JCI The Journal of Clinical Investigation

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J Clin Invest. 1977;59(5):828-840. https://doi.org/10.1172/JCI108705.

Research Article

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Biliary Lipid Secretion in Cholesterol Gallstone Disease

THE EFFECT OF CHOLECYSTECTOMY AND OBESITY

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ABSTRACT Cholesterol gallstone disease is initiated in a liver which produces abnormal bile with excess cholesterol relative to bile salts and phospholipid. To define the responsible secretory mechanism(s), the rate of biliary lipid secretion was measured by a duodenal marker perfusion technique, while the bile salt pool was simultaneously estimated by isotope dilution. Two groups of control patients expected to have normal biliary lipid composition—14 subjects without hepatobiliary disease and 6 patients with pigment gallstones, were compared to two experimental groups expected to have abnormal bile—10 nonobese patients with cholesterol gallstones and 7 obese subjects without gallstones. Both control groups had nearly identical biliary lipid secretion rates, and a corresponding low relative molar concentration of cholesterol. Two different secretory mechanisms were found to be responsible for the abnormal bile in the experimental groups. In the nonobese patients with cholesterol gallstones, bile salt and phospholipid secretion rates were both significantly reduced. Conversely, the grossly obese subjects had an increased cholesterol secretion.

To determine how cholecystectomy improves biliary lipid composition, three groups of gallstone patients —6 with pigment stones, 4 grossly obese with cholesterol stones, and 13 nonobese with cholesterol stones —were all examined after full recovery from surgery. In the nonobese patients with cholesterol gallstones, both bile salt and phospholipid secretion significantly increased, causing a definite improvement in bile composition. Cholecystectomy produced a similar but less marked trend in the obese patients with cholesterol stones, and in the patients with pigment stones. Cholesterol secretion, however, was unaffected by surgery. The bile salt pool was definitely small in the nonobese patients with cholesterol gallstones and became slightly smaller after cholecystectomy. The pool was significantly reduced by cholecystectomy in the patients with pigment stones and also in the obese patients with cholesterol gallstones. Removal of the gallbladder in all three groups caused a greater fraction of the pool to cycle around the enterohepatic circulation each hour. This more rapid cycling produced the increase in bile salt and phospholipid secretion, and was responsible for the improved composition found after cholecystectomy.

INTRODUCTION

Cholesterol gallstone disease is associated with the liver secreting lithogenic bile containing excess cholesterol relative to the solubilizing agents, bile salts, and phospholipids (3). The rates at which these three major components are secreted determine bile composition, while their relative composition defines cholesterol solubility in bile (4). Bile salts secreted from the liver come from two sources: one being *de novo* synthesis in the liver and the other recycled from the enterohepatic circulation. The latter is dependent on both the size of the bile salt pool and the speed at which this pool traverses the enterohepatic circulation. The reduced bile salt pool, which has been found in patients with cholesterol gallstones, has been assumed to cycle at a normal rate and result in a decreased bile salt secretion rate (5, 6). Phospholipid secretion, which depends on bile salt secretion (7–9), presumably would also be reduced.

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This work was presented in part at the Canadian Society of Clinical Investigation, January 1976, Quebec City, Quebec and at the plenary session of the American Gastroenterological Association, May 1976, Miami Beach, Fla. and reported in abstract form (1, 2).

Received for publication 6 July 1976 and in revised form 14 January 1977.

A decrease in the secretion of both bile salts and phospholipid should adversely affect the solubility characteristics of bile, predisposing to cholesterol precipitation and gallstone formation.

The available studies on biliary lipid secretion rates have come to divergent conclusions. In one study, rather than decreased bile salt secretion being the predominant feature, an increase in cholesterol secretion was the major factor causing lithogenic bile formation in obese Indian (10) and obese White women (11) with gallstones. Another investigation was unable to detect any abnormality in biliary lipid output or bile salt pool size in gallstone patients (12).

We have previously examined the composition of bile before and after cholecystectomy. These studies demonstrated that removal of the gallbladder caused bile to revert to a more normal composition (13). The results emphasized the importance of the presence of the gallbladder in the genesis of abnormal hepatic bile. We concluded that either the bile salt pool expanded after cholecystectomy, and/or it recycled more rapidly. The responsible hepatic defect was therefore inferred to be at the level of the feedback mechanism controlling bile salt synthesis and hence pool size (13). Since then, others have indicated that biliary lipid metabolism may not be improved by removal of the gallbladder (14–16).

The present study was therefore directed at investigating the factors regulating bile composition and the effect of cholecystectomy. Bile salt pool size and biliary lipid secretion rates were simultaneously measured in man under controlled conditions. These parameters were measured in four groups of patients: control subjects without any hepatobiliary disease, patients with pigment stones, nonobese patients with cholesterol gallstones, and very obese subjects without radiological evidence of gallstones. These latter two groups, including the obese subjects who had not yet developed gallstones, were expected to have lithogenic bile. The differences between obese and normal weight patients with lithogenic bile and gallstones were clearly defined and the beneficial effects of cholecystectomy elucidated.

METHODS

Patients. The clinical data on the patients studied are described in Tables I and II. The study groups were broadly divided into two major categories: control patients without cholesterol gallstones, in contrast to experimental patients with either overt cholesterol gallstones or a strong propensity to gallstone formation. The control groups consisted of 14 subjects without any evidence of hepatobiliary disease and 12 patients with pigment gallstone disease, 6 of whom were studied before and 6 after cholecystectomy. The experimental groups consisted of nonobese patients with cholesterol gallstones and morbidly obese patients. In the nonobese group with cholesterol gallstones, 10 patients with gallstones were compared to 13 similar patients who had undergone cholecystectomy. Five of these cholesterol gallstone patients were studied before and again after cholecystectomy. In the obese group, seven subjects did not have gallstones, while four had undergone cholecystectomy for cholesterol gallstone disease. All patients listed in Tables I and II were white; in addition, a 50-yr-old Mohawk Indian woman, near ideal body weight, with cholesterol gallstones, was also examined before and again after surgery. A time interval of at least 1 mo was allowed to elapse before carrying out the studies after cholecystectomy. Pigment stone patients were examined 1–4 mo after surgery (average 2 mo); nonobese patients with cholesterol gallstones, 1 mo–15 yr (average 33 mo); obese patients with cholesterol gallstones, 2–9 yr (average 69 mo). All groups were within 12% of their ideal body weight[1] except for the morbidly obese patients who were almost twice their ideal body weight. Cholecystectomy effected only minimal weight changes (less than 8%), and before the study all patients had maintained a stable weight on their routine diet for at least 1 wk. Liver function tests (including bilirubin, alkaline phosphatase, transaminase, and serum protein electrophoresis) were all normal. Oral cholecystography visualized normally in all with intact gallbladders, even when gallstones were present. The control subjects without any hepatobiliary disease were composed of either healthy volunteers or patients with functional gastrointestinal complaints. 8 of the 11 morbidly obese patients were investigated as part of their preoperative evaluation for bypass surgery. In these, liver biopsy was performed as part of the protocol, and despite the normal liver function tests, a variable degree of fatty infiltration was found. Hypertriglyceridemia and chemical diabetes mellitus were also present in four obese patients as well as the Indian woman. Verbal and written consent was obtained from all subjects.

Gallstone analysis. Gallstones, including several from patients who had saved them for 5–10 yr after cholecystectomy, were chemically analyzed by standard methods (3). Cholesterol gallstones contained predominantly (more than 50%) cholesterol whereas pigment gallstones contained only a trace (less than 5%) of cholesterol. The gallstones of four patients (nos. 7, 8, 12, and 13), who had undergone cholecystectomy 1–15 yr previously, were not available for chemical analysis but the surgical and pathological records fit the morphological criteria for cholesterol gallstones (17). Similarly, two patients with gallstones (nos. 2 and 4) who did not undergo surgery were assumed to have pigment stones because they were found to be totally radiopaque (18).

Biliary-lipid composition in gallbladder bile. Bile was directly aspirated from the gallbladder at the time of surgery from 10 patients with gallstones and 5 of the morbidly obese subjects who were undergoing ileojejunal bypass. The lipid composition was determined by analysis for bile salts, phospholipid, and cholesterol, as described previously (3).

Biliary lipid composition and secretion rates of hepatic bile. The biliary lipid composition and rates of secretion of biliary lipid into the duodenum were measured by the standard marker perfusion technique (19) based on that of Grundy and Metzger (20) and used by Gordon et al. to measure bilirubin secretion (21). Briefly, the fasting subject was intubated with a triple-lumened polyvinyl tube. The proximal orifice was positioned fluoroscopically

[1] Metropolitan Life Insurance Company. 1959. New weight standards for men and women. Statistical Bulletin 40. New York.

TABLE I
Clinical Data—Control Groups

Patient	Sex	Age	Surface area	Weight	Ideal body wt
		yr	m^2	*kg*	%
Subjects without hepatobiliary disease					
1	F	31	1.69	61.4	105.9
2	M	59	1.95	70.9	114.7
3	F	51	1.65	57.7	106.3
4	M	58	1.92	73.6	104.9
5	F	27	1.54	54.5	100.0
6	M	64	1.67	66.4	95.9
7	M	45	2.18	85.0	113.4
8	M	77	1.77	74.1	87.7
9	M	47	2.15	76.8	130.2
10	M	52	2.33	88.2	121.1
11	M	42	2.04	80.0	100.0
12	M	54	2.08	79.5	113.8
13	F	87	1.38	50.0	90.0
14	F	49	1.30	57.7	86.6
Mean		53	1.83	74.7	105.0
Pigment stone patients					
1	M	47	1.86	76.8	92.9
2	M	60	1.66	70.0	84.4
3	M	56	1.63	75.4	72.3
4	M	67	1.78	72.3	94.3
5	M	50	1.78	75.4	87.3
6	F	72	1.55	54.4	96.5
Mean		59	1.71	70.7	88.0
Pigment stone patients after cholecystectomy					
1	M	56	1.64	63.6	85.7
2	M	50	1.63	75.4	72.3
3	F	48	1.55	52.7	116.4
4	F	72	1.74	64.5	105.7
5	M	68	1.91	73.2	106.8
6	M	62	1.90	83.2	84.2
Mean		59	1.73	68.8	95.2

TABLE II
Clinical Data—Experimental Groups

Patient	Sex	Age	Surface area	Weight	Ideal body wt
		yr	m^2	*kg*	%
Nonobese cholesterol stone patients*					
1	F	61	1.79	67.1	91.9
2	M	58	2.12	86.2	97.9
3	M	66	1.70	63.6	100.0
4	M	48	2.00	81.4	100.9
5	M	52	2.01	87.2	126.3
6	M	72	1.78	64.9	117.5
7	M	38	2.18	102.0	132.4
8	M	48	2.13	89.4	107.7
9	M	31	2.00	75.0	113.3
10	M	58	2.03	79.5	108.6
Mean		53	1.97	79.6	109.6
Nonobese cholesterol stone patients after cholecystectomy*					
1	F	61	1.79	67.1	91.9
2	M	58	2.12	86.2	98.9
3	M	66	1.67	61.3	96.4
4	M	48	2.00	80.8	100.9
5	M	54	1.97	76.5	105.4
6	M	48	1.68	62.1	94.1
7	F	65	1.80	74.9	113.8
8	F	66	1.63	61.5	100.1
9	F	49	1.54	56.3	106.9
10	F	56	1.53	53.6	98.3
11	M	80	2.17	90.8	106.4
12	M	60	1.83	70.8	105.4
13	F	43	1.88	75.4	110.7
Mean		58	1.82	70.6	102.6
Obese subjects without gallstones					
1	M	54	2.88	189.6	245.9
2	F	51	2.36	136.2	215.8
3	M	34	2.50	147.5	207.0
4	F	37	2.06	99.1	167.9
5	M	29	2.50	140.3	176.6
6	F	33	2.28	111.0	141.4
7	F	25	2.13	108.0	171.2
Mean		38	2.39	133.1	189.4
Obese cholesterol stone patients after cholecystectomy					
1	F	49	2.07	113.9	188.0
2	M	54	2.22	120.1	185.0
3	F	49	2.06	110.5	199.8
4	F	23	1.98	94.3	149.2
Mean		44	2.08	109.7	180.5

* Patients 1–5 studied before and after cholecystectomy.

in the duodenum at the level of the ampulla of Vater. A proximal collecting port was also situated near the ampulla, and a more distal port 10 cm further along, past the ligament of Treitz. A nonabsorbable, water soluble sulfobromophthalein (BSP)[2] marker solution (5 mg/dl) was then constantly infused via a Harvard peristaltic pump (Harvard Apparatus Co., Inc., Millis, Mass.) at a known rate (2 ml/min) through the proximal orifice and allowed to thoroughly mix in the 10-cm mixing segment. Along with this, an essential amino acid solution[3] (22) was constantly infused, to both tonically contract the gallbladder and provide a source of calories during the study. Duodenal contents were continuously aspirated in hourly aliquots (8–10 ml) from both the proximal and distal collectors. The quantity collected represented less than 5% of duodenal output, so that the enterohepatic circulation was not significantly interrupted (23). The aspirated duodenal bile was assayed for phospholipid (24), cholesterol (25), and BSP (26). Phosphorus was absent from the amino acid infusate. Bile salts were extracted in methanol, and washed twice with diethyl ether:petroleum

[2] *Abbreviation used in this paper*: BSP, sulfobromophthalein.

[3] 5% protein hydrolysate in 5% glucose (Aminosol, Abbott Laboratories, Montreal, Canada) with added L-valine (25.6 mM), L-methionine (40.2 mM) and L-phenylalanine (18.2 mM) (Eastman Kodak Co., Rochester, N. Y.).

ether (1:1), as modified from Sjövall (27). The residue was then taken for quantitation of bile salts by the 3α-hydroxysteroid dehydrogenase assay (4).

The hourly rate of secretion of biliary cholesterol into the duodenum was determined from the sample withdrawn distally by use of the marker dilution principle (20):

$$\text{cholesterol secretion (mg/h)} = \text{cholesterol concentration (mg/ml) in distal aspirate} \times \left[\frac{\text{BSP infused (mg/h)}}{\text{BSP concentration in distal aspirate (mg/ml)}}\right]$$

From the ratios of bile salts (or phospholipid) to cholesterol determined on the duodenal samples collected from the proximal aspirating site, bile salt, and phospholipid secretion were calculated:

$$\text{bile salt (or phospholipid) secretion (mg/h)} = \text{cholesterol secretion (mg/h)} \times \left[\frac{\text{bile salt (or phospholipid)}}{\text{cholesterol}} \text{ ratio}\right]$$

The purpose of the essential amino acid infusion was to release endogenous cholecystokinin (22) and cause tonic gallbladder contraction. With the gallbladder functionally removed from the enterohepatic circulation, the entry of bile into the duodenum directly reflected hepatic secretion into the biliary tree (20). After the first 4–6 h of infusion, biliary lipid output into the duodenum became quite constant in patients with functioning gallbladders. In those whose gallbladders were removed, an equilibration period of only 2 h was required. After this equilibrium period, the secretory rate was taken as the average of the next 6 h (4–10 h) when steady secretion rates into the duodenum had been established.

Biliary bile salt composition. Two hydroxysteroid dehydrogenases were used to assay bile salts. Both the standard enzyme preparation, 3α-hydroxysteroid dehydrogenase, (Worthington Biochemical Corp., Freehold, N. J.) and a recently isolated 7α-hydroxysteroid dehydrogenase enzyme (Worthington Biochemical Corp.) (28) were used to analyze bile salt in the duodenal aspirates.

Bile salt pool size. The size of the circulating bile salt pool was estimated during the perfusion study. Briefly, 50 μCi of [2,4-^{3}H]cholic acid and 10 μCi of [24-^{14}C]-chenodeoxycholic acid (New England Nuclear, Boston, Mass.) in 10 ml ethanol were flushed with 100 ml 5% $NaHCO_3$ solution (pH 7.6) through the duodenal tube in the 1st h of starting the perfusion study. During this period, the maximal bile salt output into the duodenum occurs as gallbladder contraction ejects most of the bile salt pool into the duodenum. Bile was not collected for the next 2 h to allow for mixing and eliminate any disproportionate withdrawal of labeled bile salts. Thereafter, hourly duodenal bile aspirates were analyzed for total bile salt mass as described above, and ^{3}H and ^{14}C radioactivity (in disintegrations per minute) ascertained by liquid scintillation counting by using internal standardization to correct for quenching. The specific activity, which represents the ratio of isotope (disintegrations per minute) to total bile salt mass (millimoles), became constant after 3–4 h. The negligible hour-to-hour change after this period indicated that complete mixing had occurred. Similar results have recently been reported by Grundy and Bennion, and Grundy (29, 30). The specific activity determined on hourly samples was averaged, and the total circulating pool calculated by dividing the total dose of radioactivity given by the average specific activity:—

$$\text{bile salt pool (millimoles)} = \frac{\text{total radiolabel given (dpm)}}{\text{average specific activity (dpm/millimoles)}}$$

The pool size determinations showed excellent agreement with less than 5% difference between the two labeled, primary bile salts.

Frequency of cycling. The mean rate at which the total bile salt pool cycles around the enterohepatic circulation during the experimental period is equal to the bile salt secretion rate divided by the pool size:

$$\text{frequency of cycling } (h^{-1}) = \frac{\text{bile salt secretion rate}\left(\frac{\text{millimoles}}{\text{hour}}\right)}{\text{bile salt pool size (millimoles)}}$$

Data computation. The lipid composition of bile is expressed in terms of molar percent bile salts, phospholipid, and cholesterol; according to Admirand and Small (4). Percent saturation of cholesterol in stimulated hepatic bile from each patient was calculated (31) by using the maximum cholesterol solubility at 3% total solid concentration of Carey and Small (32, 33). A 3% line for total solids was chosen because this is close to the total solid concentration of human hepatic bile secreted from the liver. The total concentration of phospholipid plus cholesterol plus bile salts was found to have a mean (±SE) of 3.5 g/dl±0.5 in hepatic biles directly aspirated at surgery from 27 patients without hepatic disease (13).

Statistical significance of data was evaluated by the Student's *t* test, and expressed as mean±SEM. Correlation coefficients and regression lines were computed and plotted by using polynomial regression analysis and a commercially available least squares fit program (Hewlett-Packard model 9810A Stat Pac. volume 1. Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Biliary lipid composition. The molar percentages of cholesterol in gallbladder bile directly aspirated at surgery were compared with the stimulated hepatic bile obtained from the same patients preoperatively during the duodenal perfusion studies (Table III). In all cases, the relative molar concentration of cholesterol was significantly ($P < 0.01$) lower in the hepatic bile collected under conditions of the essential amino acid infusion. The obese subjects without gallstones had by far the highest molar percentage cholesterol, the nonobese patients with cholesterol gallstones were next, and the pigment stone patients had the lowest relative concentration.

The mean relative lipid composition of hepatic bile for each group, has been plotted on triangular coordinates (Fig. 1) as percentage total moles of bile salts, cholesterol, and phospholipid (4). The experimental groups, overall, secreted bile which contained more cholesterol and less bile salts ($P < 0.01$) than the

TABLE III

Molar Percentage of Cholesterol in Hepatic Bile vs. Gallbladder Bile

Subjects	Hepatic bile*	Gallbladder bile*
	molar % cholesterol	
Pigment stone patients	5.3	7.9
	4.9	8.0
	4.8	7.3
Mean±SEM	5.0±0.3	7.7±0.4
Nonobese cholesterol stone patients	6.2	12.6
	5.9	7.3
	8.2	8.5
	7.2	11.8
	6.1	15.4
	4.3	9.6
	4.7‡	9.0‡
Mean±SEM	6.1±1.3	10.6±2.8
Obese subjects without gallstones	12.2	13.9
	9.5	16.9
	12.9	14.3
	6.4	12.7
	11.0	15.5
Mean±SEM	10.4±2.6	14.7±1.6

* Hepatic bile represents the average molar percentage cholesterol obtained before surgery during the perfusion studies. Gallbladder bile was directly aspirated at surgery. In each and every case hepatic bile had a lower cholesterol content than the corresponding gallbladder bile ($P < 0.01$ by paired t test for each group).

‡ Mohawk Indian woman.

control groups. Specifically, patients with cholesterol gallstones produced hepatic bile with significantly ($P < 0.01$) more cholesterol than either the subjects without any hepatobiliary disease or the patients with pigment stones. The obese subjects without demonstrable gallstones had a twofold elevation ($P < 0.001$) of molar cholesterol over the controls. Indeed, it was even higher in these obese subjects than in the nonobese patients who had already developed cholesterol gallstones. After cholecystectomy, all three groups demonstrated some improvement in biliary lipid composition with a lower percent of cholesterol. This improvement was only significant ($P < 0.01$) in the nonobese patients with cholesterol gallstones. Despite some reduction in percentage cholesterol ($P = 0.05$), the obese patients with cholesterol gallstones still produced bile with a very high cholesterol content after surgery. The nonobese patients who underwent cholecystectomy for cholesterol gallstones formed bile which although improved, still had a higher molar percent cholesterol ($P < 0.001$) than either the group without hepatobiliary disease or the pigment stone patients after their surgery.

Biliary lipid secretion. The hepatic secretion rates of bile salts, phospholipid, and cholesterol are given in Tables IV and V. Control groups without any hepatobiliary disease or with pigment stones are contrasted to the experimental groups with cholesterol gallstones or gross obesity. The secretion rates are expressed in absolute terms per hour, and also in terms of body weight (per kilogram body weight per hour).

Biliary lipid secretion in the 14 subjects without any hepatobiliary disease was nearly identical to that in 6 patients with pigment stones, both in absolute terms and when calculated on a body weight basis (Table IV). In contrast to the absence of any difference within the control groups, the experimental groups (Table V) markedly differed from one another. The 10 nonobese patients with cholesterol gallstones had a significantly ($P < 0.001$) reduced bile salt and phospholipid secretion in comparison to the controls. Cholesterol secretion, however, was not significantly different. The seven grossly obese subjects without gallstones demonstrated a distinctive pattern of biliary secretion. Their bile salt secretion, in absolute terms, did not differ from controls although the phospholipid secretion rate was definitely higher ($P < 0.02$). Cholesterol secretion in these obese sub-

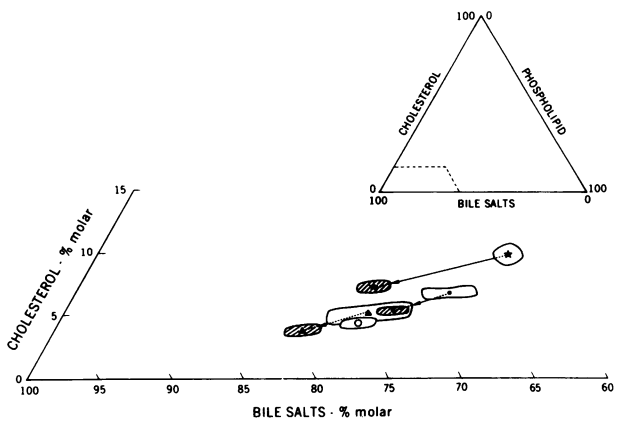

FIGURE 1 Mean relative lipid composition of biles obtained during the secretory studies. The triangle at the upper right represents the three component system for describing biliary lipid composition in bile (4). The area bounded represents±1 SE for each component. Open areas represent patients with functioning gallbladders; hatched areas for patients after cholecystectomy. The respective groups are joined by arrows. Mean composition for the groups are represented by the following symbols: open circle for subjects without hepatobiliary disease, closed triangles for patients with pigment gallstones, closed circles for nonobese patients with cholesterol gallstones, and stars for morbidly obese patients.

TABLE IV

Biliary Lipid Secretion Rates—Control Groups

Patient	Bile salts*		Phospholipid*		Cholesterol*	
	μmol/h	*μmol/kg-h*	*mg/h*	*mg/kg-h*	*mg/h*	*mg/kg-h*
Subjects without hepatobiliary disease‡						
1	1,730	24.6	346	5.29	36	0.56
2	1,056	13.0	249	3.06	35	0.43
3	1,247	20.3	228	3.73	16	0.26
4	970	12.6	227	2.94	26	0.34
5	1,141	21.0	218	4.00	23	0.42
6	779	12.6	353	5.56	22	0.35
7	1,483	15.4	300	3.12	27	0.28
8	1,298	20.0	195	3.01	33	0.51
9	831	8.3	223	2.24	29	0.29
10	1,366	12.8	233	2.19	28	0.26
11	943	11.8	208	2.61	38	0.48
12	1,672	18.0	306	3.39	32	0.36
13	1,824	40.5	249	5.54	23	0.50
14	958	19.2	244	4.89	17	0.33
Mean±SEM	1,236±91	17.9±2.1	256±13	3.68±0.32	27±2	0.38±0.03
Pigment stone patients§						
1	1,964	27.6	245	3.44	22	0.31
2	884	15.0	291	4.93	28	0.48
3	1,274	23.4	187	3.44	33	0.60
4	1,244	18.3	209	3.07	43	0.63
5	605	9.2	243	3.70	18	0.28
6	1,344	25.6	289	5.51	46	0.89
Mean±SEM	1,219±188	19.8±2.9	244±17	4.02±0.40	32±5	0.53±0.09
Pigment stone patients after cholecystectomy§						
1	1,435	26.4	177	3.26	27	0.50
2	1,534	23.3	244	3.71	18	0.27
3	1,708	27.8	343	5.59	43	0.70
4	1,921	28.2	267	3.92	29	0.43
5	1,352	17.3	300	3.84	35	0.45
6	1,576	22.5	272	3.89	36	0.54
Mean±SEM	1,588±83	24.3±1.7	267±23	4.04±0.33	31±4	0.48±0.06

* Each value of bile salts, phospholipid, and cholesterol represents an average of six consecutive hourly measurements in which biliary lipid secretion was steady.

‡ Group *t* test of subjects without hepatobiliary disease versus nonobese cholesterol stone patients demonstrated a significant ($P < 0.001$) reduction in both bile salt and phospholipid secretion. In contrast, the obese subjects showed a significantly higher ($P < 0.005$) absolute rate of cholesterol and also phospholipid ($P < 0.02$) secretion.

§ Group *t* test comparison of pigment stone group versus pigment stone patients after cholecystectomy showed no significant ($P > 0.2$) effect on lipid secretion.

TABLE V

Biliary Lipid Secretion Rates—Experimental Groups

Patient	Bile salts*		Phospholipid*		Cholesterol*	
	μmol/h	*μmol/kg-h*	*mg/h*	*mg/kg-h*	*mg/h*	*mg/kg-h*
Nonobese cholesterol stone patients‡						
1	670	10.0	201	3.00	24	0.35
2	409	4.7	225	2.61	17	0.20
3	365	5.7	109	1.72	17	0.27
4	1,045	12.6	216	2.60	40	0.48
5	452	5.2	153	1.75	22	0.26
6	302	4.7	133	2.05	12	0.19
7	824	8.1	173	1.70	32	0.32
8	1,158	13.0	210	2.35	36	0.40
9	693	8.2	205	2.42	17	0.20
10	683	9.4	159	1.95	34	0.39
Mean±SEM	660±91	8.2±1.0	178±12	2.22±0.14	25±3	0.31±0.03
Nonobese cholesterol stone patients after cholecystectomy‡						
1	881	13.1	216	3.22	24	0.35
2	835	9.7	213	2.47	18	0.21
3	922	15.0	201	3.28	27	0.45
4	1,910	23.6	308	3.82	40	0.50
5	2,118	27.7	353	4.61	43	0.56
6	660	10.6	172	2.77	23	0.37
7	1,619	21.6	287	3.84	44	0.58
8	1,254	20.4	283	4.60	38	0.63
9	987	17.5	319	5.67	37	0.66
10	852	15.9	301	5.62	31	0.59
11	1,087	12.0	279	3.08	43	0.47
12	1,034	14.6	241	3.41	29	0.48
13	777	10.3	268	3.56	28	0.37
Mean±SEM	1,149±126	16.3±1.6	265±15	3.84±0.28	33±2	0.48±0.04
Obese subjects without gallstones§						
1	1,439	7.5	450	2.37	109	0.57
2	811	6.0	301	2.21	49	0.36
3	787	5.3	226	1.53	62	0.42
4	803	8.1	260	2.60	48	0.49
5	1,246	8.9	536	3.82	64	0.46
6	1,016	9.2	362	3.26	40	0.36
7	807	7.5	326	3.02	59	0.55
Mean±SEM	987±99	7.5±0.5	352±41	2.69±0.28	66±8	0.46±0.03
Obese cholesterol stone patients after cholecystectomy§						
1	1,153	10.2	220	1.94	47	0.42
2	1,696	14.1	407	3.39	78	0.65
3	1,600	14.5	409	3.70	63	0.57
4	1,661	17.8	321	3.45	51	0.55
Mean±SEM	1,528±126	14.2±1.56	339±45	3.11±0.40	60±7	0.55±0.05

Patients 1–5 studied before and after cholecystectomy.

* Each value of bile salts, phospholipid, and cholesterol represents an average of six consecutive hourly measurements in which biliary lipid secretion was steady.

‡ Group *t* test of cholesterol stone group versus cholesterol stone patients after cholecystectomy revealed a significant ($P < 0.01$) rise in both bile salt and phospholipid secretion.

§ Group comparison of obese subjects without gallstones versus obese patients after cholecystectomy showed a significant ($P < 0.01$) increase in the bile salt secretion rate.

jects was strikingly elevated ($P < 0.001$), being more than twice the control values. Many of these differences disappeared or were changed when the secretion data were weight-related for these obese subjects. Differences within the experimental group, however, persisted. Bile salt secretion per kilogram body weight fell to the low level of nonobese patients with cholesterol gallstones. Cholesterol secretion, even in terms of body weight, was still higher ($P < 0.001$) in the obese subjects in comparison to the nonobese patients with cholesterol gallstones. Even when secretion was calculated in terms of ideal body weight, obese patients remained distinctive in having a very hi h cholesterol secretion.

The effects of cholecystectomy on mean rates of biliary lipid secretion have been illustrated in Fig. 2.

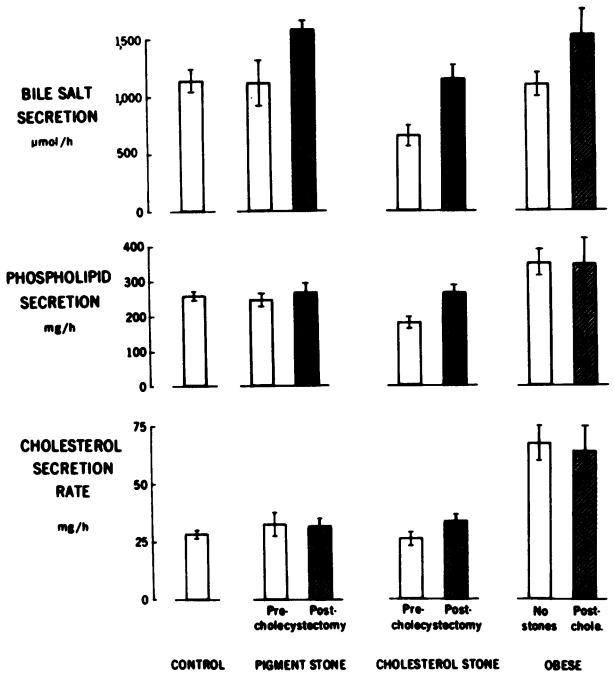

FIGURE 2 Mean biliary lipid secretion (±SEM) before and after cholecystectomy. Bile salt and phospholipid secretion rates significantly ($P < 0.01$) increased after cholecystectomy in the nonobese patients with cholesterol gallstones. Bile salt secretion also increased in the very obese patients ($P < 0.01$), but the increase was not significant in patients with pigment stones. Cholesterol secretion was not affected by cholecystectomy.

Patients with pigment gallstones were not significantly ($P > 0.2$) affected by cholecystectomy despite a small increase in both bile salt and phospholipid secretion. By contrast, the nonobese patients who underwent cholecystectomy for cholesterol gallstones demonstrated a striking ($P < 0.01$) increase in both bile salt and phospholipid secretion. This brought their secretory rates up to the level of the control subjects without any hepatobiliary disease. Even in the small number of very obese patients studied after surgery for cholesterol gallstones, bile salt secretion was significantly ($P < 0.01$) elevated in comparison to the obese subjects without gallstones. Cholesterol secretion was not affected by cholecystectomy in any group, and still remained high in the obese patients.

The effect of cholecystectomy on biliary lipid secretion and composition is shown in Fig. 3 for six patients with cholesterol gallstones (including one Indian female), who were studied before and again after complete recovery from surgery. Both bile salt and phospholipid secretion were increased whereas cholesterol secretion was not significantly changed. This resulted in a significant reduction in percent molar cholesterol.

Bile salt pool size and cycling frequency. The size of the circulating bile salt pool, as determined during the perfusion study, is given in Table VI for the patient groups examined. Mean bile salt secretion rates and pool sizes are given in absolute (millimoles) as well as weight related (micromoles/kilogram) terms. Both the patients with pigment gallstones and the obese subjects had large bile salt pools which were significantly ($P < 0.05$) reduced in the corresponding groups after surgery. In contrast. the nonobese patients with cholesterol gallstones had a significantly ($P < 0.05$) decreased bile salt pool in comparison to the controls. After cholecystectomy there was a slight further fall in this already small pool. When viewed with respect to ideal body weight, the obese subjects had large pools. When calculated in terms of per kilogram absolute body weight, the bile salt pools appeared reduced, as in the nonobese patients with cholesterol gallstones.

The frequency of cycling, included in Table VI, was obtained by dividing the secretion rate by the pool size. The resulting figure represented the fraction of the pool which was secreted and traversed the enterohepatic circuit each hour. Under the conditions evoked by the amino acid infusion, there was a rather narrow range of recycling (0.12–0.19) in the four major groups with functioning gallbladders. The cycling frequency after cholecystectomy more than doubled to over 0.30 in both gallstone groups and also the obese patients. This was especially true ($P < 0.001$) in the nonobese patients with cholesterol gallstones. Patients whose gallbladders were removed even cycled a greater fraction of their bile

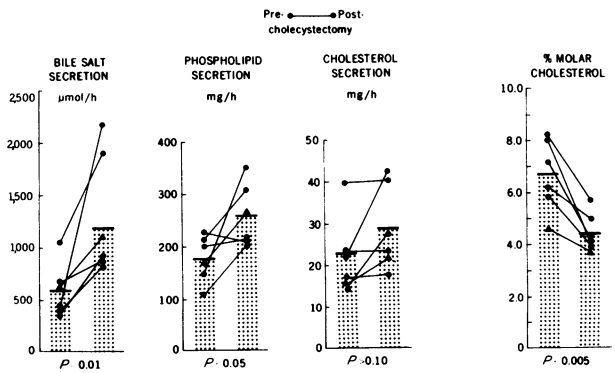

FIGURE 3 Patients studied before and after cholecystectomy for cholesterol gallstones. Individual data are plotted for five whites (closed circles) and an Indian female (closed triangles). Preoperative secretion rates and percent molar cholesterol are connected to the corresponding postoperative results. The means are represented by the shaded bars. Analysis of paired differences by *t* test showed a significant ($P < 0.05$) increase in bile salt and phospholipid secretion, but not in cholesterol secretion. Percent molar cholesterol was consequently reduced ($P < 0.005$) after cholecystectomy.

TABLE VI

Effect of Cholecystectomy on Mean Bile Salt Secretion, Pool Size, and Cycling Frequency

Patient	No.	Bile salt secretion		Bile salt pool size		Cycling frequency
		μmol/h	*μmol/kg-h*	*mmol*	*μmol/kg*	*fraction cycled/h*
Subjects without hepatobiliary disease	11	1,246	17.7	6.48*	92.1	0.19
Pigment stone patients	4	1,219	19.3	7.03	111.8	0.17
Pigment stone patients after cholecystectomy	5	1,628	24.0	4.58‡	67.5	0.36§
Nonobese cholesterol stone patients	5	660	6.6	4.52	54.7	0.12
Nonobese cholesterol stone patients after cholecystectomy	12	1,149	16.9	3.64*	52.8	0.32§
Obese subjects without gallstones	7	987	7.5	7.07	53.1	0.14
Obese cholesterol stone patients after cholecystectomy	4	1,528	14.2	4.88‡	45.1	0.32§

* Group *t* test comparison that nonobese cholesterol stone patients had a significantly ($P < 0.05$) smaller bile salt pool than subjects without hepatobiliary disease.

‡ Group *t* test comparison of bile salt pool size in pigment stone and obese patients with cholesterol gallstones showed a significant ($P < 0.05$) reduction in each group after cholecystectomy.

§ Group *t* test comparison of cycling frequency in all three groups demonstrated a significant ($P < 0.05$) increase after surgery.

salt pool ($P < 0.01$) than the subjects without any hepatobiliary disease. The direct relationship between bile salt pool size and bile salt secretion rate is illustrated in Fig. 4. The 27 patients with radiologically functioning gallbladders (16 with gallstones, 11 without) demonstrated a significant linear correlation ($r = 0.63$ $P < 0.001$) between bile salt secretion and bile salt pool size. A different, but also significant ($r = 0.74$ $P < 0.001$) linear correlation existed in the 21 patients who had undergone cholecystectomy. For the same bile salt pool size, removal of the gallbladder results in a significantly increased bile salt secretion rate.

Biliary bile salt composition. The bile salt composition of hepatic bile was estimated by comparing the results of the two enzyme assays. The 3α-hydroxysteroid dehydrogenase measured bile salts possessing the 3α hydroxyl group—the primary bile salts, cholic acid, and chenodeoxycholic acid, plus the secondary species, deoxycholic acid and lithocholic acid. The 7α-hydroxysteroid dehydrogenase measured the primary bile salts, but not secondary bile salts which lack the 7α hydroxyl group. The proportion of primary bile salts was therefore determined from the ratio of primary (measured by 7α-hydroxysteroid dehydrogenase) to total (measured by 3α-hydroxysteroid dehydrogenase). Any sulfated or keto bile acids present would have been missed, but this assay provided a reasonable indication of bile salt composition.

The subjects with functioning gallbladders had hepatic bile with a bile salt composition distinctly different from patients whose gallbladders had been removed. In the presence of a functioning gallbladder, 77.8%±2.3% (mean±SE) of bile salts were primary

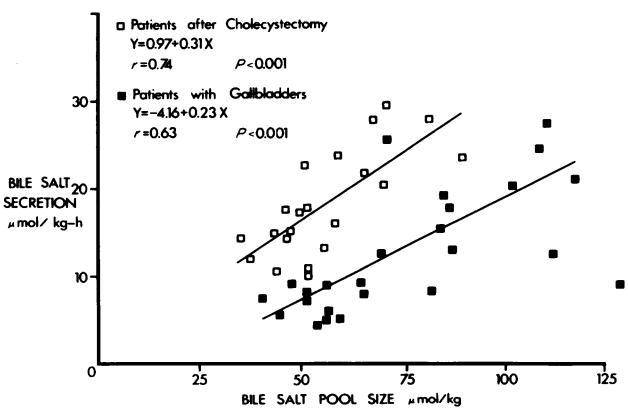

FIGURE 4 Relation of bile salt secretion rate to bile salt pool size. Patients with intact gallbladders (closed symbols) are compared to patients after cholecystectomy (open symbols). Linear regression lines were fitted by least squares with a model 9810A Hewlett-Packard calculator (Hewlett Packard Co.). A significant but different linear correlation existed in both groups.

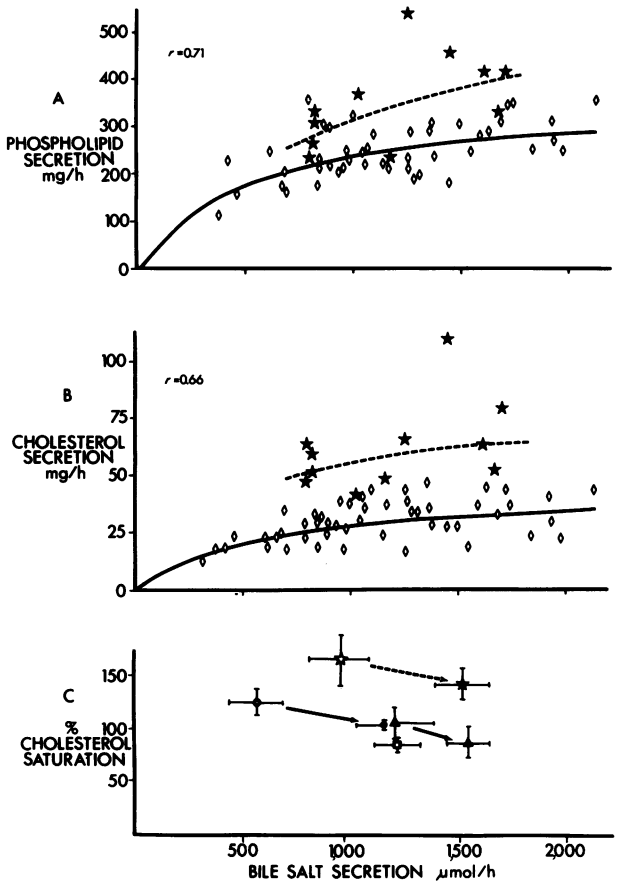

FIGURE 5 Interrelations between bile salt and biliary lipid secretion; the effect of cholecystectomy. Comparison of hepatic secretion of bile salts to that of phospholipid and cholesterol is shown respectively in A and B. Data points represent individual secretory rates for all studies. The 11 very obese patients are depicted by stars with a dashed line. All others are represented by diamonds; the r values shown have been calculated for the corresponding solid regression lines. The nonlinear regression used was in the form $y = x/(a + bx)$ (8). In these nonobese subjects, a curvilinear relation was found between bile salt secretion and both phospholipid and cholesterol secretion. The rates of secretion were quantitatively different in the patients with morbid obesity, with higher phospholipid and especially cholesterol secretion. In C, the percent cholesterol saturation in bile is plotted against the bile salt secretion rate for each patient group. Mean values±SEM are shown as open symbols for patients with functioning gallbladders, and as closed symbols for patients after cholecystectomy. The respective groups are joined by arrows. Stars represented obese patients; circles, nonobese cholesterol gallstone patients; triangles, pigment stone patients; and squares, non-stone control subjects. The percent saturation was calculated (31) by using the maximum cholesterol solubility in systems with 3.5% solid (32,33), which is the total concentration for the three components in hepatic bile (13). An inverse relation existed between bile salt secretion rate and cholesterol saturation. The obese patients, however, formed more saturated bile at any given rate of bile salt secretion. Cholecystectomy in all groups resulted in higher bile salt secretion rates and hence less saturated bile.

(containing the 7α hydroxyl group); the remaining 22.2% were secondary bile salts (lacking the 7α hydroxyl group). After cholecystectomy, the percentage of primary bile salts decreased significantly ($P < 0.001$) to 60.0%±2.7% (mean±SE) with a corresponding increase in secondary bile salts.

Interrelations between bile salt and biliary lipid secretion. The relation between the hepatic secretion of bile salts, and that of cholesterol and phospholipid is shown in Fig. 5A and B. The lipid secretion data overall appeared curvilinear, like an ascending hyperbola. A nonlinear regression of the form $y = x/(a + bx)$, originally used by Wheeler and King (8), was applied to fit the curves to biliary lipid secretion. In the patients who were not obese, both phospholipid and cholesterol secretion were related to bile salt secretion. After a rapid initial increase, phospholipid and cholesterol secretion slowed when bile salt secretion was over 1,000 μmol/h. For the 11 morbidly obese patients, the secretion of phospholipid and especially cholesterol tended to be higher. For the same level of bile salt secretion, the cholesterol secretion rate was double that of the normal weight patients. The influence of obesity on cholesterol secretion was further emphasized by the definite linear relationship ($r = 0.79$, $P < 0.001$) found between the extent of obesity, in terms of percent ideal body weight, and the absolute cholesterol secretion rate. A similar relationship between cholesterol secretion and body surface area was demonstrated, but with a lower correlation coefficient ($r = 0.61$, $P < 0.001$). Grossly obese patients certainly had the highest cholesterol secretion rate.

The effect of the bile salt secretion rate on bile composition was further indicated by a plot of the bile salt secretion rate versus the percent saturation of cholesterol in bile (Fig. 5C). A relationship between the bile salt secretion rate and the percent saturation is quite evident. The greater the secretion rate, the less saturated the bile. The nonobese patients with cholesterol gallstones had a low bile salt secretion rate and hence formed bile saturated with cholesterol. Obese patients, on the other hand, had a nearly normal bile salt secretion rate, but yet much greater cholesterol saturation. This is due to their increased cholesterol secretion rate. Cholecystectomy increased the bile salt secretion rate and lowered the cholesterol saturation in all three groups.

DISCUSSION

A major discovery in our understanding of cholesterol gallstone formation was that cholesterol solubility in bile could be defined by the relative molar proportions of the major biliary constituents, cholesterol, bile acids, and phospholipids (4). Admirand and Small

thus proposed that the underlying defect in cholesterol gallstone formation was the production of bile containing an excess of cholesterol in relation to the solubilizing capacity of bile salts and phospholipid. The liver was then shown to be the source of this abnormal bile, and cholesterol gallstone disease became a metabolic disorder (3). A primate model was developed to further examine the enterohepatic circulation of bile salts and the relation between the bile salt pool size, secretion rate and the resultant bile composition (34). A low pool size gave rise to a low secretion rate of bile salts, resulting in a more saturated bile. Meanwhile, Vlahcevic and co-workers found that patients with cholesterol gallstones had a significantly decreased pool size of bile salts (5). They postulated that if the pool cycled at a normal rate, then the abnormal bile resulted from a decrease in the bile salt secretion rate (6). Such a calculation, of course, assumed that the pool recycles at a known rate (which really must be measured) and that gallbladder bile was representative of bile composition throughout the day (which it is not). To measure the biliary lipid secretion rate in man, Grundy and Metzger developed a duodenal perfusion technique (20). When American Indian women were examined, they found an increase in cholesterol secretion and a moderate decrease in bile salt secretion (10). Further studies showed that obese white females with cholesterol gallstones had markedly increased cholesterol secretion but normal bile salt secretion (11). Therefore, it appeared that there was a discrepancy between patients who were obese, American Indians, and the patients previously studied by Vlahcevic et al. Subsequent confusion arose when Northfield and Hofmann suggested that there was neither a low bile salt nor a high cholesterol output (12). Indeed, no clearcut explanation for gallstone formation was forthcoming, but this study was peculiar in two respects. The bile salt pool was not significantly reduced in their cholesterol gallstone patients; also, at low bile salt outputs, phospholipid secretion fell well below that of cholesterol, a situation not noted in either animals or man (7–11, 31). Altogether, these studies did not clarify whether the basic problem was an increase in cholesterol secretion, or a decrease in the bile salt pool and secretion rate. In a similar vein, our initial observations showed that cholecystectomy improved biliary lipid composition in patients with cholesterol gallstones (13). The attention focussed on the gallbladder led to some reports that surgery improved bile composition (35, 36) and that even gallstone patients with radiologically nonfunctioning gallbladders formed a more normal bile (37–40). Others have suggested that lithogenic bile was not consistently transformed to normal (41, 42), or else remained continuously abnormal despite cholecystectomy (14–16). Thus, the questions confronting us were: (*1*) what were the mechanisms for cholesterol gallstone formation in normal nonobese whites?, (2) how did obese patients differ?, and (3) what were the effects of the standard surgical treatment, cholecystectomy?

These apparent discrepancies can now be largely resolved. Firstly, our findings clearly elucidate two different mechanisms by which abnormal bile is produced. Nonobese patients with cholesterol gallstones show an absolute reduction in both bile salt and phospholipid secretion. Obese subjects, in contrast, have as their primary defect, an excessive rate of cholesterol secretion, rather than any profound change in bile salt and/or phospholipid secretion. Further, the circulating bile salt pool measured in the nonobese patients is reduced, confirming previous observations by Vlahcevic et al. and Swell et al. (5, 6). In the presence of extreme obesity, the bile salt pool is not reduced. Instead, the cholesterol secretion rate is increased and directly related to the extent of obesity. This high cholesterol output into bile agrees with the increased cholesterol turnover documented in obesity (43, 44), the source of which presumably arises in the liver or intestine rather than from adipose tissue (45).

A graduation of secretory defects may therefore exist between the massively obese and the nonobese (46). At one extreme lie the grossly obese subjects whose main problem is excessive cholesterol secretion (30). Next come the obese white women with gallstones, who not only have a raised cholesterol secretion but also a marginally decreased bile salt output (11). Then the obese Indian women follow with combined defects: increased cholesterol secretion, plus a reduced bile salt pool and secretion (10). Finally, at the other extreme, are the nonobese whites whose gallstone disease is manifest by a decreased secretion of the solubilizing lipids in bile, bile salts, and phospholipids. Where the typical, slightly overweight female with cholesterol gallstones would be is, as yet, unknown.

As for the effect of cholecystectomy on bile composition, the divergent findings can be reconciled by considering the conditions under which bile is sampled, —specifically the changing enterohepatic circulation of bile salts and the resultant effect on bile composition (47). Indeed, the present study reaffirms the close association between bile salt secretion and the resultant bile composition (9, 48). At high rates of bile salt secretion, as occurs postprandially, cholesterol and phospholipid secretion plateau so that bile becomes unsaturated. At lower bile salt secretion rates, as might occur with fasting, bile becomes more saturated. Bile therefore exhibits a diurnal variation in composition related to conditions of fasting and feeding, even in patients who have undergone chole-

cystectomy (13, 49). As well, gallbladder bile contains a higher proportion of cholesterol than the duodenal bile collected during the amino acid infusion, a situation mimicking the postprandial state. Because the compositions of fasting hepatic, gallbladder, and stimulated postprandial biles all differ, one must compare hepatic bile composition and secretion under identical conditions. This obviates the potential error introduced by comparing gallbladder bile composition in one (preoperative) state to fasting hepatic bile in another (post cholecystectomy) (14). Certainly, our patients with cholesterol gallstones produced hepatic bile saturated with cholesterol, even under the influence of a stimulated enterohepatic circulation. The very obese subjects without overt gallstones secreted the most saturated bile, and obviously were at great risk of developing gallstones. In the study on obese Indian patients with gallstones, their inordinate propensity to form abnormal bile plus a high proportion of nonfunctioning gallbladders may have nullified any effect of cholecystectomy on biliary lipid secretion (15). The present investigation has shown that obesity alters the relation between bile salt secretion and biliary lipid composition, so that both cholesterol and phospholipid secretion are elevated at corresponding rates of bile salt secretion. That is, obesity is a different disease.

The present results establish that cholecystectomy has a beneficial effect on bile composition. In the nonobese patients, surgery improved the relative lipid composition but not to the level found in either the control group without hepatobiliary disease, or the comparable pigment stone patients who had also undergone cholecystectomy. The morbidly obese patients, moreover, continued to produce quite abnormal bile, despite some reduction in cholesterol saturation. The defect causing lithogenic bile formation is therefore ameliorated but not eliminated by cholecystectomy.

This improvement clearly results from an increase in bile salt and phospholipid secretion, rather than any effect on cholesterol. The increased bile salt secretion rate produced by removal of the gallbladder could only have occurred because either the bile salt pool increased in size, traversed the enterohepatic circuit more rapidly, or both (13). Previous investigations have given conflicting results. Some have suggested that a normal bile salt pool exists after cholecystectomy (50, 51), implying that the pool must have increased; others have noted that the small pool persists, unchanged (14). In animal models, the bile salt pool is reduced by cholecystectomy (52, 53). Our findings are consistent with the latter. They show that cholecystectomy produces a significant reduction of the bile salt pool in patients with a large pool size, and a small but further decline of the already reduced pool in nonobese patients with cholesterol gallstones. The rate at which this small pool cycles, however, doubles after cholecystectomy, and this increase is the prime factor responsible for enhancing bile salt secretion. More rapid recycling of the pool also increases the exposure of bile salts to intestinal bacterial degradation and results in a higher proportion of secondary bile acids (52, 53). In the overall economy of the enterohepatic circulation, a more rapidly circulating pool with increased hepatic return and secretion should cause a reduction in pool size (13). Nevertheless, the decrease in pool size is more than offset by the increase in cycling.

After cholecystectomy, the frequency of enterohepatic cycling increases because the bile salt pool is never trapped in the gallbladder. Even during an overnight fast, a portion may normally continue to circulate but at a slow rate (54). Paradoxically, the primary tenet of perfusion techniques such as the one used here, is that a continuous release of cholecystokinin tonically contracts and hence functionally inactivates the gallbladder (20). Under these conditions, the secretion rate might be expected to merely reflect the bile salt pool size (12). Even though such a relationship was found in our patients with functioning gallbladders, a different interdependence was demonstrated in those patients who had undergone cholecystectomy. Although the gallbladder may not spasmodically fill and empty, it is unlikely to completely empty without any residual volume. More likely it remains at a low fixed volume with constant ingress and egress of bile. Mixing must be complete because the labeled bile salts remain uniformly distributed. Hence, under the experimental conditions, perhaps the enterohepatic circuit becomes longer in the presence of the gallbladder, with some bile always entering and leaving it. Alternatively, rather than a mechanical change, the increased secondary bile salts present after surgery may be handled differently within the enterohepatic circulation. For example, at the level of the liver, hepatic transport of various bile salt species differ (55). Within the small intestine, proximal reabsorption of conjugated secondary bile salts may cause shortcircuiting, such as the jejunohepatic shunting associated with bacterial overgrowth (56).

In conclusion, while all patients with cholesterol gallstones form bile containing a relative excess of cholesterol, the mechanisms in nonobese and obese patients are different. The basic secretory defect in nonobese patients is not excess cholesterol secretion, but rather decreased bile salt and phospholipid secretion. Conversely, in grossly obese subjects with very abnormal bile, cholesterol secretion is greatly increased without any absolute reduction in

bile salt or phospholipid secretion. The low bile salt pool previously reported in most gallstone patients (5) is confirmed by using a different technique, but it is found only in nonobese patients. Further, since the cycling frequency in these patients is low or normal, the small pool size must be the major factor leading to the decreased bile salt secretion rate. The overall homeostatic mechanism regulating the bile salt pool presumably is abnormal. Such patients must have an abnormal suppression of hepatic synthesis of bile salts to result in such a decreased pool size (13, 46). After cholecystectomy, biliary lipid composition distinctly improves. The improvement occurs because, although the bile salt pool remains small (or is even reduced further), it cycles more frequently around the enterohepatic circulation to increase bile salt secretion. In effect, increased cycling offsets the reduction in pool size. Cholecystectomy does not eliminate but merely masks the underlying hepatic defect.

ACKNOWLEDGMENTS

We wish to thank Ms. Ann Tuohy and Ms. Rosalind O'Brien for their excellent technical assistance, and Ms. Anne-Marie Mattyasovszky for typing this manuscript. Appreciation is also expressed for the support and advice given by Dr. Carl Goresky and Dr. Ellen Gordon, and the encouragement of Dr. Eli Schimmel. This work could not have been done without the kind cooperation of the staff of the Montreal General Hospital, Montreal, Quebec, specifically the nursing help of Mrs. Olga Clarke.

Dr. Shaffer was supported in part by the Medical Research Council grant MA-5080 and Dr. Small by the U. S. Public Health Service grant AM 11453.

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