Enlargement of taurocholate micelles by added cholesterol and monoolein: self-diffusion measurements

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ABSTRACT The effect of solubilized cholesterol and 1-monoolein on the size of micellar aggregates of sodium taurocholate (3α,7α,12α-trihydroxy-5β-cholanoyl taurine) has been determined in vitro.

Measurements of the self-diffusion coefficient of sodium taurocholate (0.15 M in Na^+) at 37°C and pH 7.4 led to the conclusion that at concentrations above the critical micelle concentration (6.7 mM) the solutions contain, besides monomeric ions, a single micellar species containing five taurocholate ions. In the presence of cholesterol, much larger micelles are formed, apparently containing one molecule of cholesterol and 25 of taurocholate. These mixed micelles coexist with small micelles of pure taurocholate as well as the taurocholate monomers. The addition of 1-monoolein increases the solubility of cholesterol in the taurocholate solution, but not by reducing the size of the micelle into which the cholesterol will fit: three-component micelles (monoolein–taurocholate–cholesterol) are, if their diffusion coefficients are any guide, still larger than taurocholate–cholesterol micelles. The molar ratio of cholesterol to taurocholate is higher in these solutions than in the absence of monoolein.

Comparison with work by other authors on taurodeoxycholate–cholesterol micelles suggests that more than 25 molecules of either dihydroxy or trihydroxy bile salts are needed to transport each molecule of cholesterol through an aqueous solution in the absence of other amphipathic molecules.

SUPPLEMENTARY KEY WORDS particle weight · open-ended capillary method · mixed micelles · solubilization · micellar size · critical micelle concentration

BILE SALT MICELLES, which solubilize cholesterol and monoglycerides, are interesting in connection with the intestinal absorption of cholesterol and of lipolytic breakdown products of fat (1, 2). Pure conjugated bile salts form very small micelles, with aggregation numbers ranging (2, 3) from 4.5 (sodium taurocholate) to 18 (sodium taurodeoxycholate and glycochenodeoxycholate) under physiological conditions. The structure and composition of the mixed micelles formed by conjugated bile salts with cholesterol and monoglycerides are unknown, although the size of the micelles has been estimated by gel filtration (4, 5). Because cholesterol and bile acids have the bulky, rigid cyclopentanophenanthrene nucleus in common, one likely structure for the mixed cholesterol–bile salt micelle would be that in which one molecule of bile acid in the simple micelle had been replaced by one molecule of cholesterol. If this hypothetical structure were the correct one, the mixed micelle would have approximately the same size as the pure micelle.

I have examined this hypothesis for the sodium taurocholate–cholesterol mixed micelle by comparing its self-diffusion coefficient in free solution with that of pure sodium taurocholate and found that, contrary to expectation, the mixed micelle is far larger than the pure micelle. Thus, the unmixed micelle seems to consist of 5 molecules of taurocholate whereas the mixed micelle contains 1 molecule of cholesterol and 25 of taurocholate. Addition of monoolein does not cause rearrangement to smaller mixed micelles but leads to a still larger mixed micelle, whose structure is as yet unknown.

MATERIALS

Sodium Taurocholate

Sodium taurocholate from Maybridge Research Chemicals, Tintagel, Cornwall, England was shown by TLC

Abbreviations: CMC, critical micelle concentration; NaTC, sodium taurocholate, i.e., sodium 3α,7α,12α-trihydroxy-5β-cholanoyltaurine. The convenient term "pentamer" is used to denote a micellar aggregate of five ions or molecules, although of course the molecules are not covalently linked.

(system S-VIII of Hofmann [6]) to contain about 5% of cholate and taurodeoxycholate. These were removed by recrystallization (7). The yield was variable (65–85%), but the product consistently pure, as judged by TLC in the same system.

Sodium taurocholate-24-^{14}C, 6.75 mc/mmole, synthesized from sodium cholate-24-^{14}C, was obtained from Tracerlab, Waltham, Mass., and shown to be 99% pure by TLC on Silica Gel G in toluene–acetic acid–water 15:25:2.

Cholesterol

Cholesterol was purchased from Matheson, Coleman and Bell. Cholesterol-4-^{14}C, 58 mc/mmole, was from New England Nuclear Corp., Boston, Mass. It was 97% pure according to reversed-phase chromatography (95% methanol vs. mineral oil) and 98% pure according to TLC in diethyl ether–heptane 55:45 on Silica Gel H.

1-Monoolein

Myverol ("distilled glycerol monooleate Type 18–71-E, prepared from oleic acid," Distillation Products Industries, Rochester, N.Y.) was purified by chromatography on neutral alumina, activity I, in diethyl ether previously freed from peroxides by passage over active alumina. The ether eluate (600 ml/12 g of Myverol applied to the column) was evaporated to dryness at room temperature under reduced pressure; the oil was dissolved in ethanol–water 7:3, and the monoglyceride was extracted with chloroform. TLC (petroleum ether–diethyl ether–acetic acid 95:5:1 on Silica Gel G) showed that all traces of fatty acid and higher glycerides had been removed. Saponification and methylation of a sample followed by GLC showed that oleic acid constituted 90% of the fatty acids present.

Buffer

Sodium phosphate salts and sodium chloride were analytical grade. Sterile, triple-distilled water was obtained from Abbott Laboratories, North Chicago, Ill.

METHODS

Measurement of Diffusion Coefficients

Self-diffusion coefficients were determined by the open-ended capillary method (8). Into each of eight thick-walled precision-bore tubes, 25 mm × 0.5 mm I.D., sealed flat at one end, was injected the ^{14}C-labeled solution under investigation (sodium taurocholate-^{14}C alone, sodium taurocholate-^{14}C solution containing solubilized unlabeled cholesterol, or cholesterol-^{14}C solubilized in unlabeled sodium taurocholate, at various concentrations). The small drop of excess solution remaining on the slightly bevelled open end was removed not by blotting (which often leaves a small air gap at the top of the tube) but by brief immersion (1 min) in a large volume of stirred buffer. The capacity of each tube was then determined as follows. The tubes' contents were centrifuged out into counting vials. For this operation each tube was mounted in a special split-ring holder attached to the cap of the counting vial, with its open end down and in contact with two small (2-cm diameter) filter-paper discs. Centrifugation for 1 min at low speed drew out the solution (approximately 8 μl) fully onto the filter paper. The capillary tubes were removed, and 0.5 ml of distilled water was added to each vial to ensure efficient distribution of the radioactive substance into the scintillation fluid. 18 ml of scintillation fluid (9) containing 2.3% (w/v) Cab-O-Sil was added and the radioactivity of the samples was determined in a Packard Tri-Carb spectrometer. The radioactivity in 100 μl of the test solution was similarly determined.

The capillary tubes were filled and emptied four times in all, as a check on the reproducibility of filling and as a test for any adsorption of radioactive material to the walls of the capillary, which is manifested by irregular results from one filling to another. (Fatty acids, for example, adsorb in this way and the method cannot be applied to them.)

The tubes were finally filled once more and suspended (open end upward) in 250 ml of an outer solution of exactly the same composition as the radioactive diffusing solution except that no radioactivity was present. This solution was contained in a large (30 cm × 5 cm I.D.) stoppered test-tube, containing a magnetic stirring bar and suspended in an oil-bath maintained at 37 ± 0.05°C. The outer solution was stirred at a rate sufficient to mix diffused solution efficiently with the outer solution but insufficient to lead to any swirling out of the contents of the tubes. This rate was arrived at by trial and error and judged to be satisfactory if the values obtained in the two halves of the experiment, about to be described, were in agreement. Diffusion was allowed to proceed for 3–14 days (depending on the expected diffusion rate), and four of the tubes were then removed. After a similar (also accurately known) time the remaining four tubes were removed. The tubes were emptied by centrifugation as above; the radioactivity remaining in them was measured and expressed as a fraction of the amount originally contained in the tube. From this fraction the corresponding value of Dt/l^2 (D = diffusion coefficient, t = time, l = length of the capillary) can be obtained from the theoretical plot for one-dimensional diffusion (10). D is then readily calculated. The values of D at the two time points agree if the experiment has proceeded satisfactorily.

Preparation of Solutions

All solutions were made in 0.01 M sodium phosphate buffer, pH 7.4, 0.15 M in NaCl (sterile, triple-distilled water). During protracted diffusion at 37°C, unprotected taurocholate solutions were invaded by a microorganism, identified as *Pseudomonas subtilis*, which led to cloudy solutions and possibly a decrease in taurocholate concentration. All solutions were therefore autoclaved at the beginning of the experiment and sterile conditions were maintained in sterilized glassware.

Sodium taurocholate solutions of the desired concentration (1.0–100 mM) were made by weighing the dry powder (kept over P_2O_5) and making up to volume with the buffered NaCl solution. Cholesterol was solubilized in one of two ways: (*a*) the taurocholate solution was stirred with excess crystalline cholesterol for 1–3 days and the excess was filtered off; (*b*) weighed amounts of cholesterol and of sodium taurocholate in the molar ratio of about 1:3 were dissolved in ethanol–water 4:1 and the solution was evaporated to dryness under nitrogen. The intimate mixture thus obtained was taken up in buffered taurocholate solution of the appropriate concentration. Procedure (*b*) increased the amount of cholesterol solubilized as much as 10-fold compared to procedure (*a*); for this suggestion I am indebted to Dr. Donald M. Small (Boston University Medical Center).

For all experiments the final molar ratio of cholesterol:taurocholate was determined independently by taking nonradioactive taurocholate and cholesterol-^{14}C of known specific activity through exactly the same procedure as used in the preparation of the solution for the diffusion experiment. The radioactivity of the resultant solution was then measured after filtration and the molar ratio was calculated from the known concentration of taurocholate and the concentration of cholesterol deduced from the radioactivity per milliliter.

RESULTS

Diffusion of Sodium Taurocholate in Monomeric and Micellar Forms

Self-diffusion coefficients (D) for sodium taurocholate at concentrations from 1 mM to 100 mM are shown in Fig. 1, where they are plotted against the reciprocal of concentration. The intersection of horizontal and sloping lines gives the CMC for taurocholate under these conditions. It is seen to be 6.7 mM. At concentrations below the CMC, D is constant at 5.90×10^{-6} cm²/sec; this represents the diffusion of single ions. The radius of the particle, assumed to be spherical, was calculated from Stokes' formula to be 5.5 A. The partial specific volume for sodium taurocholate, measured by pycnometry, was 0.761 ml/g (in agreement with that found by Laurent and Persson [11] for sodium taurodeoxycholate). Calculation from these values leads to a mol wt of 560, the small difference from 515 (the ionic weight for taurocholate) being attributable either to a deviation from sphericity (very probable, even certain), or to the presence of water of hydration (three molecules if the bile salt molecule were spherical), or both. At all concentrations below 6 mM the diffusion coefficient was the same; no evidence was obtained for dimer formation.

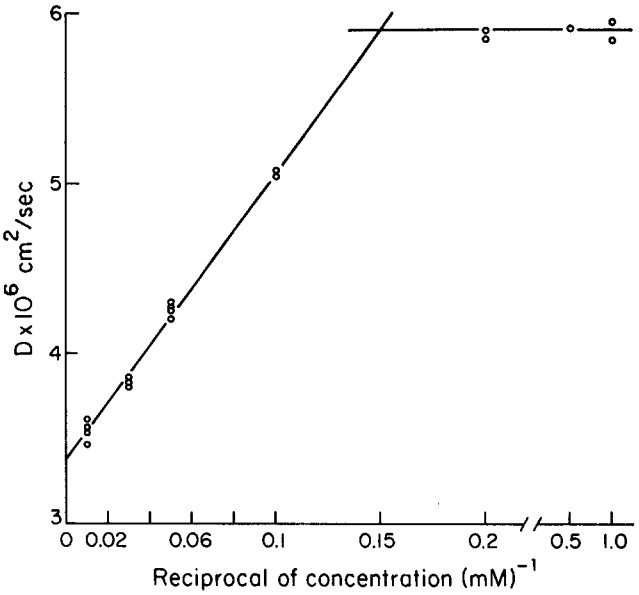

FIG. 1. Apparent self-diffusion coefficient for sodium taurocholate solutions (pH 7.4, 0.15 M Na^+, 37°C) as a function of concentration. The horizontal line gives the diffusion coefficient of the monomeric species below the CMC; this is unaffected by concentration. The sloping line represents apparent diffusion coefficients that are weighted means for mixtures of monomeric and micellar forms above the CMC. The point of intersection gives the CMC (6.7 mM) for taurocholate under these conditions. Extrapolation to infinite concentration ($1/c = 0$) gives the diffusion coefficient (3.4×10^{-6} cm²/sec) for taurocholate micelles.

Above the CMC, the apparent diffusion coefficient (D_{app}) is the weighted mean of the coefficients for monomolecular and micellar particles and is given (12) by the equation

$$cD_{app} = c_1D_1 + c_mD_m,$$

where c is the total concentration, c_1 and D_1 represent the concentration and diffusion coefficient for monomer, and c_m and D_m those for the micelles. If, as the concentration is increased, the number of micelles increases but the size of each remains constant, D_m remains constant and the following equation represents a straight line (13), provided c_1 is constant:

$$D_{app} = D_m + (c_1/c)(D_1 - D_m).$$

Although the assumption of a constant monomer concentration above the CMC represents an oversimplification, and theoretical considerations (14) would predict some curvature in the plot of D_{app} against $1/c$, especially in the immediate neighborhood of the CMC, in practice this curvature was not detectable in the system examined.

If the line is extrapolated to infinite concentration ($1/c = 0$) it will intersect the ordinate at D_m. Such extrapolation yields a D value of 3.4×10^{-6} cm²/sec. This corresponds to $r = 9.6$ A, particle wt 2950, aggregation number (assuming no water of hydration) 5.5. Small's measurements (3) by ultracentrifugation (which gives the anhydrous micellar weight) lead to an aggregation number of 4.5. Thus, the micelle probably consists of a pentamer, with the hydrophilic groups pointing outward like those in the tetramer visualized by Dietschy (15). Although quantitative speculation on the difference between Small's estimate and mine may not be justified, I tentatively suggest that each pentamer bears 15 molecules of water of hydration (possibly 3 for each taurocholate ion).

Mixed Micelles of Cholesterol-^{14}C and Unlabeled Taurocholate

The diffusion coefficient for a mixed micelle containing unlabeled taurocholate and cholesterol-^{14}C can be determined in a single measurement, since the disappearance of radioactivity from the capillary tube reflects the diffusion of only one species, namely the labeled mixed micelle (the monomolecular taurocholate ions, being unlabeled, are undetected). The coefficient was measured for 20, 40, and 100 mM taurocholate solutions loaded with cholesterol-^{14}C and was found to be 2.0×10^{-6} cm²/sec for all concentrations. A particle with this diffusion coefficient would have a radius of 16.3 A and, if $\bar{v}$ is unchanged by the presence of cholesterol, a particle weight of 14,500. If each particle contained one cholesterol molecule, almost five of the hydrated pentamers (particle wt 2950) would be needed to make up the total. Of course, the composition of the micelle could be quite different from that envisaged—the micelle might contain two or more cholesterol molecules enclosed by a correspondingly smaller number of taurocholate ions—but consideration of the maximum solubility of cholesterol in taurocholate (see below) suggests that a molar ratio of 1:25 in the micelle is, in fact, not unreasonable and that a higher ratio is unlikely. In any case, it is clear that a simple expansion of the pentamer by the inclusion or substitution of one cholesterol molecule is not the mechanism of mixed micelle formation, since this would lead to a particle wt of not more than 3500.

Mixed Micelles of Unlabeled Cholesterol and Taurocholate-^{14}C

Further support for the idea that cholesterol radically changes the organization of taurocholate micelles is afforded by a consideration of solubilization ratios. If the mixed taurocholate–cholesterol micelle were formed by a slight change of the pentamer, the maximum molar ratio of cholesterol to (micellar) taurocholate in mixed micellar solution should approach 1:4 or 1:5, whereas if the micelle contains 25 molecules of taurocholate, the ratio will approach only 1:25. The maximum ratio is, in fact, about 1:50 (my own measurements confirm those of Niederhiser and Roth [16]). This suggests not only that large mixed micelles are present but also that only about half the micellar taurocholate ions are clustered around cholesterol, the other half forming pure pentameric micelles that are in dynamic equilibrium with the large, mixed micelles.

We can test this hypothesis by determining the effect of known amounts of solubilized, unlabeled cholesterol on the apparent diffusion coefficient of taurocholate-^{14}C at different concentrations of the latter. The results of such a test are shown in Table 1. Crystalline cholesterol was stirred with taurocholate solutions to produce the solutions for diffusion; the amount of cholesterol taken up *under these conditions* (which did not in general lead to saturation) was measured as described under Methods. Several reasonable assumptions are made in calculating D_{app} from the equation

$$cD_{app} = c_1D_1 + c_mD_m + c_{mm}D_{mm},$$

TABLE 1 CALCULATED AND OBSERVED APPARENT DIFFUSION COEFFICIENTS FOR SODIUM TAUROCHOLATE-^{14}C IN THE PRESENCE OF SOLUBILIZED CHOLESTEROL

Total Concn (c) of NaTC	Solubility of Cholesterol*	Calculated c_{mm}†	Calculated c_m‡	D_{app} Calculated§	D_{app} Observed
mM	mM	mM	mM	$cm^2/sec \times 10^6$	
20	0.05	1.25	12.0	4.15	4.16 ± 0.03 (8)‖
40	0.21	5.25	28.0	3.63	3.59 ± 0.03 (4)
75	1.43	35.8	32.6	2.96	2.99 ± 0.08 (2)

NaTC, sodium taurocholate.

* Independently determined under the same conditions as those used to make up the diffusing solution.

† Concentration of NaTC present in the form of mixed micelles, calculated on the assumption that these all contain 25 molecules of NaTC and 1 of cholesterol.

‡ Concentration of NaTC present in the form of pure micelles, calculated by difference: $c_m = c - c_{mm} - c_1$, where c_1 = concentration of NaTC present in monomeric form = 6.7 mM, the CMC.

§ Calculated from the equation $cD_{app} = c_1D_1 + c_mD_m + c_{mm}D_{mm}$, where $D_1 = 5.90 \times 10^{-6}$ (Fig. 1), $D_m = 3.4 \times 10^{-6}$ (Fig. 1), and $D_{mm} = 2.0 \times 10^{-6}$ cm²/sec (determinations with cholesterol-4-^{14}C solubilized in unlabeled NaTC).

‖ SEM (n in parentheses).

where c_{mm} and D_{mm} represent the concentration and diffusion coefficient for the mixed micelles. First, the critical micelle concentration is assumed to be unchanged, so that $c_1 = 6.7$ mM. Second, the mixed micelles are assumed to contain 25 molecules of taurocholate and one of cholesterol, so that $c_{mm} = 25c_{cholesterol}$. Third, the species are assumed to be in stable equilibrium, so that c_m and c_{mm} remain constant. c_m is calculated by difference ($c - c_1 - c_{mm}$). $D_1 = 5.9$, $D_m = 3.4$, and $D_{mm} = 2.0 \times 10^{-6}$ cm²/sec as before.

The observed values for D_{app} (Table 1) were found to agree closely with those calculated in this way.

Three-Component Micelles of Sodium Taurocholate, 1-Monoolein, and Cholesterol-^{14}C

During intestinal absorption of cholesterol, the micelles in which the sterol is solubilized usually contain monoglycerides (as well as soaps, phospholipids, and other substances) in addition to bile salts (1). It is reasonable to assume that the mixed micelles formed by monoglycerides with bile salts (17) will be different in structure from the micelles formed by bile salts alone. Dreher, Schulman, and Hofmann suggest (18) that the bile salt molecules attach themselves as a hydrophilic shell to a nonpolar core consisting of monoglyceride, in which nonpolar solutes such as cholesterol could "dissolve."

The resultant mixed, three-component micelle (taurocholate–monoolein–cholesterol) might well be smaller than the taurocholate–cholesterol micelle whose size is discussed above. The diffusion coefficients of some examples of such three-component micelles were therefore examined (Table 2). For three different ratios of taurocholate to monoolein and for several modes of preparation of the solutions the diffusion coefficients were all lower than for the taurocholate–cholesterol micelle, so that the monoglyceride seems to expand, not contract the cholesterol-bearing micelle. The diffusion coefficient seems characteristic of the ratio taurocholate:monoolein and to be independent of the concentration of cholesterol, at least in the small amounts solubilized in these experiments.

TABLE 2 DIFFUSION COEFFICIENTS FOR TAUROCHOLATE–MONOOLEIN–CHOLESTEROL MICELLES

Mode of Preparation of Solution*	Taurocholate	1-Monoolein	Cholesterol	D
	mM	mM	mM	$cm^2/sec \times 10^6$
A	40	5	0.03	1.52
B	40	5	0.40	1.57
C	40	5	0.44	1.62
C	40	10	0.92	1.28
D	40	10	0.66	1.28
C	40	10	0.92	1.19
B	20	3.3	0.10	0.95
B	20	3.3	0.10	1.05

* A, cholesterol solubilized in NaTC by stirring at room temperature, excess filtered off, and monoolein added after autoclaving.

B, cholesterol solubilized in NaTC by stirring at 37°C, monoolein added in presence of slight excess of cholesterol, and mixture stirred before filtration.

C, cholesterol solubilized in NaTC by stirring at room temperature, solution then autoclaved in presence of excess cholesterol, monoolein added, and mixture stirred at 37°C before filtration.

D, monoolein added before cholesterol and whole stirred at 37°C before filtration and autoclaving.

DISCUSSION

It can hardly be claimed that the determination of self-diffusion coefficients by the use of open-ended capillary tubes is rapid, although it is fairly simple. The bore of the capillary tube must be narrow if swirling out of the contents is to be avoided; this limits the volume of radioactive solution per unit length of tube. The tube cannot, therefore, be conveniently much shorter than 2.5 cm, and for a diffusion coefficient of the order of 2×10^{-6} cm²/sec, this means a diffusion time of 7–12 days to reduce the amount of diffusing substance in the tube to 35–50% of the initial quantity, the range in which the coefficient can be most accurately deduced. The undesired microbiological intervention I encountered during these long experiments at 37°C may be a phenomenon peculiar to this laboratory; the possibility of such contamination should be kept in mind.

The method does, however, have the advantage over light-scattering and ultracentrifugation that the diffusing characteristics of monomers, micelles, and mixed micelles of the bile acids can all be examined by a single technique. Further, if the solubilized nonpolar substance (cholesterol in this case) is radiolabeled, the mixed micelles in which it is included can be distinguished with certainty from all other particles. Gel filtration has the same advantages, although that method is complicated by the possibility of adsorption to the large surface of the gel to which the solution is exposed.

Critical Micelle Concentration

The horizontal and sloping lines in Fig. 1 intersect at a point corresponding to 6.7 mM, which is therefore the CMC for sodium taurocholate at 37°C, pH 7.4, 0.15 M Na^+. This is in good agreement with that determined by measurement of surface tension (19), namely 7.4 mM, but somewhat lower than the value (10 mM) obtained by Hofmann (20). The latter value resulted from measurement of the lowest concentration at which azobenzene is solubilized, and might be more representative of a system in which cholesterol (a hydrophobic inclusate like azobenzene) is present. However, substitu-

tion of $c_1 = 10$ in the equation for D_{app} (Table 1) gives values that are much higher (outside the experimental error) than those observed and it seems that 6.7 mM is, after all, a better estimate for the CMC in the cholesterol–taurocholate system. Hofmann noted (20) that his method would tend to overestimate the CMC.

Micelles of Taurocholate Alone

The straightness of the line for D against $1/c$ above the CMC (Fig. 1) indicated that micellar solutions of this bile salt are monodisperse. The size obtained for sodium taurocholate micelles at 37°C and pH 7.4 was in fair agreement with that deduced from ultracentrifugation measurements (3), which led to the aggregation number of 4.5, interpreted by Small (2) as indicating a pentamer. My measurements also suggest an aggregate of five taurocholate ions, each bearing three molecules of water of hydration, the same degree of hydration calculated from the diffusion coefficient of the monomer. Perhaps one H_2O is associated with each hydroxyl group on the steroid nucleus. All these conclusions about structure are highly speculative except for the extremely small micellar size, which is characteristic of trihydroxy bile salts and contrasts with the micellar size of the dihydroxy bile salt taurodeoxycholate, which has an aggregation number of 18 at the same temperature and ionic strength (3). The pH in my measurements (7.4) was higher than is usual in the intestinal lumen (6.3), but Small's investigation of this variable (3) for taurodeoxycholate shows that for the strongly ionized taurine conjugates the aggregation number is unchanged from pH 4 to 9.

Taurocholate Micelles with Cholesterol

Measurement of the diffusion coefficient of cholesterol-^{14}C-labeled mixed micelles indicated that in the presence of cholesterol the pure micelles undergo radical rearrangement to form a much larger aggregate containing the cholesterol molecule. The effects of unlabeled cholesterol on the apparent diffusion coefficient of labeled taurocholate (Table 1) support the idea that a small number of large aggregates is present together with a larger number of small, pure micelles; although the calculations are based on several unsupported assumptions, they are in good agreement with the experimental results. The large, mixed aggregates are presumably in dynamic equilibrium with the small, pure micelles just as the latter are in equilibrium with monomeric taurocholate ions.

The "theoretical" maximum solubility of cholesterol in taurocholate, calculated from the size of the large aggregate, would be 1/25 of the micellar taurocholate concentration, yet the most that can be solubilized in practice is 1/50. Hence, even in saturated solutions, each large aggregate (25 molecules of taurocholate plus one of cholesterol) will be accompanied by five small ones (each containing five molecules of taurocholate alone).

Feldman and Borgström (5), using gel filtration to determine particle size, reported only a "slight expansion" on adding cholesterol to bile salt, but comparison of the present results with theirs is difficult and treacherous since they worked at room temperature, used the dihydroxy bile salt taurodeoxycholate, and added other lipids in various proportions in different experiments. It is important to recognize that their "slight expansion" actually refers to an increase of one-quarter of the radius, which corresponds to a *doubling* of the particle weight. Further, these authors accept 24 A as the radius of the pure taurodeoxycholate micelle although their gel filtration experiments indicate a range of 20–24 A and, in an earlier paper by Borgström alone (4), 16–20 A. More puzzling is the fact that Table I of ref. 4 shows K_{av} values for cholesterol-^{3}H-labeled mixed micelles that are *higher* than those for the pure micelle. These values would lead to the unexpected—and unsubmitted—conclusion that the mixed micelles are actually only about half the size of the pure micelles. Nevertheless, the conclusion of "slight expansion" could indeed be valid for taurodeoxycholate: it is quite possible that the already large taurodeoxycholate micelle (18 molecules) can, unlike the taurocholate micelle, accommodate cholesterol without great expansion, but this is not adequately established by the published data (4, 5). Taurodeoxycholate has not yet been examined by the techniques described in the present report.

Ultracentrifugal measurements of taurocholate solutions saturated with cholesterol suggest (Small, personal communication) only a small expansion of the taurocholate micelle in the presence of cholesterol, in accordance with the original hypothesis. I cannot explain this interesting disagreement between diffusion and ultracentrifugation measurements as yet.

Taurocholate Micelles with Cholesterol and Monoolein

Inclusion of monoolein with the cholesterol molecule leads to a still larger micelle (Table 2), in agreement with all authors' findings. The increased solubility of cholesterol induced by monoglycerides in bile salt solutions is frequently referred to; values are given by Simmonds, Hofmann, and Theodor for a mixture of taurocholate and taurodeoxycholate (1), but none have been published for taurocholate alone. My approximate measurements show that the ratio of cholesterol to micellar taurocholate can be increased from 1:50 to as much as 1:12 in the presence of monoolein. Particle weights derived from diffusion coefficients for the mixed micelle cannot be interpreted in terms of the number of cholesterol, taurocholate, and monoolein molecules, since (*a*)

there may be more than 1 molecule of cholesterol per micelle, and (*b*) the relative numbers of taurocholate molecules in three-component (taurocholate–monoolein–cholesterol), two-component (taurocholate–cholesterol), and pure (taurocholate) micelles are unknown. Even the concentration of monomeric taurocholate is unknown, since the CMC is known to change in the presence of monoolein (18) and may change again with the addition of cholesterol.

These preliminary studies of the three-component micelle were designed only to decide whether monoolein induced the formation of a small aggregate in which cholesterol was, as it were, more comfortably lodged than in the absence of monoglyceride. Apparently it does not. Satisfactory investigation of the structure of the three-component micelle requires a more extensive investigation in which (*a*) the CMC of taurocholate in the presence of both monoolein and cholesterol is ascertained, (*b*) the diffusion coefficients of monoolein-^{14}C-labeled taurocholate micelles are determined at various concentrations of monoolein and of taurocholate, (*c*) the maximum solubility of monoolein in taurocholate is determined (for higher taurocholate concentrations than in ref. 19) as well as the solubility of cholesterol in monoolein–taurocholate solutions, and (*d*) the diffusion coefficients are determined for series of solutions in which the taurocholate:monoolein ratio is kept constant while the concentration of cholesterol is increased. Labeling of the bile salt as well as the insoluble components in such an investigation will help to elucidate equilibria between different types of mixed micelles, as it has done in the present investigation.

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