Gallbladder function, cholesterol stones, and bile composition

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SUMMARY Gallbladder bile obtained at operation from five patients with no symptoms of biliary disease was undersaturated with cholesterol in every case. However, gallbladder bile from patients with stones composed of 97-100% crystalline cholesterol was on average just saturated with cholesterol when the gallbladder was functioning and undersaturated when it was not. Regardless of gallbladder function, the patients with stones had on average significantly more cholesterol in their bile than the control group, but the differences between the mean composition of bile from functioning and non-functioning gallbladders were not significant. Common duct bile from patients with non-functioning and functioning gallbladders was on average supersaturated with cholesterol, but there was significantly more bile salt and significantly less cholesterol in the bile from patients with non-functioning gallbladders. Only in the case of patients with functioning gallbladders did the mean composition of the common duct and gallbladder biles differ significantly. The former contained significantly more cholesterol and less bile salt than the latter. It is suggested that patients with non-functioning gallbladders may be 'autocholecystectomised' with the duct bile reverting to a more 'normal' composition.

Small and Rapo (1970) and Vlahcevic *et al.* (1970) showed that hepatic bile in their group of patients undergoing cholecystectomy for cholesterol gallstones was always supersaturated with cholesterol. Other workers, however, have sometimes found hepatic bile of normal composition in similar stone-formers under the same conditions (Mackay *et al.*, 1972; Smallwood *et al.*, 1972; Cahlin *et al.*, 1973). Such different results could reflect variations in the methods used in measuring gallstone and bile composition, or, represent real differences in the groups of patients studied, since production of a supersaturated bile by the liver may not always be the primary abnormality in cholesterol gallstone formation.

In order to understand gallstone formation, bile composition must be known in different circumstances. Few (Bell *et al.*, 1973; Cahlin *et al.*, 1973) studies have attempted to relate bile composition and gallbladder function, so we have investigated this in patients with pure cholesterol stones (97-100% crystalline cholesterol).

Methods

Thirty-two patients undergoing surgery for cholesterol gallstones formed the basis of this study. Preoperatively, liver function tests and serum cholesterol estimations were performed on all patients. Gallbladder function was assessed by oral cholecystography. At operation the gallbladder was aspirated completely to avoid stratification errors (Tera, 1960), having first clamped the cystic duct to minimize mixing of gallbladder bile with that from the common duct. Thereafter, the cystic duct was divided and bile obtained by cannulation of the common duct before the performance of operative cholangiography. Of 32 gallbladders aspirated, four contained insufficient bile for analysis, and another five contained white bile and these were excluded from this study. Bile from the common bile duct was not obtained from 18 patients for technical reasons. After cholecystectomy, stones were removed from the gallbladder for subsequent analysis.

At the conclusion of the operation, bile samples were stored at -14 °C. Before chemical analysis, all the samples were thawed slowly to room temperature and vigorously shaken. Total bile salts were estimated by the steroid dehydrogenase method

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of Talalay (1960) as modified by Admirand and Small (1968). Cholesterol concentrations were estimated by the methods described by Babson *et al.* (1962) and Leffler and McDougald (1963). Phospholipids were estimated as inorganic phosphorus (Fiske and Subbarow, 1925), after extraction of the bile with chloroform and methanol, and digestion of the lipids with sulphuric acid. The results were expressed as percentages of the total molar concentration of cholesterol, phospholipid, and bile salts, and plotted on a three-component diagram showing the micellar zone of cholesterol solubility as determined by Admirand and Small (1968).

The gallstones were soaked in formaldehyde for 24 hours, washed well with water and air dried. They were analysed by the x-ray powder method using the techniques described by Sutor and Wooley (1969, 1971).

Phospholipid and bile salt results were statistically analysed by multiple comparisons at the 5% level

(Scheffé, 1959), while the cholesterol results were analysed using Welch's approximate *t* test on the transformed data (Scheffé, 1970).

Bile was obtained only from the gallbladders of five patient undergoing operations for other conditions—namely, duodenal ulceration, Crohn's disease, carcinoma of the stomach, and pancreatic disease (two cases). None of these patients had symptoms of biliary disease.

Results

No patient had any biochemical evidence of altered or impaired liver function or an abnormal serum cholesterol.

CONTROLS

The mean molar percentage composition of gallbladder bile from the five control subjects is given in Table 1 together with other results from the

Author	No.	Origin of sample	Percentage molar lipid composition (mean $\pm$ SEM)		
			Cholesterol	Phospholipids	Bile salts
Present study	5	Gallbladder bile	5.1 $\pm$ 0.4	11.3 $\pm$ 2.9	83.6 $\pm$ 2.7
Simmons <i>et al.</i> (1972)	10	Duodenal aspirate	8.0 $\pm$ 1.2	14.0 $\pm$ 2.5	78.0 $\pm$ 3.4
Mackay <i>et al.</i> (1972)	21	Duodenal aspirate	5.1 $\pm$ 0.4	18.7 $\pm$ 1.5	76.1 $\pm$ 1.7
Heller and Bouchier* (1973)	12	Gallbladder bile	6.9 $\pm$ 1.0	17.6 $\pm$ 0.8	75.3 $\pm$ 1.6
Vlahcevic <i>et al.</i> (1972)	7	Duodenal aspirate	7.9 $\pm$ 1.0	23.2 $\pm$ 3.4	68.9 $\pm$ 3.7

Table 1 Percentage molar lipid composition in control subjects

*SEM calculated from original data.

Authors	Gallbladder function	No.	Percentage molar lipid composition (mean $\pm$ SEM)						
			Gallbladder bile			Common duct bile			
			Cholesterol	Phospholipids	Bile salts	No.	Cholesterol	Phospholipids	Bile salts
Present study	Yes	15	9.2 $\pm$ 0.7	14.3 $\pm$ 2.2	76.4 $\pm$ 2.4	5	22.9 $\pm$ 5.6	13.9 $\pm$ 3.1	63.2 $\pm$ 4.9
Present study	No	8	8.4 $\pm$ 1.5	16.2 $\pm$ 2.9	75.4 $\pm$ 3.8	9	10.7 $\pm$ 1.2	11.7 $\pm$ 2.6	77.6 $\pm$ 3.3
Vlahcevic <i>et al.</i> * (1970)	Not specified	14	12.5 $\pm$ 1.1	23.8 $\pm$ 1.3	63.7 $\pm$ 1.9	11	14.5 $\pm$ 1.5	23.9 $\pm$ 1.6	61.6 $\pm$ 2.4
Small and Rapo (1970)	Yes	18	8.6 $\pm$ 0.5	19.7 $\pm$ 0.4	71.6 $\pm$ 0.6	21	16.6 $\pm$ 1.2	25.1 $\pm$ 1.5	58.2 $\pm$ 2.5
Mackay <i>et al.</i> (1972)	Yes	17	7.2 $\pm$ 0.5	18.6 $\pm$ 1.5	74.2 $\pm$ 1.8	17	9.1 $\pm$ 0.7	21.8 $\pm$ 1.7	69.1 $\pm$ 2.2
Vlahcevic <i>et al.</i> (1972)	Yes	8	14.9 $\pm$ 3.3	25.8 $\pm$ 2.9	59.3 $\pm$ 4.5	8	15.5 $\pm$ 3.8	25.4 $\pm$ 4.7	59.1 $\pm$ 6.5
Simmons <i>et al.</i> (1972)	Yes	—	—	—	—	23	13.0 $\pm$ 1.1	14.0 $\pm$ 1.0	73.0 $\pm$ 1.3
Heller and Bouchier* (1973)	Yes	16	8.6 $\pm$ 1.0	17.6 $\pm$ 1.5	73.7 $\pm$ 1.9	—	—	—	—

Table 2 Percentage molar lipid composition in gallbladder and common duct bile of cholesterol stone-formers

*SEM calculated from original data.

Fig. 1 Mean $\pm$ SEM and individual results for biliary lipid composition in gallbladder bile from five control subjects

Fig. 2 Mean $\pm$ SEM for biliary lipid composition in gallbladder and common duct bile from patients with cholesterol gallstones

literature. Only one of these refers to bile obtained by the same method, the remainder being an analysis of duodenal aspirate. The individual results are plotted in Fig. 1 together with the mean and one standard error. All of these points were within the

micellar zone representing the composition of solutions undersaturated with respect to cholesterol.

STONE-FORMERS

The mean molar percentages for the three main

biliary lipids in gallbladder and common duct bile for patients with functioning and non-functioning gallbladders are given in Table 2 together with some other published results. Our mean values with one standard error are plotted in Fig. 2. The points representing the mean composition of the common duct bile from patients with either functioning or non-functioning gallbladders fell outside the micellar zone, the latter point lying closer to the boundary. Bile from functioning gallbladders had a mean value which was on the boundary of the micellar zone and was thus just saturated with cholesterol, while that from non-functioning gallbladders had a mean composition represented by a point lying just within the micellar zone.

There was, however, considerable variation in the individual values. In the group with functioning gallbladders, the composition of 60% gallbladder biles and 100% common duct biles fell outside the micellar zone, while, in the group with non-functioning gallbladders, the composition of 50% gallbladder biles and 78% common duct biles were outside this area.

Table 3 shows the statistical significance in a comparison of the mean molar percentages of the three main biliary lipids. There was significantly more cholesterol in the gallbladder bile of stone-formers than in that of the controls regardless of gallbladder function ($P < 0.001$ for patients with functioning and $P < 0.05$ for those with non-functioning gallbladders). Comparing the bile from the common duct and the bile from the gallbladder in stone-formers with functioning gallbladders, there was significantly more cholesterol ($P < 0.01$)

and less bile salt ($P < 0.05$) in the common duct bile. Similarly, there was significantly more cholesterol ($P < 0.05$) and less bile salt ($P < 0.05$) in the common duct bile of stone-formers with functioning gallbladders as compared with common duct bile from patients with non-functioning gallbladders.

Discussion

This study shows that the composition of the common duct bile in patients with cholesterol gallstones is potentially lithogenic, as is the composition of the gallbladder bile of those patients whose gallbladder is functioning. However, if the gallbladder is non-functioning, the bile contained within it is more 'normal'. When points representing the molar ratios of the lipids in these biles are plotted on triangular coordinates, only the biles from the gallbladders of patients with non-functioning gallbladders fall within the micellar zone of cholesterol solubility. Despite considerable variation in individual cases, it is evident from our results that the difference chiefly responsible for this abnormality is a relative increase in the proportion of cholesterol and a fall in the proportion of bile salt from the more normal levels seen in the controls. The molar ratios seen in this series for control patients are similar to those reported by others (Table 1), although there is a somewhat higher proportion of bile salt and lower proportion of phospholipid. However, the analyses in other series were performed chiefly on bile obtained by duodenal aspiration and are not strictly comparable. Table 2 shows that the biles from patients with cholesterol stones also have rather more bile salt and rather less phospholipid but otherwise compare well in most circumstances with bile compositions reported by others. The striking difference is the considerable increase in the cholesterol content of the bile from the common duct in our patients with functioning gallbladders. This finding is unlikely to be due to differences in methodology, since the analyses compare well in other circumstances. However, it is possible that cholesterol crystals may have been present in the bile from the common duct of patients with functioning gallbladders and not filtered before analysis. Van der Linden and Nakayama (1974) have shown that an excess of crystals may be present in the hepatic bile of such patients compared with patients whose gallbladder is not functioning.

In patients forming cholesterol gallstones, the cholesterol content of gallbladder bile is significantly greater than normal controls whether the gallbladder is functioning ($P < 0.001$) or non-functioning ($P < 0.05$). Normal common duct bile could not be obtained. Common duct bile from patients with

Compared groups	Level of significance		
	Cholesterol	Phospho-lipids	Bile salts
Control, GB-bile vs Functioning GB, GB-bile	$P < 0.001$	NS	NS
Control, GB-bile vs Non-functioning GB, GB-bile	$P < 0.05$	NS	NS
Functioning GB, GB-bile vs Functioning GB, CBD-bile	$P < 0.01$	NS	$P < 0.05$
Non-functioning GB, GB-bile vs Non-functioning GB, CBD-bile	NS	NS	NS
Functioning GB, CBD-bile vs Non-functioning GB, CBD-bile	$P < 0.05$	NS	$P < 0.05$
Non-functioning GB, CBD-bile vs Functioning GB, GB-bile	NS	NS	NS
Non-functioning GB, GB-bile			

GB: gallbladder; CBD: common bile duct

Table 3 Statistical testing between individual groups

GB: gall bladder. CBD: common bile duct.

functioning gallbladders contained significantly more cholesterol than the gallbladder bile ($P < 0.01$), but no such difference occurred in patients with non-functioning gallbladders (Table 3). These statistical differences, however, do not necessarily indicate the lithogenic potential of bile. It is only on consideration of the phase diagrams (Figs. 1 and 2), that it is evident that the most grossly lithogenic bile comes from the common duct in patients with functioning gallbladders. Since all patients had virtually pure cholesterol stones—and can therefore be presumed to be a population virtually with the same defect of bile composition—the conclusion must be that progressive deterioration in gallbladder function has the effect of reducing the lithogenic potential of common duct bile.

Admirand and Small (1968) suggested that the lithogenic changes in gallbladder bile composition could be detected in all cases, but others have been unable to confirm this (Smallwood *et al.*, 1972; Heller and Bouchier, 1973). In this series only 60% of the gallbladder biles tested were potentially lithogenic, although 78% of the common duct biles when the gallbladder was non-functioning and 100% of the common duct biles when the gallbladder was functioning had molar ratios which could be plotted above the micellar line. These findings do, however, support the view that the primary biochemical abnormality occurs during the hepatic production of bile (Small and Rapo, 1970; Vlahcevic *et al.*, 1970). Mackay *et al.* (1972), while supporting this view, point out that there must be other factors of importance since, although most abnormal biles contain more cholesterol and less bile salt, many have a composition still within the micellar zone.

Malagelada *et al.* (1973) showed that cholecystectomy, which changes the dynamics of the enterohepatic circulation from an intermittent process to a continuous one, results in a 24 hour output of bile which exceeds that which occurred preoperatively. This was disputed by Adler *et al.* (1974) who reported no change in secretion rates but their study was confined to a very small group of American Indians. Support for Malagelada's view came from Shaffer *et al.* (1972) who reported that interference with the enterohepatic circulation resulted in a fourfold increase in bile salt synthesis. The importance of the rate of bile salt secretion was shown recently by Scherstén *et al.* (1974) who showed that a minimum rate of secretion existed below which the bile became saturated with cholesterol. Fasting is known to affect bile composition in Rhesus monkeys (Redinger *et al.*, 1971), although the bile never becomes oversaturated, and in man (Metzger *et al.*, 1973), in whom the bile becomes more lithogenic. This may be a function of the fall in bile salt secretion rate or

a rise in cholesterol. After cholecystectomy, the composition of common duct bile becomes more normal (Nilsson and Scherstén, 1969; Small and Rapo, 1970; Scherstén *et al.*, 1971; Simmons *et al.*, 1972) and this is probably a result of the change in enterohepatic circulation dynamics. Recently, Nakayama and Van der Linden (1974) have again reported this finding and in their cases the gallbladder bile at operation was not oversaturated, while the common duct bile was. They suggested that preoperative fasting might account for this difference.

Recently, Pederson *et al.* (1974) reported the effect of small doses of chenodeoxycholate given to four patients with radiolucent gallstones and functioning gallbladders. It was shown that there was on average a 40% reduction in the 'input of cholesterol into the rapidly miscible pool'. If this effect of bile salt on cholesterol metabolism is confirmed, it might explain the findings in the present series of a lower cholesterol level in bile if the gallbladder is non-functioning and the findings of a 'normalisation' of bile composition as reported by others after cholecystectomy.

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