Gallbladder function, cholesterol stones, and bile composition

G. ANTSAKLIS, M. R. LEWIN1, D. JUNE SUTOR, A. G. A. COWIE, AND C. G. CLARK

From the Department of Surgery, University College Hospital Medical School, London, and the Department of Chemistry, University College, London

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.

1Address for correspondence: M. R. L., Department of Surgery, University College Hospital Medical School, University Street, London WC1.

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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 Subbarrow, 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

<table>
<thead>
<tr>
<th>Author</th>
<th>No.</th>
<th>Origin of sample</th>
<th>Percentage molar lipid composition (mean ± SEM)</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Bile salts</th>
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</thead>
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<td>Present study</td>
<td>5</td>
<td>Gallbladder bile</td>
<td>5-1 ± 0-4</td>
<td>11-3 ± 2-9</td>
<td>8-3-6</td>
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<tr>
<td>Simmons et al. (1972)</td>
<td>10</td>
<td>Duodenal aspirate</td>
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<td>14-0 ± 2-5</td>
<td>7-8-0</td>
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<td>Mackay et al. (1972)</td>
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<td>5-1 ± 0-4</td>
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<td>7-6-1</td>
<td></td>
</tr>
<tr>
<td>Heller and Bouchier* (1973)</td>
<td>12</td>
<td>Gallbladder bile</td>
<td>6-9 ± 1-0</td>
<td>17-6 ± 1-6</td>
<td>7-5-3</td>
<td></td>
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<tr>
<td>Vlahcevic et al. (1972)</td>
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<td>Duodenal aspirate</td>
<td>7-9 ± 1-0</td>
<td>23-2 ± 3-4</td>
<td>6-8-9</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Percentage molar lipid composition in control subjects

*SEM calculated from original data.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Gallbladder function</th>
<th>No.</th>
<th>Percentage molar lipid composition (mean ± SEM)</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Bile salts</th>
<th>Common duct bile</th>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<td>15</td>
<td>9-2 ± 0-7</td>
<td>14-3 ± 2-2</td>
<td>76-4</td>
<td>5 ± 2-2</td>
<td>22-9 ± 3-1</td>
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<tr>
<td></td>
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<td>8</td>
<td>8-4 ± 0-7</td>
<td>16-2 ± 3-8</td>
<td>75-4</td>
<td>9 ± 1-7</td>
<td>10-7 ± 2-6</td>
</tr>
<tr>
<td>Vlahcevic et al.* (1970)</td>
<td>Not specified</td>
<td>14</td>
<td>1-1 ± 0-5</td>
<td>1-3 ± 0-4</td>
<td>62-3</td>
<td>11 ± 2-6</td>
<td>14-5 ± 3-8</td>
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<tr>
<td></td>
<td>Yes</td>
<td>18</td>
<td>8-6 ± 0-5</td>
<td>19-7 ± 3-8</td>
<td>71-6</td>
<td>21 ± 1-5</td>
<td>16-6 ± 2-6</td>
</tr>
<tr>
<td>Mackay et al. (1972)</td>
<td>Yes</td>
<td>17</td>
<td>7-2 ± 0-5</td>
<td>18-6 ± 3-8</td>
<td>74-2</td>
<td>17 ± 1-2</td>
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<tr>
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<td>8</td>
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<td>25-8 ± 4-5</td>
<td>59-3</td>
<td>8 ± 1-5</td>
<td>15-5 ± 5-5</td>
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<td>23</td>
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<td>23 ± 3-3</td>
<td>13-0</td>
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<td>73-0 ± 3-3</td>
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<td>Heller and Bouchier* (1973)</td>
<td>Yes</td>
<td>16</td>
<td>8-6 ± 1-0</td>
<td>17-6 ± 1-5</td>
<td>73-7</td>
<td>7-3 ± 1-0</td>
<td>7-3 ± 1-0</td>
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</tbody>
</table>

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

*SEM calculated from original data.
Gallbladder function, cholesterol stones, and bile composition

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

Fig. 2 Mean ± 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 composi-
tion 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 bile fell outside the
micellar zone, while, in the group with non-function-
ing gallbladders, the composition of 50% gall-
bladder biles and 78% common duct bile 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 gal-
bladders as compared with common duct bile from
patients with non-functioning gallbladders.

Discussion

This study shows that the composition of the com-
mon 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 gall-
bladders fall within the micellar zone of cholesterol
solubility. Despite considerable variation in individ-
ual 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 circum-
stances 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 func-
tioning 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 signifi-
cantly 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
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 bile tested were potentially lithogenic, although $78\%$ of the common duct bile when the gallbladder was non-functioning and $100\%$ of the common duct bile 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 bile 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 radioolucent 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 ‘normalisation’ of bile composition as reported by others after cholecystectomy.

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