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# Variation in Hepatic Bile Composition Following Cholecystectomy in Patients with Previous Gallstones[1]

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The composition of fasting hepatic bile was analyzed in 63 samples from 8 patients following cholecystectomy to determine if bile was lithogenic in patients with previous cholesterol gallstones after removal of the gallbladder. Bile specimens were obtained from *t*-tubes over a 7–20 day study period following re-establishment of the enterohepatic circulation. Bile composition varied on a day to day basis in each patient. 18 of 63 samples were lithogenic according to criteria of Admirand and Small while 35 of 63 samples were lithogenic according to criteria of Hegardt and Dam. Variations in the composition of hepatic bile appeared related to changes in the excretion rate of bile acids. These studies demonstrate that hepatic bile may be lithogenic after cholecystectomy and indicate that factors other than sequestration of the bile acid pool in the gallbladder influence the enterohepatic circulation of bile acids and the lithogenicity of bile.

## INTRODUCTION

Many patients with cholesterol gallstones have abnormalities in the chemical composition of bile characterized by an excess of cholesterol (1). Indeed, at the time of cholecystectomy, hepatic bile is often supersaturated with cholesterol (2, 3). These findings led to the concept that the liver was the primary source of lithogenic bile and that the gallbladder played a passive role in the formation of cholesterol gallstones.

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However, because studies of hepatic bile were made in the fasting state when the majority of the bile acid pool was sequestered within the gallbladder, hepatic secretion of lithogenic bile resulted in part from the interruption of the enterohepatic circulation of bile acids rather than just reflecting an intrinsic metabolic defect in the liver (4, 5). However, if the lithogenicity of hepatic bile were due entirely to a physiologic interruption of bile acid circulation by the gallbladder, hepatic bile should be continuously normal in composition once the gallbladder was surgically removed.

We examined this problem of the determinants of lithogenic bile by measuring the composition of serial samples of hepatic bile in the same patients following cholecystectomy for previous gallstones.

## MATERIALS AND METHODS

Eight patients who underwent common duct exploration were subjects for this study (Table 1). In each case, the gallbladder had been removed shortly before study or at a previous operation. Cholesterol was extracted in chloroform and methanol (6) from available stones or sludge from 6 of the 8 patients, and was the major constituent detected qualitatively by thin layer chromatography in all but one, where bilirubin was predominant (A. G.) (7). To avoid studying these patients in the immediate postoperative period when the bile acid pool was depleted by *t*-tube drainage, samples were obtained 2–5 wk after surgery, at a time when liver function was normal, and the enterohepatic circulation had been progressively reestablished. When the *t*-tube was clamped for at least 2–3 days, patients who consented to the study were transferred to a metabolic research ward where they consumed a normal hospital diet. The *t*-tube was opened between 8 and 9 A.M. after fasting from midnight. Three to 5 ml of dead space bile was drained and discarded, and a 5–10 ml sample of freshly secreted hepatic bile was collected. Bile was obtained daily or every other day over 4–20 days and less than 100 mg of bile salts were removed during each collection. All samples were immediately frozen at —20°C and analyzed subsequently after agitation, for cholesterol by the method of Abell *et al.* (8), phospholipids by the method of Bartlett (9), and bile salts by the hydroxysteroid dehydrogenase method (Boyer, Scheig and Klatskin) (10). The composition of each sample was then determined and plotted on triangular coordinates as described by Admirand and Small (1). Criteria for cholesterol solubility were based on both Small, Bourges and Dervichian's (11), and Hegardt and Dam's phase equilibria (12), and were calculated from mixtures containing 95% $H_2O$ since determinations of % $H_2O$ of 12 different hepatic bile samples from two patients averaged 95.4 ± 2.75%. There was no significant difference in % solids between samples that were saturated or unsaturated with cholesterol. Complete collections of bile could be obtained in two studies where *t*-tubes containing an inflatable balloon on the distal arm (Baldwin *t*-tubes) were inserted at the time of surgery. The balloon on the distal arm of the *t*-tube was inflated to occlude the lumen only during the period of bile collection. In these studies, samples of bile were obtained for approximately 1 hr after an overnight fast, and bile salt and cholesterol secretion rates were measured and compared with the lithogenic index as described by Metzger, Heymsfield and Grundy (13). Routine tests of liver and pancreatic function were obtained three to four times each week to avoid analysis of bile samples from patients with impaired hepatic or pancreatic function. One patient was excluded from the study on this basis.

## RESULTS

Table 1 illustrates the incidence of lithogenic samples observed in these eight patients following cholecystectomy, as judged by both the criteria of Admirand and Small (1) and Hegardt and Dam (12). In two patients, daily bile composition varied, but always remained within the line of cholesterol solubilization by either criteria (Fig. 1). In the remaining six patients, the composition also varied on a day to day basis but some or all of the samples were lithogenic depending on the criteria selected (Fig. 2).

In two patients who secreted lithogenic bile (W. G. and A. G.), complete collections were obtained for approximately 1 hr every other day after inflation of a Baldwin *t*-tube. In these patients, bile lithogenicity, expressed as the lithogenic index (13), was compared with the rate of bile acid excretion. The lithogenic index was always increased on days when bile acid secretion rates were diminished. No significant changes in composition were observed when sequential samples of bile were obtained on a given day over a 30 min period in any patient.

TABLE 1[a]
BILE LITHOGENICITY AFTER CHOLECYSTECTOMY

| Patient | Age | Sex | Problem | Bile composition | No. lithogenic / No. samples *A* | No. lithogenic / No. samples *B* |
|---|---|---|---|---|---|---|
| 1. S. T. | 31 | Black female | Acute cholecystitis. Cholesterol stones in gallbladder | —Normal | 0/7 | 0/7 |
| 2. L. C. | 76 | White female | Cystic duct remnant 35 yrs after cholecystectomy for gallstones. Sludge with cholesterol crystals in common duct. | —Normal | 0/6 | 0/6 |
| 3. V. W. | 37 | White male | Transient obstructive jaundice 2 mo prior to operation. Cholesterol stones in gallbladder. | Int. Lithogenic | 0/4 | 2/4 |
| 4. G. L. | 34 | Black female | Chronic cholecystitis and gallstones 6 yrs prior to operation for cystic duct remnant—no stones at 2nd operation. | —Int. Lithogenic | 0/6 | 6/6 |
| 5. C. M. | 80 | White female | Common duct stones reformed 4 yrs after cholecystectomy for stones. | —Int. Lithogenic | 4/9 | 4/9 |
| 6. W. G. | 50 | White female | Recurrent pancreatitis for 6 yrs. Sludge containing cholesterol crystals in gallbladder and common duct. | —Int. Lithogenic | 9/12 | 12/12 |
| 7. L. S. | 28 | White female | Stricture of common duct two years after acute cholecystitis for gallstones. | —Int. Lithogenic | 3/9 | 5/9 |
| 8. A. G. | 46 | White male | Choledochoduodenal fistula and formation of common duct stones predominantly bilirubin. | —Int. Lithogenic | 2/10 | 6/10 |
| | | | | | 18/63 | 35/63 |

[a] Lithogenicity of daily samples of *t*-tube bile according to criteria of Admirand and Small (A) and Hegardt and Dam (B).

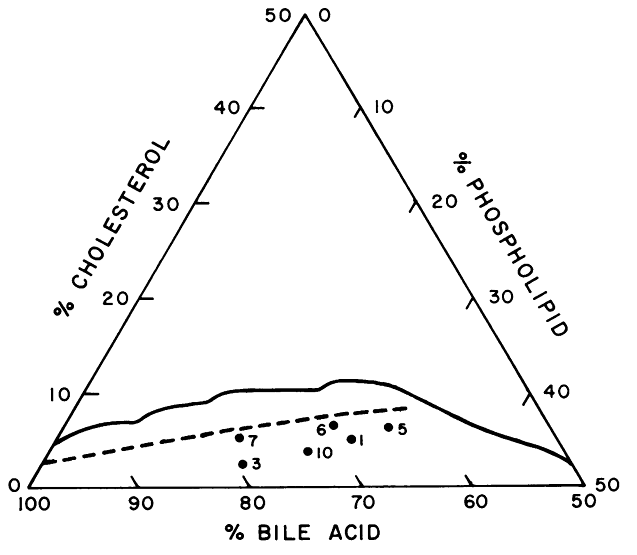


FIG. 1. Variation in the composition of post-cholecystectomy hepatic bile from a fasting patient (L. C.) where cholesterol always remained within the range of micellar solubility. The triangle represents the lower left hand corner of triangular coordinates. The solid line is the line of cholesterol saturation defined by Admirand and Small, while the dotted line represents the limit of solubility defined by Hegardt and Dam. The numbers represent the day of the study that samples were obtained.

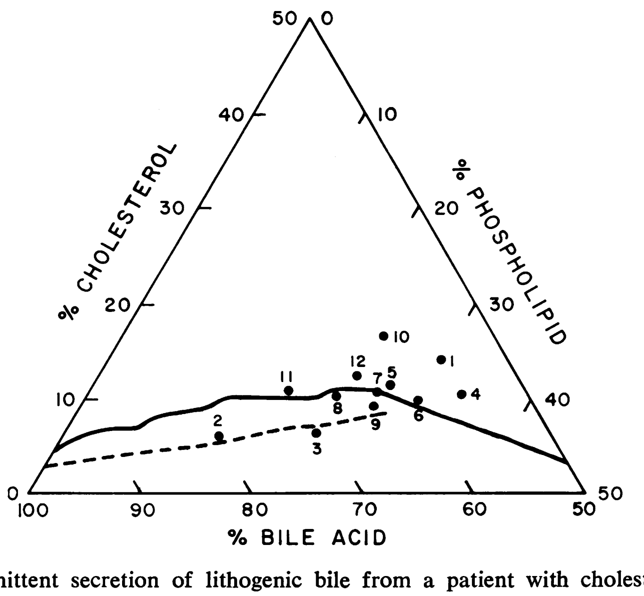


FIG. 2. Intermittent secretion of lithogenic bile from a patient with cholesterol gallstones following removal of the gallbladder (W. G.).

## DISCUSSION

The line of cholesterol solubility defined by Admirand and Small, and the one proposed by Hegardt and Dam, and more recently confirmed by Holzbach, Marsh, Olsewski and Holan (14), roughly define an area where cholesterol is metastably supersaturated. Thus, cholesterol may or may not be in micellar solution when the composition of bile falls below the Admirand and Small line, but above the Hegardt and Dam line, and such bile may support the formation of cholesterol crystals only if sufficient time has passed for the supersaturated solution to come to equilibrium (14, 15). Therefore, it seems reasonable to refer to both lines in determining the significance of the physical state of cholesterol in a given sample of bile, realizing that cholesterol supersaturation is not necessarily synonymous with the formation of cholesterol precipitates. Despite this difficulty, it is generally agreed that bile from patients with cholesterol gallstones contains relatively more cholesterol than bile from patients without stones (1, 3, 14).

Debate exists, however, as to the significance of the gallbladder in the pathogenesis of gallstones and the formation of lithogenic bile since it is capable of sequestering a large portion of the bile acid pool and, therefore may enhance the lithogenicity of hepatic bile. However, the observations in the present study indicate that the composition of hepatic bile varies from day to day in a given patient when analyzed in the fasting state 2–5 wk after cholecystectomy. Since cholesterol was saturated or supersaturated in many of these bile samples irrespective of two different published criteria for cholesterol solubility in bile, factors other than sequestration of bile acids in the gallbladder must influence the lithogenicity of hepatic bile. Similar findings have also been reported by Smallwood, Jablonski and McKwatts (16), using criteria of Admirand and Small (1). On the other hand, Simmonds, Ross and Bouchier (4) and Shaffer, Braasch and Small (5) have demonstrated that the lipid composition of hepatic bile obtained from cholesterol gallstone formers returns toward normal after cholecystectomy suggesting that the gallbladder influences the degree of hepatic bile lithogenicity in many instances. However, these findings were not confirmed by Almond *et al.* (17) who found that cholecystectomy produced no significant effect on bile lipid composition or pool size and that bile remained lithogenic when examined 3–41 mo after operation. These authors suggest that the results of Simmonds *et al.* might be explained by technical problems in studying *t*-tube patients too soon after operation when biliary lipid metabolism may be nonphysiologic. Nevertheless, it is apparent from the present studies, and those of Smallwood *et al.* (14), that a significant proportion of *t*-tube bile samples in post cholecystectomy patients with cholesterol stones continue to be lithogenic. Lithogenic hepatic bile may also be observed following cholecystectomy in patients who do not have cholesterol stones (patient #8, Table 1). The cause of the variation in lithogenicity is unlikely to be related to a depletion of the bile acid pool from *t*-tube drainage since samples were obtained 2 or more wk after surgery when the enterohepatic circulation had been reestablished, and in the present study less than 100 mg of bile salts were removed in any one collection. Furthermore, there was no time order relationship in the daily composition of samples (Figs. 1 and 2) as would be expected if the bile acid pool were progressively altered. Since liver function tests were consistently normal, impairment of hepatic function is also unlikely to explain these observations. Rather, the results of the studies in two patients where complete collections of bile were obtained suggest that the variations in postcholecystectomy bile composition were related to fluctuations in the hepatic secretion of bile salts (Table 2). Since we did not observe a correlation between cholesterol excretion and lithogenicity in these studies the secretion rate of bile acids was the important determinant. Variations in bile acid secretion in the post-cholecystectomy state might result from alterations in the rate of hepatic synthesis or changes in the rate of intestinal transit and absorption, or some combination

TABLE 2[a]
LITHOGENIC INDEX AS A FUNCTION OF BILE ACID EXCRETION RATES

| | Lithogenic index (range) | Bile acid secretion (μmoles/min) |
|---|---|---|
| I. | 0.33–0.95 (12) | 34.5 ± 19.9 |
| II. | 1.0–1.5 (10) | 15.5 ± 7.0 |
| | | $P < .01$ |

[a] Lithogenic Index (Metzger, Heymsfield and Grundy, 1972) and Bile Acid Secretion rates in 22 samples of hepatic bile from 2 patients. Group I samples are non-lithogenic while Group II samples are saturated or supersaturated with cholesterol according to Admirand and Small. Numbers of samples in each group is given in parenthesis.

of both. Significant enhancement of bile acid excretion and improvement of hepatic bile composition has been observed in cholecystectomized patients after eating (18), which suggests that feeding enhances while fasting diminishes bile acid circulation even in the absence of the gallbladder. Thus, variations in the enterohepatic circulation of bile acids may continue to influence the lithogenicity of hepatic bile in cholecystectomized patients.

Whether or not continued excretion of lithogenic bile predisposes to common duct stone formation in the post-cholecystectomy patient is not clear although in two of our patients (W. G. and C. M.) either common duct stones or sludge containing cholesterol crystals were removed from the common duct 4 and 35 years respectively after a previous cholecystectomy.

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