Table IV—Analysis of Iodochlorhydroxyquin Bulk Drug

Lot	Purity, %	
	Peak Area Calc.	Peak Height Calc.
A	98.9, 98.3	99.4, 98.9
B	99.2, 99.9	99.5, 99.1
C	97.4, 98.7	98.9, 99.4
D	98.5, 98.4	98.3, 98.9
E	99.9, 99.4	99.2, 98.7
F	98.3, 98.9	98.2, 99.2

of a standard preparation before and after the addition of III in an amount equivalent to 3% of I. Baseline resolution was achieved and recovery of III was quantitative over the range of 1.5–5.0% expressed on the basis of I. While 5,7-dichloro-8-hydroxyquinoline elutes near the peak for I, its relative response at 254 nm is very low and should not interfere at low concentrations. Furthermore, no changes in assay results for I were observed with samples spiked with as much as 6% of III.

Typical chromatographic tracings for standard and sample preparations are shown in Fig. 3. No interferences were observed from formulation excipients, even though no sample clean-up steps were employed. No late eluting peaks were observed over an 8-hr period.

Placebo samples with added I were analyzed by this method to determine recovery efficiency. The recovery data (Table II) indicate that the procedure is quantitative for I over the range of 22–37 mg/g. This range corresponds roughly to 75–125% of label for the typical 3% cream formulations (30 mg/g). Replicate analysis of a single lot (n = 8) at 30 mg/g gave a 1.1% *RSD*. Results from the analyses of several lots of cream formulations from five manufacturers are shown in Table III. Good agreement with label content was observed in all cases.

A slightly modified procedure was used to analyze samples of bulk drug. Five samples of I (18–43 mg) were analyzed according to this procedure. The weight of I found was plotted against the weight of I added. The resulting linear regression equation had a slope of 1.00, an intercept of −0.02, and a correlation coefficient of 0.999. One lot of bulk drug was analyzed 10 times to determine the precision of the bulk drug assay. Using peak area ratios as the basis for calculation, the average value was 99.12% purity with a 1.1% *RSD*. Using peak height ratios, the mean was 99.21% purity with a 0.6% *RSD*. Six additional lots of iodochlorhydroxyquin bulk drug were analyzed in duplicate. The results of these assays are shown in Table IV.

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Distribution of Bile Salts Between 1-Octanol and Aqueous Buffer

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Abstract □ The distribution of four bile salts: sodium cholate (I), sodium deoxycholate (II), sodium chenodeoxycholate (III), and sodium ursodeoxycholate (IV), between aqueous buffer and 1-octanol has been measured as a function of temperature between 25 and 55° and as a function of bile salt concentration at concentrations <0.1 mole/liter in the aqueous phase. The distribution isotherms obtained have been explained on the basis of reversible association in the aqueous phase. The treatment assumes that the bile acid exists as a monomer in the organic phase, which is verified by vapor pressure osmometry. A graphical method has been employed to estimate the association constants in the aqueous phase for the various equilibria encountered. An aggregation number of four for IV and 12 for I, II, and III has been estimated. From the results, thermodynamic functions associated with the transfer of each of the bile salts from water to octanol and those associated with association processes in the aqueous phase were calculated. These results are consistent with previous findings that the premicellar association of bile salts occurs by hydrophobic interaction. The thermodynamics of transfer of bile salts revealed an unfavorable enthalpic and favorable entropic contribution for all four bile salts. However, for IV, which is an epimer of III, both enthalpic and entropic contributions are reduced, compared to III, suggesting a pronounced effect of stereochemical orientation on hydrophobic interaction.

Keyphrases □ Partition coefficient—distribution of bile salts between 1-octanol and aqueous buffer □ Thermodynamics—distribution of bile salts between 1-octanol and aqueous buffer □ Surfactants—distribution of bile salts between 1-octanol and aqueous buffer

Bile salts are biological detergents which play an important role in the dissolution or dispersion of cholesterol and other lipids in the body (1, 2). The solubility of cholesterol in the sodium cholate–water and the sodium cholate–lecithin–water system was studied (3). It was shown (4) that bile salts were capable of solubilizing a large number of poorly water soluble organic and inorganic compounds. The solubilization of various steroidal hormones in bile salt solutions was investigated (5–7). It was demonstrated (8, 9) that the solubilities and dissolution rates of several unrelated poorly water-soluble drugs were

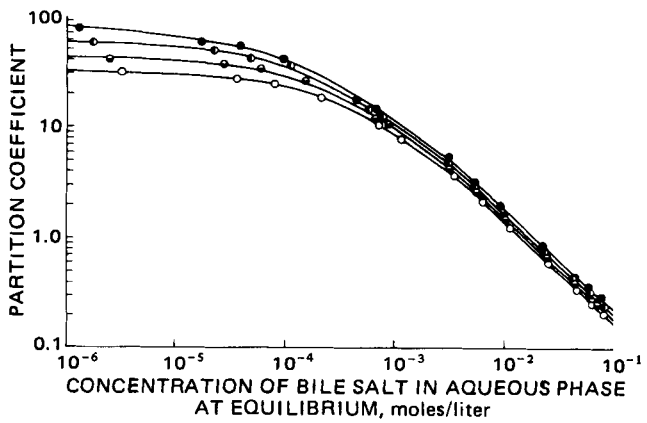

Figure 1—*Distribution isotherms for sodium deoxycholate at 25° (○), 35° (◒), 45° (◐), and 55° (●) between 1-octanol and 0.02 M trometh-amine buffer (pH 8). The solid line is calculated according to Eqs. 15a and b.*

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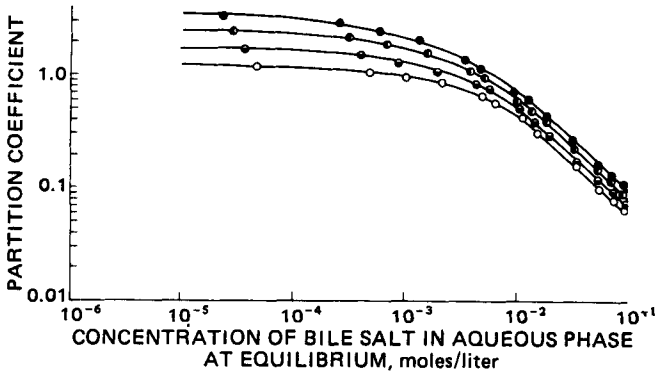


Figure 2—*Distribution isotherms for sodium cholate at 25° (○), 35° (◒), 45° (◐), and 55° (●) between 1-octanol and 0.02 M tromethamine buffer (pH 8). The solid line is calculated according to Eqs. 15a and b.*

increased by the presence of bile salts. Recently, it was reported (10) that the solubility of diazepam increased in bile salt solutions. This ability of bile salts to dissolve water-insoluble material has been attributed to the presence of micelles (2). A knowledge of factors that govern the micellization, therefore, is of great importance in understanding the role of these biological surfactants in the dissolution of poorly water-soluble substances.

As a part of the program to study the aggregation pattern of bile salts in aqueous solution, the partition method offered an interesting tool. The partition method has been used (11–13) to determine the critical micelle concentration of the quaternary ammonium salts. The distribution of four bile salts[1]: sodium cholate (I), sodium deoxycholate (II), sodium deoxychenocholate (III), and sodium ursodeoxycholate (IV) between 1-octanol and aqueous buffer was measured, and the results of these studies have been used to give information on the state of aggregation of bile salts in aqueous solution and the thermodynamics of the transfer process.

EXPERIMENTAL

Materials—The sources of bile acids and their purification are given elsewhere (14). [2,4-^{3}H]Cholic acid in ethanol[2], specific activity 1 mCi/6.7 × 10^{-4} mmoles; [^{3}H,(G)]deoxycholic acid in ethanol[2], specific activity 4 Ci/mmole; and [11,12(H)-^{3}H]chenodeoxycholic acid[3] in toluene–ethanol, specific activity 10.4 Ci/mmole were used without further purification. A sample of [^{14}C(G)]ursodeoxycholic acid[4] was purified by TLC (purity >99%). Benzil[5] was recrystallized several times from methanol, giving a melting point of 95.0 ± 0.5°. 1-Octanol[6] was used as obtained. The water was double deionized. The same liquid scintillation cocktail[7] was used for both aqueous and organic samples.

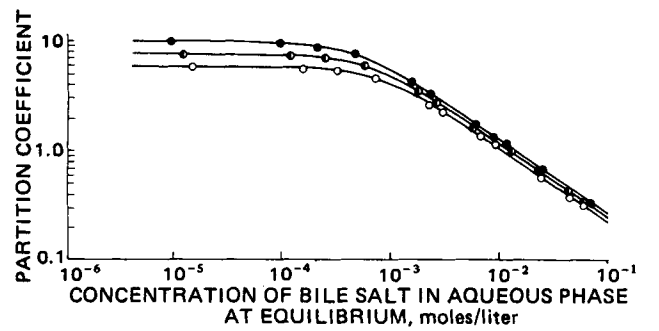


Figure 3—*Distribution isotherms for sodium ursodeoxycholate at 25° (○), 40° (◐), and 55° (●) between 1-octanol and 0.02 M tromethamine buffer (pH 8). The solid line is calculated according to Eqs. 15a and b.*

[1] These compounds exist largely as the free acids in 1-octanol.
[2] New England Nuclear Corp., Boston, Mass.; radiochemical purity was guaranteed to be >98%.
[3] Amersham, Arlington Heights, Ill.; purity >98%.

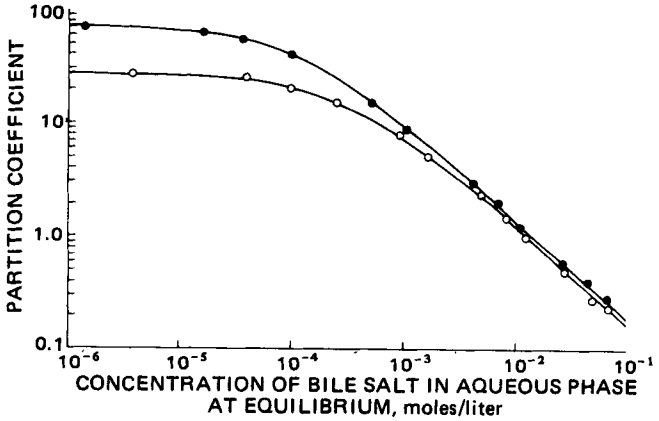


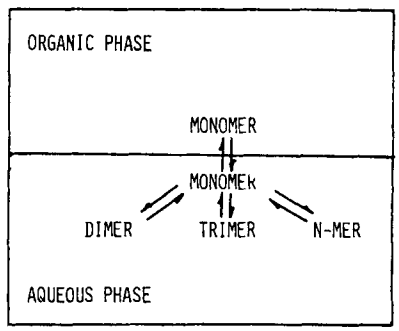
Figure 4—*Distribution isotherms for sodium chenodeoxycholate at 25° (○) and 55° (◐) between 1-octanol and 0.02 M tromethamine buffer (pH 8). The solid line is calculated according to Eqs. 15a and b.*

Partition Coefficient Measurements—The aqueous phase was 0.02 *M* tromethamine buffer (pH 8) and the organic phase was 1-octanol. To minimize volume changes due to mutual solubility, the aqueous and organic phases were presaturated with each other. The aqueous solutions of various concentrations of bile salt were prepared by dilution using a stock solution. ^{3}H-Labeled bile salt was incorporated for analytical determination.

Vials containing 4 ml of octanol and 4 ml of aqueous phase with various amounts of bile salt were shaken for 12 hr in a thermostated water bath. The temperature of the water bath was controlled to ±0.4°. Two samples of 1 ml each from both phases were withdrawn for analysis while keeping the vials thermostated. Scintillation cocktail[7] (10 ml) was added to each sample and mixed thoroughly. Count rates were measured on a liquid scintillation system[8]. The mass balance in the two phases was accounted for within ±2%. The maximum estimated error in the partition coefficient measurements was within ±5%.

Vapor Pressure Osmometry—Vapor pressure osmometry was used to determine the state of association of the bile acids in the organic phase. In this method (15), a steady-state temperature difference between a reference solution and a test solution and the pure solvent was determined. Thermistors were employed as temperature sensors and their electric resistance measured with an osmometer[9]. A solid-state null detector[10] was substituted to improve stability and sensitivity, and the temperature was controlled with a proportional thermistor temperature controller.

HYPOTHETICAL EQUILIBRIA



Scheme I

[4] Received from A. F. Hofmann, University of California, San Francisco, Calif.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Fisher Scientific Co., Pittsburgh, Pa.
[7] Aquasol, New England Nuclear Corp., Boston, Mass.
[8] Model LS 7500, Beckman Instruments, Southfield, Mich.
[9] Wheatstone bridge, Mechrolab Model 301 Vapor Pressure Osmometer.
[10] Keithley, 150B Microvolt Ammeter.

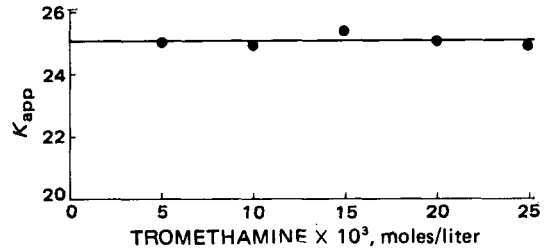


Figure 5—*Effect of concentration of tromethamine buffer on partition coefficient of sodium chenodeoxycholate at 25°, organic phase: 1-octanol, aqueous phase: 0.02 M tromethamine buffer (pH 8).*

RESULTS AND DISCUSSION

The experimental results for the partition of the sodium salts of the bile acids between 0.02 *M* tromethamine buffer (pH 8) and 1-octanol are summarized in Figs. 1–4. Here the partition coefficient, defined as the ratio of the bile salt concentration in the organic phase to the concentration of bile salt in the aqueous phase, is plotted as a function of the equilibrium bile salt concentration (moles/liter) in the aqueous phase. It can be shown that if a solute exhibits ideal behavior in each of two liquids in contact, and in equilibrium, then the partition coefficient is constant, independent of the concentration. It can be seen from Figs. 1–4 that the partition coefficient of all four bile salts studied varies with the concentration. At higher concentrations of bile salt much more solute is found in the aqueous phase than would be expected from a consideration of the partition behavior at lower concentrations.

A sharp break at the critical micelle concentration (CMC) in a plot of C_{org} *versus* C_{aq} was observed (11–13) for quaternary ammonium compounds. A phase separation model for the quaternary ammonium compounds based on the partition behavior was proposed. Unlike quaternary ammonium compounds, bile salts do not exhibit a sharp break in the C_{org} *versus* C_{aq} plot; instead the change in the slope is gradual. This behavior can be explained on the basis of the reversible association of bile salts in the aqueous phase.

Theoretical Considerations—The following treatment of the experimental data can be made by assuming that, although the bile salt present in the aqueous phase is composed of monomers and aggregates of bile salt, only monomeric bile salt is distributed between the two solvents. The hypothetical equilibria involved are presented in Scheme I.

It is conceivable that the monomeric form is partitioned as one or more of three species: free acid; sodium–bile salt ion pair; and tromethamine–bile salt ion pair.

Figure 5 shows the independence of partition coefficient on the tromethamine buffer concentration, indicating that bile salt is not partitioned as tromethamine–bile salt ion pair at pH 8. Figure 6 shows the effect of sodium ion concentration at pH 8 and 9.2. It is evident that at higher pH values, where the bile acid exists mostly in the ionized form, there is a linear dependence of the partition coefficient on the sodium ion concentration; whereas, at pH 8 there is virtually no sodium ion concentration dependence indicating negligible partitioning of bile salt

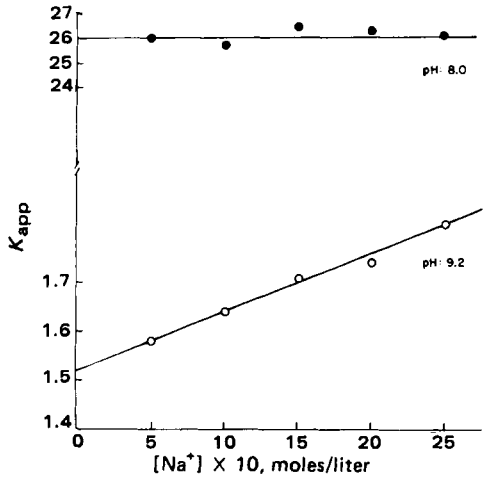


Figure 6—*Effect of sodium ion concentration on partition coefficient of sodium chenodeoxycholate at pH 8.0 (●) and pH 9.2 (○), 25°; organic phase: 1-octanol, aqueous phase: 0.02 M tromethamine buffer.*

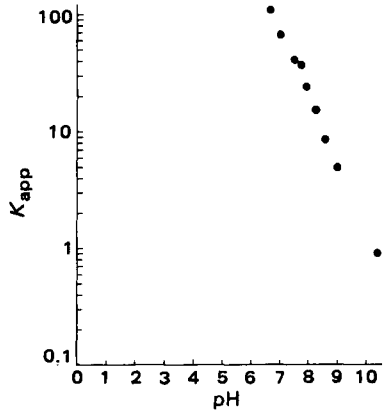


Figure 7—*Effect of hydrogen ion concentration on partition coefficient of sodium chenodeoxycholate at 25°; organic phase: 1-octanol, aqueous phase: 0.02 M tromethamine buffer.*

as the sodium salt ion pair compared to free acid. The marked dependence of partition coefficient on hydrogen ion concentration is seen in Fig. 7.

The concentration in the aqueous phase may be written as[11]:

$$[C]_{aq} = [\text{Monomer}] + 2[\text{Dimer}] + 3[\text{Trimer}] + \ldots \quad \text{(Eq. 1)}$$

where, $[C]_{aq}$ represents the total equilibrium molar concentration of bile salt in the aqueous phase.

Assuming that the association equilibria follow the mass law, the equilibria in the aqueous layer can be described in terms of association constants. Thus:

$$2\ \text{Monomer} \overset{K'_2}{\rightleftharpoons} \text{Dimer}$$

$$3\ \text{Monomer} \overset{K'_3}{\rightleftharpoons} \text{Trimer}$$

Therefore, Eq. 1 may be written as:

$$[C]_{aq} = [M]_{aq} + 2K'_2[M]^2_{aq} + 3K'_3[M]^3_{aq} + \ldots \quad \text{(Eq. 2)}$$

where, $[M]_{aq}$ is the monomer concentration in the aqueous phase. The concentration in the organic phase can be described by the following relationship:

$$[C]_{org} = K\frac{[H^+]}{[H^+] + K_a}[M]_{aq} \quad \text{(Eq. 3)}$$

where K is the intrinsic or true partition coefficient of the acid form, and K_a is the dissociation constant of the bile acid. Let:

$$K\frac{[H^+]}{[H^+] + K_a} = K^0_{app} \quad \text{(Eq. 4)}$$

where K^0_{app} is the apparent partition coefficient at a given pH in the infinitely dilute solution. Then Eq. 3 may be written as:

$$[C]_{org} = K^0_{app}[M]_{aq} \quad \text{(Eq. 5)}$$

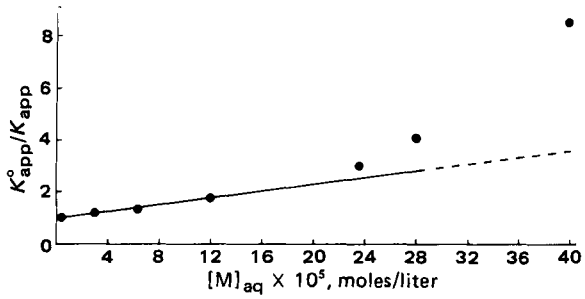


Figure 8—*A plot testing the possible occurrence of dimers in aqueous solutions of sodium deoxycholate at 25° according to Eq. 7.*

[11] The different forms identified as monomer, dimer, trimer, *etc.*, may consist of the free acids, charged anions, or mixtures of the two.

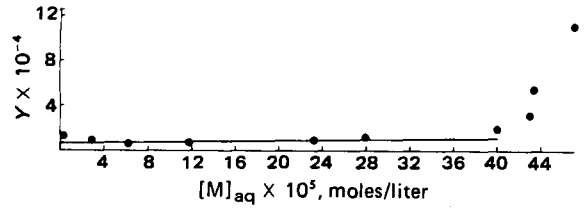


Figure 9—*A plot testing the possible occurrence of dimers and trimers in aqueous solutions of sodium deoxycholate at 25° according to Eq. 10.*

from Eqs. 2 and 5 the following can be written:

$$\frac{[C]_{aq}}{[C]_{org}} = \frac{1 + 2K'_2[M]_{aq} + 3K'_3[M]^2_{aq} + \ldots}{K^0_{app}}$$

or:

$$\frac{K^0_{app}}{K_{app}} = 1 + 2K'_2[M]_{aq} + 3K'_3[M]^2_{aq} + \ldots \quad \text{(Eq. 6)}$$

where $K_{app} = [C]_{org}/[C]_{aq}$ is the partition coefficient at the same given pH but at some finite concentration.

Proposed Testing of Assumptions—Since reversible associations of the type discussed are markedly dependent on the concentration of associating solute, it would be expected that in relatively dilute solutions, dimerization would be the primary equilibrium.

As the concentration of bile acid is increased, the tendency to form higher aggregates would be expected to be more pronounced. With this consideration, a simple test of the proposed relationship can be made.

If, at lower concentration of bile salt, the formation of dimers is the primary equilibrium, then to a first approximation, Eq. 6 can be reduced to:

$$K^0_{app}/K_{app} = 1 + 2K'_2[M]_{aq} \quad \text{(Eq. 7)}$$

From Eqs. 3 and 4, $[M]_{aq}$ can be obtained:

$$\frac{[C]_{org}}{K^0_{app}} = [M]_{aq} \quad \text{(Eq. 8)}$$

Figure 8 summarizes the results obtained when Eq. 7 was tested. The straight line relationship expressed by Eq. 7 is found to exist at lower concentration of II. The curvature apparent at higher concentration is due to the formation of higher polymeric species. The same trend is found for all four bile salts studied.

The proposition that higher polymeric species are found at higher concentration can be tested in a similar manner, for if the bile acid found in the aqueous phase is primarily present as monomer, dimer, and trimer, then Eq. 6 can be approximated by:

$$K^0_{app}/K_{app} = 1 + 2K'_2[M]_{aq} + 3K'_3[M]^2_{aq} \quad \text{(Eq. 9)}$$

or:

$$\frac{(K^0_{app}/K_{app}) - 1}{[M]_{aq}} = Y = 2K'_2 + 3K'_3[M]_{aq} \quad \text{(Eq. 10)}$$

The linearity expressed by this equation is tested in Fig. 9. Here, Y is plotted as a function of monomeric bile salt concentration in the aqueous phase. The expected intercept $2K'_2$ agrees within 5% with the value obtained from Fig. 8. A slope of 2.17×10^7 with correlation coefficient of 0.86 is obtained for this relationship.

By assuming that dimerization and tetramerization are the important equilibria, Eq. 6 may be reduced to:

$$K^0_{app}/K_{app} = 1 + 2K'_2[M]_{aq} + 4K'_4[M]^3_{aq} \quad \text{(Eq. 11)}$$

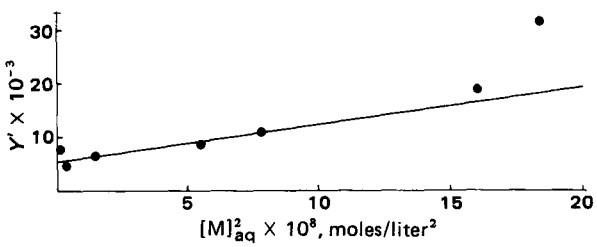


Figure 10—*A plot testing the possible occurrence of dimers and tetramers in aqueous solutions of sodium deoxycholate at 25° according to Eq. 12.*

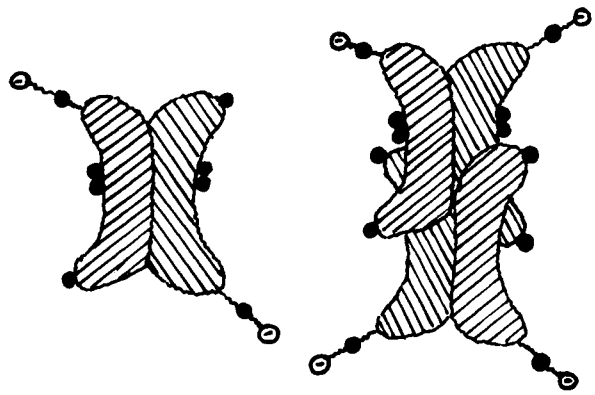


Figure 11—*Longitudual sections of dimers and tetramers; (●) OH groups; (⊖) negatively charged ionic group of bile salt.*

or:

$$\frac{(K^0_{app}/K_{app}) - 1}{[M]_{aq}} = 2K'_2 + 4K'_4[M]^2_{aq} \quad \text{(Eq. 12)}$$

Figure 10 illustrates a plot of this relationship. The theoretically expected straight line relationship is found to exist, with a correlation coefficient of 0.97. The linear relationship for 1-2-3 and 1-2-4 model extends over to the same concentration range with a somewhat better correlation for the latter one, however. Therefore, a monomer–dimer–tetramer species was considered to be prominent in this region. However, the existence of the trimer cannot be ruled out. A preponderance of tetramer over trimer is expected since a pair of dimers can come together with back-to-back hydrophobic interaction to form a tetramer with wide separation of charges (Fig. 11) (16), whereas in the trimer, charges would be expected to be closer, thus rendering it relatively less stable.

A curvature at higher concentration once again indicates that aggregates greater than tetramer are very likely. If it is assumed that, at higher concentration only monomer, dimer, tetramer, and 'n'mer are predominant species, then Eq. 2 reduces to:

$$[C]_{aq} = [M]_{aq} + 2K'_2[M]^2_{aq} + 4K'_4[M]^4_{aq} + nK'_n[M]^n_{aq} \quad \text{(Eq. 13)}$$

Rearranging and taking logs:

$$\log Y'' = \log nK'_n + n \log [M]_{aq} \quad \text{(Eq. 14)}$$

where:

$$Y'' = [C]_{aq} - [M]_{aq} - 2K'_2[M]^2_{aq} - 4K'_4[M]^4_{aq}$$

Thus, a plot of log Y'' *versus* log $[M]_{aq}$ should yield a straight line with slope equal to n, the aggregation number. The linearity of this double log

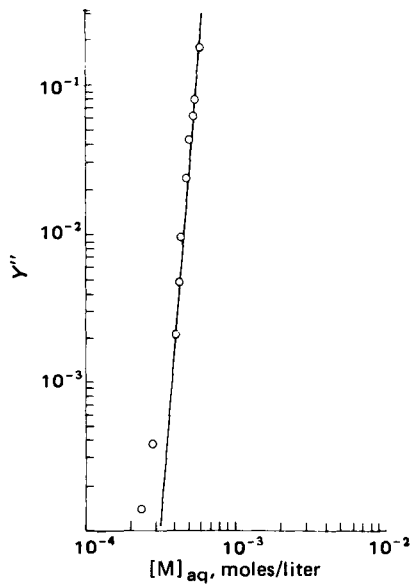


Figure 12—*A plot testing the possible occurrence of dimers, tetramers, and n-mers in aqueous solutions of sodium deoxycholate at 25° according to Eq. 14.*

Table I—Association Constants

Bile Salt (Aggregate Number)	Temp	log K'_2, M/liter^{-1}	log K'_4, M/liter^{-3}	log K'_{12}, M/liter^{-11}
Cholate	25°	2.20	4.00	25.70
(12)	35°	2.39	4.46	26.61
	45°	2.57	4.80	27.48
	55°	2.74	5.30	28.26
Deoxycholate	25°	3.48	10.30	36.78
(12)	35°	3.60	10.54	37.92
	45°	3.72	10.78	39.04
	55°	3.88	11.04	40.08
Ursodeoxycholate	25°	2.35	8.15	—
(4)	40°	2.51	8.45	—
	55°	2.65	8.78	—
Chenodeoxycholate	25°	3.43	10.30	36.78
(12)	55°	3.85	11.60	40.78

plot is tested in Fig. 12. The aggregation number and all the association constants are summarized in Table I. The aggregation number of 12 for II obtained in this study is in close agreement with the value 13 reported previously (17). However, the association constant value, log K'_{13} = 27.4, differs from log K'_{12} = 36.78, obtained in the present study. This discrepancy may be due to the octanol dissolved in the aqueous phase in this study.

It is recognized that the distribution of water and octanol in the equilibrium phases might change with the concentration of bile salt, in which case K^0_{app} would no longer be constant. In this treatment, it is assumed for the sake of simplicity that the composition of the equilibrium phases is unchanged by the presence of bile salt. This assumption may have a small effect on the values of the association constants derived in the treatment.

The partition isotherms were generated using the following:

$$K_{app} = [C]_{org}/[C]_{aq} \qquad \text{(Eq. 15}a\text{)}$$

and:

$$[C]_{aq} = \left(\frac{[C]_{org}}{K^0_{app}}\right) + 2K'_2\left(\frac{[C]_{org}}{K^0_{app}}\right)^2 + 4K'_4\left(\frac{[C]_{org}}{K^0_{app}}\right)^4 + 12K'_{12}\left(\frac{[C]_{org}}{K^0_{app}}\right)^{12} \qquad \text{(Eq. 15}b\text{)}$$

and association constants from Table I. Figures 1–4 show that there is good agreement between the calculated and experimental values.

On the basis of the preceding discussion, it is also possible to calculate the distribution of bile salt/acid in its various forms in aqueous solution. The results of such calculations are illustrated in Fig. 13. Here, the concentration of bile salt as a particular species has been plotted as a function of total concentration of bile salt in solution. The concentration of bile salt as monomers and dimers reaches a plateau, whereas dodecamer is seen to increase as the concentration of total bile salt in the system increases.

All the association constants, K'_2, K'_4, K'_{12}, follow a Van't Hoff type relationship which is seen in Fig. 14. From the slope of these lines it is possible to estimate ΔH^0 for association. The results of such calculations are listed in Table II. These results are in agreement with the expectations that hydrophobic interactions lead to a positive enthalpy change (18).

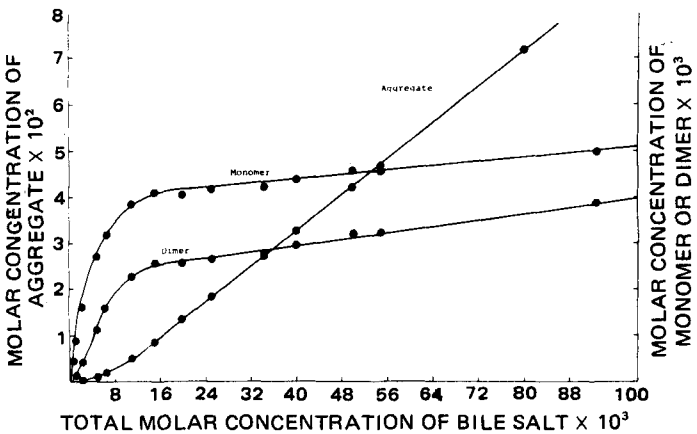


Figure 13—*A plot showing the distribution of sodium cholate among its various forms in aqueous solution at 25°.*

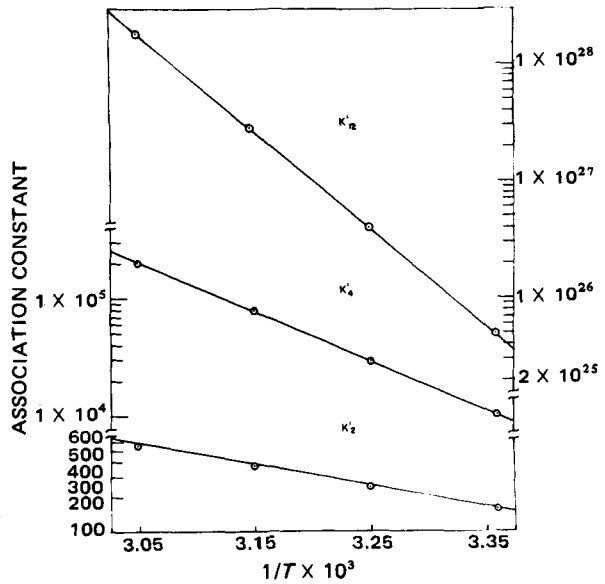


Figure 14—*A plot showing Van't Hoff type relationship for the association constants of sodium cholate.*

Figure 15 shows plots of log K^0_{app} *versus* $1/T$ for all the bile salts studied. If the activity coefficients in the two phases are assumed to be unity in very dilute solution, then from the slope of the plot log K^0_{app} *versus* $1/T$, estimates of ΔH^0 can be obtained. Also by using:

$$-\Delta G^0 = RT \ln K^0_{app}$$

and

$$\Delta G^0 = H^0 - T\Delta S^0$$

all of the thermodynamic functions for the transfer process can be obtained. Table III lists these thermodynamic transfer functions.

The values for the enthalpy of transfer of bile salt from the aqueous to the organic phase are all positive (unfavorable) (Table III). Positive values for ΔH^0 of transfer of hydrocarbon and aliphatic alcohols are also obtained (18). Positive values of ΔH^0 for this process are expected on the basis of the hydrophobic properties of these solutes. In aqueous solution the water molecules in the vicinity of these nonpolar molecules or moieties are more strongly hydrogen bonded so that a region of higher local order exists than in pure water. In partitioning out of the aqueous phase, the normal hydrogen bonded structure resumes, accompanied by a decrease in the amount of hydrogen bonding and a less ordered state. These processes are accompanied by an increase in enthalpy (the energy required to break hydrogen bonds) and an increase in entropy as expected for a loss in order. The data show that the entropy gain is greater for the more hydrophobic molecules. The dihydroxy bile salts, II and III, show a larger entropy change than I: the more polar tri-hydroxy bile salt. The data for IV demonstrate that it is not only the number of hydroxyl groups on the molecule that determine its hydrophobic character, but the orientation is also important. In IV, the —OH groups are no longer all in the α configuration, the —OH group in the 7 position is oriented toward the back of the molecule so that there is no longer a clearly hydrophobic side to the structure. As a consequence, even though it is a dihydroxy bile salt, IV shows a smaller positive change in entropy for the aqueous–organic transfer process than either of the other dihydroxy salts (II and III) or even the trihydroxy salt (I). Even for IV, however, hydrophobic forces appear to predominate since positive values of ΔH^0 and ΔS^0 are still obtained for the transfer process.

One of the assumptions in the foregoing model was that the bile acids do not associate in octanol. This was verified by using vapor pressure osmometry.

Table II—ΔH^0 for Association Process [a]

Bile salt	ΔH^0_{dimer}	$\Delta H^0_{tetramer}$	$\Delta H^0_{dodecamer}$
Cholate	4.0	4.8	3.2
Deoxycholate	3.0	2.8	4.0
Ursodeoxycholate	2.2	2.3	—
Chenodeoxycholate	3.1	4.8	4.9

[a] Kilocalories per mole of bile salt in the aggregate.

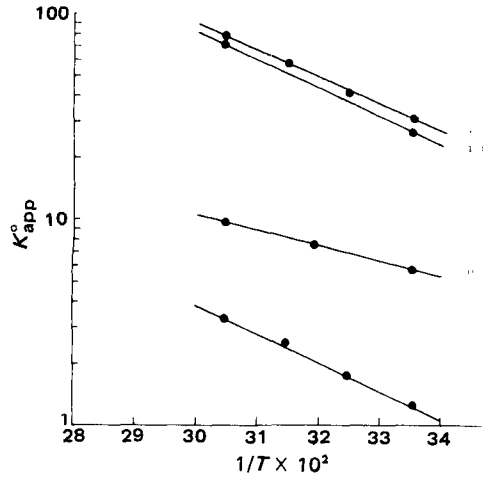


Figure 15—*A plot showing Van't Hoff type relationship for the* K^0_{app}, *sodium deoxycholate (II), sodium cholate (I), sodium ursodeoxycholate (IV), and sodium chenodeoxycholate (III).*

The osmolality of the test solution ($m_x\phi_x$), the reference solution ($m_r\phi_r$) and the measured resistance ratio ($\Delta R_x/\Delta R_r$) are related by:

$$\frac{m_x\phi_x}{m_r\phi_r} = \frac{\Delta R_x}{\Delta R_r}$$

where m is the molality, ϕ is the osmotic coefficient, ΔR is the change in resistance, and x and r refer to test and reference solutions, respectively.

If a nonassociating solute such as benzil is used as a reference, then the deviation of the ratio (ϕ_x/ϕ_r) from unity is related to the extent of association.

Table IV shows the ratio (ϕ_x/ϕ_r) for cholic and deoxycholic acids at 25°. It is seen that the ratio is ~0.8 and above in all cases, suggesting that under the conditions of the partition studies, the bile acid exists mainly as the monomer in the organic phase. It is likely that there is some association in the organic phase. However, for simplicity of calculation, this was assumed to be zero. This approximation may have an effect on the magnitude of the association constants.

Bile Acid–Octanol Complex—Figure 16 shows the effect of varying the ratio of octanol to isooctane in the organic phase, and it can be seen that octanol complexes with bile acid. A quantitative treatment may be obtained by considering that each mole of bile acid $(BA)_{aq}$ complexes with n moles of octanol (X) to give a complex $(BA\ X_n)_{org}$. Thus:

$$(BA)_{aq} + n\ X \overset{K_{eq}}{\rightleftharpoons} (BA\ X_n)_{org} \qquad \text{(Eq. 16)}$$

Accordingly:

$$K_{eq} = \frac{[(BA\ X_n)_{org}]}{[(BA)_{aq}][X]^n} \qquad \text{(Eq. 17)}$$

From Eqs. 15*a* and 17 it follows:

$$\log K_{app} = \log K_{eq} + n \log [M] \qquad \text{(Eq. 18)}$$

Table III—Thermodynamic Transfer Functions

Bile Salt	K^0_{app} 25°	ΔG^0 25°	ΔH^0 Kcal/mole	$T\Delta S^0$
Deoxycholate	32.0	−2.05	+6.0	8.05
Chenodeoxycholate	27.0	−1.95	+6.1	8.05
Ursodeoxycholate	5.8	−1.04	+3.4	4.44
Cholate	1.2	−0.11	+6.3	6.41

Table IV—ϕ_x/ϕ_r Ratio at 25°

Concentration of Bile Acid in Octanol	$\phi_c{}^a/\phi_b{}^b$	$\phi_{dc}{}^c/\phi_b$
0.005 *M*	0.83	0.87
0.010 *M*	0.81	0.83
0.020 *M*	0.76	0.80

[a] cholic acid. [b] benzil. [c] deoxycholic acid.

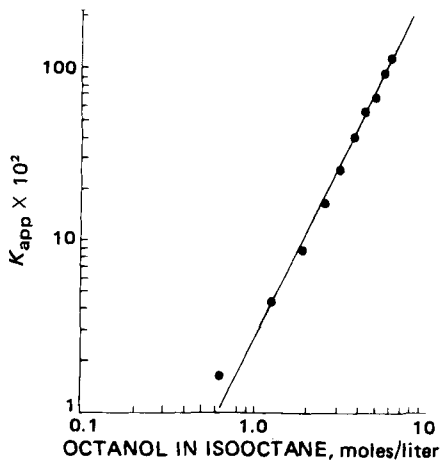


Figure 16—*A plot testing the possible complexation of sodium cholate with octanol according to Eq. 18.*

The linearity predicted in this double log plot is realized in Fig. 16. Binding numbers of 2, 1.7, and 1.8 are found for I, II, and III, respectively, suggesting that each mole of bile acid is solvated or complexed by 2 moles of octanol.

Since the solvation is the same in the organic phase for all bile acids studied, the differences in the free energy of partitioning results entirely from the differences in their interactions with water. The solvation of bile acids by octanol provides further support for the finding that bile acids are monomeric in the organic phase. There is evidence which suggests (19, 20) that in nonhydrogen bonding solvents, bile acids and their esters tend to form dimers and higher aggregates. Studies to determine the nature of the aggregates in nonhydrogen bonding solvents are presented in the following report (21).

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Association of Deoxycholic Acid in Organic Solvents

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Abstract □ The distribution of deoxycholic acid (I) between aqueous buffer and an organic phase consisting of isooctane–1-octanol (70:30, v/v) (System A) or isooctane–chloroform (80:20, v/v) (System B) was studied. The distribution isotherms suggested that I associates strongly in the organic Systems A and B unlike in pure 1-octanol. Therefore, a previous model, describing distribution of bile salts between 1-octanol and aqueous buffer, was modified to include association of I in the organic phases to describe distribution behavior. The treatment suggested that I exists as monomer and dimer in System A with a dimerization constant of 820 M^{-1}. A model consisting of monomer–tetramer–hexamer in the organic phase best describes the data for System B. The data support the view that association in the organic phase is due to hydrogen bonding between bile acid molecules.

Keyphrases □ Deoxycholic acid—association in organic solvents □ Bile salts—association of deoxycholic acid in organic solvents □ Partition coefficient—association of deoxycholic acid in organic solvents

In the preceding paper (1), the distribution behavior of bile salts between aqueous buffer and 1-octanol was reported. By vapor pressure osmometry data, it was shown that bile acids exist primarily as monomers in 1-octanol[1]. A recent paper (2) reported strong association of bile acid esters in nonaqueous solvents such as carbon tetrachloride and chloroform using vapor pressure osmometry. The association in carbon tetrachloride is much stronger than in chloroform. The relatively low association in chloroform was attributed to the hydrogen bonding ability of chloroform. This probably also explains the monomeric state of bile acids in 1-octanol. If this were true, then modifying the hydrogen bonding solvent (*e.g.*, 1-octanol or chloroform) by addition of a nonhydrogen bonding nonpolar solvent like isooctane should result in an increased association of bile acid in such organic systems. In the present report the state of association of deoxycholic acid (I) is examined in two such modified solvent systems: isooctane–1-octanol (70:30) (System A) and isooctane–chloroform (80:20) (System B) using the distribution behavior of I between the organic phase and aqueous buffer. Partition equilibria were used to study the association of solutes in the organic phase on the basis of the previously determined association pattern in the aqueous phase.

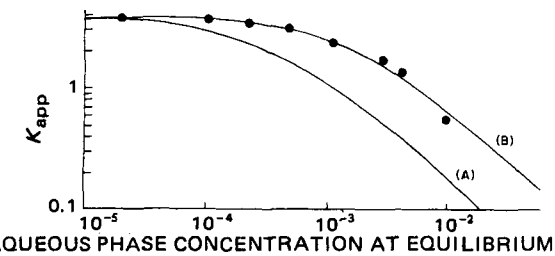

Figure 1—*Distribution isotherm for sodium deoxycholate at 25°. Curve A is based on the model in Scheme IA. Curve B is calculated on the basis of the model in Scheme IB. Key: (●) experimental data.*

[1] It was found that bile salts exist primarily in the acidic form in 1-octanol and in the anionic form in the aqueous phase under the experimental conditions of this investigation.

EXPERIMENTAL

Materials—The purity of sodium deoxycholate[2] (>99%) was confirmed by TLC and titration with perchloric acid in glacial acetic acid [^{3}H,(G)]deoxycholic acid in ethanol, specific activity 4 Ci/mmole[3], radiochemical purity 98%; 1-octanol[4]; 2,2,4-trimethylpentane (isooctane)[5] were used as obtained. The same scintillation cocktail[6] was used for both aqueous and organic samples.

Experimental details of partition coefficient measurements are given in the preceding paper (1).

RESULTS AND DISCUSSION

The experimental results obtained for the partition of I between 0.02 *M* tromethamine buffer (pH 8) and two mixed organic phases are summarized in Figs. 1 and 2. Here, the partition coefficient defined as the ratio of the bile salt (and acid) concentration in the organic phase to the concentration of bile salt (and acid) in the aqueous phase, is plotted as a function of the equilibrium total bile salt and acid concentration (moles/liter) in the aqueous phase.

The nature of the distribution isotherm obtained for System A is similar to one obtained for 1-octanol in the previous study (1). The distribution of I between 1-octanol and aqueous buffer was explained on the basis of reversible association of I in the aqueous phase and no association in the organic phase as shown in Scheme IA. In the present case, since

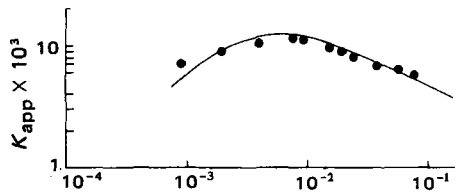

Figure 2—*Distribution isotherm for sodium deoxycholate at 25°. Organic phase isooctane–octanol (80:20, v/v). Aqueous phase 0.02 M tromethamine buffer (pH 8.0). Solid line represents distribution isotherm calculated on the basis of the 1-4-6 association model in organic phase. Key: (●) experimental data.*

[2] Calbiochem, LaJolla, Calif.
[3] New England Nuclear, Boston, Mass.
[4] Fisher Scientific Co., Pittsburgh, Pa.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Aquasol, New England Nuclear, Boston, Mass.

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