Effects of Acute Changes of Bile Acid Pool Composition on Biliary Lipid Secretion

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Abstract. To elucidate the mechanism responsible for the bile acid-induced changes of biliary lipid secretion, we evaluated bile flow and biliary output of bile acids, cholesterol, phospholipids, and alkaline phosphatase activity in seven cholecystectomized subjects with a balloon occludable T-tube during two experimental periods: (*a*) depletion of the endogenous bile acid pool and (*b*) replacement of the pool by means of duodenal infusion with individual bile acids, such as deoxycholic (DCA), chenodeoxycholic (CDCA), cholic (CA), and ursodeoxycholic (UDCA) acids. Bile flow, cholesterol, and phospholipid output were linearly related to bile acid secretion in all experimental periods. During the replacement periods, the amount of cholesterol and phospholipids coupled to bile acids was significantly different (at 1% level at least) for each individual bile acid secreted; it was the highest during DCA secretion (slope value: 0.209 for cholesterol and 0.434 for phospholipids) followed, in the order, by CDCA (0.078 and 1.794), CA (0.044 and 0.127), and UDCA (0.030 and 0.122).

The phospholipid to cholesterol ratio was higher during secretion of CA and UDCA as compared with DCA and CDCA. The secretion of CA seemed to stimulate a greater bile flow than the other bile acids did.

The infusion of all bile acids, except UDCA, induced an increase of biliary alkaline phosphatase activity as compared with the values of the depletion period. The mean highest increase (13-fold the pretreatment value) was observed during DCA secretion followed by CDCA (fivefold) and CA (1.5-fold).

These results would suggest that the physical chemical properties, namely the lipid-solubilizing capacity, of bile acids could directly contribute to the regulation of biliary lipid secretion. The observed changes in biliary alkaline phosphatase activity lend support to the view that bile acid-induced lipid secretion may be, at least in part, contributed by membrane solubilization.

Introduction

It is well established that the biliary secretion of cholesterol and phospholipid is closely linked to that of bile acids (1–4). Although the precise mechanism responsible of this coupling is not defined, kinetics of bile lipid secretion have been characterized in many animal species including man (5–8). Biliary output of cholesterol and phospholipid is described as a curvilinear function of bile acid secretion. At low bile acid output, as it occurs during bile acid depletion, cholesterol and phospholipid to bile acid ratio is far higher than that observed at higher bile acid output when both cholesterol and phospholipid secretion tend to plateau. This finding suggests that, besides bile acid secretion, other factors influence the secretion of cholesterol and phospholipid into the bile. This concept is also supported by the observation that for a given bile acid output, cholesterol secretion may vary among different animal species and also within human subjects (9). In fact, obese subjects secrete more cholesterol than nonobese suggesting that total body synthesis of cholesterol may bear relevance on its biliary secretion (10) and that other determinants should be considered in the regulation of cholesterol secretion into the bile.

Portions of these studies were presented at the 17th Meeting of the European Association for the Study of the Liver, 9–11 September 1982, and at the Autumn Meeting of the British Society of Gastroenterology, 22–24 September 1982.

An Appendix has been deposited with the National Auxiliary Publications Service (NAPS) as NAPS document 04195. This information may be ordered from ASIS/NAPS, Microfiche Publications, P. O. Box 3513, Grand Central Station, New York 10017. Remit in advance, in U. S. funds, $4.00 for microfiche copy, or for photocopy, $7.75 up to 20 pages plus 30¢ for additional pages. Outside the U. S. and Canada add postage of $4.50 for photocopy and $1.50 for microfiche. Checks should be made payable to Microfiche Publications.

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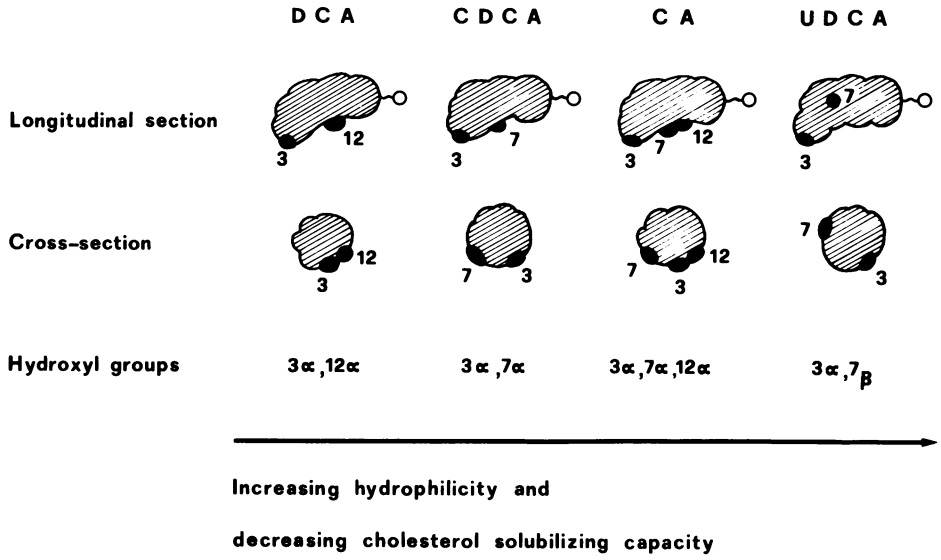


Figure 1. Schematic view of space-filling molecular models of bile acids naturally occurring in man. The dashed body represents the steroid nucleus; black dots, the hydroxyl groups; and the wiggly line ending with a circle, the side chain of the molecule.

Factors contributing to the hydrophilicity of the different bile acids are in order of importance: (*a*) the spatial orientation of the hydroxyl groups; the equatorial 7β-OH of UDCA is more hydrophilic than the axial 7α-OH of CDCA and CA; (*b*) the number of axial hydroxyl groups; trihydroxy (CA) are more hydrophilic than dihydroxy (CDCA and DCA) bile acids; (*c*) the position of the axial hydroxyl groups; 3α,7α-OH (CDCA) are more hydrophilic than 3α,12α (DCA) (ref. 39 and 50).

The introduction of chenodeoxycholic (CDCA)[1] and ursodeoxycholic (UDCA) acids in the medical treatment of cholesterol gallstones has permitted us to gain new information on this problem. Chronic administration of these bile acids, in appropriate dosage, reduces biliary cholesterol secretion so that bile becomes undersaturated with cholesterol (11–14). Attempts to correlate the desaturating effect of these bile acids to the changes in hepatic synthesis (15–21) and/or the accompanying intestinal absorption of cholesterol (22–27) have produced controversial results in man.

However, a direct link between synthesis and/or absorption of cholesterol and its biliary secretion has been undermined by the observation that the administration of deoxycholic acid (DCA), while depressing both hepatic synthesis (28) and absorption (29, 30) of cholesterol, does not change (28, 31, 32) or actually increases bile saturation (30, 33). Moreover, under appropriate conditions, feeding of cholic acid (CA) depressed cholesterol saturation (34) without affecting hepatic synthesis (19) or increasing the absorption of cholesterol (30). Finally, the fact that changes of cholesterol secretion have been observed after acute administration of CDCA and UDCA in T-tube patients (35, 36) and healthy subjects with intact enterohepatic circulation (37) would suggest that the effect of bile acids on biliary lipid secretion might be a direct one and not be mediated by changes of sterol metabolism.

Based on these findings, we postulated that one of the factors contributing to the regulation of biliary lipid secretion might well be the physical chemical characteristics of the bile acids present within the pool (38). Due to their structural differences, natural bile acids of man, such as CA, CDCA, DCA, and UDCA, have a different balance between the hydrophobic (steroid nucleus) and the hydrophilic (hydroxyl groups and the carboxyl group of the side chain-linked aminoacid) part of the molecule. Evaluation of this balance, by means of high performance liquid chromatography (HPLC), has shown that DCA is the least hydrophylic (or the most hydrophobic) followed, in order of increasing hydrophylicity, by CDCA, CA, and UDCA (39) (Fig. 1).

The hydrophylic-hydrophobic balance seems to dictate the capacity of the bile acid to solubilize insoluble lipids such as cholesterol in the sense that if the bile acid is less hydrophylic, its capacity to dissolve cholesterol will be greater.

To test the hypothesis that the solubilizing properties of bile acids could bear a relevant relationship to the biliary lipid secretion, we investigated the effect of acute replacement of the endogenous bile acid pool by the individual bile acids, DCA, CDCA, CA, and UDCA.

Parameters investigated included bile flow, bile lipid output, and biliary alkaline phosphatase activity.

Methods

Patients. Seven patients, three males and four females, admitted to the surgical ward of Castelfranco Hospital, Modena, for uncomplicated gallstones disease were studied. Clinical relevant data of the subjects are reported in Table I. At the time of cholecystectomy, exploration of the common bile duct was required, and then, a balloon occludable T-tube was positioned.

Laboratory tests did not reveal any abnormality indicating liver or gastrointestinal disease. Liver biopsy taken at the time of surgery showed a normal liver structure in all patients. None of the subjects had been on medication for at least 15 d before entering the study. The protocol of the study was explained and all subjects gave their consent.

Design of the study. For 7–10 d after cholecystectomy, the patients

1. *Abbreviations used in this paper:* AP, alkaline phosphatase; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HPLC, high performance liquid chromatography; SI, saturation index; UDCA, ursodeoxycholic acid.

Table I. Clinical Findings of the Investigated Subjects

Name	Sex	Age	Weight	Percent ideal body weight	Blood lipids	
					Cholesterol	Triglycerides
					mg/dl	*mg/dl*
B.R.	F	56	53	98	180	95
M.C.	F	57	56	96	220	110
S.V.	F	61	67	110	265	150
M.A.	M	63	76	106	220	135
A.C.	F	45	54	97	195	97
C.R.	M	56	70	99	250	170
P.R.	M	48	74	108	260	126
Mean ±SD		55±6.5	64.2±9.7	102±5.8	227±32.6	126±27.8

were administered 2 g/d intramuscularly of ampicillin to reduce bacterial degradation of primary bile acids. The T-tube was kept open to deplete the endogenous bile acid pool. Patients were given a standard diet adequate to keep their weight constant and the calories were equally distributed between the three main meals. Each meal consisted of 15% protein, 45% carbohydrate, and 40% fat. The patients were kept fasting 10 h before and throughout the study. At 7 A.M. in the morning, the study (Fig. 2) was begun by inserting, under fluoroscopic control, a duodenal catheter; the duodenal arm of the T-tube was occluded by inflating the balloon and the occlusion checked to be complete by means of trans T-tube cholangiogram. The occlusion interrupted completely the enterohepatic circulation.

After 5 h of unstimulated bile secretion (depletion period), individual bile acids in free form, dissolved in saline, and brought to pH 9–9.5 with $NaHCO_3$ were infused through the duodenal catheter at a rate of ~1 g (about 2.5 mmol)/h for 5 h (replacement period). The first subject (not included in the study) infused 1 g/h of DCA, which was manifested as a transient rise in serum transaminase. Thereafter, in the remaining subjects, DCA was infused at a rate of 700–800 mg/h and no similar side effects were noted. None of the subjects that infused CDCA, CA, and UDCA complained of any side effect including diarrhea.

Each patient had two studies performed with 2 different bile acids at 3 d apart one from the other. Three patients received CDCA and CA, three received DCA and UDCA, and one received CA and UDCA. The sequence of administration was CDCA-CA in one, CA-CDCA in two, DCA-UDCA in one, UDCA-DCA in two, and UDCA-CA in one. During each study, bile was collected hourly, and the specimen was measured and immediately frozen.

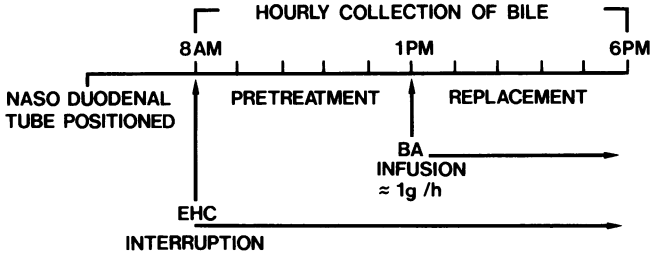


Figure 2. Experimental design. Enterohepatic circulation (EHC) was completely interrupted by inflating the balloon in the duodenal arm of the T-tube. Pretreatment indicated the period during which the endogenous bile acid (BA) pool was depleted so that at the beginning of the bile acid infusion only primary bile acids were present in bile.

Absorption of bile acids. In four experiments (two with UDCA, one with CDCA, and one with DCA), the infused bile acids were labeled with the respective radioactive tracers (5 μCi) in order to evaluate the amount of administered bile acid excreted into the bile during the replacement period.

Materials. Of the bile acid used for infusion, CDCA, CA, and DCA were purchased from Sigma Chemical Co. (St. Louis, MO); [^{14}C]CDCA and [^{14}C]DCA were purchased from Amersham Corp., Arlington Heights, IL; UDCA used for infusion and [^{14}C]UDCA were kindly supplied by Gipharmex Co., Milan, Italy. All bile acids were found by thin layer chromatography to be >95% pure. All other materials used for analytical purposes was reagent grade.

Analytical procedure. Total bile acids were assayed by the 3α-hydroxysteroid dehydrogenase method of Talalay (40), cholesterol was measured with the Abell's procedure (41), and phospholipid was measured as inorganic phosphorus according to Bartlett's method (42). The relative proportion of the individual bile acids in the bile was evaluated by gas-liquid chromatography as described previously (43). Bile saturation index was calculated according to Carey (44) without correcting for UDCA content. In the experiments with radioactive bile acids, a 0.5-ml aliquot of bile was bleached with hydrogen peroxide, and after standing overnight, the liquid scintillation mixture (Picofluor 15, Packard Instrument Co., Downers Grove, IL) was added and the sample counted in a Packard 3320 counter (Packard Instrument Co.). Quenching was corrected for by the external standard channel ratio method. Alkaline phosphatase activity was evaluated on diluted bile samples by means of standard automated technique.

Statistical analysis. Linear regression lines of the relationship between bile lipid secretion values were obtained by the least square method. During replacement periods, the values used were those observed when most of the endogenous pool was replaced by the infused bile acid. Comparison of the slopes was made using the variance analysis. Where appropriate, Student's t test was used for statistical significance.

Results

Biliary bile acid composition. Fig. 3 shows the proportions of individual bile acids in the bile during the experimental periods of the study. In the pretreatment period, the mean proportion of CA was 73.2±14.7% and that of CDCA was 26.8±13.4% of the pool, whereas secondary bile acids were virtually undetectable. Such a composition reflected the interruption of the enterohepatic circulation with the consequent increased synthesis of primary bile acids and the absence of intestinal metabolism of bile acids, partly contributed by the antibiotic treatment. During replacement, the percentage of the infused bile acid increased steadily and in most of the cases 70–90% of the pool was made in 2 h by the administered bile acid. Some difference was noted among the individual bile acids with regards to the completeness of pool substitution. Namely, UDCA increase was less steep than that observed with the other bile acids and pool substitution tended to plateau at the third hour with a mean percentage of 75%.

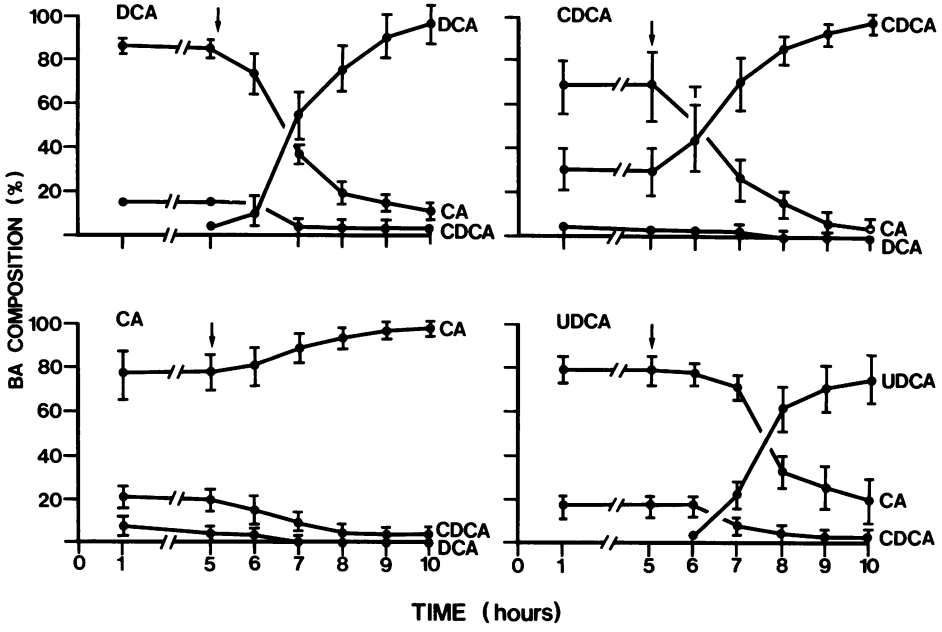


Figure 3. Effect of bile acid infusion on bile acid (BA) pool composition. Proportions of individual bile acids, expressed as means of all studies±SD are plotted against time. Arrows indicate the point at which infusion of bile acid was started. On the left is the composition during the depletion period; on the right, changes in composition during the bile acid infusion.

Biliary bile acid output. Table II reports the mean values of bile acid output during the experimental periods of the study. In the replacement periods, although bile acids were infused approximately at the same rate, a striking difference was observed in the biliary output of DCA, CDCA, and CA as compared with UDCA. During UDCA infusion, bile acid output was roughly ¼ of that observed during the infusion of the other three bile acids. In the experiments with radioactive tracers, illustrated in Fig. 4, the amount of UDCA excreted in the 5 h of the replacement period was <5% of the administered dose; at 16 h after the start of the infusion, the cumulative excretion of the radioactivity reached 16% of the dose. When radioactive DCA or CDCA were used, a far higher portion of the administered dose was excreted either during the 5 h of the replacement period (nearly 50% for DCA and 80% of CDCA) or at 16 h (80% for both bile acids).

Bile flow. Bile flow correlated positively with bile acid output during all experimental periods. As shown in Fig. 5, for similar bile acid output, bile flow was significantly higher during replacement with CA than that observed during the secretion of the other bile acids, which did not differ one from the other.

Bile lipid secretion relationship. Fig. 6 shows the effect on the bile acid-cholesterol secretory coupling of the acute pool replacement with DCA, CDCA, CA, and UDCA.

Table II. Hourly Bile Acid Output (μmol) Observed during the Two Experimental Periods

	Depletion					Infusion				
	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Bile acid infused										
DCA (3 patients)	640±342	412±103	431±140	418±100	365±234	753±220	864±177	963±155	1139±440	1208±146
CDCA (3 patients)	555±417	214±110	388±78	274±143	262±70	365±75	906±176	1340±430	1209±457	1218±278
CA (4 patients)	408±209	302±202	301±188	231±142	293±172	264±185	1203±555	1899±779	1662±570	1457±572
UDCA (4 patients)	614±508	362±195	257±85	228±98	315±136	298±71	354±144	367±155	422±146	308±216

Values are expressed as mean±SD. The first hour of each period shows values different from those observed in the following hours of the respective period. This is probably due to the time needed for a steady state to be reached following the complete interruption of the enterohepatic circulation and the start of the infusion, respectively.

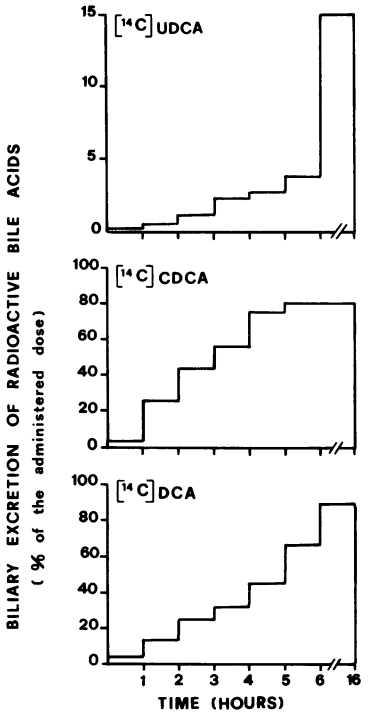


Figure 4. Cumulative biliary excretion of radioactivity at different times from duodenal infusion of individual bile acids labeled with the respective radioactive tracers. Note the different scale used for UDCA. Each curve represents one experiment except for UDCA, which is the mean of two experiments.

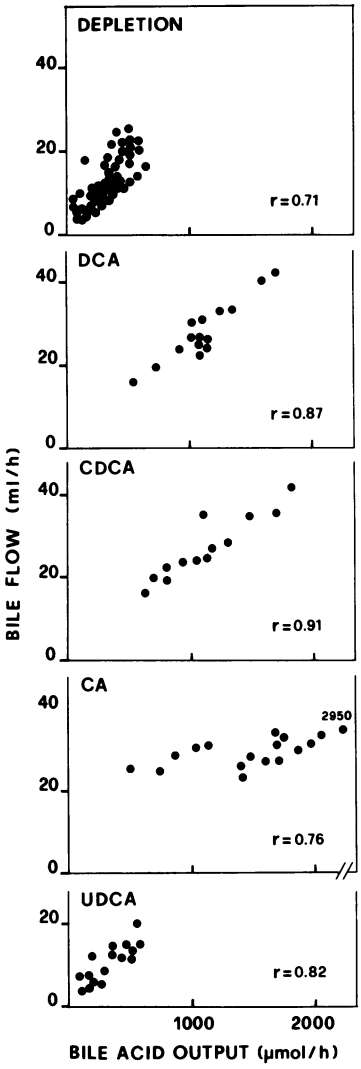


Figure 5. Relationship between bile acid secretion and bile flow during depletion of the bile acid pool and during replacement of the pool with individual bile acids. The best curve fit for the experimental data was a linear function. Regression equations for each set of points were: Depletion: $y = 0.031x + 3.69$; DCA: $y = 0.020x + 3.54$; CDCA: $y = 0.019x + 5.53$; CA: $y = 0.008x + 18.37$; UDCA: $y = 0.021x + 5.73$. The slope of the regression line of the replacement with CA is significantly different from that of the replacement with DCA, CDCA, and UDCA; $P < 0.01$.

A linear relationship between bile acid and cholesterol secretion was evident both in the depletion period when the pool was made by the primary bile acids and in each of the replacement periods with whatever bile acid was infused. In the experimental periods, the y-axis intercept of the regression lines was never significantly different from zero. However, the slopes of the regression lines were significantly different in each period. In fact, the amount of cholesterol secreted for a given bile acid output was higher during DCA secretion than that observed during CDCA followed by CA and UDCA.

In the range of bile acid output obtained in the depletion period, cholesterol bile acid coupling showed values similar to those observed during the secretion of DCA. Fig. 7 shows that, for lecithin as well as cholesterol, a linear relationship with bile acid secretion was observed in all experimental periods. The amount of lecithin coupled per mole of bile acid was significantly greater during replacement with DCA, followed by that observed during replacement with CDCA, CA, and UDCA. Fig. 8 shows the coupling of lecithin to cholesterol secretion. It can be seen that the amount of lecithin per mole of cholesterol was lower during depletion, DCA replacement, and CDCA replacement as compared with that observed during CA and UDCA secretion.

Bile cholesterol saturation. Fig. 9 shows the effect of the infusion of the individual bile acids on the cholesterol saturation of the bile expressed as percent variation of the mean pretreatment value. It can be seen that during the replacement, the saturation index (SI) tended to decrease to reach a plateau. The curves of the replacement periods were shifted progressively downward on the y-axis from DCA to UDCA. Table III reports the mean absolute values of the SIs calculated according to Carey and using the data obtained when the pool was substituted for nearly 80% by the infused bile acid. A significant difference can be noted between the value of the depletion period and those observed during replacement. Among these values, the one observed during DCA secretion is the highest followed by that of CDCA, CA, and UDCA. Only during UDCA, bile appeared to be undersaturated.

Biliary alkaline phosphatase activity. Fig. 10 illustrates the pattern of the biliary alkaline phosphatase (AP) during the different replacement periods expressed as variations of the mean value observed during depletion. AP described a parabolic curve with its zenith of activity exhibited at the second hour after

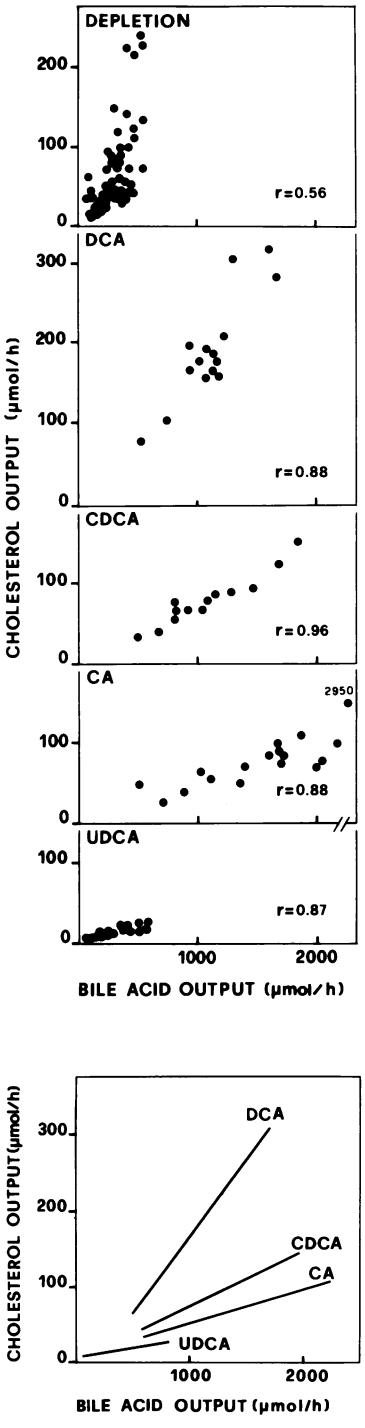


Figure 6. (*Top*) Relationship between biliary bile acid and cholesterol secretion during depletion of the bile acid pool and during replacement of the endogenous pool with individual bile acids. The best curve fit for the experimental data of each period was a linear function. Regression equations for each set of points were: Depletion: $y = 0.222x + 3.54$; DCA: $y = 0.209x - 52$; CDCA: $y = 0.078x - 3.52$; CA: $y = 0.044x + 7.89$; UDCA: $y = 0.030x + 3.24$. The slope of the regression line for each replacement period is significantly different from the others at 1% level at least in the order: PRT > DCA > CDCA > CA > UDCA. (*Bottom*) Slopes of cholesterol-bile acid coupling showing the ordering of biliary cholesterol output in response to the secretion of individual bile acids.

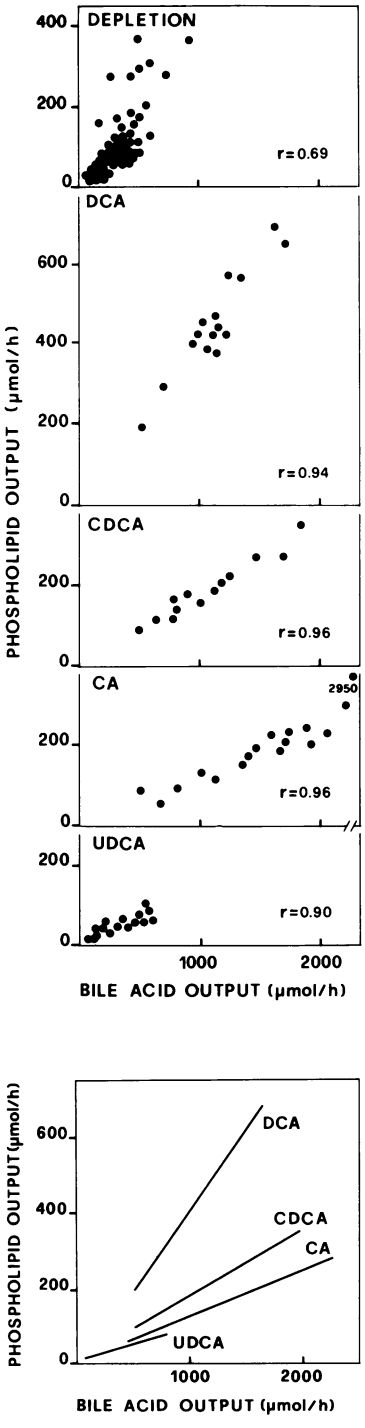
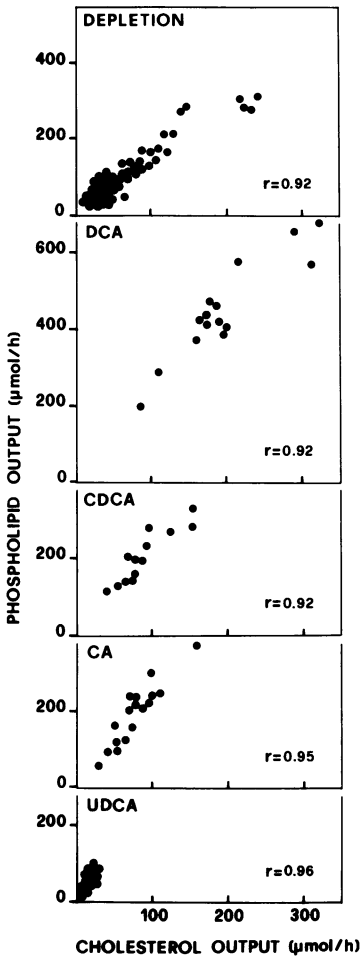


Figure 7. (*Top*) Relationship between bile acid and phospholipid secretion during depletion of the endogenous bile acid pool and during replacement of the pool with individual bile acids. The best curve fit for the experimental data of each period was a linear function. Regression equations were: Depletion: $y = 0.414x - 12.84$; DCA: $y = 0.434x - 32.3$; CDCA: $y = 0.177x + 1.9$; CA: $y = 0.127x - 5.44$; UDCA: $y = 0.122x + 12.58$. The slope of the regression line for each replacement period differs significantly from the others at 1% level at least except for that of CDCA vs. CA. The order is DCA > PRT > CDCA, CA > UDCA. (*Bottom*) Slopes of lecithin-bile acid coupling showing the ordering of biliary lecithin output in response to the secretion of individual bile acids.

starting the infusion of bile acids. Thereafter, it declined to reach the pretreatment level at 4 h. The absolute levels of activity were greater during replacement with DCA followed by CDCA and CA. In contrast with these three bile acids, during replacement with UDCA, the changes of AP activity were negative as compared with pretreatment.



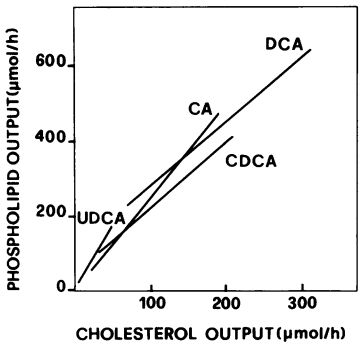


Figure 8. (*Top*) Relationship between cholesterol and phospholipid secretion during depletion of bile acid pool and during replacement of the pool with individual bile acids. The best curve fit for the experimental data of each period was a linear function. Regression equations for each set of points were: Depletion: $y = 1.319x + 20.36$; DCA: $y = 1.766x + 133$; CDCA: $y = 1.794x + 45.74$; CA: $y = 2.508x - 0.073$; UDCA: $y = 3.711x + 3.38$. The slope of the regression line for each period is significantly different from the others at the 1% level at least, except for that of DCA vs. CDCA. The order is UDCA > CA > CDCA, DCA > PRT. (*Bottom*) Slopes of lecithin-cholesterol coupling during individual bile acid secretion. Note the ordering of bile acids, which is the opposite of that of Fig. 6 (*Bottom*) and 7 (*Bottom*).

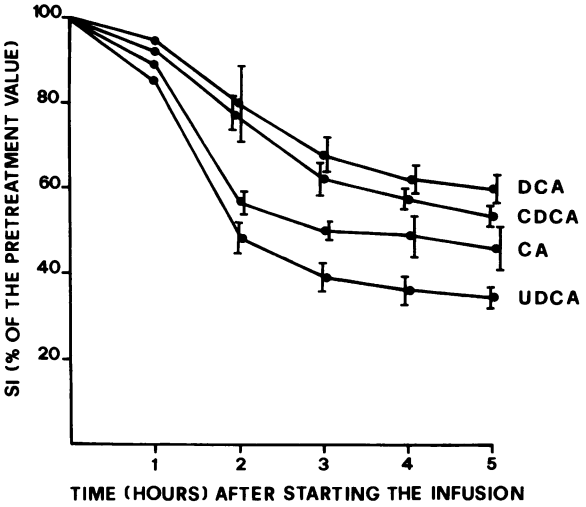


Figure 9. Relationship between changes in cholesterol saturation and time following the infusion of individual bile acids. Saturation is expressed as percent variation from the mean value observed during the depletion period.

Discussion

This study was performed to determine whether changes of bile lipid secretion occur in response to acute changes of bile acid pool composition. The results show that following replacement of the endogenous pool with individual bile acids, the biliary lipid output varies depending on the structure of the bile acid being secreted.

Of the various bile acids investigated, DCA was found to stimulate the highest cholesterol and phospholipid output, followed in decreasing order by CDCA, CA, and finally UDCA. The observed ordering of bile lipid secretion appears to be related to the hydrophilic-hydrophobic balance of the different bile acids in the sense that the secretion of the less hydrophilic (or more hydrophobic) bile acid, such as DCA, was associated with a lipid output far higher than that observed with the more hydrophilic one (UDCA). In vitro studies on model systems have shown that hydrophilicity correlates inversely with the cholesterol-solubilizing capacity of a given bile acid (39); thus, our

Table III. Saturation Indices during Depletion and Bile Acid Pool Replacement with Individual Bile Acids

	Number of observations	SI	*P*
Depletion	52	2.58±0.94	
DCA	15	1.69±0.33	<0.01
CDCA	13	1.45±0.15	0.02
CA	17	1.28±0.23	<0.05
UDCA	16	0.96±0.16	<0.01

The values of the replacement periods are means±SD of all the values calculated when replacement with the infused bile acid was nearly 80%.

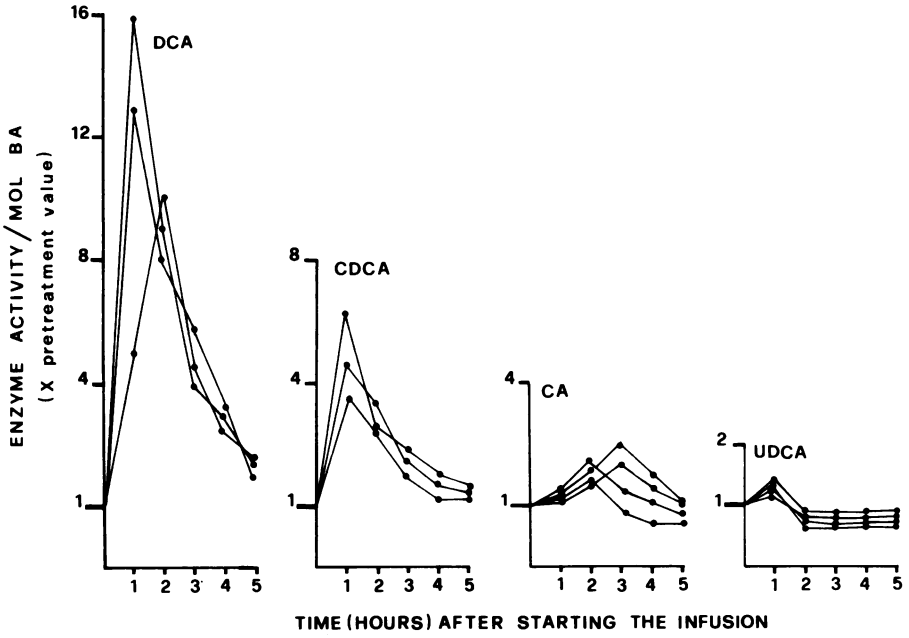


Figure 10. Changes of biliary alkaline phosphatase activity following duodenal infusion of individual bile acids (BA). Each curve represents one subject. Enzyme activity/mol of bile acid secreted is expressed as a variation with respect to the mean values of the depletion period taken as 1.

results would suggest that the solubilizing properties of bile acids could have a direct bearing on the biliary secretion of lipids.

Although the absolute values of both cholesterol and phospholipids were directly proportional to the solubilizing capacity of each of the bile acids, the amount of lecithin coupled to a given output of cholesterol was greater during the secretion of CA and even more so of UDCA than during that of DCA and CDCA. In other words, it seems that increasing the hydrophilicity of the bile acid decreases the capacity to stimulate the secretion of cholesterol more than that of lecithin.

The cholesterol saturation of bile reflected the different lipid secretion obtained in the various experimental periods of the study. Following bile acid infusion, bile saturation tended to decrease as compared with the prereplacement period; however, this decrease was less pronounced during the secretion of DCA and CDCA than during that of CA and UDCA. Only during UDCA secretion did the bile become undersaturated.

In theory, the state of the hepatic cholesterol and phospholipid metabolism preceding the study and/or changes in their hepatic synthesis, which might have occurred as a consequence of bile acid infusion, could have played a role in determining the observed differences of bile lipid secretion. Although the first possibility cannot be controlled, it seems an unlikely theory to us considering that the infusion of different bile acids to individual patients caused a similarly different lipid secretion, regardless of the sequence of administration of the bile acids. As for the second possibility, we believe that for the rapidity with which changes of lipid secretion took place during the infusion, it could be reasonably excluded that synthetic changes had an important role.

Moreover, it has been shown in man that the administration of DCA (28) and CDCA (15–17, 19) seems to be more effective than that of CA (19) and UDCA (16, 21) in suppressing the hepatic synthesis of cholesterol; thus, if biliary secretion was directly influenced by the synthetic rate, we would have expected to find the output of cholesterol to be lower during DCA and CDCA than during CA and UDCA secretion, which is the opposite of what we have observed in our study. While the regulation of biliary lipid secretion remains a complex and multifactorial process, our results point to an immediate effect of bile acid structure.

The results of the present study are partially at variance with those reported by Lindblad et al. (35), Scherstèn et al. (36) and Sama et al. (37). These authors found that UDCA, as in our study, is the least effective in promoting bile lipid secretion whereas CA produced an effect similar or greater than that of CDCA. Different experimental design as well as differences of the investigated subjects may have contributed to this discrepancy; in our opinion, however, the most likely explanation is that in these studies, at variance with ours, during the infusion of CA, a consistent proportion of DCA was present in the pool and this could have concealed the effect of CA.

Our data do not reveal how bile acid structure influences bile lipid secretion since it is not known how and where within the liver cell bile acids interact with the other biliary lipids. In our study, as with lipids, it is clearly shown that alkaline phosphatase, a canalicular membrane constituent, is secreted into the bile in a fashion directly related to the solubilizing capacity of the bile acids. This finding confirms in a more systematic way previous results in man (45, 46) and animals (47) and

suggests that various bile acids could affect membrane constituents to a different extent.

In this context, it is possible that, as firstly suggested by Small (48), biliary lipid secretion may result from bile acid-induced membrane solubilization, and that if the secreted bile acid was more hydrophobic it would remove more lipids from the membranes.

In vitro studies on biological (49) as well as artificial membranes (50) support this view, but clearly more work is needed to elucidate the molecular basis of transport of biliary lipids.

Replacement of the pool with individual bile acids did not seem to alter bile flow consistently with the exception of CA, whose secretion appeared to obligate more water into the bile than other bile acids.

A comment on the bile acid secretory rates and the linear relationship of biliary lipids observed in our study is in order. Bile acid outputs were somewhat low due to our experimental conditions, for which enterohepatic circulation was completely interrupted. In such a situation, the intestine is virtually deprived of bile and orally administered free bile acids are less readily absorbed. This is clearly evident for UDCA, for which the measurements of absorption and biliary output were lower than those for the other bile acids, owing to its poor solubility and hence precipitation at intestinal pH (51).

As for the biliary lipid relationship, this is usually best described by a curvilinear function (8); our data, however, seemed to fit better a linear function either because of the observed secretory values or because we plotted data points representing only those values obtained when most of the pool was made by the infused bile acid. If the results, including those for the depletion period, had been plotted altogether, a curvilinear function would have been fitted as well.

The results of the present study, although obtained in unphysiological conditions, could yield information about the mechanism by which chronic feeding of bile acids induces changes of bile cholesterol secretion and saturation.

In physiological conditions, the bile acid pool of man is made by different proportions of bile acids, each having a given solubilizing capacity. On the basis of our results, we could assume that the influence of the bile acid pool on biliary cholesterol secretion would be the net result of the composite effect of the bile acids present within the pool. Thus, changing the composition of the pool by means of bile acid administration would lead to a decrease or an increase in bile cholesterol depending on the resulting changes in the solubilizing capacity of the pool. For instance, bile desaturation, following UDCA or CDCA administration to man, may be brought about because the pool becomes enriched with weaker detergent, such as UDCA, or because the proportion of stronger detergent, such as DCA, decreases or both. Following this reasoning, the administration of CA, a weak detergent (the terms "detergent" and "lipid solubilizing" are used interchangeably in this paper), should also produce similar effect. In earlier reports, however, it was shown that CA failed to desaturate bile (52–54). In our opinion, the most likely explanation of this failure is that during CA feeding the proportion of DCA in the pool increases and this overcomes the effect of CA. Indeed, when the increase of DCA is prevented by depressing its intestinal formation, the administered CA enriches the pool and bile desaturation ensues (34).

In conclusion, these results would suggest that bile acid pool, by virtue of its chemical physical characteristics, may play a direct role in bile cholesterol secretion. This does not mean that other factors related to cholesterol metabolism could not be relevant as well.

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