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Pigment vs Cholesterol Cholelithiasis: Comparison of Stone and Bile Composition

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This report presents a comparative study of gallstone and gallbladder bile composition from 100 unselected American patients, 23 with pigment and 77 with cholesterol cholelithiasis. Cholesterol stones were predominantly composed of cholesterol, whereas pigment stones were mainly composed of an unidentified residue, bilirubin, and bile salts. The residue in pigment stones was not calcium bilirubinate, which sharply contrasts with the composition of bile pigment calcium stones found in Japanese subjects. Bile composition of the two groups differed in that the cholesterol content of biles surrounding pigment stones was significantly less than that of biles surrounding cholesterol stones. Bilirubin in biles was conjugated, but the pigment extracted from stones was unconjugated bilirubin. This study indicates that (1) pigment stones account for an appreciable percentage of gallstone specimens found at cholecystectomy, and (2) pigment stone formation involves the precipitation of bilirubin, bile salts, and unidentified material which is not calcium bilirubinate.

Recent investigations into gallstone formation have centered on the composition of bile surrounding cholesterol stones (1, 2). These studies from selected American (3–7), Japanese (8, 9), Swedish (10), and Danish populations (11) support the concept that cholesterol stones (CS) result from the precipitation from bile of cholesterol, which exceeds its limits of solubilization by bile salts and phospholipids. By contrast, little is known about the composition of pigment stones (PS) and their surrounding biles in an American population. We therefore felt that additional information about gallstone formation might be gained by a comparative study of stone and bile composition from unselected patients with pigment or cholesterol cholelithiasis.

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PATIENTS AND METHODS

Patients. Specimens of stone and gallbladder bile were obtained from 100 unselected patients at the time of cholecystectomy for symptomatic cholelithiasis. No attempt was made to exclude patients on the basis of stone morphology. The distribution of these 100 patients by race and sex and according to stone type (PS or CS) was not significantly dif-

Table 1. Percent by Weight (w/w) of Gallstone Composition

Component	Pigment ($N = 22$)		Cholesterol ($N = 31$)	
	Mean ± SE	Range	Mean ± SE	Range
Bile salts	2.72 ± 0.4*	0.29 - 6.76	0.71 ± 0.1	0.08 - 2.66
Bilirubin	7.23 ± 1.9*	0.67 - 40.0	0.49 ± 0.1	0 - 1.43
Residue	66.4 ± 4.3*	28.9 - 94.8	7.50 ± 2.8	0 - 59.6
Cholesterol	2.16 ± 1.1*	0.09 - 24.9	77.0 ± 2.8	29.6 - 101

*$P < 0.001$

ferent ($\chi^2_3 = 3.55$). Black patients accounted for 52% of those with PS and 54% of those with CS. After excluding 11 specimens with acute bacterial cholecystitis or white bile secondary to cystic duct obstruction, 89 specimens from 21 men and 68 women were analyzed.

Bile Analysis. Before surgical manipulation of the gallbladder, gallbladder bile was obtained by needle aspiration and placed on ice. Bile was protected from light, but was not filtered or centrifuged. Within 2 hours after collection, bile was subjected to Folch solvent partition for phospholipid determination by the procedure of Fiske and Subbarow (12) and total bilirubin was estimated by the Michaelsson technique (13). Cholesterol was measured using the method of Abell et al (14). Total bile salts were measured by a fluorimetric modification of Talalay's procedure (15). Solids in bile were determined gravimetrically.

Stone Analysis. Prior to analysis, gallstones were classified by visual inspection as PS from 22 patients or CS from 67 patients. Typical PS were black to brown and on cross section were amorphous; CS were yellow to light tan and on cross section were crystalline and usually laminated. All stones within a given gallbladder were of a single type. But 2 patients had stones clearly composed of two distinct layers and were classified by the predominant component. The composition of 22 PS was compared to that of the first 31 CS, and the accuracy of visual classification was substantiated.

Stones were washed with distilled water, dried, pulverized, and desiccated to constant weight. Twenty-five milligrams of gallstone powder was extracted three times with 15 ml of acidified methanol–chloroform (1:1,v/v) (16, 17) (acidified methanol is 0.9% volume of 1 N HCl with 99.1% volume of 100% methanol). The portion of stone powder insoluble in acidified methanol–chloroform was desiccated to dry weight and was designated the residue. The extract was evaporated and partitioned between 50 ml of petroleum

Table 2. Bile Composition from Gallbladders Containing Stones

	Pigment ($N = 22$)		Cholesterol ($N = 67$)	
	Mean ± SE	Range	Mean ± SE	Range
Total solids*	109 ± 13	18.3 - 203	115 ± 5.5	28.6 - 216
Percent of solids†				
Bile salts	36.6 ± 2.5	6.70 - 60.8	34.3 ± 1.3	8.60 - 58.4
Phospholipids	17.4 ± 1.1	5.30 - 25.6	18.5 ± 0.8	3.90 - 34.6
Cholesterol	3.88 ± 0.3‡	1.20 - 6.20	5.07 ± 0.2	1.36 - 9.20
Bilirubin	1.73 ± 0.3	0.44 - 4.90	1.40 ± 0.1	0.20 - 5.90
Unmeasured solids	40.5 ± 3.2	19.7 - 70.1	40.6 ± 1.9	14.2 - 85.0
Molar lipid ratio§	12.5 ± 1.3‖	2.58 - 27.4	8.36 ± 0.3	2.50 - 12.2

*mg solid/g bile.
†Components of total solids expressed as percentages (w/w).
‡$P < 0.005$.
§Molar ratio of bile salts and phospholipids to cholesterol
‖$P < 0.001$.

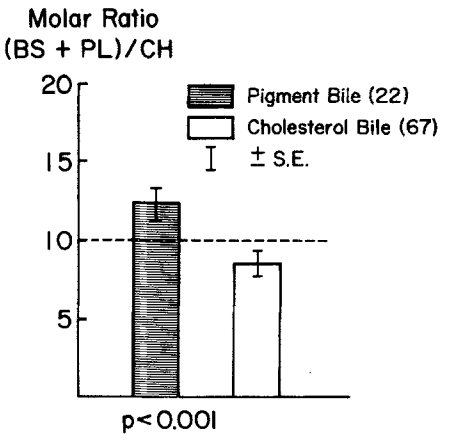

Fig 1. Comparison of mean molar lipid ratio of biles surrounding pigment stones to biles surrounding cholesterol stones. Below the dashed line biles are supersaturated with cholesterol and above the line the biles are undersaturated with cholesterol.

ether (BP 38–53° C) and successive volumes (20, 15, and 15 ml) of 70% ethanol (16). Cholesterol was determined on the petroleum ether phase and total bile salts on the ethanol phase by the methods described above. A separate aliquot of gallstone powder was extracted into acidified ethyl acetate–chloroform (1:2, v/v) (acidified ethyl acetate is 0.7% volume of 1 N HCl with 99.3% volume of ethyl acetate). Bilirubin was measured on the extract by a modification of the Weber–Schalm technique (18).

Thin Layer Chromatography. The presence of conjugated or unconjugated bilirubin in stone or bile was determined by thin layer chromatography on silica gel using a phenol-water system (19).

Statistics. The results were evaluated by Student's t test (20) and expressed as means ± standard error of the mean (SE). Distribution of patients was evaluated by the χ^2 test (20).

RESULTS

Gallstone Composition. The relative composition of PS differed strikingly from that of CS (Table 1). PS were characterized by significantly increased amounts of bile salts, bilirubin, and insoluble residue when compared to CS. The cholesterol content of PS was minimal but was the major component of CS. Furthermore, on the basis of cholesterol content, PS and CS formed mutually exclusive groups. The mean percentages of the initial sample weights accounted for by bile salts, bilirubin, residue, and cholesterol were 78.5 ± 3.1% for PS and 85.7 ± 1.4% for CS ($P < 0.025$).

Gallbladder Bile Composition. Bilirubin, bile salts, and phospholipids, when expressed as percentages of total solids in bile, were similar for both groups (Table 2). However, the cholesterol content of biles surrounding PS was significantly less than that of biles surrounding CS. The percentage of unmeasured solids in bile of PS was similar to that in bile of CS. In order to evaluate the relationship between cholesterol, bile salts, and phospholipids, the molar ratio of bile salts and phospholipids to cholesterol was calculated. Figure 1 shows that biles of PS had a mean molar ratio of 12.5 ± 1.3, indicating additional capacity to solubilize cholesterol (1), whereas biles of CS had a mean ratio of 8.36 ± 0.3 ($P < 0.001$), demonstrating cholesterol supersaturation of bile. However, the molar lipid ratios of individual specimens did not distinguish patients with PS from those with CS: 31% of the biles from patients with PS had ratios less than 10 and 22% of those from patients with CS had ratios greater than 10. Thus, the molar lipid ratio is of limited value in differentiating biles of PS from those of CS.

Thin Layer Chromatography. A representative chromatogram of bilirubin from gallbladder bile and stone of a patient with PS is shown in Figure 2. Bilirubin in stone had the same mobility as the free (unconjugated) bilirubin standard. By contrast, bilirubin in bile was identical in mobility to the conjugated bilirubin standard. By the Weber–Schalm method, at least 95% of the bilirubin in both types of stones was unconjugated, and at least 95% of bilirubin in all bile samples was conjugated.

DISCUSSION

This report presents a comparative study of stone and bile composition from unselected American patients with pigment or cholesterol cholelithiasis. Similar studies of Japanese sub-

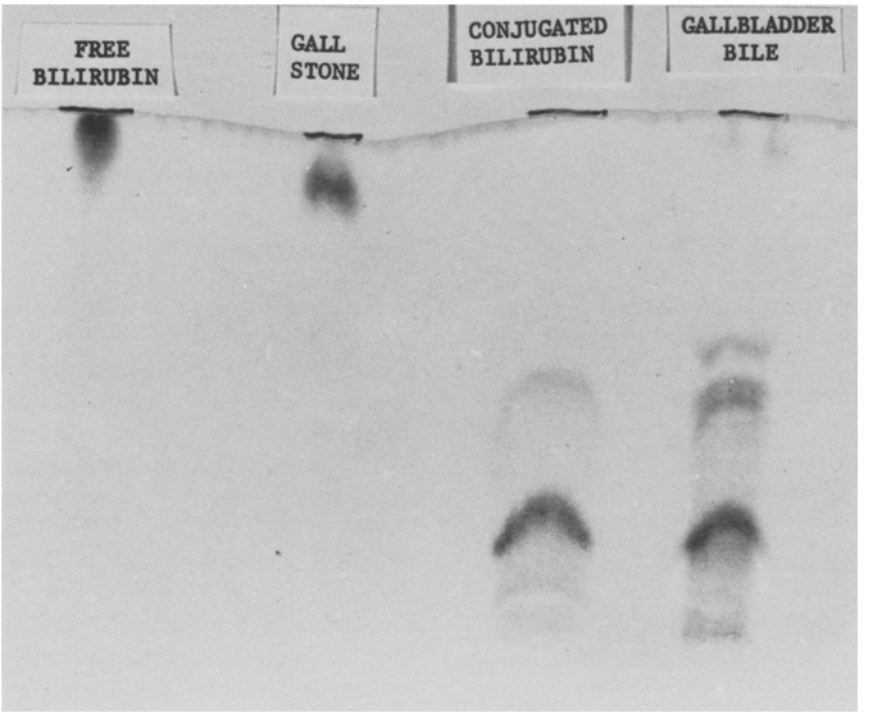

Fig 2. Chromatogram of bilirubin from gallstones and bile of a representative patient with PS. Samples of stone and bile are compared to free (unconjugated) and conjugated bilirubin standards using phenol-water (9:1) as the solvent system. Horizontal lines at the top of each channel indicate solvent front.

jects have been reported, but comparisons may not be valid because of geographic, dietary, and ethnic differences (9, 21). The population reported here was ethnically mixed and was 50% black in each stone group. PS accounted for 23% of the stones obtained. The only comparable study of gallstone incidence was the Framingham study of gallbladder disease (22). That study, among predominantly white patients, showed that PS accounted for 30% of the stones identified from pathology reports. Thus, our data suggests that race is not a significant determinant of stone type when reasonable sampling size is available. Furthermore, the findings of this study and the Framingham report indicate that PS constitute a larger percentage of gallstone disease in American blacks and whites than previously had been appreciated.

Visual distinction of PS from CS was uniformly confirmed by biochemical analysis. Therefore, the complex morphological classification of CS as proposed by Rains does not seem warranted (23). CS contained primarily cholesterol, but also contained small amounts of bile salts, unconjugated bilirubin, and residue. By contrast, PS were predominantly composed of an insoluble residue associated with unconjugated bilirubin and bile salts. The components of PS in Americans measured in this study contrast sharply with the components of bile pigment calcium stones found in Japanese subjects (9). The mean percents by weight of ten bile pigment calcium stones reported by Iz-

umi (9) were (a) bile salts, 2.5% (range 1.6–3.6%), (b) cholesterol, 11.7% (range 2.0–34.7%), and (c) bile pigment, 49.5% (range 27.1–87.9%). It is obvious that the bile pigment calcium stones of Japanese patients consisted of more soluble components than the PS from the Americans of this study. The lack of solubility of the residue of PS in dilute acidified organic solvents indicates that the majority of the material is not calcium bilirubinate (24). Thus, the abnormality leading to PS formation involves the precipitation of material as yet unidentified, most of which is not calcium bilirubinate, whereas the abnormality leading to CS formation centers on cholesterol precipitation.

The composition of gallbladder biles surrounding PS differed significantly from that of biles surrounding CS in the mean cholesterol content and molar lipid ratio, confirming a recent report by Shaffer, et al (25). However, the mean molar percentages of bile salts and phospholipids of biles surrounding the two types of stones were similar (25, 26).

Biles of PS or CS contained similar amounts of total bilirubin, which was almost entirely conjugated, agreeing with the results of a recent report (27). In all stones, however, bilirubin was essentially unconjugated, although PS have quantitatively more bilirubin than CS. Of note is the finding that no pigments other than unconjugated bilirubin were detected on thin layer chromatography, indicating that unconjugated bilirubin was the only pigment extractable, by our methods, from PS and CS. The data suggest that conjugated bilirubin is hydrolyzed during or after stone formation. Alternatively, unconjugated bilirubin could be secreted into bile, but to date this phenomenon has been observed only in the Gunn rat (28, 29). Maki proposed that conjugated bilirubin is hydrolyzed in the presence of bacterial β-glucuronidase, and unconjugated bilirubin precipitates as an insoluble calcium salt (30). But, this study did not reveal any unconjugated bilirubin in the biles of PS or CS, which suggests that unconjugated bilirubin is not present or that it is present intermittently. In addition, the unidentified residue of PS—the majority of which is not calcium bilirubinate—may be the material responsible for PS formation. Further investigations into the composition of this material will be necessary to fully explain PS formation.

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