tions just above the LAC differs somewhat in appearance from the phase formed at higher concentrations.

It may be of biological significance that the lowest concentrations at which fatty acid salts are capable of transforming solid crystalline cholesterol into the mesomorphous state varies from acid to acid and decreases regularly with increasing chain length of the parent acid. This means that aqueous solutions containing fatty acid anions in very low concentrations (only about 2.5×10^{-4} M in the case of oleate, for example) are able to effect the transformation of solid cholesterol into the mesomorphous state. If cholesterol separates from an aqueous biological medium in which the fatty acid anion concentration exceeds the LAC, it is thus probable that the cholesterol does not separate in the usual crystalline form, but rather as a water-containing mesomorphous phase.

We have found that cholesterol occurring in the mesomorphous state is not stained by oil-soluble dyes such as Sudan Red III and IV, Oil Blue N and Oil Red 4B, which are employed to detect cholesterol in tissue specimens. This was established in experiments where the dye was initially dissolved in the solid cholesterol crystals or in the micelles of the soap solutions. It therefore is doubtful whether cholesterol that occurs in the mesomorphous state in tissues can be identified by the customary staining methods.

1. Steiger, A. *Microcosmos* **35** (1941/1942) 54.
2. Dervichian, D. *Bull. soc. chim. biol.* **28** (1946) 419.
3. Lawrence, A. S. C. *Discussions Faraday Soc.* **25** (1958) 51; Durham, K. (Ed.) *Surface Activity and Detergency*, Macmillan, London 1961, p. 158.
4. Ekwall, P. *Proc. 2nd Intern. Congr. Surface Activity*, London (1957) Vol. I, 405. Butterworths 1957.

Received July 26, 1961.

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

II. The Formation of Cholesterol-Containing Mesomorphous Phases in the Presence of Protein-Association Colloid Complexes, Serum Albumin and Bile Acid Salts

PER EKWALL and HERRICK BALTSCHEFFSKY

Institute of Physical Chemistry, Åbo Akademi, Åbo, Finland

We have previously established that aqueous solutions containing protein-association colloid complexes are able to solubilize compounds such as polycyclic aromatic hydrocarbons, steroid hormones, fat-soluble vitamins and other substances which possess marked lipophilic properties [1]. When, however, cholesterol is added to these solutions, a mesomorphous water-containing phase separates in many cases. We have found that this occurs with solutions containing complexes formed by serum albumin and sodium dodecyl sulphate or fatty acid soaps, but not with solutions containing complexes formed by serum albumin and sodium taurocholate. As it is known that both sodium dodecyl sulphate and fatty acid soaps as such in aqueous solutions down to very low concentrations (as low as the LAC) are able to transform solid crystalline cholesterol into a mesomorphous phase composed of cholesterol, water and association colloid [2], it seems probable that the reason for the separation of the mesomorphous phase from solutions containing protein-association colloid complexes is the presence also in these solutions of free dodecyl sulphate and fatty acid anions in sufficiently high concentrations. The concentrations of these anions in the last-mentioned solutions, however, have not yet been determined. No formation of a mesomorphous cholesterol-containing phase has been observed to result from the interaction of cholesterol with aqueous solutions of taurocholate or other bile acid salts, and hence it seems natural that neither are their protein complexes able to effect a similar transformation of cholesterol. In these latter cases the interaction is limited to a solubilisa-

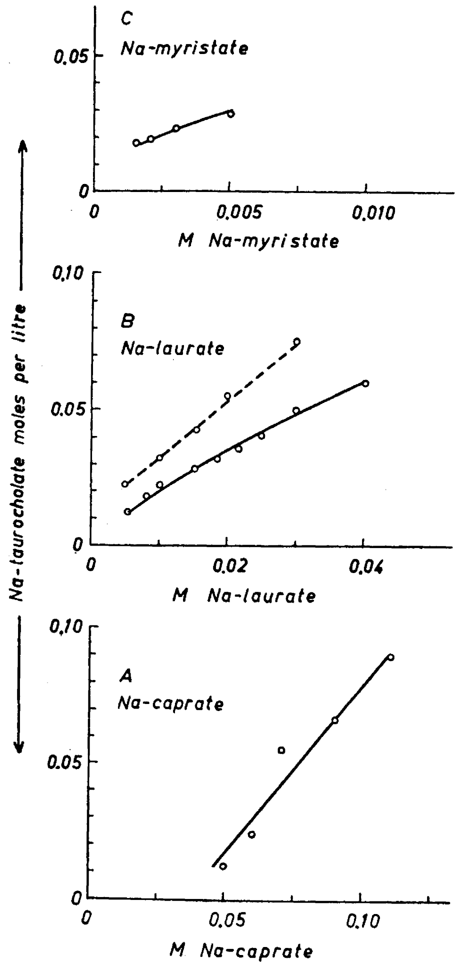

Fig. 1.

tion of cholesterol, whereupon homogeneous isotropic solutions are formed [3].

We have considered it of interest to study whether protein (serum albumin) or bile acid salts (sodium taurocholate) are able to promote or prevent the development of mesomorphous phases containing cholesterol, soap, and water by interaction with fatty acid ions. By microscope observations the minimum concentrations of fatty acid soaps that are required to transform cholesterol crystals into the mesomorphous state in the presence of various concentrations of the mentioned compounds were determined.

We have found that aqueous solutions of bovine serum albumin (Armour Laboratories) alone (0.5–2.0 %, pH 9.3) are unable to transform cholesterol into the mesomorphous state. Neither was bovine serum albumin in the mentioned concentrations found to have any effect on the interaction between sodium laurate and cholesterol; as in pure laurate solutions, the mesomorphous cholesterol-containing phase was in these cases formed as soon as the sodium laurate concentration exceeded 0.005–0.006 M.

Sodium taurocholate, on the other hand, was found to suppress the formation of the mesomorphous phase by interaction between cholesterol and fatty acid anions to some extent and necessitated an increase of the minimum concentration of soap anions to a higher value. The approximate minimum concentrations of sodium taurocholate that are sufficient to prevent the formation of the mesomorphous phase in various soap solutions are plotted in Fig. 1. The presence of 2 % serum albumin decreases the effect of taurocholate to some extent as shown by the broken line in Fig. 1 B.

It may be of biological significance that a protein such as serum albumin does not influence the separation of cholesterol as a mesomorphous phase. It is also of interest that an association colloid that does not cause such a separation to take place can obviously alter the conditions that promote the separation, but cannot completely prevent the latter from occurring.

1. Ekwall, P. *Proc. 2nd Intern. Conf. Biochem. Problems of Lipids*, Ghent 1955, 121. Butterworths, 1955.
2. Part I of this series. *Acta Chem. Scand.* **15** (1961) 1195.
3. Ekwall, P. *Proc. 1st Intern. Conf. Biochem. Problems of Lipids*. Brussels 1953, 103. *Koninkl. Vlaamse Acad. Wetenschap. Belgie*, 1953.

Received July 26, 1961.