Contribution of vesicular and micellar carriers to cholesterol transport in human bile

Giora J. Sömjen and Tuvia Gilat

Department of Gastroenterology, Ichilov Hospital and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Abstract A nonmicellar, bile salt-independent mode of cholesterol transport in human bile involving phospholipid vesicles was recently reported by our group. In the present study, we have investigated the relative contribution of the phospholipid vesicles and mixed bile salt-phospholipid micelles to cholesterol transport in human hepatic and gallbladder biles. The vesicles (ca 800 Å diameter) were demonstrated by quasi-elastic light scattering (QELS) in fresh bile and after chromatography. Gel filtration under conditions that preserved micellar integrity demonstrated that biliary cholesterol was associated with both vesicles and micelles. At low bile salt concentration, the vesicular phase was predominant and most of the cholesterol was transported by it. With increasing bile salt concentrations, a progressive solubilization of the vesicles occurred with a concomitant increase in the amount of cholesterol transported by micelles. The vesicular carrier may be of particular biological significance for cholesterol solubilization in supersaturated biles. —**Sömjen, G. J., and T. Gilat.** Contribution of vesicular and micellar carriers to cholesterol transport in human bile. *J. Lipid Res.* 1985. **26:** 699–704.

Supplementary key words quasi-elastic light scattering • gel filtration • bile salts • phospholipids • phase transition

Gallstones, most of which are cholesterol stones, are a major public health problem (1). Characterization of mixed bile salt and and phospholipid micelles solubilizing cholesterol (2) and the interaction among these three biliary lipids as described in the triangular phase diagram marked a major advance in biliary physiology (3–5). It defined a narrow micellar zone of cholesterol solubility. According to this model system, cholesterol precipitation was predicted when the solubilizing capacity of the mixed micelles was exceeded. However, considerable discrepancies between this theoretical model and the findings in human bile soon became apparent (6), and supersaturation is frequently found in healthy subjects (5). This led to a search for additional factors that cause cholesterol precipitation in bile. At present, investigations of nucleating factors in bile (7, 8) and factors affecting the motility of the gallbladder (9) are being actively pursued.

We have recently described a bile salt-independent mode of cholesterol transport in human bile (10–12). Cholesterol is carried in large (ca. 800 Å diameter) phospholipid vesicles. It was shown that under experimental conditions these vesicles were able to solubilize up to 80% of the biliary cholesterol at low bile salt concentrations (12). A lecithin lamellar phase has already been suggested as a cholesterol carrier (3), and recently vesicles were reported in model bile solutions (13) and in native bile (14). In the present work we studied the relative contribution of the vesicular and micellar carriers to cholesterol transport in human bile. The interaction between these carriers was also investigated.

MATERIALS AND METHODS

Hepatic bile was obtained from patients after choledochotomy with an indwelling T-tube. Gallbladder bile was obtained from patients during cholecystectomy after clamping of the gallbladder. Fresh bile samples were centrifuged at room temperature for 40 min at 192,000 g_{max} in an ultracentrifuge (Beckman Model L3-50 Rotor SW 50.1) and the supernatant solution was used for further analysis. Chemical analysis of cholesterol, phospholipids, and bile salts before and after centrifugation showed no differences (n = 6). Frozen aliquots were stored at −20°C for further lipid analysis as described (12). The cholesterol saturation index was calculated according to the critical tables (15). [^{3}H]Cholesterol (47.7 Ci/mmol, Amersham) in toluene was heated to 37°C in a glass tube and flushed with N_2 until dry. Biles were added to the dry cholesterol (13 nM) and incubated for 1 hr at room temperature with gentle shaking.

Chromatographic analysis was done on Sephacryl S-300 (Pharmacia) columns (12 × 260 mm except when otherwise specified) with a flow rate of 0.3 ml/min. Blue Dextran (Sigma), mol wt 2 × 10^6, was used as a marker

Abbreviations: QELS, quasi-elastic light scattering; NaTE, buffer solution containing 150 mM NaCl, 50 mM Tris-HCl, pH 8, and 1.5 mM EDTA. CMC, critical micellar concentration.

for the void volume and [^{14}C]alanine (20 mCi/mmol, Amersham) was used to detect the end of the elution from the columns. [^{14}C]Bovine serum albumin (Sigma, radiolabeled according to Rice and Means (16), mol wt 66,700); ovalbumin (Sigma, mol wt 43,000), and myoglobin (Sigma, mol wt 17,200) were used to calibrate the high resolution (60-cm long) columns. The elution buffer contained 50 mM Tris-HCl, pH 8, 1.5 mM EDTA (disodium salt), and 150 mM NaCl (NaTE). Ten mM Na-cholate was added to the buffer to preserve micellar integrity (17, 18) except when otherwise stated. The apparent particle diameter was calculated from the gel filtration according to Laurent and Killander (19). Radioactivity of the samples was measured in Hydro-Luma (Lumac) scintillation fluid by a liquid scintillation counter (Packard Model 3375).

QELS measurements were performed at the Department of Polymer Research, The Weizmann Institute of Science, as previously described (12, 20). Light scattering intensity measurements were done with the same experimental set up as the QELS measurements and the photon count was determined with a counter (Fluke, Model 1953A).

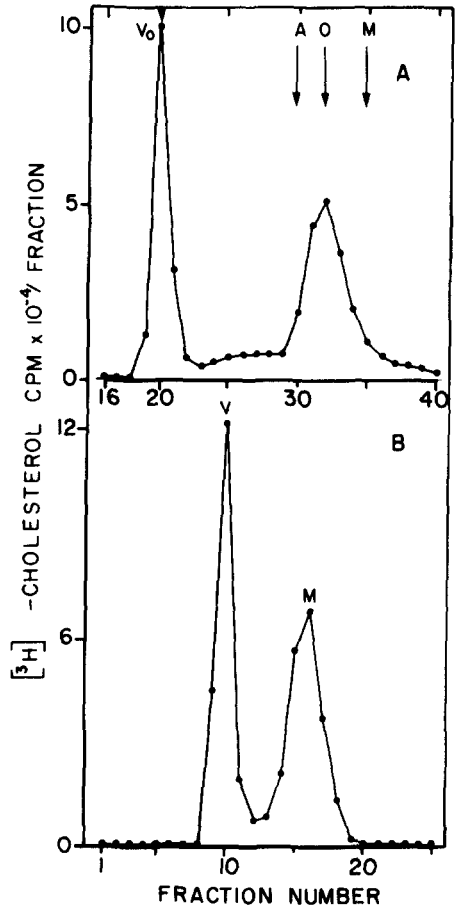

Fig. 1. Gel filtration of human hepatic bile (6.8 mM cholesterol, 7.7 mM phospholipid, and 20 mM bile salts). Following incubation with [^{3}H]cholesterol, aliquots were applied to Sephacryl columns: 60 cm long, 1.2 cm diameter (A); 26 cm long, 1.2 cm diameter (B). The elution buffer contained 10 mM Na-cholate. The void volume marker (V_o) was Blue Dextran, [^{14}C]bovine serum albumin (A), ovalbumin (O), myoglobin (M) and [^{14}C]alanine for the end of elution. Vesicular peak, (V); micellar peak, (M).

TABLE 1. Distribution of cholesterol in vesicles and micelles in bile

Bile No.	% of [^{3}H]Cholesterol in Peak		Lipids in Whole Bile			CSI
	V	M	Ch	PL	BS	
			mM			
1.	57	43	0.8	1.1	3.0	
2.	24	76	0.1	0.2	1.4	
3.	41	59	6.1	7.6	22.3	308
4.	30	70	2.2	3.5	12.8	288
5.	39	61	3.6	4.0	15.0	383
6.	43	57	3.5	4.7	16.5	320
7.	32	68	4.9	5.5	12.1	389
8.	48	52	6.8	7.7	20.0	340
9.	49	51	0.7	0.6	2.5	
10.	55	45	14.7	17.0	37.0	309
11.	52	48	4.2	4.7	13.2	375
12.	11	89	17.1	29.3	120.8	166
13.	33	67	10.3	13.0	52.8	248

Chromatographic analysis of fresh human bile was performed in the presence of 10 mM Na-cholate. Samples 1–8 were hepatic biles, samples 9–13 were gallbladder biles. V, vesicular peak; M, micellar peak; Ch, cholesterol; PL, phospholipids; BS, bile salts; CSI, cholesterol saturation index.

RESULTS

Chromatography of human hepatic and gallbladder biles (n = 13) always revealed two [^{3}H]cholesterol peaks (**Fig. 1, Table 1**). The first peak was always eluted at the position of the void volume marker and the second peak was eluted at the position of the ovalbumin marker (Fig. 1 A). The same separation of the peaks was obtained also on a shorter column (Fig. 1 B). Therefore, all the subsequent work was performed on the shorter column.

As the elution patterns of the endogenous biliary cholesterol and the radioactive cholesterol were similar (r = 0.89, n = 16), the [^{3}H]cholesterol was used as a marker for the endogenous cholesterol. The recovery of the endogenous cholesterol was 80% ± 10 (n = 5). The biliary phospholipids were eluted in both cholesterol peaks with a recovery of 82% ± 12 (n = 6). However, the endogenous bile salts were present only in the second cholesterol peak (at the position of the ovalbumin marker) with recovery of 98% ± 5 (n = 6).

Characterization of the cholesterol peaks

Examination of the first cholesterol peak by QELS revealed particles with a diameter of 720 Å ± 45 (n = 10). The size of the vesicles as measured by QELS in the peak fraction was unchanged at the various concentra-

tions of Na-cholate used in the elution buffer (0–15 mM). The quantity of photons scattered by these particles was directly proportional to the amount of [^{3}H]cholesterol in each fraction of the peak. Electron microscopic examination of this peak after freeze fracture also demonstrated similar sized particles having the appearance of unilamellar liposomes (vesicles). Since the major components of this peak were endogenous cholesterol and phospholipids, we have designated it the "vesicular peak". The size of the vesicles in the original biles (diameter of 760 Å ± 40, n = 17, by QELS) and in the vesicular peak fraction was quite similar. The EM appearance and size of the vesicles after freeze fracture or negative staining in the original biles were similar to that found in the vesicular peak fractions.

The second cholesterol peak (Fig. 1) that was eluted at the position of the ovalbumin marker contained all the endogenous bile salts, as well as part of the cholesterol and phospholipids (see Table 2). On QELS analysis this peak was devoid of vesicles. The apparent particle diameter in this peak was 55 Å as calculated from the gel filtration (19). As the endogenous bile salts also eluted in this peak, it was designated the "micellar peak".

Partition of cholesterol between the vesicular and micellar peaks

Gel filtration was performed (see Table 1) in the presence of 10 mM Na-cholate in order to preserve micellar integrity. In the hepatic bile of eight patients, 24–57% of the [^{3}H]cholesterol was in the vesicular peak. In the gallbladder bile of five patients, 11–55% of the [^{3}H]cholesterol was in the vesicular peak (Table 1). No correlation was found between the proportion of [^{3}H]cholesterol in the vesicular peak of the bile and the cholesterol saturation index of these bile samples (n = 13). Lipid analysis of the chromatographic peak fractions (**Table 2**) showed that the cholesterol phospholipid ratio in the vesicular peak was up to 2:1, whereas in the micellar peak it was 1:2 or even 1:3. The low lipid concentrations reflect the effect of chromatographic dilution.

TABLE 2. Lipid concentration in the peak fractions of biles

Bile No.	Vesicular Peak			Micellar Peak		
	Ch	PL	BS	Ch	PL	BS
		mM			*mM*	
1.	0.2	0.2	10	0.1	0.3	10.2
3.	1.2	0.7	10	0.3	1.0	11.8
9.	0.2	0.1	10	0.1	0.2	10.2
10.	3.3	2.0	10	1.1	3.4	17.3
11.	0.5	0.3	10	0.2	0.5	10.9
12.	0.2	0.2	10	1.6	2.5	21.0
13.	0.9	0.4	10	0.5	1.6	13.3

Biles were chromatographed in buffer containing 10 mM Na-cholate. The total lipid concentration and the relative cholesterol concentration are listed in Table 1. Bile No. refers to numbers in Table 1. Ch, cholesterol; PL, phospholipids; BS, bile salts.

Effect of bile salt concentration on lipid phase transitions

Gel filtration with varying bile salt concentrations was performed to investigate the behavior of the vesicular and micellar phases in bile. Aliquots of one bile sample were applied simultaneously to five identical Sephacryl columns and were eluted with NaTE buffer containing different concentrations of Na-cholate (**Fig. 2**). Without Na-cholate in the buffer almost all the [^{3}H]cholesterol was in the vesicular fraction (Fig. 2 A). With increasing concentrations of bile salts (5 to 40 mM), a progressively higher proportion of the [^{3}H]cholesterol appeared in the micellar peak (Fig. 2 B–D). At Na-cholate concentrations of 10 and 15 mM, the cholesterol appeared in both the vesicular and micellar peaks. At Na-Cholate concentration of 40 mM, the vesicular peak disappeared and all the [^{3}H]-cholesterol was in the micellar phase (Fig. 2 E). **Table 3** demonstrates the transition from the vesicular to the micellar carrier as a function of increasing bile salt concentration.

The phenomenon of phase transition was also demonstrated by QELS (**Fig. 3**). Fresh hepatic bile and the vesicular peak fraction obtained by chromatography of the same bile were incubated with increasing concentrations of Na-cholate. Light scattering measurements of partially purified vesicles originating from the gel filtration showed an almost linear disappearance curve. The diminution in number of vesicles began with the initial augmentation in bile salt concentration (10 mM total endogenous and exogenous bile salts). In the native bile the disappearance curve was linear only above 25 mM bile salts. Total bile salt concentrations up to 20 mM had little effect on the number of vesicles.

For further investigation of phase transitions, rechromatography experiments were performed (**Fig. 4**). When the vesicular peak was reapplied to a Sephacryl column in the same milieu from which it originated (10 mM Na-cholate), there was a dissociation of about 70% of the vesicles (Fig. 4 B). However, when the same vesicular peak was chromatographed in 40 mM Na-cholate, all the [^{3}H]cholesterol appeared in the micellar peak (Fig. 4 C). On the other hand, chromatography of the micellar peak in buffer devoid of bile salts resulted in almost complete transition to the vesicular phase (Fig. 4 D).

DISCUSSION

The current concept of cholesterol transport in bile is based mainly on the solubilization of cholesterol in mixed bile salt-phospholipid micelles (3–5) and mixed discs (13). Although a lamellar-liquid crystalline phase was described

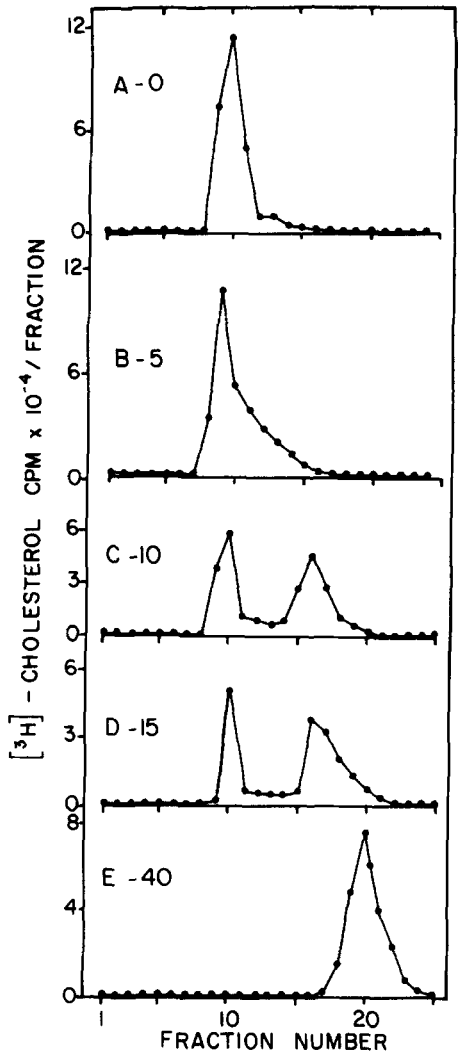

Fig. 2. The effect of Na-cholate on cholesterol carriers in bile. Aliquots of one human hepatic bile (6.1 mM cholesterol, 7.6 mM phospholipid, 22.3 mM bile salts) after incubation with [^{3}H]cholesterol were applied to Sephacryl columns and eluted with buffer only (A), with buffer containing Na-cholate: 5 mM (B), 10 mM (C), 15 mM (D), 40 mM (E).

in model systems by Small, Bourges, and Dervichian (3), its relative contribution to overall cholesterol solubilization has not been emphasized recently.

In a previous study (12), we have demonstrated the presence of large 800 Å diameter cholesterol-containing phospholipid vesicles in fresh human hepatic bile. Under experimental conditions these vesicles were able to solubilize up to 80% of the cholesterol at low bile salt concentrations in vitro.

In the present work we have analyzed the relative contributions of vesicles and mixed micelles to cholesterol solubilization and the interrelationship between these two carriers. Since gel filtration of bile causes dilution and results in micellar dissociation, 10 mM Na-cholate in the elution buffer was used to preserve micellar integrity (17, 18). This concentration of Na-cholate was chosen as being above its CMC. The exposure of the small bile samples to the large amount of bile salts in the elution buffer probably resulted in some vesicular dissociation and shifted the balance toward the micellar phase as shown in Fig. 4 B. Thus, 10 mM Na-cholate in the elution buffer probably favors the micellar carrier and underestimates the proportion of cholesterol carried by vesicles. The choice of 5 mM Na-cholate in the elution buffer might also have been valid and would have shifted the balance to vesicles. The relative contributions of vesicles and micelles to cholesterol transport in bile have not yet been directly measured. Under these experimental conditions biliary cholesterol was found in two distinct structures: the one containing phospholipid vesicles and the second devoid of vesicles and containing most of the endogenous bile salts. We thus have demonstrated and separated the vesicular and micellar cholesterol carriers in human bile. The presence of vesicles was demonstrated in both the original biles and chromatographic peaks by QELS and electron microscopy. The presence of micelles in the second chromatographic peak was postulated on the basis of particle size and lipid composition. The micelles could not be detected by QELS with our experimental setup. Under these experimental conditions 37% ± 4 (n = 15) of the cholesterol was transported by the vesicular carrier and 63% ± 4 (n = 15) by the micellar carrier, with considerable differences among the individual biles (Table 1).

Borgström (17) used gel filtration in the presence of bile

TABLE 3. Effect of Na-cholate on cholesterol distribution in vesicles and micelles

Bile No.	Concentration of Na-cholate in Buffer										Lipids in Bile		
	0 mM		5 mM		10 mM		15 mM		40 mM				
	V	M	V	M	V	M	V	M	V	M	Ch	PL	BS
	% [^{3}H]cholesterol in vesicular and micellar peaks												
1.	87	13	71	29	41	59	28	72	0	100	6.1	7.6	22.3
2.	92	8	63	32			2	98	0	100	0.4	2.0	1.9
3.	64	36	36	64	38	62	22	78	2	98	2.4	2.1	7.2
4.	64	36	72	28	52	48	34	66	2	98	4.2	4.7	13.2
5.	51	49	35	65	11	89	0	100	0	100	17.1	29.3	120.8
6.	65	35	45	55	33	67	20	80	1	99	10.3	13.0	52.8

Chromatographic separation of biles was performed as in Fig. 2. Biles 1–3 were hepatic biles and 4–6 were gallbladder biles. V, vesicular peak fraction; M, micellar peak fraction; Ch, cholesterol; PL, phospholipids; BS, bile salts.

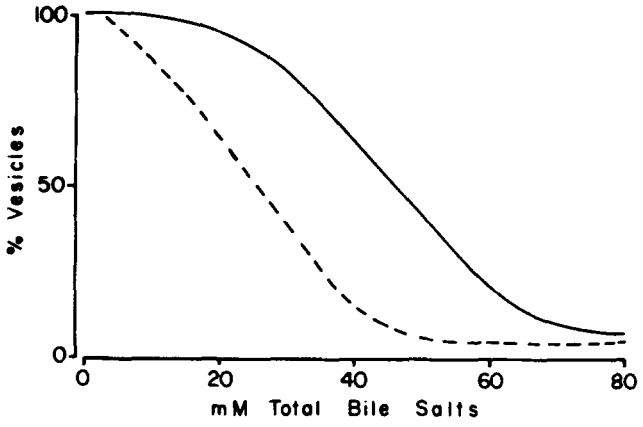

Fig. 3. Vesicle solubilization by Na-cholate. Aliquots of hepatic bile (——) and the vesicular peak fraction (- - -) of the same bile were mixed (1:3 v/v) with saline containing different concentrations of Na-cholate. The percentage of vesicles was calculated from light scattering intensity. The x-axis represents total bile salt concentration including the endogenous bile salts.

salts to measure the size of mixed micelles in model solutions. Norman (21) as well as Lafont et al. (22) performed gel filtration of human bile. They found a peak composed of cholesterol and phospholipids that was eluted at the position of the void volume marker of the columns when no exogenous bile salts were present. In the presence of exogenous bile salts, Lafont et al. (22) demonstrated a discrete peak of the three biliary lipids that eluted approximately at a molecular weight of 70,000. Since they did not use QELS and electron microscopy, they were unable to show the presence of vesicles in the first chromatographic peak and, more importantly, could not prove their presence in the fresh bile prior to any processing.

Large lamellar particles were previously noted in model systems outside the micellar zone (3). Recently, vesicles have been recognized as an important component in diluted supersaturated model systems of the three biliary lipids (13, 23). Large particles were also described as an insignificant population in dog bile (24).

The cholesterol solubilizing capacity of the vesicles is at equimolar or even higher cholesterol/phospholipid ratios (see Table 2) and thus markedly exceeds the cholesterol-carrying capacity of the mixed micelles (25, 26). Mixed micelles are known to be metastable at higher cholesterol saturation levels, however vesicles, especially charged vesicles, may be a more stable structure (27).

In the present work we have demonstrated the presence of vesicles also in gallbladder bile despite high bile salt concentrations in some samples (Table 1, bile samples 9–13). This may be explained by the binding of bile salts to mucins and other proteins (28), causing a decrease in their effective cholesterol solubilizing capacity. This notion is supported by the fact that partially purified vesicles (after gel filtration) were more susceptible to solubilization by exogenous bile salts than vesicles in the native bile (Fig. 3). The persistence of vesicles in gallbladder bile is also favored by the high cholesterol saturation of human bile.

At present the lithogenicity of bile is determined exclusively by reference to the micellar zone in the triangular phase diagram (15). This does not reflect the cholesterol solubilizing capacity of native bile. The vesicular-lamellar phase is ignored in this calculation. It is also possible that the effective solubilizing capacity of bile salts in native bile has been overestimated due to binding of part of the bile salts, probably to proteins.

The present study demonstrated that both vesicles and micelles are major cholesterol carriers in human bile. Vesicles seem to be particularly important for cholesterol solubilization in dilute and supersaturated hepatic bile. The phase transitions between these two carriers may be a physiologic mechanism for cholesterol solubilization in different biliary environments.

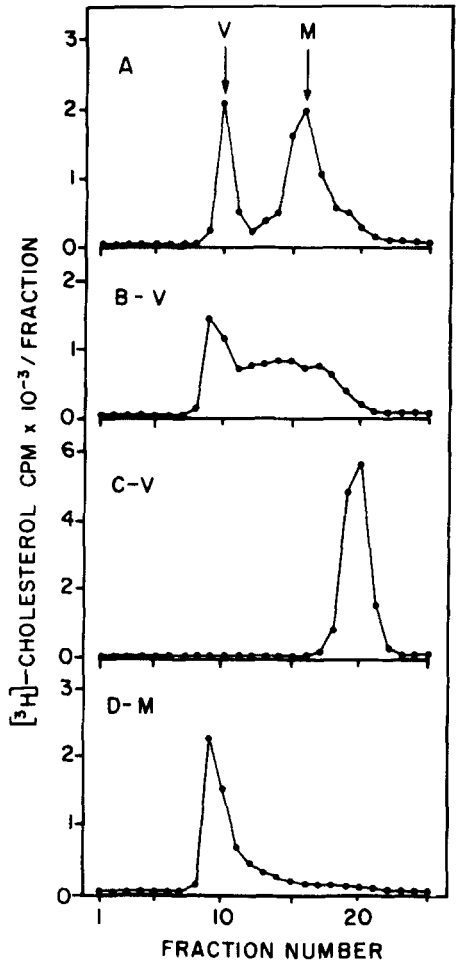

Fig. 4. Rechromatography of vesicular and micellar peaks. A sample of a hepatic bile (2.2 mM cholesterol, 3.5 mM phospholipid, 12.8 mM bile salts) was chromatographed on a Sephacryl column with buffer containing 10 mM Na-cholate (Fig. 4 A). The vesicular peak fraction (V) was reapplied to Sephacryl columns and was eluted with buffer in the presence of Na-cholate 10 mM (B) and 40 mM (C). The micellar fraction (M) was reapplied to a Sephacryl column and was eluted with NaTE buffer only (D).

We wish to thank the following members of the Weizmann Institute of Science: Drs. Henryk Eisenberg and Zvi Kam for valuable help in performing the QELS experiments and Dr. Diana Bach for advice and criticism throughout the study.

Manuscript received 22 August 1984.

REFERENCES

1. Holzbach, R. T. 1983. Pathogenesis and medical treatment of gallstones. In Gastrointestinal Disease. M. H. Sleisenger and J. S. Fordtran, editors. W. B. Saunders Co., Philadelphia. 1356–1373.
2. Isaksson, B. 1953–4. On the lipids and bile acids in normal and pathological bladder bile: a study of the main cholesterol-dissolving components of human bile. *Acta Med. Upsal.* **59:** 296–306.
3. Small, D. M., M. Bourgès, and D. C. Dervichian. 1966. Ternary and quaternary aqueous systems containing bile salt, lecithin, and cholesterol. *Nature.* **211:** 816–818.
4. Admirand, W. H., and D. M. Small. 1968. The physical chemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47:** 1043–1052.
5. 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.
6. Vlahcevic, Z. R., C. C. Bell, Jr., and L. Swell. 1970. Significance of the liver in the production of lithogenic bile in man. *Gastroenterology.* **59:** 62–69.
7. Holan, K. R., R. T. Holzbach, R. E. Hermann, A. M. Copperman, and W. J. Claffey. 1979. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology.* **77:** 611–617.
8. Gollish, S. H., M. J. Burnstein, R. G. Ilson, C. N. Petrunka, and S. M. Strasberg. 1983. Nucleation of cholesterol monohydrate crystals from hepatic and gallbladder bile of patients with cholesterol gallstones. *Gut.* **24:** 836–844.
9. Holzbach, R. T. 1983. Gallbladder stasis: consequence of long term parenteral hyperalimentation and risk factor for cholelithiasis. *Gastroenterology.* **84:** 1055–1058.
10. Sömjen, G. J., and T. Gilat. 1982. Cholesterol is transported in human bile by phospholipid vesicles. A quasi-elastic light scattering (QLS) study. Proc. VIIth Bile Acid Meet. Falk. Symp. **33:** 16 (Abstract).
11. Sömjen, G. J., Y. Peled, and T. Gilat. 1983. Bile salt-independent cholesterol transport in human bile. *Gastroenterology.* **84:** 1316 (Abstract).
12. Sömjen, G. J., and T. Gilat. 1983. A non-micellar mode of cholesterol transport in human bile. *FEBS Lett.* **156:** 265–268.
13. Mazer, N. A., and M. C. Carey. 1983. Quasi-elastic light-scattering studies of aqueous biliary lipid systems. Cholesterol solubilization and precipitation in model bile solutions. *Biochemistry.* **22:** 426–442.
14. Kibe, A., A. C. Breuer, and R. T. Holzbach. 1984. Cholesterol nucleation in human bile by video-enhanced contrast-differential interference microscopy (VEM). The role of vesicles in metastable supersaturation. *Gastroenterology.* **86:** 1326 (Abstract).
15. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
16. Rice, R. H., and G. E. Means. 1971. Radiolabeling of proteins in vitro. *J. Biol. Chem.* **246:** 831–832.
17. Borgström, B. 1965. The dimensions of bile salt micelle. Measurements by gel filtration. *Biochim. Biophys. Acta.* **106:** 171–183.
18. Roda, A., A. F. Hofmann, and K. J. Mysels. 1983. The influence of bile salt structure on self-association in aqueous solutions. *J. Biol. Chem.* **258:** 6362–6370.
19. Laurent, T. C., and J. Killander. 1964. A theory of gel filtration and its experimental verification. *J. Chromatogr.* **14:** 317–330.
20. Jolly, D., and H. Eisenberg. 1976. Photon correlation spectroscopy, total intensity light scattering with laser radiation, and hydrodynamic studies of a well-fractionated DNA sample. *Biopolymers.* **15:** 61–95.
21. Norman, A. 1964. Application of gel filtration of bile acids to studies of lipid-complexes in bile. *Proc. Soc. Exp. Biol. Med.* **116:** 902–905.
22. Lafont, H., D. Lairon, G. Domingo, and J. C. Hauton. 1974. Does a lecithin-polypeptide association in bile originate from membrane structural subunits? *Biochimie.* **56:** 465–468.
23. Müller, K. 1981. Structural dimorphism of bile salt/lecithin mixed micelles. A possible regulatory mechanism for cholesterol solubility in bile? X-ray structure analysis. *Biochemistry.* **20:** 404–414.
24. Mazer, N. A., P. Schurtenberger, M. C. Carey, R. Preisig, K. Weigand, and W. Känzig. 1984. Quasi-elastic light scattering studies of native hepatic bile from the dog: comparison with aggregative behaviour of model biliary lipid systems. *Biochemistry.* **23:** 1994–2005.
25. Horwitz, C., L. Krut, and L. Kaminsky. 1971. Cholesterol uptake by egg-yolk phosphatidylcholine. *Biochim. Biophys. Acta.* **239:** 239–336.
26. Gershfeld, N. L. 1978. Equilibrium studies of lecithin-cholesterol interactions I. Stochiometry of lecithin-cholesterol complexes in bulk systems. *Biophys. J.* **22:** 469–488.
27. Israelachvili, J. N., D. J. Mitchell, and B. W. Ninham. 1977. Theory of self-assembly of lipid bilayers and vesicles. *Biochim. Biophys. Acta.* **470:** 185–201.
28. Makino, S., J. A. Reynolds, and C. Tanford. 1973. The binding of deoxycholate and Triton X-100 to protein. *J. Biol. Chem.* **248:** 4926–4932.