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Thermal Properties of Systems Containing Cholesteryl Esters and Triglycerides

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Binary and ternary systems of the three cholesteryl esters, linoleate oleate, and stearate and the two triglycerides, triolein and tristearin were studied in order to determine the phase transitions and the conditions for the cholesteric and smectic mesophases. Phase transitions were determined using differential thermal analysis, melting point determination, and polarizing microscopy.

Of the cholesteryl esters the linoleate-oleate system showed complete miscibility in both the liquid and solid phases. The linoleate-stearate and oleate-stearate systems are of the eutectic type with limited solid solubility. The mesophases are monotropic as to the crystalline state and exist over the entire composition interval in all cholesteryl ester systems studied.

The triglycerides studied showed no mesophase transitions. In mixed systems with cholesteryl esters even low concentrations of the triglycerides removed the cholesteric transition typical for cholesteryl ester systems. At higher concentration of triolein the smectic mesophase was also removed. In systems with tristearin and cholesteryl esters an apparently smectic mesophase with mosaic texture was found. In ternary systems the property of the low melting lipids to depress the melting points of the high melting ones was found to be additive.

Cholesteryl esters and triglycerides belong to the major components of atherosclerotic deposits and serum lipoproteins and the phase conditions of these lipids serve directly to explain the state of the "oil phase" in atherosclerotic deposits and of the lipid core of serum lipoproteins.

Cholesteryl esters and triglycerides are abundant lipids in serum lipoproteins and atherosclerotic lesions.[1,2] Based upon their physical interactions with water both these lipid groups can be classified as insoluble, nonswelling amphiphilic lipids.[3] On the other hand considering their interactions with other lipids there are marked differences. Long chain cholesteryl esters have a very weak polar character, are solubilised at a low concentration in the lamellar mesophase made of egg lecithin and water [4] and do not form miscible films with surface-active lipids.[5] Therefore long chain cholesteryl esters have to be characterized as typical oil phase lipids. Triglycerides show more ambivalent interactions with other lipids. Triolein can be incorporated into the lecithin-water lamellar mesophase in rather high concentration.[6] In mixed films with surface-active lipids triolein forms partly miscible films, while tristearin forms miscible ones.[7] In mixed films with cholesteryl esters and surface-active lipids, the triglycerides together with the cholesteryl esters are squeezed out into an oil bulk phase.[8] The triglycerides then take up a mid-position between oil and interfacial lipids.

An outstanding feature of the physico-chemical properties of long chain cholesteryl esters is their tendency to exhibit thermotropic mesomorphism. In recent years the mesomorphic behaviour of biologically important cholesteryl esters has been intensively investigated.[9,10] Also binary mixtures of cholesteryl esters have been examined.[10,11]

The physico-chemical properties of cholesteryl esters and triglycerides are largely determined by the type of their fatty acid chains. Thus the melting point varies greatly between different esters: *e.g.* cholesteryl stearate 81.8 °C [11] and tristearin 73 °C [12] compared to cholesteryl oleate 50.6 °C [11] and triolein 4 °C.[10]

The physico-chemical state of lipid mixtures in biological systems is not only determined by the physical properties of the individual lipids but also by the interactions between them. An introductory study of the interactions between triglycerides and cholesteryl esters has been done by Small.[10] In order to clarify the role of the physico-chemical properties of lipids for example in the structure of lipoproteins and at the appearance of atherosclerotic plaques, systematic studies of model systems have to be done.

MATERIALS AND METHODS

The cholesteryl linoleate used was prepared by a modified acid chloride method.[13] Cholesteryl oleate and cholesteryl stearate were purchased from E. Merck AG and recrystallized from pentyl alcohol with subsequent washing in an ethanol-water solution. Triolein and tristearin were purchased from Fluka AG. The triolein was purified by Florisil column chromatography and the tristearin by recrystallization from acetone.

Mixtures of the lipids were prepared by dissolving the weighed components in chloroform which was then removed *in vacuo*. Ca 30 mg of sample were weighed in an aluminium pan which was placed in a Fisher Model 370 Differential Thermal Analyzer (DTA). The heating curves were obtained with a scan speed of 10 °C/min. until the sample was entirely melted. The sample was then cooled using a scan speed of 5 °C/min. When examining lipids with unsaturated fatty acid chains an atmosphere of N_2 gas was used.

The melting point values obtained for the lipid samples by DTA measurements were complemented by examinations with a Gallenkamp melting point apparatus. In order to identify the phase changes recorded by the DTA measurements a Wild polarizing microscope equipped with a hot stage was used.

RESULTS

Individual lipids. All three C_{18} cholesteryl esters showed monotropic behaviour; from one solid state they melt directly to an isotropic liquid. Upon cooling the isotropic liquid, cholesteric and smectic mesophases are formed before solidification. The unsaturated esters can exhibit a polymorphism in the solid state[11] but in mixtures of these esters precautions were taken to obtain the stable higher melting modification. The temperatures of the crystal-isotropic, isotropic-cholesteric, and cholesteric-smectic transitions for the three C_{18} cholesteryl esters were found to be 83, 75, 72 (stearate), 50, 44, 39 (oleate), 41, 35, 32 °C (linoleate), respectively. These transition temperatures are close to the values reported by other authors.[9–11]

Triolein melted directly to an isotropic liquid at 4 °C. Tristearin can exhibit a complex phase behaviour on heating.[12] Precautions were, however, taken to bring the tristearin into the triclinic β_L form from which it melted directly to isotropic liquid at 72 °C.

Mixtures of cholesteryl esters. On heating solid cholesteryl linoleate-cholesteryl oleate mixtures only one sharp melting endotherm was registered by DTA over the entire composition region. The phase diagram for mixtures of cholesteryl linoleate and cholesteryl oleate in Fig. 1 shows complete miscibility of the two esters in both the liquid and solid phases. On cooling from the isotropic melt, single homogeneous cholesteric and smectic mesophases were observed (Fig. 2a and b). The smectic-solid transition for mixtures of these cholesteryl esters was slow at low temperatures and could not be recorded by the DTA measurements.

In binary systems with one of the unsaturated cholesteryl esters (linoleate or oleate) and cholesteryl stearate the melting point of stearate is depressed continuously down to an eutectic point at a high concentration of the unsaturated ester (Fig. 3a and b). On heating,

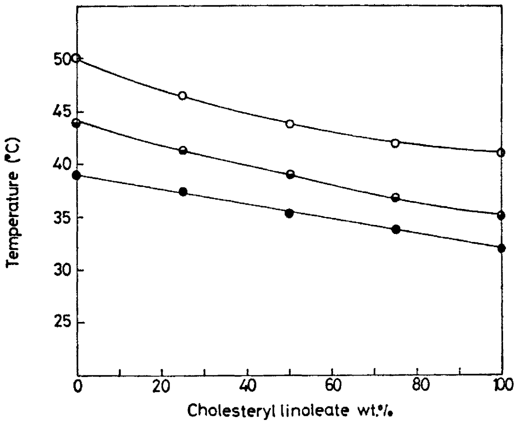

Fig. 1. Cholesteryl linoleate-cholesteryl oleate condensed binary phase diagram. Solid-liquid ○, liquid-cholesteric ◒, cholesteric-smectic ●.

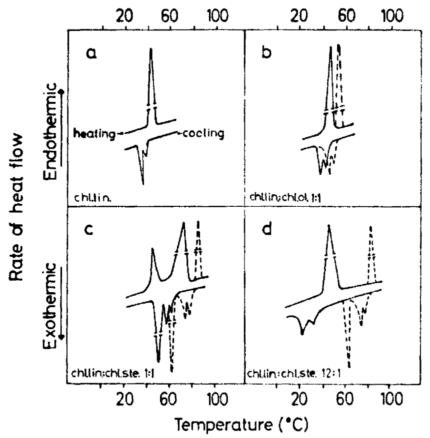

Fig. 2. DTA curves of the three C_{18} cholesteryl esters, linoleate (chl. lin.), oleate (chl. ol.), and stearate (chl. ste.) and mixtures of these. In the figures the first heating curves are above and the cooling curves below. (a) The heating curve shows the sharp melting endotherm and the cooling curve the two mesomorphic transitions (liquid-cholesteric and cholesteric-smectic) of linoleate, (b) the same transitions of oleate (dotted line) and a 1:1 mixture of linoleate and oleate (continuous line). (c) The heating curves show the sharp melting endotherm of stearate (dotted line) and two melting endotherms of a 1:1 mixture of linoleate and stearate (continuous line). The first peak in the curve of the mixture corresponds to the melting of the eutectic composition and the subsequent peak to the melting of the solid solution portion of the blend. On cooling, both stearate and the mixture show two mesomorphic transitions (liquid-cholesteric and cholesteric-smectic) and one crystallization exotherm. (d) DTA curves of a 12:1 mixture of linoleate and stearate (continuous lines) with a single melting endotherm and two mesomorphic transitions (liquid-cholesteric and cholesteric-smectic). The curves of stearate (dotted lines) are shown as a comparison.

the DTA curves for blends with composition within the miscibility gap gave two peaks; the first corresponding to the melting of the eutectic composition and the second one to the melting of the solid portion of the blend (Fig. 2c). At compositions approaching the eutectic point the second peak diminishes (Fig. 2d).

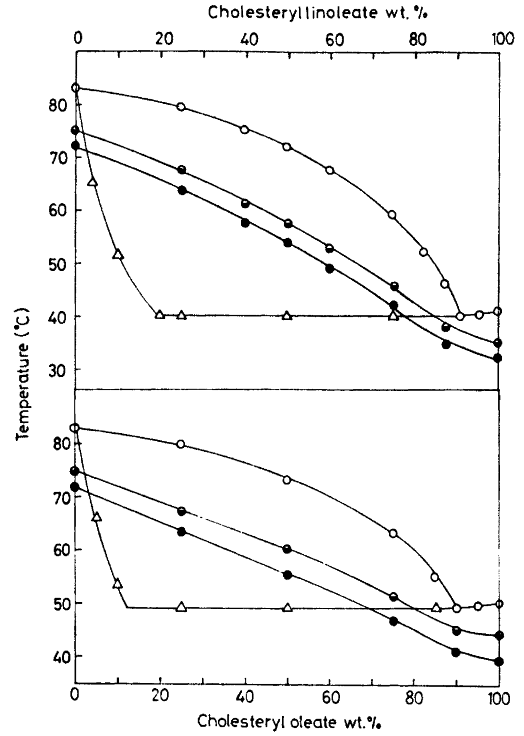

Fig. 3. Condensed binary phase diagrams for the systems (a) cholesteryl linoleate-cholesteryl stearate and (b) cholesteryl oleate-cholesteryl stearate. Solid-liquid ○, liquid-cholesteric ◒, cholesteric-smectic ●, solid-(solid+liquid) △.

On cooling isotropic melts of binary systems with one unsaturated ester and stearate, single homogeneous cholesteric and smectic mesophases were found to exist over the entire composition interval. On further cooling, crystals were formed from the smectic mesophase. For high concentrations of the unsaturated ester the smectic-solid transition took place slowly and could not be recorded by the DTA measurements.

In ternary systems the cholesteryl esters studied showed the same general features as in the binary systems. The melting point of the higher melting ester is depressed by increasing concentrations of the lower melting esters. This is shown in Fig. 4 where the proportion of cholesteryl stearate is held constant and the mutual concentrations of linoleate and oleate are changed. The linear curve for the solid-liquid transition shows that the melting point depressing property is additive in an ideal manner. On cooling from the isotropic melt, single homogeneous cholesteric and

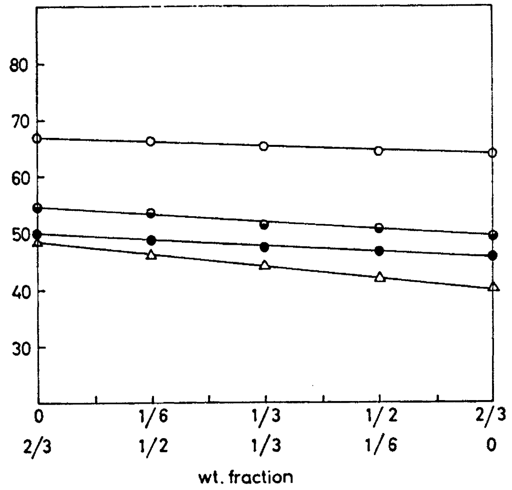

Fig. 4. Phase transitions in ternary systems of the three cholesteryl esters, linoleate, oleate, and stearate. Proportion of stearate is constant at the wt. fraction of 1/3 and the proportions of linoleate (upper abscissa) and oleate (lower abscissa) vary between 0 and 2/3. Solid-liquid ○, liquid-cholesteric ⊖, cholesteric-smectic ●, solid-(solid+liquid) △. Ordinate: Temperature (°C).

smectic mesophases, respectively, are formed. These transitions also show an ideal behaviour.

Mixtures of triglycerides. Tristearin has been reported to exhibit a complex phase behaviour on heating.[12] This property was also noted in mixtures with other lipids. In order to obtain correspondence between different measurements, attention was paid to always having the tristearin in the most stable triclinic β_L form. This form melts directly to isotropic liquid.

The melting point of tristearin is depressed by increasing concentrations of triolein (Fig. 5).

Mixtures of cholesteryl esters and triglycerides. The melting points of the cholesteryl esters are depressed by increasing concentrations of triolein down to an eutectic point at high proportion of triolein (Fig. 6).

On cooling from isotropic melt both cholesteric and smectic mesophases were recorded at very low triolein concentrations (Fig. 7a). Between 1 and 3 % triolein the cholesteric mesophase disappears. The smectic transition was recorded by DTA up to about 10 % triolein in mixtures with linoleate and up to about 25 % with oleate and stearate. By microscopic examination the smectic phase was observed at somewhat higher triolein concentrations. For mixtures with more than about 50 % triolein no mesophase can be observed and crystallization takes place directly from the isotropic liquid (Fig. 7b).

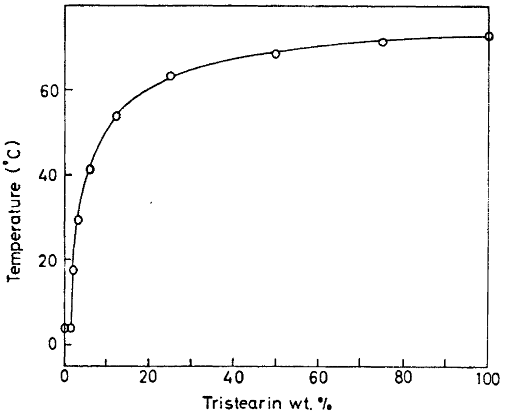

Fig. 5. The liquidus curve for the triolein-tristearin system. Tristearin melts from its β_L modification.

As seen in Fig. 8a and b the melting point of tristearin is depressed by increasing proportions of the unsaturated cholesteryl esters down to an eutectic point at high concentration of the cholesteryl ester. In mixtures of cholesteryl stearate and tristearin on the other hand the melting point of the cholesteryl ester, which is higher than that of tristearin, is depressed by increasing concentrations of tristearin down to an eutectic point at about 70 % tristearin (Fig. 8c).

On cooling from isotropic melt both cholesteric and smectic mesophases were exhibited at low tristearin concentrations (Fig. 7c). Between 3 and 6 % tristearin the cholesteric transition disappears. The smectic transition remains at high tristearin concentration but the microscopic texture changes considerably; a mosaic texture phase is formed (Figs. 9 and 10). At the same concentration as the mosaic texture appears, a peak on the DTA curve corresponding to crystallization of tristearin is noticed (Fig. 7d). The direct connection between the typical cholesteryl ester smectic mesophase in the region with low tristearin

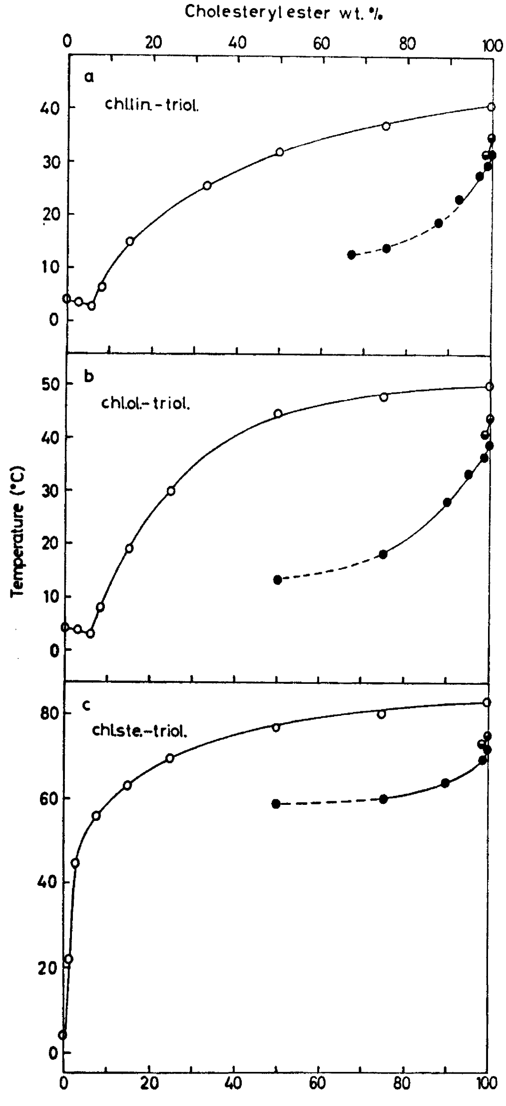

Fig. 6. The liquidus and mesomorphic transitions for the binary systems (a) cholesteryl linoleate-triolein, (b) cholesteryl oleate-triolein, and (c) cholesteryl stearate-triolein. Solid-liquid ○, liquid-cholesteric ◒, cholesteric-smectic ●.

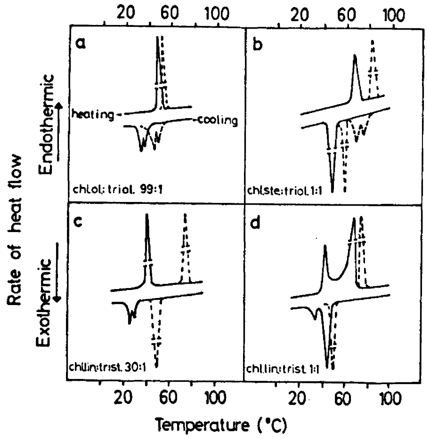

Fig. 7. DTA curves of mixtures of the three C_{18} cholesteryl esters linoleate (chl. lin.), oleate (chl. ol.), stearate (chl. ste.) and the two C_{18} triglycerides triolein (triol.) and tristearin (trist.). In the figures the first heating curves are above and the cooling curves below. (a) The curves with continuous lines show a single melting endotherm and two mesomorphic transitions (liquid-cholesteric, and cholesteric-smectic) of a 99:1 mixture of cholesteryl oleate and triolein. The curves of cholesteryl oleate are shown as a comparison (dotted lines). (b) Melting endotherm and crystallization exotherm of a 1:1 mixture of cholesteryl stearate and triolein (continuous line) compared with the curves of cholesteryl stearate (dotted lines). (c) Melting endotherm and two mesomorphic transitions (liquid-cholesteric and cholesteric-smectic) of a 30:1 mixture of cholesteryl linoleate and tristearin (continuous line). The melting endotherm and the crystallization exotherm of tristearin are shown by dotted lines. (d) the heating curves show the melting endotherm of tristearin (dotted line) and two melting endotherms of a 1:1 mixture of cholesteryl linoleate and tristearin (continuous line). The cooling curves show the crystallization exotherm of tristearin (dotted line) and two exotherms of the mixture (continuous line) the larger of which corresponds to the crystallization of tristearin and the smaller to the mosaic phase transition.

concentration and the mosaic texture phase seems to be plausible but is not structurally confirmed. At low concentration of the unsaturated cholesteryl ester the smectic transition cannot be recorded because of limited calorimeter sensitivity. However, the curve obtained by plotting composition *versus* the transition heat for the smectic transition indicated that this phase exists down to very low cholesteryl ester concentrations.

The interaction of cholesteryl stearate with tristearin is somewhat different from that of the unsaturated cholesteryl esters. At low tri-

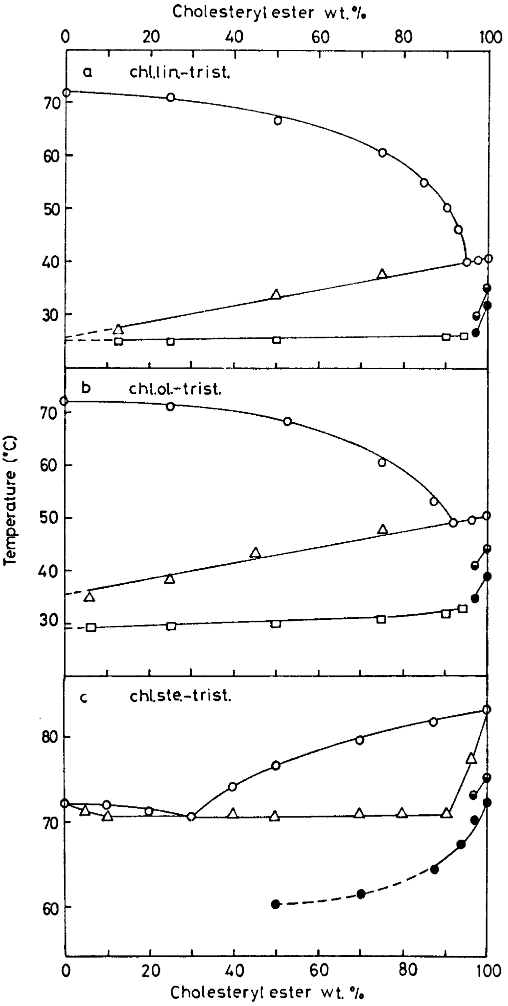

Fig. 8. Condensed binary phase diagrams for the systems (a) cholesteryl linoleate-tristearin, (b) cholesteryl oleate-tristearin, and (c) cholesteryl stearate-tristearin. Solid-liquid ○, liquid-cholesteric ⊖, cholesteric-smectic ●, liquid-mosaic smectic □, solid – (solid + liquid) △.

stearin concentrations cholesteric and smectic mesophases are exhibited. However, no mosaic type mesophase is formed at higher tristearin concentrations but the crystallization takes place directly from melt (Fig. 8c).

An addition of triolein to a binary cholesteryl ester mixture depresses the melting points of the esters and removes the mesophase transitions as for the individual cholesteryl esters.

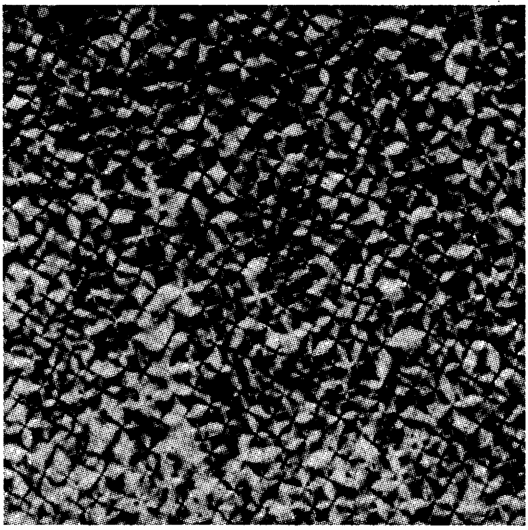

Fig. 9. Spherulitic texture of smectic phase in a system of 3 % tristearin and 97 % cholesteryl oleate. Crossed polars, × 400.

The melting point depressing effect of mixtures of triolein and either of the unsaturated cholesteryl esters on cholesteryl stearate and tristearin was found to be directly additive.

Mixtures of tristearin and the two unsaturated cholesteryl esters showed the same

Fig. 10. Mosaic texture of probably smectic phase in the stage of crystallization in a system of 10 % tristearin and 90 % cholesteryl oleate. Crossed polars, × 400.

characteristics as those of tristearin with the individual esters. The melting point depressing effect on tristearin was found to be additive.

BIOLOGICAL CONCLUSIONS

Schematically the arterial tissue and the blood can be divided into oil and water phases which are separated by specific interfaces. The cholesteryl esters and triglycerides are the major oil phase lipids. The physical state of the oil phase is determined by the physical characteristics of the lipids in it and the interaction between them.

The lipids in atherosclerotic deposits can be in a liquid, mesomorphic or solid state.[14] Living organisms require mobility which facilitates, *e.g.*, diffusion, transport, and enzyme function. This requirement for mobility is fulfilled by the liquid state. The viscosity of mesomorphic phases is usually considerably higher than that of the liquid state,[15] but it is still unclear to what extent this quality effects biological metabolization.

The physical characteristics of cholesteryl esters and triglycerides are greatly affected by the fatty acid residues bound in them. Of the lipids studied in this paper triolein is liquid, cholesteryl linoleate cholesteric and the rest solid at body temperature. A typical feature of the interaction between cholesteryl esters and triglycerides is that the low melting ones depress the melting points of the high melting ones. This effect is, however, rather small and an excessive incorporation of high melting cholesteryl esters and triglycerides causes a deposition of solid lipids in the arterial wall.[16]

A remarkable property of the triglycerides is their ability to remove the cholesteric phase in mixtures with cholesteryl esters. This is probably the explanation to why this phase has not been found in atheroschlerotic lesions.[14]

In serum lipoproteins the neutral lipids are supposed to be embedded in the lipid matrix.[17] The physical state of this can be either liquid or smectic. The cholesteric state can be excluded because of too high triglyceride contents. For low-density lipoproteins it can be concluded that the smectic state is the most probable one, because of the high concentrations of cholesteryl linoleate and cholesteryl oleate. For the chylomicrons the situation is different. They contain so much triglycerides that the liquid state is the most probable one. Thus information from model systems can elucidate the physical state of the lipids in biological systems.

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