Regulation of Bile Acid Synthesis via Direct Effects on the Microsomal Membrane†

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ABSTRACT: Rats treated with ethinylestradiol (5 mg kg^{-1} day^{-1} for 5 days) secrete de novo synthesized bile acids at a markedly reduced rate (−57%). Administration of the nonionic detergent Triton WR-1339 to estradiol-treated rats rapidly restored the rate of secretion of de novo synthesized bile acids to control levels. In contrast, when Triton was administered to control rats, the secretion rate of bile acids was unaffected. The reduction in bile acid synthesis displayed by estradiol-treated rats was similar to the 50% decrease in the activity of hepatic microsomal 7α-hydroxylase. The activity of 7α-hydroxylase was also restored to control levels by the administration of Triton to estradiol-treated rats. We examined the possibility that estradiol acts directly on the hepatic microsomes. Adding increasing amounts of estradiol to microsomes obtained from control rats resulted in decreasing activities of 7α-hydroxylase. The inhibition by estradiol of 7α-hydroxylase obtained in vitro occurred with amounts of estradiol that were found to accumulate in the liver via in vivo treatment. Double-reciprocal analysis showed that at and below 50 μg of estradiol/0.5 mg of protein uncompetitive inhibition was displayed. Additional experiments showed that adding Triton to microsomes obtained from estradiol-treated rats increased the activity of 7α-hydroxylase to control levels. In contrast, Triton did not increase the activity of 7α-hydroxylase when it was added to control microsomes. These data show for the first time that the estrogenic steroid estradiol acts directly on the microsomes and inhibits both the activity of 7α-hydroxylase and the rate of bile acid synthesis. In addition, Triton also acts on the microsomes and reverses the inhibition of both 7α-hydroxylase and bile acid synthesis. This is the first demonstration of a method to reverse the inhibition of 7α-hydroxylase and bile acid synthesis caused by estrogenic steroids.

The catabolism of cholesterol to bile acids is the predominant pathway through which cholesterol is excreted from the body. The specific regulators of 7α-hydroxylase,[1] the rate-limiting enzyme controlling the conversion of cholesterol to bile acids, are not well established (Myant & Mitropoulis, 1977). In bile fistula rats, infusion of large amounts of taurocholate into the intestine has been reported to inhibit 7α-hydroxylase and bile acid synthesis, suggesting a negative-feedback regulation (Shefer et al., 1969). However, in suspensions of hepatocytes (Botham et al., 1981) and hepatocyte monolayer cultures (Davis et al., 1983a), bile acid synthesis was unaffected by adding bile acids to the culture medium. The availability of cellular cholesterol was found to correlate with rates of bile acid synthesis in cultured hepatocytes (Davis et al., 1983b). Furthermore, while the activity of hepatic microsomal 7α-hydroxylase was not inhibited by infusing taurocholate into bile-diverted rats, it did vary in parallel with the free cholesterol content of microsomes (Davis et al., 1985). These data support the concept that cholesterol availability at least is partially responsible for regulating 7α-hydroxylase and bile acid synthesis.

It is well established that pharmacologic doses of estradiol and its synthetic derivatives increase the incidence of cholesterol gallstone disease (Ingelfinger, 1974). Cholesterol precipitates in bile when its concentration relative to that of bile acids and phospholipids is increased (Admirand & Small, 1968). We have shown that rats treated with pharmacologic doses of estradiol have markedly decreased rates of bile acids synthesis (Davis & Kern, 1976). In addition, estradiol also causes an accumulation of hepatic cholesterol esters (Davis et al., 1978a). Apparently, the positive relationship between hepatic cholesterol availability and bile acid synthesis is interrupted by estradiol. Estradiol has been shown to inhibit the activity of 7α-hydroxylase while it does not affect the activity of HMG CoA reductase (Bonorris et al., 1977).

Many of the alterations in metabolism elicited by estrogens are initiated by derepression of specific genes via estrogen–receptor complex association with genomic DNA (Clark et al., 1985). Estrogens may also act by direct effects on membrane enzymes [e.g., ACAT (Schweppe & Jungemann, 1969) and Na^+,K^+-ATPase (Simon et al., 1980)]. In this study, we examine the mechanism by which pharmacologic doses of estradiol inhibit bile acid synthesis. The results show that estradiol inhibits the activity of 7α-hydroxylase uncompetitively

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[1] Abbreviations: estradiol, 17α-ethinylestradiol; 7α-hydroxylase, cholesterol 7α-monooxygenase (EC 1.14.13.17); Triton, Triton WR-1339; TLC, thin-layer chromatography; ACAT, acylcholesterol acyl-CoA transferase; HMG CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; LDL, low-density lipoproteins; BSP, (bromosulfo)phthalein; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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by acting directly on hepatic microsomal membranes. Moreover, these data show for the first time that estrogen inhibition of 7α-hydroxylase (hence, bile acid synthesis) can be reversed by the nonionic detergent Triton both in vitro and in vivo.

Materials and Methods

Glucose 6-phosphate, NADP, bile acid standards, β-mercaptoethylamine, glucose-6-phosphate dehydrogenase, 3α-steroid dehydrogenase, Triton WR-1339, and buffers were obtained from Sigma Chemical Co., St. Louis, MO. TLC plates were purchased from Analtech, Newark, DE. Solvents were obtained from Baker Chemicals, Phillipsberg, NJ. Sterol standards were obtained from Steraloids, Wilton, NH.

Male Sprague-Dawley rats were purchased from Holtzman, Madison, WS. Rats were fed a standard chow diet ad libitum and drinking water. Rats were exposed to a reverse light cycle (light from 1600 to 0400 h) for at least a week prior to use.

Control rats were treated with the vehicle propylene glycol. Estradiol (dissolved in propylene glycol) was administered at a dose of 5 mg kg^{-1} day^{-1} for 5 days as described in detail (Davis & Kern, 1976). After 5 days of treatment, some rats were treated with either Triton WR-1339 dissolved in 0.9% saline (22.5 mg/100 g ip) or saline only (Davis et al., 1978a). After an additional 18 h, rats were either sacrificed (via exsanguination under ether anesthesia) for 7α-hydroxylase determination or were subjected to surgery.

Measurement of Biliary Bile Acid Secretion. Jugular vein catheters (using PE-50 tubing) and bile duct catheters (using PE-10 tubing) were placed as described in detail (Davis & Kern, 1976). Rats were placed in Bollman restraining cages and infused with a sterile solution containing 5% dextrose in 0.5% saline. Rats had free access to 1% $NaHCO_3$ in drinking water and rat chow throughout the entire experiment. Bile was collected in hourly samples with a fraction collector.

Bile flow was determined by weight. Bile acid concentrations were determined by the 3α-steroid dehydrogenase assay as described in detail (Davis & Kern, 1976). Cholesterol concentrations in bile and rat liver microsomes were determined by GLC as described (Davis et al., 1982).

Determination of 7α-Hydroxylase Activity. The activity of 7α-hydroxylase was determined by the procedures described in detail (Goodwin et al., 1982; Schwartz & Margolis, 1983). At the time of the experiment, appropriately treated rats (see legends for exact details) were rapidly killed by exsanguination (under ether anesthesia), and the liver was removed, placed (1 g of liver/2.5 mL) in cold homogenization buffer consisting of 225 mM sucrose, 50 mM NaF, 5 mM reduced glutathione, and 25 mM Tris (pH 7.8), and immediately homogenized in a Dounce glass apparatus (Kontes, Vineland, NJ). Microsomes were prepared by ultracentrifugation and washed once (Goodman et al., 1982; Schwartz & Margolis, 1983). The assays were performed using freshly prepared microsomes except in the experiments in which we examined the *in vitro* modulation of 7α-hydroxylase by adding estradiol and Triton. The microsomes (0.5 mg of protein) were first preincubated in 100 mM sucrose, 30 mM EDTA, 20 mM DTT, 50 mM KF, 100 mM β-mercaptoethylamine, 100 mM glucose 6-phosphate, and 40 mM potassium monobasic phosphate (final pH 7.2) for 10 min at 37 °C with 2 μg of [4-^{14}C]cholesterol (previously purified by TLC), which was solubilized in a suspension of Tween 80. (In experiments in which we examined the effect of estradiol on the activity of 7α-hydroxylase, estradiol was added as a benzene solution to the test tube along with the [^{14}C]cholesterol.) The benzene was then evaporated and solubilized with Tween. The 7α-hydroxylase reaction was initiated by adding NADP (10 mM) and glucose-6-phosphate dehydrogenase (15.6 units). Blanks were determined for each assay with microsomes that were boiled for 5 min prior to use.

After the time indicated (see legends), the reaction contents were extracted with chloroform/methanol (2:1 v/v); the organic phase was washed with 0.9% saline solution and then evaporated to dryness under nitrogen. The residues were dissolved in chloroform and spotted on TLC plates (silica gel G, 25 mm thick). The TLC plates were developed in benzene/ethyl acetate (2:3 v/v). Phosphomolybdic acid detection of standards of cholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol allowed the products of the assay to be identified (according to migration characteristics). The silica gel containing the desired product was scraped and the radioactivity quantitated by scintillation assay.

Protein content of microsomes was determined by dye binding (Bradford, 1976) with bovine serum albumin as the standard. Glucose 6-phosphatase activities of microsomes were determined as described (Simon et al., 1980).

Determination of Liver Uptake of [6,7-^{3}H]-17α-Ethinylestradiol. [6,7-^{3}H]-17α-Ethinylestradiol was mixed with unlabeled estradiol so that the specific activity was 4.08×10^6 dpm/0.2 mg. Rats were injected with this estradiol as described above. After the appropriate treatment, rats were killed by exsanguination (under ether anesthesia), and the livers were taken, homogenized, and fractionated into microsomes as described above. The amount of estradiol in total liver cytosol (105000*g* supernatant) was then determined by counting the radioactivity. Since the specific radioactivity of the estradiol was known, the amount (mass) of estradiol could be calculated.

Statistical Analysis. All values are reported as the mean ± SD. Differences were determined to be statistically significant by Student's *t* test and two-tailed *p* values. Values of $p < 0.05$ were considered to be significant.

Results and Discussion

Several studies (Shefer et al., 1969; Eriksson, 1957; Myant & Eder, 1961) including our own (Davis & Kern, 1976) have shown that after biliary diversion the excretion rate of bile acid rapidly declines for about 14 h, after which it becomes fairly stable for 12 h. The rapid decline is thought to reflect the depletion of the endogenous bile acid pool, which in the rat largely resides in the intestine (Shefer et al., 1969). Bile acids that are excreted after bile acid pool depletion are de novo synthesized. Thus, after endogenous bile acid pool depletion, rates of biliary bile acid excretion become a measure of bile acid synthesis (Davis & Kern, 1976).

The effect of estradiol on the rate of excretion of bile acids was determined 18 h after bile duct cannulation (Table I). Consistent with previous results (Davis & Kern, 1976), estradiol inhibited bile acid synthesis by 57%. However, estradiol inhibited biliary cholesterol excretion by only 25%. Thus, in estradiol-treated rats the molar ratio of biliary cholesterol to bile acid was increased by 79%.

We have shown that administering Triton to estradiol-treated rats restored the alterations in the maximum capacity to transport BSP and taurocholate (Simon et al., 1980), ACAT activity (Davis et al., 1978a), and liver surface membrane viscosity, Na^+,K^+-ATPase, and bile flow (Davis et al., 1978b). In this study, we examined the possibility that Triton might also restore bile acid synthesis to normal in estradiol-treated rats.

Administration of Triton to estradiol-treated rats increased bile acid synthesis so that the rate became similar to that of control rats (Table I). Furthermore, the molar ratio of biliary

Table I: Effect of Estradiol and Triton on Biliary Bile Acid and Cholesterol Excretion and on the Activity of 7α-Hydroxylase[a]

treatment	bile acid excretion [μmol h^{-1} (100 g)$^{-1}$]	cholesterol excretion [μmol h^{-1} (100 g)$^{-1}$]	molar ratio cholesterol/bile acid	7α-hydroxylase [pmol (mg of protein)$^{-1}$ (20 min)$^{-1}$]
control	1.51 ± 0.25 (N = 3)	0.071 ± 0.004 (N = 3)	0.047 ± 0.008	78 ± 4 (N = 6)
EE	0.65 ± 0.11 (N = 6)[b]	0.055 ± 0.003 (N = 6)[b]	0.084 ± 0.014[b]	39 ± 4 (N = 6)[b]
EE + Triton WR-1339	1.63 ± 0.15 (N = 7)	0.092 ± 0.012 (N = 7)[c]	0.056 ± 0.005[c]	63 ± 6 (N = 6)[c]
control + Triton WR-1339	1.57 ± 0.04 (N = 6)	0.076 ± 0.007 (N = 6)	0.048 ± 0.010	76 ± 3 (N = 6)

[a] Control rats and rats treated with estradiol, Triton, or estradiol + Triton (as described under Materials and Methods) were implanted with jugular and bile duct catheters. After 18 h of bile drainage, bile was collected in hourly samples. The activity of 7α-hydroxylase in hepatic microsomes is expressed as the amount of radiolabeled substrate incorporated into product. Each value represents the mean ± SD for the number of rats in each group. [b] Significant difference from the control group. [c] Significant difference between the estradiol group and the estradiol + Triton group.

cholesterol to bile acids was significantly reduced and also became similar to control values. In contrast, administering Triton to control rats did not affect the excretion rate of either biliary cholesterol or bile acids. Thus, rather than causing a generalized stimulation of bile acid secretion, Triton reversed the inhibition of bile acid synthesis caused by estradiol.

We also examined the effect of estradiol and Triton on the activity of 7α-hydroxylase using an assay developed by others (Goodwin et al., 1982). For all groups in the study, the incorporation of [4-^{14}C]cholesterol into 7α-hydroxycholesterol was linear for 20 min (data not shown). The 7α-hydroxylase activities obtained by us (for untreated rats) were in close agreement with those reported by others (Schwartz & Margolis, 1983).

Consistent with the idea that 7α-hydroxylase is the rate-determining enzyme that regulates bile acid synthesis, variations of the activity of 7α-hydroxylase paralleled those of the rates of bile acid synthesis (Table I). Moreover, compared to control microsomes, those obtained from estradiol-treated rats displayed a 50% reduction in the activity of 7α-hydroxylase (Table I). Administration of Triton to estradiol-treated rats restored the activity of 7α-hydroxylase to levels displayed by control rats. However, administration of Triton to control rats did not affect the activity of 7α-hydroxylase. The altered activity of 7α-hydroxylase was not caused by differences in the free cholesterol content of microsomes, which was similar in all groups examined: (in μg/mg of microsomal protein) 33 ± 7, control; 37 ± 8, estradiol treated; 29 ± 3, Triton; 32 ± 6, estradiol + Triton.

To examine the direct effect of estradiol on hepatic microsomal 7α-hydroxylase, it was necessary to first determine the amount that was associated with the liver in vivo. Following the administration of [6,7-^{3}H]-17α-ethinylestradiol, livers were obtained, and the amount of [^{3}H]estradiol in the cytosol was quantitated as described under Materials and Methods. This value is expressed per 0.5 mg of microsomal protein in order to directly correspond to subsequent in vitro experiments (Figure 2). After 5 days of administration, there was 16 ± 2 μg/0.5 mg of microsomal protein (N = 3). Administration of Triton WR-1339 did not reduce the amount of [^{3}H]estradiol that accumulated in the liver (25 ± 5 μg/0.5 mg of microsomal protein, N = 3). To examine the direct effects of estradiol on microsomal 7α-hydroxylase, it was added to hepatic microsomes at concentrations approximating 0.05–50 times the concentrations found to accumulate in the liver in vivo. Estradiol was added with the [^{14}C]cholesterol substrate to microsomes obtained from control rats (Figure 1). Adding only 10 μg of estradiol to 0.5 mg of microsomal protein caused a 25% decrease in the activity of 7α-hydroxylase. The inhibition was maximized (85%) at 100 μg/0.5 mg of microsomal protein). When microsomes were incubated with estradiol (10 μg/0.5 mg of protein), a time

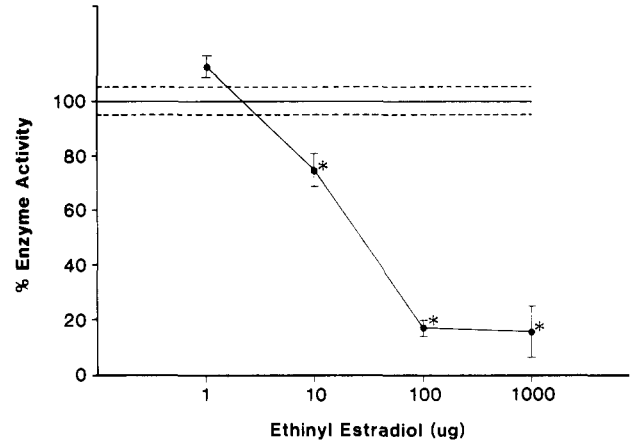

FIGURE 1: Effect of adding estradiol to hepatic microsomes on the activity of 7α-hydroxylase. Hepatic microsomes (0.5 mg of protein in all assays) were obtained from control rats (injected with propylene glycol only). Estradiol was added to microsomes with the [^{14}C]-cholesterol substrate, as described under Materials and Methods. The control values ± SD are shown by the horizontal solid and dashed lines, respectively. Points represent the mean ± SD of three individual determinations. (*) Denotes significant difference from control value (no addition).

course showed that the formation of labeled 7α-hydroxycholesterol was linear with time for 20 min (data not shown). Thus, it is unlikely that estradiol altered the rate of equilibration of labeled cholesterol with endogenous cholesterol. The in vitro inhibition by estradiol of 7α-hydroxylase occurred well within the concentrations of estradiol found to accumulate in the liver following its administration to rats. Since even at a concentration of 1000 μg/0.5 mg of microsomal protein estradiol did not inhibit the activity of glucose 6-phosphatase (data not shown), the in vitro inhibition of 7α-hydroxylase is not likely to be caused by a generalized impairment of microsomal membrane function.

Double-reciprocal analysis of the inhibition by estradiol of 7α-hydroxylase showed that at concentrations of estradiol less than 50 μg/0.5 mg of protein there was an uncompetitive inhibition (with regard to cholesterol substrate, Figure 2). At higher concentrations (i.e., 100 μg/0.5 mg of microsomal protein) of estradiol the inhibition was complex. This experiment was performed a total of 3 times, and similar double-reciprocal plots were obtained. These data show for the first time that estradiol acts directly on the microsomal membrane and inhibits the activity of 7α-hydroxylase uncompetitively.

We also examined the direct effects of Triton on the activity of 7α-hydroxlase. Similar to the results obtained from its in vivo administration, Triton stimulated the activity of 7α-hydroxylase in microsomes from estradiol-treated rats (Figure 3). Greater amounts of Triton increased 7α-hydroxylase

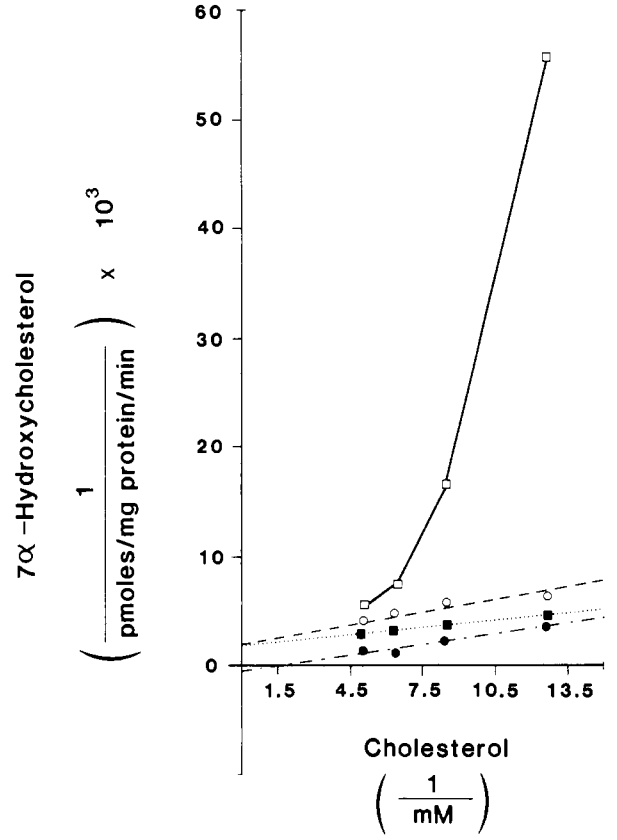

FIGURE 2: Double-reciprocal plots of estradiol inhibition of 7α-hydroxylase. Microsomes obtained from a control rat were incubated with estradiol and the indicated amount of [^{14}C]cholesterol for 5 min prior to adding the cofactors of the 7α-hydroxylase reaction. The content of microsomal cholesterol was determined by GLC. The activity was calculated as the amount of total cholesterol in the assay (microsomal + exogenous substrate) that was converted to 7α-hydroxycholesterol. Each point represents the mean of triplicate assays. Except for the solid line (100 μg of estradiol/0.5 mg of protein), lines represent the best fit linear relationship (least-squares analysis): (closed circles, dot–dashed lines) no estradiol added; (closed squares, dotted line) 10 μg of estradiol/0.5 mg of protein; (open circles, dashed line) 50 μg of estradiol/0.5 mg of protein; (open squares, solid line) 100 μg of estradiol/0.5 mg of protein. The slopes of the best fit lines calculated for 0, 10, and 50 μg of estradiol are not significantly different.

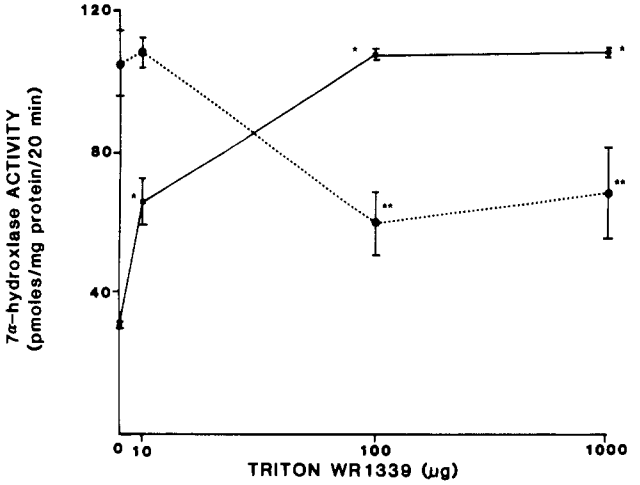

FIGURE 3: Effect of adding Triton to hepatic microsomes. Microsomes (0.5 mg of protein in all assays) were obtained from control rats (dashed lines) and estradiol-treated rats (solid lines). Values represent the mean ± SD of three individual determinations. (**) Denotes a significant difference from the value obtained from microsomes (without added Triton) of control rats, whereas (*) denotes a significant difference from the value obtained from microsomes (without added Triton) of the estradiol-treated rats.

activity until it reached the level expressed by control microsomes. At this point, additional Triton caused no further stimulation. The amount of Triton required to restore the activity of 7α-hydroxylase to control levels (Figure 3) is well within the amount of Triton found to be in the liver following its in vivo administration [i.e., 1.4% of injected dose = 159 μg/0.5 mg of microsomal protein (Henning & Plattner, 1975)]. These data show that Triton restored the activity of 7α-hydroxylase to control levels via a process not involving de novo protein synthesis. In contrast, Triton did not stimulate the activity of 7α-hydroxylase when added to control microsomes. In fact, at a concentration higher than 10 μg/0.5 mg of microsomal protein, Triton actually inhibited the activity of 7α-hydroxylase when added to control microsomes. Thus, when the response of 7α-hydroxylase to Triton between microsomes obtained from control and those obtained from estradiol-treated rats was compared, there was a striking difference.

The combined results of this study show a concordance of in vivo and in vitro observations. Estradiol treatment decreased the rate of bile acid synthesis, and Triton reversed the inhibition. Moreover, the production of similar changes in the activity of 7α-hydroxylase by adding Triton and estradiol to hepatic microsomes (Figures 1–3) suggests that these agents can determine the expression of this enzyme via direct interactions with the microsomal membrane.

It is important to consider that 7α-hydroxylase is an integral microsomal membrane enzyme that utilizes a water-insoluble substrate, which is likely to be membrane-free cholesterol. In addition, estradiol is also water insoluble, and it (like the exogenous [^{14}C]cholesterol substrate) will partition between the aqueous phase and the microsomal membrane. Therefore, derivation of values of K_m, V_{max}, and inhibition constant are complicated by the heterogeneous system intrinsic to an integral membrane enzyme assay. However, it is likely that the behavior of the membrane enzyme assay to the inhibitor is accurately depicted by the changes in K_m and V_{max}. For example, with the same assay as used in our study, the water-insoluble product 7α-hydroxycholesterol exhibited competitive inhibition of 7α-hydroxylase (Schwartz & Margolis, 1983). With some caution due to the intrinsic complications of this microsomal membrane enzyme, it is reasonable to assume that the uncompetitive inhibition by estradiol of 7α-hydroxylase reflects a mechanism not involving competition between estradiol and substrate for the active site.

The data of this study may explain the apparent dissociation of hepatic cholesterol availability and the rate of bile acid synthesis that occurs in response to estradiol treatment. By acting directly on the microsomal membrane, estradiol inhibits the activity of 7α-hydroxylase and bile acid synthesis (Table I and Figures 1 and 2). Although there is an accumulation of cholesterol in livers of estradiol-treated rats (Davis et al., 1979a), bile acid synthesis is depressed because of decreased 7α-hydroxylase activity.

The practical importance of this work is that for the first time we describe a method for reversing the inhibition of bile acid synthesis caused by pharmacologic doses of estradiol. Triton reverses this inhibition of 7α-hydroxylase both in vivo (Table I) and in vitro (Figure 3), and as a result, bile acid synthetic rates are returned to normal (Table I). In contrast, Triton does not stimulate either the activity of 7α-hydroxylase or the rate of bile acid synthesis in untreated animals. Thus, this effect of Triton is distinct from its stimulation of another

microsomal membrane enzyme [i.e., HMG CoA reductase (Goldfarb, 1978)].

Whether estradiol and/or Triton acts on 7α-hydroxylase via changes in microsomal membrane lipid structure in a manner analogous to that found for their effects on Na^+,K^+-ATPase (Davis et al., 1976b) remains to be proven. However, our results showing a direct effect of these agents on microsomal 7α-hydroxylase provide valuable information toward being able to control bile acid synthesis and cholesterol homeostasis.

Registry No. Estradiol, 57-63-6; 7α-hydroxylase, 9037-53-0; Triton, 25301-02-4; cholesterol, 57-88-5.

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Kinetic Mechanism of Ribulosebisphosphate Carboxylase: Evidence for an Ordered, Sequential Reaction

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ABSTRACT: NMR measurements and isotope-trapping experiments indicate that activated ribulosebisphosphate carboxylase binds bicarbonate anion but *not* carbon dioxide in the absence of ribulose bisphosphate. In the presence of suboptimal amounts of carbon dioxide, the enzyme also catalyzes the exchange of H-3 of RuBP with solvent faster than it catalyzes product formation. At saturating concentrations of carbon dioxide, the rate of exchange is dramatically reduced. These results indicate that the carboxylation of ribulose bisphosphate proceeds via the ordered addition and enolization of ribulose bisphosphate followed by reaction with the gaseous substrate.

Ribulosebisphosphate carboxylase/oxygenase (RuBP carboxylase)[1] catalyzes two reactions, the oxygenation and carboxylation of RuBP, which are the initial steps in the competing metabolic pathways of photorespiration and photosynthesis in higher plants. A variety of evidence suggests that the relative flux of carbon through these pathways may well determine the rate of plant growth in a wide variety of agronomically useful plant species. Accordingly, the manipulation of the kinetic properties of RuBP carboxylase is often viewed as a potentially attractive means for increasing the relative rate of photosynthesis relative to that of photorespiration.

Jordan and Ogren have shown that the specificity of RuBP carboxylase for CO_2 and O_2 is species-specific (Jordan &

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[1] Abbreviations: RuBP, ribulose 1,5-bisphosphate; XuBP, xylulose 1,5-bisphosphate; CABP, 2-*C*-(phosphohydroxymethyl)-D-ribonic acid 5-phosphate (carboxyarabinitol bisphosphate); PGA, glyceric acid 3-phosphate; Tris, tris(hydroxymethyl)aminomethane; Bicine, *N,N*-bis(2-hydroxyethyl)glycine.

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