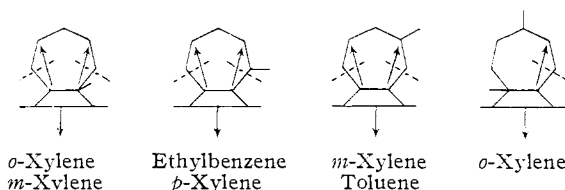

Over catalyst 2, methylcycloheptane gave the corresponding product distribution of 18:40:42. By the same reasoning as before, the aromatization proceeded essentially according to the non-ionic mechanism; however, a contribution of a carbonium ion mechanism in this case is not excluded inasmuch as catalyst 2 was able to dehydroisomerize 1,1-dimethylcyclohexane to *o*-xylene. The relatively higher yield proportion of ethylbenzene and of *o*-xylene than that obtained with catalyst 3 also suggests participation to a small extent of a carbonium ion mechanism.

The behavior of chromia alone toward the aromatization of methylcycloheptane is similar to that experienced with compounds I and II (Exps. 1 and 2). At first the chromia acted as an aromatization catalyst without showing acid catalytic tendencies. However, with time the dehydrogenation sites of the catalyst were deactivated while acid sites increased. This is indicated by the relatively greater amount of ethylbenzene formed.

Conclusion

The experimental results discussed above show: (1) that chromia is a dual action catalyst having dehydrogenation and isomerization properties; the latter are associated with the intrinsic acidity of the chromia and (2) that alumina of different intrinsic acidities influences the catalytic properties of chromia-alumina catalysts in aromatization reactions with respect to involvement of carbonium ion mechanism therein.

[CONTRIBUTION FROM THE BIOLOGY DEPARTMENT, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS]

Studies on the Formation of Helical Deoxycholate Complexes[1,2]

BY D. M. BLOW AND ALEXANDER RICH

RECEIVED NOVEMBER 12, 1959

It was previously found that under appropriate conditions sodium deoxycholate aggregates in solution to form a gelatinous complex of macromolecular dimensions; X-ray diffraction studies of fibers of this complex showed that the molecules had assumed an elongated helical configuration 36Å. in diameter. Viscometric measurements have been used for the study of the conditions favoring formation of the complex and for observing thixotropic behavior. Complexing is favored by lower *p*H (down to the point where the insoluble acid is precipitated) and by raising the ionic strength; at sufficiently high ionic strength solutions of sodium deoxycholate will gel without added acid. The results suggest that the formation of the complex is highly specific since closely related compounds do not exhibit this effect. Solutions of deoxycholate show noticeable rises of *p*H during formation of the complex; it has been shown that this absorption of hydrogen ions is correlated with the extent to which the gel is broken up by stirring. The mechanism of the thixotropic behavior and the implications of these observations with respect to the molecular structure of the complex are discussed.

Introduction

In an earlier paper, we described the various changes which occur upon the slight acidification of solutions of sodium deoxycholate.[3] When the *p*H is lowered, the solution gradually becomes viscous and ultimately gels if allowed to stand in a beaker. These effects also have been observed by Sobotka and Czeczowiczka.[4] When in the form of this viscous "complex," deoxycholate behaves in many ways like a polymer of high molecular weight. The solutions are slightly turbid, and in the ultracentrifuge show a rapidly migrating peak. The solutions can be drawn into fibers which dry into glassy, birefringent rods.

X-Ray diffraction photographs of these fibers showed a remarkably detailed pattern which is characteristic of a helical aggregate.[3] The elongated units are organized in the fiber in hexagonal packing with a distance of 36.2 Å. between the centers. This diameter increases if other molecules, such as amino acids or peptides, are in the solution during the formation of the complex. The diffraction pattern suggests that the other complexing species is attached to the outside of the helical steroid core.

In order to reach a clearer understanding of this polymer-like complex, we have studied its formation under a variety of conditions. Viscosity measurements have been used chiefly to trace the formation of the complex; a *p*H change also has been observed during its formation, indicating that hydrogen ions are being removed from the solution. The effect has been found to be rather specific for deoxycholate and most other bile salts show no sign of similar behavior.

There is evidence that the phenomenon is related to micelle-forming properties of deoxycholate but that these are greatly enhanced by lowering the *p*H. We believe that under these conditions a specific system of hydrogen bonding becomes possible within the micelle, leading to formation of huge micelles with a regular or "crystalline" internal structure.

Materials and Methods

For most experiments commercial preparations of sodium deoxycholate were used (Fisher Scientific Co. and Nutritional Biochemical Corp.). The solutions were filtered before

(1) Presented at the 135th meeting of the American Chemical Society, Boston, Mass., April, 1959.

(2) Supported in part by a Research Grant from the National Cancer Institute, Bethesda, Maryland.

(3) A. Rich and D. M. Blow, *Nature*, **182**, 423 (1958).

(4) H. Sobotka and N. Czeczowiczka, *J. Colloid Sci.*, **13**, 188 (1958).

making viscosity measurements but otherwise not purified. A few measurements were made with purified material, prepared from a commercial preparation by the procedure of Sobotka and Goldberg,[5] which gave essentially the same results. The concentrations of deoxycholate given below are calculated on the assumption that the commercial material is pure, though in fact it usually contains 2–3% sodium cholate. They are thus slightly too high.

A variety of related steroids was obtained from other sources. Their solubility at varying *p*H was studied by dissolving them in strong alkali, if necessary in the presence of alcohol, followed by stepwise neutralization with acid. If sufficiently soluble in the neutral range, viscosity measurements were made on the solutions.

Viscosities were measured in standard 5 ml. Ostwald viscometers, generally at a temperature of 24.6°. In order to cover a large range of viscosities with reasonable accuracy, viscometers were used with emptying times for water from 4 min. down to 10 sec. As will be described later, the observed viscosity depends greatly on the exact history of the solution and is therefore to some extent a function of the dimensions of the viscometer. Repeated experiments with the same viscometer, however, showed remarkably reproducible results.

The uptake of hydrogen ions during the formation of the complex was measured by using an automatic titrator (Radiometer, Copenhagen) as a *p*H-stat. In a typical experiment a 0.02 *M* deoxycholate solution was placed in a small beaker fitted with electrodes for *p*H measurement and with a magnetic stirrer. A microburet was arranged to deliver 0.05 *M* hydrochloric acid directly between the electrodes, under the control of the automatic titrator. A simple form of viscometer was arranged for this apparatus. This was the capillary tube of a 0.5 ml. pipet whose tip just dipped into the solution and which could be supplied with a reproducible slight suction, using a constant head of water. The viscosity was measured by observing the time for the solution to be drawn to the upper mark of the pipet. This method was preferred to measuring the emptying time of the pipet, because filling the pipet itself subjects the solution to additional shear. After the measurement, the sample was allowed to return to the stirred solution.

Results

Observation of Viscosity Changes.—If sodium deoxycholate is dissolved in distilled water, the *p*H of the resulting solution is found to be between about 7.4 and 8.6, depending on the concentration. This *p*H for the appropriate concentration will be referred to as the "natural" *p*H of sodium deoxycholate.

If the *p*H of a deoxycholate solution is lowered, the material may gel, even from quite dilute solutions. However, these gels are thixotropic, in that shaking such a gel may convert it into a solution. Experiments were carried out to measure the rate of formation of the complex by repeated measurement of the viscosity. The procedure adopted was to place the freshly mixed solution in the viscometer and at once begin a viscometric run. As soon as one viscosity measurement was completed, the solution would be drawn up to start another. Typical curves showing observed viscosity as a function of time are shown in Fig. 1. Under these conditions, the viscosity rises to a sharp maximum and then falls to a steady value. If a freshly mixed solution is held undisturbed in the upper bulb of the viscometer for a time, it will indicate much higher viscosities or even gel in the tube. Thus, the shear of the viscometer is used as a mechanical influence which produces a thixotropic effect during the initial gelation process. On the other hand, a solution which was approaching the final viscosity was often left in the viscometer overnight and no in-

(5) H. Sobotka and A. Goldberg, *Biochem. J.*, **26**, 555 (1932).

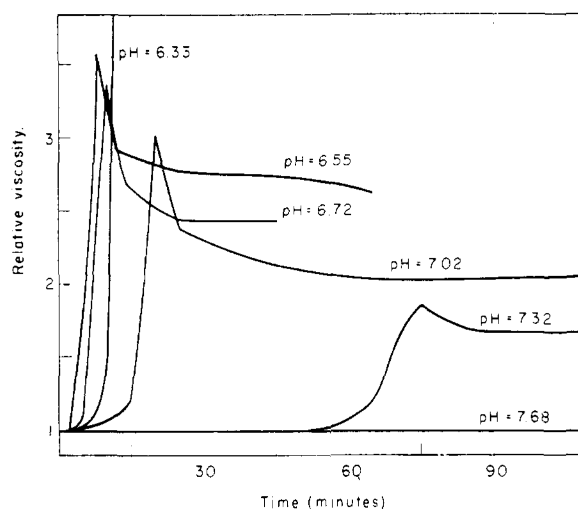

Fig. 1.—The viscosity of 0.004 *M* sodium deoxycholate solutions in 0.18 *M* phosphate buffer: relative viscosity is plotted as a function of time for different *p*H values; T = 24.6°.

crease of viscosity was observed. Hence, the mechanical effect of the measurements is significant in the early stages of complex formation but not in the final steady state. This is an example of a nonreversible thixotropy, since the final effect of the mechanical disturbance is to prevent gelation.

We can thus describe two distinct types of deoxycholate aggregation. One of these is a gel, the formative stages of which are seen in the initial spike of viscosity as a function of time. The other type of aggregation is the final or "steady" state in which the gel has been broken up by the shearing action of the viscometer, and will not reform, but the solution has a residual viscosity appreciably greater than that of unaggregated deoxycholate molecules. In forming fibers for X-ray diffraction investigation, the solution is subjected to considerable velocity gradients (stirring, pulling out on a glass rod, etc.). To determine whether the state of aggregation in the gel is similar to what is seen in a stirred solution or in an oriented fiber, we prepared two identical solutions, one of which was allowed to gel without mechanical agitation, while the other was stirred continuously to a final steady viscosity. Both of these solutions were dried down considerably in a vacuum desiccator to get rid of most, but not all, of the water, and then X-ray diffraction photographs were taken. These showed identical powder diffraction diagrams which were the same as those produced by the powdered, oriented fibers.

Other X-ray diffraction experiments were carried out to demonstrate the existence of the helical aggregate in solution, in order to rule out the influence of drying on the formation of the complex. These solutions, oriented by the shear of a fine capillary tube, also produced the characteristic helical diffraction pattern.[3]

It is possible to accelerate formation of the helical complex by "seeding," in just the same way that crystal growth may be encouraged by a seed crystal. Thus if a small amount of solution containing the complex is added to a freshly acidified deoxy-

cholate solution, the viscosity increased much more rapidly than usual.

Effect of *p*H.—A series of observations showing the effect of *p*H on the time dependent viscosity is also shown in Fig. 1. The results for *p*H 6.33 are probably anomalous due to the formation of small amounts of the insoluble deoxycholic acid. It can be seen that lowering the *p*H increases both the final viscosity and the rapidity of the interaction. Over most of the *p*H range measured, the final or equilibrium specific viscosity appears to be a linear function of the *p*H (Fig. 2).

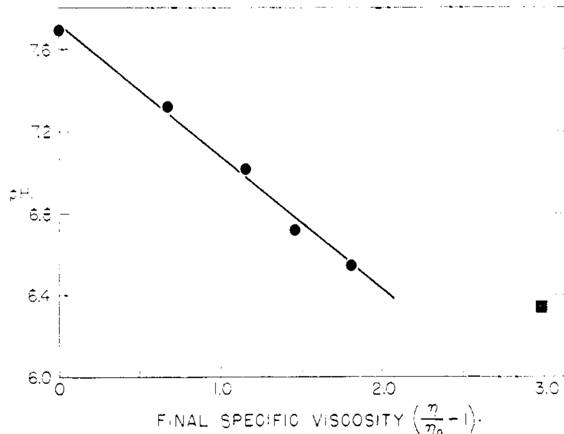

Fig. 2.—The final specific viscosity of 0.004 *M* sodium deoxycholate solutions as a function of *p*H. The buffer is 0.18 *M* phosphate; $T = 24.6°$. The measurement shown by the square at *p*H 6.33 suggests that some insoluble particles of deoxycholic acid may have formed at that *p*H.

These results may be explained by assuming that the helical aggregates grow longer as the *p*H is lowered. It is known that the viscosity of a solution of rod shaped molecules is proportional to the square of the rod length.[6] Thus the rod length may be proportional to the square root of the *p*H.

However, an alternative explanation is to assume that the extent of complex formation increases as the *p*H is lowered but that all the solutions have the same distribution of particle lengths. Further experimental work would be needed to resolve these two alternative interpretations.

Effect of Ionic Strength.—In further experiments, we studied the influence of varying salt concentrations on the viscosity. The experiments were complicated by the fact that the region of most interest was that in which the ionic strength was very low and, accordingly, the solutions were left unbuffered in order to reduce the ionic strength. Standard solutions of 0.004 *M* sodium deoxycholate were made up with a varying concentration of sodium chloride. The solutions were then acidified by the direct addition of 0.15 equivalent of HCl for each equivalent of deoxycholate. The *p*H was measured immediately on mixing, and the viscosity changes observed in the same way as before; the results are listed in Table I. It can be seen that the *p*H rose as the salt concentration increased. This effect is due to the variation in the dissociation constant of sodium deoxycholate with increasing ionic strength. At low salt concentrations there is no observable change in viscosity. The rise in viscosity at high salt concentration occurs even though the *p*H is higher. The experiments of the previous section showed that lowering the *p*H, at constant ionic strength, caused higher viscosities. This emphasized the fact that the complex is stabilized by an increase in ionic strength.

(6) M. L. Huggins, *J. Phys. Chem.*, **42**, 910 (1938); *ibid*, **43**, 439 (1939); W. Kuhn and H. Kuhn, *Helv. Chim. Acta*, **28**, 97 (1945).

TABLE I

VISCOSITY MEASUREMENTS ON 0.004 *M* SODIUM DEOXYCHOLATE WITH VARIOUS CONCENTRATIONS OF ADDED SALT, TO WHICH APPROXIMATELY 0.15 EQUIVALENT OF HCl IS ADDED; $T = 24.6°$

Concn. added salt, *M*	*p*H after mixing	Final rel. visc.
0.005 NaCl	6.8	1.00
.06 NaCl	7.0	1.00
.10 NaCl	7.1	1.00
.20 NaCl	7.1	1.20
.40 NaCl	7.2	1.45
.60 NaCl	7.3	1.75
.20 Na_2SO_4	7.45	1.66

As shown in Table I, no complexing was observed up to an ionic strength of 0.1. However, if slightly more HCl is added to a solution with 0.1 *M* NaCl, then a rise in viscosity is observed. Thus, the solution can be made to react either by raising the ionic strength or by further lowering the *p*H. When Na_2SO_4 is added instead of NaCl, the effect is similar to that of a NaCl solution with the same concentration of sodium ions. The increase in *p*H associated with increased ionic strength makes it hard to decide whether the total ionic strength or merely the cation concentration is important. We were unable to experiment with divalent cations since we could not find one whose deoxycholate was soluble.

Change of ionic strength had little effect on the rapidity of the interaction, which under these conditions was complete in about 2 hr. High ionic strength can cause complexing to occur in pure sodium deoxycholate solution without the addition of acid. For example, the "natural" *p*H of 0.06 *M* sodium deoxycholate is 8.5. A solution of 0.06 *M* deoxycholate, 0.5 *M* phosphate buffer, *p*H 8.5 and 0.8 *M* sodium chloride formed a thick gel in about 2 hr.

Effect of Deoxycholate Concentration.—A series of viscosity measurements now was made in which the deoxycholate concentration was varied. A strong phosphate buffer was used, so that the deoxycholate concentration had little effect on either the ionic strength or on the *p*H. The final viscosities reached by the deoxycholate solution are plotted in Fig. 3 against their concentration. The experiment was carried out in 0.20 *M* phosphate buffer; two different *p*H's were used to extend the range of concentration which could be used.

The two curves are very similar and show a discontinuity in the vicinity of 0.010 *M*. There is perhaps another break at 0.02 *M*, though viscosities were so high in this range that the measurements are rather uncertain. It has been shown[7] that

(7) R. R. Roepka and H. L. Mason, *J. Biol. Chem.*, **133**, 103 (1940); S. A. Johnston and J. W. McBain, *Proc. Roy. Soc.* (*London*) A, **181**, 119 (1942).

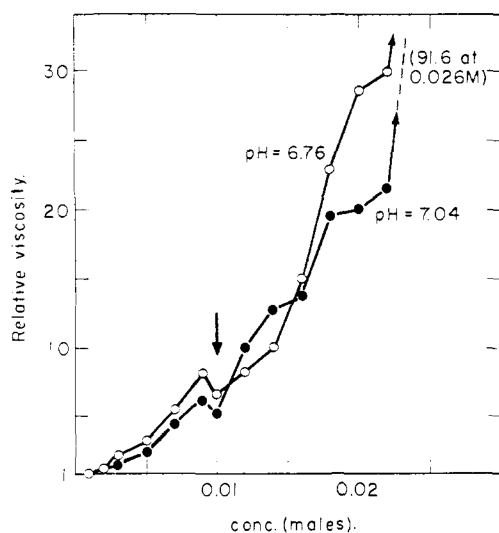

Fig. 3.—Final relative viscosity of sodium deoxycholate solutions in 0.20 *M* phosphate buffer. Various concentrations of deoxycholate are shown for *p*H 6.76 and 7.04, $T = 24.6°$.

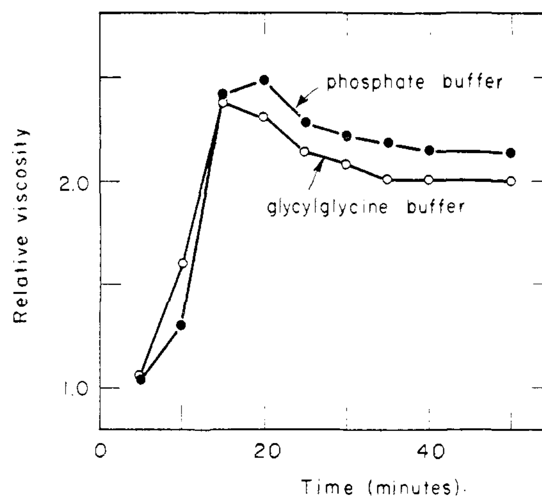

Fig. 4.—The effect of different buffers on the viscosity of sodium deoxycholate solutions. The solution had 0.004 *M* deoxycholate with 0.02 *M* buffers each adjusted initially to *p*H 6.60. Each point represents the mean of two experiments.

solutions of many bile salts, including sodium deoxycholate, form micelles in solution. Ekwall[8] has studied numerous properties of sodium deoxycholate solution which were at their "natural" *p*H and has identified a number of concentration ranges separated by discontinuities in various properties such as dissociation constant and solubilizing power. These discontinuities are attributed to changes in the type of micelle which is stable at different concentrations. The first discontinuity we observe is close to a region of altered properties which Ekwall finds near 0.01 *M*. We have also found that no complexing occurs below 0.003 *M* in salt free solutions at these *p*H values, while Ekwall gives about 0.005 *M* as the critical concentration for the sodium salt, below which there is no micellar aggregation.

Effect of Different Buffers.—In our earlier paper,[3] we reported that the diameter of the fibrous complex, as measured from the X-ray diffraction pattern, increases with increasing glycylglycine concentration when the glycylglycine is used as a buffer. It was first suspected that this was due to some kind of specific interaction between the complex and the peptide, which suggested the possibility that the presence of glycylglycine might increase the viscosity of the solutions and perhaps might also affect the rate of formation. Various experiments were designed to demonstrate some interaction in solutions. Viscosity runs were carried out in which 0.02 *M* glycylglycine and phosphate buffers were compared, using 0.18 *M* NaCl and 0.004 *M* deoxycholate. The buffers were arranged so as to have the same *p*H immediately after mixing with deoxycholate.

The results are shown in Fig. 4. The curves show the results with the two buffers for two solutions with the same deoxycholate concentrations. The rate of reactions appears identical in the two buffers, and there is only a slight difference in the final viscosity, the phosphate buffer giving the more viscous solutions. This is because rather weak buffer solutions are being used, and at this *p*H glycylglycine is a much less effective buffer than phosphate. The absorption of hydrogen ions in the course of complexing (described in the next section) causes the glycylglycine buffer to reach a higher *p*H than the phosphate buffer. There is no indication that any special interaction occurs with the glycylglycine which can be detected by a viscosity change.

Two other experiments were designed to detect a specific interaction between the deoxycholate complex and peptides or amino acids. In one series of experiments the deoxycholate complex was formed in glycylglycine buffers and sedimented in the preparative ultracentrifuge to give a paste in the bottom of the tube. The concentrations of glycylglycine and deoxycholate in the supernatant and in the paste were measured Glycylglycine was estimated by the ninhydrin method, and deoxycholate by the method of Eriksson and Sjövall.[9] The paste always showed a much higher deoxycholate concentration, but the glycylglycine concentrations were not significantly increased. A similar experiment was carried out with 0.001 *M* tyrosine, which could be detected spectrophotometrically. Both these experiments would have detected a small excess of amino acid in the paste.

In the other series of experiments conductimetric measurements were used. These showed that in dilute solutions the mobility of the deoxycholate ion was scarcely affected by the complexing, and also that the presence of an amino acid of zero net charge (glycine) had no observable effect. The experiment was repeated with lysine, which carries one positive charge at this *p*H. If interaction occurred with negatively charged deoxycholate ions, the conductivity of the solutions should be reduced. Again no significant change was observed.

(8) P. Ekwall, *J. Coll. Sci.* (*Suppl. 1*), 66 (1944); P. Ekwall, *Konikl. Wetenschap. Colloq.*, 103 (1953).

(9) S. Eriksson and J. Sjövall, *Arkiv. f. Kemi*, **8**, 303 (1955).

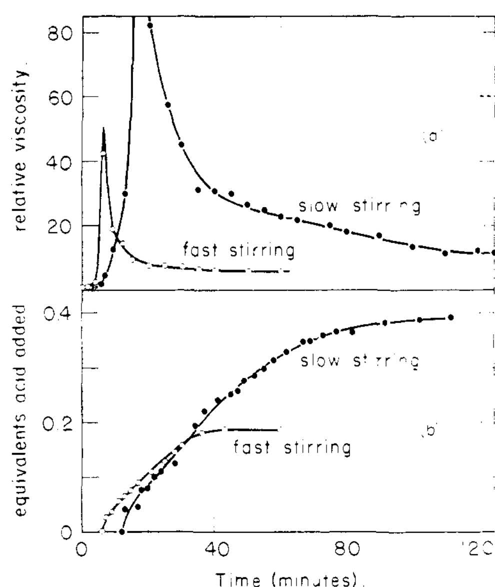

Fig. 5.—Experiments on the absorption of hydrogen ions during the formation of the helical complex: (a) relative viscosity as a function of time for 0.02 *M* solution of sodium deoxycholate in 0.2 *M* NaCl and 0.02 *M* phosphate buffer, *p*H 7.1; (b) number of equivalents of acid added to maintain the *p*H of the solution at 7.1. Curves are shown for two stirring speeds and the observations (a) and (b) were made simultaneously in each case.

Although there is good X-ray diffraction evidence that fibers drawn from these solutions are altered in structure by the presence of glycylglycine and amino acids which are evidently incorporated into the fibrous lattice, these substances do not interact strongly in dilute solution.

Absorption of Hydrogen Ions During Formation of the Complex.—During formation of the complex, the solution, if only weakly buffered, shows a noticeable increase of *p*H. A more instructive way to observe the phenomenon, however, is to use the automatic titrator. This is set to the *p*H of the solutions when first mixed, and used as a *p*H-stat, so as to follow the amount of extra acid taken up by the solution. As described in the experimental section, a system was set up to allow simultaneous measurement of the viscosity.

At the beginning of a run sufficient acid was introduced into the beaker, with slow stirring, to bring it to a *p*H where complexing would begin. With the automatic titrator set to hold the solution at this *p*H, the viscosity was observed at one or two minute intervals. The results of such an experiment are shown in Fig. 5. The viscosity rises sharply, in the usual way, and until it has almost reached its peak, the *p*H remains steady. At this point the *p*H begins to rise, and acid is added to supply additional hydrogen ions.

It was not possible to obtain entirely reproducible stirring with such large viscosity changes: however, the general conclusion emerged that the more slowly a solution was stirred, up to a certain limit, the more hydrogen ions were absorbed by the complexing system. It was also found that if a solution was allowed to stand, completely undisturbed in the *p*H meter, no change of *p*H occurred. Under these conditions, of course, no viscosity measurements could be made, but even very weak solutions slightly acidified set to a solid gel in the course of a few days. It can be seen that rapid stirring reduces the initial peak of viscosity, causing the viscosity to fall off more rapidly and preventing it from reaching the high value with slow stirring.

Study of Other Bile Salts.—We have considerably extended the search for other bile salts which might exhibit a similar phenomenon.[3] All the bile salts we have been able to examine are listed in Table II. Viscosity measurements were made on all those salts which gave clear solutions of reasonable concentration in the neutral *p*H range. In every case a control solution of sodium deoxycholate was run under identical conditions and gave an easily detectable rise of viscosity in a short time.

The results in every case were negative, with the exception of lithocholic acid.[4] This acid is practically insoluble below *p*H 12 at room temperature, and we were unable to obtain sufficiently clear solutions to give meaningful viscosity measurements. Lithocholic acid resembles deoxycholic acid in that, while going into solution, the solid particles first become gummy on the exterior and coagulate into a sticky mass. A suspension of lithocholate at *p*H 10, made by boiling to give a clear 0.01 *M* solution, followed by a cooling, formed a definite turbid gel over a period of several days. This gel is even more easily dispersed by a slight agitation than the deoxycholate gel. Lithocholic acid has a 3α-hydroxyl group in common with deoxycholic acid. This may be significant since Bernal, Crowfoot and Fankuchen[10] found that this position is very favorable for hydrogen bonding in crystalline steroids.

Discussion

Sodium deoxycholate, at its "natural," slightly alkaline *p*H, is a typical member of a series of detergent-like bile salts, which exhibit the usual properties of colloidal aggregation.[7,8] Debye[11] has shown clearly that this type of aggregation is governed by a balance between the energy gained by bringing the hydrophobic parts of the molecules together within a micelle and the work done by bringing the charged parts of the ionized molecules into proximity on the micelle surface. If the concentration of oppositely charged ions in the surrounding solution is raised, this electrostatic repulsion is partially masked, and the micelles are able to grow larger.

In the case of the deoxycholate helical complex, a further variable is introduced into this scheme. It appears that when the *p*H is lowered, some of the deoxycholate ions can take on protons which neutralize their charge but without being precipitated as the insoluble deoxycholic acid. Instead, they are arranged into the helical structure. Probably such uncharged carboxyl groups are situated in the inside, hydrophobic part of the structure; it seems likely that the extra proton is used to form a hydrogen bond. In this way the mutual repulsion within the system is reduced while the internal en-

(10) J. D. Bernal, D. Crowfoot and I. Fankuchen, *Phil. Trans. Roy. Soc. London, Ser. A*, **239**, 135 (1940).

(11) P. Debye, *J. Phys. Chem.*, **53**, 1 (1949).

TABLE II

LIST OF STEROIDS TESTED FOR COMPLEXING ABILITY[a]

Steroid	Solubility	Viscosity meas.
Cholic acid	Soluble in alkali and in neutral range	0.008 *M* solution at *p*H 7.0; no change in viscosity
Glycocholic acid	Soluble in alkali and in neutral range	0.008 *M* solution at *p*H 7.0; no change in viscosity
Dehydrocholic acid	Soluble in alkali and in neutral range	0.008 *M* solution at *p*H 7.0; no change in viscosity
$3\alpha,12\alpha$-Dihydroxy-7-ketocholanic acid[b,c]	Soluble down to *p*H 8	Insufficiently soluble at *p*H 7
Hyocholic acid	Soluble in alkali. Below *p*H 10, solubility only about 0.002 *M*	Insufficiently soluble at *p*H 7
Hyodeoxycholic acid[c]	Soluble in alkali and in neutral range	0.01 *M* solution in 0.2 *M* phosphate buffer at *p*H 7.5; no change in viscosity
Lithocholic acid	Soluble only in alcohol and strong alkali	See text
Chenodeoxycholic acid[c]	Soluble down to *p*H 8	Insufficiently soluble at *p*H 7
Apocholic acid[d]	Soluble in alkali and in neutral range	0.2 *M* solution at *p*H 7.3, 7.0 and 6.6; no change in viscosity
Nordeoxycholic acid[b]	Soluble in alkali and in neutral range	0.01 *M* solution in 0.2 *M* phosphate buffers at *p*H 7.0 and 6.6; no change in viscosity

[a] Materials are commercial preparations unless otherwise designated. [b] Sample kindly supplied by L. F. Fieser. [c] Sample kindly supplied by G. A. D. Haslewood. [d] Sample kindly supplied by E. Titus.

ergy is increased, permitting the particle to grow to great length. As in the case of normal micelles, high ionic strengths also favor particle growth; and in strong salt the helical form appears at a higher *p*H. There is an equilibrium between the complexed helix form of deoxycholate and the ion in free solution. The energy of this transition is strongly dependent on the ionic strength, which in turn affects the equilibrium.

Each individual helix has a diameter of 36 Å. and is likely to be a rather rigid structure. If the complex forms initially in an undisturbed medium, these rod-like structures will grow in all directions, forming a matted matrix of high viscosity which will eventually be recognized as a gel. Since there are no covalent bonds to hold the helix together, the rods are rather brittle and the effect of shearing forces would be to break them down into shorter pieces. Greater shear gradients will result in shorter pieces, thus accounting for the lower final viscosity in the more rapidly stirred sample of Fig. 5.

Additional hydrogens ions are absorbed by the complex when the solution is subjected to stirring action. Thus, a subsequent process must occur in addition to the initial complex formation. The nature of this secondary process is not at all clear. It is possible, for example, that the rod-like fragments of the broken matrix may aggregate together in a lateral fashion. Since these rods are negatively charged such aggregation could be accompanied by the absorption of additional protons onto carboxyl groups near the outer surface of the cylindrical particle. If a process of this type were occurring then one would expect the longer rod-like particles to aggregate laterally to a greater extent and, accordingly, to absorb more protons than a solution made of shorter particles. However, such an interpretation would have to be subjected to further experimental verification before it can be taken seriously.

Although the behavior of these helical aggregates is in many ways typical of micellar association, we should emphasize that some features are not typical of micelles. The first of these is an extremely specific requirement for molecular structure. Of the numerous similar bile salts believed to form micellar aggregates, only lithocholic acid shows any evidence for forming this type of helical complex. Removal of a methylene group from the side chain (nordeoxycholic acid) or introduction of a double bond into the steroid skeleton (apocholic acid) is sufficient to destroy all complexing properties. A second distinctive feature is the fact that the X-ray diagram shows crystalline details to a spacing of less than 2 Å. which indicates that the internal structure of the helical complex is quite definite. On the other hand, studies of soap micelles have always concluded that the internal hydrocarbon structure is "liquid." This point has been emphasized by Luzzatti and his collaborators in recently published X-ray diffraction studies.[12]

The reason for these two effects must be the formation of a highly specific, energetically favorable, helical structure in which the van der Waals stacking of the irregularly shaped steroid molecules and the O-H—O hydrogen bond must play their part. The charged carboxyl groups must lie on the cylindrical surface of the complex, but it is quite likely that some carboxyl groups are involved in hydrogen bonding and accordingly are uncharged. They may be buried in the structure. A direct confirmation of these ideas might be obtained from a detailed structure determination by X-ray diffraction.

Acknowledgments.—We are indebted to Mrs. Barbara Farley for technical assistance. We also wish to thank Dr. E. Titus, Professor L. F. Fieser and Professor G. A. D. Haslewood for their generosity in supplying us with samples of various steroids.

(12) V. Luzzatti, H. Mustacchi and A. Skoulios, *Discussions Faraday Soc.*, **25**, 43 (1958).