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Studies on Bile Acid Salt Solutions

II. The Solubility of Cholic Acid in Sodium Cholate Solutions and that of Desoxycholic Acid in Sodium Desoxycholate Solutions

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The solubility of cholic acid in sodium cholate solutions and that of desoxycholic acid in sodium desoxycholate solutions has been determined by potentiometric titration of solutions of their sodium salts and by measuring the changes in the refractive indexes when the solutions have been saturated with the acids. The former method was found suitable for the determination of the solubilities in dilute solutions where no association has yet occurred; the latter method was more appropriate for solutions containing micellar substance. The solubility product exponent of cholic acid at 20°C was found to be $pK_L = 8.72$ and that of desoxycholic acid $pK_L = 9.15$.

From bile salt solutions saturated with the corresponding acids, the acids separate in pure form. No acid salts were detected in the precipitates. The activity of undissociated cholic acid in sodium cholate solutions saturated with the acid was found to be $a_{HA} = 1.8 \times 10^{-4}$ M. Knowing this value, it has been possible to evaluate the activity of the cholate anion from the pH values of the solutions. The results obtained show that no association takes place in the cholate solutions below a cholate concentration of about 0.01 M. From this concentration up to about 0.03 M, a partial association is observed, but above the latter concentration all the added cholate ions become associated. The activity of undissociated desoxycholic acid in solutions of its sodium salt saturated with the acid is 1.05×10^{-4} M. The evaluation of the activity of the desoxycholate anion in these solutions showed that no association occurs up to a desoxycholate concentration of 0.005—0.006 M. Above the latter concentration a partial association takes place which seems to become complete at least when the desoxycholate concentration exceeds about 0.01 M.

As soon as association sets in, the bile salt solutions begin to dissolve the respective bile acids. The solubilizing power increases stepwise which shows that the micelles have different properties in different concentration ranges. The concentrations delimiting these ranges in solutions saturated with bile acids have been determined.

When an attempt is made to elucidate the structures of aqueous bile salt solutions it is desirable to have exact information about the solubilities of the respective acids in these salt solutions.

As far as the solubilities of bile acids in water are concerned, it is known that the unconjugated acids are sparingly soluble and that their solubilities increase with the number of hydroxyl groups in the molecule. Gillert [1] has reported the solubility of desoxycholic acid as 0.24 g/l and that of cholic acid as 0.28 g/l at 15°C. Also the bile acids conjugated with glycine have relatively low solubilities, whereas the taurine conjugate of desoxycholic acid and especially that of cholic acid are very soluble in water.

The bile acids have a tendency to remain in the colloidal state in solution. Josephson [2] found that when strong acid is slowly added to aqueous bile salt solutions a colloidal solution often results from which the liberated bile acid begins to crystallize out only after some time, frequently after several days. The excess of mineral acid that can be added to solutions of the same concentration of different bile salts before the crystallization begins is observed to vary with the nature of the bile acid. Also foreign salts present in the solution influence the separation of the bile acid. Ekwall and coworkers have confirmed some of these observations [3-5] and have also reported preliminary values for the solubilities of cholic and desoxycholic acids in their respective salt solutions. They have further shown that the solubility increases with the degree of micelle formation.

We have now performed a more detailed investigation of the solubility of cholic acid in sodium cholate solutions and that of desoxycholic acid in sodium desoxycholate solutions. The solubilities have been calculated from the results of potentiometric titrations of the bile salt solutions with hydrochloric acid or have been determined directly by measuring the changes in refractive indices of the saturated solutions. In addition the solubility of cholic acid in pure water has been evaluated.

The bile salts were prepared from the same acids as were employed in the experiments in Part I of this series [5].

THE SOLUBILITY OF CHOLIC ACID IN WATER

The solubility of cholic acid was determined as follows. An excess of cholic acid was added to a volume of boiling conductance water exceeding 2 litres. The resulting mixture was then allowed to stand in a closed flask at 20°C and was shaken from time to time during a period of one week. The undissolved acid was then separated from the solution by filtration and two one-litre aliquots of the solution were evaporated separately to dryness in platinum dishes. The solubilities were found to be 91.9 and 91.4 mg of cholic acid per litre.

Another determination was carried out without heating. The water and excess of cholic acid were mixed in a rocking machine at about 20°C for one week and the mixture was then allowed to stand at 20°C for a further three days. The solubility value was 92.2 mg of cholic acid per litre.

The mean of these three solubility values is 91.8 mg per litre. This value is appreciably lower than the value reported by Gillert.

POTENTIOMETRIC DETERMINATION OF THE SOLUBILITIES OF BILE ACIDS IN BILE SALT SOLUTION

Back and Steenberg [6] have shown that it is possible to determine the solubility of a sparingly soluble weak acid and hence the corresponding solubility product K_L by a potentiometric titration of the solution of the salt of the acid with a strong mineral acid up to a point slightly past that where the acid begins to precipitate. The solubility is calculated directly from the amount of strong acid added and the pH of the solution at the precipitation point where the following equation is in force.

$$L_{HA} = c'_S + c'_{OH^-} - c'_{H_3O^+} \tag{1}$$

In the solution saturated with the weak acid (HA):

$$a_{H_3O^+} \cdot a_{A^-} = K_A \cdot a_{HA} = \text{constant} = K_L \tag{2}$$

$$a_{A^-} = (c_{tot} - c_S - c_{OH^-} + c_{H_3O^+})\, f_{A^-} \tag{3}$$

$$pK_L = pH - \log(c_{tot} - c_S - c_{OH^-} + c_{H_3O^+}) + \frac{0.5\sqrt{\mu}}{1+\sqrt{\mu}} \tag{4}$$

In the equations c_{tot} is the initial salt concentration recalculated with respect to the changed volume of the titration solution, c_S the concentration of free weak acid liberated by adding hydrochloric acid. The primed concentrations, c', refer to those at the precipitation point.

$$L_{HA} = \frac{K_L}{K_A} \cdot \frac{1}{f_{HA}} \simeq \frac{K_L}{K_A} \tag{5}$$

Whereas the calculations based on eqn. 1 should always yield correct values, this is the case for the calculations based on eqns. 4 and 5 only when the activity of the anion of the weak acid can be computed from eqn. 3. In the case of association colloids, *e.g.* bile acid salts, the latter is true only when no micelle formation has taken place. After micelles have been formed eqn. 3 must be replaced by eqn. 3 a.

$$a_{A^-} = (c_{tot} - c_S - c_{OH^-} + c_{H_3O^+} - c_{mic}) \cdot f_{A^-} \tag{3 a}$$

where c_{mic} is the concentration of acid anions bound in the micelles.

If the micelle formation is disregarded, the values obtained for K_L will be too high. As long as the extent of micelle formation is unknown the above method of evaluating the solubility product will not be valid when the salt concentration is so high that micelles are present in the solution. Furthermore, as soon as micelles are formed, the bile acid will be solubilized in the solution; eqn. 5, however, gives only the concentration of unsolubilized bile acid and can hence be applied only when solubilization does not occur. Thus, when micelle formation and solubilization does occur, it is necessary to calculate the solubility with the aid of eqn. 1.

The theory presumes, of course, that the precipitate that separates from the solution when hydrochloric acid is added is actually the weak acid itself and not an acid salt. In order to confirm this in the case of bile acid salts, the precipitates formed on titration of these salts were examined under the

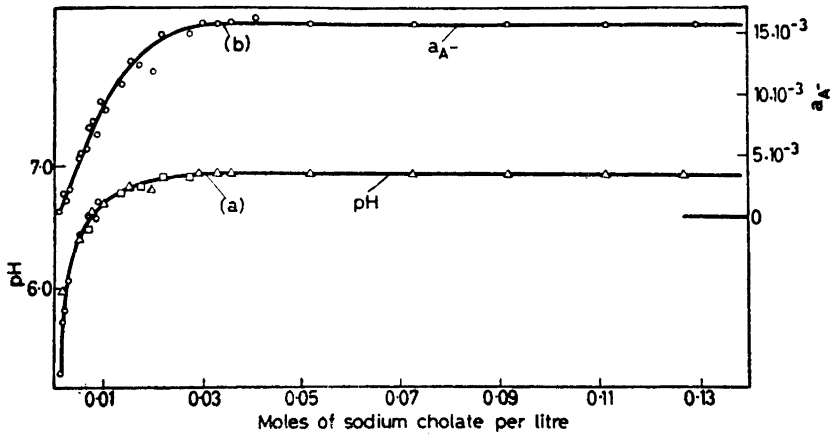

Fig. 1. pH values (curve a) and cholate anion activities, a_{A^-},(curve b) in sodium cholate solutions saturated with cholic acid at 20°C.

microscope, their melting points were determined, and, in a few doubtful cases, an analysis was carried out. All these investigations revealed that the precipitate forming during the titrations consisted only of the bile acid in question.

In titrations of bile salt solutions the applicability and reliability of the potentiometric method are limited by experimental difficulties. It may happen that supersaturated solutions of bile acids in solutions of their salts are readily formed. Sometimes the supersaturation continues also after the appearance of an opalescence or turbidity in the solution or even in solutions containing macroscopically dispersed bile acid precipitate. The calculation of the solubility and solubility product based on pH values for such supersaturated solutions will naturally lead to too high values.

In some cases it may be possible to break off the supersaturation whereupon the pH of the solution will rise abruptly to an appreciably higher value than previously. Probably this higher pH value is the theoretical value for the bile salt solution saturated with bile acid at the point in question in the titration. When no micelle formation has occurred in the solution, the experimental data for this equilibrium state can be used to calculate the solubility product from eqn. 4. It will, however, be less appropriate to use data for such a titration curve to calculate the solubility from eqn. 1 because there will be difficulties in determining at which point in the titration precipitation should have occurred if no supersaturation had taken place. (It is, of course, possible to extend the horizontal part of the curve after the abrupt rise in pH to the left to intersect the descending part of the curve (see Ref.[5], Fig. 1) and consider that part of the curve which is below the horizontal line as being due to supersaturation and to take the point of intersection to represent the saturation point. The solubility value obtained in this way will,

Table 1. The solubility and solubility product of cholic acid in sodium cholate solutions at 20°C.

Sodium cholate mole/l	pH of solution saturated with cholic acid	$pK_{I'}$	Mean $pK_{I'}$	$L_{HA} = \frac{K_{I'}}{K_A}$
0.00049	5.34	8.67		
0.00137	5.73	8.61		
0.00293	6.06	8.62		
0.00480	6.40	8.73		
0.00509	6.43	8.77		
0.00674	6.47	8.69		
0.00682	6.43	8.64		
0.00688	6.47	8.67	8.72 S.D. 0.07	$1.82 \cdot 10^{-4}$ mole/l 77.6 mg/l undissociated acid
0.00690	6.60	8.80		
0.00750	6.62	8.79		
0.00847	6.57	8.69		
0.00862	6.71	8.82		
0.0100	6.69	8.74		
0.0133	6.77	8.77		
0.0151	6.84	8.72		

however, be in error and the magnitude of the error will increase with the solubility.)

When calculating the solubilities of the bile acids we have used in addition to the experimental data previously collected in our earlier determination of the dissociation constants of cholic and desoxycholic acids data obtained in new titrations which were continued until precipitation occurred. In the titration of dilute desoxycholate solutions it was in many cases possible to avoid supersaturation and the data could be used directly in the calculation

Table 2. The solubility and solubility product of desoxycholic acid in sodium desoxycholate solutions at 20°C.

Sodium desoxy-cholate mole/l	pH of solutions saturated with desoxy-cholic acid	$pK_{I'}$	Mean $pK_{I'}$	$L_{HA} = \frac{K_{I'}}{K_A}$	Solubility at the precipitation point	
					L_{HA}	Mean L_{HA}
0.000849	6.04	9.12			$9.5 \cdot 10^{-5}$	
0.000850	6.04	9.13			$9.4 \cdot 10^{-5}$	
0.00180	6.40	9.17			$9.25 \cdot 10^{-5}$	
0.00181	6.40	9.16			$8.60 \cdot 10^{-5}$	
0.00273	6.54	9.13	9.15	$1.05 \cdot 10^{-4}$ mole/l	$10.5 \cdot 10^{-5}$	$1.05 \cdot 10^{-4}$ mole/l
0.00274	6.58	9.17	SD. 0.025		$10.4 \cdot 10^{-5}$	
0.00275	6.58	9.19		43.1 mg/l	$10.3 \cdot 10^{-5}$	43.1 mg/l
0.00367	6.65	9.12		undissociated	$10.8 \cdot 10^{-5}$	undissociated
0.00368	6.70	9.16		acid	$11.1 \cdot 10^{-5}$	acid
0.00369	6.70	9.16			$10.7 \cdot 10^{-5}$	

of the solubility and solubility product. More concentrated desoxycholate solutions containing micelles often yielded highly viscous, supersaturated systems in which the supersaturation could not be broken off and thus the solubility could not be evaluated. In the titration of cholate solutions a supersaturated state usually resulted which, however, could be broken off; in many of these cases the data could be used to calculate the solubility product.

The data obtained are presented in Tables 1 and 2. In the calculation of solubility products only values for solutions in which no micelle formation had occurred were employed. The solubility product exponent (pK_L) for cholic acid in 0.001—0.015 M sodium cholate solutions was found to be 8.72, S.D. 0.07 and that for desoxycholic acid in 0.001—0.004 M sodium descoxycholate solutions 9.15, S.D. 0.025. Using eqn. 5 and the previously determined values of the dissociation constants (cholic acid, $pK_A = 4.98$; desoxycholic acid, $pK_A = 5.17$) the following solubility values were calculated: 77.6 mg of undissociated cholic acid per litre and 43.1 mg of undissociated desoxycholic acid per litre. By direct measurement of pH at the point where precipitation begins and substitution of this value in eqn. 1, the solubility of desoxycholic acid in the above concentration range was found to be 43.1 mg per litre.

The titration method leads to higher solubility values at higher salt concentrations than the above- mentioned. These values are, however, not reliable. Nevertheless, they show that micelle formation and solubilization take plase in these solutions.

DISCUSSION

The solubility products of the bile acids. The value of the solubility of cholic acid in pure water, 91.9 mg/l or 2.15×10^{-4} mole/l, refers to both undissociated and dissociated cholic acid. When the dissociation of the acid is taken into account, the value $K_L = 1.84 \times 10^{-9}$ ($pK_L = 8.73$) is obtained for the solubility product of the acid at 20°C and the value 1.78×10^{-4} M for the activity, a_{HA}, of the undissociated acid in its saturated aqueous solution. When calculating these values it has been assumed that the dissociation equilibrium is not influenced by dissolved carbon dioxide.

This value of the solubility product is in excellent agreement with the mean value, $pK_L = 8.72$, obtained by the potentiometric method. The latter value gives for the activity of undissociated cholic acid in its saturated aqueous solution the value 1.82×10^{-4} M.

The solubility product K_L of desoxycholic acid at 20°C computed from data obtained by potentiometric titration is 7.08×10^{-10} ($pK_L = 9.15$) from which the activity a_{HA} of the undissociated acid in its saturated aqueous solutions is found to be 1.05×10^{-4} M. The direct determination of the solubility from the titrimetric data yielded the same value.

The structure of bile salt solutions saturated with bile acid. Despite the occurrence of micelle formation and solubilization the activity of cholic acid in all sodium cholate solutions saturated with cholic acid should remain constant and equal to 1.82×10^{-4} M. It should therefore be possible to calculate the activity of the cholate ion in these solutions from the data provided by the potentiometric titrations.

Table 1 contains pH values for 0.005—0.015 M cholate solutions at the points in the titration where a precipitate began to form and after equilibrium was attained. As already mentioned, above this point in the titration the titration curve runs parallel to the horizontal axis for some distance. The same is the case also in more concentrated cholate concentrations. Curve a in Fig. 1 shows how the pH at this point and on the horizontal part of the curve varies with the cholate concentration (the abscissa gives the actual cholate concentration at the point in question). In dilute solutions the pH increases with cholate concentration, remains practically constant at the value 6.94 in the 0.03—0.175 M concentration range, but tends to decrease slightly at still higher concentrations (0.3 M, pH 6.88; 0.4 M, pH 6.82; 0.8 M, pH 6.70). The constant value of the pH implies that the activity of the cholate anion is also constant.

The activity of the cholate ion in cholate solutions saturated with cholic acid has been computed from the pH values with the aid of eqns. 2 or 2 a.

$$\log a_{A^-} = \mathrm{pH} - \mathrm{p}K_{\mathrm{I}} \qquad (2\,\mathrm{a})$$

The values of the activity are plotted in curve b of Fig. 1. In the concentration range where the pH is constant the cholate ion activity is 1.57×10^{-2} M. This shows that the major proportion of the cholate ions are bound in micelles at concentrations above 0.03 M. At low concentrations up to approximately 0.01 M cholate the cholate ion activity increases linearly with the cholate concentration, and the activity values are only slightly lower than the concentration values. Between 0.01 and 0.031 M the cholate ion activity increases more slowly as the concentration approaches the upper value. These data show that all the cholate is present in form of single ions up to a concentration of about 0.01 M, but that above this concentration the ions begin to associate more and more until in solutions above 0.03 M in cholate all the added cholate is completely bound to micelles.

The data can also be used to calculate the concentration of unassociated cholate ions and the concentration of the cholate ions bound in the micelles. These calculations, which can be carried out in different ways, are subject to the uncertainties involved when calculating the activity coefficients of ions in solutions where micelle formation has taken place. Our procedure has been as follows. When data obtained for micellar solutions are substituted in eqn. 4 values $\mathrm{p}K_{\mathrm{I,\,exp}}$ are obtained, which differ from the correct values $\mathrm{p}K_{\mathrm{I,\,theor}}$. The correct value $\mathrm{p}K_{\mathrm{I,\,theor}}$ is in these cases obtained only when eqn. 4 is modified by taking into account eqn. 3 a. By combining both these modifications of eqn. 4, eqn. 6 is obtained which permits the calculation of the concentration of micellar substance in the solution.

$$c_{\mathrm{mic}} = (c_{\mathrm{tot}} - c_{\mathrm{S}} - c_{\mathrm{OH^-}} + c_{\mathrm{H_3O^+}}) \left(1 - \frac{K_{\mathrm{I,\,theor}}}{K_{\mathrm{I,\,exp}}} \right) \qquad (6)$$

The results of the calculation are given by the points of curve a in Fig. 2. Micelle formation is seen to begin at the 0.015 M cholate concentration and increases rapidly in extent. (The values in the region immediately above 0.015 M are erratic and it may be that they are slightly too high.)

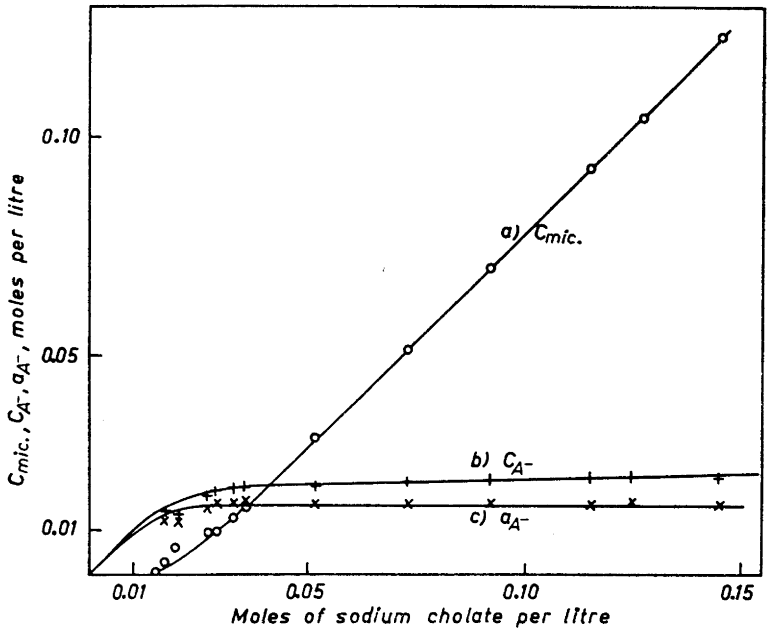

Fig. 2. Micelle formation in sodium cholate solutions saturated with cholic acid at 20°C. The concentration of micellar cholate (a) and of molecular cholate (b), and cholate anion activity (c). The points marked o, × and + have been calculated with the aid of eqn. 6, the curves are based on the activity values given in Fig. 1 b.

On the basis of these values the concentrations of unassociated cholate ions (the points on curve b) and the activity of free cholate ions (the points on curve c) have been calculated (the continuons curves a, b and c have been drawn using values calculated from the activity values in Fig. 1 b). Also these calculations show that the micelle formation begins above 0.01 M (concentration limit 1) is incomplete between 0.01 and 0.03 M, and that all added cholate forms micelles above the latter concentration (concentration limit 2). They give the constant value 1.62×10^{-2} M for the cholate ion activity in the concentration range above about 0.03 M; this value is only slightly higher value than that previously mentioned.

In sodium desoxycholate solutions saturated with desoxycholic acid, also in solutions containing micelles, the activity, a_{HA}, of desoxycholic acid is $1.05 \times 10^{4-}$ M. Using the pK_I value 9.15 we have, as above, calculated the activities of the desoxycholate anion from the pH values of desoxycholate solutions titrated to the precipitation point. It was possible, however, to carry out these calculations with satisfactory accuracy only up to a desoxycholate concentration of about 0.005 M. (At higher desoxycholate concentrations the solutions, as mentioned above, become supersaturated with respect to the acid during the titration and it was not possible to break off the supersaturation; hence exact pH values could not be determined for the saturated solutions.)

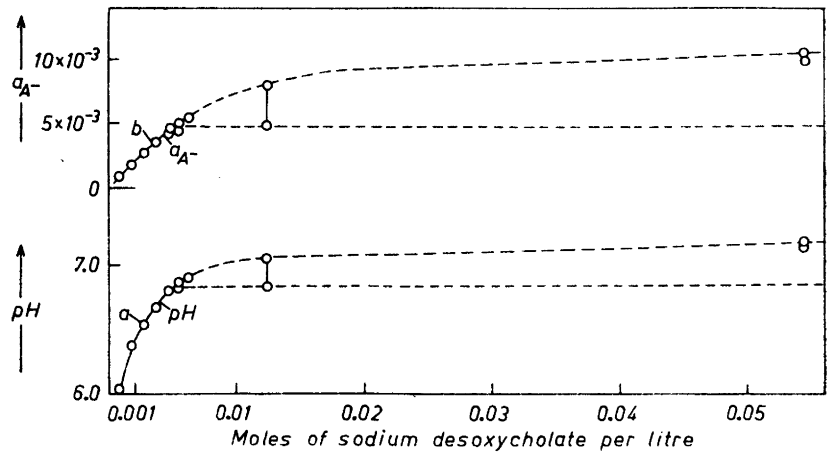

Fig. 3. pH values (curve a) and desoxycholate anion activities, a_{A^-}, (curve b) in sodium desoxycholate solutions saturated with desoxycholic acid at 20°C. In the concentration range above 0.005 M desoxycholate only approximate values lying between the two dashed curves were obtained.

The measured pH values and the respective anion activities are given by curves a and b in Fig. 3. It is possible to state with certainty only that the activity of the desoxycholate anion increases linearly up to a desoxycholate concentration of 0.005 M and that the activities are only slightly lower than the concentration values. This shows that no association takes place up to the mentioned concentration. Above this concentration (concentration limit 1) the activity of the desoxycholate anion seems to remain practically constant or to increase slowly with increasing concentration. In the 0.075 M desoxycholate solution saturated with desoxycholic acid, the anion activity does not seem to be higher than 1.1×10^{-2} M. Association hence begins at the 0.005 M desoxycholate concentration and increases rapidly to such an extent that, at least in solutions above 0.01 M, all the added desoxycholate forms micelles.

DIRECT DETERMINATION OF THE SOLUBILITIES IN BILE SALT SOLUTIONS

In order to obtain reliable information on the solubilities of bile acids in solutions of their salts at concentrations where micelles are formed we have performed a series of direct solubility measurements. In these experiments known weights of cholic and desoxycholic acids were added to measured volumes of solutions of the respective sodium salts in ampoules. After the ampoules had been sealed by fusing, they were placed in a thermostat at 40°C, in some cases at 20°C, and shaken for 5—7 days, during which time either the acid dissolved or the solution became saturated with the acid. The changes in refractive index of the solutions caused by the dissolved acid were measured with a Zeiss — Löwe liquid interferometer. As soon as the solution

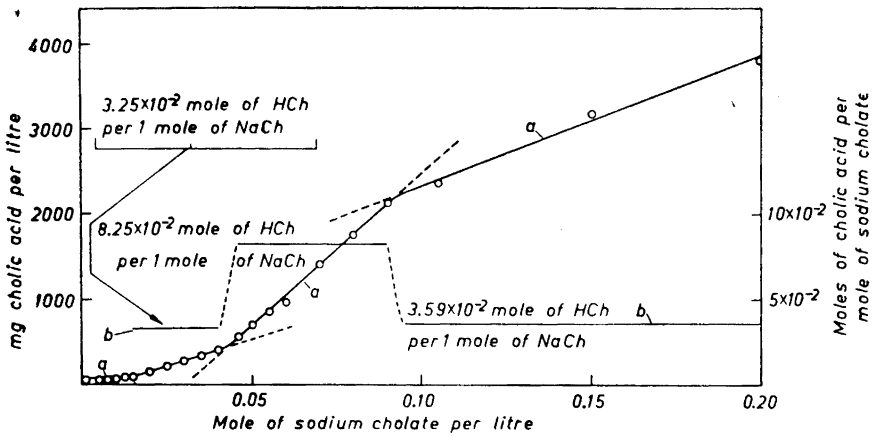

Fig. 4. The solubility of cholic acid in sodium cholate solutions at 40°C determined with the aid of liquid interferometer measurements. The solubility in mg of cholic acid per litre of sodium cholate solution (curve a). The solubility in moles of cholic acid per mole of sodium cholate in the micellar state, *i. e.* the saturation capacity of the cholate micelles (curve b).

became saturated with the acid, no further change in the refractive index was observed. At low bile salt concentrations, where very little acid dissolved, the solubility values were erratic (the maximum deviations from the mean were as large as ± 10 %. With increasing salt concentration and acid solubility more reproducible values (maximum deviations from the mean less

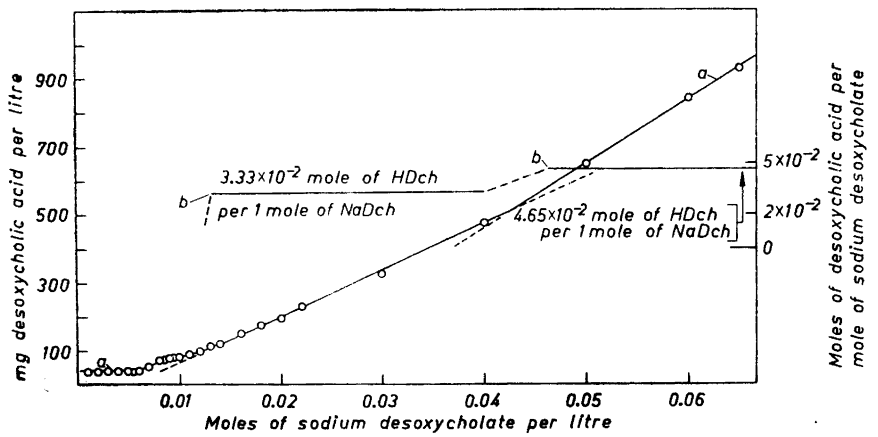

Fig. 5. The solubility of desoxycholic acid in sodium desoxycholate solutions at 40°C determined by the aid of liquid interferometer measurements. The solubility in mg of desoxycholic acid per liter of sodium desoxycholate solution (curve a). The solubility in moles of desoxycholic acid per mole of sodium desoxycholate in the micellar state, *i. e.* the saturation capacity of the desoxycholate micelles (curve b).

than ± 2 %) were recorded. The direct method is thus less suitable for measurement of the solubility in very dilute bile salt solutions than the titration method. In this concentration range the direct method gave somewhat lower solubility values (although they were measured at 40°C) than the titration method. (The values of the solubility products of the bile acids calculated from these values were: for cholic acid in 0.003—0.009 M sodium cholate solutions $pK_L = 8.90$ and in 0.010—0.015 M solutions $pK_L = 8.74—8.69$; for desoxycholic acid in 0.001—0.0055 M sodium desoxycholate solutions $pK_L = 9.23—9.24$.) In bile salt solutions where micelle formation and solubilization occurs the direct method yields more reliable solubility values than the titration method. The results obtained are shown in Figs. 4 and 5, curves a.

SOLUBILIZATION OF BILE ACIDS AND THE PROPERTIES OF THE MICELLES

In sodium cholate solutions (Fig. 4 a) up to 0.015 M in cholate (concentration limit 1) no solubilization of cholic acid could be detected; the solubility in this range is practically constant. Above limit 1 the solubility increases linearly until the cholate concentration rises to 0.04—0.045 M (concentration limit 2). Above this second limit the solubility also increases linearly but more rapidly. Above a cholate concentration of 0.09—0.10 M (limit 3) the increase of the solubility continues but less rapidly than between limits 2 and 3. The ability of cholate to solubilize cholic acid thus varies in different concentration ranges.

It should be noted that the solutions in question were not supersaturated but equilibrium systems in which the cholic acid is solubilized by cholate micelles; thus, they are thermodynamically stable, colloidal systems. Supersaturated solutions may contain appreciably larger amounts of dissolved acid than solutions in which only solubilization occurs. In the potentiometric titrations we have, for instance, encountered clear 0.012 M sodium cholate solutions containing up to 800 mg of cholic acid in one litre (no solubilized acid) and clear 0.03 M cholate solutions containing up to 3 000 mg of the acid per litre (only about 230 mg of cholic acid is solubilized). Also in these cases the cholic acid is colloidally dissolved, but the colloid formed is for the most part unstable and the excess acid will separate from the system sooner or later.

From the solubility curve we have computed the saturation capacity of the micellar cholate, *e.g.* the solubilizing power of the cholate micelles expressed in moles of cholic acid per mole of micellar cholate.

Above concentration limit 2, where all added cholate forms micelles, the slope of the solubility curve directly gives the saturation capacity per mole of micellar substance. Between limits 1 and 2, where all added cholate does not form micelles, we have computed the real saturation capacity of the micellar substance using our previous calculated values of the content of cholate anions in micellar form. The curve b in Fig. 4 shows that the power of the cholate micelles to solubilize cholic acid changes abruptly at the concentration limits but is constant in the concentration ranges between the limits. This implies

that the association leads to the formation of micelles with different properties in the different ranges and that the concentration limits represent cholate concentrations where new types of micelles begin to form in sodium cholate solutions saturated with cholic acid.

In sodium desoxycholate solutions up to about 0.006 M (limit 1) no solubilization of desoxycholic acid could be observed (Fig. 5, curve a); the solubility is practically constant in this range. Above limit 1 the solubility of desoxycholic acid increases rapidly at first, but then somewhat more slowly. Above the 0.010—0.012 M concentration (limit 2) the solubility again increases rapidly, but now practically linearly, up to 0.04—0.05 M concentration (limit 3) above which the solubility curve rises even more rapidly with increasing desoxycholate concentration.

We have shown above that all added desoxycholate forms micelles from the concentration 0.01 M upwards. Above this concentration, the slope of the solubility curve thus gives the saturation capacity of the micellar desoxycholate for desoxycholic acid (curve b in Fig. 5). (Between limits 1 and 2 it is impossible to evaluate the saturation capacity of the micellar substance with certainty because the extent of the micelle formation is not known.) Our calculations show that the solubilization power of the desoxycholate micelles changes at the concentration limits 2 and 3 but remains constant between these limits and in the range above limit 3. Hence micelles with different properties and of different types exist in different concentration ranges also in desoxycholate solutions saturated with desoxycholic acid.

We have previously come to similar conclusions with respect to association and micelle properties in cholate and desoxycholate solutions not saturated with the respective bile acids. The bile acids seem, however, to shift the limits slightly to lower concentrations.

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