4063 (Mar. 4, 1965) (applied Mar. 11, 1961); through *Chem. Abstr.*, **62**, 16119a (1965).

(4) K. Ogiu, H. Fujimura, and S. Mita, Japanese pat. 23,087 (Dec. 23, 1961) (applied Sept. 9, 1958); through *Chem. Abstr.*, **58**, 13843d,e (1963).

(5) E. C. Dodds, W. Lawson, and P. C. Williams, *Nature*, **151**, 614 (1943).

(6) E. C. Dodds, W. Lawson, and P. C. Williams, *Proc. Roy. Soc. (London)*, **B 132**, 119 (1944).

(7) M. Nakazaki, *Chem. Ind. (London)*, **1962**, 1577; through *Chem. Abstr.*, **58**, 1379b (1963).

(8) M. Nakazaki, I. Mita, and N. Toshioka, *Bull. Chem. Soc. Jpn.*, **36**, 161 (1963).

(9) "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 6th ed., C. O. Wilson, O. Gisvold, and R. F. Doerge, Eds., Lippincott, Philadelphia, Pa., 1971, p. 555.

(10) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, London, England, 1970, pp. 753, 754.

(11) N. Mann, W. Back, and E. Mutschler, *Ger. Arch. Pharm.*, **306**, 625 (1973).

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Light-Scattering Studies on Bile Acid Salts II: Pattern of Self-Association of Sodium Deoxycholate, Sodium Taurodeoxycholate, and Sodium Glycodeoxycholate in Aqueous Electrolyte Solutions

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Abstract □ The pattern of self-association of the bile salts sodium deoxycholate, sodium glycodeoxycholate, and sodium taurodeoxycholate was investigated in aqueous electrolyte solutions by the light-scattering technique. The turbidity of the bile salt solutions was obtained over the concentration range of 0–20 mg/ml at 25°. These data were analyzed according to a monomer–micellar equilibrium model and a stepwise association model. Comparison of the light-scattering data with these models suggests that the monomer–micellar model may be inappropriate. Analysis of the data according to the stepwise association model suggests that the dihydroxy bile salts associate to form dimers, trimers, and tetramers in addition to a larger aggregate which varies in size depending on the degree of conjugation of the bile salt.

Keyphrases □ Bile salts, dihydroxy—self-association in aqueous electrolyte solutions, turbidimetric study □ Self-association—various dihydroxy bile salts in aqueous electrolyte solutions, turbidimetric study □ Turbidimetry—study of self-association of various dihydroxy bile salts in aqueous electrolyte solutions

The self-association of the dihydroxy bile salts has been studied extensively (1–5). Most often, the association pattern has been interpreted in terms of a monomer–micellar equilibrium model. This model is analogous to that utilized for flexible chain surfactants and involves the existence of a critical micelle concentration (CMC) below which surfactant aggregation is assumed to be negligible (6). Above the CMC, it is assumed that nearly all added surfactant in excess of the CMC associates to form micelles. Appreciable concentrations of intermediate-sized aggregates are presumed absent, and the polydispersity of the micelles is assumed to be small (7).

Previously (8), it was noted that the conditions necessary for the highly cooperative transition observed for flexible chain surfactants may not be met by all molecular structures. For example, rigid, planar aromatic structures such as dyes and purine and pyrimidine bases seem to associate by continuous stepwise self-association in which small aggregates such as dimers, trimers, and tetramers dominate (8). Furthermore, nonrigid aromatic structures such as the antihistamines self-associate in aqueous solution. Attwood and Udeala (9–11) showed that either pattern of association may dominate depending on the specific molecular structure.

The bile salts possess rigid but nonplanar molecular structures. However, unlike the dyes and the purine and pyrimidine bases, which are approximately symmetrical with respect to hydrophobicity on both sides of the ring, the bile salts consist of a rigid, nonplanar, alicyclic ring system in which one face is hydrophobic while the remaining face is hydrophilic because of the presence of either two or three hydroxyl groups. It was argued previously (12) that the association pattern of trihydroxy bile salts is best represented by a model that assumes the existence of some small aggregates such as dimers and trimers in addition to a higher aggregate.

The present paper extends previous work on the self-association of the bile salts to the dihydroxy bile salts including sodium deoxycholate, sodium taurodeoxycholate, and sodium glycodeoxycholate. The variation of the light scattered as a function of bile salt concentration was obtained in 0.15 *M* sodium halides. These data were analyzed in terms of a monomer–micellar equilibrium model and a stepwise association model in which it is assumed that appreciable concentrations of small multimers coexist with large aggregates.

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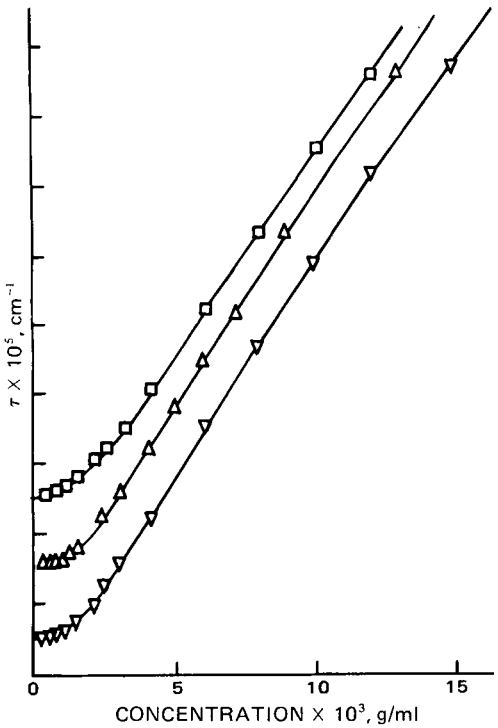

Figure 1—*Turbidity, τ, versus concentration of sodium deoxycholate in 0.15 M sodium fluoride (□), sodium chloride (△), and sodium bromide (▽). Each division on the ordinate represents 10 × 10⁻⁵ cm⁻¹. The intercepts representing the turbidity of the solvent are: sodium bromide, 5.32 × 10⁻⁵ cm⁻¹; sodium chloride, 5.07 × 10⁻⁵ cm⁻¹; and sodium fluoride, 4.85 × 10⁻⁵ cm⁻¹.*

EXPERIMENTAL

Materials—Deoxycholic acid[1] was recrystallized according to the method of Sobotka and Goldberg (13). Glycodeoxycholic acid and taurodeoxycholic acid were synthesized from deoxycholic acid according to the method of Lack *et al.* (14). All bile acids were shown to be free of other bile acids by TLC, using a 100-μg spot (15). In addition, the conjugated bile salts were found to be free of unconjugated glycine and taurine by a similar procedure, using a ninhydrin reagent for development of the chromatogram.

The sodium salts were obtained by titration of aqueous solutions of the acids to pH 10.0 ± 0.2, followed by flash evaporation of the solvent and drying to constant weight in a vacuum oven at 40 ± 2°. Sodium chloride[1] and sodium bromide[1] were reagent grade chemicals and were used as received. Sodium fluoride[1] was also reagent grade but was recrystallized twice from water before use. All water was deionized[2], distilled twice from an all-glass still, and stored in glass containers.

Apparatus—Light-scattering measurements were made with a light-scattering photometer[3] using the small dissymmetry cell[4]. The cell was aligned in the instrument as previously described (12). Refractive index measurements were obtained on a differential refractometer[5]. Temperature control of the refractometer was maintained at 25 ± 0.2° by a circulating water bath[6]. The pH measurements were made with a digital pH meter[7].

Methods—Solutions for the light-scattering measurements were made either by dilution of stock solutions or by direct weighing of the required quantities. All dilutions were made from stock solutions adjusted to pH 10.0 ± 0.1. Particulate-free solutions were obtained by filtration through porous membrane filters[8] (0.22-μm pore diameter) using the technique described previously (12). Membranes were used as received. Various control experiments indicated that detergent abstraction from these membranes was not a problem.

Only solutions that showed a dissymmetry of less than 1.03 were used for the light-scattering measurements. All light-scattering measurements were made at room temperature (24 ± 1°) at 436 nm. Depolarization and fluorescence of the scattered light were negligible.

[1] J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] Model LD-2a, Corning Glass Works, Corning, N.Y.
[3] Brice-Phoenix model BP-2000, Virtus Co., Gardiner, N.Y.
[4] Catalog No. D105, Virtus Co., Gardiner, N.Y.
[5] Model BP-2000-V, Virtus Co., Gardiner, N.Y.
[6] Lauda model K2-R, Brinkmann Instruments, Westbury, N.Y.
[7] Model 110, Corning Glass Works, Corning, N.Y.
[8] Type GS, Millipore Filter Corp., Bedford, Mass.

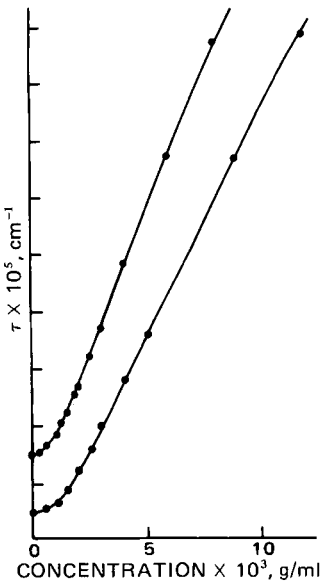

Figure 2—*Turbidity, τ, versus concentration for sodium taurodeoxycholate (upper curve) and sodium glycodeoxycholate (lower curve) in 0.15 M NaCl. The units of the ordinate and the intercepts are as in Fig. 1.*

RESULTS

The turbidity, τ, in centimeters^{-1}, as a function of the sodium deoxycholate concentration in several 0.15 *M* sodium halides is shown in Fig. 1. Figure 2 is a similar plot for sodium taurodeoxycholate and sodium glycodeoxycholate in 0.15 *M* NaCl. The refractive increments, *dn/dc*, for the various bile salts are given in Table I, together with a comparison of the values obtained by previous investigators.

DISCUSSION

To obtain an accurate representation of the self-association pattern of any compound, it is necessary to obtain information about the variation of the monomer concentration, *b*, as a function of the total concentration of the associating species (8, 16). For light scattering, Steiner (16) developed a method for obtaining this information. The weight average molecular weight of all species, M_w, is related to the weight concentration, *c*, in grams per milliliter by:

$$M_0/M_w = 1 + d \ln x/d \ln c \qquad \text{(Eq. 1)}$$

where M_0 is the molecular weight and *x* is the weight fraction of the monomer. The value of M_w can be obtained from the experimental turbidity curve by:

$$\frac{Hc}{\Delta\tau} = \frac{1}{M_w} + 2Bc \qquad \text{(Eq. 2)}$$

where $\Delta\tau$ is the excess turbidity of the solution over the solvent at concentration *c*, *B* is the second virial coefficient, and *H* is an optical constant defined by:

$$H = \frac{32\pi^3 n_0 (dn/dc)^2}{3N_A \lambda_0^4} \qquad \text{(Eq. 3)}$$

Table I—Refractive Index Increments for the Dihydroxy Bile Salts

Solvent	*dn/dc* at 436 nm, ml/g: Sodium Deoxycholate	Sodium Taurodeoxycholate	Sodium Glycodeoxycholate
0.15 *M* sodium fluoride	0.193[a]	—	—
0.15 *M* sodium chloride	0.193	0.171[a] (0.172)[b]	0.193[a] (0.2)[b]
0.15 *M* sodium bromide	0.193	—	—

[a] Present study. [b] Reference 2.

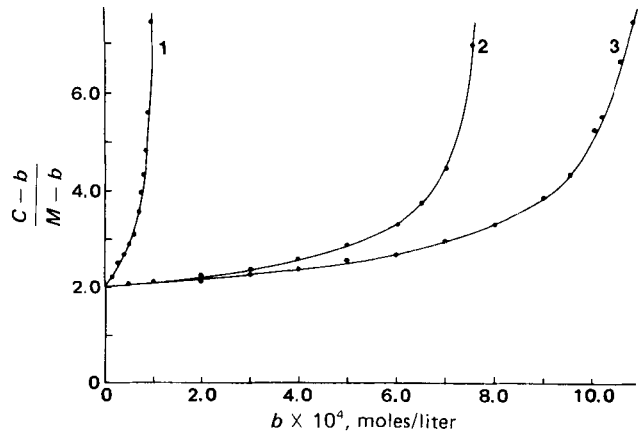

Figure 3—*Average degree of association,* C − b/M − b, versus *the monomer concentration,* b, *for sodium taurodeoxycholate (1), sodium glycodeoxycholate (2), and sodium deoxycholate (3).*

where n_0 is the refractive index of the solvent, dn/dc is the refractive increment of the solute at a constant concentration of added electrolyte, N_A is Avogadro's number, and λ_0 is the wavelength of incident light in vacuum. To obtain M_w by means of Eq. 2, it must be assumed that the second virial coefficient is zero. This approximation can affect the values of M_w calculated at a high concentration but should have relatively minor effects at a low concentration (7). The weight fraction of the monomer, x, can be obtained from:

$$\ln x = \int_0^c [(M_0/M_w) - 1] d \ln c \qquad \text{(Eq. 4)}$$

Given the weight fraction of the monomer, the molar concentration of the monomer, b, at concentration c (grams per milliliter) can be obtained from the relation $b = xc\ 1000/M_0$.

In general, the self-association pattern of a species can be represented by the stepwise addition of monomers to preexisting aggregates. The simplest of such schemes is dimerization, which can be represented by Scheme I:

$$2b \overset{K_2}{\rightleftharpoons} b_2$$

Scheme I

where:

$$K_2 = \frac{b_2}{b^2} \qquad \text{(Eq. 5)}$$

In Scheme I, b_2 is the concentration of dimer and K_2 is the association constant for dimerization. Further steps in this process can be represented by the reactions in Scheme II.

$$b + b_2 \overset{K_3}{\rightleftharpoons} b_3$$

$$b + b_3 \overset{K_4}{\rightleftharpoons} b_4$$

$$b + b_{q-1} \overset{K_q}{\rightleftharpoons} b_q$$

Scheme II

Based on Scheme II, the total equivalent concentration, C, of all species can be defined as:

$$C = b + 2K_2b^2 + 3K_2K_3b^3 + \cdots q \prod_2^q K_q b^q \qquad \text{(Eq. 6)}$$

The total molar concentration, M, of all species can be defined by:

$$M = b + K_2b^2 + K_2K_3b^3 + \cdots \prod_2^q K_q b^q \qquad \text{(Eq. 7)}$$

Finally, the number average degree of association, N_n, for all species excluding the monomer can be defined by:

$$N_n = \frac{C - b}{M - b} \qquad \text{(Eq. 8)}$$

The total equivalent concentration is known from the experimental method. Mukerjee and Ghosh (17) showed that the total molar concentration, M, can be obtained from data on C and b by means of graphical integration according to the following equation:

$$M' - M = 2.303 \int_b^{b'} C\ d \log b \qquad \text{(Eq. 9)}$$

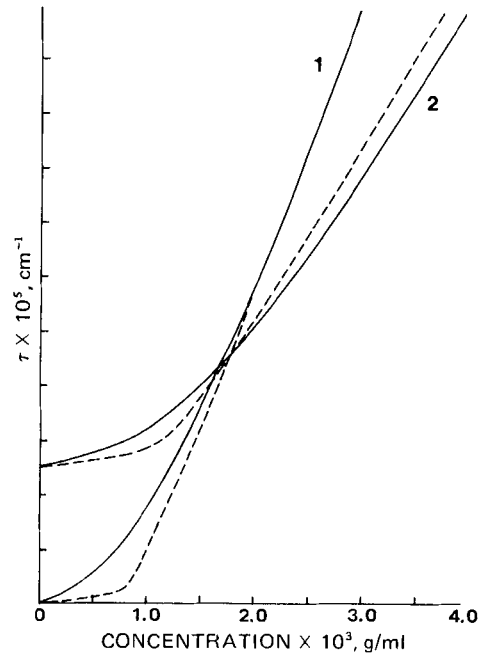

Figure 4—*Comparison of the calculated turbidity (- - -) based on the monomer–micellar model with the experimental curve (—) for sodium taurodeoxycholate (1) and sodium deoxycholate (2) in 0.15* M *NaCl. Each division on the ordinate represents* 2×10^{-5} cm^{-1}. *The intercept for each plot is* 5.07×10^{-5} cm^{-1}.

Monomer-Unique Oligomer Model—Several investigators (1–4) suggested that the dihydroxy bile salts associate in a manner similar to flexible chain surfactants. Under this assumption, the quantities C and M can be defined by:

$$C = b + q\beta_q b^q \qquad \text{(Eq. 10)}$$

$$M = b + \beta_q b^q \qquad \text{(Eq. 11)}$$

where:

$$\beta_q = \prod_2^q K_q$$

By comparison with Scheme II and Eqs. 6–8, it can be seen that, according to this model, N_n should remain nearly zero for all values of b below the CMC. Above the CMC, N_n should rise rapidly and approach a constant value equal to the average aggregation number of the micelles.

Figure 3 shows the variation of N_n with b for all three bile salts in 0.15 M NaCl. For all three bile salts, N_n approaches 2 in dilute solution. For sodium deoxycholate, N_n increases slowly as the monomer concentration increases and then increases rapidly at relatively high values of b. This slow increase in N_n at low values of b is consistent with small aggregate formation in dilute solution (17). For sodium taurodeoxycholate, N_n increases rapidly in dilute solution, suggesting that large aggregates form in relatively dilute solutions. Sodium glycocholate is intermediate between these extremes.

The smallest aggregate formed by all three bile salts is the dimer (Fig. 3). Furthermore, large aggregates must be present to account for the large increase in N_n at the higher concentrations. Thus, the results shown in Fig. 3 are inconsistent with a model that assumes the existence of only monomers plus a unique higher aggregate. This conclusion can also be reached by a comparison of the experimental turbidity curves with those calculated on the basis of a monomer–unique oligomer model. This comparison is shown in Fig. 4 for sodium taurodeoxycholate and sodium deoxycholate. For these calculations, aggregation numbers of 25.3 and 15.9 were used. These values were obtained from the usual Debye plot (12).

Stepwise Association Models—Based on Fig. 3, the limiting size aggregate formed in dilute solution must be the dimer. Moreover, some higher aggregates must form to account for the slowly increasing trend of N_n with b.

Table II—Association Constants, Second Virial Coefficients, and Aggregation Numbers for the Dihydroxy Bile Salts in 0.15 *M* Sodium Halides

Bile Salt and Electrolyte	K_2, liters/mole	K_3, liters/mole	K_4, liters/mole	B, ml-mole/g^2	Aggregation Number
Sodium deoxycholate					
Sodium fluoride	300	810	1360	1.05×10^{-3}	17.7
Sodium chloride	310	870	1290	7.82×10^{-4}	16.7
Sodium bromide	310	710	1130	8.41×10^{-4}	16.0
Sodium glycodeoxycholate					
Sodium chloride	500	780	1370	3.32×10^{-4}	18
Sodium taurodeoxycholate					
Sodium chloride	6820	7160	2.94×10^4	1.23×10^{-4}	25

To determine the nature and size of the higher aggregates formed, the method of Steiner (16) was applied. In this method, it is assumed that aggregate growth occurs in a stepwise fashion such as is given by Eq. 6. According to this method, the weight fraction of the monomer is obtained by the methods described in the previous section. The equilibrium constants K_2, K_3, *etc.*, can be obtained from:

$$[(M_w/xM_0) - 1]/(xc/M_0)$$

$$= 4K_2 + 9K_2K_3(xc/M_0) + \cdots q\prod_2^q K_q(xc/M_0)^{q-2} \quad \text{(Eq. 12)}$$

where K_2 is obtained from the intercept and K_3 is obtained from the limiting slope of a plot made according to Eq. 12. The higher association constants can be obtained by successive applications of Eq. 12.

It was shown previously (9–12) that application of Eq. 12 to light-scattering data of the type generated in the present study leads to a self-consistent set of parameters K_2, K_3, *etc.* In these systems, the association constants were relatively low and little difficulty was encountered in obtaining the values of these constants. In the present systems, however, the turbidity increases rapidly at low concentrations, so small errors in the turbidity can lead to large errors in the derived values of M_w and x. Therefore, it was necessary to develop an iterative technique to obtain K_2, K_3, *etc.*

Based on this procedure, the association constants K_2, K_3, and K_4 shown in Table II were developed. These values were generated from the best smooth curve of plots of both turbidity and molecular weight *versus* concentration. Based on these curves, values of the monomer fraction, x, and the association constants were obtained. The best fits of the association constants were developed by taking advantage of the fact that the slope of the first application of Eq. 12 must be equal to the intercept of the second application, *etc.* Although it is not possible to obtain a direct estimate of the accuracy of these values, the values for sodium deoxycholate are probably somewhat more reliable than those for sodium glycodeoxycholate and sodium taurodeoxycholate because of the slower increase in turbidity with the total concentration.

When an attempt was made to obtain the association constant for the pentamer for each bile salt, the value of the limiting slope was found to be nearly zero. This result suggests that the stability of the pentamer must be small relative to the lower aggregates. This observation that the limiting size of the smaller aggregate does not depend on the degree of conjugation of the bile is consistent with earlier work on the trihydroxy bile salts (12) where the association constants were easier to develop.

At higher values of (xc/M_0), the plots according to Eq. 12 tend to increase rapidly, indicating that some higher aggregates must exist in solution. To determine the size of the higher aggregates, it is assumed that the total equivalent concentration of the dihydroxy bile salts follows a model defined as:

$$C = b + 2K_2b^2 + 3K_2K_3b^3 + 4K_2K_3K_4b^4 + q\beta_q b^q \quad \text{(Eq. 13)}$$

where $q\beta_q b^q$ represents the concentration of the large aggregate. The molecular weight and the second virial coefficient of the large aggregate were obtained from the following equations:

$$\frac{H(c - c')}{(\tau - \tau')} = \frac{1}{M_w} + 2B(c - c') \quad \text{(Eq. 14)}$$

where:

$$c' = b + 2K_2b^2 + 3K_2K_3b^3 + 4K_2K_3K_4b^4 \quad \text{(Eq. 15}a\text{)}$$

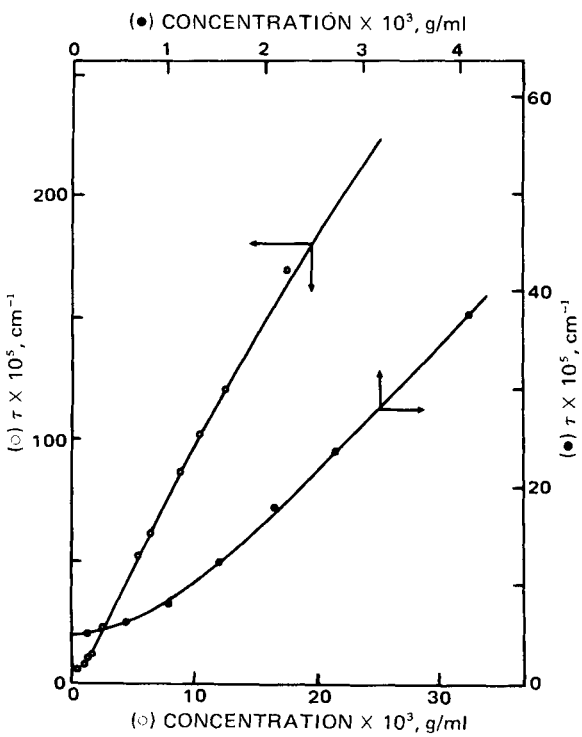

Figure 5—*Comparison of the experimental curve for sodium taurodeoxycholate in 0.15* M *NaCl (—) with the calculated values (○,●) based on the stepwise association model.*

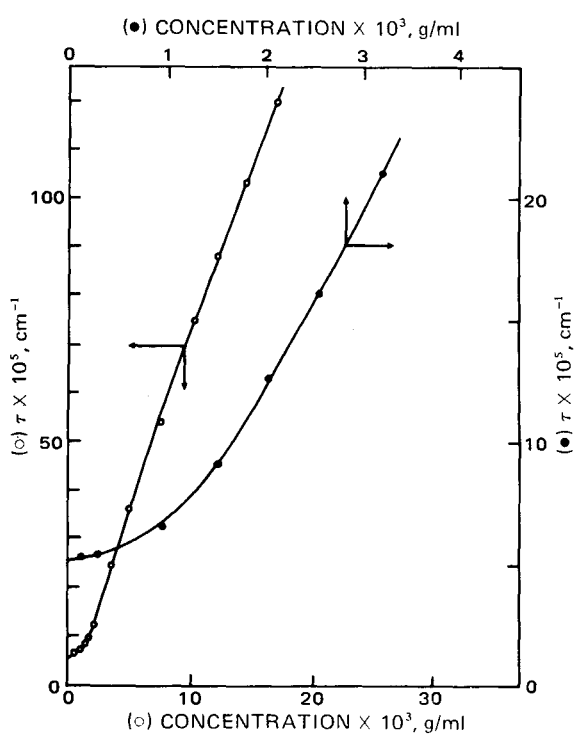

Figure 6—*Comparison of the experimental curve for sodium glycodeoxycholate in 0.15* M *NaCl (—) with the calculated values (○,●) based on the stepwise association model.*

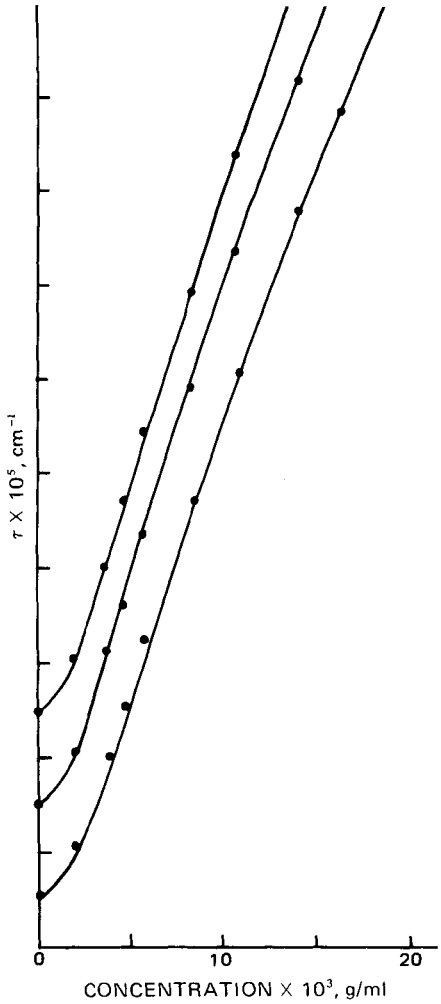

Figure 7—*Comparison of the experimental curves (—) for sodium deoxycholate in 0.15 M sodium halides with the calculated values (●) based on the Vrij–Overbeek treatment of the stepwise association model. Proceeding from top to bottom, the curves represent sodium deoxycholate in sodium fluoride, sodium chloride, and sodium bromide. The units of the ordinate and the intercepts are as given in Fig. 1.*

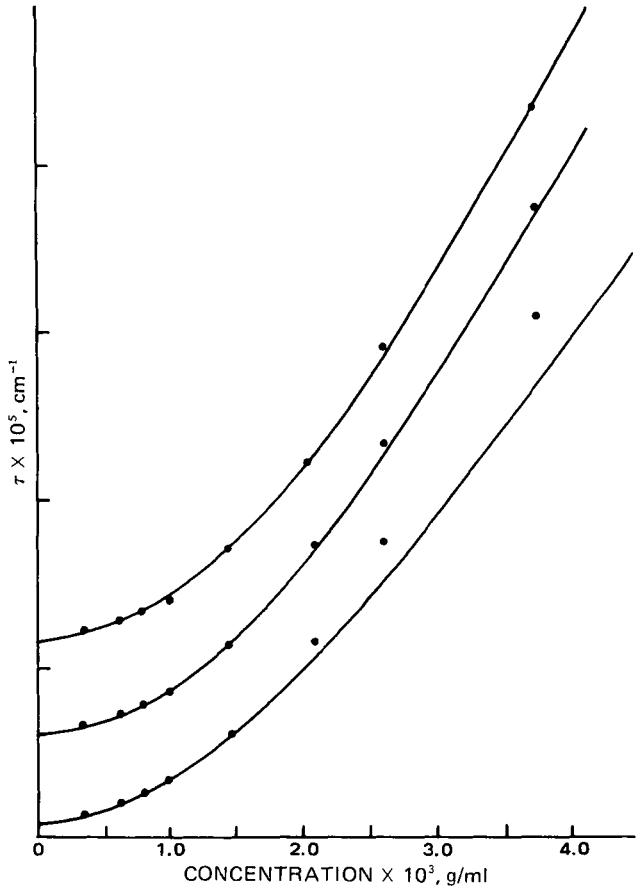

Figure 8—*Plot similar to Fig. 7 for the low concentration region. Each division on the ordinate represents 5 X 10^{-5} cm^{-1}. The intercepts are as given in Fig. 1.*

and:

$$\tau' = \tau_0 + \tau_1 + \tau_2 + \tau_3 + \tau_4 \qquad \text{(Eq. 15}b\text{)}$$

where c represents the total concentration and τ represents the total turbidity at concentration c, τ_0 represents the turbidity of the solvent, and τ_1, τ_2, *etc.*, represent the turbidity of the monomer, dimer, *etc.* The value of τ_0 is obtained experimentally. The values of τ_1, *etc.*, can be calculated according to Eq. 2, assuming that the second virial coefficients are zero. Application of these equations to the light-scattering data leads to the aggregation numbers for the high aggregates shown in Table II. These values are somewhat higher than those obtained previously by application of the usual monomer–micellar model. This trend of higher aggregation numbers from the stepwise association treatment is consistent with the results obtained previously (12, 18).

A comparison between the experimental curves and those calculated on the basis of this model for sodium taurodeoxycholate and sodium glycodeoxycholate is shown in Figs. 5 and 6. The agreement between the experimental curves and the calculated values is excellent in both the low and high concentration regions.

As discussed by Vrij and Overbeek (19), the aggregation numbers shown in Table II are apparent values uncorrected for effects arising from the negative adsorption of the co-ions. These authors (19) developed the necessary procedures to obtain the true aggregation number from the apparent values, provided that the apparent values were obtained in several different sodium halides at the same concentration.

In the present study, sufficient data for application of the procedure of Vrij and Overbeek (19) were obtained only for sodium deoxycholate. When using the apparent aggregation numbers shown in Table II, the true aggregation number was found to be 18.8. Comparisons of the calculated values with the experimental curves for the three electrolytes are shown in Figs. 7 and 8. The agreement seems to be quite good except for sodium bromide, where the calculated values lie somewhat above the experimental curve in the intermediate concentration region. These curves were calculated on the basis of the average values of K_2, K_3, and K_4 for all electrolytes shown in Table II. The lack of agreement of the calculated values with the experimental curve in sodium bromide is consistent with the lower values of K_3 and K_4 found in the sodium bromide system. The reasons for these lower values in this system are not known.

The results obtained in the present study of the dihydroxy bile salts are in agreement with those reported by previous investigators with respect to several points, including that the dihydroxy bile salts associate to form large aggregates, which are substantially larger than those found in the trihydroxy bile salts, and that conjugation affects the size of the higher aggregates, the size decreasing in the order sodium taurodeoxycholate > sodium glycodeoxycholate > sodium deoxycholate. However, in this study, evidence suggests that dimers, trimers, and tetramers are also present. This result should be compared with the results obtained on the trihydroxy bile salts where the evidence suggests that only dimers and trimers are present (12). Also, the association constants are much smaller for the trihydroxy bile salts. Thus, the number of hydroxyl groups on the bile salt molecule seems to affect not only the aggregation number of the higher aggregates but also the type and stability of the small aggregates formed. Any indepth analyses of the pattern of the association constants for the low aggregates would probably be misleading because of the uncertainties in these values for the dihydroxy bile salts.

REFERENCES

(1) D. M. Small, in "The Bile Acids," P. P. Nair and D. Kritchevsky, Eds., Plenum, New York, N.Y., 1971, chap. 8.

(2) J. P. Kratohvil and H. T. DelliColli, *Can. J. Biochem.*, **46**, 443 (1968).

(3) J. P. Kratohvil and H. T. DelliColli, *Fed. Proc.*, **29**, 1335 (1970).
(4) J. P. Kratohvil, *Colloid Polym. Sci.*, **253**, 251 (1975).
(5) K. Fontell, *Kolloid-Z. Z. Polym.*, **244**, 253 (1971).
(6) P. Mukerjee, *Adv. Colloid Interface Sci.*, **1**, 241 (1967).
(7) P. Mukerjee, *J. Phys. Chem.*, **65**, 565 (1972).
(8) P. Mukerjee, *J. Pharm. Sci.*, **63**, 972 (1974).
(9) D. Attwood and O. K. Udeala, *J. Phys. Chem.*, **79**, 889 (1975).
(10) D. Attwood and O. K. Udeala, *J. Pharm. Sci.*, **65**, 1053 (1976).
(11) D. Attwood, *J. Phys. Chem.*, **80**, 1984 (1976).
(12) Y. Chang and J. R. Cardinal, *J. Pharm. Sci.*, **67**, 174 (1978).
(13) H. Sobotka and A. Goldberg, *Biochem. J.*, **26**, 555 (1932).
(14) L. Lack, G. D. Singletary, T. Walker, and F. O. Dorrity, *J. Lipid Res.*, **14**, 367 (1973).
(15) A. F. Hofmann, *ibid.*, **3**, 127 (1962).
(16) R. F. Steiner, *Arch. Biochem. Biophys.*, **39**, 333 (1952).
(17) P. Mukerjee and A. K. Ghosh, *J. Am. Chem. Soc.*, **92**, 6403 (1970).
(18) K. J. Mysels and L. H. Princen, *J. Phys. Chem.*, **63**, 1949 (1959).
(19) A. Vrij and J. T. G. Overbeek, *J. Colloid Sci.*, **17**, 570 (1962).

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New Opiate–Receptor Model

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Abstract □ A new opiate–receptor model is proposed in which only one conformation of the receptor is needed for binding of both agonists and antagonists. There are two different spacially fixed amine-binding sites in this model: one agonist and one antagonist. The opiates undergo binding to their amine-binding sites *via* the lone electron pair on nitrogen. The role of the *N*-allyl or other such group in imparting antagonist properties is explained in terms of the steric requirements of this group. For this group to be accommodated without imposing severe steric interactions in the rest of the opiate molecule, the piperidine ring must assume a flexible (skew boat) conformation; in this conformation, the *N*-lone-pair electron lobe assumes the characteristic directionality of an antagonist toward its amine-binding site. If the *N*-lone-pair lobe is not rigorously maintained in this direction, the opiate molecule assumes both antagonist and agonist conformations and mixed antagonist–agonist activity is observed. The observed differences in the effect of sodium on the degree of binding of an agonist *versus* an antagonist can be explained in this model by the different effects of sodium on the two amine-binding sites. The antagonist activity of an *N*-methyl antagonist can be rationalized on the basis of the proposed model.

Keyphrases □ Opiate–receptor model—proposed, only one receptor conformation needed for binding both agonists and antagonists □ Model, opiate–receptor—proposed, only one receptor conformation needed for binding both agonists and antagonists □ Binding—opiate agonists and antagonists to receptor, new model proposed

Recently, Snyder and coworkers (1, 2) proposed a model of the opiate receptor to explain structure–activity relationships of opiate agonists and antagonists. They postulated that the receptor can exist in two different conformations: the antagonist conformation (a sodium-binding form) and the agonist conformation (no sodium form). On the basis of the qualitative conformational analysis of a series of opiate agonist and antagonist molecules, the following spacially fixed binding sites on the receptor were proposed (2): lipophilic, amine binding, agonist binding, and specific antagonist binding (Fig. 1).

To account for the observation that substitution of the *N*-methyl of an opiate agonist by an *N*-allyl or *N*-cyclopropylmethyl usually confers antagonist activity, Snyder and coworkers (2) suggested that: "Presumably, the *N*-allyl or other such group interacts with a portion of the opiate receptor regulating antagonist activity." This portion of the receptor in Fig. 1 is the specific antagonist binding site. Their view, therefore, is one interpretation of the data.

BACKGROUND

In the Snyder model, the amine-binding site is defined as the ionic site that interacts with the amine nitrogen. The protonated amine has been considered (3, 4) the active form at the receptor site. From the pKa of morphine (7.88), Beckett and Casy (3) estimated that it would be 80% protonated at physiological pH. However, it is not unreasonable to believe that the unprotonated amine is, in fact, the active species because of the nature of the amine-binding site. In this respect, a relatively high pKa value (a thermodynamic, not kinetic, value) may have an adverse effect on binding.

Figure 1 indicates that, in the Snyder model, the *N*-lone-pair electron lobe of naloxone is pointed away from the amine-binding site, while the *N*-lone-pair lobe of morphine is directed toward this binding site. It seems improbable that interaction between naloxone and the amine-binding site would occur in the juxtaposition shown in this figure. Only if the interaction between the amino nitrogen and the amine-binding site were chemical could a binding interaction in the conformation shown in Fig. 1 be reconcilable, and then only as a minor collision process in a cross-

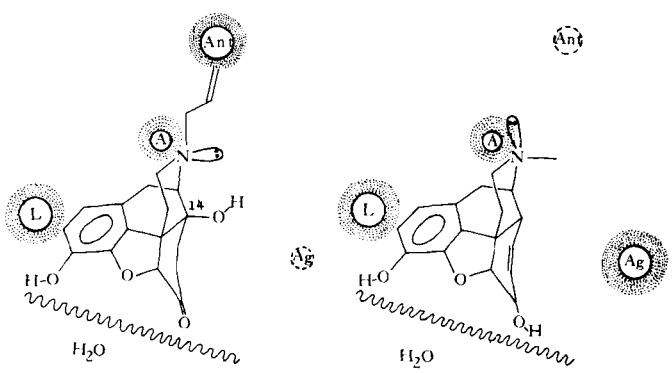

Figure 1—*Snyder's model for binding of naloxone (antagonist) and morphine (agonist) to the receptor. Key: left, naloxone bound to the antagonist conformation of the receptor; right, morphine bound to the agonist conformation of the receptor; L, lipophilic site; A, amine-binding site; Ag, agonist-binding site; and Ant, specific antagonist-binding site.*

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