

Effects of ethinylestradiol on enzymes catalyzing bile acid conjugation and sulfation

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Abstract Administration of large doses of estrogens has been shown to result in decreased conjugation and increased sulfation of bile acids as well as cholestasis. There have been no previous studies on the effects of low doses of estrogens on these parameters of bile acid metabolism. Therefore, rats were given ethinylestradiol, 0.06 to 600 $\mu\text{g}/\text{kg}/\text{day}$ subcutaneously for up to 21 days and the in vitro activity of the hepatic conjugation and sulfation enzymes was measured. Conjugating enzyme activity was unchanged at doses below 600 $\mu\text{g}/\text{kg}/\text{day}$. In contrast, hepatic sulfation of conjugated bile acids increased significantly after 21 days treatment with 0.6 $\mu\text{g}/\text{kg}/\text{day}$. The magnitude of the increase was both time- and dose-dependent. Increased sulfotransferase activity was noted only in the liver and was specific for conjugated bile acids; there was no change in the rate of sulfation of the unconjugated bile acids or in renal sulfotransferase activity. Increased hepatic bile acid sulfotransferase activity occurred in the presence of normal bile flow and bile acid secretion. These data indicate that treatment with doses of ethinylestradiol comparable to those present in oral contraceptives may lead to readily detectable time- and dose-dependent changes in bile acid sulfation without producing cholestasis. The data also suggest that there may be significant differences in the enzymatic sulfation of conjugated and unconjugated bile acids in the liver.—Kirkpatrick, R. B., and P. G. Killenberg. Effects of ethinylestradiol on enzymes catalyzing bile acid conjugation and sulfation. *J. Lipid Res.* 1980. 21: 895–901.

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Intrahepatic metabolism of bile acids is an important determinant of the physiologic action and metabolic fate of these compounds. In mammals, most of the bile acids are conjugated with amino acids in the liver (1); in addition, recent evidence suggests that lithocholate conjugates undergo further metabolism to form the 3α -sulfate esters (2). Sulfate esterification of other bile acids is not a major metabolic pathway except in the presence of cholestasis (3, 4).

Previous workers have shown that the administration of estrogens to animals has resulted in altered hepatic metabolism of the bile acids; decreased conjuga-

tion of bile acids with amino acids (5–7) and increased formation of sulfates have been recorded (8). All of these experiments have been performed using potentially toxic doses of ethinylestradiol (1–5 mg/kg day). Administration of ethinylestradiol in these doses has also resulted in cholestasis as evidenced by decreased bile flow and bile acid secretion into bile (6–10).

Recently, others have shown a sex-dependent difference in the in vitro rate of sulfation of tauro-lithocholate (11) and glycochenodeoxycholate (12); activity in female livers is approximately twice that of males. In addition, castrated female rats showed a decrease in sulfate ester formation to male levels; activity could be restored to normal female levels by administration of physiological doses of estradiol benzoate (0.15 mg/kg/day). However, administration of this dose of estradiol benzoate to male rats did not change the rate of enzymatic sulfation (11). There have been no previous studies which have attempted to define the relationship between dose and duration of estrogen administration and bile acid conjugation and sulfation.

In order to study this question, we administered a range of doses of ethinylestradiol to male rats and measured the in vitro activity of cholic acid:CoA ligase, bile acid-CoA:amino acid N-acyltransferase, and bile acid sulfotransferase. Bile acid sulfotransferase activity was increased in a time- and dose-dependent manner; significant increases in activity were observed following 14 days of administration of ethinylestradiol in doses comparable to those used in oral contraceptives. Increased bile acid sulfotransferase activity was specific for conjugated bile acids; the rate of sulfation of unconjugated bile acids was not increased. Bile flow and bile acid secretion into bile was no different from normal under conditions in which sig-

Abbreviations: EE, 17 α -ethinylestradiol; PAPs, adenosine 3'-phosphate, 5'-phosphosulfate; PG, propylene glycol.

nificant stimulation of bile acid sulfotransferase activity was noted. The activity of the enzymes catalyzing the conjugation of bile acids with amino acids was unaffected by ethinylestradiol. These data suggest that the administration of estrogens has a specific effect on the in vivo rate of sulfation of conjugated bile acids under conditions where bile acid conjugation with amino acid and bile production are unaffected.

METHODS

Reagents

Coenzyme A, ATP, and adenosine 3'-phosphate, 5'-phosphosulfate (PAPS) were obtained from PL Laboratories, Milwaukee, WI. 17 α -Ethinylestradiol was purchased from Sigma Chemical Company, St. Louis, MO. Lithocholate, glycolithocholate, tauro-lithocholate, chenodeoxycholate, and taurochenodeoxycholate were purchased from CalBiochem, San Diego, CA, as were the 3-SO₄ esters of conjugated and unconjugated lithocholate. These compounds were found to be more than 95% pure by high performance liquid chromatography (13). The 3 α - and 7 α -monosulfate esters of chenodeoxycholate and taurochenodeoxycholate were a gift from Professor Leon Lack who synthesized them as previously reported (14). Coenzyme A derivatives of bile acids were synthesized by methods previously reported (15). [24-¹⁴C]chenodeoxycholate, [24-¹⁴C]taurocholic acid, ³⁵S-labeled PAPS, and [6,7-³H]17 α -ethinylestradiol were obtained from New England Nuclear, Boston, MA. 3 α -Hydroxysteroid dehydrogenase was obtained from Worthington Biochemicals, Freehold, NJ. Propylene glycol (PG) was purchased from Fisher Scientific Company, Fairlawn, NJ. All other chemicals were of reagent grade or better and were obtained from commercial sources.

Animals

Random-bred male Sprague-Dawley rats (180–250 g initial weight) were obtained from Charles River Breeding Labs, North Wilmington, MA, housed in wire cages, and given free access to water and pelleted rat chow (Purina Company, St. Louis, MO). Each morning, animals were injected subcutaneously with 0.05 to 0.10 ml PG containing EE in doses calculated on the basis of the initial weight of the animal. Control animals were injected with equal volumes of PG. To control for an effect of PG injection, some animals were removed daily from their cages, petted, and

returned without injection. Animals subjected to hepatectomy were not injected on the morning they were killed.

Tissue preparation

Rats were stunned, exsanguinated by cervical laceration, and the livers were removed into ice cold 250 mM sucrose, 5 mM morpholinopropane sulfonic acid, pH 7.2. After 5 min, livers were blotted dry on filter paper and weighed. A small piece was placed in 10% buffered formalin. Four grams of the remainder were homogenized in 12 ml of the above buffer, diluted to 40 ml, and centrifuged at 5°C for 12 min at 500 g. A portion of the supernatant was retained as "whole cell homogenate"; the remainder was centrifuged at 20,000 g for 12 min. This second supernatant was recentrifuged at 240,000 g for 65 min. The ultracentrifugation supernatant was decanted and used for measurement of bile acid sulfotransferase and bile acid-CoA:amino acid N-acyltransferase activities. The ultracentrifugation pellet was rehomogenized in 8 ml of ice cold buffer and used for assay of microsomal cholic acid:CoA ligase. Kidney tissue was prepared in the same manner, except that the starting weight of tissue was 2.0 g and the volumes used were scaled down proportionately.

Enzyme assays

Cholic acid:CoA ligase and bile acid-CoA:amino acid N-acyltransferase activities were measured by methods previously described in detail (16).

Sulfotransferase activity was determined by duplicate incubations following the method of Chen, Bolt, and Admirand (17). The labeled substrate was either ³⁵S-labeled PAPS or the appropriate ¹⁴C-labeled bile acid. With lithocholate, glycolithocholate, tauro-lithocholate, and chenodeoxycholate, product formation was determined after thin-layer chromatography of the incubation medium on Absorbosil-1 (Applied Science Labs, State College, PA), developed with 16 cm with chloroform–methanol–acetic acid–water 65:25:15:9 as described by Cass et al. (18). The position of appropriate standards was determined by spraying one vertical lane on each plate with 3.5% phosphomolybdic acid. Migration of standards (*R_f*) was: PAPS, 0.02; tauro-lithocholate-3-SO₄, 0.33; glycolithocholate-3-SO₄, 0.45; lithocholate-3-SO₄, 0.56; chenodeoxycholate monosulfates, 0.53–0.57; tauro-lithocholate, 0.60; glycolithocholate, 0.97; chenodeoxycholate, 0.98; lithocholate, 0.99.

In taurochenodeoxycholate incubations, ³⁵S-labeled PAPS was the labeled substrate. Chromatography was performed on Absorbosil-5 (Applied Science Labs,

State College, PA) eluted with 2-butanone–methanol–2-propanol–acetic acid–water 10:1:1:1:1, as reported by Parmentier and Eysen (19). Taurochenodeoxycholate sulfates migrated as follows (R_f): -3,7-di-SO₄, 0.23; -7-SO₄, 0.38; -3-SO₄, 0.51. PAPS and taurochenodeoxycholate exhibited R_f 0.02 and 0.67, respectively.

Each vertical lane of the chromatograph was divided into five horizontal sections, the position of which was determined by the location of standards in a parallel lane. Each section was scraped into a counting vial and suspended in liquid scintillant containing Cab-O-sil (Research Products International Corporation, Elk Grove Village, IL). Counting efficiency (69–72%) was determined by addition of known ¹⁴C activity (Radiomatic Instrument and Chemical Company, Addison, IL).

Sulfation of bile acids was calculated from the radioactivity in the section(s) corresponding to the location of the intended product(s) and expressed as a percent of the total radioactivity recovered from all five sections. After several hundred assays, it was consistently observed that the sum of the radioactivity in the sections including the labeled substrate and the product(s) resulted in recovery of more than 99% of the total radioactivity on the plates. The sulfotransferase specific activity calculated on the basis of these sections differed from that determined on the basis of five sections by only 1.9% (0 to 3%, $n = 29$). Thereafter, only these sections were scraped. The coefficient of variation among replicate assays using this latter method was 5.3%.

In order to compensate for potential incorporation of ³⁵S-labeled-PAPS into unknown substrates contained in the enzyme source, controls for reactions using this labeled substrate contained no added bile acid substrate. When ¹⁴C-labeled bile acid substrates were used, control reactions were maintained at 5°C. Specific activity with ¹⁴C-labeled bile acid substrate was somewhat higher than in comparable assays using ³⁵S-labeled PAPS. However, there was excellent correlation between assays comparing the two labels ($r = 0.982$).

Ethinylestradiol sulfates did not contribute to measured product formation; incubations including [6-7,³H]-17 α -ethinylestradiol as the labeled substrate resulted in a product which migrated with an R_f of 0.39 in the system of Cass et al. (18). This product was present in the horizontal section below that containing glycolithocholate-3-SO₄ and above that containing tauroolithocholate-3-SO₄. Addition of unlabeled EE to incubations containing ³⁵S-labeled PAPS resulted in no difference in the specific activity of

sulfotransferase with conjugated or unconjugated bile acids.

Protein was determined by the biuret method using defatted bovine serum albumin as standard (20). 3- α -Hydroxysteroid content of the bile was estimated by the 3- α -hydroxysteroid dehydrogenase method as described by Talalay (21). Solvolysis of bile samples followed the procedure of Makino et al. (22).

Collection of bile

Animals used in these studies received their final injection on the morning of operation, approximately 2 hr before the start of bile collection. Under ether anesthesia, the common bile duct was ligated and a PE-50 polyethylene catheter (Clay-Adams, Inc., Parsippany, NJ) was inserted into the common bile duct proximal to the ligature. An identical catheter was inserted into a femoral vein, and the animals were placed in restraining cages. 0.78 M Sodium chloride, 0.28 M glucose was infused into the femoral vein catheter at 0.04 ml/min. After 20 min, during which time the bile was collected and retained, 0.2 ml of 2% bovine serum albumin containing 14 ng of [24-¹⁴C]-taurocholate acid (6.75 mCi/mmol) was injected into the femoral vein catheter in a single bolus. Bile was collected in 30-min intervals for a total of 4 hr after injection of taurocholate tracer. Bile volume was estimated by weighing each 30-min collection in tared vessels. Recovery of [¹⁴C]taurocholate was measured by counting an aliquot (0.02 ml) of each 30-min collection in 5 ml of liquid scintillant, correcting for quenching by addition of known ¹⁴C activity as above. Bile acid content of bile was determined on pooled specimens of the bile collected from 0–4 hr after placement of the bile duct fistula. The percent of total bile acids in pooled bile that was sulfated was measured by the differences in 3 α -hydroxysteroid dehydrogenase activity before and after solvolysis (22) and by thin-layer chromatography (18).

Histopathology

Tissues fixed in 10% buffered formalin were embedded in paraffin, cut, and stained with hematoxylin and eosin. The slides were coded and interpreted by the authors without knowledge of the origin of the specimen. The presence or absence of canalicular plugging, bile duct proliferation, periductular inflammation, fibrosis, and fatty change were recorded.

Analysis of data

Variation about means is noted as \pm SEM. Differences between means were evaluated by Student's t test (23).

TABLE 1. Effect of ethinylestradiol in the in vitro specific activity of hepatic bile acid conjugating enzymes

Enzyme Activity	Treatment		
	Propylene Glycol	Ethinylestradiol ^c	
		6 $\mu\text{g}/\text{kg}/\text{day}$	600 $\mu\text{g}/\text{kg}/\text{day}$
		<i>$\mu\text{mol}/\text{min}/\text{mg protein}$</i>	
Cholic acid:CoA ligase	(8) ^a 3.43 \pm 0.61 ^b	(6) 3.60 \pm 0.55	(7) 2.91 \pm 0.31
Bile acid-CoA:amino acid N-Acyltransferase			
Glycine-dependent	(7) 8.84 \pm 1.26	(6) 8.55 \pm 1.00	(4) 11.08 \pm 2.30
Taurine-dependent	(7) 5.33 \pm 0.70	(6) 3.95 \pm 0.58	(4) 5.71 \pm 0.62

^a Number of animals shown in parentheses.

^b Values shown are means \pm SEM.

^c Animals were treated for 21 days. The values shown for EE-treatment are not significantly different ($P > 0.05$) from PG-treated controls.

RESULTS

All animals gained weight during treatment with PG or EE. However, the relative weight gain was less in EE-treated animals at or above 6 $\mu\text{g}/\text{kg}/\text{day}$ ($P < 0.01$). Liver weight and total hepatic protein were not significantly different in EE-treated animals compared with PG-treated controls ($P > 0.05$). There were no differences in weight gain, liver weight, liver protein, or any enzyme activity between PG-treated and non-injected controls. Direct addition of EE (0.06 μg –6.0 μg) or PG (20%, v/v) to incubation mixtures did not affect the specific activities of any of the enzyme assays.

Conjugating activity

Microsomal cholic acid:CoA ligase and supernatant bile acid-CoA:amino acid N-acyltransferase specific activities were not affected by treatment with EE for 21 days (Table 1).

When these activities were assayed in whole cell homogenate, EE had no effect on N-acyltransferase activity. However, after treatment for 21 days at 600 μg EE/kg/day, cholic acid:CoA ligase activity of whole

cell homogenate was decreased, (0.73 \pm 0.06 $\mu\text{mol}/\text{min}/\text{mg protein}$ versus 1.01 \pm 0.10 $\mu\text{mol}/\text{min}/\text{mg protein}$ in controls, $P < 0.05$). This was not observed with doses of EE below 600 $\mu\text{g}/\text{kg}/\text{day}$. Total hepatic activity of each of these enzymatic reactions was the same in EE- and PG-treated animals except after 600 μg EE/kg/day where total hepatic cholic acid:CoA ligase activity was 2782 \pm 942 $\mu\text{mol}/\text{min}$ compared to 4841 \pm 1088 $\mu\text{mol}/\text{min}$ in PG controls ($P < 0.01$).

Sulfotransferase activity

In contrast to the effect on the conjugating enzymes, EE treatment resulted in a significant increase in hepatic bile acid sulfotransferase activity (Table 2). This increase was specific for sulfation of conjugated bile acids, sulfation of glycine and taurine conjugates increasing similarly. There was no increase in sulfotransferase activity with unconjugated lithocholate or chenodeoxycholate.

Increases in sulfotransferase activity with glycolithocholate were proportional to \log_{10} dose between 0.6 and 600 μg EE/kg/day (Fig. 1). Stimulation was observed after 10 days of treatment with 6 $\mu\text{g}/\text{kg}/\text{day}$ but was not apparent before 14 days at 0.6 $\mu\text{g}/\text{kg}/\text{day}$

TABLE 2. Effect of ethinylestradiol on in vitro rates of sulfation of conjugated and unconjugated bile acids

Substrate	Specific Activity		
	Propylene Glycol	Ethinylestradiol ^a	P Value
	<i>$\text{pmol}/\text{min}/\text{mg protein}^b$</i>		
Lithocholate	5.4 \pm 1.3	4.8 \pm 1.1	NS
Glycolithocholate	11.7 \pm 2.8	35.9 \pm 8.1	$P < 0.01$
Taurolithocholate	11.9 \pm 2.9	39.9 \pm 7.2	$P < 0.01$
Chenodeoxycholate	3.0 \pm 0.9	3.0 \pm 1.0	NS
Taurochenodeoxycholate	2.5 \pm 0.5	16.8 \pm 5.8	$P < 0.05$

^a EE-treated animals received 6 μg EE/kg/day for 21 days.

^b Values shown are means \pm SEM of determination on six animals.

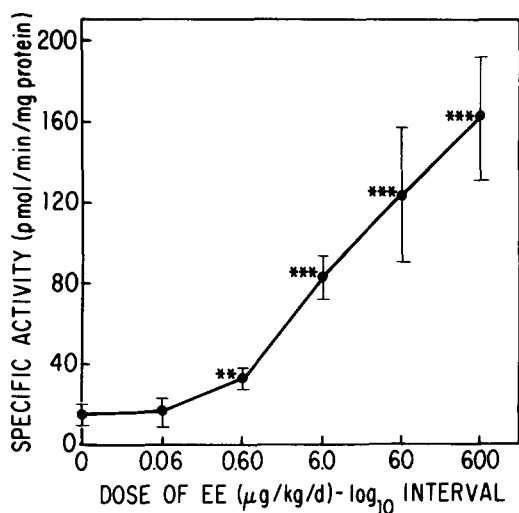


Fig. 1. Relationship between dose of ethinylestradiol and specific activity of hepatic bile acid sulfotransferase with glycolithocholate as substrate. Animals were treated for 21 days. Data points represent the mean \pm SEM of determination on six animals for each group except at 60 and 600 $\mu\text{g}/\text{kg}/\text{day}$ where three animals were in each group. Specific activity was significantly different from controls at doses of 0.60 $\mu\text{g}/\text{kg}/\text{day}$ and above; **, $P < 0.01$; ***, $P < 0.001$.

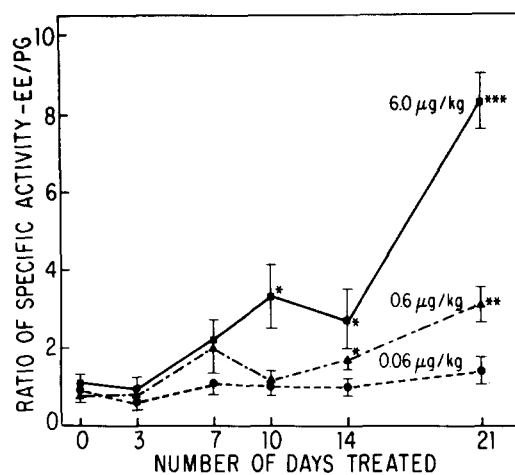


Fig. 2. Relationship between the duration of treatment and increases in the specific activity of hepatic bile acid sulfotransferase with glycolithocholate as substrate. Doses of EE are as indicated. The ratio of the specific activity of EE-treated animals to that of PG-treated controls is shown on the ordinate. Mean specific activity (pmol/min/mg \pm SEM) of PG-treated animals was 15.7 ± 1.6 at 3 days; 16.0 ± 2.6 at 7 days; 11.5 ± 2.7 at 10 days; 7.0 ± 0.4 at 14 days; and 10.0 ± 1.55 at 21 days. Data points represent the means \pm SEM for six animals in each group except at 14 days where there were three animals. Significant differences from PG treated controls are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

(**Fig. 2**). In contrast to its effect in liver, EE treatment (0.06 to 600 μg EE/kg/day for 21 days) had no effect on glycolithocholate sulfation in kidney supernatant.

The lack of an effect of EE treatment on lithocholate and chenodeoxycholate sulfation cannot be explained by limitations inherent in the routine assay system. Using protein from EE-treated animals, specific activity was constant at protein concentrations from one-half to 2.5 times that used in the routine assay (0.2 mg–1 mg), and a Lineweaver and Burk plot (21) was linear from 50 to 200 μM bile acid substrate. Under conditions of the routine assay (0.4 mg protein, 100 μM bile acid), specific activity was constant for up to 15 min, 1.5 times the usual duration of incubation. Assays including combinations of protein from EE-treated (600 $\mu\text{g}/\text{kg}/\text{day}$ for 21 days) and PG-treated animals gave the predicted specific activities; neither stimulation nor inhibition were observed.

Presence of cholestasis

Evidence of cholestasis was sought in rats treated with 6 μg EE/kg/day and PG controls. The rate of bile

flow and bile acid secretion was the same in both groups (**Table 3**). The rate of recovery of radioactivity from bile after injection of a tracer dose of [^{14}C]taurocholate into a femoral vein was also similar; after 2 hr, $81.9 \pm 6.5\%$ of the label was recovered from bile in PG-treated animals compared to $83.7 \pm 6.4\%$ in animals treated for 21 days with 6 μg EE/kg/day ($P > 0.05$). Thin-layer chromatography of this bile demonstrated that 77.6% and 76.2% of the administered radioactivity migrated as taurocholate in the PG and EE groups, respectively ($P > 0.05$). The remainder of the radioactivity in the bile was recovered in the region where more polar compounds would be expected; 6.5% and 7.7% localized to the position of taurocholate monosulfates ($P > 0.05$). Measurement of 3α -hydroxysteroid content of the bile before and after solvolysis showed no significant difference between PG- and EE-treated animals.

Microscopic analysis of liver biopsies showed no

TABLE 3. Effect of ethinylestradiol on bile flow and bile acid secretion

Treatment	Bile Flow		Bile Acid Secretion	
	g/hr	g/hr/100 g	$\mu\text{mol}/\text{hr}$	$\mu\text{mol}/\text{hr}/100\text{ g}$
Propylene glycol	0.96 ± 0.14^a	0.212 ± 0.034	19.5 ± 2.8	4.69 ± 1.22
Ethinylestradiol ^b	0.88 ± 0.10	0.204 ± 0.026	17.5 ± 2.9	4.23 ± 0.69

^a All values shown are means \pm SEM of determination on six animals.

^b EE-treated animals received 6 μg EE/kg/day for 21 days. The values shown for EE-treated animals are not significantly different ($P > 0.05$) from PG controls.

consistent differences among PG-, EE-, and uninjected animals. About 60% of animals in all groups showed mild generalized vacuolization of hepatocytes. A few biopsies showed periductular inflammation in the portal tracts. Only one animal, treated for 21 days with 600 μg EE/kg/day, showed evidence of periportal piecemeal necrosis of hepatocytes with periductular inflammation and replication of bile ductules. Distention and inspissation of bile in the lobular canaliculi was not seen in any biopsy.

DISCUSSION

The data presented here indicate that low doses of EE resulted in a time- and dose-dependent increase in the specific activity of the enzyme(s) catalyzing sulfation of conjugates of lithocholate and chenodeoxycholate. Significant increases in sulfotransferase activity occurred in the absence of changes in the conjugating enzymes. The first evidence of impaired conjugation, decreased cholic acid:CoA ligase activity, occurred at a dose which is 1000 times the lowest dose which increased sulfating activity. The basis of the decrease in this activity, seen only when measured in whole cell homogenate, is not known.

The increase in sulfotransferase activity occurred at doses which did not cause obvious cholestasis. Although others have shown that histology, including electron microscopy, may be normal in animals treated with doses of EE which cause cholestasis (24, 25), the longer time frame of our experiments justified looking at this parameter. No consistent difference was seen between EE-treated animals and controls.


At 6 μg EE/kg/day, there were no demonstrable differences in biliary function compared to PG controls; bile acid secretion and bile flow were unchanged, and the rate of recovery of labeled taurocholate was the same in both groups. Decreased bile acid clearance has been observed following much higher doses of EE (8). However, the basis for the decreases in clearance was a significant decrease in bile flow; this was not seen under the conditions of our experiments (Table 3). The effect of doses of EE above 6 μg /kg/day on bile flow and bile acid secretion was not studied.

The mechanism by which low doses of EE result in increased sulfotransferase activity is not known. Although we found no evidence for cholestasis at doses of EE which were ten times the lowest dose which resulted in increased sulfotransferase activity (Fig. 1), abnormal biliary canalicular function, defined by parameters not reflected in bile flow or bile acid secre-

tion, may yet prove to play a role. However, since estrogens have been shown to increase the rate of sulfation of other non-bile acid substrates (12, 26), it is also possible that EE-stimulation of bile acid sulfotransferase activity is independent of any effect on biliary function.

At the doses used, increased sulfotransferase activity is seen in the liver but not in the kidney suggesting that, if the effect is the result of hormonal action, the mediator is either specific for the liver or that the liver has a lower threshold for this signal. Additional specificity of the action of EE on sulfotransferase activity can be inferred from the different effects of EE on rates of sulfation of conjugated and unconjugated bile acids. Although previous reports have suggested that a single enzyme catalyzes sulfation of both conjugated and unconjugated lithocholate (17, 27, 28), treatment with EE results in clear distinction between the two substrates. We have previously noted a difference in the pattern of postnatal development of the capacity to sulfate conjugated and unconjugated lithocholate (29). The present data provide further evidence for differences in the enzymatic sulfation of conjugated and unconjugated bile acids.

The doses used in our experiments were lower than those employed by others. On a direct $\mu\text{g}/\text{kg}$ basis, our dose schedules are comparable to those experienced by women receiving oral contraceptive agents containing EE. Due to the greater rate of metabolism of drugs by rats compared with humans (30), the doses of EE used in the present experiments may in fact be relatively smaller than those used in oral contraceptive pills. Thus, the present doses may be well within the pharmacological and, in pregnancy, physiological exposure to estrogens. These considerations serve to contrast our work with most previous studies which, on the basis of doses of EE used, may have explored the toxic effects of estrogen administration.

At doses of EE which resulted in a 3- to 4-fold increase in hepatic sulfotransferase activity, there was no significant difference in the proportion of biliary bile acids which were sulfated. The physiologic importance of stimulation of this enzyme activity by EE, therefore, remains to be determined. However, it is clear that low doses of EE are capable of causing readily detectable specific biochemical changes and that the magnitude and nature of the response to EE is, in part, dependent on the dose and time of exposure to the drug. Future interpretations of the effects of estrogens on bile acid metabolism, therefore, must include these considerations. 

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