Regulation of bile acid synthesis. IV. Interrelationship between cholesterol and bile acid biosynthesis pathways

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Abstract Under most experimental conditions, the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase) and cholesterol 7α-hydroxylase, change together in parallel directions. It has been suggested that newly synthesized cholesterol may be the preferred substrate for cholesterol 7α-hydroxylase, which may account for the observed synchronous behavior of the two enzymes. To test this hypothesis, mevinolinic acid, a potent competitive inhibitor of HMG-CoA reductase, was administered as a single intravenous bolus (10 mg/kg) to rats with a chronic bile fistula. Bile acid synthesis was determined following inhibition of HMG-CoA reductase by mevinolinic acid over a 27-h time course and specific activities of HMG-CoA reductase and cholesterol 7α-hydroxylase were determined in liver microsomes. At 3, 6, and 27 h after a bolus dose of mevinolinic acid, bile acid synthesis was reduced by 54 ± 5%, 42 ± 8%, and 23 ± 13%, respectively, from preinfusion baseline. Within 30 min after administration of mevinolinic acid, HMG-CoA reductase activity was inhibited by at least 87%. At 0.5, 1.5, 3, 6, and 27 h after mevinolinic acid injection, cholesterol 7α-hydroxylase activity was decreased by 6%, 25%, 54%, 41%, and 17%, respectively. By 27 h, the activities of both enzymes had returned to baseline levels. The reduction of bile acid synthesis correlated closely with the observed changes in the activities of cholesterol 7α-hydroxylase. In vitro addition of mevinolinic acid (up to 20 μM) to rat liver microsomes failed to inhibit cholesterol 7α-hydroxylase activity, suggesting no direct effect of mevinolinic acid on enzyme activity. When a bolus dose of mevinolic acid was coupled with a continuous infusion of mevalonate, the product of the reaction catalyzed by HMG-CoA reducatse, the mevinolinic acid-induced decrease in cholesterol 7α-hydroxylase activity and bile acid synthesis was prevented. The results of this study provide evidence that, under the experimental conditions described, there is a linkage between the rates of cholesterol synthesis and the activities of cholesterol 7α-hydroxylase. The data also emphasize the importance of the newly synthesized cholesterol in the regulation of cholesterol 7α-hydroxylase activity. — **Pandak, W. M., D. M. Heuman, P. B. Hylemon, and Z. R. Vlahcevic.** Regulation of bile acid synthesis. IV. Interrelationship between cholesterol and bile acid biosynthesis pathways. *J. Lipid Res.* 1990. **31:** 79–90.

Supplementary key words bile acid • HMG-CoA reductase • cholesterol 7α-hydroxylase • cholesterol • mevinolinic acid

It has been generally agreed that flux of bile salts circulating in the enterohepatic circulation up- or down-regulates cholesterol 7α-hydroxylase, the rate-limiting enzyme in the bile acid biosynthesis pathway (1, 2). The concept of negative bile acid biofeedback of bile acid synthesis by bile salts originated from the experiments in which complete or partial interruption of the enterohepatic circulation was associated with up-regulation of cholesterol 7α-hydroxylase activity and, consequently, in an increase of bile acid synthesis (3). Bergström and Danielsson (4) demonstrated that duodenal infusion of taurochenodeoxycholic acid in rats with chronic bile fistula restored bile acid synthesis to normal. These original data were later confirmed by others (5–7). More recent studies in human subjects have shown that prolonged administration of cholic and chenodeoxycholic acids resulted in profound inhibition of bile acid synthesis (8, 9). While all these observations were consistent with the concept of negative bile acid biofeedback, the molecular basis for the regulation of cholesterol 7α-hydroxylase activity by bile salts remained uncertain. More recently, several investigators reported that bile salts at physiologically relevant concentrations neither inhibit cholesterol 7α-hydroxylase activity in in vitro assays (10), nor decrease bile acid synthesis rates in freshly suspended or cultured hepatocytes (11–13). Because of these and other inconsistencies, we (14, 15) and others (11, 16), proposed that bile acid synthesis may be regulated by bile acid-induced changes in a critical microsomal regulatory pool of free cholesterol. This hypothesis

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; HPLC, high performance liquid chromatography; ACAT, acyl CoA:cholesterol acyltransferase; CEH, cholesteryl ester transferase.

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was attractive because of the reports that suggested that cholesterol 7α-hydroxylase may not be fully saturated in in vivo; consequently the availability of cholesterol substrate could affect the activity of this enzyme (17), a claim that has not been universally agreed upon (18).

Under most physiologic and experimental conditions, the activity of HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway, changes in the same direction as cholesterol 7α-hydroxylase, the rate-limiting enzyme in the bile acid biosynthesis pathway (19–23). The most plausible explanation for the co-variation between the two enzymes is that increased HMG-CoA reductase activity leads to an increased supply of substrate for cholesterol 7α-hydroxylase (17). The findings of at least one study suggest that changes in HMG-CoA reductase might precede those of cholesterol 7α-hydroxylase activity (24). This attractive hypothesis was recently questioned by Björkhem and his colleagues (18, 25–27), who concluded that microsomal cholesterol concentration, degree of substrate saturation, and HMG-CoA reductase are not major regulators of cholesterol 7α-hydroxylase activity.

The purpose of the present study was to characterize the link between the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase in short-term acute experiments. This was accomplished by studying the effects of a single bolus of mevinolinic acid, a known potent competitive inhibitor of HMG-CoA reductase, on cholesterol 7α-hydroxylase and on the rates of bile acid synthesis in rats with a chronic bile fistula. The results of the study demonstrate that acute inhibition of HMG-CoA reductase with mevinolinic acid was followed by a rapid and profound decrease in both cholesterol 7α-hydroxylase activity and bile acid synthesis. This rapid decrease in cholesterol 7α-hydroxylase activity and bile acid synthesis was prevented by maintaining the supply of newly synthesized cholesterol with a constant infusion of mevalonate, in the face of continued inhibition of HMG-CoA reductase activity with mevinolinic acid. These data provide additional evidence for the close interrelationship between the cholesterol and bile acid biosynthesis pathways, and point out the importance of newly synthesized cholesterol in the regulation of cholesterol 7α-hydroxylase activity.

MATERIALS AND METHODS

Chemicals

Mevinolin (lovastatin) was obtained through a generous gift of Merck Sharp and Dohme Research Laboratories (Rathway, NJ). [4-^{14}C]Cholesterol (59.4 mCi/mmol), DL-3-[glutaryl-3-^{14}C]hydroxy-3-methylglutaryl coenzyme A (57.6 mCi/mmol), [^{14}C]taurocholate (46.7 mCi/mmol), and DL-[^{3}H]mevalonate (30 Ci/mmol) were obtained from New England Nuclear (Boston, MA). DL-Mevalonolactone, dithiothreitol, glucose-6-phosphate and glucose-6-phosphate dehydrogenase, HMG-CoA, NADP^{+}, and NADPH were obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals were of the highest grade available commercially. Silica gel chromatography plates were obtained from Fisher Scientific (Springfield, NJ). Intramedic polyethylene tubing (P50) and Dow-Corning silastic tubing were obtained from American Scientific (Columbia, MD).

Animals

Male Sprague-Dawley rats (Charles River, Cambridge, MA) weighing between 250 and 400 g were housed under controlled lighting conditions (0600–1800 h light phase). Groups of age- and weight-matched animals were used in all experiments. Under brief methoxyflurane anesthesia, biliary fistulas and internal jugular cannulas were placed. For biliary fistulas, the common duct was exposed and cannulated with silastic tubing (Dow-Corning, #602-135; ID 0.020-OD 0.037 inches). For intravenous infusion, a polyethylene infusion cannula (P-50) was placed into the right internal jugular vein. Cannulas were tunneled subcutaneously to the back of the head and brought out via a spring harness sutured to the skin overlying the occiput. This allowed the rats free movement and was well tolerated. After the surgery, the animals were placed in individual metabolic cages with free access to water and laboratory chow (Prolab RMH 3000, Agway Corp.). All rats received intravenous infusion of glucose–electrolyte solution at the fixed rate of 1.07 ml per h via a syringe pump (Harvard Biosciences, Boston, MA). Each liter of glucose–electrolyte solution contained 100 meq sodium chloride, 30 meq sodium acetate, 6 meq potassium chloride, and 50 g of glucose. In addition, each liter of animal drinking water was supplemented with 50 meq sodium chloride, 15 meq sodium bicarbonate, 3 meq potassium chloride, and 50 g of sucrose. This electrolyte replacement was carried out in order to restore water and electrolyte losses in the bile. Diverted bile was allowed to drain freely and was collected in timed increments with a programmed fraction collector. Throughout the experiments, the animals were carefully monitored for appearance and activity. Chow, water consumption, urine, bile, and stool outputs were determined daily. Rats consuming less than 10 g of chow, drinking less than 15 ml of water, producing less than 5 ml of urine per 24 h, or exhibiting inconsistent bile flow were not analyzed in this study.

After a 3-day recovery period, the experimental protocols outlined in **Fig. 1** were followed. In the morning on the fourth postoperative day, a 10-mg/kg IV bolus of mevinolinic acid or a control vehicle, dimethyl sulfoxide (DMSO), was administered. Bile was collected in timed increments for 2 h before and up to 27 h after the bolus injection. In some experiments, constant infusion of mevalonolactone (180 μmol/h) was administered intravenous-

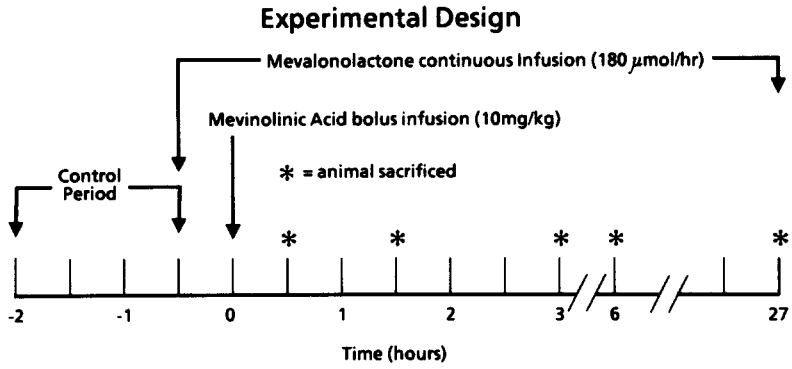

Fig. 1. Experimental protocol for in vivo studies of the effects of mevinolinic acid and mevalonolactone on bile acid synthesis and biliary lipid secretion. Time zero refers to the administration of mevinolinic acid or control vehicle via intravenous bolus injection 72 h after creation of a chronic biliary fistula. Bile was collected continuously for 2 h before and up to 27 h after administration of mevinolinic acid. When given, mevalonolactone was administered by continuous intravenous infusion beginning at −30 min and continued throughout the course of the experiment.

ly together with electrolyte solution beginning 30 min before the mevinolinic acid bolus infusion. A trace dose of [^{14}C]taurocholate (0.04 μCi; sp act 50 μCi/mmol) was administered intravenously 2 h before the mevinolinic acid bolus (internal control period), and again 1 h after mevinolinic acid administration to test for the presence or absence of impaired bile flow. At 0 (control), 0.5, 1.5, 3, 6, or 27 h after administration of mevinolinic acid, four to six rats at each time interval were killed and their liver were obtained and used for the determination of the microsomal activities of 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7α-hydroxylase, as well as for the determination of total microsomal cholesterol.

Test agent preparation and administration

Mevinolin (lovastatin) belongs to the family of substituted hexahydronapthalene lactones. Mevinolinic acid, which was used in these experiments, is the corresponding hydroxy acid (opened lactone) structure of mevinolin but with higher inhibitor activity (**Fig. 2**). Mevinolinic acid was prepared from mevinolin by incubation for 1 h with an equal volume of aqueous sodium hydroxide, as described by Alberts et al. (28). The pH was then neutralized with HCl, and normal saline was added to bring the final concentration to 3 mg/ml. Mevinolin in its lactone form cannot be infused intravenously as it is insoluble in an aqueous medium, whereas mevinolinic acid is water-soluble. The 10-mg/kg bolus infusion of mevinolinic acid was administered through the internal jugular venous cannula followed by normal saline to flush the cannula. Mevalonolactone (100 mg/ml of water) dissolved in the intravenous electrolyte solution was administered intravenously, at a rate of 180 μmol/h (calculated replacement dose accounts for approximate degradation of cholesterol to biliary cholesterol and bile acids, lipoprotein formation and synthesis of hormones). Dimethylsulfoxide, which served as the solvent in the preparation of mevinolinic acid, was incubated in the absence of mevinolin for 1 h with sodium hydroxide and used as the control vehicle solution. [^{14}C]-Taurocholate (2 μCi/ml) in a tracer dose of 0.04 μCi was administered through the internal jugular cannula.

Chemical analysis

Bile was extracted according to the method of Folch, Lees, and Sloane Stanley (29). Phospholipid phosphorus

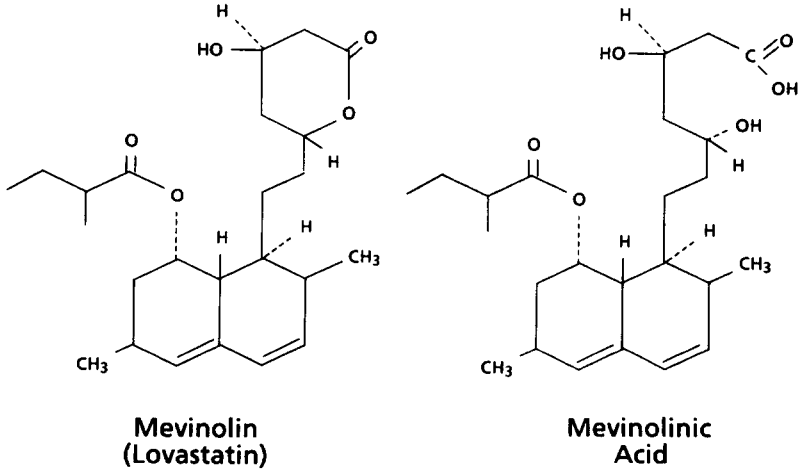

Fig. 2. Structures of mevinolin (lovastatin) (1) and its active metabolite mevinolinic acid (2).

was determined using the method of Bartlett (30), and total cholesterol was determined by a modified cholesterol oxidase method (31). Conjugated bile acids were analyzed by reverse phase HPLC using a modification of the method of Nakayama and Nakagaki (32). In rats with complete biliary fistulas, bile acid synthesis is equivalent to biliary bile acid secretion, and was therefore quantified by HPLC determination of the mass of bile salts secreted into bile. Microsomal proteins were determined by the method of Bradford (33).

Preparation of microsomes

Animals were killed by decapitation. Livers were removed immediately, rinsed in iced normal saline, homogenized in buffer A (sucrose, 100 mM; KCl, 50 mM; EDTA, 30 mM; potassium phosphate (pH 7.4) 100 mM; DTT, 3 mM; 3 mM) at 4°C. Homogenates were then centrifuged for 20 min at 12,000 *g* at 4°C. The resultant supernatant fluid was centrifuged at 105,000 *g* for 90 min at 4°C, and the sedimented membranes were washed by resuspension and recentrifugation. The final microsomal pellet was suspended in buffer A to a protein concentration of approximately 20 mg protein/ml, and frozen in aliquots until analyzed.

Microsomal HMG-CoA reductase activity was assayed by the procedure of Whitehead et al. (34). Two methods were used to determine the activities of cholesterol 7α-hydroxylase. Cholesterol 7α-hydroxylase activity of all microsomes was assayed using exogeous cholesterol as substrate by a radioisotope incorporation method of Shefer, Hauser, and Mosbach (35). In some in vivo and in vitro experiments, cholesterol 7α-hydroxylase activity was also determined by a modified HPLC method originally described by Ogishima and Okuda (36) as modified by Hylemon et al. (37).

Microsomal cholesterol

Microsomal free cholesterol was extracted with acetone–ethanol 1:1, and quantified after digitonide precipitation by the method of Abell et al. (38).

RESULTS

The data in **Fig. 3** demonstrate the effects of administration of a 10-mg/kg intravenous bolus of mevinolinic acid on rates of bile acid synthesis and biliary cholesterol and phospholipid secretion. In the bile fistula model, in which complete interruption of enterohepatic circulation takes place, bile acid synthesis is equal to bile acid secretion. Bile acid synthesis at 3, 6, and 27 h after administration of mevinolinic acid was reduced by 54 ± 5%, 43 ± 13%, and 23 ± 13%, respectively, from preinfusion baseline ($P < 0.01$). Inhibition of bile acid synthesis became apparent 1 h after administration of a bolus dose of mevinolinic acid, was maximal at 3 h, and remained relatively unchanged until 6 h. A slow recovery began after 6 h with the return to almost normal rates of bile acid synthesis by 27 h. At the same time periods, biliary cholesterol and phospholipid secretion were reduced by 39 ± 13%, 27 ± 13%, and 26 ± 13%, and 63 ± 3%, 30 ± 9%, and 8 ± 9% from the preinfusion baseline ($P < 0.01$), respectively. In contrast, bile flow remained essentially unchanged in spite of the marked decrease in bile acid secretion.

The data in **Fig. 4** demonstrate clearance of an IV bolus of [^{14}C]taurocholic acid (0.04 μCi), administered 2 h before and 1 h after mevinolinic acid administration. Peak secretion of ^{14}C radioactivity in bile was observed 60 ± 5 min after each bolus with >90% of the total dose secreted within 2 h. This 60-min delay represents the dead

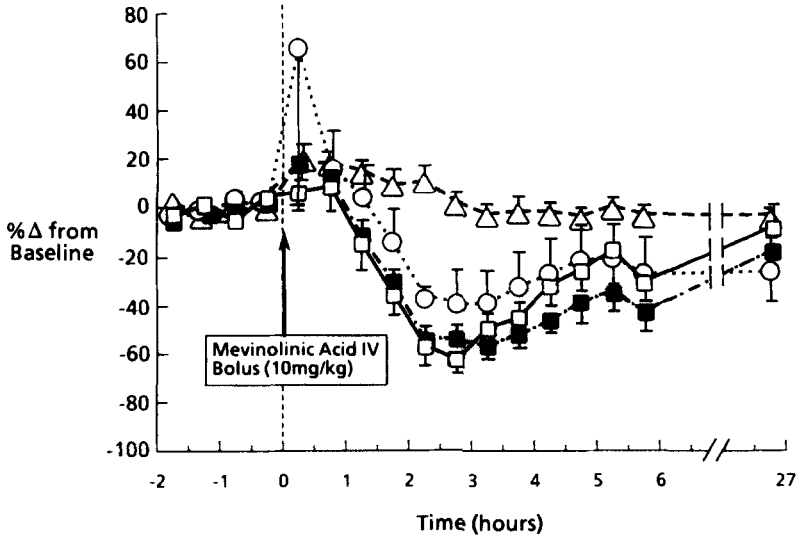

Fig. 3. Effect of mevinolinic acid on bile flow (△---△), bile acid synthesis (secretion) (■-·-■), biliary cholesterol (○· · ·○), and phospholipid (□—□) secretion. In rats with chronic bile fistula, bile acid secretion is equal to bile acid synthesis. The data are expressed as percent change from the mean pre-drug infusion baseline mean. Each point represents the mean ± SE of six determinations. The actual baseline values (mean ± SE) are: bile flow = 0.64 ± 0.02 ml/h; bile acid synthesis = 2.45 ± 0.15 μmol/100 g·h^{-1}; biliary cholesterol secretion = 0.08 ± 0.01 μmol/100 g·h^{-1}; biliary phospholipid secretion = 0.32 ± 0.02 μmol/100 g·h^{-1}.

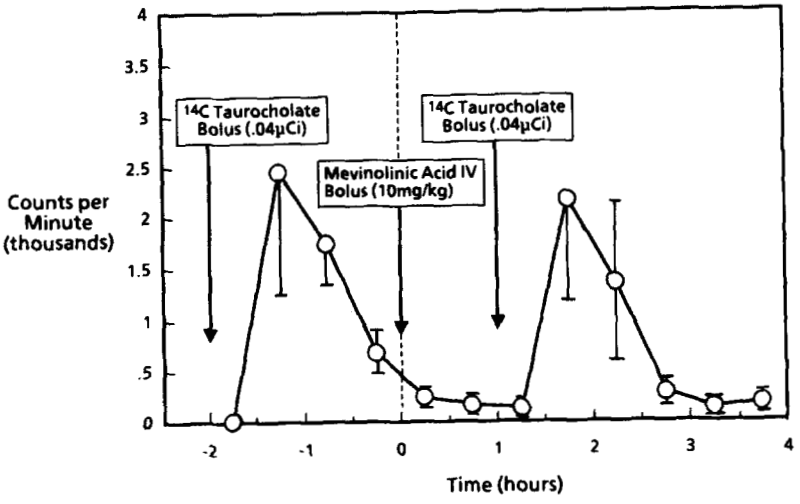

Fig. 4. Effects of mevinolinic acid on biliary excretion of [^{14}C]taurocholate. [^{14}C]Taurocholate was administered twice as a single intravenous bolus i.e., 2 h before and 1 h after mevinolinic acid. In each case, greater than 90% of the total radioactivity administered was secreted into the bile within 2 h. Each point represents the mean ± SE of four individual determinations.

space in the biliary collection system. Prompt secretion of [^{14}C]taurocholate after infusion of mevinolinic acid suggests that administration of mevinolinic acid did not cause cholestasis. Liver function tests (aspartate aminotransferase and alanine aminotransferase) of animals infused with mevinolinic acid and/or mevalonolactone were not elevated over controls (data not shown).

The percent decrease of chenodeoxycholic acid and its biotransformation products, α- and β-muricholic acids (A + B + C), were somewhat greater than that of cholic acid (**Fig. 5**). The reduction of A + B + C, was 46%, 50%, and 37% at 3, 4, and 5 h post infusion as compared to 38%, 45%, and 32% for cholic acid. During the maximal inhibition of bile acid synthesis (3–5 h post infusion of mevinolinic acid), there was a significant increase in the ratio of cholic acid to A + B + C acids. The mean ratio of cholic to A + B + C acids in control animals was 0.64 as compared to 0.81 in mevinolinic acid-treated rats. This represents a 27% increase which is statistically significant ($P < 0.05$).

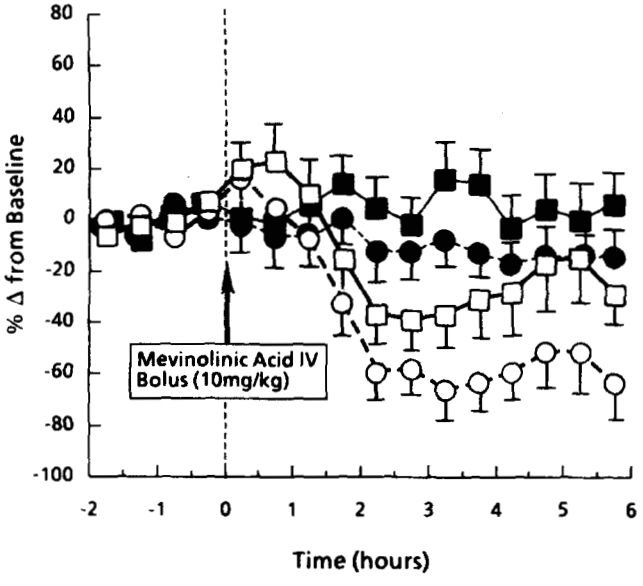

Fig. 5. Effects of mevinolinic acid on synthesis of individual bile acids. Conjugated bile acids were analyzed by reverse phase HPLC; (■—■), cholic acid synthesis in the control setting; (□—□), cholic acid synthesis after mevinolinic acid intravenous bolus; (●---●), chenodeoxycholic acid and its products of metabolism, α- and β-muricholic acid in the control setting; (○---○), chenodeoxycholic acid and its products of metabolism, α- and β-muricholic acid after mevinolinic acid intravenous bolus. Data are expressed as percent change from the pre-drug infusion baseline mean. Each point represents the mean ± SE of six determinations.

HMG-CoA reductase activity at 0.5, 1.5, 3, 6, and 27 h after mevinolinic acid infusion was suppressed by 87%, 74%, 67%, 17%, and 9%, respectively (**Fig. 6**). These are minimal values for the inhibition in HMG-CoA reductase activities since some of the inhibitor (mevinolinic acid) might have been washed out during the preparation of microsomes. The maximal inhibition of HMG-CoA reductase activity (87%) was observed at 30 min after administration of mevinolinic acid. At the same time points, the suppression of cholesterol 7α-hydroxylase activity was 6%, 25%, 54%, 41%, and 17%. These data demonstrate a delay (about 1 h) in down-regulation of cholesterol 7α-hydroxylase activity after profound inhibition of HMG-CoA reductase. The existence of this delay constitutes strong evidence against direct inhibition of cholesterol 7α-hydroxylase by mevinolinic acid.

The data in **Fig. 7** compare the percent decrease in cholesterol 7α-hydroxylase activity with the percent decrease in bile acid synthesis for the duration of the study (27 h). The data on bile acid synthesis rates were corrected for the 60-min lag period, considered as dead space in the indwelling catheter. There was a very close correlation between the measured rates of bile acid synthesis by HPLC and the cholesterol 7α-hydroxylase activities determined by the isotope incorporation method (35). In additional experiments, cholesterol 7α-hydroxylase activity was determined on these same samples using an HPLC

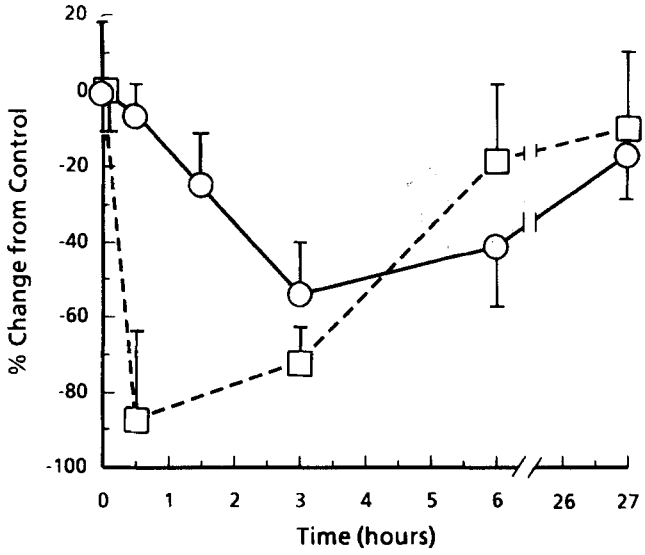

Fig. 6. Effects of mevinolinic acid on the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase. Profound inhibition of HMG-CoA reductase (□---□) occurs at 30 min after administration of mevinolinic acid. By contrast, onset of down-regulation in cholesterol 7α-hydroxylase activity (○—○) was delayed for approximately 1 h. Maximal down-regulation of cholesterol 7α-hydroxylase activity was between 3 and 6 h. Data are expressed as percent change from controls. Each point represents the mean ± SE of 6 determinations.

spectrophotometric method (36, 37). This method, which uses endogenous cholesterol substrate, showed similar inhibition when the results were expressed as percent of the control values (data not shown).

When a bolus dose of mevinolinic acid was coupled with the admistration of continuous intravenous infusion of mevalonolactone (180 μmol/h), the decrease in bile acid synthesis was prevented (**Fig. 8**). The mevalonolactone infusion was initiated 30 min before administration of a single bolus of mevinolinic acid and was continued for 6 h. The difference in bile acid synthesis at 2 to 6 h between the administration of mevinolinic acid alone and mevinolinic acid plus mevalonolactone was highly significant ($P < 0.01$). Similarly, the reduction of biliary cholesterol and phospholipid secretion that followed administration of mevinolinic acid alone was prevented by a concurrent mevalonolactone infusion (data not shown).

The effects of mevinolinic acid, mevinolinic acid plus mevalonolactone, and mevalonolactone alone on cholesterol 7α-hydroxylase activity at 6 h after administration of mevinolinic acid are shown in **Fig. 9**. Mevalonolactone infused alone decreased HMG-CoA reductase activity by 50% (data not shown), but had no effect on cholesterol 7α-hydroxylase activity. Cholesterol 7α-hydroxylase activity was significantly inhibited (43%) at 6 h ($P < 0.02$) after mevinolinic acid administration. This inhibition seen with mevinolinic acid alone was prevented by simultaneous constant infusion of mevalonolactone.

Neither mevinolinic acid alone, mevalonolactone alone, or a combination of both altered microsomal cholesterol content as compared to values in control animals (**Table 1**). Serum cholesterol levels 6 h after mevinolinic acid and/or mevalonolactone infusions were not significantly different from those of controls (data not shown).

In vitro addition of mevinolinic acid at concentrations of 0.5, 1, 5, 10, and 20 μM caused a 49%, 81%, 94%, 95%, and 92% inhibition of HMG-CoA reductase activity, respectively. In contrast, mevinolinic acid in concentrations as high as 20 μM failed to inhibit cholesterol 7α-hydroxylase activity (**Fig. 10**). The concentration of mevinolinic acid at the hepatocytes in in vivo experiments is not known since the distribution of the compound after intravenous administration is uncertain. It is apparent that profound inhibition of HMG-CoA reductase occurred at very low concentrations of mevinolinic acid (0.5 μM, 49% inhibition), whereas no inhibiton of cholesterol 7α-hydroxylase activity occurred at a 40-fold higher concentration (20 μM). This would suggest that mevinolinic acid does not directly affect cholesterol 7α-hydroxylase activity. More importantly, in in vivo experiments, addition of mevalonolactone to mevinolinic acid (Fig. 9) prevented the decrease in the activity of cholesterol 7α-hydroxylase seen with administration of mevinolinic acid alone. This could not have happened if mevinolinic acid inhibited cholesterol 7α-hydroxylase activity directly.

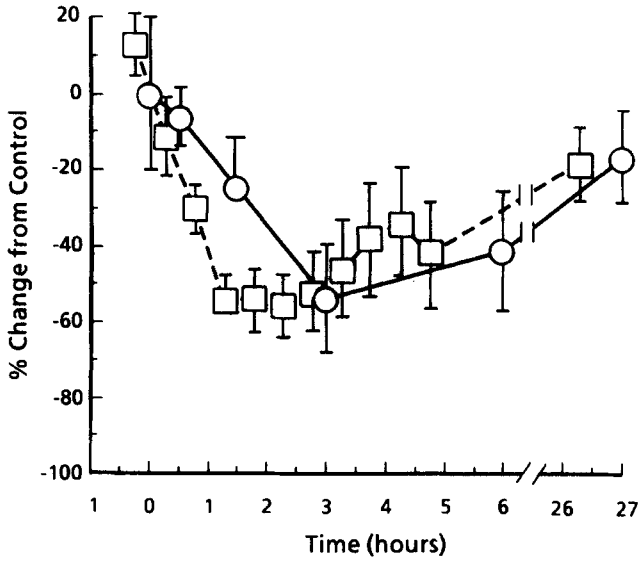

Fig. 7. Effect of mevinolinic acid on cholesterol 7α-hydroxylase and bile acid synthesis. After administration of mevinolinic acid, an excellent correlation between the decrease in bile acid synthesis (□---□) and cholesterol 7α-hydroxylase activity is seen (○—○). Data for cholesterol 7α-hydroxylase activity are expressed as percent change from controls and represent mean ± SE of six determinations at each point. Bile acid synthesis data are expressed as percent change from pre-drug infusion baseline mean. The data on bile acid synthesis were corrected for the 60-min lag period which corresponds to the "dead space" in the indwelling catheter. Each point represents the mean ± SE of six determinations.

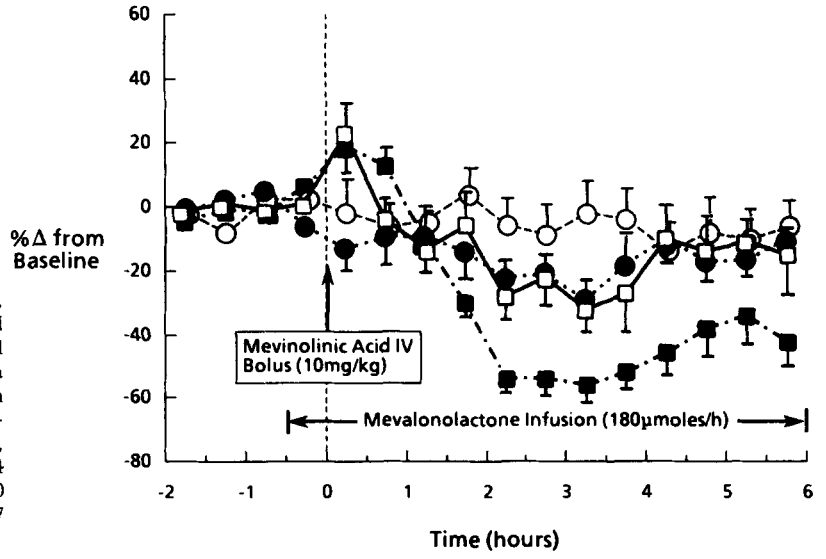

Fig. 8. Effects of a bolus of mevinolinic acid (■· ·■), constant infusion of mevalonolactone alone (●· · ·●), and mevinolinic acid plus mevalonolactone (□—□) on bile acid synthesis (secretion) as compared to controls (○---○). Data are expressed as percent change from pre-drug infusion baseline. Each point shown is the mean ± SE of four determinations. Actual baseline values (mean ± SE) are: control, 1.98 ± 0.11 μmol/100 g·h^{-1}; mevalonolactone, 2.81 ± 0.24 μmol/100 g·h^{-1}; mevinolinic acid, 2.45 ± 0.15 μmol/100 g·h^{-1}; mevinolinic acid plus mevalonolactone, 2.35 ± 0.17 μmol/100 g·h^{-1}

DISCUSSION

The purpose of the present study was to determine, in closely monitored experiments, the interrelationship between the cholesterol and bile acid biosynthesis pathways. Specifically, we were interested in establishing the temporal relationship and the magnitude of changes in the activity of cholesterol 7α-hydroxylase after the administration of a bolus dose of mevinolinic acid, a known competitive inhibitor of HMG-CoA reductase. Profound inhibition of HMG-CoA reductase with mevinolinic acid was followed by a >50% decrease in cholesterol 7α-hydroxylase activity and bile acid synthesis. A decrease in cholesterol 7α-hydroxylase activity became apparent after 90 min, with maximal down-regulation occurring at 3 h. Bile acid synthesis similarly declined in response to mevinolinic acid administration. When cholesterol 7α-hydroxylase activity and bile acid synthesis rates were expressed as percent change from baseline, changes in the two measurements as a function of time were essentially identical. Thus, the observed changes in bile acid synthesis are entirely explained by the decreases in cholesterol 7α-hydroxylase activity. Reduction of bile acid synthesis after administration of mevinolinic acid was coupled with similar declines in biliary cholesterol and phospholipid secretion. Similar interrelationships between bile acid, biliary cholesterol, and phospholipid secretion have been previously reported by Kempen et al. (39) after administration of compactin, another known inhibitor of HMG-CoA reductase.

Alteration of cholesterol 7α-hydroxylase activity after administration of mevinolinic acid does not appear to result as a consequence of direct inhibition of the enzyme with mevinolinic acid, or as a result of nonspecific toxici-

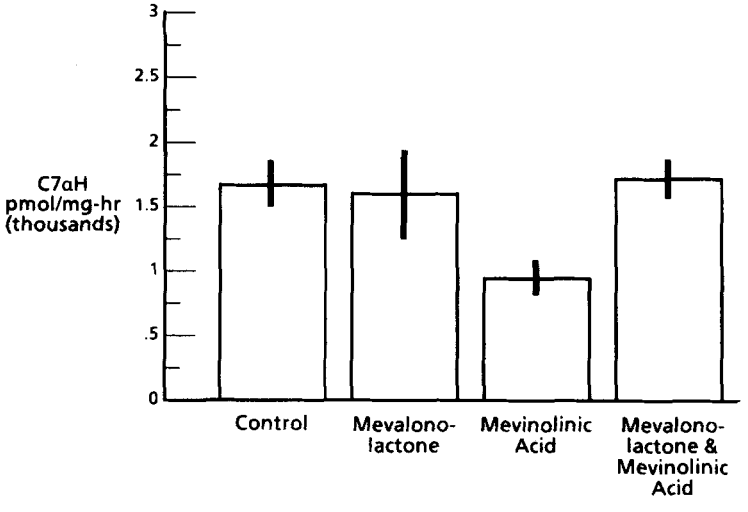

Fig. 9. Cholesterol 7α-hydroxylase activity after administration of mevinolinic acid alone (10 mg/kg intravenous bolus), mevalonolactone (180 μmol/h continuous intravenous infusion), and mevalonolactone (180 μmol/h continuous infusion) plus mevinolinic acid (10 mg/kg intravenous bolus) at 6 h after initiation of experiment. Each point is the mean ± SE of four determinations.

TABLE 1. Effect of mevinolinic acid (intravenous bolus) and/or mevalonolactone (constant infusion) on microsomal cholesterol

Condition	Time after Administration of Bolus (Mevinolinic Acid) or Control Vehicle	Microsomal Cholesterol	
		n	μmol/g of Microsomal Protein[a]
	h		
Control	0	6	47.8 ± 8.24
Mevinolinic acid	0.5	6	45.6 ± 4.01
Mevinolinic acid	3	6	42.0 ± 17.60
Mevinolinic acid	6	6	53.2 ± 12.20
Mevinolinic acid	27	6	42.8 ± 13.60
Mevalonolactone	6	4	59.6 ± 12.90
Mevinolinic acid	6	6	54.4 ± 10.70
Both	6	5	58.7 ± 7.54

[a]Results given as mean ± SD.

ty. Direct inhibition of cholesterol 7α-hydroxylase by mevinolinic acid is unlikely for the following reasons: *a*) cholesterol 7α-hydroxylase remained unaffected in the first hour after injection of mevinolinic acid, at the time when maximal inhibition of HMG-CoA reductase had occurred; *b*) addition of high concentrations of mevinolinic acid to rat liver microsomes markedly inhibited HMG-CoA reductase activity without affecting cholesterol 7α-hydroxylase activity; *c*) constant infusion of mevalonate, an intermediate in the cholesterol biosynthetic pathway, prevented the reduction of cholesterol 7α-hydroxylase activity and bile acid synthesis in the face of inhibition of HMG-CoA reductase activity with mevinolinic acid; and *d*) there was no evidence of nonspecific toxicity of mevinolinic acid as the bile flow, liver function tests, and clearance of [^{14}C]taurocholate all remained normal for the duration of experiments. Therefore, we believe that the decrease in cholesterol 7α-hydroxylase activity and bile acid biosynthesis after mevinolinic acid administration occurred as a result of alterations in the rates of cholesterol synthesis.

The molecular basis responsible for the down-regulation of cholesterol 7α-hydroxylase after mevinolinic acid administration remains uncertain. The regulation of cholesterol 7α-hydroxylase activity could involve changes in the differential rates of synthesis or degradation of a specific cytochrome P-450 catalyzing the 7α-hydroxylation of cholesterol, as a consequence of alteration of the size of cholesterol substrate pool available to the enzyme, or as a result of covalent modification of the enzyme. The rapidity of decline of cholesterol 7α-hydroxylase activity after inhibition of HMG-CoA reductase suggests, but does not prove, that reduction of specific activity did not result from changes in enzyme synthesis or breakdown. This possibility will remain viable until it becomes possible to measure the amount of specific cytochrome P-450 by means other than determining the specific activity.

The most likely explanation of our results is that the decrease in cholesterol 7α-hydroxylase activity took place as a result of depletion of newly synthesized cholesterol substrate which was caused by inhibition of HMG-CoA reductase with mevinolinic acid. It has been suggested that a critical microsomal pool enriched in newly synthesized free cholesterol may regulate the activity of cholesterol 7α-hydroxylase (40, 41). Such an explanation makes sense

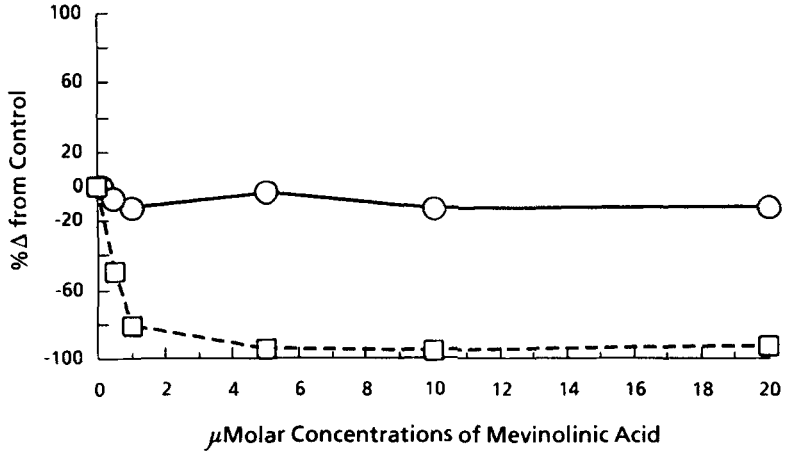

Fig. 10. In vitro data on the effects of mevinolinic acid on the activities of HMG-CoA reductase (□---□) and cholesterol 7α-hydroxylase (○—○). Data are expressed as percent change from control. Each point is the mean of two determinations.

in light of the observation that newly synthesized cholesterol may be the preferred substrate for cholesterol 7α-hydroxylase, at least in the rat (40, 41). The assumption underlying this possibility is that cholesterol 7α-hydroxylase is not fully saturated in vivo. This indeed may have been the case in our experimental model, in which the enterohepatic circulation was completely interrupted and cholesterol synthesis was markedly inhibited. The importance of newly synthesized cholestrol in the regulation of cholesterol 7α-hydroxylase is emphasized further by the experiments in which continuous administration of mevalonolactone was coupled with a single bolus dose of mevinolinic acid. As a result of constant infusion of mevalonolactone, a constant flow of newly synthesized cholesterol was established, which in turn was associated with the prevention of down-regulation of cholesterol 7α-hydroxylase activity observed with the administration of mevinolinic acid alone. Lack of measurable changes in the content of microsomal free cholesterol does not necessarily exclude this possibility, since we and others have shown no correlation between the microsomal free cholesterol pool and cholesterol 7α-hydroxylase activity (15, 18). It should be pointed out that, in the present study, the fractional changes in cholesterol 7α-hydroxylase were comparable regardless of whether exogenously added cholesterol or endogenous microsomal cholesterol was used as substrate in the cholesterol 7α-hydroxylase assay system. Consequently, reduction of cholesterol substrate cannot fully explain the observed decrease in cholesterol 7α-hydroxylase activity. An alternative explanation is that mevalonate or a metabolic product of mevalonate, rather than newly synthesized cholesterol, plays a role in the regulation of cholesterol 7α-hydroxylase. This hypothesis has merit, particularly in light of the observations of Brown and Goldstein (42) who provided convincing evidence that mevalonate or a metabolic product of mevalonate down-regulates HMG-CoA reductase. Conceivably, such an intermediate in the cholesterol biosynthesis pathway could have a role in regulation of cholesterol 7α-hydroxylase as well (**Fig. 11**).

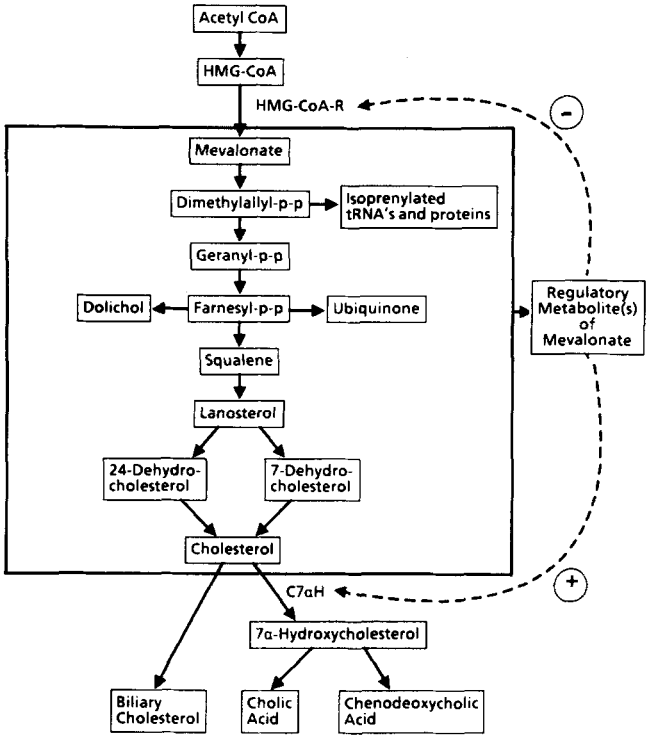

Fig. 11. The pathways of cholesterol and bile acid synthesis denoting major intermediates in both pathways. HMG-CoA reductase and cholesterol 7α-hydroxylase are two rate-limiting enzymes. Putative intermediates of the cholesterol synthesis pathway are known to down-regulate HMG-CoA reductase. It is postulated that the same (or different) intermediates of cholesterol synthesis pathway may up-regulate cholesterol 7α-hydroxylase activity.

The rapidity of down-regulation of cholesterol 7α-hydroxylase after inhibition of cholesterol synthesis could indicate that a decrease in the enzyme activity might have occurred as a result of covalent alteration of the enzyme. There are several studies suggesting that cholesterol 7α-hydroxylase activity may be activated by phosphorylation and deactivated by dephosporylation (43–45). Moreover, phosphorylation has been reported to be an important mechanism for regulating the catalytic activities of other enzymes involved in hepatic cholesterol metabolism including HMG-CoA reductase (46–49), acyl-CoA:cholesterol acyltransferase (ACAT) (50–52), and possibly cholesteryl ester hydrolase (CEH) (53, and S. Ghosh, personal communication). Scallen and Sanghvi (54) postulated that phosphorylation–dephosphorylation may explain the coordinate activities of HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT. In this regard, we have observed rapid and synchronous changes in ACAT and CEH activities in mevinolinic acid-treated rats (55). If the above interpretation of the data is correct, this would suggest that the rate of cholesterol synthesis may be metabolically linked to changes in the activities of specific protein kinases or phosphatases which recognize the above enzymes as substrates. This possibility is now being tested in our laboratories.

Our conclusions regarding the linkage between HMG-CoA reductase and cholesterol 7α-hydroxylase differ somewhat from those of Björkhem (56). Björkhem reported that feeding mevinolin in the diet for 3 days at concentrations as high as 0.2% led to a modest decrease in cholesterol 7α-hydroxylase activity (35%) while inducing a substantial increase in microsomal HMG-CoA reductase activity. The observed decrease in cholesterol 7α-hydroxylase was attributed to the direct inhibitory effect of mevinolin on the enzyme. The nature of interaction between mevinolin and the cholesterol 7α-hydroxylase was not defined in their studies. Based on these data, Björkhem concluded that no coupling exists between the induction of synthesis of HMG-CoA reductase with mevi-

nolin and the activity of cholesterol 7α-hydroxylase (56). It should be pointed out that his experiments were chronic in nature and were associated with compensatory changes in hepatic cholesterol metabolism that could affect cholesterol 7α-hydroxylase activity in many unpredictable ways. The current report specifically avoids this problem by focusing on events in the initial 6 h after the administration of the inhibitor of HMG-CoA reductase before compensatory changes such as an increase in HMG-CoA reductase synthesis could occur.

The possibility of a metabolic linkage between the pathways of cholesterol and bile acid biosynthesis is physiologically appealing. It has long been appreciated that HMG-CoA reductase and cholesterol 7α-hydroxylase activity vary in parallel under a variety of conditions, particularly with regard to changes in the enterohepatic circulation of bile salts (biliary drainage, bile acid feeding). We have recently shown a close positive linear correlation between HMG-CoA reductase and cholesterol 7α-hydroxylase in the rat after 14 days feeding of seven different bile acids (57). Only hydrophobic bile salts (cholic, chenodeoxycholic, and deoxycholic) inhibited cholesterol 7α-hydroxylase, but these bile salts also inhibited HMG-CoA reductase. Based on the data presented in this communication, it is possible that a decrease in cholesterol 7α-hydroxylase activity by hydrophobic bile salts can occur secondary to changes in HMG-CoA reductase activity. A decrease in HMG-CoA reductase activity by bile salts could result from the direct effects of bile acids on enzyme synthesis or degradation, or due to the indirect effect of bile acids on the enzyme via alteration of the rate of cholesterol absorption from the intestines and/or cholesterol flux across the hepatocyte. Recent experiments in our laboratories are consistent with the latter explanation (58).

The results of the current study demonstrate that the regulation of cholesterol 7α-hydroxylase activity and hence bile acid synthesis can occur in response to metabolic signals other than bile acid flux across the liver, a basic postulate of the "negative bile acid biofeedback" hypothesis. Although this study does not identify the underlying mechanism or mechanisms responsible for the regulation of cholesterol 7α-hydroxylase, it clearly shows that the availability of newly synthesized cholesterol plays an important role in the regulation of cholesterol 7α-hydroxylase activity and bile acid biosynthesis. Further studies aimed at identifying the mechanisms involved in the regulation of both HMG-CoA reductase and cholesterol 7α-hydroxylase are currently under way in our laboratory.

This study was supported by the Veterans Administration and NIH Grant 1-PO1-DK38030. Dr. Pandak was supported by the Veterans Administration Career Development Award and by the American Liver Foundation.

Manuscript received 9 May 1989 and in revised form 31 August 1989.

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