TAUROCHOLATE- AND TAUROCHENODEOXYCHOLATE-LECITHIN MICELLES: THE EQUILIBRIUM OF BILE SALT BETWEEN AQUEOUS PHASE AND MICELLE.

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SUMMARY. The equilibrium of bile salt between aqueous phase and mixed micelle was studied in solutions of pure bile salt and lecithin comparing taurocholate and taurochenodeoxycholate. The relationship between bile salt concentration in the aqueous phase and the ratio of bile salt/lecithin in the mixed micelle was determined by equilibrium dialysis on serial dilutions of these solutions. Extrapolation of this relationship to zero mixed-micellar bile salt permitted calculation of the critical micelle concentration (CMC) of the mixed micelle. For taurocholate, taurochenodeoxycholate, and an equimolar mix of these two bile salts, the mixed micelle CMC's were 3.1 mM, 0.47 mM, and 0.89 mM respectively. In the most concentrated solutions, aqueous phase bile salt concentration surpassed the CMC of the simple bile salt micelle by more than four-fold indicating the presence of simple micelles as well as mixed micelles. At all dilutions taurochenodeoxycholate had a much greater affinity for the mixed micelle than did taurocholate. This last finding may be the reason for the superior cholesterol solubilizing capacity of taurochenodeoxycholate-lecithin solutions compared to taurocholate-lecithin solutions.

INTRODUCTION

Bile salt-lecithin micelles are of particular interest because of their capacity to solubilize biliary cholesterol and their apparent role in biliary secretion of cholesterol and lecithin (1-4). The bile salt component of these micelles exists in dynamic equilibrium between micelles and aqueous phase. Previous study of this equilibrium in serial dilutions of human bile has provided an estimate of the affinity of bile salt for the mixed micelle and permitted measurement of the critical micelle concentration (CMC) of biliary micelles (5). In that study, as in many

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other investigations, the bile salt component of bile was regarded as a single, homogeneous chemical entity. However, bile contains several different species of bile salt, which may differ with respect to physical interactions with lecithin.

In the present study the equilibrium of bile salt between mixed micelle and aqueous phase was studied in relatively simple systems of pure bile salt and lecithin. The taurine conjugates of cholate and chenodeoxycholate, the two bile salts synthesized by the human liver, were compared both in systems where each represented the entire bile salt component and in a system where both bile salts were present in equimolar proportions. This comparison is of special interest because these two bile salts are known to have different cholesterol solubilizing capacities (6). Moreover, administration of chenodeoxycholate has been shown to dissolve cholesterol gallstones while cholate is absolutely ineffective in this regard (7).

MATERIALS AND METHODS

Bile salt and lecithin were measured as previously described (5). The sodium salts of taurocholate (TC) and taurochenodeoxycholate (TCD) as well as a chromatographic preparation of egg lecithin were obtained commercially (Sigma Chemical, St. Louis, Missouri) and checked for purity by thin-layer chromatography. TCD required further purification by recrystallization. Sterile aqueous bile salt-lecithin solutions for each of these two bile salts and for a 1:1 molar mixture of the two were prepared by dissolving the dry lipids in 0.10 N NaCl. The final solutions were adjusted to pH 7.3 ± .02. Samples of these solutions or dilutions thereof were aseptically dialyzed against 0.10 N NaCl at room temperature for measurement of the aqueous phase bile salt concentration. Bile salt and lecithin concentrations in the retentate were used to calculate the molar ratio of bile salt/lecithin in the mixed micelle by subtraction of the aqueous phase bile salt concentration. Details of this entire procedure have been published elsewhere (5).

In addition to measurement of total bile salt, retentates and dialysates of the 1:1 molar mix of TC- and TCD-lecithin were analyzed for individual bile salts. Separation of TC and TCD in these samples was accomplished by thin-layer chromatography using silica gel G as the stationary phase and isoctane:ethyl acetate: acetic acid 5:5:1 as the moving phase. Prior to chromatography the bile salts were deconjugated by hydrolysis in 1.25 N NaOH at 125°C. and 15 p.s.i. for three hours. Known amounts of ^{14}C-cholate and ^{14}C-chenodeoxycholate (New England Nuclear, Boston, Mass.) were added to each sample before application to the silica gel plates to account for losses during chromatography.

Dialysis equilibration was entensively evaluated for each type of solution studied. Dialysis of pure bile salt solutions (not containing lecithin) demonstrated complete equilibration (equal bile salt concentration in dialysate and retentate) even well above the CMC indicating that both

TABLE I

FRACTION OF BILE SALT IN THE AQUEOUS PHASE AND CONCENTRATIONS OF BILE SALT AND LECITHIN AT DIALYSIS EQUILIBRIUM

Concentrations of bile salt and lecithin in retentate ([B]r, [L]r) and concentration of bile salt in dialyzate ([B]a) are given in mmol/liter. Fa represents the fraction of total bile salt present in the aqueous phase rather than in mixed micelles. To obtain this number, total mmoles of bile salt were calculated from the bile salt concentration of the original working solution and the volume of this solution placed in the dialysis bag. Millimoles of bile salt in the aqueous phase was calculated by multiplying total volume (dialyzate + retentate) by the concentration of bile salt in the dialyzate.

Taurocholate				Taurochenodeoxycholate				Taurocholate-Taurochenodeoxycholate			
[B]r	[L]r	[B]a	Fa	[B]r	[L]r	[B]a	Fa	[B]r	[L]r	[B]a	Fa
40.08	5.92	23.63	.623	28.54	6.68	4.45	.224	20.88	6.28	5.64	.509
35.72	6.00	20.70	.682	29.34	7.15	3.95	.199	18.54	6.38	4.41	.557
30.94	5.83	17.72	.700	24.12	6.50	2.99	.251	17.33	6.66	3.99	.612
26.23	6.15	13.95	.735	23.44	6.26	2.97	.268	11.30	5.35	2.90	.668
22.48	6.26	11.81	.778	13.34	4.17	2.18	.366	7.69	4.52	2.33	.715
15.96	4.00	9.90	.783	9.08	3.84	1.81	.455	4.34	3.30	1.79	.824
13.88	3.92	8.66	.799	6.98	2.91	1.57	.526	2.51	2.76	1.39	.853
12.80	4.04	7.77	.819	3.76	2.22	1.25	.629	1.64	2.12	1.16	.890
11.61	4.02	6.98	.828	2.92	2.06	1.02	.684				
10.36	3.84	6.41	.845	1.43	1.51	0.775	.780				
7.26	2.38	5.43	.858	0.888	1.34	0.587	.787				
5.02	2.20	4.25	.896								
4.08	2.28	3.61	.951								

bile salt monomer and simple bile salt micelles were measured as dialyzable bile salt. In the bile salt-lecithin solutions equilibrium was indicated by a plateau in dialyzate bile salt concentration. In these studies the slowest equilibration time for TCD was 28 hours, for TC was 15 hours, and for the 1:1 mix of TC-TCD was 20 hours. These slowest equilibration times were noted in the most concentrated solutions (Table 1). As the solutions were diluted dialysis time quickly became shorter. Never the less all TC-lecithin solutions were dialyzed for 24 hours while all TCD-lecithin and TC-,TCD-lecithin solutions were dialyzed for 36 hours. As discussed previously (5) the speeding of dialysis with dilution is probably a result of reduction in concentration of simple bile salt micelles while the descrepancy between equilibration times for TC versus TCD is a result of the larger simple micelles formed by TCD (8). Below the CMC TC and TCD dialyze at the same rate reaching equilibrium in about 4 hours. Analysis of dialysates for phospholipid at dialysis equilibrium showed values of less than 1% of the retentate phospholipid indicating nearly complete retention of lecithin by the dialysis membrane.

RESULTS AND DISCUSSION

Primary data for each individual dialysis are given in Table 1 along with the calculated percentage of bile salt present in the aqueous phase. At dialysis equilibrium the retentate of the most dilute TC solutions was slightly turbid indicating some precipitation of lecithin. Retentates of all other solutions were clear at dialysis equilibrium. Presumably in these solutions all lecithin was present as mixed micelles despite the fact that as much as 89% of the bile salt was outside mixed micelles in the aqueous phase.

Dilution, which can be viewed simply as expansion of the aqueous phase relative to the mixed-micelle phase, promoted movement of bile salt out of the mixed micelle and progressively increased the percentage of bile salt in the aqueous phase. Viewed conversely dilution reduced the ratio of bile salt/lecithin in the mixed micelle (Fig. 1) confirming our previous observation on bile (5). Depletion of mixed-micellar bile salt may be the reason that cholesterol solubilizing capacity is reduced when bile salt-lecithin solutions are diluted (11).

From the data in Fig. 1 it is evident that TCD has a greater affinity for the mixed micelle than does TC since a much higher aqueous phase bile salt concentration is required to support any given ratio of bile salt/lecithin in the micelle for TC than for TCD. Thus for any given bile salt-lecithin

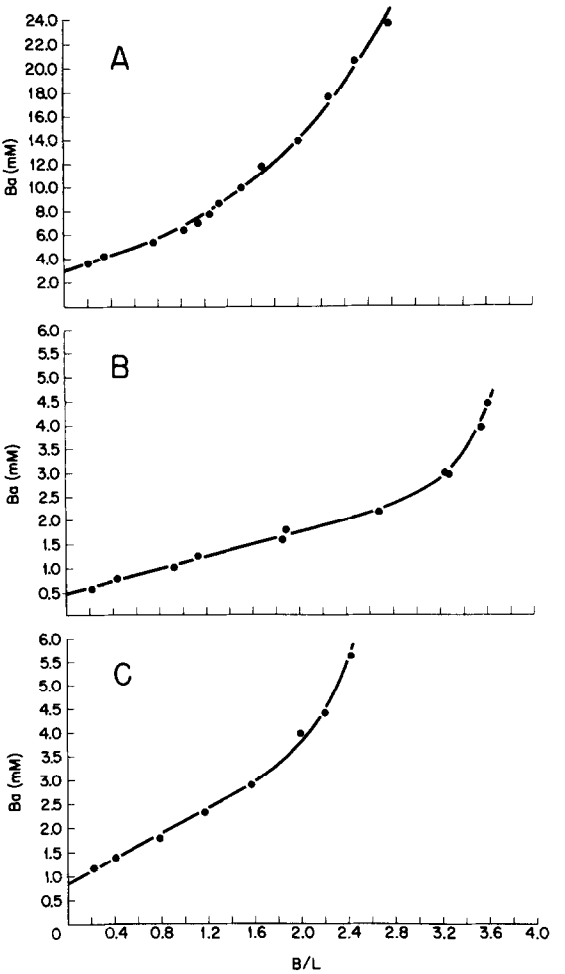

Figure 1: Aqueous phase bile salt concentration (B_a) as a function of the ratio of bile salt/lecithin in mixed micelles (B/L). Volume of solution inside the dialysis bag was 1.0 or 2.0 ml. Dilution of B_a was accomplished either by direct dilution of the initial working solution or by increasing the dialysate volume. Points to the far left of each graph represent the most dilute solutions while points to the far right represent the most concentrated solutions. Curves connecting the points were fitted by inspection. A. Taurocholate (TC). Initial working solution was 75.9 mM bile salt and 9.56 mM lecithin. Dilutions range from two-fold to 20-fold. The most dilute solution (Far left) was slightly turbid at dialysis equilibrium indicating beginning precipitation of lecithin. B. Taurochenodeoxycholate (TCD). Initial working solution was 59.6 mM bile salt and 12.02 mM lecithin. Dilution ranged from two-fold to 80-fold. There was no turbidity in any of the solutions at dialysis equilibrium. C. 1:1 molar mix of TC and TCD. Initial working solution was 55.4 mM bile salt and 10.2 mM lecithin. Dilutions ranged from 5-fold to 85-fold. No turbidity was noted in any of the solutions at equilibrium.

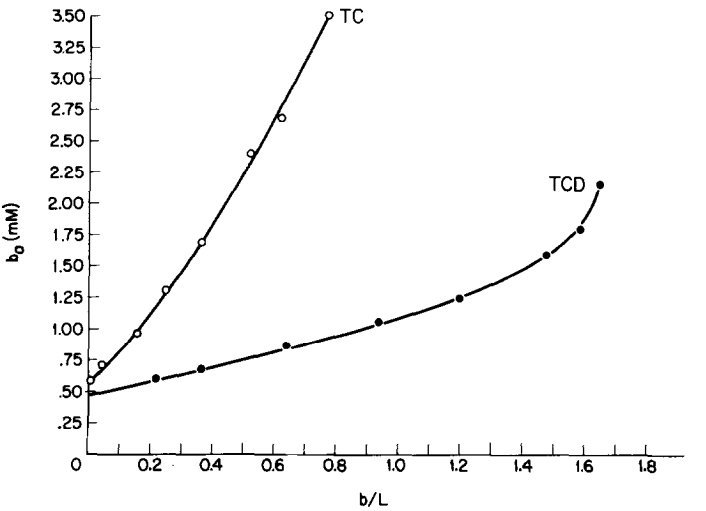

Figure 2: Aqueous phase TC and TCD concentration (b_a) as a function of the ratio of TC or TCD to lecithin in the mixed micelle (b/L) for the equimolar mix of TC-TCD (Fig. 1, C.) Curves were fitted by inspection. TCD has a greater affinity for the mixed micelle than does TC even when both bile salts are present in the same solution.

solution, substitution of TC for TCD would result in a reduction of bile salt/lecithin in the micelle. Again, depletion of mixed micellar bile salt may be the reason for the reduction in cholesterol solubilizing capacity of bile salt-lecithin solutions when TC is substituted for TCD (6). Thus two independent perturbations, dilution and substitution of TC for TCD, reduce both cholesterol solubilizing capacity and the ratio bile salt/lecithin in the micelle suggesting that bile salt content of the mixed micelle is a determinant of cholesterol solubility.

The CMC of the simple bile salt micelle is ~4.0 mM for TC and ~1.0 mM for TCD (8). The highest aqueous phase bile salt concentrations shown in Fig. 1 surpass these CMC values by more than 4-fold for both TC and TCD indicating that concentrated solutions of bile salt and lecithin contain both mixed micelles and simple micelles. The presence of simple micelles in the aqueous phase of concentrated solutions may account at least in part, for the deviation from linearity seen in all three solutions of Fig. 1.

It is possible, but not certain, that saturation of the mixed micelle with bile salt also contributes to this deviation from linearity.

Careful work by Higuchi, et al, indicates that the simple bile salt micelle solubilizes cholesterol faster than the bile salt-lecithin mixed micelle (9). The presence of simple micelles in concentrated solutions of bile salt and lecithin may therefore have unexplored implications for kinetics of cholesterol dissolution in bile and artificial bile.

The CMC of the bile salt-lecithin micelle can be estimated by extrapolating the data in Fig. 1 to zero B/L (5). This calculation gives CMC values of 3.1 mM, and 0.47 mM, for the TC-lecithin and the TCD-lecithin micelles respectively. These CMC values are below the CMC's of the corresponding simple micelles as would be predicted on theoretical grounds (10).

At the CMC of the equimolar TC:TCD mix (b/L = 0, Fig. 2), the aqueous phase concentration of TCD is 0.47 mM, a value identical to the CMC of the TCD-lecithin micelle (Fig. 1). This identity indicates that the presence of TC monomers in the mix does not alter the CMC of the TCD-lecithin micelle. Perhaps more unexpected is the presence of TC in mixed micelles at aqueous phase TC concentrations much less than 3.1 mM, the CMC of the TC-lecithin micelle. Thus once TCD-lecithin micelles are formed, entry of TC into the mixed micelle appears to be facilitated.

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