Bile from patients with mixed gallstones and controls without gallstones was analysed for relative concentrations of bile salts, phospholipids and cholesterol. A significant difference was found in the concentration of bile salts in normal controls and patients with cholelithiasis. The mean values for total bile salts, phospholipids and cholesterol in patients' bile were Mean + S.E. 84.34 ± 15.89; 19.34 ± 0.97 and 18.74 ± 1.38, and in controls the same was 169.67 ± 19.18; 27.42 ± 1.76 and 16.83 ± 2.76 respectively. (JPMA 30:60, 1980).

Abstract

Bile salts in association with phospholipids solubilize cholesterol in bile. Pure cholesterol is otherwise insoluble in water, but in normal bile it is completely solubilized in the form of mixed micelles of bile salts and phospholipids (Small, 1967; Tamgue and Juniper, 1967). A decrease in the quantity of bile salts and phospholipids, an increase in the cholesterol or combination of both, could result in the saturation of bile and the formation of gall-stones (Admirand and Small, 1968).

Studies (Admirand and Small, 1968; Small, 1967; Antasaklis et al., 1975) have shown wide variations in the concentrations of bile salts, phospholipids and cholesterol in normal human gall bladder bile. Thus the quantity of any single constituent has a little meaning without its being compared to that of the other two major components.

It is, therefore, essential to estimate the quantities of all the three major components in bile to establish a ratio between bile salts and phospholipids and cholesterol which, probably, will show the capacity of bile salts and phospholipids to solubilize cholesterol. This study was, thus, planned to evaluate this ratio in healthy subjects and patients with cholelithiasis.

Material and Methods

Twentythree samples of gall-bladder bile from patients with cholelithiasis and six from subjects with no gall bladder disease were collected at laparotomy for abdominal problems. The samples were stored at -20CC before analysis. Bile salt extraction was carried out by the combination of two methods (Andrews and Hardina, 1932; Murphy et al., 1970). The dried residue, obtained after these extractions, was mixed well with 5 ml acetic anhydride then 10 drops of concentrated sulphuric acid were added to this mixture. The colour, produced after 20 minutes, was read at 385 mu. Each set was accompanied by a standard. Cholesterol was estimated by the method of Ferro and Ham (1960), and phospholipids by that of Fiske and Subbarow (1925).

Results

The mean values of bile acids, cholesterol and phospholipids are shown in table I.
The ratio of cholesterol to bile salts + phospholipids is given in Table II.

Table II: Mean of Percent Molar Lipid Composition

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<tr>
<th>Studies</th>
<th>Controls</th>
<th>Patients</th>
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<td></td>
<td>BS</td>
<td>PL</td>
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<td>Present Study</td>
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<td>Antsaklis, et al.,</td>
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*BS=Bile Salts, PL=Phospholipids and C=Cholesterol.

Results were plotted on a triangular coordinate (Fig. 1B).
in the manner reported by Admirand and Small (1968). In this study 19 (82.6%) samples of patients lie outside the micellar zone, 3 (13%) just on the line while only 1 lies within the zone. Against this all the samples from the controls lie much within the zone. A conspicuous increase in the concentration of bile salts (169 + 19.18) and decrease in that of cholesterol (16.83 + 2.16) can very well be seen in controls as compared to the patients (84.34 ± 15.89 and 18.74 + 1.38 respectively) The mean of percent composition of these components in this study as well as from the studies by Antasaklis et al (1975) and Heller and Bouchier (1973) are compared in table II. The cholesterol levels were twice as high, bile salts were very low and no difference was observed in phospholipid levels in the present study when compared with those of the other two studies. Phospholipid levels were higher in the patients than controls in this series.

Discussion
The lithogenicity of bile in the present study may either be due to increased cholesterol (C) contents relative to the quantities of bile salts (BS) and phospholipids (PL) or due to decreased concentration of bile salts, resulting in the formation of cholesterol gallstones as has previously been observed by Admirand and Small (1968). If compared with the studies of Antasaklis et al (1975), Heller and Bouchier (1973) and Admirand and Small (1968) as shown in Fig. 1 (A,B and C), the results were similar to those observed by Antasaklis et al (1975), while the study by Admirand and Small (1968) substantially differs from these two. The samples lying within the micellar zone are from the patients having gallstones, whereas, according to Admirand and Small (1968) these must lie outside the zone and all controls should be within the zone. To explain this a modified triangular model was presented by Holzbach cholesterol solubility by describing an intermediary 'metastable' zone. The points falling within this zone represent a bile in which cholesterol exists in
micellar, liquid-crystalline and crystalline phases. The liquid crystal phase is unstable and its cholesterol may either return to the micellar form or precipitates as crystals (Coyne et al., 1977) as shown in Fig. 2.

The normal bile may also be in a 'litho-genic' phase, i.e., 'metastatic-supersaturated zone' (Antasaklis et al., 1975). The finding in the present study can also be explained on this basis. Accordingly 4 samples from the patients lie within this 'metastatic supersaturated' zone, in which bile might be thermodynamically unstable and in a state which favours the precipitation of crystalline cholesterol.

Saturated bile originates from the liver and does not result from unsaturated hepatic bile undergoing changes in the gall bladder.

The synthesis and secretion of both the bile acids and cholesterol are responsible for the saturation of the bile. Without a concomitant increase in hepatic cholesterol synthesis and secretion, a reduced bile acid pool alone may not be sufficient for the formation of the saturated bile (Coyne et al., 1977). In cirrhosis, when bile acid pool is reduced, the bile was not found saturated with cholesterol because of an associated decreased cholesterol secretion (Vlahcevic et al., 1971). The increased biliary cholesterol secretion could result from high dietary intake, as demonstrated by Den Besten et al (1973), who found that high cholesterol intake leads to the formation of saturated bile. Alternatively it could result from increased endogenous synthesis of cholesterol, and finally increased cholesterol secretion could result from decreased hepatic conversion of cholesterol to bile acids. This possibility is enhanced by the reports of Coyne et al (1976) that patients with gall-stones have a decreased bile acid synthesis despite the increased cholesterol synthesis.

The gall bladder is an important participant in the pathogenesis of cholelithiasis, since most gall-stones form in the gall bladder and seldom recur after cholecystectomy. The exact extent of this contribution of gall bladder to stone formation, however, has not yet been defined. The condition in the gall bladder such as progressive concentration, constant temperature, occasional mixing and an abundance of mucus provide an excellent milieu for gallstone formation. The gall bladder has also been postulated to induce saturated bile through enhanced phospholipids and bile acid absorption. The contribution of altered lipid absorption in gall bladder to gall stone formation remains uncertain, especially when cholesterol saturation of hepatic bile is similar to or greater than that of gall bladder in patients with gall stones (Coyne et al., 1977).

The saturation of bile and formation of gallstones could therefore be the result of many factors contributing to the decreased bile acid or increased cholesterol concentrations in the bile.

References