Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short term feeding of chenodeoxycholic and ursodeoxycholic acid[1]

N. Carulli, M. Ponz De Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria

Istituti di Clinica Medica, Clinica Chirurgica e Chimica Organica, Universita di Modena, Italy

Abstract The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and 7α-hydroxylase, the enzymes controlling the rate of hepatic synthesis, respectively, of cholesterol and bile acids, and the microsomal cholesterol content were evaluated in 25 patients with cholesterol gallstones and 17 subjects without gallstones. The same quantities were estimated in 16 additional patients with gallstones given chenodeoxycholic (CDCA) or ursodeoxycholic acid (UDCA) at a dose of 15 mg/kg per day in order to investigate the comparative effect of a short term (7 days) administration of the two bile acids on the hepatic sterol metabolism. As compared to the controls, subjects with gallstones exhibited a 36% decrease of 7α-hydroxylase (26.8 ± 6.2 versus 41.7 ± 4.2 pmol/min per mg protein) and a 24% increase of the microsomal cholesterol (78.7 ± 15.3 versus 63.1 ± 18.1 nmol/mg protein). Although higher in the gallstone patients, the activity of HMG-CoA reductase did not differ significantly in the two groups of subjects. Administration of CDCA and UDCA changed the bile acid pool composition so that the fed bile acid predominated in the bile (mean CDCA 73% and mean UDCA 54%). Bile lipid composition did not appreciably change. In the eight subjects treated with CDCA the activity of HMG-CoA reductase was reduced to 45% of the value of untreated subjects (27.9 ± 14.5 versus 63.5 ± 25.3 pmol/min per mg protein) whereas in the eight subjects treated with UDCA the same enzyme showed a twofold increase (123.5 ± 20.9). In the treated groups 7α-hydroxylase activity was somewhat decreased but the values did not differ significantly from those of the untreated subjects. Microsomal cholesterol content decreased with CDCA (64.8 ± 11.6 nmol/mg protein) as well as with UDCA (59.1 ± 10.1) treatment; however in the latter the difference attained statistical significance ($P < 0.05$). Altogether the results would suggest that in the liver of patients with gallstones the conversion of cholesterol to bile acids is somewhat reduced, and that changing the bile acid pool composition, by exogenous bile acid feeding, has disparate effects on hepatic cholesterol synthesis. The findings could represent the acute changes produced by bile acid feeding, however they could imply that the effects of two bile acids in dissolving cholesterol gallstones might not be related only to the changes in hepatic sterol metabolism.—**Carulli, N., M. Ponz De Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria.** Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* 1980. **21:** 35–43.

Supplementary key words HMG-CoA reductase · 7α-hydroxylase · microsomal cholesterol

Cholesterol saturation and desaturation of the bile are the prerequisites for gallstone formation and dissolution, respectively (1, 2). The exact mechanism(s) responsible for the biliary lipid changes, either spontaneous or induced by exogenous bile acid administration are not well defined. Salen et al. (3) reported that the liver of patients with cholesterol gallstones exhibits an increased HMG-CoA reductase activity coupled to a decreased activity of 7α-hydroxylase, attracting attention to the hepatic sterol metabolism. Because the two enzymes control, respectively, the rate of the synthesis of cholesterol (4) and the rate of the degradation of cholesterol to bile acids (5), it was suggested that alteration of the hepatic sterol synthesis could play a role in determining biliary lipid secretion abnormalities. Both CDCA and UDCA given to patients with gallstones induce cholesterol desaturation of the bile and in due time eventually dissolve gallstones (2, 6, 7). This effect has been related to a decreased hepatic synthesis of cholesterol in the treated patients (6). The few studies (6, 8, 9) reporting the effect of bile acid feeding on the rate-limiting

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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TABLE 1. Basal data of subjects studied

Group	Age	Sex		Ideal Weight	Plasma	
		M	F		Total Cholesterol	Triglycerides
	yr			*%*	*mg/dl*	*mg/dl*
Controls (duodenal ulcer) (17)	43 ± 13[a]	11	6	89 ± 18	192 ± 17	131 ± 20
Untreated gallstones (25)	47 ± 18	10	15	102 ± 23	221 ± 23	143 ± 15
CDCA-treated gallstones (8)	42 ± 9	3	5	98 ± 15	217 ± 19	150 ± 17
UDCA-treated gallstones (8)	46 ± 15	4	4	94 ± 10	205 ± 21	139 ± 21

[a] Mean ± SD.
The number of subjects in each group is in parentheses.

enzymes of hepatic sterol metabolism in man have been performed in patients treated for a period of time long enough to produce biliary lipid changes. Since there is a question as to whether bile acids affect the regulation of hepatic synthesis of cholesterol directly or indirectly, through the changes they bring about in the disposition of cholesterol (10), it seemed of interest to us to investigate earlier effects of an altered composition of the bile acid pool on sterol metabolism.

Our study consisted of two sets of investigations; the first related to the evaluation of the sterol metabolism in subjects with gallstones as compared to subjects without gallstones, both under basal conditions; the second concerned the comparative effects of short term administration of chenodeoxycholic or ursodeoxycholic acid.

Factors studied included bile lipid composition, bile acid pool composition, HMG-CoA reductase and 7α-hydroxylase activities, and finally, the microsomal content of cholesterol.

Our results showed that short term administration of the two bile acids had disparate effects on the cholesterol synthesis although both reduced the hepatic cholesterol content.

PATIENTS AND EXPERIMENTAL DESIGN

Since 1976 we have been able to study 58 subjects admitted to the policlinic of Modena for elective surgery. Forty-one subjects had cholesterol gallstones and 17 were patients with chronic duodenal ulcer refractory to medical treatment. At the time of the study, the ulcer patients were in good nutritional state and their weight had not changed appreciably in the month before the study. Of these patients 7 managed with bed-time atropine, 6 were on cimetidine, and 4 were maintained on diet and occasionally antacids to relieve abdominal pain. After the protocol had been explained to each subject, all gave consent to the treatment and to have a liver biopsy performed. The experimental protocol had been approved by the Ethical Committee of the Institute of Clinical Medicine of the University of Modena.

The first set of investigations was carried out on 17 ulcer patients and 17 gallstone subjects. The second set was done on 24 patients with gallstones; these patients were divided into three groups: *a*) 8 untreated patients, *b*) 8 treated with CDCA, and *c*) 8 treated with UDCA. Both bile acids were given at an equal dose of 15 mg/kg of body weight for 7 days. In **Table 1** the mean age, sex distribution, the mean percent ideal weight, and the plasma lipids of the groups are reported. No significant variations were observed among the patients with gallstones in the two studies, so they were grouped. From one week before their operation, all patients were given a standard diet containing approximately 500 mg of cholesterol/day. Weight, liver function tests, and serum lipids were recorded at the beginning and at the end of the study for both control and treated patients. Weight remained unchanged and the liver tests were always within normal limits. The histological examination of the liver revealed either a normal liver or minor changes in one third of the patients with gallstones. Serum lipids were comparable between the subjects of the different groups and did not change throughout the study. In both control and treated patients, bile samples were obtained before and after the period of the study. Liver specimens (100–300 mg) were obtained at surgery for subtotal gastrectomy in the 17 patients with ulcers and for cholecystectomy in gallstone patients. General anesthesia was the same in all patients and surgery was performed between 10 AM and 11 AM.

A small portion of each liver specimen, removed immediately after laparotomy, was used for morphological examination while the larger portion was placed in ice-cold homogenizing medium, transported to the laboratory and processed immediately.

MATERIALS

CDCA and UDCA were obtained from Gipharmex Co., Milano, Italy; GLC analysis showed that CDCA was 97% pure and contained less than 0.1% lithocholic acid. UDCA was 98% pure and contained traces of CDCA. CoA, DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl CoA ([3-^{14}C]HMG-CoA), and *RS* [5-^{3}H]mevalonic acid were obtained from New England Nuclear Co., Boston, MA. Sodium boro [^{3}H]hydride was purchased from Amersham, England; 5α-cholestan-3-ol-6-one from Steraloids Inc., Milwaukee, WI; DL-mevalonic acid lactone from Sigma Chemical Co., St. Louis, MO; NADP, G-6-phosphate disodium salt, and G-6-P dehydrogenase from Biochemia-Boehringer, Mannheim Germany; sucrose, nicotinamide, cysteaminium chloride, 2-mercaptoethanol, acetone, benzene, EDTA, ethyl ether, and TLC aluminum sheets with silica gel 60 F_{254} from Merck, Darmstadt, Germany; and Pico-fluor 15 from Packard Instrument Co. Inc., Downers Grove, IL.

METHODS

Preparation of liver microsomes

Surgical liver specimens were weighed and thereafter processed in a cold room (4°C). The tissue was homogenized with a Potter-Elvejem apparatus in four volumes of a medium containing sucrose 0.3 M, nicotinamide 0.075 M, EDTA 0.002 M, and mercaptoethanol 0.02 M.

Microsomal pellets, obtained by ultracentrifugation, were suspended in a volume of 0.1 M phosphate buffer, pH 7.4, to give a 25% solution. Aliquots of the microsomal suspension were used for determination of HMG-CoA reductase and 7α-hydroxylase activities and for cholesterol content. Identical yields of microsomal protein, as determined by the method of Lowry et al. (11), were obtained from the livers of the patients of all groups (23–28 mg/g wet tissue).

Assay of HMG-CoA reductase

The enzyme activity was evaluated according to Goldfarb and Pitot (12), with only minor modifications. The assay system contained, in a volume of 1.0 ml, 3 mM $MgCl_2$, 3 mM NADP, 10 mM glucose-6-phosphate, 3 units of G-6-P dehydrogenase, 20 mM 2-mercaptoethanol, 0.4 mM [3-^{14}C]HMG-CoA (750,000 dpm/μmol), 0.1–0.3 mg microsomal protein, and 100 mM phosphate buffer, pH 7.4. The reaction was carried out at 37°C in a shaking water bath for 15 min and then stopped by addition of 0.1 ml of 10 N NaOH. A control blank was run using boiled microsomes. Lactonization of biosynthesized [^{14}C]-mevalonic acid, the use of synthetic [^{3}H]mevalonic acid as an internal standard, isolation of [^{14}C]mevalonolactone using TLC, and counting of ^{3}H and ^{14}C radioactivity were carried out as described by Goldfarb and Pitot (12). The amount of [^{14}C]mevalonate formed was calculated from the equation:

$$\text{moles of [}^{14}\text{C] mevalonate formed} = \frac{\text{[}^{3}\text{H]mevalonate added (dpm)}}{\text{[}^{3}\text{H]mevalonate recovered (dpm)}} \times \frac{\text{[}^{14}\text{C]mevalonate}}{\text{sp act [}^{14}\text{C]HMG-CoA (dpm/mol)}}$$

Assay of 7α-hydroxylase

The procedure used was first described by Van Cantfort et al. (13) and validated by other investigators (14). The [7α-^{3}H]cholesterol used as substrate in our assay was synthesized by the method of Corey and Gregoriou (15). The specificity of the tritium label at the designated site was checked biologically (14, 16) and was found to be greater than 90%. The standard assay mixture (1.0 ml) consisted of 4 mM $MgCl_2$, 2 mM NADP, 20 mM G-6-phosphate, 3 units of G-6-P dehydrogenase, 20 mM cysteaminium chloride, 0.1–0.4 mg microsomal protein, 100 mM phosphate buffer, pH 7.4, and 0.3 mM [7α-^{3}H]cholesterol (sp act 4.5 $\times$ 10^{6} dpm/μmol). The cholesterol was solubilized with the aid of the Tween-80 (1 mg/ml) by the method of Karaboyas and Koritz (17).

A control blank, using boiled microsomes, was run under the identical conditions used in the experimental samples.

The reaction was carried out at 37°C for 15 min and was terminated by adding three volumes of 20% trichloroacetic acid. The mixture was then centrifuged at 3,000 *g* for 5 min and the supernatant solution was transferred into a distillation apparatus similar to that described by Hutton, Tappel, and Udenfriend (18). An aliquot of the distilled tritiated water was counted in a Packard Tri-Carb 3320 beta counter using Pico-fluor as the scintillation mixture. Quenching was corrected by the external standard method. The enzyme activity, expressed in pmol/mg protein, was calculated from the equation:

$$\frac{\text{}^{3}\text{H distilled water (dpm)}}{\text{sp act of [7}\alpha\text{-}^{3}\text{H]cholesterol}}$$

Assay of microsomal cholesterol

The microsomal preparation was extracted with petroleum ether according to Folch, Lees, and Sloane

TABLE 2. Bile lipid and biliary bile acid composition in subjects with and without gallstones. Effects of CDCA and UDCA administration.

Group		S.I.[a]	Biliary Bile Acids (% of Total)				
			LCA	DCA	CDCA	UDCA	CA
Controls (duodenal ulcer)	(17)	0.83 ± 0.12[b]	1 ± 0.8	20 ± 7	40 ± 13		39 ± 12
Untreated gallstones	(25)	1.35 ± 0.3[c]	2 ± 1.5	23 ± 7	35 ± 10		40 ± 6
CDCA-treated gallstones	(8)	1.28 ± 0.12[c]	8 ± 4	8 ± 7	73 ± 5		11 ± 4
UDCA-treated gallstones	(8)	1.30 ± 0.10[c]	2 ± 0.5	8 ± 6	22 ± 4	54 ± 5	14 ± 4

[a] Saturation index.
[b] Mean ± SD.
[c] Differs significantly from control value ($P < 0.01$).
The number of subjects in each group is in parentheses.

Stanley (19). The extracts were evaporated under nitrogen and cholesterol was determined in the residue by the method of Abell et al. (20).

Bile lipid composition

Bile rich duodenal fluid (5 ml) was obtained by duodenal intubation after stimulation of gallbladder with caerulein (Ceruletide TAKUS—Farmitalia). Total bile acids were evaluated by the 3 OH-steroid dehydrogenase method according to Talalay (21); total cholesterol was measured by the method of Abell et al. (20); phospholipids were measured as inorganic phosphorus using Bartlett's procedure (22). Bile lipid composition was expressed as "saturation index" and calculated according to the critical tables of Carey (23) using the 10 g/dl solubility limits. Where necessary, the correction factor for the presence of UDCA was taken into account.

Biliary bile acid composition

Biliary bile acid composition was evaluated by GLC. Hydrolysis of conjugated bile acids was accomplished with cholylglycine hydrolase (24), and the extracts were methylated with diazomethane and acetylated with trifluoroacetic anhydride. Bile acid derivatives were dissolved in ethyl acetate and injected in a Packard gas-chromatograph equipped with spiral column packed with QF1-OV 17 (4:1) at 230°C. 7-ketodeoxycholic acid was used as internal standard.

Statistical analysis

Data are presented as means ± SD (standard deviation) unless otherwise indicated. The significance was evaluated by Student's t test.

RESULTS

Effects of CDCA and UDCA on biliary lipids and bile acid composition

Bile saturation indices and biliary bile acid composition are summarized in **Table 2.** Patients with cholesterol gallstones had a bile supersaturated with cholesterol, the mean saturation index (1.35 ± 0.3) being significantly higher than that of patients without gallstones (0.83 ± 0.12). The short period of treatment with either CDCA or UDCA did not appreciably change the bile lipid composition of the treated subjects.

The mean biliary bile acid composition in the subjects with gallstones and those without gallstones was the same. However composition was profoundly altered by the administration of bile acids in the sense that the fed bile acid became the most abundant in the pool. In the CDCA-treated group, mean percent CDCA rose from 35 to 74, and in the UDCA group the percentage of UDCA amounted to 54% after the treatment. The administration of either CDCA or UDCA produced a marked decrease in the percentage of CA and DCA in the bile. The proportion of LCA increased after CDCA treatment but not after UDCA treatment.

Enzyme activities and microsomal cholesterol content in subjects with untreated gallstones and without gallstones

As illustrated in **Fig. 1** the activity of HMG-CoA reductase did not differ significantly in the two groups; the mean value in ulcer patients being 58.6 ± 14.7 pmol/min per mg protein as compared to 63.5 ± 25.3 in subjects with gallstones. 7α-hydroxylase activity in gallstone patients (26.8 ± 6.2 pmol/min per mg protein) was significantly ($P < 0.01$) lower than that found in the subjects without gallstones (41.7 ± 4.2). Microsomal cholesterol content was also significantly ($P < 0.05$) higher in gallstone patients (78.7 ± 15.3 nmol/mg protein) in comparison to non-gallstone subjects (63.1 ± 18.1).

Effect of CDCA and UDCA administration on enzyme activities and cholesterol content

As shown in **Table 3,** HMG-CoA reductase activity was affected by the administration of both bile acids.

Treatment with CDCA produced a striking decrease of the enzyme activity which fell to 27.9 ± 14.5, a value significantly ($P < 0.01$) lower compared to that of the untreated gallstone patients (63.5 ± 25.3). On the contrary, in the UDCA-treated group the enzyme activity (123.5 ± 20.9) was higher than in the untreated gallstone patients, the difference being significant at a 1% level.

7α-Hydroxylase activity (Table 3) was not so much affected by either CDCA or by UDCA administration. The values determined for the CDCA (25.7 ± 7.3 pmol/min per mg protein) and UDCA groups (24.6 ± 4.8) although lower did not differ significantly from the value of the untreated gallstone group (28.8 ± 6.2). In comparison to the value of microsomal cholesterol (Table 3) found in untreated gallstone patients (78.7 ± 15.3 nmol/mg protein), both CDCA (64.8 ± 11.6) and UDCA (59.1 ± 10.1) groups showed a lower value; but only in the UDCA group was the difference significant ($P < 0.05$).

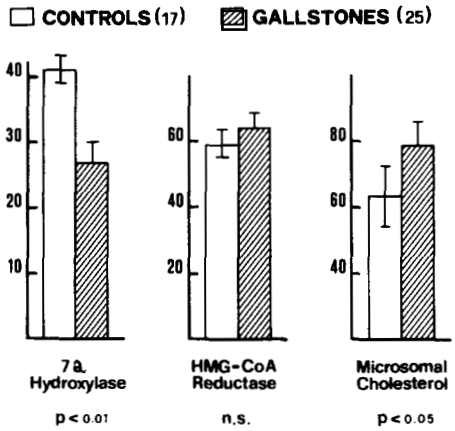

Fig. 1. 7α-hydroxylase, HMG-CoA reductase, and microsomal cholesterol in patients with and without gallstones. Enzyme activities are expressed as pmol/min/mg of protein; cholesterol is expressed as nmol/mg of protein. Results are illustrated as means ± SEM; n.s., not statistically significant.

DISCUSSION

The absolute figures of enzyme activities obtained in our study differ somewhat from those reported by others. However these differences, probably due to the different procedures, are not relevant when comparative studies are performed. With the limitations implicit in extrapolating in vitro results to the in vivo conditions, our results would suggest that: *a*) patients with gallstones have a somewhat reduced conversion of cholesterol to bile acids associated with an increased microsomal cholesterol content; *b*) in these patients the enrichment of the bile acid pool with a physiological bile acid such as chenodeoxycholic or with an unphysiological one such as ursodeoxycholic

TABLE 3. Effect of CDCA and UDCA treatment on the rate-limiting enzymes and microsomal cholesterol in patients with gallstones.

Patients No. and Treatment	HMG-CoA Reductase	Cholesterol 7α Hydroxylase	Microsomal Cholesterol
	pmol/min/mg protein		*nmol/mg protein*
CDCA			
1	20.8	20.5	74.5
2	15.2	27.2	44.3
3	54.0	27.9	66.3
4	19.6	28.8	72.4
5	17.8	12.8	67.1
6	43.3	38.0	55.7
7	35.9	22.0	80.2
8	17.0	28.5	58.2
Mean ± SD	27.9 ± 14.5[a]	25.7 ± 7.3	64.8 ± 11.6
UDCA			
1	131.0	17.5	63.0
2	160.0	22.1	54.0
3	96.1	23.8	72.0
4	111.5	27.1	67.0
5	119.0	25.0	45.0
6	146.0	31.2	50.0
7	110.0	30.8	52.0
8	115.0	20.0	70.0
Mean ± SD	123.5 ± 20.9[a]	24.6 ± 4.8	59.1 ± 10.1[b]
Untreated gallstones			
Mean ± SD	63.5 ± 25.3	26.8 ± 6.2	78.7 ± 15.3

[a] Differs significantly ($P < 0.01$) from untreated-gallstones value.
[b] Differs significantly ($P < 0.05$) from untreated-gallstones value.

has disparate effects on the HMG-CoA reductase activity without significantly affecting the first committed step of bile acid synthesis; *c*) the administration of either CDCA or UDCA seems to restore microsomal cholesterol to a normal level.

Activity of rate-limiting enzymes and microsomal cholesterol content in gallstone patients

Our results are similar to those previously reported by Salen et al. (3) and Coyne et al. (8), but we failed to observe a significantly higher HMG-CoA reductase in patients with gallstones; in the same patients, we did find a significantly augmented microsomal cholesterol. Our conditions differed from those of the two mentioned studies in that we used surgically obtained liver specimens in all cases and studied a greater number of patients.

In our study the increased content of cholesterol in the microsomes of patients with gallstones could have produced lower 7α-hydroxylase values. However, in the procedure, the amount of labeled exogenous cholesterol that we used was, according to Van Cantfort, Renson, and Gielen (13) enough to saturate the enzyme; it was exceedingly high when compared to the amount of cholesterol present in the microsomal preparation used for the assay. Thus, whatever the figures of equilibration of endogenous with labeled cholesterol are (13, 25, 26), the specific radioactivity of the latter could not have changed so much as to produce misleading results.

From the available data it can be said that a decreased 7α-hydroxylase activity is a constant finding in the liver of patients with gallstones. Speculations as to whether this represents a primary defect or is the result of an overactivity of feedback inhibition of bile acid synthesis (27) lack direct experimental proof.

Comparative effects of CDCA and UDCA on the rate-limiting enzymes and hepatic cholesterol in patients with gallstones

Under the conditions employed in our study, the administration of CDCA and UDCA produced opposite effects on the specific activity of HMG-CoA reductase. While there is sound evidence, both in animals (28, 29) and in man (6, 8), that CDCA suppresses the activity of HMG-CoA reductase, information on the effect of UDCA is scanty. Maton, Murphy and Dowling (6) reported that feeding UDCA (5 mg/kg daily) for 4 weeks or more to five patients with gallstones resulted in a HMG-CoA reductase activity significantly lower than in the untreated controls. Similarly Salen et al. (9) fed UDCA to gallstone patients for 1 year and found HMG-CoA reductase reduced to 35% of the pretreatment value. In contrast to these reports our data have shown an increased enzyme activity after short term UDCA treatment.

It must be stressed, however, that the duration of our treatment was much shorter so that a time-related effect cannot be excluded. A treatment of 7 days duration might be too short for bile acid equilibrium to have occurred. The non-steady state condition applies to UDCA as well as to CDCA-treated groups, yet the differences of HMG-CoA reductase between the two groups are too clear-cut to be due to a random phenomenon. Possibly our results represent acute changes brought about by bile acid feeding.

A knowledge of the exact mechanism by which bile acids affect the hepatic sterol metabolism would clearly be of great help in explaining apparently conflicting findings. Data have been reported which suggest a direct effect of bile acids in regulating the activity of HMG-CoA reductase (28, 30, 31). Alternatively other evidence supports the view that the inhibitory effect of bile acids can be accounted for by their ability to promote the intestinal absorption of cholesterol. This cholesterol, carried in the lipoprotein of intestinal origin, would be the ultimate effector (32, 33).

In this context some investigators found that CDCA-treated animals have a negative cholesterol balance (34), whereas in those treated with UDCA the balance was positive, possibly owing to an increased synthesis,[2] because in these animals cholesterol absorption was depressed (35). However our preliminary data on the effect of bile acids on cholesterol absorption in man have shown that either CDCA (36) or UDCA[3], in a dose of 15 mg/kg daily, depresses significantly the absorption of cholesterol, yet only UDCA-treated patients exhibit an increased HMG-CoA reductase activity. It may be speculated that both bile acids themselves and changes of cholesterol absorption could influence the enzyme activity. The prevalence of one or the other of these two effects, and hence the actual enzyme activity, depends on the administered bile acid.

Turnover studies in man have shown that the administration of the two primary bile acids (37), deoxycholic acid (38) and UDCA (39) suppresses the endogenous bile acid synthesis. This is clearly evident in the dramatic changes of the bile acid pool composi-

[2] Preliminary data in our laboratory have shown that rats fed a semisynthetic diet containing 0.5% UDCA or CDCA for 1 week exhibited different specific activity of hepatic HMG-CoA reductase. In the UDCA-treated animals the enzyme activity was increased 30%, whereas in the CDCA-treated it was decreased to 50% that of the untreated controls. Carulli, N., F. Zironi, M. Ponz de Leon and A. Pinetti. Presented at the V Bile Acid Meeting (Freiburg i.Br., West Germany, June 12–14, 1978).

[3] Ponz de Leon, M., and N. Carulli. Unpublished results.

tion following bile acid feeding. Although it is rather difficult to establish a relationship, in quantitative terms, between in vivo observations and the in vitro data, one would expect a consistently reduced 7α-hydroxylase activity to be associated with suppressed bile acid synthesis. Our results do not show a significant decrease of 7α-hydroxylase activity in contrast to the 50% decrease reported by Coyne et al. (8) after long term treatment with CDCA. Even in animal studies, the effects of bile acid feeding on the enzyme activity are controversial (28, 40). If we assume that bile acid administration could affect other steps of bile acid biosynthesis, as well as 7α-hydroxylation of cholesterol, this would explain the apparent discrepancy between changes of the bile acid pool composition and 7α-hydroxylase values.

In this respect CDCA administration to man (8) and rat (40) has been reported to reduce significantly the 12α-hydroxylase activity, a committed step in the synthesis of cholic acid. Further investigations are needed to define the relationship between exogenous bile acid feeding and their endogenous synthesis.

There are no data available in man on the effect of bile acid administration on the cholesterol content of the liver. In rats fed primary bile acids the hepatic level of cholesterol seemed to be related to the intestinal absorption more than to the hepatic synthesis (41). Our data, showing a decreased microsomal cholesterol in the liver of gallstone patients treated with either CDCA and UDCA, fit well into the observed reduction of cholesterol absorption[3] (36).

Hepatic synthesis and biliary secretion of cholesterol

Cholesterol gallstones have a supersaturated bile and an increased HMG-CoA reductase activity. This suggests a causal relationship between synthesis and biliary secretion of cholesterol. Key et al. (42) found the HMG-CoA reductase and biliary cholesterol output to be closely related; however this correlation was no longer evident during CDCA treatment (43). Turley and Dietschy (44) reported that impressive (over 400-fold) variations of cholesterol synthesis, induced by different means, were not followed by any appreciable change in biliary cholesterol output. Furthermore, Schwartz et al. (45) have presented evidence in man that the major portion of biliary cholesterol originates from lipoprotein, free cholesterol (mainly HDL) (46), while newly synthesized cholesterol contributes about one-fifth. Similar results have been obtained in the rat by Long et al. (47) using a different methodological approach. Taken together, these observations cast some doubts as to whether cholesterol synthesis is a direct determinant of biliary cholesterol secretion.

In keeping with the above-mentioned findings our results would suggest that changes of the activity of the rate-limiting enzymes of hepatic sterol metabolism, observed during CDCA and UDCA administration, are associated events which do not bear a relevant impact on the process of bile cholesterol secretion and desaturation.

Alternatively, an increased cholesterol content of the liver could be regarded as a stimulus for an increased flux of cholesterol into the bile. This would explain the increased cholesterol secretion in gallstone patients. In this respect it is noteworthy that in rats cholic acid feeding decreases HMG-CoA reductase activity and C_2 flux into cholesterol while it increases hepatic cholesterol concentration (28, 29). The same bile acid given to patients with gallstones fails to desaturate bile and induce gallstone dissolution (37).

Additional work is necessary in order to define the quantitative role of the various processes involved in cholesterol metabolism, in regulating the biliary output of cholesterol in gallstones, and during bile acid feeding.

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