Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7α-hydroxylase in the rat

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Abstract Specific activities of the hepatic microsomal enzymes 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase and cholesterol 7α-hydroxylase were studied in rats fed sterols and bile acids. The administration of bile acids (taurocholate, taurodeoxycholate, taurochenodeoxycholate) at a level of 1% of the diet for 1 wk reduced the activity of HMG CoA reductase. Taurocholate and taurodeoxycholate, but not taurochenodeoxycholate, inhibited cholesterol 7α-hydroxylase. Dietary sitosterol produced increases in the specific activity of HMG CoA reductase (3.6-fold) and cholesterol 7α-hydroxylase (1.4-fold), and biliary cholesterol concentrations in this group more than doubled. Compared with controls fed the stock diet, the simultaneous administration of sitosterol and taurochenodeoxycholate resulted in a 60% decrease of HMG CoA reductase activity and no change in cholesterol 7α-hydroxylase activity or biliary cholesterol concentration. Rats fed sitosterol plus taurocholate had nearly normal HMG CoA reductase activity, but cholesterol 7α-hydroxylase was inhibited and biliary cholesterol remained high. Bile acid secretion rates and biliary bile acid composition were similar in controls and sterol-fed animals. In all groups receiving bile acids, biliary secretion of bile acids was nearly doubled and bile acid composition was shifted in the direction of the administered bile acid. It is concluded that the composition of the bile acid pool influences the hepatic concentrations of the rate-controlling enzymes of bile acid synthesis.

Supplementary key words microsomal enzymes · rate-limiting enzymes · taurocholate · taurodeoxycholate · taurochenodeoxycholate · sitosterol · bile acid biosynthesis · cholesterol

The rate of hepatic bile acid synthesis is influenced by bile acids undergoing enterohepatic cycling (1, 2). The details of this negative feedback mechanism are not known at the molecular level, but it seems likely that the magnitude, circulation rate, and composition of the bile acid pool play a role in the regulatory process. Changes in these three characteristics of the pool apparently affect the concentrations of one or more rate-limiting enzymes of the biosynthetic pathway leading from acetyl CoA to the conjugated cholanoic acids. The rate-controlling enzyme in the formation of bile acids from cholesterol is cholesterol 7α-hydroxylase, which catalyzes the first committed step of bile acid synthesis, namely the conversion of cholesterol to 7α-hydroxycholesterol (2–4). It has recently been shown that the enzymes catalyzing the further transformations of 7α-hydroxycholesterol into bile acids are not subject to feedback control by the circulating bile acid pool (5, 6).

Since cholesterol is the obligatory precursor of the bile acids, changes in the rate of hepatic cholesterol synthesis might well affect bile acid production. Consequently, the enzyme HMG CoA reductase (EC 1.1.1.34), which is rate limiting for cholesterol biosynthesis (7), can act as a second regulatory mechanism of de novo bile acid synthesis. Participation of HMG CoA reductase in the regulatory process may also be inferred from the finding that the activity of this enzyme was reduced by exogenous cholate in rats with lymph fistulas. In such preparations absorbed cholesterol could not have reached the liver to exert a regulatory effect (8).

The present paper describes the effect of excess dietary sterols and bile acids, administered singly or in combination, on the specific activities of HMG CoA reductase and cholesterol 7α-hydroxylase in rat liver. The experiments were designed to test the hypothesis that changes in the composition of the bile acid pool produce changes in the concentrations of the two rate-limiting enzymes. It was found that the three bile acids tested, taurocholate, taurodeoxycholate, and taurochenodeoxycholate, when fed at a level of 1% of the diet for 1 wk caused a twofold increase

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Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HMG, hydroxymethylglutaryl.

of bile acid secretion and shifted the composition of the pool so that the administered bile acid predominated. Each of the three bile acids affected the two rate-determining enzymes in a different manner: taurocholate reduced the specific activities of both enzymes by about 60%, taurodeoxycholate inhibited both enzymes approximately 30%, and taurochenodeoxycholate produced a pronounced inhibition of HMG CoA reductase without affecting the activity of the 7α-hydroxylase. Cholesterol or sitosterol incorporated into the diet at a level of 2% fed for 1 wk produced no detectable change in the composition of the bile acid pool or in the secretion rate of biliary bile acids. The administration of these sterols was associated with characteristic changes in the specific activities of the rate-limiting enzymes that could be modified further by the simultaneous administration of bile acids.

EXPERIMENTAL PROCEDURES

Materials

Cholesterol, U.S.P., was recrystallized from ethanol and was used without additional purification. The sitosterol was kindly provided by Eli Lilly and Co., Indianapolis, Ind., and contained 92% sitosterol, 7% campesterol, and less than 1% stigmasterol. Taurine-conjugated bile acids were purchased from Calbiochem, Los Angeles, Calif., and from Maybridge, Tintagel, Cornwall, England, or were synthesized by the method of Norman (9) as modified by Hofmann (10). The conjugates were examined by semiquantitative TLC (11) and only those preparations containing less than 1–2% of free bile acids were used.

Animals and diets

Male Sprague-Dawley-derived rats weighing 250–300 g were purchased from the Charles River Breeding Laboratories, Wilmington, Mass. The animals were kept in individual cages and were fed a stock diet consisting of ground Purina rat chow pellets supplemented with 5% corn oil. This diet contained 0.014% cholesterol and 0.034% plant sterols. When required, sterols were added to this diet at a level of 2% and bile acids at a level of 1%. The sterols were incorporated into the diet from an ether solution, the bile acids from solution in aqueous ethanol. The rats received the diets and drinking water ad lib. for a period of 7 days. Food intake was measured daily, and the animals were weighed at the start and at the end of the feeding period. If food intake or weight gain of an experimental animal differed by more than 10% from the average of the control group, it was excluded from the study. The animals receiving 1% dietary bile acid did not develop diarrhea and gained weight at the same rate as the controls. At the end of the experimental period the animals were anesthetized with Diabutal (Diamond Laboratories, Inc., Des Moines, Iowa), cannulas were inserted into the bile duct, and bile was collected for a period of 30–60 min. When required, ether was used to maintain a light anesthesia. The rats were then killed by exsanguination, and their livers were excised and weighed. A section of liver weighing 1 g was removed for cholesterol determination and a suitable amount of the remaining liver tissue was used immediately for the preparation of the microsomal fraction. In order to eliminate as far as possible variations due to the diurnal rhythm of enzyme activity (12, 13), all animals were killed between 9 and 11 a.m., i.e., during the diurnal minimum. It was found that the relatively short period of biliary diversion had no effect on enzyme activity.

Liver cholesterol determination

A 1-g piece of liver was refluxed for 3 hr in 20% ethanolic KOH. After cooling to room temperature, an equal volume of water was added, and the sterols were extracted with *n*-hexane and analyzed by GLC exactly as described previously[1] using 5α-cholestane as internal standard.

Determination of biliary cholesterol and bile acids

Separate aliquots of bile were used for these measurements. For cholesterol determination, a 0.1- or 0.2-ml aliquot was mixed with 5 vol of 10% KOH in 95% ethanol. The solution was heated for 1 hr at 60°C, an equal volume of water was added, and the tube was allowed to cool to room temperature. The solution was extracted twice with an equal volume of *n*-hexane. The hexane layers were combined and washed three times with 1 ml of water. The hexane was evaporated and the residue was dissolved in a known volume of chloroform with 5α-cholestane as an internal standard. The cholesterol content of this solution was then analyzed by GLC.[1]

A second aliquot of the bile (0.1–0.2 ml) was deproteinized by heating with 10 vol of methanol. The precipitate was removed by centrifugation, the methanol was evaporated, and the residue was treated with 2 ml of 10% aqueous NaOH and heated in an autoclave for 3 hr at 16 lbs pressure. The hydrolyzate was cooled in ice, acidified with HCl, and extracted with ether. The ether was evaporated and the free bile acids were esterified with 2% methanolic H_2SO_4 by standing at room temperature overnight. The methyl esters of the bile acids were extracted with an ether–benzene mixture 1:2 (v/v) and dissolved in a known volume of $CHCl_3$. An aliquot containing 0.1–0.2 mg of total trihydroxy bile acids (as previously determined by GLC) was applied on a silica gel G plate (Brinkmann Instruments, Westbury, N.Y.), layer thickness 0.25 mm. The plate was developed with acetone–benzene 40:60 (v/v) (1), and the spots were made visible

[1] Nicolau, G., S. Shefer, G. Salen, and E. H. Mosbach. Unpublished data.

with 2′,7′-dichlorofluorescein. This TLC system separates methyl cholate, α-muricholate, and β-muricholate (which are not resolved by GLC on QF-1 columns), but does not separate methyl deoxycholate and methyl chenodeoxycholate (which can be resolved and determined by GLC). The spots were removed from the plate and eluted from the silica gel with methanol. Aliquots of the methanol extracts were analyzed by GLC using a 180 cm × 4 mm glass column, packed with 3% QF-1 on 80–100 mesh Gas-Chrom Q, at a temperature of 260–265°C. Known samples of sodium taurocholate and sodium taurochenodeoxycholate were carried through the entire procedure to serve as recovery standards.

Enzyme assays

The specific activities of hepatic microsomal HMG CoA reductase and cholesterol 7α-hydroxylase were determined exactly as described previously (4, 14). Since the assay conditions were such that the amount of enzyme was rate limiting, it was assumed that the specific activity of the enzyme is a measure of its concentration in the liver (15).

RESULTS

The data summarized in Table 1 deal with the effects of different bile acids on the specific activities (in pmoles of product formed per mg protein per min) of hepatic microsomal HMG CoA reductase and cholesterol 7α-hydroxylase and on liver cholesterol concentrations.

The rats were fed a stock diet or stock diet supplemented with 1% sodium taurochenodeoxycholate, sodium taurodeoxycholate, or sodium taurocholate for 1 wk. Since the animals, on the average, consumed 20–25 g of food/day, they ingested 200–250 mg of bile acid daily. At the end of the 1-wk experimental period, bile was collected for 30–60 min, the animals were killed, and the enzyme assays were carried out. Administration of the taurine-conjugated bile acids reduced the specific activity of HMG CoA reductase in all three groups, in comparison with the control group on stock diet: taurochenodeoxycholate and taurocholate produced a 60% reduction; taurodeoxycholate produced a more moderate 30% decrease. The different bile acids differed in their effect upon the specific activity of cholesterol 7α-hydroxylase: taurochenodeoxycholate did not lower the activity of this enzyme significantly, taurodeoxycholate produced a 20% decrease, and taurocholate a 70% reduction. Liver total cholesterol concentrations appeared to vary inversely as the activity of cholesterol 7α-hydroxylase: in comparison with the control group, there was no significant increase of liver cholesterol levels as a result of taurochenodeoxycholate feeding, a moderate, 50% increase in the group fed taurodeoxycholate, and a 150% increase with taurocholate.

TABLE 1. Effect of dietary bile acids on the activities of hepatic HMG CoA reductase and cholesterol 7α-hydroxylase

Diet (1 wk)	No. of Rats	Enzyme Activity[a]		Liver Cholesterol Concentration
		HMG CoA Reductase	Cholesterol 7α-Hydroxylase	
		pmoles/mg protein/min		*mg/g wet weight*
Stock[b]	6	93.9 (3.84)[c]	9.11 (0.33)	2.45 (0.081)
Stock + 1% sodium taurochenodeoxycholate	6	35.1[d] (1.95)	8.49 (0.26)	2.57 (0.092)
Stock + 1% sodium taurodeoxycholate	4	63.8[d] (4.59)	7.22[d] (0.34)	3.78[d] (0.48)
Stock + 1% sodium taurocholate	6	36.6[d] (2.16)	3.08[d] (0.18)	6.09[d] (0.19)

[a] Enzyme activities are reported throughout as pmoles of product formed (mevalonolactone or 7α-hydroxycholesterol) per mg microsomal protein per min.
[b] Ground Purina rat chow plus 5% corn oil.
[c] Standard error of the mean in parentheses.
[d] Differs significantly from control group fed the stock diet ($P < 0.01$).

Table 2 illustrates biliary cholesterol concentrations and rates of bile acid secretion in the groups of animals described in Table 1. The administration of excess dietary bile acids for 1 wk did not affect biliary cholesterol concentrations, which averaged about 0.2 mg/ml in all four groups. The ingestion of bile acids approximately doubled the secretion of total biliary bile acids, from 7.29 mg/100 g rat/hr in the control group to about 13 or 14 mg/100 g rat/hr in the experimental groups. In every group fed bile acids, the composition of the bile shifted strongly in the direction of the administered bile acid. It is of interest that in the bile of the animals fed taurochenodeoxycholate 80% of the bile acids secreted by the liver consisted of the administered bile acid, yet the production of α- and β-muricholic acids was not increased. Such an increase might have been expected in view of the increased amount of chenodeoxycholic acid reaching the liver and presumably becoming available for 6β-hydroxylation. The secretion of taurocholate in this group was reduced by about 70%, from 4.90 to 1.34 mg/100 g rat/hr. The animals fed taurodeoxycholate were apparently unable to rehydroxylate more than about a third of the absorbed bile acid to taurocholate, so that the rate of taurocholate secretion in this group, 5.11 mg/hr, did not differ from that of the control group. The rate of taurochenodeoxycholate secretion fell from 1.14 mg/100 g rat/hr in the control group to 0.011 mg/100 g rat/hr, representing a decrease of 99%. The biliary output of the tauromuricholates likewise fell, from 1.04 to 0.256 mg/100 g rat/hr, amounting to a 75% reduction. The administration of taurocholate produced a 90% decrease in the secretion of taurochenodeoxycholate, from 1.14 to 0.100 mg/100 g rat/hr, and a similar 90% fall in the tauromuricholates, from 1.04 to 0.103 mg/100 g rat/hr.

TABLE 2. Cholesterol and bile acid secretion of rats fed taurine-conjugated bile acids[a]

Diet (1 wk)	No. of Rats	Biliary Cholesterol Concentrations	Biliary Secretion of Taurine-conjugated Bile Acids				
			Total Bile Acids	Deoxycholic	Chenodeoxycholic	α- + β-Muricholic	Cholic
		mg/ml	*mg/100 g rat/hr*				
Stock[b]	6	0.21 (0.009)[c]	7.29 (0.565)	0.213 (0.010)	1.14 (0.094)	1.04 (0.104)	4.90 (0.252)
Stock + 1% sodium taurochenodeoxycholate	6	0.19 (0.095)	12.48[d] (0.497)	0.107[d] (0.009)	9.87[d] (0.356)	1.16 (0.062)	1.34[d] (0.141)
Stock + 1% sodium taurodeoxycholate	4	0.22 (0.021)	14.41[d] (0.559)	9.04[d] (0.799)	0.011[d] (0.0015)	0.256[d] (0.023)	5.11 (0.452)
Stock + 1% sodium taurocholate	6	0.22 (0.011)	13.22[d] (0.644)	0.671[d] (0.079)	0.100[d] (0.006)	0.103[d] (0.008)	12.35[d] (0.483)

[a] Based upon bile collected for 30–60 min at end of 7-day feeding period.
[b] Ground Purina rat chow plus 5% corn oil.
[c] Standard error of the mean in parentheses.
[d] Differs significantly from control group on stock diet ($P < 0.01$).

The effects of 2% dietary cholesterol and sitosterol, fed singly or in combination with sodium taurochenodeoxycholate or taurocholate, on the activities of the two enzymes and on liver total cholesterol concentrations are summarized in Table 3. The sterol intake was 400–500 mg/day, calculated on the basis of a daily food intake of 20–25 g. As expected on the basis of previous studies (e.g., Ref. 16) cholesterol feeding inhibited HMG CoA reductase activity; there was a small additional decrease in enzyme activity when bile acid and cholesterol were fed in combination. The specific activity of cholesterol 7α-hydroxylase, 9.02 pmoles/mg protein/min, in the control group, was unchanged in the animals fed cholesterol, or cholesterol in combination with taurochenodeoxycholate, but was reduced 70% to 2.74 pmoles/mg protein/min in the group fed cholesterol plus taurocholate.

TABLE 3. Effect of dietary sterols, fed singly or in combination with bile acids, on the activities of hepatic HMG CoA reductase and cholesterol 7α-hydroxylase

Diet (1 wk)	No. of Rats	Enzyme Activity		Liver Cholesterol Concentrations
		HMG CoA Reductase	Cholesterol 7α-Hydroxylase	
		pmoles/mg protein/min		*mg/g wet wt*
Stock[a]	8	87.3 (2.95)[b]	9.02 (0.26)	2.42 (0.068)
Stock + 2% cholesterol	6	19.1[c] (2.03)	8.93 (0.23)	10.13[c] (0.28)
Stock + 2% sitosterol	6	316[c] (8.52)	13.01[c] (0.31)	2.28 (0.071)
Stock + 2% cholesterol + 1% sodium taurochenodeoxycholate	4	15.3[c] (1.89)	8.05 (0.46)	7.57[c] (0.19)
Stock + 2% cholesterol + 1% sodium taurocholate	6	14.1[c] (1.93)	2.74[c] (0.33)	22.1[c] (0.62)
Stock + 2% sitosterol + 1% sodium taurochenodeoxycholate	5	34.6[c] (2.03)	8.67 (0.32)	2.17 (0.092)
Stock + 2% sitosterol + 1% sodium taurocholate	6	92.9 (3.51)	2.38[c] (0.26)	2.36 (0.069)

[a] Ground Purina rat chow plus 5% corn oil.
[b] Standard error of the mean in parentheses.
[c] Differs significantly from control group on stock diet ($P < 0.01$).

Dietary sitosterol enhanced the specific activity of both enzymes: HMG CoA reductase increased 3.6-fold to 316 pmoles/mg protein/min, and the activity of cholesterol 7α-hydroxylase averaged 13.01 pmoles/mg protein/min, which represented a 40% increase in comparison with the control group on stock diet. When sitosterol and taurochenodeoxycholate were fed in combination (Table 3), HMG CoA reductase activity averaged 34.6 pmoles/mg protein/min, a 60% inhibition in comparison with the group on stock diet, but cholesterol 7α-hydroxylase activity, 8.67 pmoles/mg protein/min, did not differ significantly from the control values. In the rats fed taurocholate plus sitosterol, the specific activity of the 7α-hydroxylase was 2.38 pmoles/mg protein/min, which represented a 75% decrease. In this group, HMG CoA reductase activity averaged 92.9 pmoles/mg protein/min and thus was similar to that of normal controls on stock diet (87.3 pmoles/mg protein/min).

Predictably, the administration of a high cholesterol diet produced a considerable increase in liver total cholesterol concentration (17) to 10.13 mg/g, as compared with 2.42 mg/g in the control group. In the animals receiving cholesterol plus taurochenodeoxycholate, the liver cholesterol level averaged 7.57 mg/g, which was less than that observed in the group fed cholesterol alone. The greatest increase in liver cholesterol concentration, 22.1 mg/g, was observed in the rats receiving cholesterol in combination with taurocholate. In all sitosterol-fed groups, liver cholesterol levels tended to be slightly below that of the stock diet controls, but these differences were not significant at the 1% confidence limit.

Table 4 summarizes concentrations of biliary cholester-

TABLE 4. Cholesterol and bile acid secretion of rats fed taurine-conjugated bile acids[a]

Diet (1 wk)	No. of Rats	Biliary Cholesterol Concentrations	Biliary Secretion of Taurine-conjugated Bile Acids				
			Total Bile Acids	Deoxycholic	Chenodeoxycholic	α- + β-Muricholic	Cholic
		mg/ml	*mg/100 g rat/hr*				
Stock[b]	8	0.20 (0.006)[c]	7.06 (0.541)	0.197 (0.011)	1.23 (0.097)	1.00 (0.088)	4.63 (0.243)
Stock + 2% cholesterol	6	0.21 (0.007)	7.18 (0.483)	0.204 (0.009)	1.05 (0.099)	1.18 (0.060)	4.75 (0.253)
Stock + 2% sitosterol	6	0.54[d] (0.015)	6.99 (0.423)	0.218 (0.023)	1.25 (0.091)	0.798 (0.086)	4.72 (0.182)
Stock + 2% cholesterol + 1% sodium taurochenodeoxycholate	4	0.19 (0.011)	14.10[d] (0.711)	0.108[d] (0.012)	9.41[d] (0.806)	2.15[d] (0.171)	2.43[d] (0.309)
Stock + 2% cholesterol + 1% sodium taurocholate	6	0.20 (0.006)	13.76[d] (0.652)	0.428[d] (0.036)	0.211[d] (0.011)	0.103[d] (0.008)	13.02[d] (0.541)
Stock + 2% sitosterol + 1% sodium taurochenodeoxycholate	5	0.19 (0.008)	12.73[d] (0.426)	0.112[d] (0.009)	8.69[d] (0.772)	1.99[d] (0.244)	1.94[d] (0.248)
Stock + 2% sitosterol + 1% sodium taurocholate	6	0.45[d] (0.016)	13.05[d] (0.691)	0.568[d] (0.066)	0.206[d] (0.022)	0.106[d] (0.011)	12.17[d] (0.587)

[a] Based upon bile collected for 30–60 min at end of 7-day feeding period.
[b] Ground Purina rat chow plus 5% corn oil.
[c] Standard error of the mean in parentheses.
[d] Differs significantly from control group on stock diet ($P < 0.01$).

ol and rates of bile acid secretion in the groups of rats described in Table 3. Biliary cholesterol concentrations were not altered by dietary cholesterol or by dietary cholesterol plus bile acid (0.19 to 0.21 mg/ml). In contrast, administration of sitosterol raised the biliary cholesterol level to 0.54 mg/ml. A similar, somewhat smaller, increase to 0.45 mg/ml was observed in the rats fed sitosterol plus taurocholate, while the animals receiving sitosterol and taurochenodeoxycholate had normal biliary cholesterol concentrations. The rate of bile acid secretion in the control group on stock diet averaged 7.06 mg/100 g rat/hr. A similar rate was observed in the animals fed 2% cholesterol or sitosterol, but bile acid secretion was approximately twice the normal rate, 13–14 mg/100 g rat/hr in all groups fed sterol and bile acid in combination. The administration of cholesterol or sitosterol plus bile acid produced similar changes in bile composition as the feeding of bile acids alone (Table 2): the composition of the bile acid pool was altered so that the administered bile acid predominated. In the groups receiving either sterol in combination with taurocholate, the secretion of taurochenodeoxycholate plus tauromuricholates was reduced from about 2 mg/100 g rat/hr to 0.3 mg/100 g rat/hr, an 85% decrease. The administration of taurochenodeoxycholate in combination with either cholesterol or sitosterol produced a much more moderate reduction in the secretion of taurocholate, from 4.8 to 2.4 mg/100 g rat/hr, a 50% decrease in the former group and a 60% fall, from 4.7 to 1.94 mg/100 g rat/hr, in the latter. In the rats fed taurochenodeoxycholate with either cholesterol or sitosterol, the biliary output of tauromuricholates was nearly doubled, both in comparison with the control group on stock diet and the group fed taurochenodeoxycholate (Table 2).

DISCUSSION

Previous studies from this laboratory demonstrated that the rate of bile acid biosynthesis in the rat, in vivo, is influenced by the magnitude and circulation rate of the bile acid pool (1). In these experiments, with bile fistula rats, sodium taurocholate was infused intraduodenally at a known rate to simulate the normally circulating bile acid pool. Other bile acids were not tested, so that the influence of changes in the composition of the bile acid pool could not be evaluated. This animal model was not very convenient for examining the effects of dietary cholesterol or sitosterol, fed singly or in combination with bile acids, on bile acid synthesis. Therefore, in the present study, intact rats were used and the sterols and bile acids were incorporated into a regular chow diet, and fed ad lib. Relatively large levels of dietary sterol (2%) and bile acid (1%) were employed to produce significant acute changes in the composition of the bile acid pool within a relatively short feeding period. The effects of the different dietary additions on bile acid synthesis were then estimated indirectly by measuring the specific activities of the two en-

zymes that are rate limiting for cholesterol and bile acid biosynthesis, namely HMG CoA reductase and cholesterol 7α-hydroxylase.

The interpretation of the results reported in this paper is consequently based upon several assumptions: first, that the enzymes HMG CoA reductase and cholesterol 7α-hydroxylase catalyze the rate-determining steps in the biosynthesis of cholesterol and bile acids, respectively (2–7); second, that the concentrations of these enzymes in the in vitro assay systems are rate limiting, so that the specific activities of the enzymes can be a measure of enzyme concentration (15); and third, that changes in the specific activities of the enzymes, determined in vitro, reflect changes in the in vivo rates of hepatic cholesterol and bile acid synthesis. (In the in vitro assay systems, substrate and cofactors are supplied in generous excess, but this situation need not necessarily apply in vivo where the availability of substrate or cofactors can assume greater importance in determining reaction rates than the concentration of the rate-limiting enzyme [18].)

Under the conditions employed in the present study, the administration of different taurine-conjugated bile acids led to increased bile acid secretion and presumably to an expansion of the circulating bile acid pool (Table 2). The administered bile acid predominated in bile, and each acid exerted different characteristic effects upon the two rate-limiting enzymes of bile acid synthesis. It may be concluded, therefore, that changes in the composition of the bile acid pool produce changes in the rates of biosynthesis of cholesterol and bile acids. The differential effects of taurocholate and taurochenodeoxycholate appear to be of particular importance: taurocholate strongly suppressed the activities of both rate-limiting enzymes while taurochenodeoxycholate inhibited HMG CoA reductase but not cholesterol 7α-hydroxylase. This finding implies that taurocholate suppresses the synthesis of both cholesterol and bile acids, while taurochenodeoxycholate controls cholesterol biosynthesis but permits a normal rate of bile acid production. These differences may explain, at least in part, why in the rats fed taurocholate the secretion of the other primary bile acid, namely taurochenodeoxycholate plus tauromuricholates, was reduced 90%, while the administration of taurochenodeoxycholate suppressed the biliary output of taurocholate plus its bacterial metabolite, taurodeoxycholate, by only 70%. Presumably, taurocholate, which suppresses both of the rate-limiting enzymes, is a better inhibitor of de novo bile acid synthesis than taurochenodeoxycholate, which interferes predominantly with cholesterol biosynthesis. It should probably not be expected that in rats receiving excess dietary taurochenodeoxycholate de novo bile acid synthesis proceeds at a normal rate. This bile acid reduces the hepatic concentration of HMG CoA reductase and therefore limits the rate of hepatic cholesterol synthesis. Once cholesterol production falls below a minimal rate it may no longer be adequate to support a normal rate of bile acid formation.

The selective inhibitory effects of the two bile acids may account for the elevated liver cholesterol concentrations in the rats fed taurocholate and the nearly normal levels in the animals receiving taurochenodeoxycholate. The accumulation of cholesterol in the livers of the taurocholate group (also observed to a milder degree in the taurodeoxycholate-fed group) can then be ascribed to a lowered activity of cholesterol 7α-hydroxylase, i.e., a relative decrease in the capacity of the liver to remove excess cholesterol by converting it to bile acids. In the light of our present knowledge it does not seem likely that the differences in liver cholesterol concentrations are due to differences in the rates of cholesterol absorption among the bile acid-fed groups. It has been shown that taurochenodeoxycholate enhanced the intestinal absorption of cholesterol more effectively than the other bile acids tested (19). However, more detailed studies of the effect of dietary bile acids on cholesterol absorption will be needed to establish this point with certainty.

In the rats fed 1% taurodeoxycholate, the biliary secretion of chenodeoxycholate and its metabolites, the muricholates, was strongly diminished, but cholic acid secretion continued at a normal rate (Table 2). This finding does not necessarily imply that taurodeoxycholate selectively inhibited the synthesis or intestinal reabsorption of taurochenodeoxycholate but not of taurocholate. It seems more likely that a certain proportion of absorbed taurodeoxycholate was 7α-hydroxylated to taurocholate (20) and that the latter was not formed de novo from cholesterol.

The data on the reduction of HMG CoA reductase activity in rats fed diets containing 2% cholesterol (Table 3) are in accord with the results of other investigators (16, 21). However, we have not been able to confirm a preliminary report by Boyd, Scholan, and Mitton (22) that cholesterol 7α-hydroxylase activity is stimulated in rats receiving a high cholesterol diet for 4 days. It seems fairly well established that rats on high cholesterol intakes exhibit an increased bile acid turnover and excrete increased amounts of bile acids in their feces (23–25). This increase in fecal bile acid output is of the order of 1–2 mg/100 g rat/day. In female rats cholesterol feeding produces an increase in the relative proportion of chenodeoxycholate in the bile acid pool, but the magnitude of the pool is not increased in animals of either sex (23, 25). In the present experiments we were unable to detect increases in the biliary secretion of cholesterol or of bile acids in response to cholesterol feeding. On the basis of the published data (23, 24), the increase in the biliary secretion of bile acid induced by excess dietary cholesterol would have amounted

to only 0.125–0.250 mg/100 g rat/hr (based upon the reported increase in daily bile acid output of 1–2 mg/100 g rat/day). This increase in bile acid synthesis is small in comparison with the normal bile acid *secretion* of 7 mg/100 g rat/hr (Table 2) and would not have been detected. Since in our cholesterol-fed rats the specific activity of cholesterol 7α-hydroxylase did not differ from that of the controls, it must be concluded that the enzyme is not induced by cholesterol during a 1-wk period, and that a small increase in bile acid output results from the mass action effect of an increased substrate concentration.[2]

The feeding of diets containing 2% sitosterol greatly enhanced the specific activity of hepatic HMG CoA reductase. An increase in the rate of hepatic cholesterol synthesis in this group was expected since the plant sterol is known to interfere with the enterolymphatic circulation of cholesterol (26). A part of the newly synthesized cholesterol was presumably secreted in the bile, since in this group biliary cholesterol concentrations were increased two- to threefold. It is possible that another part of the newly synthesized cholesterol was converted to bile acids: in the sitosterol-fed animals the specific activity of cholesterol 7α-hydroxylase was increased 40% (Table 3). However, as was the case in the rats on the high cholesterol diet, there was no detectable increase in bile acid secretion. The failure to find a correlation between the specific activity of cholesterol 7α-hydroxylase and the rate of de novo bile acid synthesis is probably inherent in the experimental design: normally, the 300-g rats used in these experiments synthesize 6–10 mg of bile acids/day,[3] or 0.25–0.42 mg/hr. A 40% increase in bile acid *synthesis* would therefore produce an increase in bile acid secretion of only 0.10–0.17 mg/100 g rat/hr. This constitutes an increase of only a few percent in the normal rate of bile acid secretion of 7 mg/100 g rat/hr and would not have been detected in the present experiments.

When cholesterol was fed in combination with taurocholate and taurochenodeoxycholate (Table 3) the differential effect of the two bile acids on the rate-controlling enzymes and on liver cholesterol concentrations was again apparent; in comparison with the stock diet controls, HMG CoA reductase activity was strongly diminished in both groups while the activity of cholesterol 7α-hydroxylase was unchanged in the rats fed taurochenodeoxycholate plus cholesterol and was reduced about 70% in animals receiving taurocholate plus cholesterol. In the latter group the hepatic cholesterol concentration averaged 22.1 mg/g, which was considerably higher than the value of 7.57 mg/g observed in the animals fed the high cholesterol diet with taurochenodeoxycholate. A similar difference between liver cholesterol concentrations of rats on high cholesterol intakes with supplements of either cholic acid or chenodeoxycholic acid has also been reported by Kritchevsky and Tepper (27). Further studies are needed to decide whether these effects can be ascribed to differences in 7α-hydroxylase activity or to differences in cholesterol absorption.

When sitosterol was administered in combination with either taurochenodeoxycholate or taurocholate the specific activities of HMG CoA reductase and cholesterol 7α-hydroxylase no longer exhibited the elevated values observed in the group fed sitosterol alone (Table 3). The two bile acids, added to the high sitosterol diet, affected the two enzyme activities in a different manner: in the group of rats receiving sitosterol plus taurochenodeoxycholate, HMG CoA reductase activity was reduced to 40% of that of the stock diet controls and the activity of the 7α-hydroxylase was identical with that of the control group. Under these conditions, i.e., a reduced rate of cholesterol synthesis coupled with a normal rate of bile acid production, there presumably exists no stimulus for an increased secretion of biliary cholesterol. In contrast, with taurocholate and plant sterol, administered simultaneously, the rate of cholesterol synthesis was within normal limits while bile acid production was relatively low. Under these conditions some of the newly synthesized cholesterol was not converted to bile acid but was eliminated via the bile. It is not known why this relative imbalance between the rates of cholesterol and bile acid synthesis results in elevated biliary cholesterol levels rather than in increased liver cholesterol concentration. In any case, under the conditions employed here all groups of rats fed sitosterol either alone or in combination with bile acid tended to have normal liver cholesterol concentrations.

The studies with sitosterol and bile acids fed in combination seem to support the hypothesis (28, 29) that bile acids are capable of inhibiting HMG CoA reductase directly, not indirectly by stimulating cholesterol absorption and thereby increasing enterolymphatic cholesterol flux (30). This is suggested but not proven by the data obtained from studies in which the animals were fed sitosterol plus taurochenodeoxycholate: HMG CoA reductase activity was lowered 60% (in comparison with the stock diet controls) yet liver cholesterol concentrations were within the normal range. It seems unlikely that in this group of rats enterolymphatic cholesterol flux was sufficiently elevated to inhibit HMG CoA reductase, even in the presence of excess dietary bile acid. This problem must be investigated further, by measurement of cholesterol absorption under conditions identical with those described here.

[2] However, recent studies in our laboratory showed that feeding of the high cholesterol diet for a period of two weeks produced a 67% increase in the specific activity of cholesterol 7α-hydroxylase (Cohen, B., R. Raicht, S. Shefer, and E. H. Mosbach. Unpublished results.).

[3] Cohen, B., R. Raicht, and E. H. Mosbach. Unpublished results.

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