

Fig. 3. Crossed Nicols. 200 \times .

The appearance of the spherical particles does not change when the temperature rises, but the spool-shaped mesomorphous substance does undergo a change. When the latter substance is warmed in its mother liquor on a heated stage between 34 and 38°C, it changes into a weakly doubly-refracting semiliquid mass (Fig. 3). The same change occurs when the mesomorphous substances that have separated from solutions containing different caprylate concentrations: it increases from about 34°C for the substance that is formed in solutions at the highest concentration studied to about 38°C for the substance formed near the CMC of sodium caprylate.

Thus cholesterol forms at least two water-containing mesomorphous phases under the influence of caprylate solutions at 20°C. The two phases are formed at different caprylate concentrations and they differ in appearance and temperature stability. Whether the mesomorphous phase that is produced when the spool-shaped liquid crystals are warmed to 34–38°C is identical with the phase that separates from highly concentrated caprylate solutions or represents a third mesomorphous phase has not yet been investigated.

These studies confirm that the chain length of the parent fatty acid is of decisive

importance in determining the properties of the water-containing cholesterol mesophases and the conditions under which these phases are produced.

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Studies on the Occurrence of Cholesterol in Water-Containing Liquid-Crystalline Form

IV. The Solubility of Cholesterol in Sodium Caprylate Solutions at 20°C

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It has been stated in the literature that cholesterol is almost insoluble in water¹ and we have been unable to find any reliable solubility data for this sterol. It is, however, known that the solubility of cholesterol in water increases appreciably in the presence of association colloids of the bile salt type owing to micellar solubilisation. Thus, Bashour and Baumann have reported rather high solubilities of cholesterol in solutions of different bile acid salts². Also Ekwall has found cholesterol to dissolve in appreciable amounts in sodium cholate solutions. However, the values of the latter author were much lower than the values given by the former³. Values of the solubility of cholesterol in solutions of fatty acid salts or other association colloids of the paraffin-chain type seem not to have been reported.

We have found that cholesterol in the finely divided form in which it exists after its solubilisation in association colloid micelles undergoes rapid oxidation at temperatures over 40°C. This confirms the observation of Bergström that cholesterol in the colloidal state (in the presence of sodium stearate) is oxidised at high tem-

peratures ⁴. In order to avoid this oxidation of cholesterol we have carried out our experiments in a nitrogen atmosphere at 20°C. We saturated the association colloid solutions with cholesterol by shaking and vigorously mixing the solutions with a magnetic stirrer over long periods (1–5 weeks). The excess of solid cholesterol or cholesterol-containing mesophases that were formed under certain conditions were separated from the solution by combined centrifugation and filtration through fine membrane filters in sealed filter tubes. These precautions were found necessary to effect a complete separation of the very finely divided matter. The cholesterol in the clear solutions was determined spectrophotometrically by the method of Zlatkis, Zak and Boyle which is suitable for the measurement of cholesterol in amounts down to about 0.01–0.05 mg.⁵

The most reliable determinations gave a solubility value of only 0.06–0.07 mg of cholesterol in 1 000 g of water. We found that the solubility of cholesterol was already slightly higher (several tenths of a milligram to about one milligram in 1 000 g of solution) in sodium caprylate solutions below the critical micelle concentration (CMC). Owing to the variation of the measured solubility values, we are unable to report any reliable solubility data for this concentration range. A definite increase in solubility is, however, observed when the sodium caprylate concentration exceeds the CMC, 0.36 *M*. (In this paper *M* = moles sodium caprylate/1 000 g of cholesterol-free solution). Already in a 0.39 *M* caprylate solution, the solubility is 2 mg of cholesterol in 1 000 g of solution and in a 0.56 *M* caprylate solution the solubility is about 60 mg of cholesterol in 1 000 g of solution. The solubility then increases more rapidly with increasing caprylate concentration and is 1 000 mg of cholesterol in 1 000 grams of the 1 *M* solution and about 5 000 mg in 1 000 grams of the 1.5 *M* solution. The solubility increases even more rapidly in more concentrated caprylate solutions, being about 20 g in 1 000 g of the 2 *M* solution and about 50 g in 1 000 g of the 2.4 *M* solution. In the latter range of caprylate solutions, the solubility of cholesterol is hence about 200 000–500 000 times as great as in pure water (Fig. 1).

This great increase in solubility depends, of course, on the solubilising power of sodium caprylate which increases markedly with increasing colloid concentration. The different solubilisation powers in various

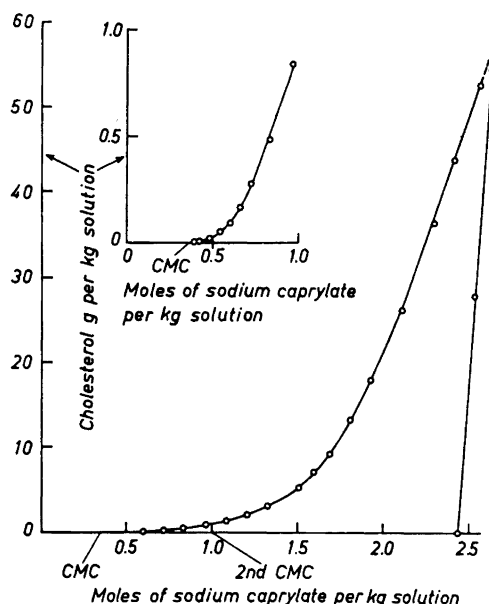


Fig. 1.

concentration ranges becomes more clearly evident if the solubility is divided by the content of micellar sodium caprylate and the increase of this ratio with the caprylate

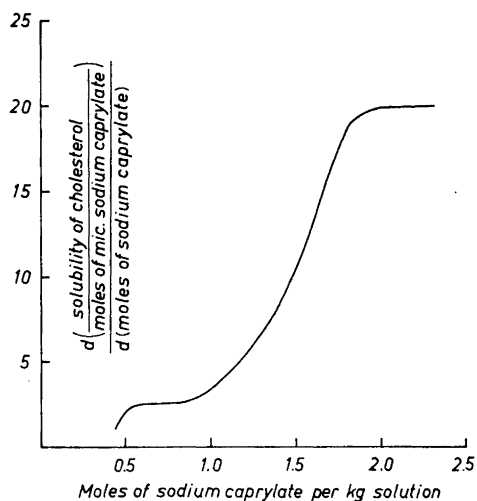


Fig. 2.

concentration is plotted as a function of the concentration (Fig. 2). A pronounced increase in the power of micellar caprylate to solubilise cholesterol is observed between 1.0 and 1.9 *M* caprylate. Below the first-mentioned and above the last-mentioned concentration the solubilising power of the micelles changes only little with the concentration. Our earlier studies have revealed that the structures of pure caprylate solutions change at approximately these same concentrations⁶. The concentration range from the CMC, at 0.36 *M*, to about 1 *M* (0.38 *M* → 1.2 *M*, *i.e.*, moles per 1 000 g of water) is known as the so-called small micelle range where the properties of the micellar substance are constant and the micelles are relative small spheres. Above the last-mentioned concentration (the so-called 2nd CMC), the properties of the micellar substance change evidently as a result of the formation of larger micelles which are no longer spherical in form. Above the concentration of about 1.9–2.0 *M* (*i.e.* 2.8–3.0 *M*) a further change has been observed to take place in the properties of the micellar substance and this has been attributed to the formation of micelles of cylindrical form which rapidly increase in length with increasing colloid concentration. It is of considerable interest that there is a relationship between the solubility of cholesterol and the micellar structure of the caprylate solution. Similar variations in the solubility of cholesterol with the structure of the solution would be expected with other association colloids.

In the solubilisation mixed micelles composed of caprylate ions and cholesterol are formed, but nothing definite is known about their composition. It is probable that there exists an equilibrium between these mixed micelles and pure caprylate micelles similarly as in the solubilisation of other lipophilic compounds with large molecules such as steroid hormones and polycyclic aromatic hydrocarbons^{7,8}. It is also likely that the mixed micelles, at least those formed in the small micelle range, do not contain more than one cholesterol molecule to a micelle. As is usual, the large hydrocarbon part of cholesterol is incorporated within the micelle and the hydrophilic hydroxyl group, like the carboxyl groups of caprylate ions, is probably located at the surface of the micelle in contact with water.

In the formation of these mixed micelles ion-dipole interactions and hydrogen-bond formation between the hydroxyl groups and the adjacent ionised carboxyl groups play a decisive part similarly as in the solubilisation of other amphiphilic compounds such as paraffin-chain alcohols and fatty acids. The mixed micelles in question are evidently sparingly soluble in water for when more cholesterol is added to the system the mixed micelle substance separates from the isotropic solution together with the excess of cholesterol as a mesomorphic phase composed of cholesterol, sodium caprylate and water. We have previously reported⁹ that the mesomorphic phase that separates from caprylate solutions between the CMC and a concentration of about 1.9–2.0 *M* (*i.e.* 2.8–3.0 *M*) differs in type from that which separates from more concentrated caprylate solutions in which the pure caprylate micelles, and probably also the mixed micelles, have a structure different from the structure of the micelles in solutions below the last-mentioned concentration limit.

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