Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster

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Abstract The effect of various dietary additions such as cholesterol, β-sitosterol, bile acids, and bile acid analogs on gallstone formation was studied in the hamster. Gallstones were formed in 50% of the animals fed a high glucose, fat-free diet. Administration of 0.2% cholesterol or 1% β-sitosterol had no effect on the incidence of gallstones. Ursodeoxycholic acid (0.5%) and its analog ursodeoxy-oxazoline [2-(3α,7β-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline] were ineffective in preventing gallstones. Hyodeoxycholic acid and hyodeoxy-oxazoline [2-(3α,6α-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline] at the same dosage effectively prevented gallstones, while the trihydroxy bile acid, hyocholic acid, was not effective. Of all the dietary regimens tested, only hyodeoxycholic acid significantly lowered serum cholesterol. The lithogenic diet produced a five-fold increase in hepatic HMG-CoA reductase activity; this activity was not affected by dietary cholesterol or β-sitosterol. Hyodeoxycholic acid and hyocholic acid feeding increased the reductase activity by an additional 50% while the other bile acids had no effect. β-Sitosterol doubled the cholesterol 7α-hydroxylase activity whereas hyodeoxy-oxazoline lowered it. Hyodeoxycholic acid-fed animals had significantly lower cholesterol absorption than the animals on the lithogenic diet alone. Biliary cholesterol content increased dramatically in the animals fed the lithogenic diet and was increased still further by ursodeoxycholic acid, hyodeoxycholic acid, and hyodeoxy-oxazoline. These data show that hyodeoxycholic acid and hyodeoxy-oxazoline do not prevent gallstones by inhibiting hepatic cholesterol synthesis or biliary cholesterol secretion.—**Singhal, A. K., B. I. Cohen, J. Finver-Sadowsky, C. K. McSherry, and E. H. Mosbach.** Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster. *J. Lipid Res.* 1984. **25:** 564–570.

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Gallstones were induced in young golden Syrian hamsters maintained on a high glucose, fat-free diet (1). The mechanism of gallstone formation in these animals is not fully understood but is associated with a striking increase in hepatic cholesterol synthesis (2, 3). Replacement of glucose in the diet by rice starch or the supplementation of the diet with essential fatty acids caused a decrease in hepatic cholesterol synthesis and prevented the formation of gallstones (3).

In the same animal model administration of 1% dietary cholesterol has been shown to prevent the formation of cholesterol gallstones, but amorphous pigment stones may be found instead (4). When the lithogenic diet contained 2.9% of a plant sterol mixture (55.2% β-sitosterol, 38.8% campesterol, and 6% stigmasterol) the cholesterol saturation of bile was reduced and no stones were found (5). More highly purified preparations of β-sitosterol have apparently not been studied so far. In this hamster model, cholic acid (CA), deoxycholic acid (DA), and lithocholic acid (LA) had no effect on gallstone formation (6). Chenodeoxycholic acid (CDA) increased the severity of gallstone formation by an unknown mechanism whereas hyodeoxycholic acid (HDA) prevented cholelithiasis (7). Wheeler (8) has pointed out that HDA may prevent gallstones by causing biliary cholesterol to be excreted "as a liquid crystal or gel". On the basis of a recent in vitro study, Salvioli, Igimi, and Carey (9) showed that hydrophilic bile acids, such as ursodeoxycholic acid (UDA), may prevent cholelithiasis by promoting the formation of lecithin-cholesterol liquid crystals and inhibiting the phase transition to monocrystalline cholesterol monohydrate.

In the present study, we examined the effect of cholesterol, β-sitosterol, and various hydrophilic bile acids on gallstone formation in the hamster. The effect of certain bile acid analogs was also studied. The bile acids examined were UDA, HDA, and hyocholic acid (HCA) and the bile acid analogs were hyodeoxy-oxazoline and ursodeoxy-oxazoline (**Fig. 1**). In addition, we studied the effect of the different dietary modifications on biliary lipid composition and attempted to determine whether

Abbreviations: HDA, hyodeoxycholic acid; HCA, hyocholic acid; UDA, ursodeoxycholic acid; CA, cholic acid; DA, deoxycholic acid; LA, lithocholic acid; CDA, chenodeoxycholic acid; ursodeoxy-oxazoline, 2-(3α,7β-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline; hyodeoxy-oxazoline, 2-(3α,6α-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline; GLC, gas–liquid chromatography.

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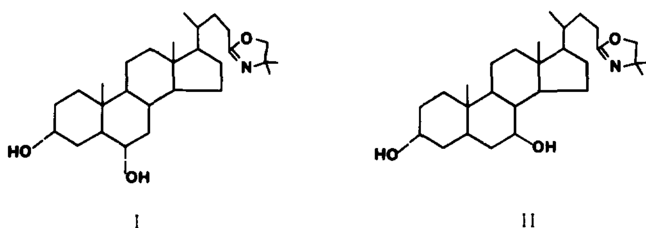

Fig. 1. I, 2-(3α,6α-Dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-2-oxazoline; II, 2-(3α,7β-dihydroxy-24-nor-5β-cholanyl)-4,4-dimethyl-oxazoline.

gallstone formation was correlated with the activity of hepatic HMG-CoA reductase and/or cholesterol 7α-hydroxylase, and cholesterol absorption.

MATERIALS AND METHODS

Animals and diets

Growing male golden Syrian hamsters of the Lakeview strain, weighing 50 g, were obtained from Charles River Breeding Labs, Wilmington, MA. The animals were fed a lithogenic diet containing glucose, 74.3%; casein, 20%; salt mixture, 5%; vitamin mix, 0.5%; choline chloride, 0.2%, (Dyets, Inc., Bethlehem, PA); and water ad libitum. The test diets were supplemented with either 0.2% cholesterol, 1% β-sitosterol, or 0.05% of one of the following: UDA, HDA, HCA, hyodeoxy-oxazoline, ursodeoxy-oxazoline. The animals were maintained on the various diets and water ad libitum for a period of 30–40 days, and were kept under an alternating 12-hr-light and 12-hr-dark cycle.

Cholesterol adsorption studies were carried out by a procedure described by Quintão, Grundy, and Ahrens (10). Two groups of six animals each were fed the lithogenic diet with or without HDA for 5 weeks. The animals were then given by stomach tube 2.87 μCi of [^{3}H]cholesterol (sp act 0.553 mCi/mmol) and 1.00 μCi of [^{14}C]sitosterol (sp act 0.415 mCi/mmol) in 0.5 ml of olive oil. The feces were quantitatively collected for 5 days and were analyzed for radioactivity in neutral sterols. The following equation was used: percentage of dietary cholesterol absorbed = [1 − (radioactivity in fecal cholesterol ÷ radioactivity in fecal β-sitosterol) × (radioactivity in administered β-sitosterol ÷ radioactivity in administered cholesterol)] × 100 (10).

At the end of the feeding period, the animals were injected with 7.5 mg of ketamine hydrochloride (Bristol Labs, Syracuse, NY). Blood was withdrawn by cardiac puncture for the determination of serum cholesterol. The gallbladder was exposed and examined visually. Gallstones, when present, were frequently visible through the gallbladder wall. Bile was aspirated as quantitatively as possible with a 100-μl Hamilton syringe. One drop was immediately examined under a polarizing microscope for the presence of cholesterol crystals. The remainder of the bile was used for chemical analysis. The gallbladder was then excised and opened and the presence of gallstones was determined by direct inspection and under a low-power microscope. The gallstones were usually an amorphous solid, pale yellow to white in color, 1–2 mm in diameter. They contained 90–95% cholesterol on a dry weight basis, as determined by GLC. The interior wall of the gallbladder was then scraped carefully with a square coverslip to detect small stones (less than 1 mm in diameter) which are sometimes embedded in the mucosal folds. The liver was excised and aliquots were used for cholesterol analysis and for the preparation of microsomes.

Compounds

Cholesterol was obtained from Sigma Chemical Co., St. Louis, MO. β-Sitosterol was a gift from Dr. E. R. Diller, Eli Lilly & Co., Indianapolis, IN, and contained 95% β-sitosterol, 4% campesterol, and 1% stigmasterol. UDA (Tokyo Tanabe, Tokyo, Japan), HDA, and HCA (Canada Packers, Inc., Ontario, Canada) were found to be 99% pure by GLC analysis. Hyodeoxy-oxazoline and ursodeoxy-oxazoline were synthesized in our laboratory (11) and were 99% pure as determined by GLC. 5α-Cholestane was used as an internal standard in all GLC analyses. 3α,7α-Dihydroxy-12-keto-5β-cholanoic acid (Steraloids, Wilton, NH) was used as a recovery standard for bile acid analyses.

The radioactive compounds, [1α,2α(n)-^{3}H]cholesterol, and β-[4-^{14}C]sitosterol were obtained from Amersham, Arlington Heights, IL. DL-Hydroxymethyl-[3-^{14}C]-glutaryl-CoA was obtained from New England Nuclear, Boston, MA.

Enzyme assays

Liver microsomes were prepared by a procedure described earlier (12). HMG-CoA reductase was assayed by a modified micro procedure (13). Incubations were carried out at 37°C in a total volume of 255 μl in Beckman 1-ml microfuge tubes. The assay system consisted of 0.38 mmol of phosphate buffer, 0.25 μmol of $MgCl_2 \cdot 6H_2O$, 0.8 μmol of $NADP^+$, 4.6 μmol of glucose-6-phosphate, 1 I.U. of glucose-6-phosphate dehydrogenase, 0.018 μmol of mercaptoethanol, and 25 μmol of NaCl in a total volume of 140 μl. An aliquot of the microsomal suspension, 100 μl, containing 0.3–1.0 mg of protein, was added to each tube. After 2 min of preincubation, 168 nmol (15 μl) of DL-hydroxymethyl-[3-^{14}C]glutaryl-CoA (sp act 0.9 mCi/mmol) was added to the incubation tubes. The tubes were further incubated for 25 min. The reaction was terminated by the addition of 25 μl of 10 N HCl. Un-

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labeled mevalonolactone, 20 μl (150 mg/ml), and 100,000 dpm of [5-^{3}H]mevalonolactone were added as recovery standards. The samples were lactonized at 37°C for 1 hr and then centrifuged in a Beckman microfuge B for 4 min to sediment the proteins. Aliquots (100 μl) of the supernatants were streaked on silica gel G plates and developed in benzene–acetone 1:1 (v/v). The plates were sprayed with 2′,7′-dichlorofluorescein and the mevalonolactone spots were scraped off and counted for ^{14}C and ^{3}H. Cholesterol 7α-hydroxylase activity was determined as described previously by a method employing exogenous cholesterol as substrate (14). TLC separation of cholesterol and 7α-hydroxycholesterol was carried out under nitrogen.

Analytical procedures

Determination of the cholesterol content of liver, plasma, and bile was carried out as described previously (15). The gallstones were dried in vacuo at 60°C and were analyzed for cholesterol content by the same procedure as used for liver cholesterol. Biliary bile acids were quantitated by GLC using 0.5% OV-210 and 1% HiEff 8BP columns. Relative retention times of bile acid methyl ester acetates on the 0.5% OV-210 column (T_c = 220) were as follows: LA, 0.47; DA, 1.0; CDA and HDA, 1.30; UDA, 1.51; CA and HCA, 2.47; allocholic acid, 2.55; internal standard, 3.47. The bile acid pairs, CDA-HDA and CA-HCA, were not separated on OV-210 column and were separated and quantitated as methyl ester TMS derivatives on a 1% HiEff 8BP column (T_c = 245). Relative retention times of these bile acids were as follows: CA, 1.0; HCA, 1.24; CDA, 1.53; and HDA, 1.78. Identification of the biological bile acids was confirmed on a Hewlett-Packard 5992B GLC–mass spectrometer. Biliary phospholipids were determined by a colorimetric procedure (16, 17). The methods used for the extraction and analyses of fecal neutral sterols have been described in detail (18).

Calculations

Cholesterol saturation of bile was calculated using the tables of Carey (19); no correction for biliary UDA, HDA, or HCA concentrations was made. The significance of differences among the various groups studied was calculated using one-way analysis of variance followed by Dunnett's test for multiple comparisons. Differences in the incidence of gallstones and biliary cholesterol crystals were determined by chi-square (20, 21).

RESULTS

Studies were carried out to examine the effect of cholesterol, β-sitosterol, bile acids, and bile acid analogs on gallstone formation in hamsters fed Dam's lithogenic diet. The animals weighed approximately 60–70 g at the start of the experiment and there was no significant weight gain during the experiment. The average food intake of the animals was 7.5 ± 1.2 g/day.

The effect of various diets on the formation of gallstones, the presence of cholesterol crystals in the gallbladder, and tissue cholesterol levels are presented in **Table 1.** Gallstones and crystals developed in 50% of the animals fed the unsupplemented lithogenic diet. Addition of 0.2% cholesterol or 1% β-sitosterol to the lithogenic diet produced no significant change in the incidence of gallstones or crystals. UDA and its analog ursodeoxy-oxazoline did not prevent gallstone or crystal formation, while HDA and its oxazoline derivative completely prevented cholelithiasis and biliary cholesterol crystals. The

TABLE 1. Effect of dietary sterols and bile acids on tissue cholesterol and incidence of gallstones in hamsters

Group	Diet	Incidence of		Cholesterol[b]	
		Gallstones[a]	Crystals[a]	Serum	Liver
				mg/dl	*mg/g*
I	Lithogenic diet (32)[c]	6/12[d]	7/12[d]	144 ± 10[d]	2.60 ± 0.15
II	+0.2% Cholesterol (15)	2/6[d]	2/6[d]	161 ± 25[d]	2.42 ± 0.18
III	+1% β-Sitosterol (20)	3/6[d]	4/6[d]	162 ± 14[d]	2.39 ± 0.20
IV	+0.05% Hyodeoxycholic acid (33)	0/17[e]	0/17[e]	107 ± 7[e]	2.44 ± 0.20
V	+0.05% Hyocholic acid (20)	6/11[d]	6/11[d]	163 ± 8[d]	1.99 ± 0.40
VI	+0.05% Ursodeoxycholic acid (28)	7/8[d]	7/8[d]	160 ± 34	2.63 ± 0.07
VII	+0.05% Ursodeoxy-oxazoline (8)	4/6[d]	4/6[d]	166 ± 8	2.71 ± 0.19
VIII	+0.05% Hyodeoxy-oxazoline (8)	0/8[e]	0/8[e]	116 ± 14	2.30 ± 0.23
IX	Rodent chow (10)	0/10	0/10	114 ± 5	1.80 ± 0.10

[a] Incidence of gallstones and crystals in surviving animals. Statistical significance determined by chi-square.
[b] Mean ± SEM.
[c] Number of animals in each group at the start of experiment.
[d] Differs from group IX, $P < 0.05$.
[e] Differs from groups I through III, and V through VII, $P < 0.05$.

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TABLE 2. Effect of dietary sterols and bile acids on hepatic enzymes[a]

Group	Diet	Hepatic HMG-CoA Reductase	Cholesterol 7α-Hydroxylase
		pmol per mg protein per min	
I	Lithogenic diet (12)[b]	1050 ± 127[c]	32.8 ± 2.0
II	+0.2% Cholesterol (6)	921 ± 281[c]	41.5 ± 6.5
III	+1% β-Sitosterol (6)	1074 ± 176[c]	64.0 ± 14.8[d]
IV	+0.05% Hyodeoxycholic acid (17)	1591 ± 204[c,e]	35.0 ± 3.1
V	+0.05% Hyocholic acid (11)	1500 ± 161[c,e]	30.3 ± 5.2
VI	+0.05% Ursodeoxycholic acid (8)	1078 ± 170[c]	36.7 ± 9.5
VII	+0.05% Ursodeoxy-oxazoline (6)	1008 ± 160[c]	36.7 ± 3.6
VIII	+0.05% Hyodeoxy-oxazoline (8)	1081 ± 65[c]	23.8 ± 3.9[f]
IX	Rodent chow (10)	204 ± 35	27.1 ± 3.5

[a] Mean ± SEM.
[b] Number of animals in each group in parentheses.
[c] Differs from group IX, $P < 0.01$.
[d] Differs from groups I and IV through IX, $P < 0.05$.
[e] Differs from groups I through III, and VI through VIII, $P < 0.05$.
[f] Differs from groups I through VII, $P < 0.05$.

hydrophilic trihydroxy bile acid, HCA, however, did not lower the incidence of gallstones compared to the controls on the lithogenic diet.

Administration of the lithogenic diet to the hamsters increased serum cholesterol by 21% compared to the chow-fed animals. Of all the administered bile acids, only HDA caused a decrease in serum cholesterol of 25.6%. Compared to chow-fed controls, the lithogenic diet raised liver cholesterol content, but none of the dietary supplements changed the liver cholesterol concentrations significantly.

There was a fivefold increase of hepatic HMG-CoA reductase activity in the animals fed the lithogenic diet compared to the animals fed rodent chow (**Table 2**). The addition of 0.2% cholesterol or 1% β-sitosterol to the lithogenic diet did not alter reductase activity. HDA and HCA stimulated the enzyme activity about 50%, whereas UDA, ursodeoxy-oxazoline, and hyodeoxy-oxazoline were ineffective. Cholesterol 7α-hydroxylase activity increased twofold in the animals fed β-sitosterol compared to the lithogenic controls. Hyodeoxy-oxazoline lowered 7α-hydroxylase activity by 27.4%.

The effect of the lithogenic diet and various steroid additions on biliary lipid composition is summarized in **Table 3.** Biliary cholesterol was elevated to 8.3 mol % in the animals fed lithogenic diet as compared to 1.2 mol % in chow-fed animals. Addition of cholesterol or β-sitosterol to the lithogenic diet did not significantly alter the cholesterol content of the bile. Interestingly, HDA, UDA, and hyodeoxy-oxazoline significantly increased the cholesterol concentration of bile compared to the animals fed lithogenic diet alone, while HCA and ursodeoxy-oxazoline had no significant effect. None of the dietary regimens produced significant changes in biliary bile acid or phospholipid concentration of the bile. In all groups of animals fed lithogenic diet the bile remained supersaturated with cholesterol. Lithogenic indices of the bile increased in the animals fed cholesterol, HDA, HCA, UDA, and hyodeoxy-oxazoline; however, these increases were not statistically significant. Although there was a

TABLE 3. Effect of dietary sterols and bile acids on biliary lipids in hamsters[a]

Group	Diet	Cholesterol	Phospholipids	Bile Acids	Lithogenic Index	Total Lipid
			mol percent			*g/dl*
I	Lithogenic diet (12)[b]	8.3 ± 1.8[c]	12.0 ± 2.4	79.7 ± 2.7	1.70 ± 0.33[c]	3.79 ± 0.50
II	+0.2% Cholesterol (6)	9.0 ± 0.5[c]	8.6 ± 1.2	82.4 ± 2.1	2.47 ± 0.03[c]	4.81 ± 0.61
III	+1% β-Sitosterol (6)	6.6 ± 1.2[c]	13.9 ± 3.4	79.5 ± 3.4	1.84 ± 0.39[c]	5.30 ± 1.44
IV	+0.05% Hyodeoxycholic acid (17)	13.4 ± 1.3[c,d]	14.1 ± 3.2	72.5 ± 4.2	2.37 ± 0.18[c]	7.48 ± 1.15[e]
V	+0.05% Hyocholic acid (11)	9.2 ± 1.0[c]	13.4 ± 2.4	77.4 ± 4.4	2.52 ± 0.18[c]	4.40 ± 0.36
VI	+0.05% Ursodeoxycholic acid (8)	16.3 ± 1.9[c,d]	13.7 ± 4.1	70.0 ± 3.9	2.64 ± 0.21[c]	—[f]
VII	+0.05% Ursodeoxy-oxazoline (6)	8.2 ± 2.0[c]	9.4 ± 0.5	82.4 ± 2.1	1.29 ± 0.28[c]	—[f]
VIII	+0.05% Hyodeoxy-oxazoline (8)	17.2 ± 1.4[c,d]	13.9 ± 3.4	68.9 ± 3.4	2.56 ± 0.22[c]	—[f]
IX	Rodent chow (10)	1.2 ± 0.2	10.9 ± 2.3	87.9 ± 2.3	0.20 ± 0.03	—[f]

[a] Mean ± SEM.
[b] Number of animals in each group in parentheses.
[c] Differs from group IX, $P < 0.001$.
[d] Differs from groups I through III, V and VII, $P < 0.05$.
[e] Differs from groups I through III, and V, $P < 0.05$.
[f] Total lipid concentration assumed to be 6.8 g/dl for calculation of lithogenic index (27).

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TABLE 4. Effect of dietary sterols and bile acids on biliary bile acid composition in hamsters[a]

Group	Diet	Biliary Bile Acid Composition, %							
		CA	CDA	UDA	HDA	HCA	DA	LA	Allocholate
I	Lithogenic diet (12)[b]	60.5 ± 3.3	32.4 ± 1.6				4.6 ± 1.2	0.6 ± 0.2	1.9 ± 1.3
II	+0.2% Cholesterol (6)	40.3 ± 1.9[c]	41.4 ± 4.9				3.5 ± 3.3	0.6 ± 0.1	14.2 ± 0.2[c]
III	+1% β-Sitosterol (6)	50.8 ± 3.6	28.8 ± 5.0				7.1 ± 2.4	0.7 ± 0.4	12.6 ± 4.1[c]
IV	+0.05% Hyodeoxycholic acid (16)	15.9 ± 1.9[c]	12.9 ± 1.5[c]		67.2 ± 2.7[c]		3.1 ± 0.6	0.9 ± 0.8	
V	+0.05% Hyocholic acid (10)	30.8 ± 3.1[c]	17.2 ± 1.7[c]		21.9 ± 4.0[c,d]	23.3 ± 5.7[c]	5.1 ± 0.8	1.7 ± 0.7	
VI	+0.05% Ursodeoxycholic acid (8)	35.3 ± 8.7[c]	39.2 ± 4.5	21.8 ± 5.8[c]			3.5 ± 1.5	0.2 ± 0.2	
VII	+0.05% Ursodeoxy-oxazoline (6)	61.1 ± 15.4	22.0 ± 8.9	6.0 ± 7.3[c,e]			10.9 ± 2.0		
VIII	+0.05% Hyodeoxy-oxazoline (8)	21.4 ± 5.9[c]	19.6 ± 3.1[c]		56.7 ± 7.8[c]		2.3 ± 1.4		
IX	Rodent chow (10)	69.2 ± 2.0	27.0 ± 1.9				2.9 ± 0.9	0.9 ± 0.1	

[a] Mean ± SEM.
[b] Number of animals in each group in parentheses.
[c] Differs from groups I and IX, $P < 0.05$.
[d] Differs from groups IV and VIII, $P < 0.01$.
[e] Differs from group VI, $P < 0.05$.

twofold increase in mol % cholesterol in HDA-fed animals, the increase in lithogenic index was not dramatic because HDA-fed animals also had a twofold increase in total biliary lipid concentration.

The biliary bile acid composition of the lithogenic controls (**Table 4**) indicated that cholic acid was the major bile acid present (60.5%), and that CDA accounted for 32%. LA, DA, and allocholic acids accounted for 0.6%, 4.6%, and 1.9% of the total bile acids, respectively. In the animals fed cholesterol, there was an increase in biliary CDA of approximately 28%, while CA decreased. In both of the sterol-fed groups, allocholic acid comprised 12–14% of the total bile acids. In the animals receiving UDA, this bile acid accounted for only 22% of the total, whereas CDA and CA accounted for 39% and 35%, respectively. Presumably, UDA was transformed to CDA in these hamsters either in the liver, or by intestinal bacteria, or both. In ursodeoxy-oxazoline-treated animals, total bile acids contained only 6% UDA while CA was the major bile acid present (61.1%). In HDA and hyodeoxy-oxazoline-treated animals, bile acid composition was shifted toward the administered bile acid, which accounted for 57–67% of the total. In HCA-treated animals, HCA comprised only 23% of the total bile acids; HDA, the 7α-dehydroxylation product of HCA, was present in approximately equal concentration (22%).

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Cholesterol absorption studies were carried out following the administration of a single dose of [^{3}H]cholesterol and [^{14}C]β-sitosterol in olive oil. Under the conditions employed, cholesterol absorption was 50.0 ± 5.5% in the animals maintained on the lithogenic diet alone. Cholesterol absorption was dramatically reduced by the administration of HDA (12.1 ± 2.5%).

DISCUSSION

Dam and Christensen (1) discovered that cholesterol gallstones can be induced in golden Syrian hamsters by feeding a semisynthetic diet containing high proportions of glucose and no fat. Although intensive work has been carried out on this hamster model, the mechanism of gallstone formation has remained unclear. In the hamsters fed the lithogenic diet, there is a striking increase in hepatic cholesterol synthesis (2, 3). It was assumed that this increased hepatic cholesterol synthesis caused the increased secretion of cholesterol in the bile and subsequent formation of gallstones. However, Turley, Spady, and Dietschy (22) reported that the increased secretion of cholesterol in the bile did not result directly from newly synthesized cholesterol and that the biliary cholesterol may be derived from an unknown preformed cholesterol pool. The nature of this pool remains to be determined.

In the present study, 0.2% cholesterol feeding did not

inhibit hepatic HMG-CoA reductase activity and the incidence of gallstones was 33% (lithogenic controls, 50%). It was shown in a previous study that the administration of 0.15% cholesterol in rodent chow (which contained 5% fat) caused a 90% inhibition of the reductase activity in the hamster (12). The lack of inhibition by cholesterol in animals on the fat-free diet may be due to *1*) lack of cholesterol absorption in the absence of fat, and/or *2*) the lack of feedback control, caused perhaps by hepatic membrane damage preventing the binding of lipoproteins to membrane receptors. However, in a study by Iijima, Yamazaki, and Maruyama (23), there was approximately 60% inhibition of HMG-CoA reductase activity by feeding a large proportion of cholesterol (2%) in the lithogenic diet. This would suggest that the lack of inhibition observed in the present study is due to the lower cholesterol content of the diet (0.2%) associated with reduced absorption. β-Sitosterol, which presumably reduces the absorption of cholesterol even further, did not stimulate the reductase activity, although this would have been possible in the light of the HDA experiments.

In the animals treated with UDA, ursodeoxy-oxazoline, and hyodeoxy-oxazoline, the latter prevented gallstone formation yet none of the groups exhibited changes in HMG-CoA reductase activity. Similarly, HDA and HCA caused a 50% increase in the reductase activity but only the former prevented the cholelithiasis. The results with HDA are similar to those of Beher, Baker, and Penney (24) who observed a significant increase of hepatic cholesterol synthesis in hamsters on a chow diet supplemented with 1% HDA. It is therefore clear that HDA and hyodeoxy-oxazoline prevent gallstones by a mechanism not directly related to the synthesis or secretion of cholesterol. It is not possible to state whether in the experiments with HCA this bile acid itself affected reductase activity since HDA was also present in appreciable concentrations in the bile.

Biliary lipid composition indicates that cholesterol concentration was elevated in all groups of animals maintained with the lithogenic diet. β-Sitosterol caused a decrease in mol % of biliary cholesterol; while this decrease was not statistically significant, it suggests some inhibitory effect on the absorption of cholesterol from the intestine. However, 1% β-sitosterol did not lower biliary cholesterol sufficiently to affect the incidence of gallstones. In contrast, the mixture of plant sterols administered by Kubota et al. (5) significantly lowered biliary cholesterol concentration and prevented the formation of gallstones.

UDA, HDA, and hyodeoxy-oxazoline caused a considerable increase in biliary cholesterol. Such an effect of HDA had previously been reported by Wheeler (8). In a recent in vitro study, Salvioli et al. (9) pointed out that the more hydrophilic bile acids induce the formation of liquid crystals containing lecithin and cholesterol while preventing the nucleation and crystallization of cholesterol monohydrate. Since the liquid crystals can hold large amounts of cholesterol, gallstone formation is prevented even though the bile is supersaturated. In our study, the other hydrophilic bile acids administered, namely, HCA and ursodeoxy-oxazoline, did not increase the cholesterol concentration of bile, probably because they are poorly absorbed (see below). Gallstones were prevented only by the administration of HDA and hyodeoxy-oxazoline and not by UDA, HCA, and ursodeoxy-oxazoline.

Biliary bile acid composition provides a clue to the ineffectiveness of UDA, HCA, and ursodeoxy-oxazoline in preventing cholelithiasis. In the UDA-fed animals, a large amount of the administered bile acid was converted to CDA, which has been shown to increase the severity of gallstone formation in this hamster model (7). In the animals fed ursodeoxy-oxazoline, CA was the major bile acid, as in the lithogenic controls, and UDA accounted for only 6% of the total biliary bile acids. This suggests that ursodeoxy-oxazoline, in contrast to hyodeoxy-oxazoline, may not have been efficiently absorbed in this animal model. This is in contrast to our finding in the prairie dog on a 0.4% cholesterol diet where ursodeoxy-oxazoline administration resulted in significant amounts of UDA (30%) and CDA (38%) in the bile and an inhibition of cholelithiasis (25). Upon administration of HDA and hyodeoxy-oxazoline, HDA was the major biliary bile acid, accounting for 57–67% of the total, most of which was present as the glycine conjugate. Upon administration of HCA to hamsters, HCA accounted for 23% and HDA for 22% of the total bile acids. In the HCA-fed animals, the bile contained 31% CA (twice the amount of the HDA group). The presence of large proportions of CA in the bile may indicate that insufficient amounts of HCA were absorbed to prevent cholelithiasis.

HDA prevented cholesterol absorption significantly, as determined by the dual isotope ratio method. This finding is supported by the lower serum cholesterol levels and increased fecal neutral sterol output of these hamsters. However, HDA presumably does not prevent gallstones by preventing cholesterol absorption, as evidenced by the increased cholesterol saturation of the bile. Salvioli et al. (9) predicted that only taurine conjugates of HDA should be effective in preventing cholelithiasis, but in our hamsters, where more than 90% of the HDA was glycine conjugated, complete gallstone prevention was achieved. HCA would have been expected to prevent gallstone formation by a mechanism similar to that of HDA since both bile acids are highly hydrophilic. Apparently, the present in vivo experiments do not correlate entirely with the in vitro studies of Salvioli et al. (9), presumably because under the conditions employed HCA was not absorbed efficiently by the hamster. UDA, another relatively hydrophilic bile acid, did not prevent the cholelithiasis be-

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cause it was largely converted to CDA which is not effective in promoting the formation of the liquid crystalline phase (9).

Hyodeoxy-oxazoline was not only as effective as HDA in preventing gallstones, but it also prolonged the life span of the animals. Dam and Christensen (1) pointed out that premature deaths of hamsters on the fatty acid-deficient diet are probably due to bacterial infection leading to diarrhea. An antibiotic effect of bile acid oxazoline derivatives in pure cultures of anaerobic bacteria was previously observed by Hylemon, Fricke, and Mosbach (26). It seems possible, therefore, that the HDA derivative exerted an antibiotic effect, prevented the diarrhea, and increased the survival rate of the animals.

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