Cholesterol (Thermodynamic) Activity Determinations in Bile Salt–Lecithin–Cholesterol Systems and Cholesterol-Rich Liquid Crystalline Mesophase Formation

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Previous *in vitro* studies have shown that tauroursodeoxycholate (TUDC)–lecithin (L) micellar solutions solubilize cholesterol (Ch) poorly compared to its 7α-epimer, taurochenodeoxycholate (TCDC). However, in clinical studies ursodeoxycholic acid (UDC) has been found to be as effective as chenodeoxycholic acid (CDC) in Ch gallstone dissolution, and it has been suggested that, during UDC therapy, liquid crystalline mesophase formation may be involved in enhancing micellar Ch dissolution and dispersion. The purpose of the present study was to investigate whether measurements of the Ch thermodynamic activity (A_T) would provide new insights into the problem of Ch solubilization and mesophase formation in bile salt–lecithin–Ch systems. Using the silicone polymer uptake method developed in this laboratory, A_T was measured as a function of Ch concentration in the TUDC-L-Ch and TCDC-L-Ch model bile systems. In the TCDC systems Henry's law was obeyed almost up to unit activity (i.e., A_T was proportional to Ch concentration almost up to $A_T = 1.0$). However, in many of the TUDC-containing systems negative deviations from Henry's law were observed well below unit activity and these systems became visibly turbid before saturation with respect to cholesterol monohydrate (ChM) was reached. The effects of varying the TCDC/TUDC ratio upon the A_T behavior were also studied. With increasing TCDC/TUDC ratio, the onset of mesophase formation was shifted to higher A_T values. A_T measurements were also conducted in BS-L-Ch mixtures simulating biles of patients undergoing UDC therapy. The results obtained suggest that mesophase formation may not always occur in biles of patients undergoing UDC therapy. However, it is suggested that, even in cases where mesophase formation may not take place in the bulk solution phase, mesophase formation may occur on ChM crystallite surfaces during micellar dissolution.

KEY WORDS: thermodynamic activity; cholesterol; bile salt; lecithin; mesophase; liquid crystalline.

INTRODUCTION

Chenodeoxycholic acid (CDC) and ursodeoxycholic acid (UDC), the 7β-hydroxy epimer of CDC, are effective in inducing cholesterol (Ch) gallstone dissolution in humans (1–9) when orally administered either alone or in combination. Interestingly, both agents demonstrate comparable clinical efficacy (10). This is noteworthy and important because the ability of the micelles of UDC and its conjugates to solubilize Ch is much poorer than that of CDC and its conjugates (11–14) either alone or as mixed bile acid–lecithin micelles. Furthermore, it has been shown recently (14–16) that the initial dissolution rates of cholesterol monohydrate (ChM) disks in conjugated UDC–lecithin (L) solutions are significantly slower than in conjugated CDC-L solutions.

Corrigan *et al.* (17) first made the observation that, during dissolution of ChM crystallites in conjugated UDC-L solutions, a nonfilterable cloudiness developed in the solution after several days. Microscopic examination and chemical analysis revealed a globular phase of liquid-crystalline nature, rich in Ch and L. Later studies by Su *et al.* (18,19) showed that such mesophase formation may also occur during *in vitro* dissolution of human Ch gallstones obtained at surgery from patients: the initial *in vitro* dissolution rates of the Ch gallstones were slower in glycoursodeoxycholate (GUDC)-L solutions than in glycochenodeoxycholate (GCDC)-L solutions of the same concentrations, but mesophase developed in the GUDC-L solution phase after a few days and Ch dissolution and dispersion in the GUDC-L experiments eventually far exceeded micellar Ch dissolution in the GCDC-L experiments.

In the studies by Corrigan *et al.* (17) and in the more recent investigations by Salvioli *et al.* (20), mesophase formation on the ChM disks or on ChM crystallite surfaces appears to occur much earlier (in time) than mesophase formation in the bulk solution. This suggests that, via a fortuitous and interesting combination of kinetic events at the crystal–solution interface, mesophase may form and exist at the ChM crystal surface, while the bulk solution may still be thermodynamically unsaturated with respect to it (the mesophase).

The purpose of the present research was to employ a newly developed technique (21) for measuring the thermodynamic activity (A_T) of Ch in bile salt (BS)-L-Ch solutions with a special focus upon those systems in which mesophase formation occurs in the bulk solution before $A_T = 1.0$, i.e., before saturation with respect to ChM occurs. The concept of relating A_T to the properties and the behavior of BS-L-Ch solutions is new (21) and, as will be seen, this approach should ultimately provide new insights relative to the importance of simultaneous micellar dissolution and mesophase formation occurring during Ch gallstone dissolution in patients undergoing UDC therapy. Results of A_T measurements are reported here for conjugated UDC-L systems and for mixtures of conjugated UDC-L with "normal" BS species, including compositions which simulate biles of patients undergoing UDC therapy.

MATERIALS AND METHODS

Chemicals

The sodium salts of taurocholic acid, tauroursodeoxycholic acid, taurochenodeoxycholic acid, glycoursodeoxycholic acid, glycochenodeoxycholic acid, taurodeoxycholic acid, and taurolithocholic acid were purchased from Behring Diagnostics, La Jolla, CA. The purity (>97%) of these chem-

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icals was confirmed by a thin-layer chromatography procedure (22). Egg yolk lecithin was obtained from Lipid Products, Surrey, UK. Cholesterol (Sigma Chemical Company, St. Louis, MO) was recrystallized as ChM three times with 95% ethanol before use. Radiolabeled ChM crystals were prepared by dissolving 6 g of the recrystallized ChM in 400 ml of acetone at 48°C. This solution was filtered and 40 ml of water was slowly added to the filtrate, followed by the addition of 50 μCi of [^{14}C]cholesterol (New England Nuclear, Boston, MA) with occasional stirring, maintaining the temperature at 48°C during the entire process. The solution was then allowed to stand for 48 hr at room temperature. The radiolabeled ChM crystals obtained were collected and stored in an amber-colored bottle in a closed chamber saturated with water vapor. Silicone polymer (Silastic Sheeting No 502-1) was obtained from Dow Corning Company, Midland, MI. Prior to use, the polymer was vigorously washed with boiling 95% ethanol for about 15 sec, followed by a thorough rinse with boiling deionized water. All other chemicals were analytical grade and used as received.

ChM Solubility Determination

The equilibrium solubility ($C_{S,Aq}$) of ChM in aqueous BS or BS-L micellar solutions was determined by the addition of an excess amount of radiolabeled [^{14}C]ChM crystals to four vials, each containing 1.5 ml of the solvent (BS or BS-L mixtures in 0.1 *M* NaCl and 0.01 *M* sodium phosphate buffer, pH 7.4). After flushing with nitrogen for 30 sec, the sample vials were sealed tightly and shaken by a wrist-action shaker in a water bath maintained at 37°C. The contents of the four vials were filtered through a 0.22-μm membrane filter (preheated to 37°C) at day 7, 14, 21, and 28, respectively, before analysis for radioactivity in a liquid scintillation counter (Beckman Instruments Inc., Irvine, CA). The equilibrium solubility value was obtained when no further increase in radioactive counts was observed in the assayed solutions.

"Calibration" Plot for Determination of A_T

Two methods were employed in the calibration studies.

Method A. Taurocholate (TC), TCDC, and TCDC-L solutions were allowed to equilibrate with excess radiolabeled ChM crystals in sealed vials (flushed with nitrogen before sealing) at 37°C for 10 days. All BS and BS-L solutions used for activity measurements were prepared in 0.1 *M* NaCl and 0.01 *M* sodium phosphate buffer (pH 7.4). The excess crystals were filtered and an aliquot of the filtrate (1.5 ml) was transferred to 4-ml sample vials, each containing 250 mg of the silicone polymer (cut into small pieces, approximately 1 mm in size). After flushing with nitrogen, the vials were shaken at 37°C for 48 hr, during which the systems reached equilibrium. At the end of 48 hr, an aliquot of the solution was taken and assayed for the Ch concentration ($C_{Aq,Eq}$). The silicone polymer was then separated from the bulk solution by filtration and washed thrice with 10-ml portions of ethanol/water (20/80, v/v). The washed polymer was then carefully transferred to a vial and extracted repeatedly (normally three or four times) with 3-ml portions of reagent alcohol until free of radioactivity. The ethanol from the extracts was allowed to evaporate and the radioactivity in the residue of each extract was assayed separately. The radioactive counts were then combined to determine the concentration of Ch in the polymer ($C_{Sp,Eq}$). The $C_{Sp,Eq}$ values were plotted as a function of cholesterol saturation index (CSI), which is defined by Eq. (1),

$$\text{CSI} = C_{Aq,Eq}/C_{S,Aq} \quad (1)$$

The thermodynamic activity (A_T) of Ch was calculated based on the equation,

$$A_T = C_{Sp,Eq}/C^{\circ}_{Sp,Eq} \quad (2)$$

where $C^{\circ}_{Sp,Eq}$ is the $C_{Sp,Eq}$ value at ChM saturation (i.e., unit activity).

In a variation of this method, a predetermined amount (less than saturation solubility) of radiolabeled Ch was shaken in 1.5 ml BS or BS-L solution at 37°C until the Ch was completely solubilized. Silicone polymer (250 mg) was added to the above solution. After flushing with nitrogen, the solutions were allowed to equilibrate at 37°C for 48 hr and the Ch A_T was determined as described above.

Method B. This method differs slightly from Method A. A stock solution of known concentration of the radiolabeled ChM in ethanol was prepared and known aliquots of this solution were transferred to several vials. The ethanol was allowed to evaporate by overnight incubation in an oven maintained at 37°C and the Ch residue in each vial was dispersed in BS or BS-L solution (1.5 ml). Silicone polymer (250 mg) was added to each vial, and after flushing with nitrogen the vials were heated to 80°C in a water bath until the solution was isotropically clear (approximately 4 hr). The temperature of the bath was adjusted to 37°C and the vials were allowed to cool gradually to that temperature. The sample vials were maintained at that temperature with constant shaking for 48 hr. The Ch concentration in the silicone polymer and in the aqueous phase was determined as described above. This method greatly reduced the time required to prepare the BS-L-Ch solutions and was routinely used to determine the Ch activity in subsequent experiments.

Quasi-Elastic Light Scattering (QLS) Measurements on BS-L Solutions

Details of the procedure for the QLS measurements are described elsewhere (23). Briefly, BS-L solution (4 ml) was pipetted into each of several vials containing predetermined amounts of Ch to yield a series of Ch concentrations. The sample vials were heated to 80°C until the Ch was completely solubilized. While hot, the BS-L-Ch solutions were filtered through a 0.22-μm filter and quasi-elastic light scattering (Brookhaven Instruments Corporation, Holtsville, NY) measurements were made on the filtered solutions (argon laser, 514.5 nm, 37°C).

RESULTS AND DISCUSSION

Calibration Plot for Ch Thermodynamic Activity (A_T)

Table I presents the solubility data for four BS and BS-L systems involved in the calibration studies. Solubility

equilibrium was reached in less than 7 days. The solubility data were in general agreement with earlier literature (15,20).

The $C_{Sp,Eq}$ (and the A_T) values obtained are plotted as a function of CSI in Fig. 1 for the TC, TCDC, TUDC, and TCDC-L solutions. As can be seen, irrespective of the method used, all the data fall close to the same straight line. This indicates that Henry's law is obeyed (or approximately obeyed) in these systems (up to the point of Ch saturation), a result consistent with our previous finding (21). Figure 1 may then serve as a "calibration" for Ch activity in the silicone polymer and these results are used to determine the A_T values for systems containing conjugated UDC/L and where mesophase formation may occur. A brief discussion on the applicability of Henry's law with the silicone polymer system and the validity of using the data in Fig. 1 as a "calibration" plot should be instructive here. First, it was shown in our previous study (21) that neither BS (TC or TCDC) nor L appears to partition into silicone polymer under the conditions of these experiments. There may be a very small amount of water uptake by the polymer [less than one-tenth of 1% (24)], but it is likely to be relatively constant as the thermodynamic activity of water is relatively constant over the range of these studies. Therefore the situation is believed to be approximated by only Ch partitioning into a relatively constant phase (silicone polymer) from variable BS-L-Ch solutions. Now Henry's law is likely to be obeyed when a solution is dilute and when solute–solute association tendencies are small. In the neat TUDC systems, it was estimated (14) that only 1–2% of the micelles contain one molecule of Ch at unit activity (i.e., micellar Ch saturation). Since the TUDC micelles carry a small load of Ch, it would be reasonable to assume that this system would bind Ch in direct proportion to the thermodynamic activity of Ch in solution, i.e., Henry's law must be obeyed (Fig. 1). Further, it would be reasonable to expect Henry's law to be obeyed for Ch in the silicone polymer as $C°_{Sp,Eq}$ is small, i.e., ~2.8 m*M*. There is experimental evidence in the literature (25) which suggests that at such low concentrations in the organic phase, alcohols exist predominantly in the monomeric form. Also, due to the ability of the siloxane bond to form hydrogen bonds with proton donors (26), significant Ch–Ch hydrogen bonding is believed to be improbable in silicone rubber,

Table I. Solubility of ChM in BS and BS-L Media[a] of Varying Compositions for Calibration Studies

Solvent composition[b]	ChM solubility, $C_{S,Aq}$ (m*M*) Day 7	Day 14	Day 21	Day 28	Avg.
TC (68.2 m*M*)	0.951	0.914	0.954	0.973	0.948 ± 0.02
TCDC (73.7 m*M*)	1.44	1.47	1.45	1.41	1.44 ± 0.02
TCDC-L (75.1/21 m*M*)	5.28	5.22	5.23	5.33	5.27 ± 0.05
TUDC (334.7 m*M*)	0.615	0.631	0.602	0.621	0.617 ± 0.01

[a] In 0.1 *M* NaCl, 0.01 *M* sodium phosphate buffer, pH = 7.4.
[b] TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TUDC, tauroursodeoxycholic acid; TCDC-L, taurochenodeoxycholic acid–lecithin.

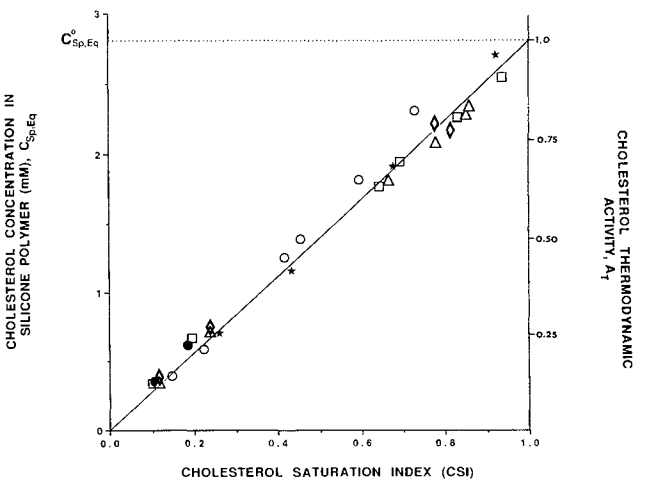

Fig. 1. "Calibration" plot for the determination of cholesterol thermodynamic activity. (○) TC = 68.2 m*M* (Method A); (□) TCDC = 73.7 m*M* (Method A); (●) TCDC = 73.7 m*M* (Method B); (△) L = 21 m*M*, TCDC = 75.1 m*M* (Method A); (◇) L = 21 m*M*, TCDC = 75.1 m*M* (Method B); (★) TUDC = 334.7 m*M* (Method B). Solid line denotes Henry's law.

especially for dilute solutions. Until there is more work on this point, it shall be assumed that Henry's law is a good first approximation (for Ch in silicone rubber) and therefore $C_{Sp,Eq}$ should be a good proportionate measure of A_T.

A_T Measurements in Conjugated UDC-L-Ch Solutions

The results of A_T determinations in tauroursodeoxycholate (TUDC)-L-Ch solutions as a function of Ch concentration are presented in Figs. 2 and 3. Both Method A and Method B were used for A_T measurements; however, since the activity data were independent of the method used

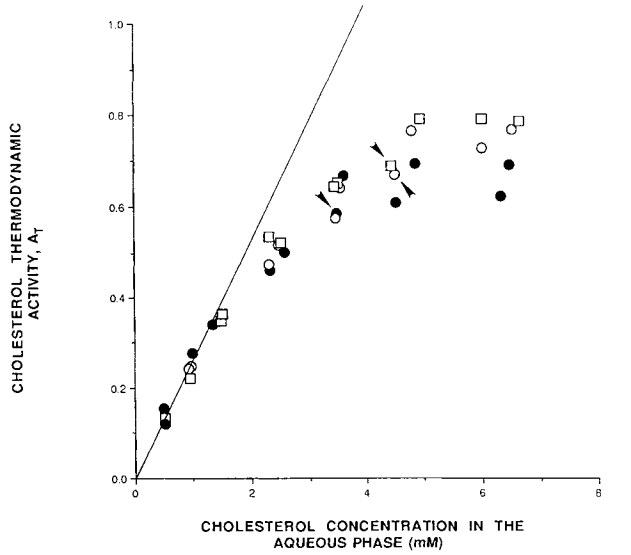

Fig. 2. The relationship between cholesterol thermodynamic activity (A_T) and cholesterol concentration ($C_{Aq,Eq}$) in the tauroursodeoxycholate–lecithin–cholesterol systems (constant lecithin). (●) L = 20 m*M*, TUDC = 50 m*M*; (○) L = 20 m*M*, TUDC = 100 m*M*; (□) L = 20 m*M*, TUDC = 150 m*M*. Arrowheads indicate onset of mesophase formation; solid line denotes Henry's law.

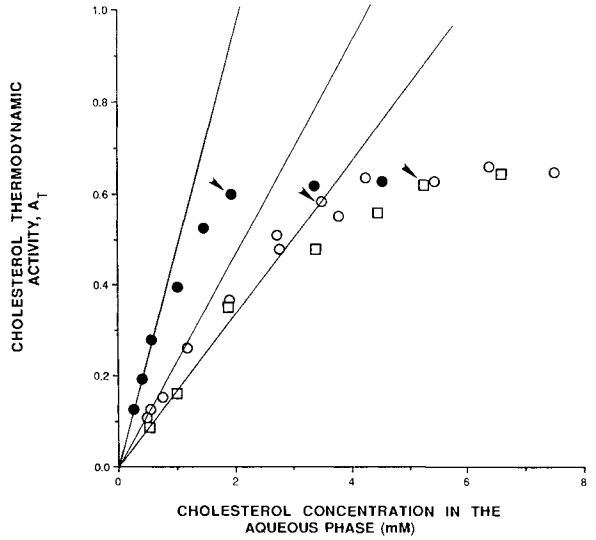

Fig. 3. The relationship between cholesterol thermodynamic activity (A_T) and cholesterol concentration ($C_{Aq,Eq}$) in the tauroursodeoxycholate–lecithin–cholesterol systems (constant bile salt). (●) L = 10 m*M*, TUDC = 80 m*M*; (○) L = 20 m*M*, TUDC = 80 m*M*; (□) L = 30 m*M*, TUDC = 80 m*M*. Arrowheads indicate onset of mesophase formation; solid lines denote Henry's law.

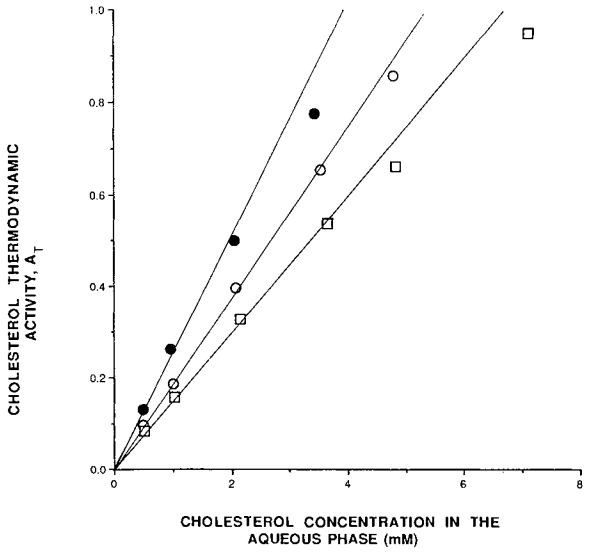

Fig. 4. The relationship between cholesterol thermodynamic activity (A_T) and cholesterol concentration ($C_{Aq,Eq}$) in the taurochenodeoxycholate–lecithin–cholesterol systems (constant lecithin). (●) L = 20 m*M*, TCDC = 50 m*M*; (○) L = 20 m*M*, TCDC = 100 m*M*; (□) L = 20 m*M*, TCDC = 150 m*M*. Solid lines denote Henry's law.

(within experimental error), only the data obtained by Method B is presented in Figs. 2 and 3. L concentration was held constant at 20 m*M* and the TUDC level was varied in one set of experiments (Fig. 2) and TUDC was held constant at 80 m*M* while L was varied in the other study (Fig. 3). The most striking aspect of these data (compare with Fig. 1) are (a) the early (A_T ~0.4) deviations of A_T from Henry's law and (b) the appearance (indicated by arrowheads) of the mesophase in the range of $0.5 < A_T < 0.8$. This behavior contrasts sharply from that for "normal" BS-L-Ch systems (Fig. 1; see also Ref. 21) or that of TUDC-Ch solutions in the absence of L (21).

Looking at the results in Fig. 2 a little more closely, it is first noted that, within the scatter of the data, all of the A_T values at a low Ch concentration (Henry's law region) fall on top of each other. It is instructive to compare this behavior of the TUDC-L-Ch system with that for the taurochenodeoxycholate (TCDC)-L-Ch system in the Henry's law region under the same conditions (see Fig. 4). For the TCDC-L-Ch system, the A_T values for the three TCDC levels fall on different Henry's law lines. This difference in the behavior between the TUDC and the TCDC systems may be explained on the basis of (a) the coexistence of two micellar species (simple bile salt micelles and the BS-L mixed micelles) and (b) the affinity for Ch by the TUDC simple micelle being negligibly small while that by the TCDC simple micelle is not so. The single Henry's law slope for the Fig. 2 data is close to the Henry's law slope of the 50/20 TCDC-L experiments. This is consistent with the contribution to Ch solubilization by the simple TCDC micelles being small compared to the mixed micelles for the 50/20 TCDC-L case because there is a relatively low concentration of simple TCDC micelles at this low BS/L ratio (27,28) and the affinity of the mixed micelle for Ch being similar for the TUDC-L and the TCDC-L cases. It follows from this that the lower slopes of the Henry's law line for the 100/20 and the 150/20 TCDC-L cases (compared to the 50/20 TCDC-L case) are due to the TCDC simple micelle contributions to Ch solubilization. The above explanation is in agreement with our recent work (27,28) on the coexistence of simple and mixed micelles in BS-L solutions and on Ch solubilities in BS solutions (15).

Further, on the preceding point, the Henry's law lines as shown in Fig. 3 correspond exactly, i.e., the slopes are exactly in the ratio 3.0:1.5:1.0 for the 80/10, 80/20, and 80/30 cases, respectively. This is in quantitative agreement with the TUDC-L mixed micelles dominating Ch solubilization in the TUDC-L system (and, therefore, the TUDC simple micelles contributing negligibly).

Since the purpose in varying the TUDC/L ratio was to determine the extent to which this variable may influence the mesophase formation tendency, it is interesting to note that the minimum A_T for mesophase formation appears to be affected only little (or none at all) by this ratio. The data in Fig. 2 indicate that the lower the TUDC/L ratio, the lower the minimum A_T value for mesophase formation; but it is seen that this effect is marginal and within the scatter of the data.

This rather small influence of the TUDC/L ratio upon the minimum A_T value for mesophase formation suggests the following: either the thermodynamic activity of TUDC is dependent only weakly upon the TUDC concentration above the critical micelle concentration for the simple TUDC micelles, or the TUDC activity has little influence upon the (thermodynamic) stability of the TUDC-L mixed micelle, or both. That the Henry's law slopes are the same for the three sets of data in Fig. 2 is consistent with the view that there is little or no effect of varying the TUDC simple micelle concentration on the thermodynamic stability of the TUDC-L mixed micelles (here Ch plays the role of a probe). It follows then, presuming little or no involvement of TUDC in the

mesophase composition, that the point of appearance of the mesophase should be dependent primarily (or only) on A_T.

In Fig. 5, data taken from a recent QLS study (23) are presented to give some insight into the sizes of the mesophase particles near the minimum A_T for mesophase formation. TUDC-L of 80/32 corresponds to the same TUDC/L ratio as the 50/20 case (Fig. 2) and the minimum A_T for mesophase formation here is in the range, 0.5 to 0.6. The numbers given next to the data points are the mean hydrodynamic radii (R_H as Å). As can be seen, the R_H values for the TUDC-L system are in the range 21–25 Å (and consistent with only micelles being present), up to (but not including) the first experiment (concentration of Ch = 5.76 m*M*, A_T = 0.525) where visible turbidity was noted and where R_H = 258 Å. At a concentration of Ch = 7.48 m*M* and A_T = 0.57, R_H = 588 Å. For comparison, R_H values are also given for the TCDC-L system and the R_H values here were found to be 21–22 Å over the entire range studied ($0 \leq A_T \leq 1.0$); this corresponds to only micelles being present for the TCDC-L system over the entire range ($0 \leq A_T \leq 1.0$). Liu *et al.* (23) have carried out a detailed analysis of their QLS data for the TUDC-L-Ch system taking polydispersity into account; their findings suggest that the R_H = 258 Å may be in a region of coexistence between mixed micelles of TUDC-L and mesophase vesicles of much larger sizes (500–600 Å). It may be noted here that small deviations from Henry's law occur in the TCDC-L system at A_T values greater than ~0.8 and that these solutions may no longer follow ideal behavior. This probably is the result of the high load of Ch carried by the mixed micelles in these systems, and as expected from regular solution theory, deviations from Henry's law may occur.

Finally, the results of a comparative study conducted with GUDC-L and GCDC-L systems are presented in Fig. 6.

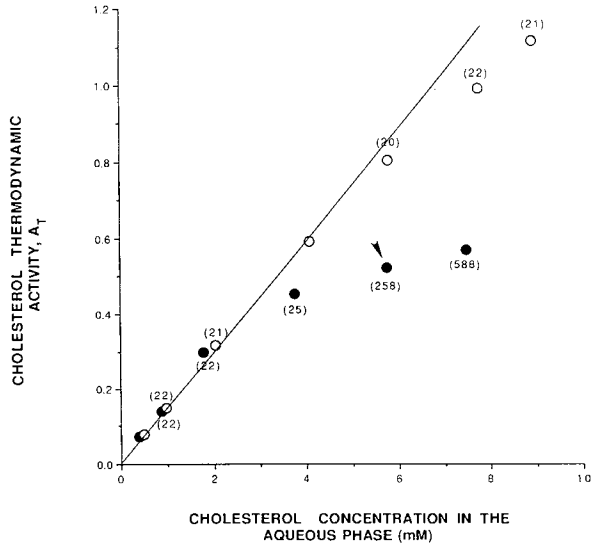

Fig. 5. Comparison of the cholesterol thermodynamic activity (A_T) in the tauroursodeoxycholate–lecithin–cholesterol and the taurochenodeoxycholate–lecithin–cholesterol systems. Numbers in parentheses are the mean hydrodynamic radii of the species present in the solution. (●) L = 32 m*M*, TUDC = 80 m*M*; (○) L = 32 m*M*, TCDC = 80 m*M*. Arrowhead indicates onset of mesophase formation; solid line denotes Henry's law.

The main point to be made here is that the TUDC-L and GUDC-L systems, under this set of conditions, behaved similarly, i.e., Henry's law is obeyed up to A_T ~0.4 and mesophase formation begins at an A_T of about 0.5 to 0.55 in both cases and at around the same aqueous phase Ch concentration. The TCDC-L and the GCDC-L results are also similar.

A_T Measurements in BS-L-Ch Systems with TUDC-TCDC Mixtures

The effects of varying the TCDC/TUDC ratio upon A_T and the mesophase formation behavior are presented in Fig. 7, for the case in which the total bile salt concentration of 80 m*M* and L concentration of 32 m*M* were held constant. With increasing TCDC/TUDC ratio, where deviations from Henry's law begin and the point of first appearance of mesophase (indicated by arrowheads) were both shifted to higher A_T values. For the 40/40 TCDC/TUDC case, mesophase formation began at about A_T ~0.9, which is very near the point of ChM saturation (A_T = 1.0). For the TCDC/TUDC ratio of 56/24, mesophase formation was not observed at all in the range $0 \leq A_T \leq 1.0$. These results suggest that the ratio of UDC conjugates to normal BS conjugates is monotonically related to the propensity of the system to allow mesophase formation.

A_T Measurements in BS-L-Ch Mixtures Simulating Biles of Patients Undergoing UDC Therapy

Typical compositions of BS-L-Ch mixtures simulating biles of patients undergoing UDC therapy are presented in

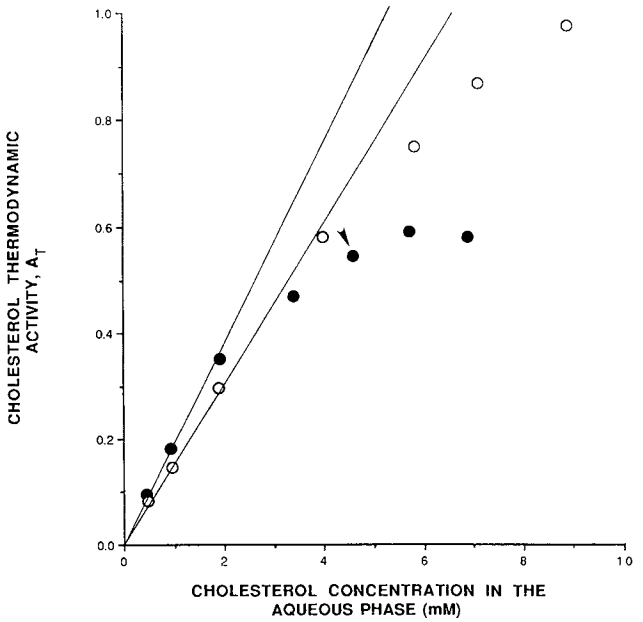

Fig. 6. Comparison of the cholesterol thermodynamic activity (A_T) in the glycoursodeoxycholate–lecithin–cholesterol and the glycochenodeoxycholate–lecithin–cholesterol systems. (●) L = 32 m*M*, GUDC = 80 m*M*; (○) L = 32 m*M*, GCDC = 80 m*M*. Arrowhead indicates onset of mesophase formation; solid lines denote Henry's law.

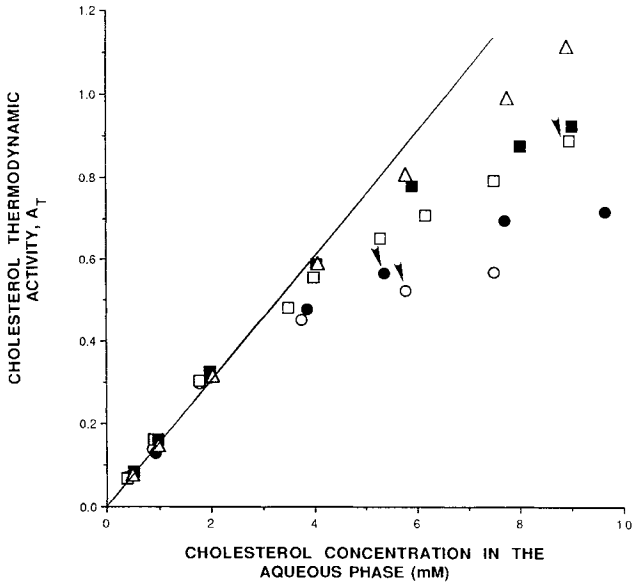

Fig. 7. The relationship between cholesterol thermodynamic activity (A_T) and cholesterol concentration ($C_{Aq,Eq}$) in the tauroursodeoxycholate–lecithin–cholesterol, taurochenodeoxycholate–lecithin–cholesterol, and tauroursodeoxycholate–taurochenodeoxycholate–lecithin–cholesterol systems (constant lecithin). (○) L = 32 m*M*, TUDC = 80 m*M*; (●) L = 32 m*M*, TUDC = 56 m*M*, TCDC = 24 m*M*; (□) L = 32 m*M*, TUDC = 40 m*M*, TCDC = 40 m*M*; (■) L = 32 m*M*, TUDC = 24 m*M*, TCDC = 56 m*M*; (△) L = 32 m*M*, TCDC = 80 m*M*. Arrowheads indicate onset of mesophase formation; solid line denotes Henry's law.

Table II. Composition A may be somewhat on the high side, and composition C somewhat on the low side, of a median value [approximately 3.0 (29)] for the total BS-to-L ratios estimated from pooling of patient population data (6,7,10,11, 20,29–34).

Table II. Typical Compositions[a] of Gallbladder Bile of Patients Undergoing UDCA Therapy

Bile composition[b]	Total BS (m*M*)	L (m*M*)	Total BS/L
(A) 60.2 m*M* GUDC, 9.8 m*M* TUDC, 23.8 m*M* GCDC, 4.2 m*M* TCDC, 16.8 m*M* TDC, 22.4 m*M* TC, 2.8 m*M* TLC	140	35	4/1
(B) 55 m*M* GUDC, 8.96 m*M* TUDC, 21.76 m*M* GCDC, 3.84 m*M* TCDC, 15.36 m*M* TDC, 20.48 m*M* TC, 2.56 m*M* TLC	128	42.6	3/1
(C) 51.6 m*M* GUDC, 8.4 m*M* TUDC, 20.4 m*M* GCDC, 3.6 m*M* TCDC, 14.4 m*M* TDC, 19.2 m*M* TC, 2.4 m*M* TLC	120	48	2.5/1

[a] For all cases, total lipids (i.e., total BS + L concentration) = 9.5 g/dl (20,29).

[b] BS, bile salt; L, lecithin; GUDC, glycoursodeoxycholic acid; TUDC, tauroursodeoxycholic acid; GCDC, glycochenodeoxycholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; TC, taurocholic acid; TLC, taurolithocholic acid.

The results of A_T determinations with biles A, B, and C are presented in Fig. 8. For mixture C, which had the lowest total BS-to-L ratio of 2.5, mesophase formation was observed at A_T = 0.84. For the intermediate mixture B (with a total BS-to-L ratio of 3.0), mesophase was observed very close to A_T = 1.0. With mixture A, mesophase formation could not be detected over the entire region, $0 \leq A_T \leq 1.0$. When these results are compared to those obtained above with the simple TUDC-TCDC mixtures (Fig. 7), it is apparent that the simple TCDC-TUDC-L mixtures (Fig. 7) may be a good model for these more complex mixtures: the A_T value for the beginning of mesophase formation for the TCDC/TUDC (40/40) mixture with a BS/L ratio of 2.5 was about 0.87; for mixture C (UDC conjugates = 50%), the A_T was found to be about 0.84.

Significance of the Present Findings

The present results contribute to a better understanding of Ch dissolution behavior in BS-L solutions rich in conjugates of UDC. The activity (A_T) data provide insights into propensities for the systems to deviate from ideal behavior (i.e., from Henry's law, for which the Ch escaping tendency is simply proportional to its solution concentration). This tendency to deviate from the ideal behavior, if it is sufficiently great, leads to mesophase formation before ChM saturation is reached. The present study has shown, for example, that when the conjugates of UDC are the only bile salts present, then mesophase formation may occur at relatively low A_T values (A_T = 0.6); when the UDC conjugates represent about 50% of the total bile salts, then the A_T value needs to be about 0.85 for mesophase formation to occur; and at lower percentages of UDC conjugates, mesophase formation may not occur at all before ChM saturation is reached.

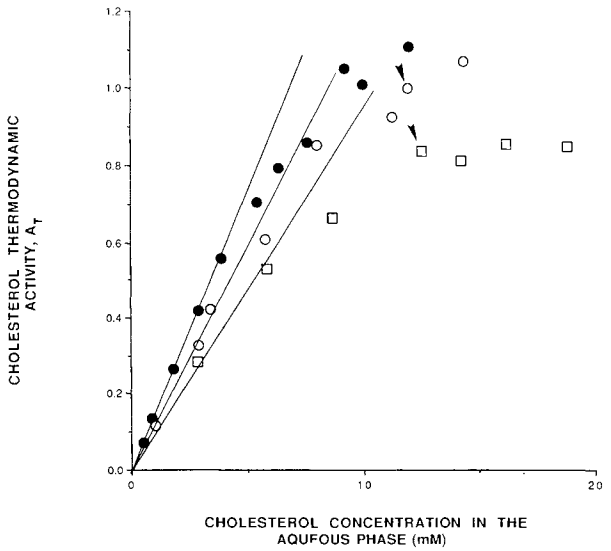

Fig. 8. The relationship between cholesterol thermodynamic activity (A_T) and cholesterol concentration ($C_{Aq,Eq}$) in bile salt–lecithin–cholesterol mixtures simulating biles of patients undergoing UDC therapy. Compositions as shown in Table II. (●) Composition A, BS/L = 4/1; (○) Composition B, BS/L = 3/1; (□) Composition C, BS/L = 2.5/1. Arrowheads indicate onset of mesophase formation; solid lines denote Henry's law.

From the standpoint of possible clinical significance, the data in Fig. 8 are very interesting because they suggest that the situation is only marginal with regard to mesophase formation in biles of patients undergoing UDC therapy: only a fraction of the patients undergoing UDC therapy may possess biles that, from a thermodynamic point of view, are able to produce liquid crystalline mesophase at or near ChM saturation. Recently, Dr. Alan Hofmann (35) stated, "When patients with gallstones take UDC, the enrichment of UDC in biliary bile acids plateaus about 40% to 50% and is lower than the comparable enrichment that one gets when one takes CDC. I asked Dr. Einarsson if they have even seen liquid crystals in patients on UDCA therapy; he said 'No.' "

The foregoing represents a thermodynamic equilibrium viewpoint. In both the studies by Corrigan *et al.* (17) and those by Salvioli *et al.* (20) on ChM dissolution in conjugated UDC enriched systems, the evidence is compelling that liquid crystalline mesophase may occur on ChM crystal surfaces when A_T in the bulk solution phase is probably far too low for bulk solution mesophase formation to take place. Salvioli *et al.* (20) have suggested that mesophase formation "is first an intermediate-interfacial phenomenon and then a bulk phenomenon." Mesophase formation on the Ch crystal rather than in the bulk aqueous phase is possible if we assume an unstirred boundary layer around the crystal, the Ch activity in the vicinity of the crystal being greater (high enough to form mesophase) than in the bulk solution. Alternately, but perhaps unlikely, there may be collisions of the BS-L mixed micelles with ChM crystal surfaces leading to deposition of L upon the ChM surfaces (where Ch and water may be at or near unit thermodynamic activity); this may be followed by the formation of the Ch-L liquid crystalline mesophase on ChM surfaces (20), provided that the rate of micellar solubilization of this thermodynamically unstable phase is kinetically slow enough. Clearly more work needs to be done on this question, especially (a) to determine more quantitatively the relationships between mesophase formation on the ChM crystal surfaces during dissolution and mesophase formation tendencies in the bulk solution and (b) the extent to which mesophase formation on the ChM surfaces under these conditions appreciably contribute to Ch gallstone dissolution and dispersion in "urso-rich" BS-L solutions.

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