for the natural product [1]. The two specimens gave identical R_F-values and infra-red spectra. The mother liquors from the purification gave crystalline fractions presumably representing partly racemized willardiine as estimated from infrared data.* Unchanged, crystalline L-2-(*p*-toluenesulphonamido)-3-(1-uracyl)-propionic acid (120 mg) separated from the original reaction mixture.

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* *Added in proof:* On comparison of the infra-red spectra with one of authentic, synthetic DL-willardiine, kindly furnished by Dr. G. Shaw, these fractions in fact appeared to be racemic willardiine. The infra-red spectrum of the latter deviated notably from that of the L-isomeride in the solid state.

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

I. The Minimum Fatty Acid Anion Concentrations Able to Transform Cholesterol Crystals into a Water-Containing Mesomorphous Form

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Information about the different forms in which cholesterol separates from aqueous solutions and in which cholesterol can occur in aqueous systems is of both physical-chemical and biological interest.

It has long been known that certain derivatives of cholesterol undergo a transformation into the liquid-crystalline state on heating (thermotropic mesomorphism). Cholesterol is also known to be transformed into the liquid-crystalline state by the action of solvents (lyotropic mesomorphism). Thus, for example, Steiger stated that cholesterol is transformed into a myelinic mesomorphous phase by water containing fatty acids and alkali [1]. Dervichian reported the formation of myelinic figures in the presence of water by mixtures of cholesterol with a number of biologically active long-chain substances, such as lysolecithin, alkylcholine chlorides, fatty acid salts, alkyl sulphates and alkyl phosphates [2]. Lawrence recently reported a series of observations on the transformation of solid crystalline cholesterol into a water-containing mesomorphous phase by the action of relatively concentrated association colloid solutions [3]. The liquid-crystalline substance that is formed in a 20 % sodium dodecyl sulphate solution containing an equimolar amount of cholesterol is stable on heating up to 195°C, where it separates into two isotropic liquids. When a solution of an association colloid such as sodium dodecyl sulphate flows over a cholesterol crystal, a gelatinous membrane composed of doubly-refracting matter is seen under the microscope to be formed immediately when the two phases come into contact. The membrane gradually increases in thickness and the solid crystal decreases in size, while at the same time the outer parts of the mesomorphous layer dissolve in the surrounding solution until finally a homogeneous solution results. Since 1956 similar observations have been made in our laboratory on the behaviour of cholesterol crystals in contact with solutions of sodium dodecyl sulphate, fatty acid soaps and albumin-dodecyl sulphate and albumin-soap complexes. We were first mainly interested in determining the lowest concentrations of these substances that interact and reported briefly a few years ago that the interaction between solid cholesterol crystals and aqueous solutions of various alkali metal salts of fatty acids can be observed until the solutions are diluted down to the limiting association concentrations (LAC) of the soaps [4]. In the first two papers of this series we present experimental data that define the limiting conditions necessary for the conversion of cholesterol into the mesomorphous state. In later papers the results of a more systematic study of the processes in question will be reported.

The cholesterol employed (Hoffmann-La Roche) melted at 148–149°C. The cholesterol was used partly in the anhydrous form and partly as the crystalline monohydrate. The salts of the fatty acids were prepared by dissolving the pure fatty acids in absolute ethanol and neutralising them with a solution of sodium ethylate in ethanol. The isolated dried soaps were dissolved in twice distilled water to obtain solutions of known concentrations. The interaction between the solid cholesterol crystals and soap solutions was followed with a microscope between crossed nicols and in ordinary (unpolarized) light with or without using a phase contrast condenser. The solutions were usually allowed to enter between the object and cover slides from one end until they came into contact with the cholesterol crystals. As the observations were made over long periods the cholesterol crystals were placed in thin plane-parallel cuvettes which were tightly sealed to prevent any evaporation.

The cholesterol crystals underwent no change when they were immersed in water, alkaline solutions containing sodium hydroxide or sodium carbonate, and sufficiently dilute aqueous soap solutions, but a strong interaction was observed when the crystals came into contact with soap solutions whose concentrations exceeded the respective critical micelle concentrations (CMC). In the latter experiments a layer of mesomorphous matter rapidly formed on the surfaces of the cholesterol crystals and increased in thickness until the whole crystal was transformed. The mesomorphous phase is, however, also produced in soap solutions with concentrations below the CMC, but the process slows down as the soap concentration decreases until it ceases altogether. Table 1 gives the concentration ranges where the formation of the mesomorphous phase definitely did not occur at 20°C. A weak formation of mesomorphous substance was still noted at the upper concentration limit, but no signs of the transformation were noted at the lower concentration limit although the crystals and solutions were in contact for long periods (24 h).

From the standpoint of the phase rule, this means that in the phase diagram of the three-component system cholesterol-soap-water one of the corners of a three-phase triangle lies in the given concentration range of homogeneous soap solutions, whereas the two other corners represent solid crystalline cholesterol and the mesomorphous cholesterol-soap-water phase in equilibrium with the soap solution in question. The triangle separates two two-

Table 1.

Association colloid	Concentration range below which no mesomorphous matter was formed.	LAC
Sodium nonylate	0.11 –0.14 M	0.06 M
Sodium caprate	0.020 –0.033 »	0.025 »
Sodium laurate	0.005 –0.0068 »	0.006 »
Sodium myristate	0.001 –0.0013 »	0.0011 »
Sodium oleate	0.00004–0.0003 »	0.00025 »

phase regions, one consisting of aqueous solutions of low concentration in equilibrium with solid crystalline cholesterol and the other of more concentrated aqueous solutions in equilibrium with the mesomorphous cholesterol-containing phase.

Values of the so-called limiting association concentrations (LAC) for the soaps in question are also given in Table 1. These have been determined by studying the interactions between aqueous solutions containing fatty acid salts and the respective undissociated fatty acids or these salts and liquid paraffin chain alcohols and represent the lowest fatty acid anion concentrations where such interactions are observed. The interaction of fatty acid anions and cholesterol referred to above which leads to formation of an aqueous mesomorphous phase containing cholesterol thus takes place down to approximately the same concentrations as these other processes.

The microphotographs in Fig. 1 illustrate the formation of the mesomorphous phase on cholesterol crystals immersed in 0.052 M (a) and 0.103 M (b) sodium caprate solutions. The mesomorphous phase grows out forming myelin-like figures, sausage- and thread-like formations. In Figs. 2a and 2b relating to a 0.30 M sodium caprate solution the mesomorphous phase occurs in the form of doubly-refracting spheres with characteristic black crosses. The mesomorphous phase formed at low concentra-

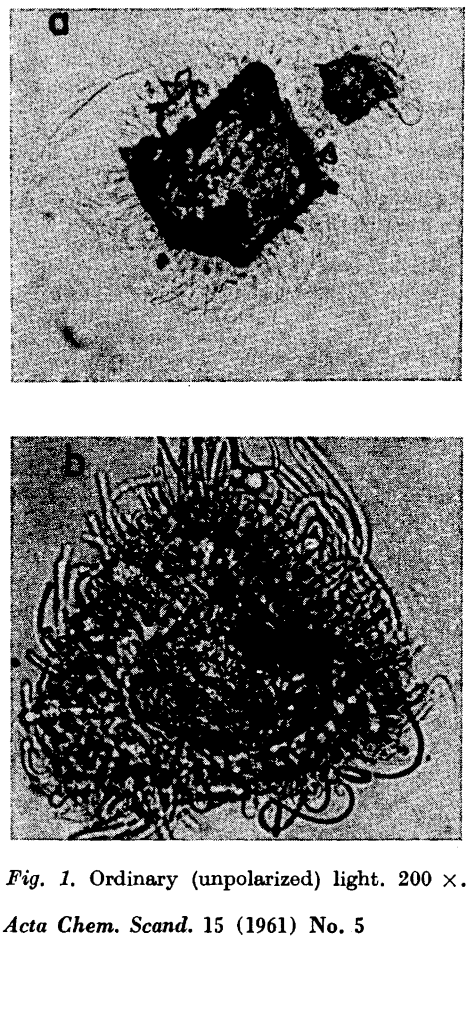

Fig. 1. Ordinary (unpolarized) light. 200 ×.

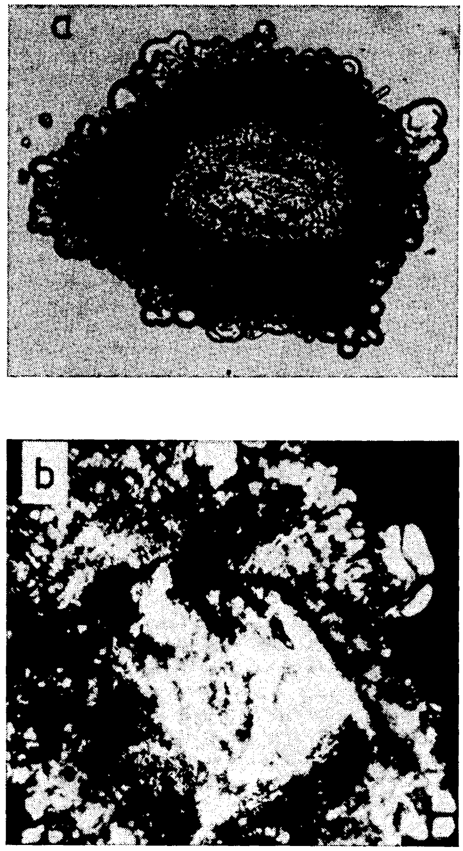

Fig. 2. a) Ordinary (unpolarized) light. 200 ×.
b) Crossed nicols. 200 ×.

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.

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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

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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-