# Enthalpy of Bile Salt–Lecithin Mixed Micelle Formation

**RUPERT O. ZIMMERER, Jr. *, and SIEGFRIED LINDENBAUM ˣ**

Received July 17, 1978, from the *Department of Pharmaceutical Chemistry, McCollum Laboratories, University of Kansas, Lawrence, KS 66044.* Accepted for publication October 20, 1978. *Present address: Analytical Research Department, Endo Laboratories Inc., Garden City, NY 11530.

**Abstract** □ The enthalpies for the dissolution of lecithin by sodium salts of cholic, deoxycholic, and chenodeoxycholic acids and their glycine and taurine conjugates are reported. Exothermic enthalpies were found in each case. It is suggested that heat evolution is due to a bile salt–lecithin interaction other than hydrophobic interactions. These results provide strong support for the "mixed disk" model for the complex lecithin–bile salt micelle, which requires that a substantial fraction of the bile salt molecules be incorporated within a lecithin bilayer where hydrogen-bonded pair formation can occur. Calorimetric studies of the interaction between sodium cholate and nonionic, cationic, and anionic detergents yielded exothermic heats. These results suggest that these bile salt molecules partition into the detergent micelle interior as hydrogen-bonded pairs.

**Keyphrases** □ Enthalpy—dissolution of lecithin in aqueous bile salt solutions, micelle formation, hydrogen bonding □ Lecithin—dissolution in aqueous bile salt solutions, micelle formation, enthalpy, hydrogen bonding □ Bile salts, solutions—dissolution of lecithin, micelle formation, enthalpy, hydrogen bonding

Recent clinical success in dissolving cholesterol gallstones by chenodeoxycholic acid administration has renewed interest in the role of bile salts in the dissolution of cholesterol and other lipids (1, 2).

Whereas bile salts are essential for cholesterol solubilization, bile salts alone in aqueous solution increase cholesterol solubility only slightly: 30–100 bile salt molecules are required to solubilize one cholesterol molecule (3). Therefore, bile salts by themselves cannot account for lipid solubilization and transport in digestion or for maintenance of cholesterol in solution in the gallbladder. Lecithin (phosphatidylcholine) is an essential component of bile; in fact, the bile salt–lecithin combination is required for the solubilization of cholesterol and other lipids (5). Bile

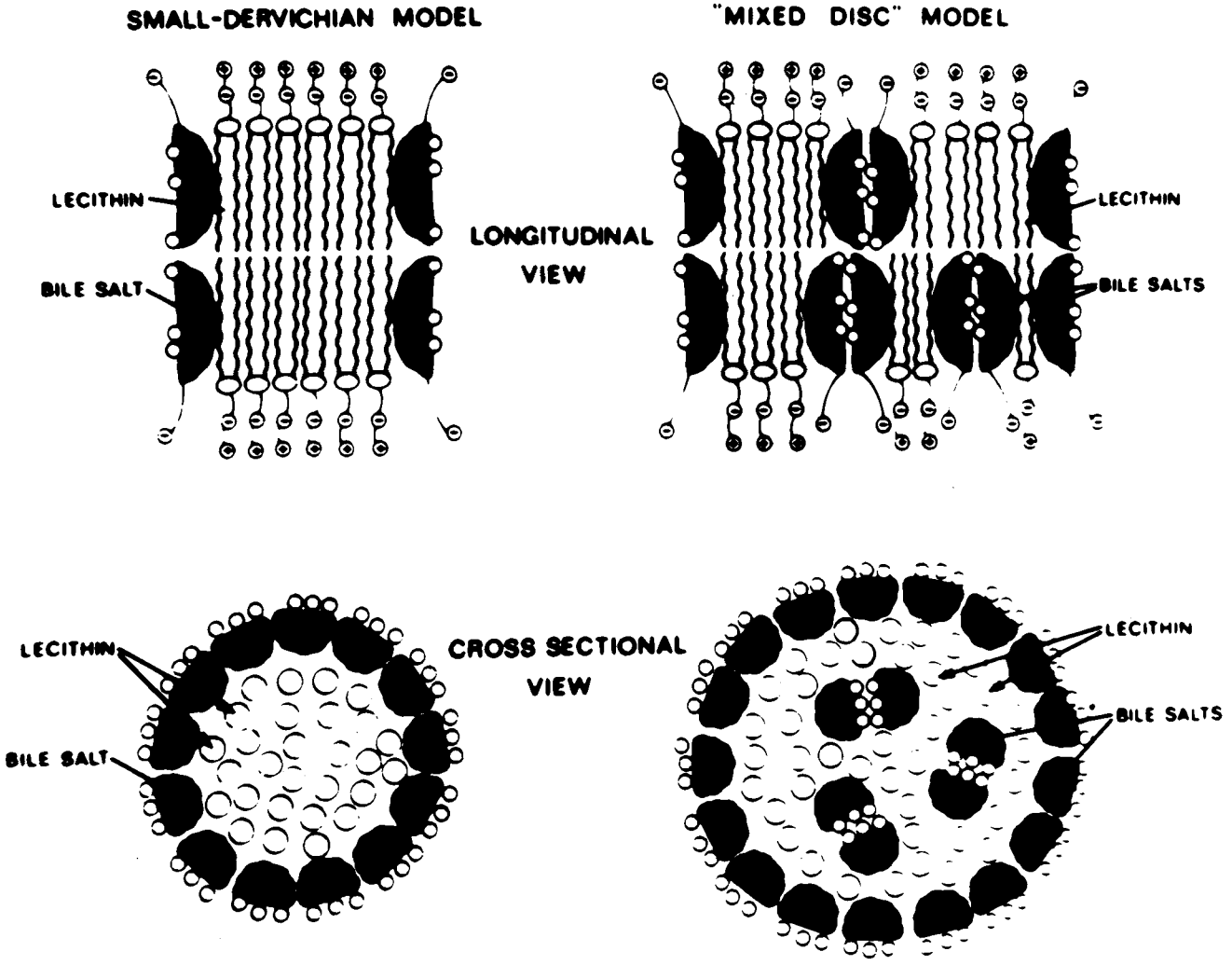


**Figure 1**—*Schematic models for the structure of the bile salt–lecithin mixed micelle. (Reprinted from Ref. 8 with permission of Plenum Press.)*

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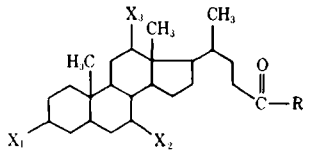
**Table I—Structures and Common Names of Bile Salts Used in This Study**



| Common Name | $X_1$ | $X_2$ | $X_3$ | R |
|---|---|---|---|---|
| Cholic acid | OH | OH | OH | OH |
| Glycocholic acid | OH | OH | OH | $NHCH_2CO_2H$ |
| Taurocholic acid | OH | OH | OH | $NHCH_2CH_2SO_3H$ |
| Chenodeoxycholic acid | OH | OH | H | OH |
| Glycochenodeoxycholic acid | OH | OH | H | $NHCH_2CO_2H$ |
| Taurochenodeoxycholic acid | OH | OH | H | $NHCH_2CH_2SO_3H$ |
| Deoxycholic acid | OH | H | OH | OH |
| Glycodeoxycholic acid | OH | H | OH | $NHCH_2CO_2H$ |
| Taurodeoxycholic acid | OH | H | OH | $NHCH_2CH_2SO_3H$ |

salts have been shown to solubilize lecithin efficiently, forming a micellar solution (3, 5).

On the basis of phase equilibrium and X-ray diffraction studies, a model for the bile salt–lecithin mixed micelle was proposed (6, 7). According to this model (Fig. 1), a disk-shaped micelle is formed on the association of lecithin with bile salt. The disk core consists of the hydrocarbon alkyl chains of the lecithin molecules surrounded by a ring of bile salt molecules. Thus, the disk-shaped micelle exterior presents to the aqueous solvent only the hydrophilic end groups of the phosphatidylcholine and the hydrophilic sides of the bile salt.

Recently, a modified model was proposed on the basis of quasielastic light-scattering data (8). The revised model allowed for the presence of both lecithin and bile salt molecules within the micelle interior (Fig. 1). The bile salt molecules within the micelle interior are presumed to exist as hydrogen-bonded dimers. Recently, other researchers suggested that hydrogen-bonded dimer formation also occurs in dilute aqueous bile salt solutions (9).

The purposes of this study were to measure the enthalpy accompanying the formation of the lecithin–bile salt mixed micellar solution and to correlate the results with the proposed models for these complex micelles.

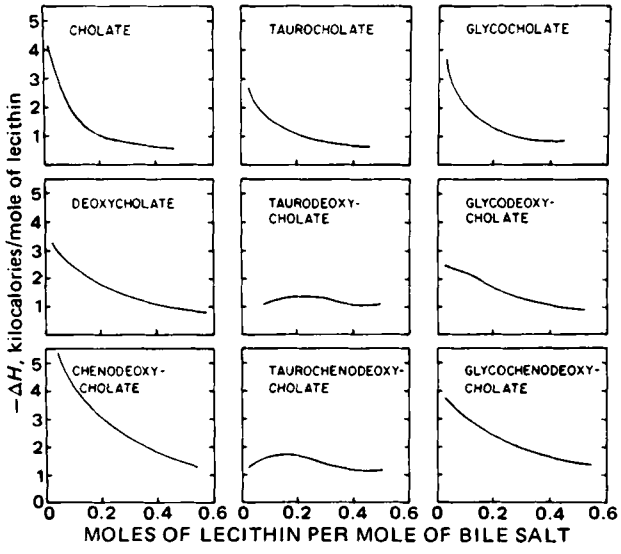



**Figure 2**—*Enthalpy per mole of lecithin, −ΔH, as a function of the molar ratio of lecithin to sodium salt of bile acid at 25°.*

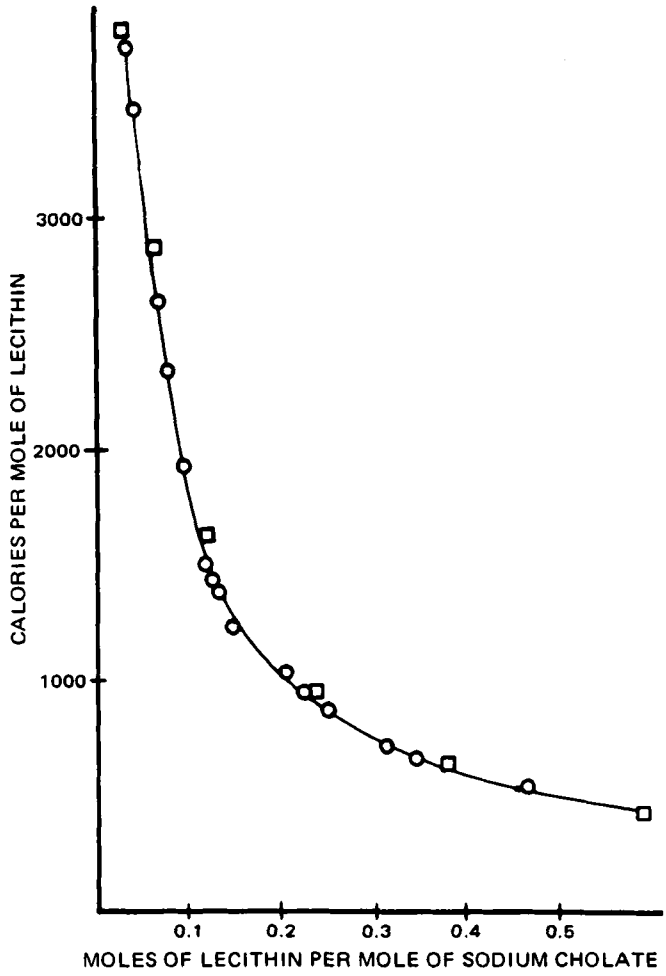



**Figure 3**—*Enthalpy for the dissolution of lecithin by sodium cholate in water (○) and deuterium oxide (□) solutions at 25°.*

## EXPERIMENTAL

**Materials**—L-α-Lecithin (egg phosphatidylcholine), obtained commercially[1], had been purified by chromatography over alumina and silica gel. The lecithin yielded a milky white suspension when mixed with water on a vortex mixer[2]. TLC showed one spot after visualization with iodine vapor. The bile salts used (Table I) were the highest purity available commercially[3]. They were purchased as the sodium salts, with the exception of glycocholic acid, which was obtained as the free acid. The free acid in 25% ethanol was titrated to pH 9 with sodium hydroxide and lyophilized.

Each bile salt was tested by TLC to estimate the number and approximate concentration of the impurities present. Quantitative TLC was performed using a densitometer[4] in the diffuse reflectance mode. The only major impurities were other bile acids, and in no case did the impurity represent greater than 1% of the total bile salt. Some salts were titrated with percholic acid in acetic acid as a further check on purity. Sodium taurocholate was recrystallized from ethanol–ether before use.

Deuterium oxide, 99.8 atom %D, was used as received[5].

Sodium lauryl sulfate[6] was recrystallized from ethanol. Cetrimonium bromide[3] was recrystallized from water–ethanol. "Scintillation grade" polyoxyethylated *tert*-octylphenol[7] was used without further purification. All other chemicals were reagent grade and were used as received. Aqueous solutions were prepared with deionized water.

**Apparatus**—Heats of dissolution of lecithin in aqueous bile salt sus-

[1] Avanti Biochemicals Inc., Birmingham, AL 35226.
[2] Vortex Genie, Scientific Products, McGaw Park, IL 60085.
[3] Sigma Chemical Co., St. Louis, MO 63178. Calbiochem, Dallas, TX 75247.
[4] Chromaflex K49500, Kontes, Vineland, NJ 08360.
[5] Aldrich Chemical Co., Milwaukee, WI 53233.
[6] Eastman Organic Chemicals, Rochester, NY 14650.
[7] Triton X-100, Yorktown Research, South Hackensack, NJ 07606.

**Table II—Enthalpies of Dissolution of Lecithin in Aqueous Solutions of Sodium Salts of Bile Acids at 25°**

| Cholate | | Deoxycholate | | Chenodeoxycholate | | Taurocholate | | Taurodeoxycholate | | Taurochenodeoxycholate | | Glycocholate | | Glycodeoxycholate | | Glycochenodeoxycholate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R^a$ | $-\Delta H^b$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ |
| 0.4671 | 550 | 0.5777 | 776 | 0.5411 | 1285 | 0.4569 | 663 | 0.6135 | 1036 | 0.5036 | 1118 | 0.4563 | 834 | 0.5226 | 882 | 0.5464 | 1329 |
| 0.3492 | 665 | 0.4626 | 924 | 0.4414 | 1595 | 0.3514 | 751 | 0.5000 | 1055 | 0.4012 | 1254 | 0.4278 | 846 | 0.3222 | 1275 | 0.4489 | 1563 |
| 0.3139 | 714 | 0.3920 | 1082 | 0.3830 | 1992 | 0.2565 | 932 | 0.4510 | 1078 | 0.3195 | 1335 | 0.3943 | 893 | 0.2559 | 1489 | 0.3640 | 1750 |
| 0.2520 | 870 | 0.3414 | 1224 | 0.3455 | 2156 | 0.1409 | 1329 | 0.3926 | 994 | 0.2842 | 1467 | 0.3688 | 907 | 0.2065 | 1635 | 0.3087 | 1897 |
| 0.2249 | 954 | 0.2865 | 1433 | 0.2860 | 2232 | 0.1170 | 1459 | 0.3531 | 1233 | 0.2053 | 1616 | 0.3438 | 950 | 0.2047 | 1622 | 0.2718 | 1929 |
| 0.2048 | 1040 | 0.2026 | 1813 | 0.1929 | 2948 | 0.0957 | 1600 | 0.2949 | 1354 | 0.1376 | 1794 | 0.3039 | 1074 | 0.1539 | 1859 | 0.2105 | 2392 |
| 0.1495 | 1257 | 0.1886 | 1875 | 0.1221 | 4035 | 0.0706 | 1935 | 0.2877 | 1240 | 0.1376 | 1634 | 0.2681 | 1003 | 0.1506 | 1925 | 0.1904 | 2510 |
| 0.1332 | 1391 | 0.1581 | 2051 | 0.0884 | 4652 | 0.0458 | 2291 | 0.2588 | 1242 | 0.0918 | 1586 | 0.2302 | 1135 | 0.1187 | 2144 | 0.1567 | 2723 |
| 0.1262 | 1446 | 0.1391 | 2040 | 0.0427 | 5381 | 0.0229 | 2709 | 0.2256 | 1213 | 0.0773 | 1671 | 0.2049 | 1274 | 0.1035 | 2276 | 0.0957 | 3103 |
| 0.1210 | 1511 | 0.1011 | 2880 | 0.0369 | 5613 | | | 0.2124 | 1083 | 0.0610 | 1457 | 0.1684 | 1362 | 0.0862 | 2291 | 0.0943 | 3161 |
| 0.0955 | 1940 | 0.0734 | 2913 | 0.0178 | 5704 | | | 0.1887 | 1263 | 0.0545 | 1430 | 0.1522 | 1470 | 0.0704 | 2252 | 0.0746 | 3388 |
| 0.0784 | 2352 | 0.0702 | 2942 | | | | | 0.1885 | 1333 | 0.0426 | 1522 | 0.1296 | 1595 | 0.0543 | 2333 | 0.0478 | 3352 |
| 0.0684 | 2644 | 0.0690 | 3347 | | | | | 0.1457 | 1544 | 0.0268 | 1257 | 0.0970 | 2017 | 0.0485 | 2422 | 0.0264 | 3627 |
| 0.0424 | 3485 | 0.0365 | 3230 | | | | | 0.1317 | 1270 | 0.0240 | 1028 | 0.0787 | 2340 | 0.0455 | 2443 | | |
| 0.0350 | 3751 | 0.0287 | 3197 | | | | | 0.1298 | 1173 | 0.0229 | 1069 | 0.0518 | 2821 | 0.0440 | 2237 | | |
| 0.0266 | 4084 | 0.0282 | 3195 | | | | | 0.1099 | 1277 | | | 0.0462 | 2813 | 0.0288 | 2374 | | |
| 0.0235 | 4233 | | | | | | | 0.1013 | 1096 | | | 0.0252 | 3682 | 0.0224 | 2425 | | |
| | | | | | | | | 0.0960 | 1180 | | | 0.0129 | 3700 | | | | |
| | | | | | | | | 0.0756 | 1014 | | | | | | | | |
| | | | | | | | | 0.0718 | 921 | | | | | | | | |
| | | | | | | | | 0.0688 | 1087 | | | | | | | | |
| | | | | | | | | 0.0670 | 1099 | | | | | | | | |
| | | | | | | | | 0.0508 | 782 | | | | | | | | |
| | | | | | | | | 0.0508 | 782 | | | | | | | | |
| | | | | | | | | 0.0345 | 767 | | | | | | | | |
| | | | | | | | | 0.0203 | 683 | | | | | | | | |

[a] Moles of lecithin per mole of bile salt. [b] Calories per mole of lecithin; 1 calorie = 4.184 joules.

**Table III—Heat of Mixing of Lecithin Suspended in Deuterium Oxide with Sodium Cholate in Deuterium Oxide Solution at 25°**

| $R^a$ | $-\Delta H^b$ |
|---|---|
| 0.5955 | 461 |
| 0.3817 | 642 |
| 0.2399 | 954 |
| 0.1213 | 1635 |
| 0.0629 | 2878 |
| 0.0306 | 3819 |

[a] Moles of lecithin per mole of sodium cholate. [b] Calories per mole of lecithin.

pensions were measured in a batch microcalorimeter[8] described previously (10). The normal calorimeter strip-chart recorder with an integrator was replaced with a digital output device. The potential from the calorimeter thermopile was amplified[9], and the output was fed sequentially to a voltage-to-frequency converter[10] and to a counter[11].

Heats of mixing of aqueous sodium cholate solutions with aqueous solutions of nonionic, cationic, and anionic detergents were measured with a titration calorimeter[12] interfaced with a minicomputer[13]. The computer counted and recorded the number of pulses representing the calorimeter heat output. It also controlled the on–off timing of the buret and calibration heater[14].

**Methods**—The techniques and methods of calibration of the batch microcalorimeter were described previously (11). Heats of dissolving aqueous suspensions of egg lecithin with bile salt solutions were obtained by mixing 1–4 ml of 0.1 molal solutions of sodium bile salt with 0.25–2 ml of 0.01–0.05 molal suspensions of lecithin in the batch microcalorimeter. Solutions and suspensions were prepared by weight and injected into the calorimeter cell with syringes, which were weighed before and after delivery of solution.

Enthalpies for the dissolution of lecithin by sodium cholate in the presence of sodium chloride were measured in the same way, except that aqueous sodium chloride solutions were substituted for distilled water in the preparation of lecithin suspensions and sodium cholate solutions.

[8] Model 10700-2, LKB Instruments, Rockville, MD 20852.
[9] Model 150 B microvolt ammeter, Keithley Instrument Co., Cleveland, OH 44139.
[10] Designed by Dr. Wesley White, Chemistry Department, University of Kansas.
[11] Model 5330 B preset counter, Hewlett-Packard, Santa Clara, CA 95050.
[12] Model 550 isothermal titration calorimeter, Tronac Inc., Orem, UT 84057.
[13] Model 2100A, Hewlett-Packard, Cupertino, CA 95014.
[14] The computer interface and programs for the operation of the calorimeter were provided by Dr. Wesley White.

**Table IV—Heat of Dissolution of Lecithin by Sodium Cholate in Aqueous Sodium Chloride Solutions at 25°**

| 0.1 $M$ NaCl | | 0.2 $M$ NaCl | |
|---|---|---|---|
| $R^a$ | $-\Delta H^b$ | $R$ | $-\Delta H$ |
| 0.4904 | 589 | 0.5021 | 596 |
| 0.4061 | 676 | 0.3894 | 703 |
| 0.3082 | 824 | 0.2787 | 923 |
| 0.2661 | 931 | 0.2483 | 1036 |
| 0.1988 | 1219 | 0.2009 | 1270 |
| 0.1511 | 1569 | 0.1437 | 1703 |
| 0.1192 | 1953 | 0.1184 | 2055 |
| 0.0890 | 2466 | 0.0894 | 2557 |
| 0.0827 | 2617 | 0.0753 | 2786 |
| 0.0515 | 3348 | 0.0426 | 3501 |
| | | 0.0240 | 3945 |

[a] Moles of lecithin per mole of sodium cholate. [b] Calories per mole of lecithin.

Likewise, the enthalpy measurements in deuterium oxide were performed with deuterium oxide substituted for distilled water in the solutions and suspensions.

Heats of mixing aqueous solutions of sodium cholate with aqueous surfactant solutions were measured by placing 20 ml of 0.03 molal sodium cholate in the isothermal reaction vessel and titrating with 0.05 molal surfactant with the motor-driven syringe buret. Detailed procedures for the operation and calibration of the titration calorimeter were published (12, 13). The batch and titration calorimeter gave results in good agreement with literature values for the heat of dilution and heats of mixing for standard reactions.

## RESULTS

Heats of dissolution of suspended lecithin by aqueous bile salt solutions are given in Table II and Fig. 2 as calories per mole of lecithin as a function of the final system composition expressed as moles of lecithin per mole of bile salt. For the dissolution of lecithin by sodium cholate solutions, these experiments were repeated in deuterium oxide (Table III and Fig. 3) and in 0.1 and 0.2 $M$ NaCl (Table IV and Fig. 4). In all cases, the initially cloudy lecithin suspension formed a clear solution after mixing in the calorimeter with the bile salt solution. Over the concentration range studied, the measured heats were a function only of the lecithin to bile salt ratio and not of the total lipid concentration.

Enthalpy values for the mixing of surfactant solutions with aqueous sodium cholate solutions are given in Table V and Fig. 5 as calories per mole of surfactant as a function of moles of surfactant per mole of sodium cholate.

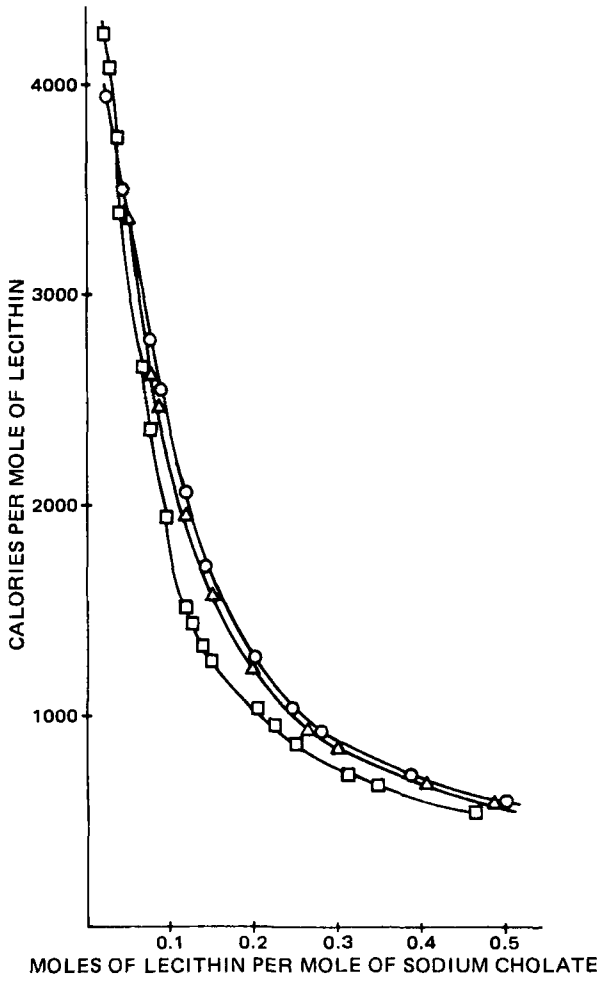



**Figure 4**—*Enthalpy of dissolution of lecithin by sodium cholate in the presence of added sodium chloride at 25°. Key: □, water; △, 0.1 M NaCl; and ○, 0.2 M NaCl.*

## DISCUSSION

The dissolution of suspended hydrated lecithin by bile salt solutions was accompanied in every case by the evolution (negative $\Delta H$) of large amounts of heat (Fig. 2). Previous observations of heats of micelle formation generally yielded small and positive values for the enthalpy (14–18). It has generally been assumed, therefore, that the driving force for micelle formation is the positive entropy change associated with a hydrophobic species leaving the aqueous environment to form a micelle (17). Micelle formation of surfactants is attributed to the hydrophobic effect phenomenon, similar to the stabilization of proteins in their native configuration in an aqueous environment (17, 19).

The large exothermic heats obtained in these studies suggest that the process is not simply "hydrophobic" micelle formation. The process investigated here is shown in Scheme I:

$$x\mathrm{L}(\mathrm{H_2O}) + y(\mathrm{BS})_{\mathrm{sol}} \rightarrow \mathrm{L}_x(\mathrm{BS})_y$$

*Scheme I*

in which $x$ moles of hydrated lecithin (L) interact with $y$ moles of bile salts (BS) in aqueous solution to form a complex micellar solution. In an aqueous dispersion, lecithin is presumably in the form of liposomes (20–22) consisting of concentric envelopes of lipid bilayers. The milky-white lipid bilayer dispersions are solubilized in the presence of bile salt solutions to form optically clear micellar solutions. Under the experimental conditions, the heat measured may be seen as the sum of the heat of simple bile salt micelle dissociation and the heat of mixed micelle formation with lecithin.

Dilution heat studies on sodium cholate solutions showed that the enthalpy of simple micelle dissociation is negligible compared to the total heat observed for the dissolution of lecithin by bile salt solution. The observed heat is, therefore, virtually entirely the heat of mixed micelle formation. Heat evolution during this process is difficult to understand

**Table V—Heat of Mixing Aqueous Surfactant Solutions with Aqueous Sodium Cholate at 25°**

| Anionic[a] Detergent | | Nonionic[b] Detergent | | Cationic[c] Detergent | |
|---|---|---|---|---|---|
| $R$[d] | $-\Delta H$[e] | $R$ | $-\Delta H$ | $R$ | $-\Delta H$ |
| 0.0352 | 4784 | 0.0247 | 6474 | 0.0244 | 3779 |
| 0.0704 | 4186 | 0.0495 | 6200 | 0.0489 | 3323 |
| 0.1056 | 3870 | 0.0743 | 5398 | 0.0733 | 2936 |
| 0.1408 | 3637 | 0.0990 | 4794 | 0.1222 | 2601 |
| 0.2112 | 3203 | 0.1485 | 3974 | 0.1711 | 2344 |
| 0.2816 | 2899 | 0.1980 | 3473 | 0.2200 | 2082 |
| 0.3520 | 2697 | 0.2476 | 3152 | 0.2689 | 1883 |
| 0.4224 | 2553 | 0.2971 | 2922 | 0.3178 | 1754 |
| 0.4928 | 2408 | 0.3961 | 2578 | | |
| 0.5632 | 2289 | 0.4950 | 2315 | | |

[a] Sodium lauryl sulfate. [b] Polyoxyethylated *tert*-octylphenol. [c] Cetrimonium bromide. [d] Moles of surfactant per mole of sodium cholate. [e] Calories per mole of surfactant.

in terms of the Small–Dervichian model (Fig. 1) since no new chemical bonds are formed in the mixed micelle. In the mixed disk model, however, a substantial fraction of the bile salt molecules are partitioned into the bilayer interior as hydrogen-bonded pairs. It may be assumed that in the low dielectric micelle interior, the bile salt molecules are hydrogen bonded to each other to a much greater extent than in the aqueous phase. The partition of bile salt molecules from the aqueous phase to the micelle interior would be accompanied by increased hydrogen bonding and heat evolution.

Recently, Oakenfull and Fisher (9) suggested that bile salts are associated by hydrogen bonding in aqueous solution. This finding, based on conductance and volume studies, is in conflict with earlier models for bile salt association. It has generally been assumed that premicellar association of bile salts occurs by hydrophobic interaction between the nonpolar flat surfaces of the bile salt molecules. This hydrophobic association model was suggested on the basis of NMR studies of aqueous bile salt solutions (23). This conflict remains to be resolved. It seems reasonable to assume that any hydrogen-bonded association in the aqueous phase will be enhanced in a less polar medium. Hydrogen bonding between bile salt molecules in a lecithin bilayer also would be consistent with spectroscopic evidence for hydrogen-bonded association of di- and trihydroxy methylcholanoates in carbon tetrachloride (24).

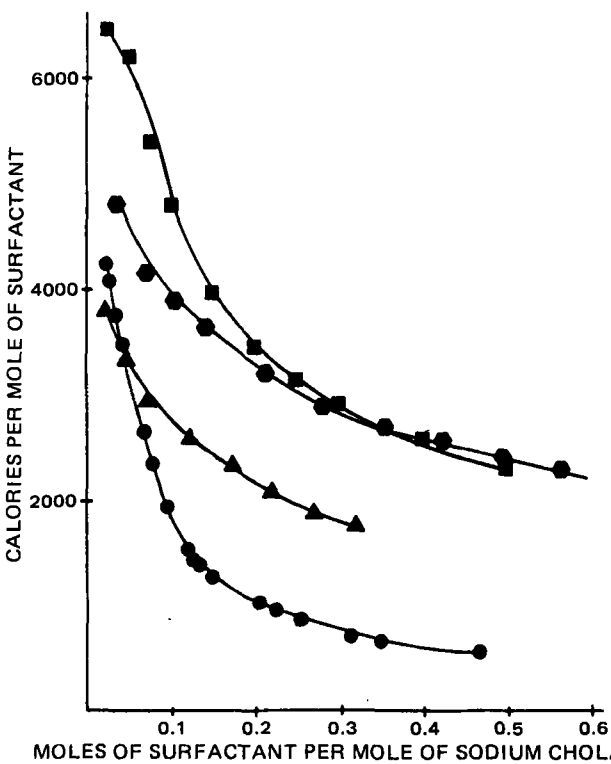



**Figure 5**—*Enthalpy of mixing surfactant solutions with sodium cholate solution at 25°. Key: ●, lecithin; ■, polyoxyethylated* tert*-octylphenol; ▲, cetrimonium bromide; and ●, sodium lauryl sulfate.*

According to the mixed disk model (8), the ratio of lecithin to cholate within the micelle interior is 2:1, not including the cholate molecules that make up the circumference of the disk.

This 2:1 ratio is also the proportion observed at the phase limit (7, 25, 26). Therefore, for each mole of lecithin solubilized, there is 0.5 mole of bile salt within the micelle. If each pair of bile salt molecules is attached by two hydrogen bonds, then one-half of a hydrogen bond is formed for each lecithin molecule solubilized. The enthalpy for hydrogen bond formation in alcohols varies from −3 to −10 kcal/mole (27, 28). If a value of −6 kcal/mole is assumed, then the heat accompanying the dissolution of 1 mole of lecithin would be −3 kcal/mole. This crude estimate of the enthalpy change expected assumes that hydrogen bonding is the most important contribution. Since other processes such as hydrophobic effects and changes in hydration also contribute, the expected enthalpy value cannot be estimated more accurately.

Comparison of the dissolution heat of lecithin by sodium cholate in water and deuterium oxide (Fig. 3) shows that the results are indistinguishable. These results also suggest that the extent of interaction of the bile salt in the micellar interior determines the enthalpy observed and that hydrophobic forces in the aqueous phase play only a minor role.

In several cases (Fig. 2), the enthalpy per mole of lecithin dissolved increased sharply as the lecithin to bile salt ratio decreased. This result suggests that as this ratio decreases, more bile salt partitions into the micelle phase, with a concomitant increase in hydrogen bonding. An increase in bile salt partition into the micellar phase as the aqueous bile salt concentration increases would be expected to occur if the distribution of bile salt between the micelle interior and the aqueous phase is seen as a mass action phenomenon. For taurocholate and taurochenodeoxycholate, equilibrium dialysis studies have shown that the micellar concentration of bile salt increases with the aqueous bile salt concentration (29).

The effect of added electrolyte on the enthalpy of dissolution of lecithin by sodium cholate is shown in Fig. 4. The enthalpy values become more negative as the concentration of added sodium chloride increases. This enhancement may be caused by increased partition of bile salt into the micelle due to "salting out" of the bile salt from the aqueous phase. The increased penetration of bile salt into the micellar phase is expected to lead to increased hydrogen-bonded association and, therefore, to more heat evolution.

Bile salts form complex micellar solutions with ampiphilic substances other than lecithin (3). It was hoped that an examination of the thermodynamic properties of the interaction between bile salts and other ampiphiles would shed further light on the bile salt–lecithin association.

Enthalpies of mixing sodium cholate solutions with solutions of a nonionic detergent (polyoxyethylated *tert*-octylphenol), a cationic detergent (cetrimonium bromide), and an anionic detergent (sodium lauryl sulfate) were measured. The results (Fig. 5) were compared with the lecithin data. In each case, even larger, more exothermic values for the complex micelle formation heat were obtained.

A detailed discussion of the differences in enthalpies between the different detergents would be highly speculative on the basis of the limited information available. However, these results are consistent with the suggestion that, in each case, the heat evolution is due to enhanced hydrogen-bonded association of the bile salt molecules in the micellar interior.

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