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The Stability of the Micelles in Bile Acid Salt Solutions of Different Acidities

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Recent research [1–6, 17] has proved that micelle formation occurs in aqueous solutions of many of the bile acid salts (sodium cholate, desoxycholate, glycocholate, taurocholate). This micelle formation begins at relatively low critical concentrations and progresses slowly at first with increasing salt concentration [6, 17]. Upon addition of a strong acid to the solutions of these salts, the bile acids are liberated sooner or later. The possibilities for the preservation of the micelles formed by bile acid anions or the formation of micelles by the undissociated bile acid molecules or by both anions and acid molecules together are determined primarily by the strengths and solubilities of the bile acids. In respect of these latter properties, wide variations are noted [7–11]. By following potentiometrically the variation of the hydrogen ion concentration on addition of hydrochloric acid and by taking note of the other changes occurring in the solutions we have attempted to obtain knowledge of the pH ranges in which micelles are able to exist and in which micelles are no longer stable.

The pH values of the solutions were measured using a glass electrode and Radiometer valve potentiometer, Type PHM 3. Figs. 1 and 2 show typical titration curves for sodium desoxycholate, sodium cholate, sodium glycocholate and sodium taurocholate. In the first two cases the liberated bile acids separated as crystals from the solutions after a relatively small addition of hydrochloric acid, in the case of sodium glycocholate a fairly large amount of hydrochloric acid was necessary, whereas with sodium taurocholate no separation occurred. This is in accordance with the acid strengths and solubilities of these acids [10, 7], the former increasing and the latter decreasing in the order mentioned. Josephson has pointed out that some of the bile acids

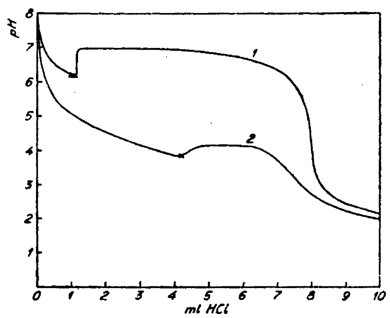

Fig. 1. Potentiometric titration of sodium cholate and sodium glycocholate with 0.1 N hydrochloric acid.

1. 20 ml 0.04 M Sodium cholate.
2. 20 ml 0.04 M Sodium glycocholate.

△ *The solution becomes turbid.*
× *Crystals begin to precipitate.*

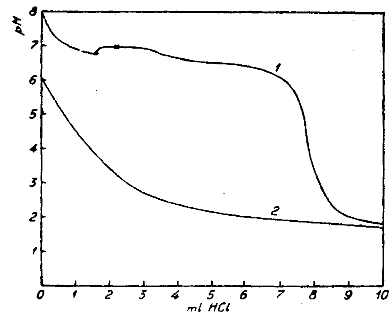

Fig. 2. Potentiometric titration of sodium desoxycholate and sodium taurocholate with 0.05 N hydrochloric acid.

1. 20 ml 0.02 M Sodium desoxycholate.
2. 10 ml 0.1 M Sodium taurocholate.

△ *The solution becomes turbid.*
× *Crystals begin to precipitate.*

(cholic, desoxycholic, glycocholic acid) easily form supersaturated, sometimes colloidal solutions. In our experiments this tendency was appreciable. When the bile acid salt concentration is above the critical concentration for micelle formation the liberated bile acid obviously is solubilized in the micelles, but also in more diluted salt solutions the acids are not always precipitated immediately.

From the titration curves it is seen that desoxycholate and cholate micelles are not generally able to exist in solutions whose pH values are below 6.5—6. In cholate solutions which temporarily were supersaturated with cholic acid, however, we have measured pH values down to 5. — Glycocholate-glycocholic acid micelles are stable down to pH 4.5—4.0 and taurocholate-taurocholic acid micelles still at pH 2—1.

The bile acid salt micelles are good solubilizers of various hydrocarbons [5, 6, 12, 13, 17]. The different stabilities of the micelle systems in media of different acidities become evident as a variation in the solubilizing power at pH values below 7. This is shown by experiments with bile acid salt solutions containing the strongly fluorescent polycyclic hydrocarbon 3,4-benzpyrene solubilized in the micelles. In a 0.1 *M* sodium cholate solution the fluorescence remains unaltered on adding hydrochloric acid even after cholic acid has begun to precipitate (fluorescence measured in the filtered solution). A continued further addition of hydrochloric acid resulted in a lowering of the concentration of the micelles and a precipitation of increasing amounts of the benzpyrene along with the cholic acid. Only after the pH had fallen below 6 did the fluorescence begin to decrease more rapidly and it disappeared altogether when the concentration of undecomposed cholate decreased below the critical concentration. In a similar experiment in which the solution studied was a 0.05 *M* sodium glycocholate solution containing benzpyrene, the fluorescence remained constant to pH 4.4. At this pH the glycocholic acid began to precipitate and this resulted in a gradual lowering of the fluorescence intensity (of the filtered solution). The fluorescence disappeared altogether at about pH 3.5 when the greater part of the salt had been decomposed. — In taurocholate solutions (0.1 and 0.05 *M*) the fluorescence of benzpyrene remained unchanged even after three times the equivalent amount of hydrochloric acid had been added and the pH had fallen below 1.5.

Bile is an association colloid solution in which the micelle-forming substances are various bile acid and fatty acid salts (in human bile primarily taurocholate, glycocholate, oleate), probably also cholesterol and lecithin. When hydrochloric acid is added to fresh ox bile, a titration curve with two jumps (Fig. 3, 1) is obtained and precipitation occurs at a pH below 4.5. As the bile becomes older, it becomes more alkaline and the titration curve passes through higher pH values, without, however, changing its form (Fig. 3, 2). The fluorescence of benzpyrene solubilized in bile did not disappear on the addition of hydrochloric acid, even when the pH attained the value 1.

These results indicate that the micelle systems in solutions of certain bile acid salts and in bile itself are stable at the low pH values which prevail in the stomach and consequently that even in such media they are still able to solubilize fat-soluble substances. This is the case at least with the taurocholate-taurocholic acid micelles. At the higher pH values which may sometimes prevail in the stomach in conditions of low acidity, the same may also apply to the micelle system glycocholate-glycocholic acid. When bile regurgigates into the stomach, an association colloid solution enters the stomach, which, thus, in spite of the acidity, is able to solubilize and keep solubilized fat-soluble substances such as the carcinogenic polycyclic hydrocarbons.

After our previous studies had shown that carcinogenic hydrocarbons solubilized in aqueous solutions of different association colloids easily penetrate into the skin of mice [14], the question arose whether these aqueous solutions are also able to assist the penetration of carcinogenic hydrocarbons into the walls of the glandular stomach through the protecting mucin layer. We continued our studies with the working hypothesis that such a penetration should be possible if the micelles are stable in the acid media of the stomach [15]. The experiments described above show that as far as the stability of the micelles

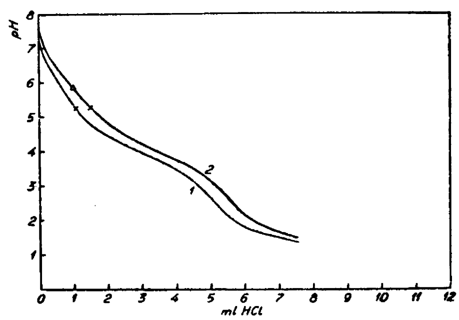

Fig. 3. Potentiometric titration of ox bile (25 ml) with 1 N hydrochloric acid.

1. Fresh ox bile.
2. Four hours' old ox bile.

is concerned certain bile acid salts and bile itself should be able to assist the penetration of carcinogenic hydrocarbons. Experiments which have been conducted later with animals have confirmed that such a penetration can be effected with solutions of these substances [16].

SUMMARY

A potentiometric titration study has been conducted to determine the pH ranges at which micelles are stable in aqueous solutions of the bile acid salts. In solutions of desoxycholate and cholate, micelles are generally not able to exist below pH 6.5—6.0, but in glycocholate solutions micelles are present down to pH 4.5—4.0 and in the taurocholate solutions even to pH 2—1, primarily owing to the greater strengths and solubilities of glycocholic and taurocholic acids.

Our studies of the ability of these solutions to solubilize the polycyclic hydrocarbon 3,4-benzpyrene confirm these findings.

Ox bile also contains micelles which are able to solubilize benzpyrene, even at pH 1.

The results have a bearing on the possible significance of the bile for the development of gastric cancer via chemical carcinogenesis.

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