Molecular species of biliary phosphatidylcholines in gallstone patients: the influence of treatment with cholic acid and chenodeoxycholic acid

Jon Ahlberg, Tore Curstedt, Kurt Einarsson, and Jan Sjövall

Department of Medicine and Surgery, Serafimerlasarettet, Department of Medicine, Huddinge University Hospital, and Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

Abstract Molecular species of phosphatidylcholines were analyzed in hepatic and gallbladder bile obtained from six subjects with adenomyoma of the gallbladder (gallstone-free controls) and 27 gallstone patients undergoing cholecystectomy. Seven of the gallstone patients had been treated with cholic acid and seven with chenodeoxycholic acid for at least 8 weeks before operation. The two predominant species were 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoyl-*sn*-glycerophosphocholines which together accounted for 75–80% of the total amount of phosphatidylcholines. Minor species were 1-palmitoyl-2-palmitoleoyl-, 1-stearoyl-2-linoleoyl-, 1-oleoyl-2-linoleoyl-, and 1-palmitoyl-2-arachidonoyl-*sn*-glycerophosphocholines. Gallstone patients had a higher proportion of the 1-palmitoyl-2-oleoyl species and a concomitant lower proportion of the 1-palmitoyl-2-linoleoyl species than gallstone-free subjects. The ratio between the two species was about 0.7 and 0.4, respectively, in the hepatic bile of the two groups of patients. Treatment with bile acids was associated with a normalization of the pattern of phosphatidylcholines.—**Ahlberg, J., T. Curstedt, K. Einarsson, and J. Sjövall.** Molecular species of biliary phosphatidylcholines in gallstone patients: the influence of treatment with cholic acid and chenodeoxycholic acid. *J. Lipid Res.* 1981. **22**: 404–409.

Supplementary key words phospholipids · lecithin · bile acids

Cholesterol is rendered soluble in bile by forming micelles with bile salts and phospholipids. During the last decade a great deal of research interest has been focused on the composition of biliary lipids in relation to gallstone formation (1). It has been established that different bile acids influence the hepatic secretion and biliary concentration of cholesterol to various degrees. Administration of chenodeoxycholic acid, but not cholic acid, has been shown to make bile unsaturated with respect to cholesterol and to induce gallstone dissolution (2, 3). Little attention has been paid to patterns of phosphatidylcholines. Thus, it is not known whether the relative amounts of different phosphatidylcholines are the same in patients with and without gallstones or whether treatment with bile acids may influence the composition of phosphatidylcholines in man.

The present report describes the results of analyses of molecular species of phosphatidylcholines in hepatic and gallbladder bile of healthy subjects and gallstone patients with and without treatment with cholic acid or chenodeoxycholic acid.

MATERIALS AND METHODS

Subjects

The study comprised 33 normolipidemic patients undergoing elective cholecystectomy. In six of the subjects the indication for operation was suspected adenomyoma of the gallbladder wall. The other patients had gallstone disease; seven had been treated with cholic acid and seven with chenodeoxycholic acid prior to operation. Basal data on the patients are given in **Table 1.**

There were no significant differences with regard to age, weight or relative weight between the four groups of patients.

Experimental procedure

Seven patients were treated with cholic acid in a dose of 15 mg/kg body weight per day for at least 8 weeks before operation, and seven were given chenodeoxycholic acid in the same dose. The medication was well tolerated, and body weight remained constant. The patients were hospitalized 2–3 days before operation. They were given the regular hospital diet in which 35, 20, and 45% energy is supplied as fat, protein, and carbohydrate, respectively. The daily intake of cholesterol was about 0.5 mmol/day (193.5 mg). Liver function tests were within normal limits.

Abbreviations: GLC, gas–liquid chromatography; MS, mass spectrometry.

All operations were performed between 8 and 9 AM after an overnight fast. Standardized anesthesia was given. After the abdomen was open, a liver biopsy was obtained, a specimen of which was sent for histological examination. Liver morphology was normal in all cases.

The cystic duct was clamped, and bile from the gallbladder and from the common duct was obtained by aspiration and kept on ice. A regular cholecystectomy was then performed. None of the patients had stones in the common duct as judged by preoperative cholangiography. Histological examination of the gallbladder confirmed the suspected diagnosis of adenomyoma in all six subjects. In about 85% of the patients with cholesterol gallstones, histology showed a slight, chronic cholecystitis but no other pathological findings were encountered. Analysis of the stones showed them in all cases to consist mainly of cholesterol.

Materials

Cholic acid was obtained from Sigma Co., St. Louis, MO, and given in 250-mg (0.64 mmol) capsules. Chenodeoxycholic acid (Chendal®) was obtained from Draco, Sweden, and administered in 125-mg (0.32 mmol) capsules. Both bile acids were shown by thin-layer chromatography to be more than 98% pure.

Analysis of biliary lipid composition

Aliquots of the bile samples were extracted with chloroform–methanol 2:1 (v/v) immediately after aspiration. The concentrations of cholesterol and phospholipids were determined in the chloroform phase of the bile extracts as described by Hanel and Dam (4) and Bartlett (5), respectively. The total bile acid concentration in the bile samples was estimated by a 3α-hydroxysteroid dehydrogenase assay method (6). The relative lipid composition of bile was calculated as moles of cholesterol, bile acids, and phospholipids per 100 moles of total lipids (7). The total lipid concentration, expressed as g/dl, was determined using molecular weights of 387 for cholesterol, 391 for bile acids, and 775 for phospholipids (8). The saturation of gallbladder bile with cholesterol was calculated according to Carey (9).

Analysis of molecular species of phosphatidylcholines

After extraction of the bile samples, small aliquots of the organic phases were analyzed by thin-layer chromatography in the system chloroform–methanol–water 65:25:4 (by vol.). After development, the plates were sprayed according to Dittmer and Lester (10). If

TABLE 1. Basal data of the patients and lipid composition in hepatic and gallbladder bile

Patient Group (Number of Subjects)	Age	Body Weight		Hepatic Bile			Gallbladder Bile		
				Total Lipids	Bile Acids	Phospholipids	Total Lipids	Bile Acids	Phospholipids
	yr	*kg*	*relative*[a]	*g·dl*$^{-1}$	*μmol·ml*$^{-1}$	*μmol·ml*$^{-1}$	*g·dl*$^{-1}$	*μmol·ml*$^{-1}$	*μmol·ml*$^{-1}$
Gallstone-free									
Females (6)	51 ± 4[b]	65 ± 3	111 ± 4	3.3 ± 1.4	42.9 ± 19.5	12.7 ± 5.4	14.3 ± 2.1	182.6 ± 29.1	51.0 ± 8.1
Gallstone									
Females (9)	56 ± 6	66 ± 3	102 ± 3	3.8 ± 0.6	51.9 ± 10.1	13.1 ± 1.8	6.5 ± 1.5[e]	91.7 ± 21.2[d]	23.3 ± 6.3[d]
Males (4)	60 ± 5	69 ± 3	98 ± 6	3.9 ± 2.1	51.0 ± 27.2	15.2 ± 9.4	7.8 ± 2.9	109.2 ± 36.7	35.1 ± 21.5
Total (13)	57 ± 4	67 ± 3	101 ± 3	3.8 ± 0.7	51.6 ± 10.5	13.8 ± 3.1	6.8 ± 1.3[e]	96.5 ± 17.6[d]	25.8 ± 6.2[e]
Cholic acid treatment									
Females (5), males (2)	44 ± 4	70 ± 4	100 ± 5	3.6 ± 1.7	49.5 ± 32.1	12.4 ± 4.8	8.9 ± 1.2[e]	116.1 ± 17.6	36.1 ± 7.8
Chenodeoxycholic acid treatment									
Females (6), male (1)	46 ± 4	62 ± 4	97 ± 4	3.0 ± 0.9	40.6 ± 12.2	12.0 ± 3.1	11.1 ± 2.1	132.4 ± 28.5	57.4 ± 10.0[f]

[a] Calculated as $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100\%$.
[b] Mean ± SEM.
[c] Significantly different from the gallstone-free group, $P < 0.05$.
[d] Significantly different from the gallstone-free group, $P < 0.025$ or $P < 0.02$.
[e] Significantly different from the gallstone-free group, $P < 0.01$.
[f] Significantly different from the gallstone groups, $P < 0.02$.

no lysophosphatidylcholines were detected, the extracted bile samples were analyzed by chromatography on a column of dibutylaminohydroxypropyl Sephadex LH-20 (115 mm × 4.4 mm) in the acetate form, using the solvent system chloroform–methanol–water 15:65:35 (by vol.) (11). The fractions containing lysophosphatidylcholines (0–4.5 ml) and phosphatidylcholines (4.5–13.5 ml) were isolated and the amount of phospholipids in each fraction was determined as described by Bartlett (5). The phosphatidylcholine fractions were hydrolyzed with phospholipase C from *C1. perfringens* (11) and the 1,2-diacylglycerols formed were analyzed as trimethylsilyl ethers. These derivatives were separated according to number of carbon atoms by GLC on a column, 2.5 m × 3.5 mm, filled with 1% SE-30 on 80–100 mesh Chromosorb W HP. The molecular species with 36 carbon atoms in the two acyl chains were also partly separated according to number of double bonds into 1-palmitoyl-2-arachidonoyl-, 1-oleoyl-2-linoleoyl-, and 1-stearoyl-2-linoleoylglycerol trimethylsilyl ethers. These species were completely separated on an OV-1 capillary column (30 m × 0.3 mm) (12) at 280°C. Furthermore, 1,2-dipalmitoyl- and 1-palmitoyl-2-palmitoleoylglycerol trimethylsilyl ethers could also be separated by the capillary column.

The trimethylsilyl ethers of the predominant species, 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoylglycerol, were not separated by GLC. The relative amount of these two species was determined by gas–liquid chromatography–mass spectrometry on an LKB 9000 instrument (LKB-produkter, Bromma, Sweden) using a 1% SE-30 column (1 m × 3.5 mm) at 270°C. The energy of the bombarding electrons was 22.5 eV. Mass spectra taken by repetitive magnetic scanning were recorded on magnetic tape and data were evaluated off-line on an IBM 1800 computer (13, 14). The intensities of ions specific for trimethylsilyl ethers of 1-palmitoyl-2-oleoyl (m/z 410) and 1-palmitoyl-2-linoleoylglycerol (m/z 408) (11, 15) were determined. The ratios between the intensities were calculated and the relative amounts of the two species were determined from a standard curve. This curve was obtained by analysis of mixtures with known amounts of trimethylsilyl ethers of 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoylglycerols by GLC/MS.

GLC/MS was also used to identify molecular species in the different GLC peaks. The analyses showed that each peak, with the exception of that containing the C_{34} species, was due to one major component. Although minor species were detected in each peak, these were added to the amount of predominant compound when the percentage composition of molecular species was calculated.

Analysis of biliary bile acid composition

An aliquot of gallbladder bile was hydrolyzed in 1 M KOH at 110°C for 12 hr. After acidification with hydrochloric acid to pH 1, the deconjugated bile acids were extracted with diethyl ether. The bile acids were methylated and trimethylsilylated and analyzed by GLC using a column, 1.5 m × 4 mm, packed with 1% HiEff 8 BP on 80–100 mesh. Responses of the individual bile acids were checked repeatedly.

Statistical analysis

Data are presented as mean ± SEM. The significance of differences between means was determined by Student's t-test.

RESULTS

Bile acid composition

The relative amounts of cholic, chenodeoxycholic, and deoxycholic acids were 36 ± 4%, 37 ± 3%, and 25 ± 4% in the gallbladder bile of the gallstone-free subjects, respectively. Corresponding values in the gallstone group were 35 ± 4%, 35 ± 3%, and 28 ± 3%, respectively. The percentage composition in the group treated with cholic acid was 56 ± 7%, 12 ± 2%, and 32 ± 9% and in the group treated with chenodeoxycholic acid 6 ± 2%, 82 ± 5%, and 7 ± 2%, respectively. Lithocholic and ursodeoxycholic acids were present in only trace amounts except in the group treated with chenodeoxycholic acid where they averaged 3 ± 1% and 2 ± 1%, respectively.

Biliary lipid composition

The total concentration of lipids in hepatic bile averaged 3.3 ± 1.4 g/dl in the gallstone-free subjects. No differences were noted between this group and other groups of patients (Table 1). The bile acid and phospholipid concentrations averaged 42.9 ± 19.5 μmol/ml and 12.7 ± 5.4 μmol/ml, respectively, in the gallstone-free group and were about the same in other groups of patients (Table 1).

The concentration of total lipids in gallbladder bile was significantly higher in the gallstone-free subjects, 14.3 ± 2.1 g/dl, than in those with gallstones, 6.8 ± 1.3 g/dl, ($P < 0.01$). This led to lower concentrations of bile acids and phospholipids in the patients with gallstones than in those without gallstones (Table 1). The phospholipid concentration was normal in the group treated with chenodeoxycholic acid (Table 1).

The gallbladder bile was saturated with cholesterol in the gallstone patients (133 ± 14%) compared with the gallstone-free subjects (83 ± 10%, $P < 0.05$).

Treatment with cholic acid made the gallbladder bile unsaturated in three of the seven patients (89 ± 11%). Chenodeoxycholic acid-feeding was associated with highly unsaturated bile in all seven patients (65 ± 7%).

Molecular species of phosphatidylcholines in hepatic bile

The results of the analyses are summarized in **Table 2.** The two predominant species were 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoyl-*sn*-glycerophosphocholines, accounting for 22.8 ± 1.2% and 55.4 ± 1.4%, respectively, in the gallstone-free subjects. Minor species were 1-palmitoyl-2-palmitoleoyl- (5.1 ± 1.3%), 1-stearoyl-2-linoleoyl- (6.3 ± 0.6%), 1-oleoyl-2-linoleoyl- (7.0 ± 0.6%), and 1-palmitoyl-2-arachidonoyl- (3.7 ± 0.5%) *sn*-glycerophosphocholines. Only trace amounts of 1,2-dipalmitoyl species were isolated.

The relative amount of the 1-palmitoyl-2-oleoyl species was higher, and that of the 1-palmitoyl-2-linoleoyl species lower in patients with gallstones. The proportions of the other phosphatidylcholines were not significantly different from those in the gallstone-free patients.

The composition of phosphatidylcholines was normal in patients treated with chenodeoxycholic acid, and nearly normal in patients given cholic acid. Patients treated with chenodeoxycholic acid also had a significantly lower proportion of the 1-palmitoyl-2-arachidonyl-*sn*-glycerophosphocholines than untreated patients.

Molecular species of phosphatidylcholines in gallbladder bile

The results are summarized in Table 2. The relative amounts of phosphatidylcholines were about the same in gallbladder bile as in hepatic bile. Gallstone patients had a higher proportion of the 1-palmitoyl-2-oleoyl species and a lower proportion of the 1-palmitoyl-2-lineoyl species than the gallstone-free subjects. Treatment with bile acids was associated with a normalized pattern of the phosphatidylcholines.

DISCUSSION

The present study confirms the well-known fact that gallstone patients have a bile more saturated with cholesterol than bile from gallstone-free subjects (8). In accordance with previous reports, chenodeoxycholic acid made gallbladder bile unsaturated with respect to cholesterol (3). The saturation was also reduced during treatment with cholic acid, which has not previously been reported for gallstone patients (3). However, our group has observed similar changes of the

TABLE 2. Molar percentage composition of molecular species of phosphatidylcholines in hepatic bile and gallbladder bile[a]

	Hepatic Bile						Gallbladder Bile					
Patient Group	16:0, 16:1	16:0, 18:1	16:0, 18:2	18:0, 18:2	18:1, 18:2	16:0, 20:4	16:0, 16:1	16:0, 18:1	16:0, 18:2	18:0, 18:2	18:1, 18:2	16:0, 20:4
Gallstone-free	5.1 ± 1.3	22.8 ± 1.2	55.4 ± 1.4	6.3 ± 0.6	7.0 ± 0.6	3.7 ± 0.5	4.1 ± 0.6	25.3 ± 0.9	51.7 ± 1.1	6.3 ± 0.3	7.7 ± 0.5	4.1 ± 0.7
Gallstone												
Female	3.8 ± 0.5	30.8 ± 1.0[d]	47.1 ± 0.7[d]	5.4 ± 0.6	7.7 ± 0.8	5.2 ± 0.6	4.5 ± 0.6	31.8 ± 2.3	45.9 ± 1.9[b]	6.1 ± 0.7	7.6 ± 0.7	4.6 ± 0.6
Male	5.5 ± 1.4	32.8 ± 4.1	43.9 ± 5.3	6.8 ± 0.7	6.8 ± 1.6	4.5 ± 1.1	5.6	28.9	48.1	—	10.1	6.3
Total	4.7 ± 0.7	31.4 ± 1.4[c]	46.0 ± 1.7[c]	5.7 ± 0.5	7.5 ± 0.7	5.0 ± 0.5	4.6 ± 0.6	31.5 ± 2.1[b]	46.2 ± 1.7[b]	6.1 ± 0.7	8.0 ± 0.7	4.8 ± 0.6
Cholic acid treatment	3.3 ± 0.6	26.1 ± 2.1[e]	52.8 ± 2.4[e]	4.9 ± 0.2	8.2 ± 0.7	4.0 ± 0.7	4.0 ± 0.9	24.5 ± 2.4	54.7 ± 4.2[e]	5.7 ± 0.8	7.9 ± 0.3	3.7 ± 0.5
Chenodeoxycholic acid treatment	4.1 ± 0.8	24.8 ± 3.8	56.0 ± 4.3[f]	6.6 ± 0.5	6.3 ± 0.9	3.2 ± 0.5[e]	4.0 ± 0.9	24.6 ± 4.7	53.7 ± 3.7	5.7 ± 0.8	8.3 ± 0.4	3.7 ± 0.5

[a] Mean ± SEM.
[b] Significantly different from the gallstone-free group, $P < 0.05$.
[c] Significantly different from the gallstone-free group, $P < 0.005$.
[d] Significantly different from the gallstone-free group, $P < 0.001$.
[e] Significantly different from the gallstone group, $P < 0.05$.
[f] Significantly different from the gallstone group, $P < 0.025$.

biliary lipid composition in some hyperlipidemic patients treated with cholic acid (16).

In accordance with previous knowledge, phosphatidylcholines accounted for more than 95% of the biliary phospholipids. Under the conditions used, only trace amounts of lysophosphatidylcholines were detected. The importance of immediate extraction of the bile samples should be stressed. Lysophosphatidylcholines appeared rapidly in unextracted samples even when kept at −20°C. As expected (17), the predominant phosphatidylcholines were the 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoyl species. These species are the major ones in bile from most animals (17). In some animals, 1-palmitoyl-2-oleoyl-*sn*-glycerophosphocholine is the major component whereas in others, as in man, the 1-palmitoyl-2-linoleoyl species predominates. The reason for these differences between animal species is unknown.

Only minor amounts of species containing stearic or arachidonic acids were found in human bile. Bile from animals also contains comparatively small amounts of these species, which constitute a relatively large part of the hepatic phosphatidylcholines. The different pattern of phosphatidylcholines in liver and bile has led to the suggestion that there exists a compartmentalized pool of hepatic phosphatidylcholines that is secreted in bile (17). However, Curstedt (18) has shown that the fractional turnover rates for corresponding phosphatidylcholines in rat liver and bile are the same, but that different phosphatidylcholines have different fractional turnover rates. This finding supports the view that hepatic and biliary phosphatidylcholines are derived from a common pool in the liver. Therefore it is more likely that a selection of certain species from a common pool takes place during bile formation.

Gallstone patients had an increased percentage of 1-palmitoyl-2-oleoyl species and a concomitantly decreased percentage of 1-palmitoyl-2-linoleoyl species. The reason for this is not obvious. A decrease of linoleic acid and an increase of oleic acid after sucrose feeding has previously been noted in analyses of fatty acids obtained after hydrolysis of phosphatidylcholines from human bile (19). An increase of 1-palmitoyl-2-oleoyl species and a concomitant decrease of 1-palmitoyl-2-linoleoyl species has also been observed in bile of rats fed glucose or fructose (20). Similar changes have been seen in liver of rats fed a fat-free diet (21). Thus, it is obvious that administration of glucose or fructose increases the percentage of fatty acids derived from de novo synthesis. It could therefore be speculated that the gallstone patients had a diet with more carbohydrates and less fat during the period before admission. However, the normalized pattern of phosphatidylcholines in gallstone patients treated with bile acids makes this explanation less likely.

Another possibility is that gallstone patients have a metabolic defect that is corrected for by bile acid feeding. It is well recognized that bile acids play an important role in the biliary secretion of phosphatidylcholines and probably also regulate the rate of synthesis of phosphatidylcholines (22–24). Studies in rats have indicated that taurocholic acid preferentially stimulates the synthesis of 1-palmitoyl-2-linoleoyl species via the cytidine diphosphate choline way (23, 24). It is also of interest that the ethynylestradiol which decreases secretion of taurocholic acid in rats increases the secretion of 1-palmitoyl-2-oleoyl *sn*-glycerophosphocholine relative to that of the 1-palmitoyl-2-linoleoyl and 1-stearoyl-2-linoleoyl species (25). Since gallstone disease in nonobese subjects is often associated with a decreased secretion of bile acids (26), it is possible that this metabolic defect causes the abnormal pattern of phosphatidylcholines seen in gallstone patients.

Whether the pattern of phosphatidylcholines may be of pathogenetic importance for gallstone formation cannot be decided from this study. Previous studies have not detected any significant influence of the phosphatidylcholines on cholesterol solubility in mixed bile salt micelles, provided that mixtures of saturated and unsaturated long-chain fatty acids have been present in the phosphatidylcholine preparations used (27, 28). However, further studies are needed to clarify the physiological significance of different phosphatidylcholines in human bile in more detail.

The skillful technical assistance of Ms. Gudrun Green, Ms. Margret Wahlström, Ms. Eija Varhenmaa, and Ms. Margareta Viestam is gratefully acknowledge. The study was supported by grants from the Swedish Medical Research Council (Projects no. 19X-04793 and 03X-219). The ethical aspects of the study were approved by the Ethical Committee of Karolinska Institutet.

Manuscript received 22 May 1980 and in revised form 16 October 1980.

REFERENCES

1. Bennion, L. J., and S. M. Grundy. 1978. Risk factors for the development of cholelithiasis in man. *N. Engl. J. Med.* **299:** 1161–1167, 1221–1227.
2. Thistle, J. L., A. F. Hofmann, B. J. Ott, and D. H. Stephens. 1978. Chenotherapy for gallstone dissolution. I. Efficacy and safety. *J. Am. Med. Assoc.* **239:** 1041–1046.
3. Hofmann, A. F., J. L. Thistle, P. D. Klein, P. A. Szczepanik, and P. Y. S. Yu. 1978. Chenotherapy for gallstone dissolution. II. Induced changes in bile composition and gallstone response. *J. Am. Med. Assoc.* **239:** 1138–1144.
4. Hanel, H. K., and H. Dam. 1955. Determination of small

amounts of total cholesterol by the Tschugaeff reaction with a note on the determination of lathosterol. *Acta Chem. Scand.* **9:** 677–682.
5. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
6. Fausa, O., and B. A. Skålhegg. 1974. Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3α-hydroxysteroid dehydrogenase. *Scand. J. Gastroenterol.* **9:** 249–254.
7. Admirand, W. H., and D. M. Small. 1968. The physical chemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47:** 1043–1052.
8. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
9. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
10. Dittmer, J. C., and R. L. Lester. 1964. A simple specific spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* **5:** 126–127.
11. Curstedt, T., and J. Sjövall. 1974. Analysis of molecular species of ^{2}H-labeled phosphatidylcholines by liquid-gel chromatography and gas chromatography–mass spectrometry. *Biochim. Biophys. Acta.* **360:** 24–37.
12. Rutten, G. A. F. M., and J. A. Luyten. 1972. Analysis of steroids by high resolution gas–liquid chromatography. I. Preparation of apolar columns. *J. Chromatogr.* **74:** 177–193.
13. Reimendal, R., and J. Sjövall. 1972. Analysis of steroids by off-line computerized gas chromatography–mass spectrometry. *Anal. Chem.* **44:** 21–29.
14. Reimendal, R., and J. Sjövall. 1973. Computer evaluation of gas chromatographic–mass spectrometric analyses of steroids from biological materials. *Anal. Chem.* **45:** 1083–1089.
15. Curstedt, T. 1974. Mass spectra of trimethylsilyl ethers of deuterium-labelled mono- and diglycerides. *Biochim. Biophys. Acta.* **360:** 12–23.
16. Angelin, B., and B. Leijd. 1980. Effects of cholic acid on the metabolism of endogenous plasma triglyceride and on biliary lipid composition in hyperlipoproteinemia. *J. Lipid Res.* **21:** 1–9.
17. Holub, B. J., and A. Kuksis. 1978. Metabolism of molecular species of diacylglycerophospholipids. *Adv. Lipid Res.* **16:** 1–125.
18. Curstedt, T. 1974. Biosynthesis of molecular species of phosphatidylcholines in bile liver and plasma of rats given [1,1-$^2H_2$]ethanol. *Biochim. Biophys. Acta.* **369:** 196–208.
19. Alling, C., E. Cahlin, and T. Scherstén. 1973. Relationships between fatty acid patterns of serum hepatic and biliary lecithins in man. Effect of sucrose feeding. *Biochim. Biophys. Acta.* **296:** 518–526.
20. Curstedt, T., and J. Sjövall. 1974. Biosynthetic pathways and turnover of individual biliary phosphatidylcholines during metabolism of [1,1-$^2H_2$]ethanol in the rat. *Biochim. Biophys. Acta.* **369:** 173–195.
21. Lands, W. E. M., and P. Hart. 1966. The control of fatty acid composition in glycerolipids. *J. Am. Oil Chem. Soc.* **43:** 290–295.
22. Nilsson, S., and T. Scherstén. 1969. Importance of bile acids for phospholipid secretion into human hepatic bile. *Gastroenterology.* **57:** 525–532.
23. Balint, J. A., D. A. Beeler, E. C. Kyrakides, and D. H. Treble. 1971. The effect of bile salt upon lecithin synthesis. *J. Lab. Clin. Med.* **77:** 122–133.
24. Gregory, D. H., Z. R. Vlahcevic, P. Schatzki, and L. Swell. 1975. Mechanisms of secretion of biliary lipids. I. Role of bile canalicular and microsomal membranes in the synthesis and transport of biliary lecithins and cholesterol. *J. Clin. Invest.* **55:** 105–114.
25. Kern, F., Jr., H. Eriksson, T. Curstedt, and J. Sjövall. 1977. Effect of ethynylestradiol on biliary excretion of bile acids, phosphatidylcholines, and cholesterol in the bile fistula rat. *J. Lipid Res.* **18:** 623–634.
26. Shaffer, E. A., and D. M. Small. 1977. Biliary lipid secretion in cholesterol gallstone disease: the effect of cholecystectomy and obesity. *J. Clin. Invest.* **59:** 828–840.
27. Hegardt, F. G., and H. Dam. 1971. The solubility of cholesterol in aqueous solutions of bile salts and lecithin. *Z. Ernährungswiss.* **10:** 228–233.
28. Neiderhiser, D. H., and H. P. Roth. 1972. The effect of modifications of lecithin and cholesterol on the micellar solubilization of cholesterol. *Biochim. Biophys. Acta.* **270:** 407–413.