sorption mechanism. No particular mechanism is concluded, nor has an enzymatic mechanism been conclusively eliminated. If the interaction with red cells is physical in nature, it is possible that interaction with other biotissues can be quite significant and that what has been found with red blood cells is only one of many important interactive phenomena. Indeed, McNiff *et al.* have recently suggested that the target tissue (blood vessels) for nitroglycerin might have a higher concentration of drug than found in plasma (13). Furthermore, Armstrong *et al.* conclude that the intact *nitroglycerin molecule is essential* for initiation of relaxation based on dose-response curves of nitroglycerin effects on phenylephrine-contracted canine dorsal pedal arteries and medial saphenous veins (14). These workers found that relaxation occurred without the release of detectable amounts of metabolites into the incubation medium.

REFERENCES

(1) J. A. Armstrong, S. E. Slaughter, G. S. Marks, and P. A. Armstrong, *Can. J. Physiol. Pharmacol.*, **58**, 459 (1980).

(2) H. L. Lee, *Biochem. Pharmacol.*, **22**, 3122 (1973).

(3) C. C. Wu, T. D. Sokoloski, M. F. Blanford, and A. M. Burkman, *Int. J. Pharm.*, **8**, 323 (1981).

(4) F. J. DiCarlo and M. D. Melgar, *Biochem. Pharmacol.*, **19**, 1371 (1970).

(5) G. A. Maier, A. Poliszczuk, and H. L. Fung, *Int. J. Pharm.* **4**, 75 (1979).

(6) I. Dunstan, J. V. Griffiths, and S. A. Harvey, *J. Chem. Soc. Rev.*, 1319 (1965).

(7) P. H. Yuen, S. L. Denman, T. D. Sokoloski, and A. M. Burkman, *J. Pharm. Sci.*, **68**, 1163 (1979).

(8) C. C. Wu, T. D. Sokoloski, A. M. Burkman, and L. S. Wu, *J. Chromatogr.*, **216**, 239 (1981).

(9) C. C. Wu, T. D. Sokoloski, A. M. Burkman, M. F. Blanford, and L. S. Wu, *J. Chromatogr., Biomed. Appl.*, **228**, 333 (1982).

(10) P. Needleman, *Annu. Rev. Pharmacol. Toxicol.*, **16**, 81 (1976).

(11) P. A. Armstrong, J. A. Armstrong, and G. S. Marks, *La Nouvelle Presse. Medicale*, **9**, 2429 (1980).

(12) C. J. Marcus, W. H. Habig, and W. B. Jakoby, *Arch. Biochem. Biophys.*, **188**, 287 (1978).

(13) E. F. McNiff, A. Yacobi, F. M. Young-Chang, L. H. Golden, A. Goldfarb, and H. L. Fung, *J. Pharm. Sci.*, **70**, 1054 (1981).

(14) J. A. Armstrong, G. S. Marks, and P. A. Armstrong, *Mol. Pharmacol.*, **18**, 112 (1980).

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Liquid Crystal Solubilization of Cholesterol: Potential Method for Gallstone Dissolution

JOSEPH B. BOGARDUS

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Abstract ◻ Solubilization rate and phase equilibrium studies were conducted for cholesterol in aqueous sodium oleate solutions. The components interacted to form a lamellar liquid crystalline phase, and this phenomenon was investigated as a potential method for cholesterol gallstone dissolution. Phase equilibria data for cholesterol–sodium oleate–water showed that the mesophase contained approximately equimolar amounts of cholesterol and oleate with large amounts of water. The cholesterol solubilization rate from a static pellet in sodium oleate solutions was much faster than dissolution in sodium cholate solutions and was independent of oleate concentration from 2.5 to 10%. In these experiments, the medium became a cloudy disperson of liquid crystalline phase in the micellar solutions. The rate-limiting step in the solubilization process appears to be dispersion of fragments from the liquid crystalline layer on the cholesterol surface. This hypothesis was consistent with the kinetic effects of viscosity, stirring rate, and oleate concentration. By converting cholesterol to a liquid crystalline phase, the solubilization process avoids the limitations of micellar solubility and interfacial resistance which control cholesterol dissolution in bile salt-containing media.

Keyphrases ◻ Cholesterol—solubilization in fatty acid salt solutions, dissolution in bile salt solutions, potential method for gallstone dissolution ◻ Solubilization—cholesterol in fatty acid salt solutions, bile salt solutions, potential method for gallstone dissolution

Cholecystectomy is the primary method for elimination of gallstones, which are composed primarily of cholesterol. In ~5% of cases, because of size or location, some stones in the ductal system cannot be removed using physical extraction techniques and are retained (1). A number of approaches have been tried for *in situ* dissolution of common bile duct stones using solvents such as ether and chloroform and solutions of bile salts or heparin (2). Recent clinical studies have shown that infusion of monooctanoin (glyceryl monooctanoate), an excellent solvent for cholesterol *in vitro* (3), dissolves common duct stones in 50–70% of patients, although treatment for 2–3 weeks is required (4, 5). The solution is infused into the bile duct *via* a T-tube drain in postcholecystectomy patients or through a nasobiliary tube inserted using a duodenoscope.

This paper reports initial investigations toward development of liquid crystal solubilization as a method for cholesterol gallstone dissolution. Ekwall *et al.* (6, 7) reported the formation of liquid crystalline droplets on the surface of cholesterol monohydrate crystals suspended in fatty acid salt solutions. Longer chain-length salts interacted more strongly with cholesterol than did shorter ones. In the present study, the rate of solubilization of cholesterol was much faster than simple dissolution in sodium cholate solutions. The cholesterol was present in the medium primarily in the form of droplets of liquid crystalline phase dispersed in the micellar solution.

EXPERIMENTAL

Phase Equilibria—Mixtures of cholesterol (1.6–11.8%), sodium oleate (1.2–14.8%), and water were weighed into glass ampules. The ampules were flushed with nitrogen, sealed, warmed to 80° in a water bath, cooled to room temperatures (22–24°), and then allowed to stand for 3 weeks with periodic shaking. A sample of the contents of each ampule was ob-

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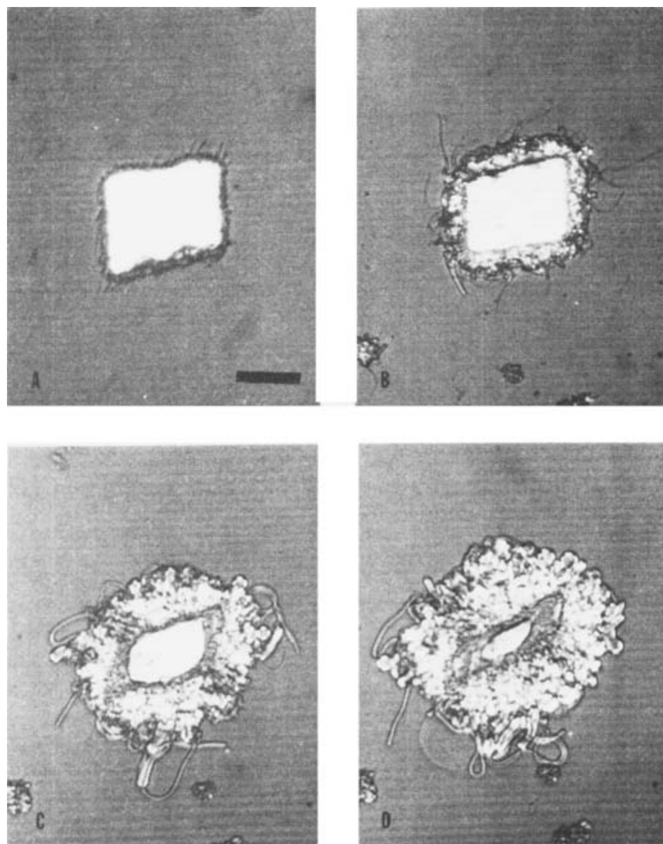

Figure 1—*Liquid crystal solubilization of cholesterol monohydrate in a 5% sodium oleate solution. Key: (A) 5–10 sec after mixing (bar = 100 μm), (B) after 1 min, (C) after 5 min, and (D) after 10 min.*

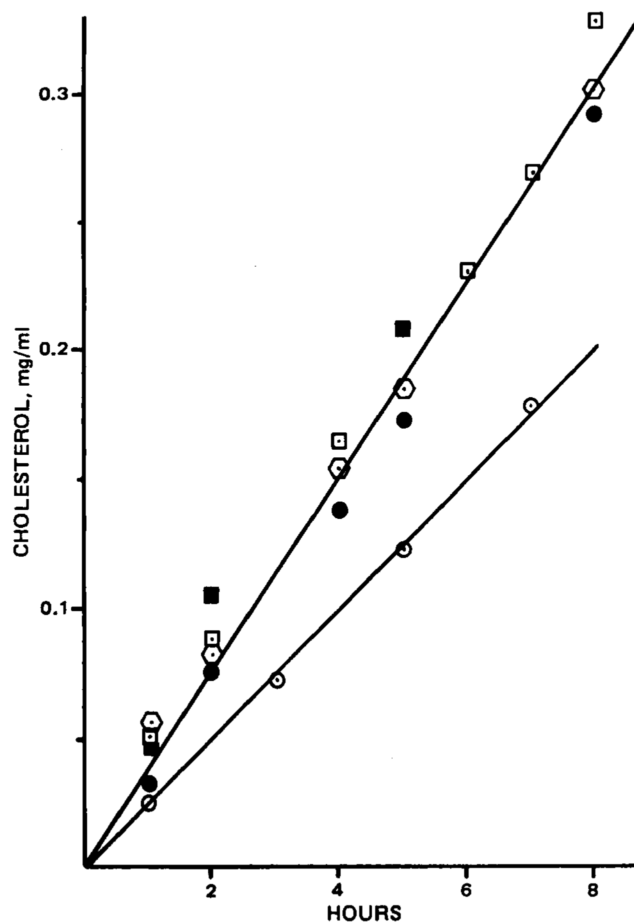

Figure 2—*Cholesterol solubilization in sodium oleate solutions at 37°, 150 rpm. The line for 2.5–10% data has slope = 25×10^{-4} mg cm^{-2} sec^{-1} and zero intercept. The slope for the 1% oleate data is 16×10^{-4} mg cm^{-2} sec^{-1}. Percent oleate key: (⊙) 1.0%, (●) 2.5%, (⊡) 5.0%, (■) 7.5%, and (⊙) 10%.*

served on a polarizing microscope[1], and the remaining material was centrifuged[2] at 22–25° for 18 hr at 25,000×g. The phases were carefully separated using a syringe, checked microscopically for homogeneity, and analyzed by high-performance liquid chromatography (HPLC).

Solubilization Rate—Pellets of cholesterol monohydrate were compressed in a 1.27-cm diameter die with a laboratory press[3]. The pellet-die was placed in the bottom of a vessel containing 300 ml of medium thermostated at 37°. A flat-faced paddle and constant-speed stirrer[4] agitated the medium at the desired rate. Samples were periodically removed, extracted with ether, and assayed by HPLC.

HPLC—An HPLC method was developed for direct separation of cholesterol–oleic acid mixtures using a C_{18} column[5]. The compounds were detected at 205 nm[6] due to the weakly absorbing UV chromophores present. Quantitation was by peak height measurement. To obtain adequate retention of oleic acid, mobile phases previously reported for cholesterol, 100% acetonitrile (8) or 50:50 isopropyl alcohol–acetonitrile (9), were modified. Optimum separation was obtained with 3:97 methanol–acetonitrile at 1.5 ml/min. Oleic acid eluted as a doublet at 3.0 and 3.6 min followed by cholesterol at 9 min.

Samples of cholesterol–sodium oleate mixtures from equilibrium or solubilization rate experiments containing 5–50 mg of the components were weighed accurately in a screw-cap tube and 2 ml of 0.5 *M* citric acid was added. The resulting oily emulsion was then extracted with 10 ml of ether. The ether phase was appropriately diluted with acetonitrile, and this solution was analyzed by HPLC. Preliminary studies indicated that both components were quantitatively extracted by this procedure. Standards containing both compounds were prepared in ether, diluted with acetonitrile, and injected periodically for peak height standardization.

Microscopy—Samples of the phases in the equilibrium study and from the pellet surface during solubilization were examined by polarizing light microscopy. Based on the classifications of Rosevear (10), the liquid crystalline phase appears to be of the lamellar (or neat soap) type.

Materials—Cholesterol USP[7], oleic acid[8], and lauric acid[7] were obtained commercially. Chromatographic solvents were HPLC grade and the source did not affect the assay.

Cholesterol was recrystallized from 10% aqueous acetone which gave the monohydrate. Problems in removal of ethanol were encountered after recrystallization from 95% ethanol. The vacuum-drying conditions needed to remove residual ethanol lowered the water content to ~1% (Karl Fischer titration) and presumably formed anhydrous cholesterol, which did not readily revert to the monohydrate in the presence of water (11). Using the acetone–water method, the solvent evaporated (air-drying for 0.5–1 hr at ambient temperature and humidity), and the water content was near the theoretical value for the monohydrate (4.4% water).

Sodium oleate and sodium laurate were prepared in ethanol from the respective acids by neutralization with 25% sodium methoxide in methanol[9]. The end point was confirmed with bromothymol blue and the solvents were removed using a rotary evaporator. Purity of the sodium oleate was checked by HPLC comparison with oleic acid.

RESULTS AND DISCUSSION

Phase Equilibria—Figure 1 is a photomicrograph of the liquid crystalline phase forming on the surface of crystalline cholesterol monohydrate. The mesophase forms rapidly upon contact with sodium oleate solutions, and smaller crystals are solubilized on the microscope slide within a few minutes. In a separate experiment, a coarse suspension of 300 mg of cholesterol with 300 mg/10 ml sodium oleate solution was solubilized within 24 hr at room temperature with occasional shaking.

[1] Zetopan, Reichert.
[2] Beckman J2-21.
[3] Carver.
[4] Hanson Research Corp.
[5] Waters Associates.
[6] Varian Varichrome.
[7] Sigma Chemical Co.
[8] Fisher purified grade.
[9] Aldrich Chemical Co.

Table I—Preliminary Phase Equilibrium Data for Cholesterol–Sodium Oleate–Water at 22°

Original Mixture (w/w) Cholesterol, %	Original Mixture (w/w) Oleate, %	Phases Present at Equilibrium[a]	Phase Composition: Isotropic Cholesterol, %	Phase Composition: Isotropic Oleate, %	Phase Composition: Liquid Crystalline Cholesterol, %	Phase Composition: Liquid Crystalline Oleate, %	Molar Ratio Cholesterol–Oleate[b]
1.6	14.8	I + L	1.6	7.9	19.6	14.6	1.01
1.6	4.9	I + L	0.16	2.8	10.2	10.6	0.72
1.6	1.2	I + (L + V) + C	0.36	1.4	3.8	4.2	0.68
3.2	14.5	I + L	2.3	5.9	26.3	16.3	1.21
3.2	4.8	I + L	0.43	2.1	9.6	8.7	0.83
3.2	1.2	I + (L + V) + C	0.02	0.59	3.2	3.1	0.78
6.2	14.1	I + L	1.3	5.8	18.8	13.0	1.09
6.2	4.7	L + C	(Phase Absent)		6.9	4.0	1.30
6.2	1.2	L + C	(Phase Absent)		4.1	1.8	1.71
11.8	13.2	I + L	0.59	2.6	9.1	7.5	0.91
11.8	4.4	L + C	(Phase Absent)		9.0	5.6	1.21

[a] Abbreviations: (I) Isotropic micellar solution, (L) Liquid crystalline phase, (V) Vesicles, and (C) Cholesterol monohydrate crystals. [b] In the liquid crystalline phase.

These observations are consistent with previous work on the interaction between fatty acid salt solutions and cholesterol (6). The minimum concentration at which the mesophase was observed decreased as fatty acid chain length increased from C_9 to C_{18}. For sodium oleate, the mesophase formed at concentrations as low as 3×10^{-4} *M*; for sodium nonanoate, it was present only at concentrations >0.14 *M*. This stronger interaction of cholesterol with longer chain-length compounds was not observed in solubility studies in homologous normal alkanols or fatty acid ethyl esters (3). For these solvents, cholesterol solubility was maximum at a chain length of ~6–7 carbon atoms. However, crystalline solvates of cholesterol were observed in some of the solvents which influenced the solubility trends. In addition, the energetic and structural requirements for mesophase formation in water are likely to be quite different than those for solvation and interaction in the nonpolar solvents.

Preliminary phase equilibrium studies were conducted in the dilute solution region for cholesterol–sodium oleate–water, as shown in Table I. Additional data will be necessary before the phase diagram can be constructed. The system viscosity increased at higher component concentrations making equilibration and phase separation more difficult. The phases present at equilibrium and their composition are dependent on the amounts of cholesterol and oleate in the original mixtures. When the cholesterol–oleate ratio was <1, the cholesterol was completely solubilized, and the system consisted of isotropic micellar solution and liquid crystalline phase, which were readily separated by centrifugation. Vesicles were observed in samples with low lipid concentrations and ratios approaching 1:1. The existence of cholesterol–oleate vesicles was reported by Hargreaves and Deamer (12) who investigated the potential use of these systems for drug delivery and as model biomembranes. In Table I, the liquid crystalline phase and vesicles are considered as a single phase, since the two cannot be readily differentiated microscopically. The lipid concentrations in the isotropic solution reflect the solubilization of cholesterol in sodium oleate micelles but are generally lower than the initial amount due to presence of the mesophase or undissolved cholesterol.

As shown in Table I, the relative amounts of cholesterol–oleate in the liquid crystalline phase ranged from 0.68 to 1.71. The lower ratios in the mesophase occurred with low initial concentration of lipids; the higher ratios were observed when the isotropic phase was absent and the mesophase was saturated with cholesterol. The structure of the mesophase is likely to be a bilayer of alternating cholesterol and oleate molecules. Thus, it is not solubilization in the micellar sense in which a number of surfactant molecules are present for each substrate molecule. Small angle X-ray diffraction data would be necessary to determine the structure of the mesophase.

Solubilization Rate—Cholesterol solubilization rate plots from a static compressed disk of cholesterol monohydrate are shown in Fig. 2. After a short time, the medium became a cloudy-milky fluid dispersion of liquid crystalline phase in the isotropic micellar solution. The amount of cholesterol solubilized, and thus the approximate amount of oleate precipitated as mesophase, was much less than the original oleate concentrations in all cases. A single line was drawn through the data at 2.5–10% sodium oleate. The solubilization rate in this range was essentially independent of oleate concentration. The slower rate with 1% oleate may be indicative of a change in the rate-limiting step for the solubilization process. Microscopic observations confirm that the mesophase forms at oleate concentrations <1% (6, 7).

Table II—Effect of 0.9% NaCl on Cholesterol Monohydrate Solubilization in Sodium Oleate Solutions at 37°, 150 rpm

Oleate, %	Rate, $\times 10^4$ mg cm^{-2} sec^{-1} Control	Rate, $\times 10^4$ mg cm^{-2} sec^{-1} With 0.9% NaCl
2.5	23.3	15.3
5	23.6	6.8
10	23.2	8.5

Table III—Effect of Stirring Rate on Cholesterol Solubilization in Sodium Oleate Solutions at 37°

Oleate, %	Rate, $\times 10^4$ mg cm^{-2} sec^{-1} 75 rpm	Rate, $\times 10^4$ mg cm^{-2} sec^{-1} 150 rpm	Rate Ratio
2.5	14.7	23.3	1.59
5	15.1	23.6	1.56

Least-squares analysis of individual runs showed a slight positive intercept which increased with concentration, although the terminal slopes were constant within experimental error. The apparently higher initial rates may be due to the initial dissolution of cholesterol prior to establishment of the liquid crystalline surface layer. Early in the experiment, the mesophase would be expected to dissolve in the bulk medium until its saturation level was reached. Despite the high oleate–cholesterol ratio in the bulk medium, saturation apparently occurred early in the experiments, judging from the turbidity.

Addition of electrolytes is known to increase the cholesterol dissolution rate in simulated bile solutions (13). In these systems, the salt decreases the interfacial resistance to dissolution, a dominant factor in the dissolution rate process. In sodium oleate solutions, sodium chloride decreased the solubilization rate of cholesterol, as shown in Table II. The origin of this effect may be related to the viscosity, the nature of the micellar solution, or that of the mesophase. The viscosity of sodium oleate solutions with added salt was noticeably higher at 5 and 10% oleate, although quantitative data were not obtained. The higher viscosity would be expected to decrease the shear at the pellet surface.

Since the solubilization process is physically different from dissolution, limited data were obtained on the effect of stirring rate (Table III). The ratio of the rates at the two rotational speeds was similar to the square root dependence (1.414) predicted by the Levich equation for a diffusion-controlled process (14). This result was surprising because cholesterol dissolution in bile salt or bile salt–lecithin solutions is relatively insensitive to stirring rate. In this case, interfacial resistance to micellar solubilization of cholesterol is a dominant characteristic (14, 15). The stirring rate dependence in the present experiments probably does not imply that the rate process is diffusion controlled. The presence of the liquid crystalline surface layer and the rate independence of oleate concentration also must be considered in the description of the solubilization process. Experiments using a rotating disk apparatus would be required

Table IV—Comparison of Solubilization Rate in Sodium Oleate Solution with Other Media at 37°

Solution, 5%	Rate, $\times 10^4$ mg cm^{-2} sec^{-1}
Sodium oleate	23.6
Sodium laurate	28.3
Sodium cholate	0.11

to quantitatively evaluate the relative importance of diffusional, interfacial, and surface shear processes in this system.

The solubilization rate of cholesterol monohydrate, prepared by recrystallization from 10% water–acetone, was not sensitive to the compression load used to make the pellet. At loads of 1364, 2045, and 3182 kg (3000, 4500, and 7000 lb, respectively) and oleate concentrations of 2.5, 5.0, and 10%, the rate was essentially unchanged. In preliminary studies, however, problems were encountered with reproducibility at the lower pressures; therefore, 3182 kg was used as a standard compression load for this investigation.

In Table IV, the solubilization rate in 5% sodium oleate is compared with 5% sodium laurate and 5% sodium cholate. The two fatty acid salts show similar solubilization rates. Previous work (6) found that the minimum concentration of sodium laurate which formed a mesophase with cholesterol was 0.007 M, approximately 20-fold higher than the minimum for sodium oleate. Thus, it appears that the concentration for mesophase formation and solubilization rate cannot be directly correlated. The solubilization rates are clearly much faster than dissolution in micellar sodium cholate solutions. The sodium cholate rate is somewhat slower than that reported by Feld and Higuchi, 0.73×10^{-4} mg cm^{-2} sec^{-1}, (15) in 5% sodium cholate with 0.1 M phosphate buffer, pH 8.0. Buffer effects (13), different assay methods, apparatus, and method of cholesterol monohydrate preparation are potential explanations for the difference in rate. The last factor was recently found to affect strongly the dissolution rate (15). Due to these differences, further comparisons with literature data are not likely to be meaningful.

Solubilization Mechanism—The solubilization mechanism of cholesterol in fatty acid salt solutions is considerably different from dissolution in bile salt solutions. Mesophase formation generally does not occur in dilute bile salt or bile salt–lecithin solutions. Formation of a liquid crystalline phase has been observed only with ursodeoxycholate and then only after several hours of contact with cholesterol (16, 17). In dissolution studies with this bile salt, cholesterol release did not level off at the apparent micellar solubility but continued to higher values with formation of the mesophase. An equilibrium model was suggested whereby cholesterol could be dissolved in the micellar solution or directly incorporated into the mesophase (17). After several days when the micelles were saturated with cholesterol, the primary mass transport process was thought to proceed by liquid crystal solubilization. For sodium oleate, the mesophase forms immediately (Fig. 1), and the system has a lower capacity for micellar solubilization of cholesterol.

A hypothetical model for cholesterol solubilization in fatty acid salt solutions is shown in Fig. 3. Rapid formation of a multilamellar region of liquid crystalline material on the crystal surface was observed microscopically. This surface layer grows by diffusion of cholesterol away from the crystal and diffusion of oleate and water from the bulk solution. Figure 1 shows that buildup of a significant surface layer under static conditions does not strongly inhibit solubilization of additional cholesterol from the crystal. Diffusion within the mesophase layer must be rapid in both the inner (cholesterol-rich) and outer (oleate-rich) regions. Scrapings from the pellet surface during the rate studies confirmed the presence of mesophase, even at 150 rpm. The primary mode of mass transfer of cholesterol to the bulk medium is thought to be by dispersion or shearing of liquid crystalline fragments from the surface, as shown in Fig. 3. These so-called myelin figures can be composed entirely of mesophase (spherulites) or have an aqueous core (vesicles or tubules). After dispersal into the bulk solution, the fragments may either dissolve into the micellar solution (which would occur early in the experiment) or remain in the liquid crystalline state.

The solubilization mechanism in this system can be considered as two major steps: (a) diffusional processes involved with formation and growth of the liquid crystalline surface layer and (b) physical dispersion or shearing of liquid crystalline fragments into the bulk medium. The present data support the proposal that the second step is rate limiting in the 2.5–10% oleate solutions. Under these conditions, the rate of surface mesophase formation is assumed to be faster than its removal by stirring. Reasons for this proposal are:

1. The observed relationship between stirring and solubilization rates (Table III) is probably due to the effect of higher shear on the surface layer, increasing the dispersal rate of the mesophase into the bulk solution. The alternate explanation of diffusional control does not seem to be consistent with the physical system under study.

2. If the first step of the solubilization mechanism were rate limiting, a kinetic dependence on oleate concentration but independence on stirring rate would be expected. Both these conditions are in disagreement with the data. Under static conditions on the microscope, however, the rate of solubilization was clearly increased with increasing oleate con-

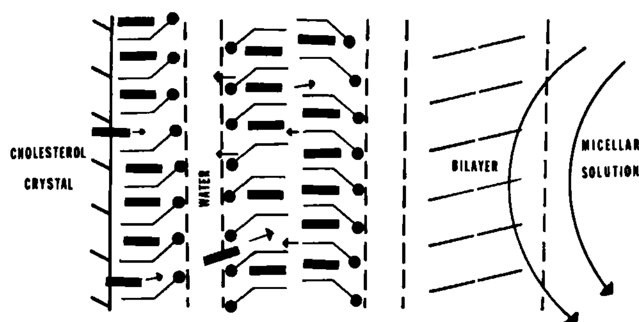

Figure 3—*Schematic diagram for the liquid crystal solubilization of cholesterol in sodium oleate solutions (bars represent cholesterol, angles represent oleate). Small arrows represent diffusion of the species within the mesophase layer. Large arrows indicate dispersion of surface material into the medium.*

centrations. Except for viscosity effects, the rate of a hydrodynamically controlled process (step b) should not be dependent on oleate concentration. This assumes that the process in the surface layer occurs much faster than the mass transport of fragments away from the surface. The slower solubilization rate with 1% oleate may be indicative of a change from hydrodynamic to surface control. As the oleate concentration is reduced, the rate of step a should decrease until this process is similar in rate to step b. At this point, a crossover between the two processes would have to occur.

3. The effect of salt (Table II) may be primarily due to the higher viscosity of the medium rather than to significant changes in the solution or the mesophase. Increased viscosity would reduce the shear on the rate-controlling surface layer.

4. Finally, the similarity in rates with oleate and laurate, but not cholate (Table IV), is consistent with a hydrodynamically controlled process. Laurate does not interact with cholesterol as strongly as oleate (6). Despite this difference in affinity, the solubilization rates were almost equal. This suggests a common rate-controlling step for both salts, which is independent of mesophase formation on the cholesterol surface.

REFERENCES

(1) F. Glenn, *Ann. Surg.*, **179,** 528 (1974).

(2) R. W. Motson, *Br. J. Surg.*, **68,** 203 (1981).

(3) G. L. Flynn, Y. Shah, S. Prakongpan, K. H. Kwan, W. I. Higuchi, and A. F. Hofmann, *J. Pharm. Sci.*, **68,** 1090 (1979).

(4) J. L. Thistle, G. F. Carlson, A. F. Hofmann, N. F. LaRusso, R. L. MacCarty, G. L. Flynn, W. I. Higuchi, and V. K. Babayan, *Gastroenterology*, **78,** 1016 (1980).

(5) J. Schenk, B. Schmack, W. Rösch, J. F. Riemann, H. Koch, and L. Demling, *Dtsch. Med. Wochenschr.*, **105,** 917 (1980).

(6) P. Ekwall, H. Baltscheffsky, and L. Mandell, *Acta Chem. Scand.*, **15,** 1195 (1961).

(7) P. Ekwall and L. Mandell, *ibid.*, **15,** 1403 (1961).

(8) E. Hansbury and T. J. Scallen, *J. Lipid Res.* **19,** 742 (1978).

(9) I. W. Duncan, P. H. Culbreath, and C. A. Burtis, *J. Chromatogr.*, **162,** 281 (1979).

(10) F. B. Rosevear, *J. Am. Oil Chem. Soc.*, **31,** 628 (1954).

(11) C. R. Loomis, G. G. Shipley, and D. M. Small, *J. Lipid Res.*, **20,** 525 (1979).

(12) W. R. Hargreaves and D. W. Deamer, *Biochemistry*, **17,** 3759 (1978).

(13) K. H. Kwan, W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann, *J. Pharm. Sci.*, **66,** 1094 (1977).

(14) S. Prakongpan, W. I. Higuchi, K. H. Kwan, and A. M. Molokhia, *ibid.*, **65,** 685 (1976).

(15) K.M. Feld and W. I. Higuchi, *ibid.*, **70,** 717 (1981).

(16) O. I. Corrigan, C. C. Su, W. I. Higuchi, and A. F. Hofmann, *ibid.*, **69,** 869 (1980).

(17) C. C. Su, J. Y. Park, W. I. Higuchi, M. H. Alkan, O. I. Corrigan, and A. F. Hofmann, *ibid.*, **70,** 713 (1981).

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