Determinants of fatty acid and alcohol monomer activities in mixed micellar solutions

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Abstract The determinants of monomer activities of lipids dissolved in micellar bile salt solutions have been studied using polyethylene discs as the organic phase of a partitioning system. The studies show that fatty acids and alcohols interact with micelles as a partitioning system so that the monomer activity is determined by micelle volume and the lipid's partition coefficient as well as mass of lipid in the solution. Influence of the partition coefficient is seen in the dependence of monomer activity on chain length, unsaturation and carboxyl or alcohol polar groups. Dependence on chain length is equivalent to an incremental free energy of approximately -700 cal $\cdot$ mol^{-1} per methylene group. Substitution of an alcohol group for the carboxyl group at pH 7.4 decreases monomer activity by a factor of 900. Expansion of taurodeoxycholate micelles with 5mM monooleoylglycerol slightly decreases monomer activity whereas solutions of lipids in taurocholate have relatively greater monomer activities, demonstrating the influence of volume of the micelle organic phase. With constant micelle structure, monomer activity was linearly dependent on lipid mass in the system as predicted by partitioning theory. Addition of low concentration of lecithin, lysolecithin, or monoacylglycerol to the solutions had only small effects on the monomer activities consistent with the small change in total micelle organic phase. Data provided allow calculation of monomer activities of fatty acids and alcohols in many complex micellar solutions. Such data are important for evaluating such processes as intestinal absorption and gallstone formation and dissolution.

Supplementary key words cholesterol $\cdot$ partition $\cdot$ distribution coefficient $\cdot$ incremental free energy

Bile salt micellar solutions are found in intestinal contents during absorption and in the gallbladder when the bile is stored before release into the intestine (1–3). Despite the importance of these solutions for the overall physiology of the body, very little is known regarding chemical activities of lipids in such solutions (1, 2, 4). Studies indicate that micelles act as mass storage forms of lipids in aqueous solutions and facilitate intestinal absorption by increasing the aqueous diffusion capability of the lipids (2, 4). Since micelles almost certainly do not interact with the intestinal brush border membrane during fat absorption (2, 4–6), determinations of lipid monomer activities are very important for understanding absorption.

Using the technique previously described for measurement of the apparent monomer activity of lipids in micellar solutions (4), various complex micellar systems have been studied to elucidate the determinants of the monomer activity. Two relationships crucial to partitioning are (*1*) a distribution ratio independent of concentration except for deviations explainable by solute–solute interactions, and (*2*) incremental free energy changes for molecular substituent groups quantitatively appropriate for the solvents. Adherence of partitioning of fatty acids with polyethylene to these relationships has been shown previously where decanoic acid (FA 10:0) and palmitic acid (FA 16:0) had distribution coefficients independent of concentration and the incremental free energy for the methylene group was -820 cal/mol for fatty acids of 9–17 carbons. Similarly, the coefficients for distribution of FA 14:0 and FA 16:0 between aqueous monomer solution and taurodeoxycholate micelles were shown to be independent of fatty acid concentration, and the influence of the addition of methylene groups on the distribution coefficient was shown to be very high, as expected for the hydrophobic micelle core. In any partitioning system the aqueous chemical activity or monomer activity is a function of three factors, the mass of the compound in the system, the partition coefficient between the two phases, and the relative volume of the aqueous and organic (micelle) solutions. Two types of concentration curves have been used to distinguish between the effects of the partition coefficient and the total micelle volume; these present studies, which are a more rigorous test of partitioning as the basis for monomer–micelle interaction, support the previously suggested hypothesis.

Data presented in this paper may be utilized to calculate monomer activities of lipids in many micellar systems, which will be useful for study of the determi-

Abbreviations: FA, fatty acid; alc, alcohol.

TABLE 1. Coefficients for partitioning of lipids between discs of polyethylene and aqueous solution

Lipid	Partition Coefficient
	μl/disc[a]
FA 9:0[b]	2.03 ± 0.09 (5)
FA 10:0[c]	10.6 ± 1.5 (10)
FA 11:0[c]	20.2 ± 2.3 (10)
FA 12:0[c]	74.4 ± 5.6 (10)
FA 13:0[c]	321 ± 21.2 (10)
FA 14:0[c]	1,334 ± 106 (10)
FA 15:0[c]	5,637 ± 363 (9)
FA 16:0[c]	21,440 ± 2,070 (10)
FA 17:0[d]	62,220 ± 15,200 (21)
FA 18:0[c]	151,000 ± 62,000 (20)
FA 14:1[d]	263.6 ± 21 (15)
FA 16:1[e]	2,280 ± 348 (14)
FA 18:1[e]	16,540 ± 2,280 (15)
alc 10:0[e]	9,990 ± 964 (20)
alc 12:0[e]	104,200 ± 8,330 (10)
alc 14:0[e]	928,800 ± 131,700 (17)
alc 16:0[e]	1,455,000 ± 516,000 (17)
cholesterol[e]	3,016,000 ± 381,000 (9)

[a] Values are means ± SD; number of determinations in parentheses.
[b] Value from ref. 4.
[c] Determined by standard method using heptane extracted isotope.
[d] Determined by standard method.
[e] Determined by transfer method.

nants of intestinal absorption as well as other phenomena.

MATERIALS AND METHODS

Distribution of ^{14}C-labeled isotopes between aqueous solution and a thin polyethylene disc was determined after equilibration in 3–10 ml of solution in a 25 × 100 mm screw-cap tube. Tubes were placed in a shaking incubator maintained at 37°C with a motor speed of approximately 100 oscillations per min. After 24–72 hr of equilibration, the disc was removed from the tube with forceps, rinsed vigorously in either buffer or bile salt solution, and the radioisotope content was determined by liquid scintillation counting. Isotope activity in the aqueous phase was determined using an aliquot of the solution, and both disc and solution samples were counted under identical conditions. Liquid scintillation counting parameters were as previously described (4).

Partition coefficients for some compounds were determined by a modification of the above method. Following 24-hr equilibration, the discs were removed and transferred to a new tube with fresh buffer. After re-equilibration for 24–72 hr the disc and solution aliquots were analyzed as above.

The partition coefficient for each compound was usually determined by equilibration of a polyethylene disc in an aqueous buffer solution containing isotope at the supplied specific activity. Units for the coefficients are expressed as μl/disc whether the coefficient was calculated as counts/min per disc divided by counts/min per μl of solution or as nmol/disc when equilibrated in a 1 mM solution.

Solutions of isotopes in complex micellar solutions were prepared by stirring all components together in the aqueous buffer until the solution was clear. Since adherence of lipid crystals to the surface of the polyethylene disc would invalidate the results, studies were limited to solutions that dissolved readily with overnight stirring.

Labeled and unlabeled fatty acids were obtained from Applied Science Laboratories, Inc., State College, PA, and verified by thin-layer chromatography to be greater than 97% pure before use. Alcohols were also obtained from Applied Science Laboratories, Inc. and were used as supplied. Bile salts were obtained from Calbiochem, San Diego, CA, or Sigma, St. Louis, MO. Many fatty acid isotopes were suspended in NaOH solution and washed with heptane, then acidified and extracted into fresh heptane immediately before use in partition coefficient experiments.

Lecithin was prepared from fresh egg yolks according to the procedure of Singleton, et al. (7). Egg lysolecithin was prepared by the method of Hanahan, Rodbell, and Turner (8) as modified by Saunders and Thomas (9) using 15 mg of *Crotalus atrox* venom supplied by Sigma. Thin-layer chromatography of the products was performed and purity was estimated to be in excess of 95% and 90% for lecithin and lysolecithin, respectively. Purity estimation was by scanning densitometry following charring. The primary impurity in the lysolecithin was lecithin.

Polyethylene discs 0.5 inch in diameter were punched from polyethylene film of 6 mil thickness and prepared as described in previous studies (4).

Solutions were prepared utilizing a phosphate buffered saline, pH 7.4, that contained 20 meq/l PO_4 as the sodium salt and 120 meq/l NaCl.

RESULTS

Equilibrium partition coefficients for compounds between polyethylene discs and aqueous phosphate buffer solution are listed in **Table 1** and plotted in **Fig. 1.** Although these results are essentially identical to those presented previously (4), values for FA 17:0 and 18:0 are higher while those for most of the shorter chain FA are slightly lower. Earlier low values for 17:0 and 18:0 were caused by a small amount of water-

soluble impurity which produces a large error when the partition coefficient is quite large. The extended linear relation of saturated fatty acids shows the improvement in isotope purity, while the insignificant change in K for most fatty acids validates the general procedure.

In Fig. 3 of reference (4), the apparent pKa for FA 16:0 was about 6.8; however, this experiment has been repeated using the transfer method. In **Fig. 2** are plotted partition coefficients for FA 16:0 vs. the pH of the aqueous solution. The midpoint of this curve is at pH 5 as expected for a carboxylic acid, and the maximum K is 7×10^6 instead of 2×10^5 as seen previously. Thus, removal of the minute amount of water-soluble impurity reveals the full pH relationship of FA 16:0 that is appropriate for the fatty acid.

Partition coefficients for fatty alcohols are also presented in Table 1 and Fig. 1 and it can be seen that the absolute partition coefficient for a given chain length alcohol is much higher than that for the respective chain length saturated fatty acid, but that the slope of the alcohol relationship is essentially identical to that of the fatty acid relationship. The relationship for mono-unsaturated fatty acids is similarly shifted lower. The very high partition coefficient for cholesterol of 3×10^6 is listed in Table 1.

It was previously shown that saturated fatty acids 14:0 and 16:0 dissolved in 20 mM taurodeoxycholate solution gave a linear relationship of monomer activity with the total concentration of fatty acid (4). This relationship has been validated as a general relationship and **Table 2** presents data for several fatty acids in three different bile salt solutions. It should be noted

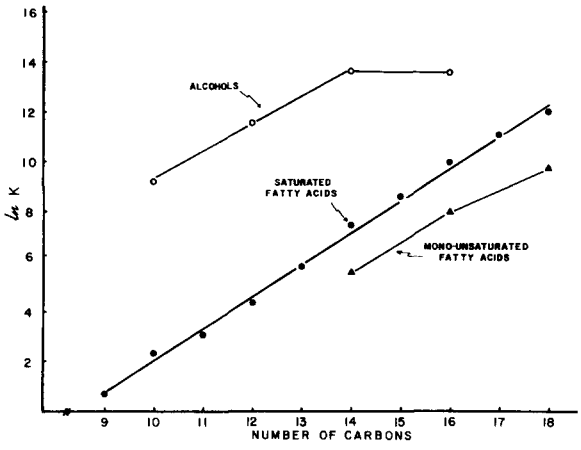

Fig. 1. Partition coefficients for fatty acids and alcohols between discs of polyethylene and aqueous buffer. Values from Table 1 are plotted as a function of the natural logarithm of the partition coefficients relative to the compound chain length. Slope of Sat FA line is equivalent to a 3.59-fold increase in K for each methylene group, or $\delta\Delta F_{w\to l} = -787$ cal·mol^{-1} per methylene group.

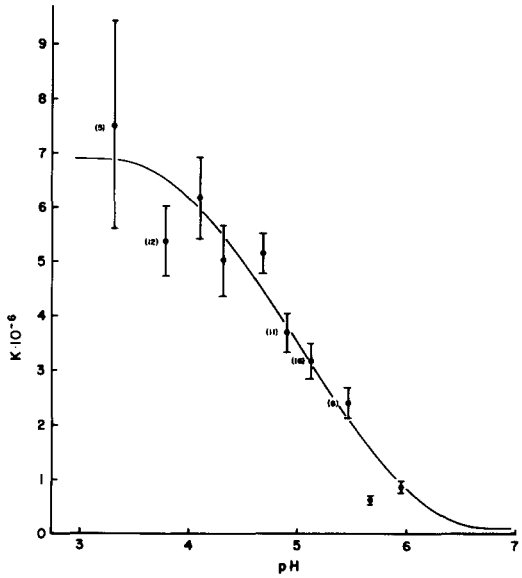

Fig. 2. Partition coefficients for FA 16:0 determined in buffer as a function of pH. Values are means ± SE for four determinations except where stated in parentheses. Buffer was 25 mM citrate in 150 mM NaCl.

that monomer activity is only 0.1–0.0001 of the total fatty acid concentration in the solution, that the intercept of each linear regression is essentially equal to zero, and that the linearity of each of these relationships is extremely high. These data support the concept of a partitioning type of relationship for fatty acid with bile salt micelles. Monomer activities of each of these fatty acids may be readily calculated for the three model solutions at any concentration.

If the proportion of fatty acid to bile salt is maintained constant, that is, the concentration of fatty acid in the bile salt micelle is constant, then the partitioning relationship would predict an essentially constant monomer activity in the solution. Results of this constant ratio type of experiment are shown for several fatty acids in **Fig. 3.** Except for concentrations near the critical micelle concentration of the bile salt, the monomer activity is essentially constant for approximately a 3-fold concentration range. Monomer activities for this plateau level of many fatty acid–bile salt combinations are presented in **Table 3.**

Studies of the monomer activities of lipid constituents of micellar solutions are important because of the critical role of micelles in intestinal absorption of lipids. In addition, understanding the formation and dissolution of gallstones requires understanding of the nature of the chemistry of complex micellar solutions. Such naturally occurring micellar solutions are exceptionally more complex than the model system described in the preceding section. Therefore, studies were conducted utilizing systems that included mono-

TABLE 2. Monomer activities of lipids in solutions containing bile salts at 20 mM

Lipid	Max. Conc.	Bile Salt	Linear Regression Slope	Linear Regression Intercept	r	N
	mM					
FA 12:0	1	TDC	0.2510	7.6×10^{-6}	0.995	20
13:0	1	TDC	0.0291	-2.5×10^{-7}	0.990	19
14:0	1	TDC	0.0208	1.2×10^{-6}	0.995	53
15:0	1	TDC	0.00233	2.5×10^{-8}	0.991	40
16:0	1	TDC	0.0010	6.9×10^{-8}	0.994	20
17:0	0.5	TDC	0.000342	4.3×10^{-9}	0.998	20
18:0	0.2	TDC	0.000115	1.8×10^{-9}	0.955	28
FA 13:0	0.5	TCA	0.0468	8.2×10^{-7}	0.983	46
14:0	0.5	TCA	0.0363	1.8×10^{-7}	0.955	46
15:0	0.5	TCA	0.00329	1.1×10^{-8}	0.980	24
16:0	0.5	TCA	0.00177	2.8×10^{-8}	0.995	52
17:0	0.1	TCA	0.000571	4.1×10^{-10}	0.980	24
18:0	0.1	TCA	0.000264	2.4×10^{-9}	0.988	21
FA 13:0	0.5	TDC/TCA[a]	0.0376	6.6×10^{-7}	0.932	24
14:0	1	TDC/TCA[a]	0.0233	1.3×10^{-6}	0.989	24
15:0	0.5	TDC/TCA[a]	0.00220	1.3×10^{-7}	0.990	23
16:0	0.4	TDC/TCA[a]	0.00117	3.9×10^{-9}	0.976	20
17:0	0.2	TDC/TCA[a]	0.000332	-1.1×10^{-9}	0.996	23
FA 14:1	0.5	TDC	0.0952	8.0×10^{-7}	0.991	20
16:1	0.5	TDC	0.00390	7.3×10^{-8}	0.990	20
18:1	0.5	TDC	0.000399	1.0×10^{-7}	0.937	19
alc 10:0	0.5	TDC	0.0476	-5.5×10^{-7}	0.997	18
alc 12:0	0.5	TDC	0.00288	4.1×10^{-8}	0.990	20
alc 14:0	0.2	TDC	0.000288	3.0×10^{-9}	0.980	20

[a] Concentrations of taurodeoxycholate (TDC) and taurocholate (TCA) were 10 mM each.

glycerides as well as lecithin and cholesterol. In **Table 4** are presented linear regression analyses of solutions containing 20 mM bile salts, and in **Table 5** are presented averages for the plateau region of constant-

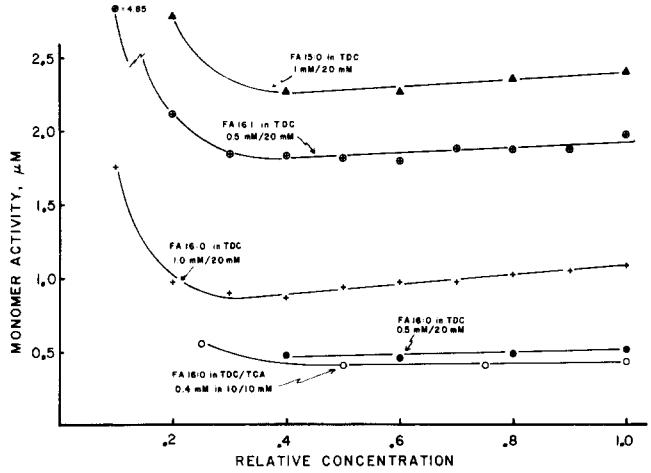

Fig. 3. Monomer activities of fatty acids in bile salt solutions with constant ratio of fatty acid and bile salt concentrations. Stated concentrations are applicable to a relative concentration of 1.0, but ratios hold for all concentrations.

ratio experiments. In **Fig. 4** are representative studies that illustrate the qualitative similarity of these values with monomer activities in the simpler model solutions. Again it may be concluded that the relationship between fatty acid and the micelle is a partitioning system and, therefore, may be compared in quantitative terms to the other systems.

A partitioning relationship would predict that the slope of the linear regression between monomer activity and total fatty acid concentration in a micellar solution would be inversely proportional to the partition coefficient between monomer and micelle and also inversely proportional to the total micellar lipid volume. This relationship may be expressed by the equation:

TABLE 3. Monomer activities of lipids in plateau region of constant lipid/bile salt ratio experiments

Lipid	TDC Conc.[a] Ratio	TDC Monomer Activity	TCA Conc.[a] Ratio	TCA Monomer Activity	TDC/TCA Conc.[b] Ratio	TDC/TCA Monomer Activity
		M		*M*		*M*
FA 12:0	1/20	2.4×10^{-4}				
13:0	1/20	3.2×10^{-5}	0.5/20	2.6×10^{-5}	0.5/20	2.0×10^{-5}
14:0	1/20	2.1×10^{-5}	0.5/20	1.7×10^{-5}	1/20	2.3×10^{-5}
15:0	0.5/20	1.2×10^{-6}	0.5/20	1.7×10^{-6}	0.5/20	1.3×10^{-6}
16:0	0.5/20	5.2×10^{-7}	0.2/20	4.5×10^{-7}	0.4/20	4.6×10^{-7}
17:0	0.2/20	6.7×10^{-8}	0.1/20	6.2×10^{-8}	0.2/20	6.6×10^{-8}
18:0	0.2/20	2.4×10^{-8}	0.1/20	3.0×10^{-8}	0.2/20	3.2×10^{-8}
FA 14:1	0.5/20	4.5×10^{-5}				
16:1	0.5/20	1.9×10^{-6}				
18:1	0.5/20	3.1×10^{-7}				
alc 10:0	0.5/20	2.0×10^{-5}				
12:0	0.5/20	1.4×10^{-6}				
14:0	0.2/20	6.0×10^{-8}				

[a] Ratio of lipid concentration to bile salt concentrations.
[b] Ratio of lipid concentration to total bile salt concentration. Concentration of TDC was equal to concentration of TCA.

TABLE 4. Monomer activities of lipids in complex micellar solutions

Lipid	Bile Salt	Other Components	Linear Regression		r	N
			Slope	Intercept		
				M		
FA14:0	TDC	MP[a]	0.0251	4.3×10^{-7}	0.996	20
FA16:0	TDC	MP[a]	0.00115	1.7×10^{-8}	0.989	19
FA18:0	TDC	MP[b]	0.00142	-7.5×10^{-10}	0.931	19
FA14:0	TDC/TCA	MP[a]	0.0314	6.7×10^{-7}	0.997	20
FA16:0	TDC/TCA	MP[a]	0.00125	2.0×10^{-8}	0.990	20
FA18:0	TDC/TCA	MP[b]	0.000178	2.9×10^{-9}	0.989	20
FA14:0	TDC	MO[c]	0.0194	8.8×10^{-7}	0.989	20
FA16:0	TDC	MO[c]	0.00928	-2.0×10^{-9}	0.996	20
FA18:0	TDC	MO[c]	9.28×10^{-5}	2.2×10^{-9}	0.986	20
FA16:0	TDC	1mM MP 1mM LL	0.00109	-3.5×10^{-8}	0.993	20
cholesterol	TDC	(2mM FA18:1, 1 mM MO)	9.91×10^{-6}	1.4×10^{-10}	0.963	20
cholesterol	TDC/TCA	(5mM L, 2mM LL)	1.05×10^{-5}	3.7×10^{-10}	0.931	20

[a] FA/MP concentration ratio was 2/1. MP, monopalmitoylglycerol.
[b] FA/MP concentration ratio was 1/1.
[c] Monooleoylglycerol concentration was 5 mM.

$$[FA]_{mon} = \frac{\text{FA mass}}{K \cdot V_{mic.\,lip.}} \qquad \text{Eq. 1}$$

To illustrate these relationships, the natural logarithm of the slope of the linear regression data has been plotted in **Fig. 5** relative to the number of carbons in the fatty acid or alcohol. This relationship should be linear for a given micellar volume since the dependency of ln K with chain length is linear. Experimental data fit this linear relationship very well as shown by the series of data for taurocholic acid and for taurodeoxycholic acid. The displacement of one line from the other is due to the difference in the micelle lipid volume available for partitioning and this is also revealed by data for expanded micelles containing monooleoylglycerol in addition to taurodeoxycholate. The slope of ln K versus the number of carbons is essentially the same for all systems, indicating that the monomer/micelle partition coefficients are a function only of the fatty acid or alcohol. Slopes are equivalent to incremental free energies of −770, −686, −767, and −843 cal/mol per methylene group for saturated fatty acids in taurodeoxycholate, in taurocholate, unsaturated fatty acids in taurodeoxycholate, and alcohols in taurodeoxycholate, respectively.

TABLE 5. Monomer activities of lipids in constant ratio plateau region of complex micellar solutions

Lipid	Conc.	Bile Salt	Other Components	Monomer Activity
	mM			*M*
FA14:0	2.0	TDC	MP[a]	4.67×10^{-5}
FA16:0	2.0	TDC	MP[a]	2.21×10^{-6}
FA18:0	1.0	TDC	MP[b]	1.20×10^{-7}
FA14:0	2.0	TDC/TCA	MP[a]	5.74×10^{-5}
FA16:0	2.0	TDC/TCA	MP[a]	2.57×10^{-6}
FA18:0	1.0	TDC/TCA	MP[b]	1.91×10^{-7}
FA16:0	2.0	TDC	MP, 1mM; LL 1mM	2.04×10^{-6}
FA18:1	2.0	TDC	MO[c],L,LL,chol.	1.46×10^{-6}
cholesterol	0.5	TDC	FA18:1[c], MO,L,LL	4.44×10^{-9}
cholesterol	0.5	TDC/TCA	FA18:1[c], MO,L,LL	4.45×10^{-9}

[a] FA/MP concentration ratio was 2/1.
[b] FA/MP concentration ratio was 1/1.
[c] Composition at highest concentration was bile salt, 20 mM; FA18:1, 2 mM; cholesterol, 0.5 mM; monooleoylglycerol. 1 mM; lecithin (L), 5 mM; lysolecithin (LL), 2 mM.

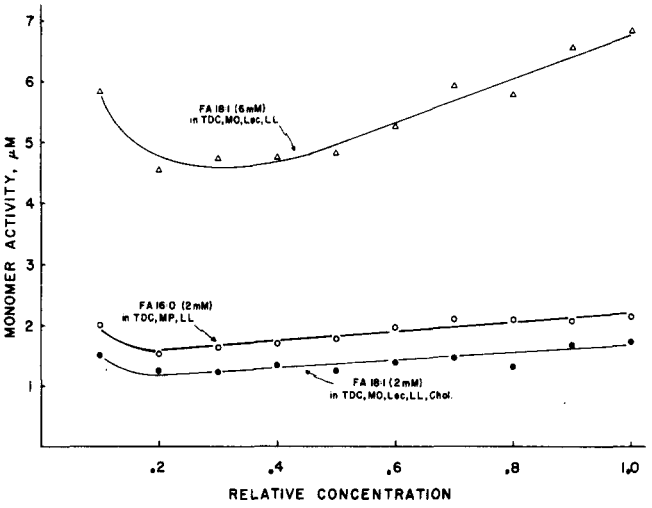

Fig. 4. Monomer activities of fatty acid in mixed micellar solutions with constant ratio of fatty acid to all other constituents. Composition of solutions at relative concentration 1.0 is as follows: △—6 mM FA 18:1, 20 mM TDC, 3 mM MO, 2 mM lecithin, 1 mM lysolecithin; ○—2 mM FA 16:0, 20 mM TDC, 1 mM MP, 1 mM lysolecithin; ●—2 mM FA 18:1, 20 mM TDC, 5 mM MO, 5 mM lecithin, 2 mM lysolecithin, 0.5 mM cholesterol.

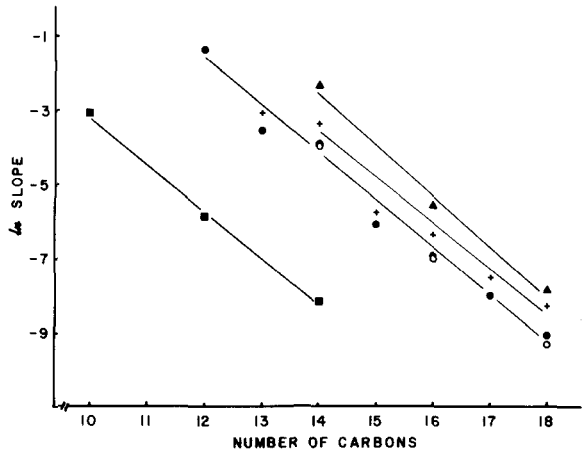

Fig. 5. Interaction of fatty acids and alcohols with bile salt micelles. Slopes of linear regressions from Table 2 have been plotted as the natural logarithm relative to the number of carbons in the compound. Series are as follows: ■—alcohols in 20 mM TDC; ●—saturated FA in 20 mM TDC; ○—saturated FA in 20 mM TDC plus 5 mM MO; +—saturated FA in 20 mM TCA; ▲—monounsaturated FA in 20 mM TDC.

DISCUSSION

Partitioning with polyethylene

The characteristics of fatty acid partitioning between aqueous phase and polyethylene was described in a previous publication (4). In that study the polyethylene partitioning system was shown to compare in all respects with classical partitioning between an aqueous phase and liquid organic solvents. A commonly seen characteristic of partitioning is the deviation by the long chain fatty acid from the anticipated linear relationship of natural logarithm of the partition coefficient vs. the number of carbons in the molecule (4, 10, 11). During an evaluation of the influence of isotopic impurities in the compounds being studied, it was found that at least part of the deviation in previous studies was experimental artifact. In the present study, errors in partition coefficient values were minimized by two procedures. Preloading the polyethylene disc with isotope and allowing reequilibration to take place in fresh aqueous buffer washes away the relatively more water-soluble impurities and gives a more accurate partition coefficient determination, as does purification of the isotope by initial heptane extraction. Utilizing these procedures, partition coefficients of longer chain length fatty acids are substantially higher so the linear region of the partition coefficient relationship has been extended. No alteration in interpretation of data is necessitated by these corrections although the more accurate partition coefficients should be used to calculate monomer activities of fatty acids. In this study the incremental free energy of partitioning of saturated fatty acids with polyethylene is equal to −788 cal/mol per methylene group, and excluding FA 18:0 is −804 cal/mol.

It has been shown that the previous evaluation of the effect of pH on the partition coefficient was influenced by water-soluble impurities as well. Since the maximum partition coefficient, achieved for fatty acid 16:0 as the pH was reduced, was limited by the impurity, the apparent pK_a for FA 16:0 was artifactually high. With the transfer technique, the full range of FA 16:0 partition coefficients is visible in Fig. 2 and the midpoint of the pH relationship is at pH 5, appropriate for the fatty acid series. This is an important observation in light of titration studies (12) performed in bile salt–micellar solutions that suggested that long chain fatty acids had abnormal ionization constants. Although ionization of fatty acid in a micelle may be altered by the surface charge, the free monomer form of fatty acid will be ionized according to a pK_a of about 5.

Studies with fatty alcohols with chain lengths of 12–16 carbons show that these molecules readily partition into polyethylene as predicted, so the line describing the logarithm of the partition coefficient relative to the number of carbons is higher than that of the fatty acids. It should be pointed out, however, that this relationship is still essentially linear and the incremental free energy for this partitioning is nearly identical to that of the fatty acids. The vertical shift of the line is appropriate for the change in molecular structure brought about by substitution of a hydroxyl group for the ionized carboxyl group, although it is not meaningful to calculate an incremental free energy for this shift since most of the difference in polarity is due to ionization of the carboxyl group.

The partition coefficient for cholesterol is significantly higher than any of the other values obtained. The values for alcohols and cholesterol were determined by the transfer technique to minimize errors due to impurities. Thus these values should be very close to the real values. The partition coefficient of cholesterol is important not only for comparison with fatty acids and alcohols but also for the determination of monomer activities as will be shown later.

Lipid–micelle interactions

A previous publication (4) suggested partitioning as a determinant of fatty acid monomer activity in a micellar solution. The present data amplify this suggestion by showing more explicitly the determinants of the monomer activity in micellar solutions. Two types of concentration curves have been utilized for these studies. First, effective volume of micelles was maintained essentially constant by making the concentration of bile salt constant. When the fatty acid mass

in this system is varied, monomer activity is directly proportional to the total fatty acid concentration. The second protocol is to maintain the fatty acid concentration in the micellar lipid volume as constant as possible by making the ratio of fatty acid concentration to bile salt concentration as a constant. As can be seen from Equation 1, K is constant for the single fatty acid and the ratio of fatty acid mass to micellar lipid volume is also constant. Thus, the fatty acid monomer activity should remain constant. That the monomer activity obeys the predicted linear relationship in the first case, and is relatively constant over a broad concentration range in the second case is clear support for the hypothesized partitioning relationship.

No significant alteration of these concentration relationships was anticipated by the addition of monoglycerides or phospholipids, but it was nevertheless important to validate that no specific interactions between individual components was present. This is shown by the similarity of the concentration curves in Fig. 4 to those previously described as well as by the predicted effect of the micellar lipid volume, which will be discussed later. The determined monomer activities are listed in Tables 4 and 5 and, surprisingly, show very little influence of these added components over the concentrations seen in the simpler model solutions.

The partitioning relationship between lipid and micelle means that primarily two factors determine the equilibrium monomer activity in association with the micelle. These two factors are the partition coefficient of the lipid with the micelle and the effective micellar lipid volume. For a given fatty acid mass, the aqueous monomer activity will be inversely proportional to both the partition coefficient and the micellar lipid volume; thus, in this analysis, the slope of the linear regression between monomer activity and total fatty acid concentration was used. When the natural logarithm of this slope is plotted relative to the number of carbons in the lipid, the partition coefficient should be a linear function of chain length, and a change in the micellar volume should be seen as a relative vertical displacement of one line from the other. In addition, relationships for saturated fatty acids, unsaturated fatty acids, alcohols, or other lipids should be vertically displaced from one another due to the influence of their partition coefficients.

In Fig. 5, the dependence of the partition coefficients of saturated fatty acids on the chain length of the molecule may be seen as the predominant linear relationship. The slope of this line is equivalent to an incremental free energy of partitioning of approximately −700 calories per mol per methylene group. The vertical displacement of the three lines representing solutions in taurocholate, taurodeoxycholate, and taurodeoxycholate plus monooleoylglycerol indicate the influence of the micelle lipid volume, which is accessible for partitioning by the fatty acids. The difference in micelle volume explains the increased solubilizing capacity of solutions containing monooleoylglycerol, because the ultimate limit to the total capacity is the solubility of the fatty acid in the aqueous phase, that is, the maximum solubility in the absence of any micelle.

A change of the fatty acid structure to an unsaturated fatty acid shows that the partition coefficient is reduced by the double bond, thus increasing the slope of the linear regression. Despite the somewhat reduced partition coefficient, the maximum amount that may be solubilized in a micelle system is increased because of the greater aqueous solubility of the unsaturated fatty acids. The influence of chain length on monomer activity is the same as for saturated fatty acid as shown by the slope of the ln K vs. number of carbons regression. Alcohols have a similar relationship; however, these values are below the line for saturated fatty acids, indicating larger partition coefficients. Again, the dependence of the partition coefficient on the number of methylene groups is esentially identical to that of the saturated fatty acids. Finally, the monomer activity for cholesterol (Tables 4 and 5) indicates an exceptionally high micelle/monomer partition coefficient resulting in a small monomer activity, which is consistent with the solubility of cholesterol in aqueous solution.

These experiments have shown that equilibrium monomer activities in micellar solutions may be relatively easily determined and that the determinants of these monomer activities may be evaluated in terms of simple theoretical considerations. These studies have immediate application to evaluating the role of monomer activity as a determinant of the intestinal absorption of lipids and of gallstone formation and dissolution.

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