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## Precision Scanning Calorimetry of Bile Salt-Phosphatidylcholine Micelles<sup>†</sup>

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**ABSTRACT:** Precision scanning calorimetry has been used to examine the thermal behavior of mixed micelles formed between bile salts and dipalmitoylphosphatidylcholine (DPPC). Complex thermal transitions are observed which change dramatically with the mole ratio of bile salt to DPPC, dilution, and ionic strength. Comparison of the behavior of sodium taurocholate (TC) mixed micelles with sodium taurodeoxycholate (TDC) mixed micelles indicates similarity in the thermal transitions at high dilution or when the actual micellar composition is similar. It was found through equilibrium dialysis that considerably less TC than TDC is incorporated into mixed micelles with DPPC at a given bile salt concentration. Accounting for these concentration differences provides a means for more direct analysis of changes in the

thermal transitions with mole ratio and dilution for the two bile salt components. Resolution of the thermal transitions into several component contributions is employed as an aid to interpretation of the differential scanning calorimetry curves. The curve resolutions lead to estimates of van't Hoff and calorimetric enthalpies of the individual contributions. The results of the curve resolutions, along with the behavior of the total enthalpies of the transitions, are consistent with a transformation in micellar structure occurring when the actual micellar composition is a mole ratio of bile salt to DPPC of about 1 to 1. The transformation region is near that found from X-ray evidence and is thought to correspond to a change from disk-shaped to spherical micelles.

**B**ile salts are physiological detergents whose solubilizing properties are important in several fundamental biochemical processes. Through the formation of mixed micelles, bile salts render lecithin and cholesterol soluble in aqueous media, providing a mechanism for transport of these compounds from the liver to the gall bladder, and finally to the intestinal milieu (Carey & Small, 1978). The properties of mixtures of bile salts with various lipidic substances have been reviewed by Small (1971). Since a number of studies indicate that the bile salt-lecithin micellar phase may be quite complex, it seemed appropriate to apply the technique of differential scanning

calorimetry (DSC)<sup>1</sup> to bile salt-lipid systems, using the high-sensitivity Privalov calorimeter (Privalov et al., 1975). The synthetic lecithin dipalmitoylphosphatidylcholine (DPPC) was chosen for the studies, since it has a well-defined gel to liquid-crystal transition (Mabrey & Sturtevant, 1976) at a convenient temperature. The effects of variables such as the bile salt to DPPC ratio, structure of the bile salt, counterion concentration, and dilution have been studied. As an aid to interpretation of the thermal transition curves, some equilibrium dialysis experiments were carried out with the DPPC-bile salt mixtures.

### Materials and Methods

**Materials.** Synthetic L- $\alpha$ -dipalmitoylphosphatidylcholine was purchased from Calbiochem (San Diego, CA) and used

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<sup>1</sup> Abbreviations: cmc, critical micelle concentration(s); DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; TDC, sodium taurodeoxycholate; TC, sodium taurocholate; TLC, thin-layer chromatography.

as received. The bile salts, sodium taurodeoxycholate (TDC) and sodium taurocholate (TC), were purchased from Sigma Chemical (St. Louis, MO) and recrystallized from absolute ethanol by slow addition of cold ether to the ethanol solution in an ice bath. The crystals formed were dried for 48 h at 50 °C in vacuo. Thin-layer chromatography of the recrystallized salts showed only a single spot. The organic solvents used in preparation of the mixtures were spectral-grade chloroform and absolute ethanol. The disodium phosphate and sodium chloride used for buffer solutions were reagent grade, and the water was doubly deionized.

**Differential Scanning Calorimetric Measurements.** Appropriate volumes of stock solutions of DPPC in  $\text{CHCl}_3$  and bile salt in absolute ethanol were mixed to provide the desired mole ratio. The organic solvents were then removed by a stream of dry nitrogen, followed by heating to 50 °C in vacuo for about 2 h. The dried solids were suspended in 2 mL of buffer solution at pH 7.4 by shaking on a vortex mixer for about 30 s after the solution had been heated above 42 °C for a minimum of 10 min. This procedure in all cases yielded solutions without visible turbidity. In most of the DSC experiments, a scan rate of 0.5 K  $\text{min}^{-1}$  was used, and the noise level did not exceed 0.02 cal  $\text{K}^{-1} \text{g}^{-1}$ . For a large number of the scans, the samples were recooled in the calorimeter cell, and a second scan was initiated to check on the reversibility and reproducibility of the transition curves. The transition curves observed upon cooling and immediate rescanning were virtually identical with the original traces. In a few cases, the samples were removed and stored at about 7 °C for periods of up to several weeks. These samples were turbid and gave scans which were different from the original scans. However, preheating these samples above 42 °C led to transition curves which were identical with the original scans. We conclude that there is slow equilibration to a new phase at low temperatures, the nature of which is unknown.

**Equilibrium Dialysis Experiments.** For determination of the actual amount of bile salt incorporated into the mixed micelles, a series of equilibrium dialysis experiments was performed by using the method of Duane (1975, 1977). After equilibration, the concentration of bile salt was determined in the dialyzate and in the retentate solution. Since only the mixed micelles do not pass through the membrane, the analyses allowed determination of the actual stoichiometry of the micellar complexes and the amount of bile salt in the aqueous phase (Duane, 1977). The buffer solutions contained 0.01 M phosphate and 0.15 M NaCl at pH 7.4. Experiments were performed at  $38 \pm 1$  and  $21 \pm 1$  °C.

The analytical procedure for determination of the bile salts in the retentate and dialyzate solutions was based on the method reported by Singer & Fitschen (1961). For TDC, equilibrium was attained in about 36 h, while TC-DPPC micelles required 40–60 h, depending on the ratio of bile salt to DPPC. A number of dialyzate samples were analyzed by TLC for the presence of DPPC, which would indicate passage of the lipid through the membrane. While the retentate showed two intense bands, corresponding to bile salt and DPPC, the dialyzate showed only the bile salt band in all cases studied. We assume, then, that DPPC was retained.

## Results and Discussion

As an aid to the interpretation of the DSC results, information on the actual composition of the mixed micelles is useful. We therefore first present the results of the equilibrium dialysis experiments.

**Equilibrium Dialysis.** Figure 1 presents results of the dialysis experiments at 38 and 20 °C for compositions of

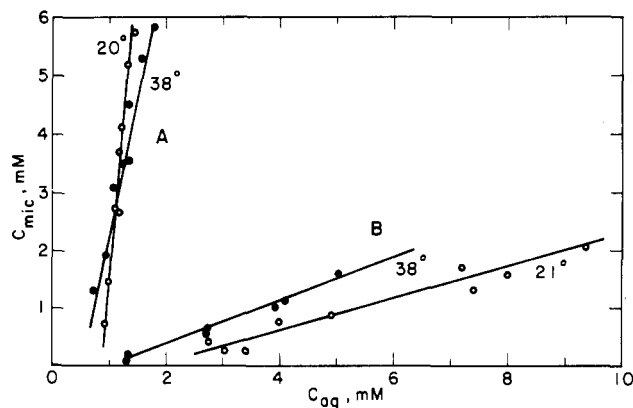


FIGURE 1: Results of equilibrium dialysis experiments showing the concentration of bile salt in the aqueous phase ( $C_{aq}$ ) and in the mixed micelles ( $C_{mic}$ ) for TDC-DPPC micelles (A) and TC-DPPC micelles (B). The concentrations of DPPC ranged from 1.3 to 1.9 mM for the TDC experiments and from 1.5 to 2.0 mM for those with TC.

DPPC in the range of 1–1.6 mg/mL, with varying ratios of bile salt to DPPC. The difference in behavior of the two bile salts is pronounced. For TDC, the dihydroxy analogue, addition of bile salt to the systems containing DPPC results in incorporation of a large percentage of the salt into the micelles, as indicated by the steep slope of the plot. The data at 38 °C show that 70–78% of the bile salt is associated with the micelles in the range studied. In contrast, TC has only 15–25% of the total bile salt within the micellar structures at 38 °C. At 21 °C, the percentage is even smaller, ranging from 7 to 18%. Comparable data obtained by Duane (1977) for TC-egg lecithin show 10–25% incorporation in the same range of lecithin concentrations and mole ratios and at room temperature. From the curves in Figure 1, critical micelle concentrations (cmc) for the formation of the mixed micelles can be obtained. Extrapolation to zero bile salt in the micellar phase gives about 2.0 and 0.86 mM, respectively, for TC and TDC at 20 °C. These values may be compared with values of 2.7 and 0.80 mM reported for the cmc for the formation of the pure bile salt micelles (Small, 1971) under the same conditions. Thus, to a first approximation, the tendency to form micelles, as measured by the cmc, is about the same for simple bile salt micelles as for the mixed micelles. Interestingly, the data at 38 °C indicate reverse trends, the cmc decreasing to 1.1 and 0.50 mM for the TC and TDC mixed micelles, respectively, compared to 4.0 and 1.2 mM for the simple micelles.

There are important consequences related to the cmc behavior of the mixed micelles. The results of the dialysis study indicate that for TC in most solutions studied both simple and mixed micelles coexist. As the bile salt concentration increases, more is incorporated into the mixed micelles, but also considerably larger quantities exist in the simple micelles of TC. For example, at a total TC concentration of about 10 mM and DPPC at 2 mM, about 82% of the bile salt is in the aqueous phase of which 67% is in the form of simple micelles at 20 °C. Thus, a little over half of the total bile salt concentration is in the form of simple bile salt micelles, even at this high ratio. From the data of Duane (1977), one can calculate the percentages of TC in the aqueous phase for egg lecithin, and the results are similar. These dialysis experiments, then, confirm that TC is incorporated into the mixed micelles with relatively low efficiency. TDC, on the other hand, is considerably more concentrated into the mixed micelles, as indicated by the dialysis data in Figure 1. Again, for 2 mM DPPC and a 1:1 ratio of TDC, 35% of the bile salt is in the aqueous phase. At a ratio of 2.6, the percentage has decreased to a little over 20%.

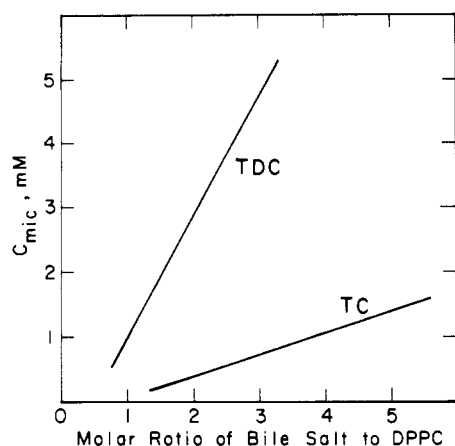


FIGURE 2: Calculated concentration of bile salt in mixed micelles with DPPC for TDC and TC at different stoichiometric ratios of bile salt to DPPC. Concentration of DPPC is 1.50 mg/mL.

The cmc data for the simple micelles indicate that only 7–8% of the total bile salt is in the form of simple micelles in either solution.

The results shown in Figure 1 are based on measurements at several different DPPC concentrations. For purposes of direct comparison, it is convenient to show the micellar bile salt concentration for a fixed amount of DPPC with increasing stoichiometric ratios of bile salt to DPPC. Figure 2 shows two such curves, calculated from the data in Figure 1, based on 1.5 mg/mL DPPC and varying mole ratios of added TC and TDC. The curves again show that one needs a considerably larger stoichiometric ratio of bile salt to DPPC for TC in order to attain the same micellar bile salt content as for TDC. For example, a 4:1 ratio of TC to lecithin gives about the same micellar composition as a 1:1 mixture with TDC. As will be seen later, these considerations are important in understanding the comparative thermal behavior of the two bile salts.

**DSC Behavior of TDC–DPPC Mixtures.** Figure 3 presents data relative to observed base lines from a series of DSC traces for  $1.0 \pm 0.05$  mg/mL DPPC, containing varying mole ratios of TDC to DPPC. At high mole ratios, the transitions start at such low temperatures that their initial parts could not be directly observed. In such cases, the base lines were assumed to be similar to those observed at lower mole ratios.

It is apparent from the data in Figure 3 that changing the mole ratio has an important effect on the sizes and shapes of the thermal transition curves. An attempt was made to interpret the transitions in terms of component transitions, each contributing independently to the total excess heat capacity and each obeying the van't Hoff equation. While it is difficult to give a definitive interpretation of these component curves in terms of the nature of the bile salt–DPPC domains responsible for them, resolution into a series of component transitions provides a mechanism for analyzing the variations that occur in the experimental curves. A computer program was written which varies the van't Hoff and calorimetric enthalpies and  $T_{\max}$ , the temperature of maximal excess specific heat, for each component curve in succession until the standard deviation of the sum of the calculated component contributions from the experimental curve is minimal. The component curves are shown as dashed lines in Figure 3 and subsequent figures; the solid lines are the calculated summation curves, and the circles are points read from the original DSC curves. In most cases, the standard deviation between the summation curve and the experimental data was in the range of 10–20 mcal K<sup>-1</sup> g<sup>-1</sup>, which corresponds to 1–3% of the maximal excess specific heats for the observed transitions.

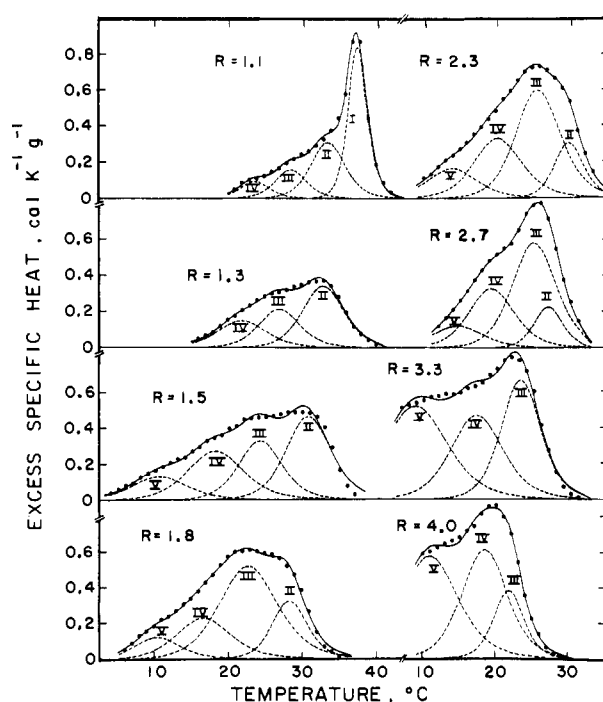


FIGURE 3: Excess heat capacity vs. temperature for varying mole ratios ( $R$ ) of sodium taurodeoxycholate to dipalmitoylphosphatidylcholine. Roman numerals identify resolved component peaks (see text). The circles are experimental points, the dashed curves are the resolved components, and the solid curve is the sum of the component curves. Concentration of DPPC is 1.10 mg/mL, and the buffer is 0.15 M NaCl–0.02 M Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4. Excess specific heat is given per gram of DPPC.

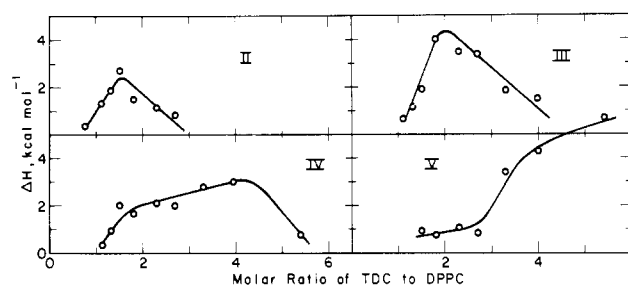


FIGURE 4: Calorimetric enthalpies of the resolved transitions for TDC–DPPC mixtures at different stoichiometric ratios of bile salt to DPPC. Roman numerals correspond to resolved peaks in Figure 3.

As can be seen in Figure 3, three or four components are required to define the overall transitions. Although this situation is quite complex, there are some systematic trends in the properties of the resolved transition curves which are of particular interest. In the lowest ratio, 1.1 mol of bile salt per mol of lipid, there is a prominent high-temperature peak, which disappears at a ratio of 1.3 and higher, leaving the broader and less intense components predominant. As the bile salt to DPPC ratio increases, there are progressive shifts in the relative intensities of the remaining components. The two higher temperature components (those labeled II and III in Figure 3) increase up to a ratio of 1.7 and then begin to decrease in size. At the higher ratios, those above about 2.7, the two lower temperature peaks are the most intense ones. The behavior of the calorimetric enthalpy for each transition is shown in Figure 4, except that of peak I, the sharp, high-temperature peak, is not shown since it was observed in only two of the mixtures. When peaks I and V are excluded for the moment, there is a general increase in the transition enthalpies up to mole ratios in the range of 1.6–2.0, with particularly abrupt

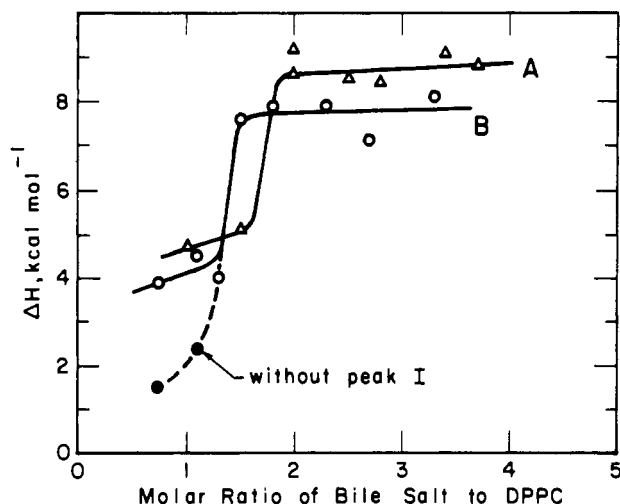


FIGURE 5: Total calorimetric enthalpies of the thermal transitions for TDC-DPPC mixed micelles at different stoichiometric ratios of bile salt to DPPC. Open circles are for  $1.1 \text{ mg mL}^{-1}$  DPPC in each case and triangles for  $1.5 \text{ mg mL}^{-1}$  DPPC. The dashed curve at low mole ratios shows the effect of omitting peak I from the total in the  $1.1 \text{ mg mL}^{-1}$  DPPC example.

changes occurring for peaks II and III. Then as the ratio increases further, the decreases in peaks II and III are compensated by increases in IV and V. The net effect on the total enthalpy of transition, that is, the sum of all of the components, is shown in Figure 5. Also shown are data from another series of curves at higher DPPC concentrations. The compensating nature of the high- and low-temperature transitions above a mole ratio of 2 is clear in Figure 5. There thus appears to be a pronounced change in the nature of the micellar system in the vicinity of 2 mol of TDC to 1 mol of DPPC. The changes occurring near a mole ratio of 2 also show up in the values of  $T_{\text{max}}$ , for each component, as shown in Figure 6. Peaks II-IV show significant changes in the slope of  $T_{\text{max}}$  vs. mole ratio at about a 2:1 ratio of bile salt to DPPC.

While it is not possible to give an exact interpretation of the physical basis of the component transitions, it seems likely that they result from specific structural entities associated with bile salt-*lecithin* micelles. Pure bile salt micelles or simple monomers show no significant changes in heat capacity in the temperature range studied, so it is the mixed micellar aggregates which are responsible for the observed transitions. Both the X-ray studies of Müller (1981) and the laser light scattering results of Mazur et al. (1980) indicate that large changes occur in the sizes of the mixed micelles at lower ratios of bile salt to *lecithin*. Light scattering data show that the hydrodynamic radius of TDC-*lecithin* mixed micelles decreases by a factor of almost 4 between mole ratios of 0.8 and 2.0. The X-ray results confirm the rather large decreases in size of the micelles as bile salt concentration increases. The structural model for the micelles proposed by Mazur et al. (1980) is a *lecithin* bilayer disk surrounded by and interspersed with bile salt. As the concentration of bile salt increases, these disks decrease in radius. The DSC data show that in the region in which size is decreasing, the transition enthalpies of several of the components (peaks II-IV) increase, while that of peak I decreases rather abruptly (Figure 3). Peak I resembles the transition observed for pure DPPC, which although much sharper and with higher enthalpy (Mabrey & Sturtevant, 1978) has a specific heat maximum at  $41.6^\circ\text{C}$ . It is possible that peak I is a perturbed DPPC transition which can only occur in the larger micelles present at lower amounts of bile salt. These perturbed regions within the micelles disappear

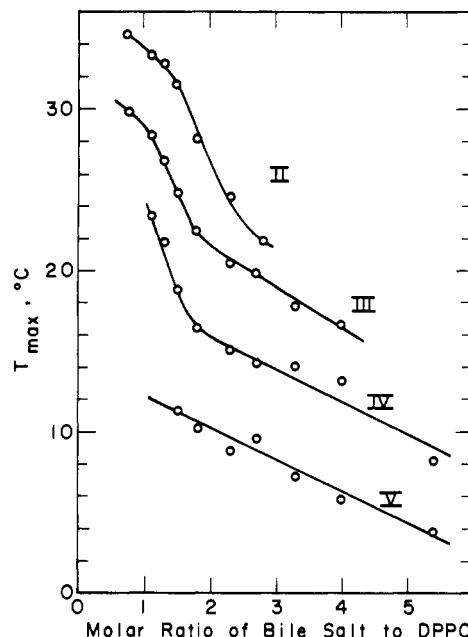


FIGURE 6: Temperature,  $T_{\text{max}}$ , of the transition maximum for the resolved components of TDC-DPPC mixed micelles as a function of the stoichiometric ratio of bile salt to DPPC. Roman numerals refer to the peaks identified in Figure 3.

rapidly as the size of the micelles decreases. On the other hand, peaks II-IV increase in intensity with increasing bile salt, suggesting that the smaller size micelles provide better structural features for the domains responsible for the observed thermal behavior. The changes which occur near a mole ratio of 2:1, however, show that additional factors are involved in the stability of the domains.

It is seen in Figure 5 that in the vicinity of the 2:1 mole ratio, TDC-DPPC mixtures show a transformation from generally low to high total enthalpy values over a range of composition in which the micellar size is approaching a minimum as determined from light scattering. This change occurs when the *actual* mixed micellar composition is about 1:1 in the range of total lipid concentrations used in these studies. Two views of what actually happens in this transition region have been presented. According to the arguments of Mazur et al. (1980), as the bile salt ratio increases in the mixed micelles, a point is reached at which simple micelles of bile salts begin to form in significant quantities along with the mixed micelles. It is further assumed that above this specific mole ratio, the hydrodynamic radius of the mixed micelles is constant as the simple micelles increase in number and in size. The DSC data shown in Figure 5 could be reconciled with these arguments, since the level region of the total enthalpy coincides nicely with the region of small, relatively constant hydrodynamic radius. However, the behavior of the individual, resolved components would seem to contradict the assumption that a constant mixed micellar structure persists in media above the transformation ratio. As the curves in Figures 3 and 4 show, even at mole ratios much higher than the region of relatively constant size, there are still rather significant changes occurring in the component contributions to the total thermal transitions. It seems doubtful that such significant changes in the shapes of the component peaks would occur if the micelles are of constant size and shape, as proposed by Mazur et al. (1980). Another argument against the transformation region corresponding to the emergence of simple bile salt micelles relates to the equilibrium dialysis results of this study and those of Duane (1977). In both instances, the concentrations of bile

salt found in the aqueous phase in equilibrium with the mixed micelles were such as to exceed the cmc of the simple bile salt micelles even at rather low stoichiometric ratios of bile salt to lecithin. Since many of the measurements of Duane (1977) were in total lipid concentrations similar to those used in the light scattering studies, it would seem that mixed micelles with egg lecithin would coexist with simple bile salt micelles over quite wide ranges of concentration, and not just above the transition region. The deduction of the intermicellar concentrations by Mazur et al. (1980) assumes that in the low ratio region the aqueous phase concentration of bile salt is constant, which seems very unlikely in light of our dialysis experiments.

The second view of the transition region is that of Müller (1981) and Claffey & Holzbach (1981), which proposes that conversion from disk-shape to spherical micelles occurs at the specific composition mentioned above. It would seem easier to correlate the DSC and dialysis data with such a conversion. The behavior of the individual component enthalpies and values of  $T_{\max}$  near the transition region suggest basic alterations in the domains responsible for the thermal transitions. The ultraviolet spectral data of Claffey & Holzbach (1981) show that marked changes in absorptivity of the bile salt occur at the transition mole ratio of 1.8 for mixtures of glycochenodeoxycholate and egg lecithin. The marked change in absorptivity indicates that significant alterations of the environment of the bile salt chromophore occur, and the changes are consistent with a shift to a basically new configuration of the micellar structure. Although these results could be interpreted as a direct result of the formation of simple bile salt micelles, the dialysis data show that even at much lower ratios simple micelles would be present. Thus, it is more likely that the abrupt changes that occur at the 1.8 stoichiometric ratio result from transformation to a new form. The centrosymmetric structure deduced from X-ray scattering (Müller, 1981) was found at mole ratios greater than 2 and showed small increases in size as the micellar mass increased from 20 to 35 kdalton. It thus seems reasonable to associate the low-temperature thermal components of the DSC curves with the emerging new spherical mixed micelles which form at higher mole ratios. While it is not possible, as mentioned earlier, to give explanations of the origins of the resolved transitions, it does seem likely that the gross size and shape of the micelles contribute to the observed behavior.

**DSC Behavior of Taurocholate-DPPC Mixtures.** The micellar properties of pure TC solutions are markedly different from those of the disubstituted compounds (Small, 1971). Critical micelle concentrations for TC are generally higher than those for TDC at equivalent ionic strengths, and micellar sizes are smaller. Compared with TDC, mixed micelles of DPPC and TC contain lower amounts of bile salt at a given mole ratio. For these reasons, and because Mazur et al. (1980) have done extensive laser light scattering on TC mixed micelles with egg lecithin, it was of interest to examine the DSC behavior of TC-DPPC mixed micelles. Figure 7 shows a comparison of TC and TDC calorimetric scans at 1.50 mg/mL DPPC, and at four different mole ratios of bile salt to lecithin. Also shown are the DSC curves for 50 mol % mixtures of TC and TDC with DPPC at the same mole ratios. These data show clearly that the observed DSC behavior is quite different for the two bile salts. The TC micelles show much more intense, higher temperature transitions than the TDC solutions at a given mole ratio. For example, at the equimolar ratio, the major TC peak has a maximal excess specific heat of over  $4 \text{ cal K}^{-1} \text{ g}^{-1}$ , compared with less than  $1 \text{ cal K}^{-1} \text{ g}^{-1}$  for the

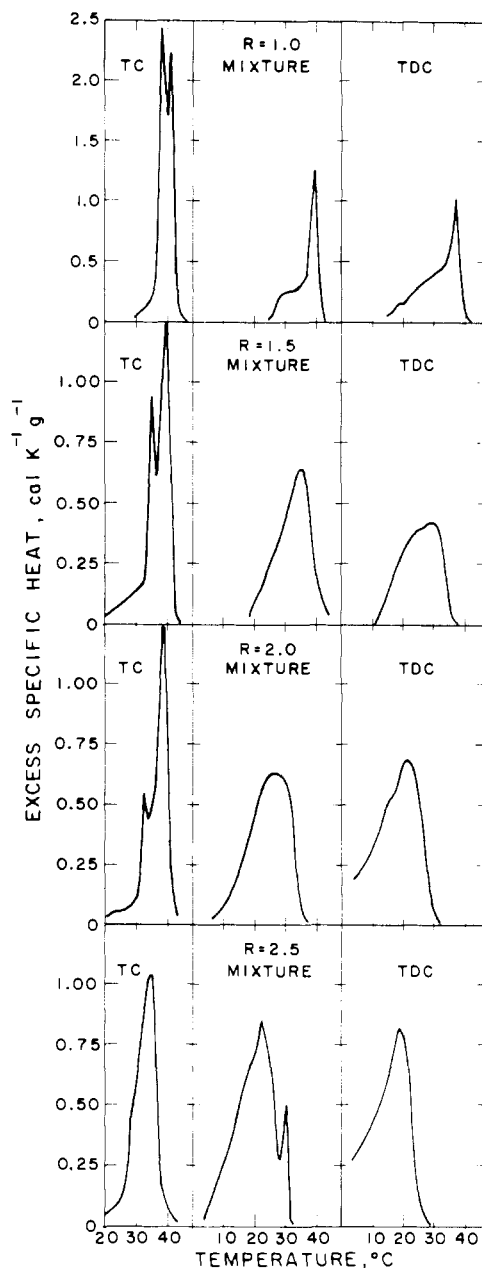


FIGURE 7: Thermal transition curves for TC-DPPC micelles (left), TDC-DPPC micelles (right), and 50 mol % TC-TDC micelles with DPPC (middle) at different mole ratios of bile salts to DPPC. The concentration of DPPC in each case was  $1.5 \text{ mg mL}^{-1}$ , and the solutions contained  $0.02 \text{ M Na}_2\text{HPO}_4$  at pH 7.4.

most intense TDC transition. The transitions for the 50% mixtures of the two bile salts more closely resemble TDC rather than TC transitions, although the resolved components in the mixtures tend to be more intense and to occur at higher temperatures than in the TDC mixed micelles. At higher ratios, the TC peaks are also larger than for TDC although they are significantly decreased in height and are broadened.

In view of the striking differences in the mixed micelles shown in the equilibrium dialysis experiments, it is perhaps not surprising that the DSC behavior for the two salts is so different. The curves in Figure 2 showing the actual micellar composition as a function of the stoichiometric ratio are for conditions identical with those used in the DSC experiments of Figure 7. The dialysis curves of Figure 2 indicate that a ratio of TC to DPPC equal to 2.5 would correspond to an actual mixed micellar composition of about  $0.5 \text{ mM}$  bile salt. The corresponding ratio for TDC-DPPC micelles is 0.75.

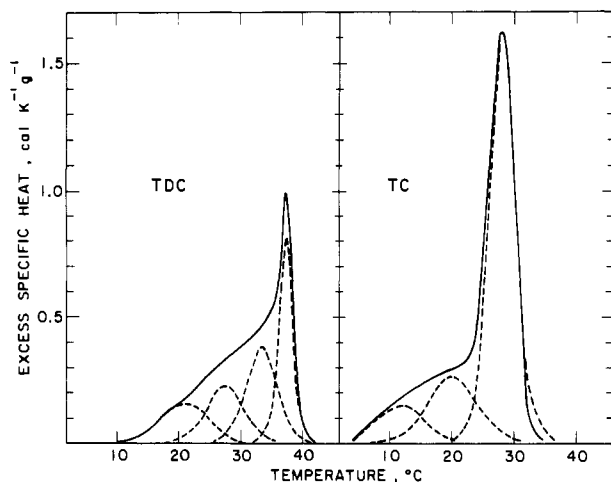


FIGURE 8: Comparison of thermal transition curves for DPPC mixed micelles with TDC and TC at mole ratios of bile salt to DPPC of 4 for TC and 1 for TDC. The total DPPC concentration in each case is  $1.50 \text{ mg mL}^{-1}$ , and the solutions contain  $0.15 \text{ M NaCl}$ – $0.02 \text{ M Na}_2\text{HPO}_4$  at pH 7.4. The solid curves are the experimental curves, and the dashed curves are the resolved components.

Thus, the curves for TC in Figure 7 are actually for micelles containing lower amounts of bile salt in the mixed micelles than for any curve shown for TDC. A point of interest is whether the DSC traces of TC and TDC which have the same amount of bile salt associated with the micelles show identical thermal components. Figure 8 compares curves for a 4 to 1 mixture of TC with a 1 to 1 mixture of TDC, which according to the data in Figure 2 should incorporate similar amounts into the mixed micellar structures. While the gross features of the two curves are similar, the individual component contributions are not the same. TC shows the strong peak near  $30^\circ\text{C}$  which seems to dominate at the higher ratios. The TDC components include the sharp high-temperature peak, which disappears at slightly higher ratios. If the high-temperature peak is excluded, the remaining components are roughly similar in the two cases, except for the differences in maximal values for the excess specific heat. The peaks are different enough, though, to suggest that the TC and TDC mixed micelles differ significantly in size or other structural features even with the same amount of bile salt incorporated in the mixed micelles.

Another comparison between TC and TDC of interest is the behavior of the total enthalpies of the transitions in the two cases. At mole ratios larger than those shown in Figure 7, the total enthalpy of the TC transitions increases significantly and then approaches a constant value, as was observed for the TDC mixed micelles. Figure 9 shows a comparison of the total enthalpies of the thermal transitions as a function of the stoichiometric mole ratio. At mole ratios just above 1 in the TDC case, the total enthalpy begins to increase, leveling at a mole ratio of about 2, as discussed above. For TC, the total enthalpy increases at ratios between 3.5 and 4.0 and appears to be leveling off in the vicinity of a ratio of 6. Referring to Figure 2 again, it is seen that the enthalpy increases in both cases seem to emerge when the actual concentration of bile salt in the mixed micelles is about  $1\text{--}1.5 \text{ mM}$  and become constant when  $C_{\text{mic}}$  is near  $2.0 \text{ mM}$ . Thus, the changes in total enthalpy seem to correlate with a specific compositional characteristic that is relatively independent of which bile salt is present. The leveling of the enthalpy occurs when the actual mixed micellar composition is about  $2 \text{ mM}$  bile salt and  $2 \text{ mM}$  DPPC in the experiments described above. From the arguments presented previously regarding the formation of spherical micelles, it appears that both bile salts exhibit the

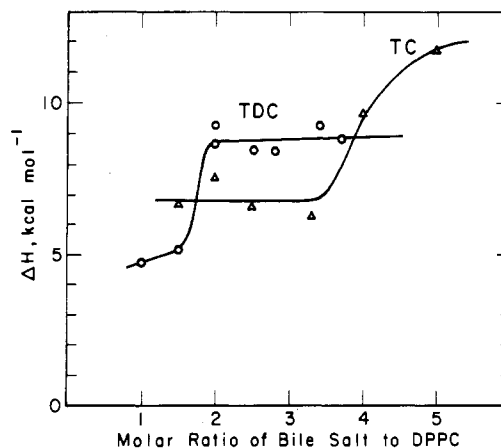


FIGURE 9: Comparison of the total enthalpies of thermal transitions for TC and TDC mixed micelles with DPPC at different mole ratios of bile salt to DPPC. The total DPPC concentration in each case is  $1.50 \text{ mg mL}^{-1}$ , and the solutions contain  $0.15 \text{ M NaCl}$ – $0.02 \text{ M Na}_2\text{HPO}_4$  at pH 7.4.

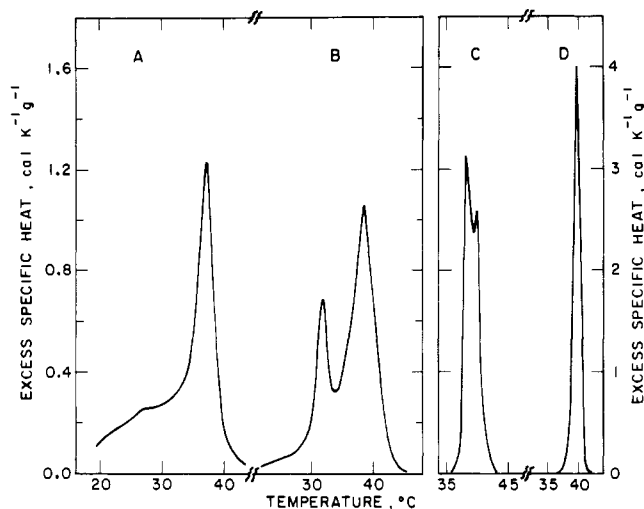


FIGURE 10: Thermal transition curves for TDC-DPPC mixed micelles at a constant stoichiometric mole ratio of bile salt to DPPC of 0.76, but at varying amounts of DPPC. Curve A is for  $1.59 \text{ mg mL}^{-1}$  DPPC, curve B is for  $0.79 \text{ mg mL}^{-1}$ , curve C is for  $0.27 \text{ mg mL}^{-1}$ , and curve D is for  $0.13 \text{ mg mL}^{-1}$ . The solutions contain  $0.15 \text{ M NaCl}$  and  $0.02 \text{ M Na}_2\text{HPO}_4$  at pH 7.4.

same conversion when the mixed micellar composition is a molar ratio of 1.

**Effects of Dilution.** The process of diluting a solution of mixed micelles with buffer shifts the micellar equilibrium in the direction of more bile salt in the aqueous phase (Shankland, 1977; Duane, 1977). That the actual amount of bile salt incorporated into the mixed micelles is fundamentally important to their thermal behavior is supported by DSC studies of a series of dilutions of mixed micellar solutions. Figures 10 and 11 show DSC curves for progressive dilutions of several solutions of TDC and TC containing DPPC. First, at the 0.75 mole ratio TDC curves, there is a steady loss of the lower temperature components at higher dilutions. In addition, a sharp transition begins to appear at  $10.6 \text{ mg mL}^{-1}$  near  $30^\circ\text{C}$ , which increases significantly with dilution to become the prominent peak in the most dilute case. The general shift to higher temperatures and to narrower, more intense peaks is apparent. A similar trend is indicated in the 2.5 mole ratio TC dilutions. In fact, there are striking similarities between the individual component peaks that emerge at the higher dilutions. One could conclude that the mixed micelles at  $0.22 \text{ mg mL}^{-1}$  DPPC with a 2.5 molar ratio of TC to DPPC are

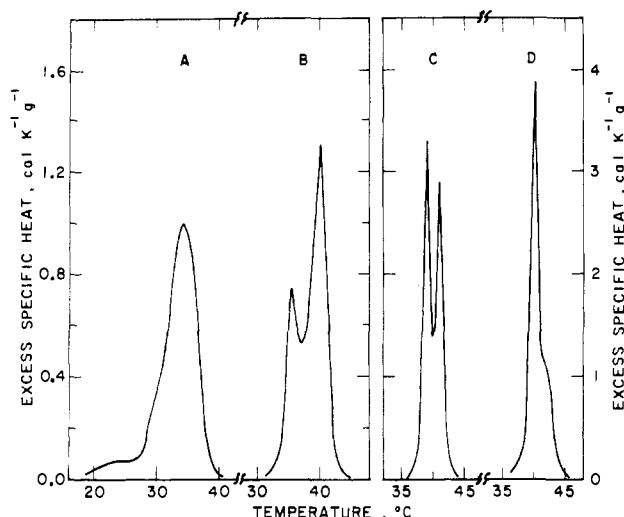


FIGURE 11: Thermal transition curves for TC-DPPC mixed micelles at a constant stoichiometric ratio of bile salt to DPPC of 2.5 at varying amounts of DPPC. Curve A is for  $1.49 \text{ mg mL}^{-1}$  DPPC, curve B is for  $0.75 \text{ mg mL}^{-1}$ , curve C is for  $0.48 \text{ mg mL}^{-1}$ , and curve D is for  $0.22 \text{ mg mL}^{-1}$ . The solution conditions are the same as in Figure 10.

quite similar to those at  $0.13 \text{ mg/mL}$  DPPC and a 0.75 ratio of TDC. According to the dialysis data presented in Figure 2, the amounts of TDC and TC associated with the mixed micelles at the 0.75 and 2.5 ratios, respectively, should be about the same. The results of the dilution experiments indicate that while the features of the DSC behavior are somewhat different at higher total lipid, the more dilute solutions show quite similar thermal transitions. Since the ratios chosen correspond to compositions where the disk-shaped micelles persist, it appears that at low dilutions the actual identity of the bile salt is not so important to the thermal behavior of the disk structures. At higher total concentrations, there are significant enough differences to suggest that the di- and trihydroxy salts associate somewhat differently in the mixed micelles. It was also found that for TDC dilution experiments at higher mole ratios (a series at a ratio of 2.7 is not shown), there is somewhat less sensitivity to the actual dilution, most likely as a result of higher total bile salt concentrations both in the aqueous phase and associated with the micelles.

A final comment regarding the comparative behavior of TC and TDC should be made. The dialysis results show that there is a small temperature dependence for the extent of dissociation of bile salt from the mixed micelles. A consequence of this temperature dependence is that there will be a contribution to the excess heat capacity due to the heat effect resulting from variations with temperature of the bile salt association. For TC mixed micelles, the temperature dependence of the equilibrium constant for binding bile salt to the micelles yields an enthalpy effect of about  $-4700 \text{ cal mol}^{-1}$ . Using the molar quantities typical of a DSC experiment, for a 1-mL sample the contribution to the excess specific heat would be less than  $1 \text{ mcal K}^{-1} \text{ g}^{-1}$ , which is well below the experimental error of the measurements. The DSC differences between TC and TDC thus cannot result from this effect.

**Effects of Counterion Concentration.** For simple bile salt micelles, particularly those involving the dihydroxy salts, the critical micelle concentration has been found to depend markedly on the counterion concentration (Carey & Small, 1970). There is a general decrease in the cmc above about  $0.3 \text{ M NaCl}$  for both TDC and taurochenodeoxycholate, a dihydroxy isomer of TDC. Correlated with cmc changes with salt concentration are changes in aggregation number, as determined by equilibrium ultracentrifugation (Small, 1968).

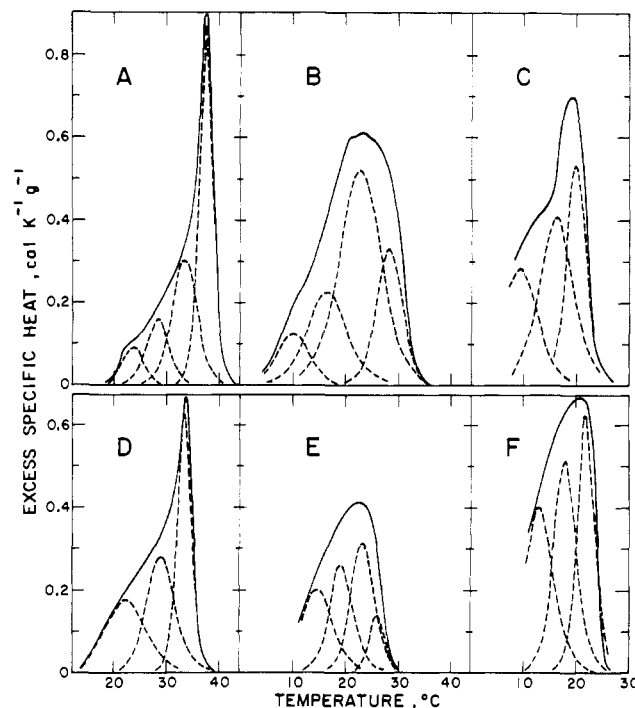


FIGURE 12: Comparison of thermal transition curves for TDC-DPPC mixed micelles in  $0.15 \text{ M NaCl}$  (top) and  $0.60 \text{ M NaCl}$  (bottom). Curves A and D are for mole ratios of 1.1, curves B and E for 1.8, and curves C and F for 2.5. Concentrations of DPPC are  $1.0 \text{ mg mL}^{-1}$ , and the solutions contain  $0.02 \text{ M Na}_2\text{HPO}_4$  at pH 7.4. The solid curves are the experimental curves, and the dashed curves the resolved components.

These studies show a progressive increase in the aggregation number for TDC micelles as the concentration of NaCl is increased from zero to  $1.0 \text{ M}$ , while there is only a slight change for TC micelles. There have been few studies on the behavior of mixed micelles as counterion concentration is altered. Mazur et al. (1980) showed that the hydrodynamic radii of TDC-egg lecithin micelles increased substantially when the NaCl concentration was increased from  $0.15$  to  $0.60 \text{ M}$ . It was thus of interest to examine the DSC behavior of TDC mixed micelles with DPPC to see what changes occur with increased counterion concentration. Figure 12 shows DSC scans for three mole ratios of TDC to DPPC, and for  $0.15$  and  $0.60 \text{ M NaCl}$  contained in the buffer solutions. The lower temperature components are somewhat more intense, and the higher temperature components are reduced in size or are missing in  $0.60 \text{ M}$  as compared with  $0.15 \text{ M NaCl}$ . This behavior suggests, in light of the previous results reported here, that more bile salt is associated with the mixed micelles at higher NaCl concentration. However, if the size of the micelles is larger at higher counterion concentrations, as determined from light scattering, one might expect that the sharper, high-temperature peaks would be observed as discussed in connection with the TC mixed micelles. These apparent contradictions are reconcilable if, indeed, the larger micelles in this case are able to incorporate more bile salt. Mukerjee (1967) has pointed to two factors which operate in determining the aggregation characteristics of ionic micelles. First, there is a "salting out" effect, which tends to increase association of large, hydrophobic moieties in the presence of increased salt concentrations. The extent of the salt effect depends on the size of the nonpolar groups in the molecule and on basic properties of the salt involved (Deno & Spink, 1963). The second factor, which also tends to favor association, is that increased numbers of counterions allow closer packing of the

ionic components of the micelles (Mukerjee, 1967). That is, the repulsive forces between, for example, the TDC molecules on the perimeter and within the disks of the DPPC mixed micelles would to some extent be neutralized by larger concentrations of  $\text{Na}^+$  ions in the aqueous phase. This neutralization allows more bile salt to associate into the mixed micelles, stabilizing larger structures.<sup>2</sup> That the larger micelle sizes persist in high salt concentration is also indicated by the behavior of the total enthalpy of the thermal transitions. As was pointed out above, the total enthalpy of the transition shows a sharp increase at about a mole ratio of 1.5 in 0.15 M salt (Figure 5). This sharp increase also occurs in 0.60 M NaCl but is delayed until the mole ratio is 2.5. If, as was argued earlier, the enthalpy increases are associated with decreasing size and transformation to more compact structures, this transformation occurs at higher amounts of bile salt when larger counterion concentrations are present. The higher salt concentration seems to stabilize the larger, but more highly associated, structures, leading to a requirement of even more TDC to disperse the structures to smaller aggregates. The behavior of the individual components of the transitions in 0.60 M NaCl supports these observations. While the higher temperature components are decreased more in 0.60 than in 0.15 M NaCl, indicating more bile salt in the micelles, the lowest temperature peaks do not begin to increase substantially until the mole ratio is over 2.0. The effects of counterion concentration demonstrate again the sensitivity of the DSC technique to subtle changes in basic micellar properties.

The results presented here have implications which suggest directions for further application of the DSC technique to the examinations of lecithin mixed micelles. A study of the effects

of cholesterol on the thermal transitions of the micelles is in progress. The behavior of mixed micelles of other phospholipids with bile salts would be of interest, as would mixed micelles of other detergents with the phospholipids. The examination of vesicles prepared by cholate dialysis and related techniques might also be useful.

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<sup>2</sup> Preliminary results of equilibrium dialysis experiments in 0.6 M NaCl indicate a greater association at the higher ionic strength (C. H. Spink and K. Springsteen, unpublished experiments).