A BSTRACT 6,7-3H-Estriol-16α-glucosiduronate-3C was administered to eight women (nine studies) by several routes: both injection and infusion (300 min) into the cubital vein, injection into the portal vein system, ingestion and instillation into the duodenum, jejunum, and ileum. Urine, collected from 0-2, 2-4, 4-8, 8-12, and 12-24 hr, was analyzed by countercurrent distribution for its content of radioactive 3- and 16-glucosiduronate (E3-3G1,E-16G1) and sulfoglucosiduronate (E3-3S16G1) of estriol as well as for 3H/3C ratio of each conjugate. After peripheral injection 50-60% of the injected E3-16G1 was excreted unchanged along with about 5% as E3-3S16G1 with an unchanged 3H/3C ratio, indicating direct sulfation of the injected E3-16G1. During a 300 min infusion, urinary excretion closely resembled that following injection. But 2-4 hr after the end of the infusion excretion of E3-3S, 16G1 stopped, excretion of E3-3G1 (17%/24 hr) with an elevated 3H/3C ratio started, and excretion of E3-16G1 continued (70%/24 hr), but with a rapidly increasing 3H/3C ratio. This indicated sequestration in a sluggishly metabolizing compartment where two processes occurred: (a), extensive hydrolysis of E3-16G1 followed by recombination at either C3 or C16 with unlabeled uridine diphosphate glucuronic acid (UDPGA), thereby increasing the 3H/3C ratio; and (b) transconjugation from C16 to C3, thereby producing E3-3G1 with finite 3H/3C ratios. Instillation into various segments of the small intestine produced results qualitatively similar to those after intravenous infusion, whereas ingestion and intraportal injection resembled perihenal intravenous injection. Therefore, we have postulated the possibility of an enteric circulation (in addition to an enterohepatic circulation) in which the steroid or its conjugates are transported into the small intestine in the success entericus, modified, and then reabsorbed and excreted in the urine—a process which requires several hours.

INTRODUCTION

The concept that the 16-glucosiduronate of estriol was destined to be solely a urinary excretory product has undergone revision as a result of studies with this conjugate. Recently, Goebelsmann, Sjoberg, Wiqvist, and Diezfalusy (1) reported the results of experiments in which E3-16G1 was infused intravenously into human subjects for about 5 hr. This conjugate was rapidly excreted into the urine and appeared to be the only conjugate of the labeled estriol found during and for 2-3 hours after cessation of the infusion. Then, E3-3G1 appeared in the urine and increased in relative amounts with time. The authors offered no suggestions to account for the delay in appearance of E3-3G1. The experiments described in this paper were designed to expand the original observations of Goebelsmann et al. and, if possible, to provide a tentative hypothesis which would give a rational explanation for the delay period in the

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1The following abbreviations and trivial names are used: estriol (E3), estra-1,3,5(10)-triene-3,16α,17β-triol; estriol-16α-glucosiduronate (E3-16G1), 3,17β-dihydroxyestra-1,3,5(10)-tien-16α-y1-β-D-glucopyranosiduronate; estriol-3-glucosiduronate (E3-3G1), 16α,17β-dihydroxyestra-1,3,5(10)-tien-3-yl-β-D-glucopyranosiduronate; estriol-3-sulfate-16α-glucosiduronate (E3-16G1), 3-sulfo-17β-hydroxyestra-1,3,5(10)-tien-16α-y1-β-D-glucopyranosiduronate; estriol-3-sulfate-16α-17β-diglucosiduronate (E3-16G1), estriol-3,16α-bis-(β-D-glucopyranosiduronate); estriol-3-sulfate (E3-3S), 16α,17β-dihydroxyestra-1,3,5(10)-tien-3-yl sulfate; UDPGA, uridine diphosphoglucuronic acid; CCD countercurrent distribution.
appearance of E₃-3GI in the urine. Data will be presented which possibly point to the partial excretion of E₉-16GI into the succus entericus, hydrolysis of the conjugate in the small intestine, and conjugation in the intestine with glucuronic acid at the 3-position, in part through trans-conjugation from the 16-position.

METHODS

6,7-³H-Estriol-16α-glucosiduronate-¹⁴C was administered to eight women. The route of administration and other pertinent data related to these subjects are presented in Table I. All the subjects were in good nutritional status and none had evident renal or hepatic dysfunction.

6,7³H-Estriol-16α-glucosiduronate (794 μc/mg) and estriol-16α-glucosiduronate-¹⁴C (21 μc/mg) were prepared in our laboratory separately by biosynthetic means (2), using, in the latter case, uridine diphosphate glucuronic acid (UDPGA) uniformly labeled in the glucuronic acid moiety with ¹⁴C. The ¹⁴C- and ³H-labeled estriol glucosiduronates were mixed and purified to a constant ratio of ³H to ¹⁴C by countercurrent distribution (CCD). The doubly labeled conjugate was also shown to be pure by paper chromatography. The glucosiduronate was injected by methods described previously (3), except the last subject (Table I) who was infused continuously for 300 min.

Urine samples were collected after administration of labeled conjugates as follows: 0-2, 2-4, 4-8, 8-12, 12-24, 24-48, and 48-72 hr. Urine specimens were mixed with an equal volume of 95% ethanol and kept in a cold room. Each specimen was taken almost to dryness under reduced pressure at a temperature not exceeding 40°C and then diluted with 300 ml of 95% ethanol. The precipitates were washed three times with 100 ml portions of 70% ethanol, so that they were essentially free of radioactivity. The ethanol extracts were stored at -15°C and brought to dryness just before analysis by CCD. The solvent systems employed and the partition coefficients of estriol conjugates in these systems are presented in Table II. The initial distribution consisted of 49 transfers in solvent system A. About 35 mg of sodium sulfate were added to tubes 0 and 1 in order to suppress emulsification of the phases. Two and sometimes three peaks were obtained, i.e., an extremely polar conjugate (N = 5, where N is the peak tube), a relatively polar conjugate (N = 10), and the same conjugate as that injected (N = 33). No detectable amount of material less polar than the injected conjugate was observed.

Radioactivity was measured by a Packard Model 3375 liquid scintillation spectrometer, with a dioxane-ethoxy-ethanol system (4) and an automatic external standard. The efficiency of counting unquenched samples is 29% for tritium and 70% for carbon-14. Therefore, the maximum sensitivity of detection of a compound after CCD is about 1000 dpm, assuming that the entire contents of each tube of the CCD apparatus is analyzed. More conventionally, when 10% of the contents is removed for analysis, 15,500 dpm is required for identification with assurance.

RESULTS

I. Injection of E₃-16GI into the cubital vein

The cumulative radioactivity and ³H/¹⁴C ratio of the materials excreted in the urine as estriol-16α-glucosiduronate and estriol-3-sulfo-16α-glucosiduronate during the initial 24 hr after a single injection of doubly labeled E₃-16GI into a normal and a pregnant woman are shown in Fig. 1. In both subjects the ³H/¹⁴C ratio of these two conjugates in the urine did not deviate significantly from that of the injected conjugate, indicating no cleavage of E₃-16GI before sulfation at the C-3 position. Initially, (0-4 hr) more than 99% of the radioactive products excreted was E₃-16GI with the same ³H/¹⁴C ratio as that of the E₃-16GI injected; at 24 hr after injection, approximately 7% of the injected conjugate had appeared as E₃-3S,16GI. The 24-48 and 48-72 hr samples were processed also and no detectable amounts of E₃-3GI were found.

Since the results obtained by us after a single, rapid injection of E₃-16GI differed from those of Goebelsmann et al. (1), i.e. no E₃-3GI was found in the urine, a prolonged infusion of E₃-16GI was administered to a normal woman in a manner similar to that used by the above authors. More than 95% of the radioactivity recovered in the urine during the 300 min infusion period was excreted as E₃-16GI and about 5% as E₃-3S,16GI (Fig.

Estriol Glucosiduronate Metabolism 381
TABLE II

Partition Coefficients of Some Estriol Conjugates

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>System A</th>
<th>System B</th>
<th>System C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-E-16G1</td>
<td>2.07</td>
<td>0.76</td>
<td>1.07</td>
</tr>
<tr>
<td>E-E-3G1</td>
<td>0.21</td>
<td>0.64</td>
<td>0.10</td>
</tr>
<tr>
<td>E-E-3S-16G1</td>
<td>0.10</td>
<td>0.25</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E-E-3S</td>
<td>4.0</td>
<td>—</td>
<td>1.9</td>
</tr>
</tbody>
</table>

A = n-butanol:ethylacetate:0.2% ammonium hydroxide (3:1:4); B = sec-butanol:10% ammonium hydroxide (1:1); C = n-butanol:ethylacetate:0.2% ammonium hydroxide (1:1:2).

2). During the 1st 4 hr after cessation of the infusion, E-E-3S,16G1 amounted to 10% of the urinary radioactivity. The $^3$H/$^{14}$C ratio of the two conjugates did not reveal any change from that of the infused E-E-16G1 during the infusion period and during the 1st 2 hr postinfusion.

Es-3-glucosiduronate first appeared 4 hr after cessation of the infusion with a $^3$H/$^{14}$C ratio of 22:1. Thereafter, the proportion of Es-3G1 increased to 16%, whereas Es-3S,16G1 disappeared. Concomitantly, the $^3$H/$^{14}$C ratio of both Es-3G1 and Es-16G1 increased until it was 30-50 times that of the injected conjugate. No detectable amounts of estriol (Es) or estriol-3-sulfate (Es-3S) were excreted in the urine of these three subjects.

II. Ingestion

Injection into a peripheral vein and, hence, into the systemic circulation permits all the organs access to the injected material in proportion to their blood flow. In an effort to afford the liver more immediate access to the Es-16G1, it was administered orally with the expectation that it might be rapidly absorbed unchanged from the intestine and delivered directly to the liver via the portal vein. The results reported below were unexpected.

The excretion pattern and $^3$H/$^{14}$C ratio of the radioactive metabolites excreted in the urine differed from those obtained after intravenous administration even though the urine contained the same two conjugates, i.e., E-

![Figure 1](image1.png)  
**Figure 1** The cumulative urinary excretion of radioactive estriol conjugates after peripheral intravenous and rapid injection of $^3$H-E-E-16G1-$^{14}$C into a normal woman ($^3$H/$^{14}$C = 2.9) and into a woman in the third trimester of pregnancy ($^3$H/$^{14}$C = 31). The number associated with each point is the $^3$H/$^{14}$C ratio of the material isolated at the indicated time period. In this and in the following four figures the curves have been arbitrarily drawn through the zero point, but it should be understood that the points of the curves between 0–2 hr have no significance or meaning regarding the rate of excretion of radioactivity during that period.

![Figure 2](image2.png)  
**Figure 2** The cumulative urinary excretion of radioactive estriol conjugates after continuous infusion ($^3$H/$^{14}$C = 3.7) and after ingestion ($^3$H/$^{14}$C = 2.9) of $^3$H-E-E-16G1-$^{14}$C.

382 N. Inoue, A. A. Sandberg, J. B. Graham, and W. R. Slaunwhite
16Gl and E-3S,16Gl (Fig. 2). After a latent period of 2 hr, a slow excretion and a rapid elevation of \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio of the two conjugates were observed, indicating that more than 90% of the ingested E-16Gl had been hydrolyzed, reconjugated, and excreted as the two conjugates. No significant amounts of E, E-3S, or E-3Gl were found in the urine.

III. Injection into the portal vein system

Since extensive hydrolysis of E-16Gl occurred after ingestion, injection into the portal vein was performed. At abdominal operation, E-16Gl was injected in 20 ml of saline in a period of a few minutes into the portal veins of two subjects, with cystic ovaries and carcinoma of the uterus, respectively. Their postoperative courses were uneventful. Unlike the results after the single injection into the cubital vein, E-3S,16Gl was excreted immediately (0–2 hr) (Fig. 3). The \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio of both estriol conjugates was the same as that of the injected E-16Gl. E-3S,16Gl accounted for about 12% of the urinary radioactivity excreted during the initial 24 hr after injection. In these experiments, as well as in the one after ingestion of the E-16Gl, it is possible that E-3Gl may have appeared in the urine after 24 hr, since the recovery of the total radioactivity ranged about 50%. It was felt, however, that the radioactivity was being excreted at such a slow rate after 24 hr that it would have precluded the detection and identification of any compounds.

IV. Instillation into intestine

Intestinal instillation was tried next for two reasons. First, the failure to find E-3Gl in the preceding experiment made it unlikely that the liver was involved in the conjugate transformation. Second, the 4–8 hr delay in appearance of E-3Gl eliminated the entire vascular compartment and the liver from consideration. Among the more sluggishly exchanging compartments, such as the lymphatic circulation, spinal fluid and small intestine, the last appeared most attractive. It is commonly known to be both metabolically active and to require several hours to complete the process of digestion and absorption. As will be pointed out in the Discussion, there is also a substantial inward flow into the intestine. In addition, the excretion of substantial amounts of estriol conjugates in the bile raised the possibility that the hepatointestinal circulation may play a part in the metabolic transformation of E-16Gl.

The excretion pattern and \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio of the urinary radioactive materials after injection of doubly labeled E-16Gl into the small intestinal lumen of three subjects at abdominal operation are shown in Figs. 4 and 5. After injection into the duodenum, jejunum, and ileum urinary radioactivity was excreted slowly and the \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio remained unchanged for 8, 12, and 24 hr postinjection, respectively, with the next collection in each instance showing a sudden elevation of the \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio (Fig. 4).

Initially, nearly 90% of the estriol conjugates excreted was as E-16Gl and about 10% as E-3S,16Gl with an unchanged \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio. The preponderant part of the tritium radioactivity was excreted after the break in the \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio, and it was during this period that E-3Gl was excreted. As in the case of continuous infusion, excretion of E-3Gl was accompanied by an abrupt cessation of E-3S,16Gl excretion (Figs. 4 and 5). Nearly 50% of the urinary \(^3\text{H}\) excreted during 72 hr after injection was E-3Gl with a \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio 10–20 times that of the injected conjugate. The \(^3\text{H}/\text{\textsuperscript{14}C}\) ratio of the E-16Gl closely approximated that of the E-3Gl formed during the same period.

V. Characterization of the urinary estriol conjugates

A. E-3S,16Gl

1) Partition Coefficient

A separation of the two more polar conjugates was to some extent incomplete whenever both polar conjugates occurred in the same extract. Fortunately, this
FIGURE 4 The cumulative urinary excretion of tritium after instillation of $^3$H-Ea-16G1-$^{14}$C into the duodenum, jejunum, and ileum ($^3$H/$^{14}$C = 3.7, 4.4, and 4.0, respectively). In this case, the solid line indicates the time during which the urinary $^3$H/$^{14}$C ratio did not deviate significantly from that of the injected E3-16G1. Bottom: the cumulative urinary excretion of radioactive estriol conjugates after instillation into the ileum.

![Figure 4](image)

2) Enzymatic Hydrolysis

(i) Bacterial $\beta$-glucuronidase. A portion of the E3-3S,16G1-like material, dissolved in 15 ml of 0.1 M acetate buffer at pH 7.0, was incubated with bacterial $\beta$-glucuronidase (100 U/ml) for 20 hr at 37°C. After incubation, the mixture was evaporated to dryness and the residue, dissolved in the lower phase, was submitted to CCD for 29 transfers in solvent system C with 5 mg of authentic estriol-3-sulfate (E3-3S)4 (Fig. 6). The distribution of tritium matches that of the carrier, measured as Kober chromogen, which produced a specific activity that remained nearly constant over the entire distribution. All of the $^{14}$C in tube 0 is presumably free glucuronic acid-$^{14}$C (or a metabolite). More than 95% of the incubated material was hydrolyzed by $\beta$-glucuronidase and converted to E3-3S.

(ii) Phenolsulfatase (Mylase-P). A portion of the E3-3S,16G1-like material was incubated with Mylase-P (2 mg/ml) in 15 ml of 0.1 M phosphate buffer (pH 6.0) for 20 hr at 37°C and was submitted to CCD in solvent system C (Fig. 6). More than 95% of the E3-3S,16G1-like material was hydrolyzed by phenolsulfatase and converted to E3-16G1.

(iii) Sequential enzymatic hydrolysis. The material from tubes 13–25 (Fig. 6, $\beta$-glucuronidase) was pooled.

384 N. Inoue, A. A. Sandberg, J. B. Graham, and W. R. Slaunwhite
and hydrolyzed with phenolsulfatase and the material from tubes 11–21 (Fig. 6, phenolsulfatase) was pooled and hydrolyzed with β-glucuronidase. The combined extracts of these two incubations were submitted to CCD for 49 transfers in solvent system D (methanol: water: chloroform: carbon tetrachloride, 70: 30: 80: 20). More than 90% of the tritium radioactivity and all of the carrier showed a single peak, K, 1.04, consistent with that of estriol. The specific activity of the distributed mixture was constant.

### TABLE III

<table>
<thead>
<tr>
<th>Solvent of crystallization</th>
<th>Es-3S,16GI Crystals</th>
<th>Mother liquor</th>
<th>Es-3GI Crystals</th>
<th>Mother liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>3885</td>
<td>2985</td>
<td>3005</td>
<td>2970</td>
</tr>
<tr>
<td>Acetone-α-β-hexane</td>
<td>3619</td>
<td>3836</td>
<td>3005</td>
<td>2970</td>
</tr>
<tr>
<td>Methanol-water</td>
<td>3607</td>
<td>3707</td>
<td>3106</td>
<td>3200</td>
</tr>
<tr>
<td>Ethanol-water</td>
<td>3381</td>
<td>3745</td>
<td>2960</td>
<td>3100</td>
</tr>
</tbody>
</table>

* Specific activity of crystals and mother liquor are in dpm/mg.

### 3) Reverse Isotopic Dilution

The estriol-like material, which was formed by sequential enzymatic hydrolysis (see preceding paragraph), was further identified by reverse isotopic dilution from three different solvents to constant specific activity as shown in Table III.

In summary, upon partial hydrolysis of the Es-3S, 16GI-like material with β-glucuronidase or phenolsulfatase, more than 95% of the radioactivity was released as Es-3S and Es-16Gl, respectively. Upon sequential hydrolysis with both enzymes, more than 90% of the radioactivity was released as estriol, which was characterized by reverse isotopic dilution.

### B. Es-3GI

A portion of the combined Es-3GI-like material was submitted to CCD for 99 transfers in three different solvent systems (A, B, and C) (Table II). In each instance, radioactivity followed a theoretical distribution with a constant $^{3}H/^{14}C$ ratio as shown in Fig. 7. The ratio was also the same as that of the undistributed material.

The Es-3GI-like material was incubated with bacterial β-glucuronidase (200 U/ml) in 15 ml of 0.1 M acetate buffer (pH 7.0) for 20 hr at 37°C and was then submitted to CCD for 49 transfers in solvent system D with 5 mg of authentic estriol. More than 90% of the radioactivity distributed as a single peak with a constant specific activity. The partition coefficient (1.04) was identical with that of estriol. No hydrolysis occurred during incubation with phenolsulfatase.

The estriol-like material from the CCD was recrystallized from three different solvents to a constant specific activity (Table III).

### DISCUSSION

The metabolic disposition of estriol in human subjects is characterized by a number of features which include rapid conjugation of estriol with glucuronic acid and primary excretion of the conjugate in the urine, substantial excretion of estriol conjugates in the bile (about 20%), the preponderant excretion of Es-3S,16Gl in the bile, negligible metabolic transformation of the aglycone and an enterohepatic circulation of estriol and its conjugates. Even though Es-16Gl has been identified and rigorously proven as the major conjugate in pregnancy urine (6), the presence of small amounts of Es-3Gl (7, 8), as well as Es-3S,16GI (7), has been reported. We have previously published data on the metabolic disposition of Es-16Gl (labeled only in the steroid moiety) and concluded that the conjugate was excreted almost exclusively in the urine (0% in the bile) (3), but recent investigations with Es-16Gl performed by...
continuous infusion of the material indicate that the conjugate may, in fact, undergo cleavage and re-conjugation, including glucuronidation at the 3-position (1). In addition, the methods used in our previous studies (3) would not have detected the small amounts of Es-3Gl or Es-3S,16Gl possibly present in the urine, particularly following a single, rapid injection. The studies of Goebelsmann et al. (1) and the data of the present investigations further indicate that the metabolism of Es-16Gl is significantly modified by the duration of the infusion. Prolonged infusion intravenously apparently allows the conjugate sufficient time to enter a compartment not accessible to it following a rapid, single injection.

The investigations reported in this paper utilize a conjugate labeled with tritium in the estriol moiety and carbon-14 in the glucuronic acid portion. Thus, the ratio of the two radioactivities serves as a measure of the metabolism of the conjugate. If the ratio is unchanged from that of the injected material, no metabolism has occurred. If, as frequently happened, urinary Es-16Gl with a changed ratio was isolated, the labeled glucuronic acid must have been removed and replaced by an unlabeled glucuronic acid. If 14C-labeled Es-3S,16Gl is obtained, the original injected molecule of Es-16Gl was sulfated; otherwise, the injected molecule was hydrolyzed before reconjugation (9). If carbon-14-labeled Es-3Gl is obtained, tranconjugation must have occurred. Thus, the use of the double label has allowed us to make several conclusions which would have been impossible with the use of a single label.

After a single, rapid injection into the cubital vein of a normal and a late-pregnant woman, most of the urinary conjugate was Es-16Gl accompanied by a small amount of Es-3S,16Gl after a delay of 4–8 hr (Fig. 1). The 3H/14C ratio of these two conjugates did not show any significant deviation from that of the injected conjugate, indicating no cleavage of Es-16Gl, even during sulfation at the C-3 position. The fact that Es-3S,16Gl was formed from Es-16Gl without cleavage is in accord with conclusions by Emerman, Twombly and Levitz (9). It is interesting to note that there was no difference in the results obtained in a pregnant and a non-pregnant woman, indicating that the foeto-placental
unit does not play a significant role in the above reaction.

After continuous infusion, most (60%) of the infused conjugate was excreted unchanged accompanied again by a small amount of E₃-3GI with unchanged ³H/¹⁴C ratio. Levitz and coworkers also observed the excretion of E₃-3GI in nearly identical amounts after either the infusion of ³H-E₃-3⁸S (9) or the intravenous or intraduodenal administration of labeled estriol (10). Starting 4 hr after cessation of the infusion, however, three things happened: (1) a slow excretion of E₃-16GI continued, but with a rapidly increasing ³H/¹⁴C ratio; (2) cessation of E₃-3GI, ¹⁴C excretion; and (3) commencement of E₃-3GI excretion with a ³H/¹⁴C ratio much like that of the E₃-16GI. The delay in excretion of E₃-³GI is in accord with the observation of Goebelmann et al. (1).

The long lag period associated with E₃-3GI formation may be related either to a slow excretion of a metabolite into the intestine, either via the bile or succus entericus, or to a slow hydrolysis of estriol conjugates and glucuronidation at position-3 in the intestine. It is possible that during prolonged infusion of E₃-16GI, sufficient amounts of the conjugate are excreted in the bile, in contrast to an apparent absence of such excretion after a single injection (3), as to result in ultimate formation of E₃-3GI. The hydrolysis of E₃-3S,16GI and E₃-16GI in the intestine with subsequent formation of E₃-3GI appear to be rather slow reactions in the intestinal tract, as is evident from the results obtained after instillation of E₃-16GI into various parts of the intestine (see Figs. 5-7).

Even though the above explanation for the time lag in the excretion of E₃-3GI is likely and plausible, the sequestration of estriol (or one of its conjugates) in an anatomical pool with an extremely slow turnover should not be lost sight of. Such a possible pool may be the succus entericus, which flows at the rate of about 3 liters/day. Nearly all of this fluid and presumably most of its solutes are reabsorbed, although not necessarily unchanged, during passage along the intestine. Therefore, E₃-16GI was administered by us by different gastroenteric routes in order to ascertain whether an enteric route might exist. After oral administration, most of the E₃-16GI was hydrolyzed, reconjugated, and excreted in the urine at a slow rate as E₃-3S,16GI with a greatly elevated ³H/¹⁴C ratio. Extensive hydrolysis may have been due primarily to gastric juice. Regardless of the mode of hydrolysis, however, it is clear from this result that neither the stomach nor the liver is the site of E₃-3GI formation.

After injection into the portal vein system a significant amount of the injected E₃-16GI was converted to E₃-3S, 16GI without cleavage of the glucuronic acid moiety. The prompt excretion of E₃-3S,16GI indicates that the sulfation reaction is rapid in the liver. We failed, however, to detect E₃-3GI during the time of this study, indicating, again, that the liver is not the site of transconjugation from E₃-16GI to E₃-3GI.

**Estriol Glucosiduronate Metabolism** 387

**FIGURE 7** Characterization of ³H-E₃-3GI-¹⁴C by CCD in two systems.
After instillation into the duodenum, jejunum, and ileum, the urine showed a sudden elevation of the $^4$H/$^{14}$C ratio at 8, 12, and 24 hr postinjection, respectively. After the break in the $^4$H/$^{14}$C ratio, Es-3GI was excreted in the urine, while before that time the urine contained Es-3S,16GI as the sole metabolite. Nearly 50% of the urinary $^4$H excreted during 72 hr after injection was Es-3GI with a $^4$H/$^{14}$C ratio 10–20 times that of the injected conjugate. The $^4$H/$^{14}$C ratio of Es-3GI was different from those of injected Es-16GI and of Es-3S,16GI and was quite similar to the results after prolonged intravenous infusion.

These results strongly suggest that the intestine or the content of the intestinal lumen is the site of transconjugation from Es-16GI to Es-3GI. The mechanism is unknown. One possibility is hydrolysis by the $\beta$-glucuronidase present in various bacteria (e.g., E. coli) in the intestinal lumen followed by absorption by the mucosal cells and reconjugation at C3 and at C16 before release into the bloodstream. This is in accord with the observations of Stoa and Levitz (10) and Levitz and Katz (11) in vivo and of Dahm and Breuer (12) in vitro.

Another possibility, which probably is operating to some extent, is direct transconjugation. Fishman and Green (13, 14) demonstrated that purified mammalian or crude bacterial (E. coli) $\beta$-glucuronidase can act as a transferase, i.e., transferring glucuronic acid from a number of phenols or menthol to 18 different alcohols and glycols in yields varying from 7–89%. Crucial to the success of this in vitro reaction was a high concentration (about 1 M) of acceptor. (Even so, the concentration of water for the competing reaction of hydrolysis was about 56 M.) Phenols and terpene alcohols could not be used as acceptors due to a combination of insolubility and inhibitory action on the enzyme. Such limitations may not exist in vivo.

The metabolic disposition of estriol and its conjugates presents a complex situation with several reactions and processes proceeding simultaneously. Some Es-16GI is absorbed unchanged. This definitely occurs because the Es-16GI excreted shortly after intestinal injection has the same $^4$H/$^{14}$C ratio as the injected material. Some Es-16GI is hydrolyzed. The released Es competes with other aglycones, mostly nonsteroidal, in a transferase reaction which produces Es-3Gl. Presumably Es-16GI would not be the sole source of glucuronic acid. The transfer reaction would tend to conserve the glucuronic acid–$^{14}$C and produce a lower $^4$H/$^{14}$C ratio in the product than a de novo synthesis employing unlabeled UDPGA.

Fig. 5 shows that the Es-3GI did have a lower $^4$H/$^{14}$C ratio than the Es-16GI isolated in the same time period. Finally, the rest of the Es would be absorbed and reconjugated in the intestinal mucosa (or liver), mostly as Es-16GI (15, 16).

The difference between a rapid injection and a prolonged infusion of Es-16GI into a peripheral vein may be one of time. We would like to stress that we do not propose that there is a qualitative difference between the metabolism of injected vs. infused Es-16GI. The difference may be merely quantitative and related to the relative amounts of the administered Es-16GI that are sulfated in the 3-position and, thus, may be excreted in the bile and further metabolized in the intestine and the relative amounts available to the intestine via the general circulation or in the succus entericus. It is entirely possible that Es-3GI was present in the urine of the subjects given a single injection, e.g., Levitz and Katz (11) found that about 3% of Es-16GI appeared in the urine as the Es-3GI in one of their subjects, but the amounts present were too small to be detected by our methods. It has been shown that Es-16GI (3) as well as Es-3GI (17) are cleared from the blood with great rapidity. On the other hand, the flow of succus entericus is very slow—125 ml/hr or 2.5% of the blood volume/hr. The rapid clearance of injected Es from the blood (3), the delayed appearance of Es-3GI in the urine after instillation of Es-16GI into the intestine, and the rate of flow of the succus entericus result in insufficient secretion of the labeled conjugate to permit detection after a rapid injection. After a 5 hr infusion about 1/4 of the infused conjugate should have entered the intestine. This number is in approximate agreement with the excretion of 16% of the infused tritium radioactivity as Es-3GI.

In support of intestinal secretion of estrogen conjugates are a number of unrelated observations. We found that injected Es-16GI was not excreted in the bile (3) and Emerman et al. (9) demonstrated that Es-3S was nearly excluded from the bile. A significant amount (9%) was, however, hydrolyzed, reconjugated, and excreted in the bile as Es-3S,16GI (9). Others have shown that unconjugated estrogens (18, 19), fat (20), and manganese (21) can be found in the intestinal lumen in the absence of bile. Furthermore, recent studies on intestinal absorption in man have revealed that there are functional differences at different levels of the human small intestine (22, 23): It is most likely that Es-16GI is partly absorbed intact, presumably at the proximal segment, while the unabsorbed Es-16GI reaches the distal segment where it is subjected to further metabolism. Thus, it appears that intestinal secretion should be considered as an additional parameter of estrogen metabolism. In addition, we have shown that the glucuronic acid moiety of Es-16GI may be reutilized and we have postulated that the ubiquitous enzyme, $\beta$-glucuronidase, acting as a transferase is responsible.

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REFERENCES


