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THE SIGNIFICANCE OF BILE ACIDS FOR BILE FLOW AND BILIARY LIPID SECRETION IN MAN

A clinical and experimental study with special reference to biliary cholesterol saturation.

By
Leif Lindblad

Göteborg 1976

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AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen med vederbörligt tillstånd av Medicinska Fakulteten vid Universitetet i Göteborg, kommer att offentligen försvaras i föreläsningssal F 3, Sahlgrenska sjukhuset, fredagen den 29 oktober 1976 kl. 9 F.M.

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GÖTEBORG 1976

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This thesis is based on the following papers:

I Supersaturated bile - Is it due to a metabolic disorder or to an impaired gallbladder? T. Scherstén, E. Cahlin, J. Jönsson, L. Lindblad & Sv. Nilsson. Scand. J. Gastroent. 9, 501-506, 1974.

II Bile flow and biliary lipid secretion following release of biliary obstruction in man. L. Lindblad, J. Hammarsten & T. Scherstén. Scand. J. Gastroent. 10, 633-639, 1975.

III Influence of cholic and chenodeoxycholic acid on canalicular bile flow in man. L. Lindblad & T. Scherstén. Gastroenterology 70, 1121-1124, 1976.

IV Influence of cholic and chenodeoxycholic acid on biliary cholesterol secretion in man. L. Lindblad, K. Lundholm & T. Scherstén. Submitted for publication.

V Incorporation rate in vitro of choline and methyl-methionine into human hepatic lecithins. L. Lindblad & T. Scherstén. Scand. J. Gastroent. in press.

These papers will be referred to in the text by their Roman numerals.

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PREFACE

Knowledge of organ functions under physiological and pathological conditions is a prerequisite for progress in surgery. Surgery today implies a wide range of processes. Preoperative investigations intended to reveal aetiological factors and concomitant interrelated diseases constitute an important part of the surgeons´task.

Several complex factors contribute to the formation of cholesterol gall stones. These factors are only partly mapped out and moreover our knowledge of the basic physiological mechanisms involved in bile flow and biliary lipid secretion in man is limited. Findings from experiments in animals are not always directly transferable to man. Hence, studies in man are necessary to clearify these basic mechanisms and such studies call for access to the biliary tree, which can only be achieved in patients undergoing bile duct surgery.

The present work is an attempt to evaluate some physiological and pathophysiological mechanisms concerning bile flow and biliary lipid secretion in man.

INTRODUCTION

The incidence of gall stones in different populations varies widely, from a low of 1.35 % in Kampala, Uganda, to a high of 73 % in American female Pima indians (cf 28). Direct comparisons between reported incidence figures are difficult to make because of factors such as divergent grouping of patients and varying completeness of the study material. In an autopsy study in Malmö and Prague of all deaths during the years 1963 - 1966 the prevalence of gall stones was similar in the two cities: 29-32 % in males and 52 % in females in the age group 40-99 years (91).

The predominating type of gall stone differs in different parts of the world (81). In western Europe and the USA cholesterol gall stones are most common, constituting from 73 % (85) to 90 % (68) of all stones. Cholesterol gall stones and pigment gall stones probably represent at least two quite different pathological conditions (85). Although most gall stones probably remain undetected because they are asymptomatic (28), gall stone disease constitutes an important economic burden for the community and gives rise to much individual suffering. In Sweden around 27.000 cholecystectomies were performed in 1974. Cholecystectomies constituted 11 % of all operations performed that year. The total duration of stay in hospital for these operations amounted to 207.000 days, representing a cost of about 100 milj. Swedish Crowns per year. The mean period of stay in hospital for uncomplicated cholecystectomy was 7.9 days. The mean period of stay in hospital for patients with chronic cholecystitis, acute cholecystitis and bile duct gall stones was 9.4, 11.7 and 15.7 days, respectively (Scherstén, personal report).

Among possible factors determining the prevalence of gall stones that have been discussed are sex, age, social class and body weight (28, 85, 91). Associations between gall-stone formation and certain diseases have also been reported e.g. Crohn´s disease of the terminal ileum (29) and diabetes (28).

Spontaneous occurrence of gall stones has been reported in many species apart from man (47). Spontaneous cholesterol gall-stone formation in baboons has been used as an experimental model (35). Cholesterol gall-stone formation can be induced in several species, for example in the hamster, prairie dog and Rhesus monkey. Results

obtained from these experimental animal models cannot, however, be directly transferred to man.

It has been clearly established that excess of cholesterol in relation to bile acids and phospholipids in bile is the main physicochemical prerequisite for cholesterol gall-stone formation (3, 65, 66, 74, 75). The biliary lipids form mixed micelles in water with a cholesterol-holding capacity depending mainly on the relative concentrations of bile acids and lecithin. Variation in the amount of solids in bile is of little significance within the range of concentrations existing in human bile (3). The micellar cholesterol-holding capacity is also dependent on the type of bile acid that is taking part in the micelle formation. The fatty acid pattern of lecithin may also play a role for the cholesterol-holding capacity even though this has not been convincingly shown (31, 50, 62).

The limit for biliary micellar cholesterol saturation has been estimated by Admirand & Small (3) and by others (30, 33, 45). Bile supersaturated with cholesterol is not always associated with stone formation, as demonstrated by the finding that cholesterol-supersaturated bile is frequent in healthy humans (33). This may partly be explained by the fact that cholesterol can be held in a metastable solution for some time at concentrations above the micellar concentration (32). Hence, the time bile remains in the gall bladder or the bile ducts may be of great importance. This time can probably be increased because of disturbances in the gall bladder emptying function, during pregnancy (54), after gastric surgery (41) and because of stasis in the bile ducts. Presence of nucleating substances as desquamated cells, bacteria, mucus substances, calcium salts and pigments in the gall bladder has also been discussed (34, 75).

Although many factors may interact in the formation of cholesterol gall stones, the secretion of bile supersaturated with cholesterol seems to be a prerequisite and a common phenomenon (3, 65, 66). Because of this supersaturated bile has been designated "lithogenic bile" (cf 65).

Theoretically, bile can be converted from being unsaturated with cholesterol to being saturated in the gall bladder. This conversion might occur because of deconjugation of bile acids (88), because of bile acid absorption (56) or because of lecithin deacylation or absorption (73). However, in the normal gall bladder the degree of

saturation is rather slightly decreased. The reason for this phenomenon is that bile acids are absorbed to a very slight extent. Intact lecithin is appreciably absorbed (49) but the absorption of cholesterol is higher (48). It has been shown repeatedly that bile supersaturated with cholesterol can be secreted from the liver (51, 67) and at present it is generally accepted that this is the main reason for the occurrence of "saturated" bile (26, 65, 75, 76, 79, 86, 87).

It is now generally accepted that the biliary bile acid secretion rate determines the biliary bile flow, lecithin secretion rate and to some extent also the cholesterol secretion rate. It has been shown in man (51, 67), and later in several animal species, that the degree of cholesterol saturation of hepatic bile is inversely related to the bile acid secretion rate (17, 27, 77, 89). Factors influencing the biliary bile acid secretion rate may thus determine the "saturation" of hepatic bile. Thureborn showed that interruption of the enterohepatic circulation (EHC) of bile acids in man caused a pronounced reduction of the bile acid to cholesterol ratio and the phospholipid to cholesterol ratio in hepatic bile (84). Moreover, bile acids returning to the liver via the EHC exert a negative feedback effect on bile acid synthesis, which was first shown in the rat (7) and later confirmed in man (25, 40, 82). It has been suggested that this inhibitory effect is located at the 7-α-hydroxylation of cholesterol (70). The hepatic synthesis rate of cholesterol - the precursor for bile acid synthesis - is also influenced by the circulating bile acid pool (70). In addition, bile acids are known to stimulate hepatic lecithin synthesis in man (10, 53) and in the rat (4).

The amount of bile acids returning to the liver via the EHC thus influences the degree of cholesterol saturation in hepatic bile. The size of the bile acid pool and the rate of bile acid pool circulation are of importance. Both the size of the pool and its rate of circulation may be influenced by other factors primarily acting on gall bladder function. Among these factors are sex hormones (54), gastrointestinal hormones and gall bladder innervation (41), and eating habits and diet, which can influence the degree and frequency of gall bladder emptying. It has been shown that the hepatic bile in gall stone patients with nonfunctioning gall bladder is less saturated with cholesterol than that of gall stone patients with functioning gall bladder (11).

The enterohepatic circulation of bile acids may also be influenced in the intestine, i.e. by non-nutritive fibres (37) and ileal dysfunction (16).

The regulation of cholesterol and bile acid synthesis may be disturbed, as in dyslipoproteinaemia (36). Moreover, it has been reported that gall stone patients have a higher liver cholesterol concentration, higher 3-hydroxy-3-methylglutaryl-CoA reductase activity (HMG-CoA reductase activity) and lower cholesterol 7-α-hydroxylase activity as compared with normal controls (61). These findings suggest that gall stone disease is a disease of liver metabolism.

The aims of the present investigation were:

1. to study the role of bile acids for bile flow in man,
2. to study the role of bile acids for biliary lipid secretion and to evaluate the role of bile acids for hepatic lecithin synthesis in man, and
3. to evaluate the importance of hepatic secretion of cholic acid (CA) and chenodeoxycholic acid (CDCA) for the degree of biliary cholesterol saturation.

TABLE I

Diagnosis	no of patients			Age (years)	
	male	female	total	Mean (± SD)	range
Cholelithiasis	24	31	55	51 ± 14 (f=48±15, m=54±13)	21 - 80
Gall bladder carcinoma		1	1	64	
Choledochal carcinoma		1	1	59	
Pancreatic carcinoma	2	1	3	79	73 - 83

PATIENTS

Details of the patients included in the study are listed in table I. All the patients taking part in the experiments were thoroughly informed about the details and the aims of the study. Participation in the study was voluntary and the patients were told that they were free to stop the experiment at any time. No complications of the experiments were observed.

METHODOLOGICAL CONSIDERATIONS

Experimental procedures

The time of the experiment (I, III, IV), 7-12 days postoperatively, was chosen because the liver function, bile flow and biliary lipid composition are considered to be restituted and stabilized about 7 days after operation (44, 57, 63, 84). The EHC was kept intact until the experiments were performed and the patients were mobilized and had an ordinary hospital diet. They were fasted for 12 hours before the start of the experiment. In ten patients (I) the EHC was kept intact during the study. In these patients bile samples were taken amounting altogether to not more than 50 ml of bile, which means that less than 10 % of the bile was diverted (51). A diversion of bile of this magnitude does not influence the secretion of biliary lipids (17).

The catheterization technique (I, II, III, IV) in the experiments with interrupted EHC has previously been reported in detail (51). Instead of the t-tube previously used, a babyfeeding-tube (size 8) was inserted in a proximal direction into the common hepatic duct through the cystic duct. It might be questioned whether such a catheter is of sufficient diameter not to influence the bile flow. Since the relationship between the bile acid secretion rate and bile flow was linear throughout the measured range of bile flow rates in all the experiments, it seems unlikely that the catheter limited the flow. The position of the catheters in the bile duct was checked by contrast X-ray examination in all cases. When the balloon catheter was inflated with saline (o.7 - 1.0 ml) the patients described slight discomfort of short duration. During the experiment small amounts of colourless fluid were obtained from the balloon catheter, which had its tip open

distally to the balloon, indicating that the balloon effectively prevented bile from passing the balloon.

Duodenal administration of cholic or chenodeoxycholic acid (III, IV) might cause release of gastrointestinal hormones such as secretin. A release of gastrointestinal hormones would be expected to enhance the ductular bile flow (9) during bile acid administration but this was not observed.

In the ^{14}C-mannitol experiments (III) the ^{14}C-mannitol clearance (C_m) was calculated according to the formula: $C_m = F \cdot \frac{B_m}{P_m}$ (F = bile flow; B_m = DPM in bile; P_m = DPM in plasma). The biliary clearance of mannitol and erythritol has been used as a measure of the canalicular bile flow in different species (9, 20, 21, 57, 90). The method is based on the fact that the hepatocytes are permeable to inert carbohydrates and on the assumption that they are excreted through the bile ducts without passing the ductular membranes (64). Mannitol was chosen as a marker instead of erythritol on the basis of reports from studies in dogs indicating that erythritol, in contrast to mannitol, enters the bile at the secretin responsive area (5).

^{14}C-mannitol in saline was administered intravenously at a constant infusion rate. Thereby, the blood concentration of ^{14}C--mannitol was kept fairly constant, without rapid changes, throughout the experiments. This means that the passage time for the ^{14}C-mannitol molecule from the canaliculi to the extrahepatic bile ducts can be neglected when the clearance is calculated. The liver biopsy technique (V) has previously been described in detail (8). The liver biopsies were taken immediately after opening of the abdominal cavity and the piece of liver was placed in ice-chilled Krebs Ringer Phosphate (KRP) buffer, pH 7.4, and immediately transported to the laboratory for further processing ($<$ 5 min). The conditions for determination of lipid synthesis in liver slices have been described previously (52).

Analytical procedures

Bile acids in bile (I, II, III, IV) were extracted according to the method of Schoenfield et al. (69) and the bile acids were quantitated and separated as described previously (51). Lipid extraction and quantification were performed as described previously (6, 23, 24, 78, 80).

Radioactivity was determined in a Packard Tri-Carb (3320) liquid scintillation spectrometer. Correction for quenching was performed by the external standard method. The incorporation of choline and methyl-methionine into lecithin (V) can be regarded as a special application of a known technique (52).

Statistics

(I, II) Analysis of variance was performed to test the variations in individual biliary lipid concentrations. Bivariate regression was calculated according to the method of least squares. Student´s T-test was applied to test differences between groups.

(III) Linear regressions were calculated according to the method of least squares. Differences between regression coefficients and intercepts were tested by analysis of variance.

(IV) The Mann-Whitney U-test was used to test differences between groups (71). Differences between regression coefficients and intercepts were tested by analysis of variance. Experimental data were tested for best fit to eight equations: $y = a + bx$; $y = a + bx + cx^2$; $y = a + bx + cx^2 + dx^3$; $y = Ae^{bx}$; $y = Ax^b$; $y = Ax^be^{cx}$; $y = a + b/x$; $y = x/(a + bx)$. Curvilinear multiple regression analysis was performed as described by Draper & Smith (19).

(V) The T-test was applied to test differences between groups. To test the difference between two small groups the Wald-Wolfowitz nonparametric test was applied (71).

The choice between parametric and nonparametric tests has in all cases been based on the size of the groups compared. In papers III-V calculations have been performed by means of a programmable computer (Olivetti P652).

RESULTS AND DISCUSSION

1. Bile acids and bile flow (II,III)

During depletion of the bile acid pool (secretion of mixed bile acids) as well as during CA administration (secretion of mainly CA; 73 ± 3 % (SEM)) and CDCA administration (secretion of mainly CDCA; 88 ± 2 % (SEM)) the relationships between the bile acid secretion rate and bile flow and bile acid secretion rate and biliary mannitol clearance were of the same linear type (III).The regression coefficients in these relationships were not significantly different from each other, indicating that CA, CDCA or a mixture of CA, CDCA and deoxycholic acid promoted the same bile flow per umol of bile acid (≈ 0.011 ml . $umol^{-1}$). The calculated bile acid independent canalicular flow (≈ 0.17 ml . min^{-1}) and the ductular flow (≈ 0.08 ml . min^{-1}) did not differ significantly between the three different experimental conditions. The interindividual variations were small. The calculated bile acid independent flow (≈ 0.025 ml . min^{-1}) and bile acid dependence are in striking agreement with the previous findings of Scherstén et al (67) who reported a bile acid independent flow of 0.20 ml . min^{-i} and bile acid dependency of bile flow of 0.014 ml . $umol^{-1}$ bile acid.

The average bile flow at intact EHC in this study was 0.43 ml . min^{-1} which is in good agreement with previous reports of 24 h bile secretion of 500 - 600 ml in cholecystectomized patients (44, 84). At this bile flow the calculated ductular contribution is 20 % and canalicular contribution 80 %, half of which is bile-acid independent. All patients in the study had a normal biliary tree as judged from peroperative cholangiograms and it seems likely that the canalicular and ductular contributions to bile flow are of the same magnitude even in normal subjects (Fig. 1).

Boyer and Bloomer and Prandy et al (9, 57) have reported corresponding values of canalicular and ductular bile flow in man. The small discrepancies between their figures and ours can be ascribed to differences in methods and the patients studied.

In the postcholestatic patients (II) statistically significant linear relationships between the bile acid secretion rate and bile flow were obtained in four patients, indicating that bile acids determine bile flow even at the low bile acid secretion rates obtained

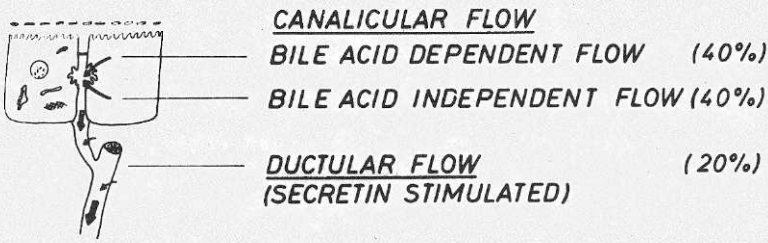

Fig. 1: Relative contributions to hepatic bile flow from canalicular bile-acid-dependent and independent flow and from ductular flow during intact enterohepatic circulation of bile acids.

in these patients. Great interindividual variations in bile acid dependency and calculated bile acid independent flow were registered. These variations may partly be ascribed to interindividual differences in the width of the bile ducts (58).

2. Bile acids and biliary secretion of lipids

A. Biliary bile acids (I, IV)

During duodenal administration of CA or CDCA to patients with depleted bile acid pool (IV) the biliary bile acid secretion rate increased. After 4 h of CA administration this acid constituted 69 ± 5 % (SEM) of the total bile acids in bile. The corresponding value for CDCA in the CDCA experiments was 87 ± 2 % (SEM). Both during CA and CDCA infusion the glycine to taurine ratio increased from 4.5 ± 0.6 (SEM) to 15.6 ± 2.9 (SEM). This increase can be explained by the greater hepatic availability of glycine as compared to taurine (cf 22).

It has been suggested that the inhibitory effect of bile acids returning to the liver via EHC on the biliary bile acid synthesis is different for CA and CDCA, owing to different effects on the rate-limiting step in bile acid synthesis (cholesterol-7-α-hydroxylase) (70). This difference in inhibitory effect has, however, not been found by Danielsson (14). The reason for this discrepancy may be due to differences in methodology and to diurnal variations (13). The

TABLE II Serum bile acid concentration before and after bile acid pool depletion and after CA and CDCA administration. (Bile acids were extracted according to Makino and Sjövall (47) and quantitated by use of fluorescencespectrophotometry (46).)

Depletion of bile acid pool		4 hr CA infusion	4 hr CDCA infusion
9 AM	1 PM	5 PM	5 PM
5.4 ± 0.5	3.7 ± 0.7	7.4 ± 1.0	23.1 ± 5.8
(n = 13)	(n = 13)	(n = 7)	(n = 6)
	$P < 0.02$	$P < 0.05$	$P < 0.001$

4 hr CA vs 4 hr CDCA, $P < 0.03$

The Mann-Whitney U -test was applied (71).

biliary bile acid concentration did not show statistically significant diurnal variations in the ten gall stone patients studied (I). During bile acid pool depletion (IV) there was a statistically significant decrease in the serum bile acid concentration in systemic blood. During administration of CA or CDCA there was a statistically significant increment in the serum bile acid concentrations in systemic blood. This increase was more pronounced in the CDCA experiments than in the CA experiments (Table II). The mean biliary bile acid secretion rate was slightly higher in the CDCA experiments (11.9 $\pm$ 0.9 (SEM) umol . min^{-1}) as compared with the CA experiments (8.8 $\pm$ 0.8 (SEM) umol . min^{-1}) ($P < 0.01$). Factors influencing the intestinal and hepatic uptake of bile acids are probably the main determinants for these variations. In a current study we found diurnal variations in serum bile acid concentrations in portal and systemic blood in five patients supporting this hypothesis (39). In these patients the pattern of variation was similar in portal and systemic blood with postprandial elevations (Fig. 2).

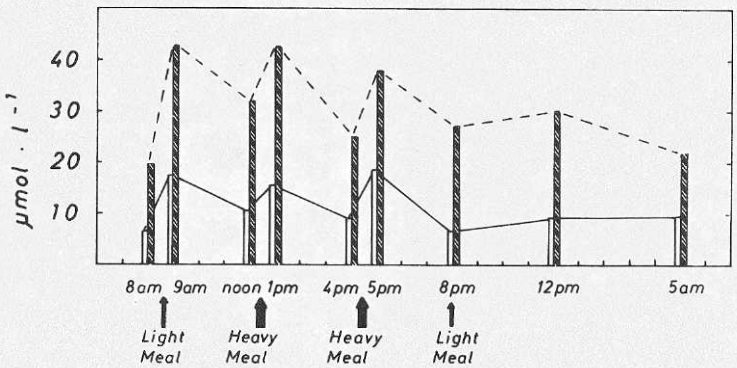

Fig. 2: Mean values of bile acid concentrations in systemic (▯) and portal (▨) serum in five patients - diurnal variation.

B. Biliary lecithin (I, II, IV, V)

A curvilinear relationship between bile acid and lecithin secretion rates with the best fit to a parabolic function was obtained in the three experimental situations - depletion of the bile acid pool ($r = 0.91$), CA infusion ($r = 0.95$) and CDCA infusion ($r = 0.85$)(IV).

The y-intercept at extrapolation to zero bile acid secretion rate was not statistically significantly different from zero, indicating that lecithin is not secreted without bile acids. In the postcholestatic situation (II) the relationship between the bile acid secretion rate and the lecithin secretion rate showed the best fit to a linear function ($r = 0.74$). This discrepancy may be explained by the smaller range of bile acid secretion rates in these patients. The present data confirm previous reports of the role of bile acids in determining lecithin secretion (51, 67) and this role seems to be valid even in the postcholestatic situation. Furthermore, the mean lecithin secretion rate per umol of bile acids was statistically significantly higher during CA secretion (0.45 ± 0.03 (SEM) umol lecithin . umol bile acids^{-1}) than during CDCA secretion (0.30 ± 0.03 (SEM) umol lecithin . umol bile acids^{-1})($F = 13.7$, $P < 0.01$). It has been shown that bile acids exert a stimulatory effect on hepatic lecithin synthesis (4, 10) and that bile acids returning to the liver influence the synthesis of biliary lecithin (53). Evidence has been presented that biliary lecithins are mainly derived from the CDP choline (CytidineDiPhosphate) synthesis pathway (2). In view of these results a stimulatory effect of bile acids on the incorporation of choline into lecithin would have been expected. In the present study we did not find any effect of bile acids on the in-vitro rate of incorporation of choline into hepatic lecithins (V). Nor did we find an effect of bile acids (CA or CDCA) on the rate of incorporation of methyl--methionine into lecithins. The reason for their lack of stimulation is not known. One possibility is that the bile acids might exert their stimulation on earlier steps in the synthesis pathway. Whatever the reason may be, the different effects of CA as compared to CDCA on biliary lecithin secretion rate cannot be explained by the present results.

C. Biliary cholesterol (I, II, IV)

The secretory association between bile acid secretion and cholesterol secretion could be demonstrated during bile acid pool depletion and during cholic acid administration (IV). The relationship between bile acid and cholesterol secretion rates was curvilinear with the best fit to a hyperbolic function during bile acid pool depletion ($r = 0.70$) and during mainly CA secretion ($r = 0.86$). This type of function indicates a theoretical maximum for the cholesterol secretion rate,

which was calculated to be 2.8 umol . min^{-1} and 8.3 umol . min^{-1} for bile acid pool depletion and CA secretion, respectively. Extrapolation to zero bile acid secretion rate in these experiments gave a y-intercept which was not statistically significantly different from zero, indicating that cholesterol is not secreted without bile acids.

In the postcholestatic situation (II) a linear relationship between the bile acid secretion rate and the cholesterol secretion rate was obtained ($r = 0.78$), with a y-intercept not statistically significantly different from zero. The relatively narrow range of bile acid secretion rates in these postcholestatic patients may explain this difference in the type of function of the relationships between the bile acid and cholesterol secretion rates in the different experimental situations.

During CDCA infusion no statistically significant relationship between the bile acid and cholesterol secretion rates was found. Long-term administration of CDCA reduces the hepatic secretion of cholesterol (1, 38) and it has been suggested that the mechanism for this reduction might be decreased activity of HMG-CoA-reductase, the rate-limiting enzyme in cholesterol synthesis (12, 60). It cannot be determined whether such a mechanism is of any importance during short-term administration of CDCA and thus of any relevance for our results.

In these studies of the diurnal variation of biliary lipid concentrations (I) statistically significant variations were found for cholesterol. The values during the day were lower than those in the night. No statistically significant variations could be shown for bile acids or lecithin. This finding is in line with the strong correlation between bile acid and lecithin secretion rates as compared with the weaker correlation between bile acid and cholesterol secretion rates.

During bile acid pool depletion and during biliary secretion of, mainly, cholic acid (IV) the correlation between the lecithin secretion rate and the cholesterol secretion rate was strong, with the best fit to the parabolic function ($r = 0.72$ and $r = 0.94$, respectively). The multiple correlation coefficient was not increased by addition of the bile acid secretion rate as another independent variable. In the depletion experiments the correlation between the

lecithin and cholesterol secretion rates was even stronger at bile acid secretion rates giving cholesterol-supersaturated bile (< 14.4 umol . min^{-1}) ($r = 0.80$). During mainly CDCA secretion a statistically significant curvilinear relationship was found between the lecithin and cholesterol secretion rates, with the best fit to a parabolic function ($r = 0.50$). Addition of the bile acid secretion rate as a second independent variable to this relationship significantly raised the multiple correlation coefficient ($R = 0.71$). This means, in statistical terms, that the lecithin and bile acid secretion rates together accounted for 50 % of the total variation in the cholesterol secretion rate. At bile acid secretion rates (mainly CDCA) giving bile supersaturated with cholesterol (< 10.7 umol . min^{-1}) the correlation between the lecithin and cholesterol secretion rates was strong ($r = 0.80$). Within this range of bile acid secretion rates the correlation coefficient was not significantly changed by addition of the bile acid secretion rate as a second independent variable.

The biological implications of these statistical relationships are by no means clear. However, they suggest that the secretory association between lecithin and cholesterol is strong and that it is stronger at low bile acid secretion rates and during CDCA secretion. To summarize, it may be assumed that the bile acids to a certain degree determine cholesterol secretion directly but also indirectly via the association between them and the lecithin secretion rate.

In the postcholestatic situation (II) a curvilinear relationship with the best fit to a parabolic function was found between the lecithin and cholesterol secretion rates ($r = 0.75$). Addition of the bile acid secretion rate as a second independent variable significantly raised the multiple correlation coefficient ($R = 0.84$). The basic secretion mechanisms in the postcholestatic situation thus seem to be unaffected by the disease. On the basis of the present results and calculations the role of lecithin for the biliary cholesterol secretion cannot be assessed.However, they imply that lecithin may at least partly determine the mass of cholesterol which is secreted at low bile acid secretion rates. This interpretation finds some support in the recent work by Robins and Armstrong,in which manipulation of the lecithin secretion rate only affected the rate of cholesterol secretion (59).

3. Bile acids and biliary cholesterol saturation

The degree of cholesterol saturation (I, II, IV) can be expressed as the molar ratio $\frac{\text{cholesterol}}{\text{BA + lecithin}}$. The approximate limit for cholesterol solubility according to Admirand & Small (3) expressed in this way is 0.10. From a physicochemical point of view this limit is probably an overestimation of the cholesterol-holding capacity. According to Hegardt & Dam, Holzbach et al, and Mufson et al (30, 33, 45) the true limit is about 0.6 within the range of bile acid concentrations in human hepatic bile. However, if the transient metastable phase (32) is taken into account microcrystallization of cholesterol in human hepatic bile probably does not occur at molar ratios lower than 0.10.

The relationship between the bile acid secretion rate and the ratio of the cholesterol secretion rate and the sum of the bile acid and lecithin secretion rates was studied.

In all the experimental situations (I, II, IV) a statistically significant relationship was found between the bile acid secretion rate and the degree of cholesterol saturation. The type of function varied between the groups but in all cases the relationship was inverse, i.e. cholesterol saturation decreased with increasing bile acid secretion rate.

During bile acid pool depletion (mixed biliary bile acids), during CA administration(biliary bile acids mainly consisting of CA) and during CDCA administration (biliary bile acids mainly consisting of CDCA), the cross-over point for the saturation limit according to Small was 14.4 $\pm$ 1.6 (SEM), 15.6 $\pm$ 0.5 (SEM) and 10.7 $\pm$ 0.3 (SEM) umol bile acid . min^{-1}, respectively (Fig. 3). The cross-over point during mainly CDCA secretion was statistically significantly at a lower bile acid secretion rate than for the two other experimental situations ($P < 0.01$).

In five gall stone patients, who were studied during depletion of the bile acid pool and subsequent duodenal administration of a mixture of cholic and chenodeoxycholic acid (I), the corresponding cholesterol saturation cross-over limit was 21.7 $\pm$ 5 (SEM) umol . min^{-1}.

The diurnal variation of biliary cholesterol saturation showed a similar pattern for nine out of ten patients (I). These nine patients had a higher cholesterol saturation degree during night-time as compared to day-time. Two patients produced bile supersaturated with cholesterol during the entire 24 h period. Seven patients

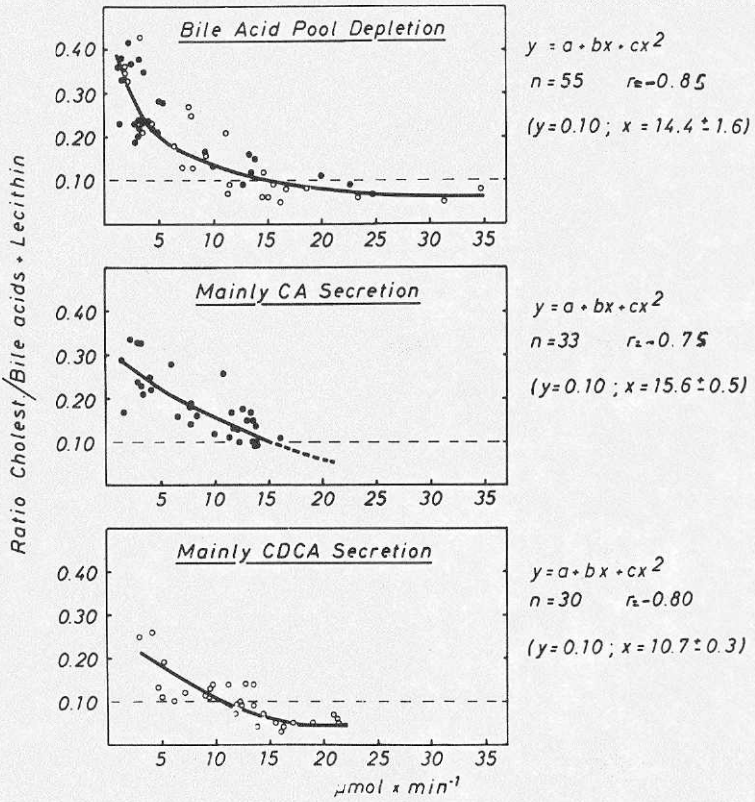

Fig. 3: Relationships between the bile acid secretion rate and cholesterol saturation during depletion of the bile acid pool (mixed bile acid secretion), during duodenal infusion of cholic acid (mainly cholic acid secretion) and during duodenal infusion of chenodeoxycholic acid (mainly chenodeoxycholic acid secretion) after bile acid pool depletion. The dashed line indicates the approximate limit of cholesterol solubility according to Admirand and Small (3).

produced bile supersaturated with cholesterol most of the time (13 h) and in these patients the bile was unsaturated with cholesterol only for one period during the day. In five cholesterol gall stone patients with functioning gall bladder before the operation the degree of cholesterol saturation of hepatic bile showed a significant decrease postoperatively. This finding is in line with previous reports (69, 72). In spite of the postoperative "normalization" of cholesterol saturation these gall stone patients produced supersaturated bile most of the time. For the time being we cannot determine the bile acid secretion rate at which the cholesterol saturation limit is crossed in normals. The present findings (IV) that the cholesterol saturation limit is crossed at a lower bile acid secretion rate when the bile acids consist mainly of CDCA as compared to CA or mixed bile acids are in line with the reports of effects of CDCA administration on biliary cholesterol saturation (1, 15, 38, 43, 55, 82, 83). It must be emphasized that the present experiments differ in one important respect from the studies described, viz the administration time period. Moreover, in the present experiments the bile acid pool was almost completely depleted when CDCA was administered. The mechanisms for cholesterol desaturation after CDCA administration in these short--term experiments may be quite different from the mechanisms for cholesterol desaturation during long-term administration of CDCA. When the cholesterol saturation line was crossed after two hours of CDCA administration, CDCA constituted 79 per cent of the bile acids. This is in good agreement with the findings of a saturation limit cross-over at approximately 70 per cent in 80 patients during CDCA treatment (18).

General conclusion

1. The two primary bile acids promote the same canalicular bile flow.
2. Cholic acid secretion promotes a higher lecithin secretion rate than chenodeoxycholic acid does.
3. Biliary cholesterol secretion in man is related to both the bile acid secretion rate and the lecithin secretion rate.
4. Cholic acid secretion promotes a higher cholesterol secretion rate per umol of secreted bile acid than chenodeoxycholic acid does.
5. The mass of cholesterol secretion may be dependent on the secretion rate of lecithin, especially during periods of low bile acid secretion rates and during chenodeoxycholic acid secretion.

1. Bile flow in man is made up of a bile-acid-dependent and an independent canalicular flow and a bile-acid-independent ductular flow. During intact enterohepatic circulation of bile acids the canalicular flow constitutes 80 per cent, half of which is bile--acid-dependent, and the ductular flow constitutes 20 per cent. Cholic and chenodeoxycholic acid promoted the same canalicular bile flow in gall stone patients.

   Bile acids determine bile flow in the early phase after release of complete extrahepatic bile duct obstruction.

2. Bile acids determine the biliary secretion of lecithin in gall stone patients during biliary bile acid secretion of mixed bile acids, mainly cholic acid and mainly chenodeoxycholic acid. More lecithin is secreted per umol of cholic acid than per umol of chenodeoxycholic acid after administration of cholic and chenodeoxycholic acid, respectively, to gall stone patients with depleted bile acid pool.

   The role of bile acids as a determinant for the biliary cholesterol secretion rate is more pronounced when the biliary bile acids mainly consist of cholic acid than when they mainly consist of chenodeoxycholic acid.

   The role of bile acids as a determinant for biliary lecithin and cholesterol secretion is maintained in the postcholestatic phase. Biliary lecithin secretion influences the mass of biliary cholesterol secretion in gall stone patients during bile acid pool depeltion and when the biliary bile acids mainly consist of cholic acid and of chenodeoxycholic acid. This effect is maintained in the postcholestatic phase after release of extrahepatic biliary obstruction.

   Bile acid stimulation of the rate of incorporation of choline and methyl-methionine into human hepatic lecithins in vitro could not be demonstrated.

3. The degree of cholesterol saturation was inversely related to the rate of biliary secretion of bile acids. The bile acid secretion rate at which the cholesterol saturation limit was reached was significantly lower when the biliary bile acids mainly consisted of chenodeoxycholic acid than when they mainly consisted of cholic acid in gall stone patients.

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