and soluble) were incubated separately and extracted as described under “Enzyme Assay”: the n-butyl alcohol extracts were chromatographed as described under “Identification of 17β-estradiol 3-monoglucuronide.” All four chromatograms showed a single peak of radioactivity in the position of 17β-estradiol 3-monoglucuronide. The results of the incubation experiments are presented in Table III.

**Effect of pH**—The activity of the estrogen glucuronyltransferase at different pH values is shown in Fig. 1. The optimum pH in Tris-HCl and glycine-NaOH buffers was found to be between 8.0 and 8.6. The activity of the enzyme was somewhat lower in Tris-maleate and phosphate buffers. Most probably,
maleate and phosphate act as inhibitors of the estrogen glucuronyltransferase. All buffers were 0.05 M.

Amount of Enzyme and Formation of 17β-Estradiol 3-Monogluconuride—A linear relationship between the amount of enzyme used and the formation of the glucuronide was observed.

Effect of Time—The formation of 17β-estradiol 3-monogluconuride is given even after 5 hours.

Heat Denaturation of Enzyme—The enzyme, in the absence of the substrates, was held for 15 min at different temperatures at pH 8.0, cooled in ice-water, and then incubated with 17β-estradiol, UDP-glucuronic acid, Mg++, and cysteine for 60 min. As is evident from Fig. 2, the activity begins to fall at a few degrees above room temperature (22°C).

From these experiments ("Effect of Time" and "Heat Denaturation of Enzyme") it appears that the substrates protect the enzyme against temperature inactivation.

Effect of Temperature on Enzyme—As can be seen from Fig. 3, the formation of 17β-estradiol 3-monogluconuride increases with rising temperatures and reaches a maximum at 42°C, after which there is an abrupt fall. The activation energy, calculated by the formula for two temperatures, 22° and 42° (15), was 15 kca per mole.

Effect of Metal Ions—At a final concentration of 3.4 mM, copper, mercury, and zinc totally inhibited the enzyme activity, while cobalt showed an inhibition of 50 to 60%. Calcium, manganese, and iron (Fe++), at a final concentration of 0.67 mM, did not have any influence; magnesium (3.4 mM) increased the enzyme activity by 65%, whereas EDTA (3.4 mM) did not have any significant effect. The total inhibition of activity, produced by copper, could be partially prevented by the addition of EDTA (final concentration 6.8 mM) to the incubation medium.

Effect of Cysteine and Propylene Glycol—In preliminary experiments, cysteine (final concentration 3.4 mM) showed a stimulation of the enzyme activity and hence was included in the incubations. The amount of propylene glycol, used in the incubations to dissolve 17β-estradiol, did not affect the activity of the glucuronyltransferase. However, with increasing amounts there was a tendency toward inhibition.

Effect of Sulfhydryl Reagents—p-Chloromercuribenzoate, in different concentrations, proved to be totally inhibitory when cysteine (3.4 mM) was absent while, in its presence, 3.4 mM p-chloromercuribenzoate inhibited the enzyme activity only by 47%. Iodoacetamide, on the other hand, was less inhibitory, even in the absence of cysteine.

Effect of Uridine and Other Nucleotides—Table IV shows the influence of UDP-N-acetylglucosamine, cyclic 3',5'-AMP, and nicotinamide adenine nucleotides on enzyme activity.

Effect of Uridine Diphosphate—Since uridine diphosphate is
one of the products of the enzymatic reaction between 17β-estradiol and UDP-glucuronic acid, its effect on glucuronidation was studied. Fig. 4 shows a double reciprocal plot of UDP-glucuronic acid and the reaction velocity in the presence of uridine diphosphate (final concentration \(25 \times 10^{-5} \text{ M}\)). The decrease in the reaction velocity appears to be due to a noncompetitive inhibition of the enzyme glucuronyltransferase. A similar effect was also observed with 17β-estradiol. The \(K_i\) calculated according to the formula given by Dixon and Webb (16) was 2.05 \(\times\) \(10^{-5}\) M.

Effect of Adenosine Triphosphate—Adenosine triphosphate (final concentration \(2.5 \times 10^{-4} \text{ M}\)) was added to incubations with different concentrations of UDP-glucuronic acid. Fig. 5 shows the results in the form of a double reciprocal plot. A \(K_i\) of 0.48 \(\times\) \(10^{-4}\) M was calculated according to Dixon and Webb (16) for adenosine triphosphate from the value obtained from the intercept on the abscissa.

Determination of \(K_m\) for 17β-Estradiol—A double reciprocal plot, according to Lineweaver and Burk (17), of the concentration of 17β-estradiol and the reaction velocity (Fig. 6) gave a \(K_m\) value of 0.74 \(\times\) \(10^{-5}\) M.

Determination of \(K_m\) for UDP-glucuronic Acid—Fig. 7 shows the double reciprocal plot of the concentration of UDP-glucuronic acid and the reaction velocity. The \(K_m\) value was 0.94 \(\times\) \(10^{-4}\) M.

Substrate Specificity—All radioactive steroids used as substrates were in a solution of alcohol. The appropriate amount was pipetted into incubation tubes; 2 drops of propylene glycol were added, and the alcohol was evaporated under nitrogen. The incubation and extraction procedures were done as described under “Enzyme Assay.” For each substrate a control incubation was simultaneously carried out, but without UDP glucuronic acid. The results are presented in Table V.

Determination of \(K_i\) for Estrone—As is evident from Table V, estrone is conjugated by the glucuronyltransferase to an appreci-
TABLE V
Substrate specificity of estrogen glucuronyltransferase
of pig intestine

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Radioactivity</th>
<th>Conjugation</th>
<th>Relative conjugation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^6 cpm</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>17β-Estradiol-4-14C</td>
<td>1.92</td>
<td>5.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Estrone-4-14C</td>
<td>1.83</td>
<td>3.9</td>
<td>78.0</td>
</tr>
<tr>
<td>Estriol-4-14C</td>
<td>1.44</td>
<td>0.2</td>
<td>4.0</td>
</tr>
<tr>
<td>1α-Estradiol-6-7-3H</td>
<td>4.33</td>
<td>0.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Dehydroepiandrosterone-4-14C</td>
<td>1.50</td>
<td>0.03</td>
<td>0.6</td>
</tr>
<tr>
<td>Testosterone-1,2-3H</td>
<td>6.00</td>
<td>0.01</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Taking the conjugation of 17β-estradiol-4-14C to be 100%.

Fig. 8. Double reciprocal plots of velocity against varying concentrations of 17β-estradiol in the presence of fixed definite amounts of estrone. Experimental details are given in the text.

The soluble glucuronyltransferase of the intestine of the pig is highly specific for conjugating the phenolic 3-hydroxyl group of 17β-estradiol and estrone; no glucuronidation of the alcoholic 17β-hydroxyl group of 17β-estradiol was observed. The presence of either a 17-oxo or a 17β-hydroxyl group does not influence the conjugation of the hydroxyl group at carbon atom 3. However, 17α-estradiol was not conjugated by the enzyme, thus showing that the 17α-hydroxyl group prevented glucuronidation of the 3-hydroxyl group. It is very interesting to know that, in contrast to the soluble estrogen glucuronyltransferase of human intestine (6), estril is not conjugated by the enzyme from pig intestine. The 16α-hydroxyl group, present in the estril molecule, appears to hinder the attachment of the enzyme to this phenolic steroid. Therefore, it may be surmised that the enzyme binds to the substrate from the α side (rear side).

Dehydroepiandrosterone and testosterone were not substrates for the glucuronyltransferase from pig intestine. The same is true for p-nitrophenol and bilirubin. This again demonstrates the substrate specificity of the estrogen glucuronyltransferase.

Studies on the subcellular distribution revealed that all fractions investigated contained the phenolic estrogen glucuronyltransferase. The highest specific activity was found in the microsomal fraction, while only one-tenth of this specific activity was present in the soluble fraction. The possibility cannot be excluded that the glucuronyltransferase activity found in the soluble fraction is similar to that present in the microsomes. This important aspect needs to be investigated.

The enzyme from pig intestine differs from the microsomal glucuronyltransferases present in the tissues of other animals which also conjugate the phenolic 3-hydroxyl group of 17β-estradiol. Thus, the microsomal 17β-estradiol glucuronyltransferase from rabbit liver (18) had a pH optimum between 7.6 and 7.8, while the optimum reported by Breuer and Wessen-dorf (3) was 8.7. This value comes close to the observed value (pH 8.0 to 8.6) for the soluble glucuronyltransferase. The Michaelis constant was found to be 0.74 × 10^{-6} M for 17β-estradiol and 0.84 × 10^{-6} M for UDP-glucuronic acid. The Km value for 17β-estradiol is appreciably lower than that reported for the microsomal glucuronyltransferase (3).

Inhibition of enzyme activity by p-chloromercuribenzoate and iodoacetamide indicates the presence of reduced disulfide groups in the soluble enzyme. Addition of cysteine led to a moderate stimulation, suggesting that some disulfide groups could still be reduced with concomitant increase in enzymatic activity and that cysteine was required to maintain the reduced nature of the disulfide groups.

Estriol, which is also conjugated by the estrogen glucuronyltransferase, proved to be a competitive inhibitor of the 3-glucuronidation of 17β-estradiol, thus indicating that the phenolic ring A is taking part in the conjugation process. The inhibition constant, Ki, for estrone was calculated to be 0.79 × 10^{-8} M; this value is almost identical with the Km value of 0.76 × 10^{-8} M for 17β-estradiol. It has been pointed out (19) that, if a competitive inhibitor gives a Ki value which is similar to the Km value of the substrate, then this reaction is catalyzed by a single enzyme. Previously, similarity of Ki and Km values has been used as an indication that one enzyme catalyzes the reaction of different substrates in impure enzyme preparations (20-22). These results indicate the possibility that a single enzyme may be responsible for the glucuronidation of estrone and 17β-estradiol.

Since ATP has been shown to stimulate the glucuronidation of
p-nitrophenol by rat liver microsomes (23), its effect on the estrogen glucuronyltransferase was studied. Enzymatic activity is inhibited by ATP at a concentration four times higher than that of UDP-glucuronic acid. At a concentration of $13.3 \times 10^{-8}$ M, ATP was found to inhibit totally the activity of the glucuronyltransferase. The low affinity of ATP and the relatively large amount of this nucleotide necessary to obtain significant inhibition, indicate that ATP may act as a competitive inhibitor of the enzymatic glucuronidation.

In fact, it was found that ATP competes with UDP-glucuronic acid for the same enzyme, although the $K_i$ value for ATP ($0.48 \times 10^{-8}$ M) and the $K_m$ value for UDP-glucuronic acid ($0.84 \times 10^{-4}$ M) differ considerably. These observations make it likely that ATP combines with the enzyme at a site close to the binding site of UDP-glucuronic acid, thereby decreasing the rate of glucuronidation (16). The present findings are contradictory to those of Pogell and Leloir (23). These authors found that ATP and UDP-$N$-acetylglucosamine, when used either singly or simultaneously, had a stimulatory effect on the glucuronidation of p-nitrophenol by rat liver microsomes; this was attributed to the inhibition of the breakdown of UDP-glucuronic acid by the microsomal pyrophosphatase. The enzyme preparation described in this paper did not contain any detectable pyrophosphatase. The enzyme preparation described in this paper did not contain any detectable pyrophosphatase activity, when determined according to the method of Ogawa, Sawada, and Kawada (24). In view of this fact it is not surprising that UDP-$N$-acetylglucosamine, which has been shown to stimulate enzymatic glucuronidation by inhibition of the breakdown of UDP-glucuronic acid, does not influence the activity of the soluble estrogen glucuronyltransferase.

Since UDP is one of the products which are formed during the enzymatic glucuronidation, it seemed appropriate to study its effect on the conjugation of $17\beta$ estradiol. As shown by the results, UDP inhibits the estrogen glucuronidation at varying concentrations of $17\beta$-estradiol and UDP-glucuronic acid. This indicates that UDP binds at a site on the enzyme different from the binding sites of $17\beta$-estradiol and UDP-glucuronic acid; UDP thereby influences the maximum reaction velocity for both substrates. The nicotinamide adenine dinucleotides and adenosine $3',5'$-monophosphate added in two different concentrations (one 10 times higher than the other) also inhibited the glucuronidation of $17\beta$-estradiol. The fact that the extent of inhibition was independent of the concentration of the nucleotides indicates a noncompetitive type of inhibition.

The physiological significance of a highly specific conjugating enzyme present in the intestine of the pig toward $17\beta$-estradiol, estrone, and UDP-glucuronic acid is not yet clear. It has been postulated that glucuronidation is a true detoxication mechanism; furthermore, it may facilitate active transport of steroids across the cell membrane and render the compounds more soluble in body fluids (25). It is left to further experimentation to evaluate the significance of the soluble estrogen glucuronyltransferase in the gastrointestinal tract in connection with the enterohepatic circulation of estrogens and to investigate the specificities of the different glucuronyltransferases.

Acknowledgments—The expert technical assistance of Mrs. M. L. Rao and Miss D. Urbach is gratefully acknowledged. We wish to thank Mrs. I. Drossé for her skillful help during the earlier part of the investigation.

REFERENCES

Partial Purification and Kinetic Properties of a Soluble Estrogen Glucuronyltransferase from Pig Intestine
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J. Biol. Chem. 1969, 244:5521-5527.

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