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Phase Studies on Binary Systems of Cholesteryl Esters B. Three C_{18} Ester Pairs

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Phase Studies on Binary Systems of Cholesteryl Esters
B. Three C_{18} Ester Pairs

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Abstract—Three binary systems for the cholesteryl esters of C_{18} aliphatic acids, the stearate plus oleate, the stearate plus linoleate, and the oleate plus linoleate were studied in order to determine the phase transitions and the conditions for the cholesteric and smectic mesophases. Phase diagrams were determined using Differential Scanning Calorimeter (DSC–1B) and a polarizing microscope with hot stage.

Both individual unsaturated esters exhibit a polymorphism in the solid state. The metastable modifications are formed from highly supercooled melts. Prolonged standing produces recrystallization in the stable form. The formation and stability of metastable modifications are enhanced in investigated binary systems and result in complex behavior with solid–solid transitions and regions of metastability.

Both stearate systems are of a eutectic type with a limited solid solubility. A continuous series of a solid solutions over the entire composition interval was found in the oleate–linoleate system.

The mesophases in binary blends are monotropic with respect to the crystalline state. The mesophase transition temperatures are linear functions of the composition of the mixtures. The cholesteric and smectic mesophases exist over the entire composition interval in both oleate systems. No sufficient supercooling was achieved to observe mesophases in the midconcentration range for the stearate–linoleate system.

There are concentration regions in all three binary systems where mesophases can exist at body temperatures. The unsaturated and saturated cholesterol esters belong to the major components of artheriosclerotic deposits. From this standpoint, the mesomorphic behavior of mixtures of cholesteryl esters can be very important.

1. Introduction

A. Background

The esters of cholesterol are the prime series exhibiting the cholesteric mesophase. They represent a thoroughly studied class of mesomorphic substances. In the last years, the behavior of this ester series of aliphatic acids has been very intensively investigated. The temperatures, heats, entropies of transition, as well as heat capacities, are now available for many of the saturated esters from the C_1 to the C_{20} ester.(1–4) Thermodynamic data for the biologically important C_{18} unsaturated esters of cholesterol have also been recently published.(5) First studies have also been developed on behavior of multicomponent systems of mesomorphic substances.(6–10) Some studies of binary systems forming a cholesteric mesophase have also been reported.(11,12) Thermal transitions, phase relationships, and conditions for mesophase formation were determined for binary mixtures of cholesteryl esters, specifically for binary systems of myristate with acetate, nonanoate,(12) undecanoate, and stearate.(11)

B. Purpose

The properties of multicomponent ester systems are deserving of attention for several reasons.

1. *Purity.* The steroids are derived from natural products and thus are inherently susceptible to purity problems.

2. *Thermodynamics.* Thermodynamic indications also suggest purity could be crucial in determining the properties and stabilities of mesophases. The transition temperatures can vary sensitively with composition and the mesophase transition heats are uniformly small. Thus, thermodynamic studies of mixtures composed of individual pure compounds are from this standpoint, very important.

3. *Indicators.* The prediction of temperature ranges for mesophase stability in multicomponent systems is also significant for the development of the now widely-used cholesteric ester blends as temperature indicators.

4. *Health.* Multicomponent systems involving esters of cholesterol and possessing mesophase properties, are known to be of importance in arteriosclerosis. The deposits are predominantly complexes containing cholesterol, its saturated and unsaturated esters and lipoproteins in relative concentrations changing with age and disease.(13,14)

C. Plan

For the present study, three binary systems were chosen for the cholesteryl esters of C_{18} aliphatic acids: stearate–oleate, stearate–linoleate, and oleate–linoleate. Systematic studies on these esters are rare.(1,5,15,16) The physical chemistry and phase behavior of these esters and their mixtures is also of likely importance for understanding biological processes. Both unsaturated esters, for example, form a substantial part of cholesteryl esters content in human β-lipoprotein blood serum. Cholesteryl oleate represents 24% and cholesteryl linoleate 47% of the cholesterol present in the esterified state.(13)

2. Experimental

All three esters were obtained from the source in good purity, reportedly higher than 99%. Transition characteristics and other tests in this laboratory confirmed a purity in this range.(5) The cholesteryl stearate was from Applied Science Laboratories, State College, Pennsylvania, and cholesteryl oleate and linoleate from the Hormel Institute, Austin, Minnesota.

The solid–isotropic liquid, smectic–cholesteric and cholesteric–isotropic transition temperatures and heats were determined on Differential Scanning Calorimeter, Model DSC–1B, Perkin–Elmer Corporation, Norwalk, Connecticut.

Blends were prepared by directly weighing the pure esters in DSC sample cups on a Mettler balance to 0.001 mg. The composition was calculated on the basis of absolute purity for the individual esters. Because of similar molecular weights, 651.1, 649.1 and 647.1, weight and molar percentage are virtually the same. The blends were homogenized by heating at 95 °C for 30 minutes in a nitrogen atmosphere within sealed sample holder in DSC unit. The sample size, scanning rate and sensitivity were adjusted to achieve optimal data quality. Generally the sample size varied from 2 to 12 mg, sensitivity from 1 to 8 mcal per second per full scale deflection (10 mV) and scanning rates from 0.6 to 10 °C per minute.

The DSC was calibrated using the known transition temperatures and heats for high-purity indium, benzoic acid, naphthalene, and *p*-nitrotoluene. Temperatures for peak maxima have been taken as the transition temperatures. Transition heats were determined by measuring the areas under transition peaks with a planimeter (Gellman Instruments, Germany). The chart area was calibrated in calories per unit area using the standard samples. Recorder Model 56, Perkin–Elmer Corporation, was used for thermogram registration, DSC unit was operated with the low temperature cover using methanol and dry ice as coolant.

The transition temperatures and heats reported represent an average of several determinations. The mesophase transitions were determined both on cooling and on reheating from smectic mesophases. Because of temperature lags due to the instrument and samples, transition temperatures on cooling are lower than those on heating. The extent of this deviation varies with scanning rate.(12) Only the mesophase transition temperatures obtained on reheating from smectic mesophases are reported in this study.

In our experiments, and consistent with results of others, all three C_{18} esters are found to be monotropic with regard to the mesophases. On heating from solid state they melt directly to isotropic liquid; both cholesteric and smectic mesophases can be observed only upon cooling the isotropic liquid or on reheating from the mesophase. The transition temperatures and heats are presented in Table 1, together with previously reported values. The solid–isotropic liquid transition temperatures were obtained on heating after cooling the sample to -30 °C.

3. Individual Esters

Cholesteryl Stearate

Only a single endotherm was observed on heating from solid state (monotropic behavior). No effect of melt crystallization was noticed. The same solid–isotropic liquid transition, 81.8 °C, was found on heating solid recrystallized from *n*-pentanol. Temperature and heat of solid–isotropic liquid transition are in good agreement with previously reported values from this laboratory.(5) Both mesophase transition temperatures are also very close to the values reported by Sell and Neumann(16) and by Gray.(1)

Cholesteryl Oleate

These new results have shown monotropic polymorphism in the solid state. Two crystal modifications with different melting points were observed. Solid–isotropic liquid transition temperature is found to be 50.6 °C for the stable higher melting form and 49.1 °C for the metastable modification. These temperatures are close and the

TABLE 1 Cholesteryl Esters of C_{18} Aliphatic Acids

Compound transition	Sell and Neumann(16) Temp. (°C)	Gray(1) Temp. (°C)	Barrall *et al.*(15) Temp. (°C)	Boston University(5)		University of Massachusetts(5)		This Work	
				Temp. (°C)	Cal/g	Temp. (°C)	Cal/g	Temp. (°C)	Cal/g
Cholesteryl stearate									
Crystal–isotropic	82	83	85.0	82.0	21.4	81.8	25.8	81.8	25.35
Smectic–cholesteric	74	75.5	—	71.2	0.57	69.6	0.60	74.3	—
Cholesteric–isotropic	78	79.5	71.0	75.5	0.66	74.4	0.60	78.3	—
Cholesteryl oleate									
Crystal–isotropic				50.5	8.6	50.0	11.2	50.6 (49.1)[a]	11.2 (10.6)[a]
Smectic–cholesteric				42.0	0.56	41.8	0.37	39.25	—
Cholesteric–isotropic				47.5	0.25	46.4	0.18	45.10	—
Cholesteryl linoleate									
Crystal–isotropic				42.0	8.0	42.6	11.0	41.3	11.0
Smectic–cholesteric				34.0	0.78	34.0	0.54	32.6	—
Cholesteric–isotropic				36.5	0.27	35.1	0.20	33.5	—

[a] Lower melting metastable form.

endothermal peaks are difficult to separate in the thermograms when both forms are present. The higher melting modification was observed on first heating of the slowly solidified melt and was also in this state as received from the Hormel Institute. For this case an endotherm was observed over a narrow temperature range with sharp peak at 50.6 °C. Different DSC traces resulted on heating after premelting and cooling to − 30 °C. After very quick cooling followed by immediate reheating as soon as the temperature reached − 30 °C only the melting of the metastable form was observed with a peak maximum at 49.1 °C. The corresponding endotherm was lower and broader, the heat of fusion, 6.89 kcal/mole, was 0.38 kcal/mole lower than the heat of the stable form. When viewed as an entropy change, the lower melting form is 1.1 cal/mole/°K less ordered than the higher melting form. A similar difference 2 cal/mole/°K was found for two crystal forms of cholesteryl heptadecanoate.(17) This difference of entropy change is of the order of magnitude for a rotational disorder which could be present in the lower melting form.(17)

The overlapping endotherms for both forms were evident in thermograms obtained on reheating samples very slowly cooled (0.6 and 1.25 °C/min) to − 30 °C. They could be partially resolved on reheating at low scanning rates and high sensitivity; higher scanning rates resulted in one peak with a well-defined break at its leading edge. Both forms were also present when isotropic liquid was left to crystallize freely at ambient temperature. The relative amount of higher melting form slowly increased with time, however the lower form was still detectable even in samples after one month's storage. These tests indicated the slow transformation of the metastable form into the stable form. On simple recrystallization of the sample from *n*-pentanol only the higher melting form reappeared.

In systems with monotropic polymorphism in the solid state, it is generally easy to prepare the metastable forms. It is only necessary to prevent thermodynamic equilibrium by fast cooling which is more easily achieved with small samples. The DSC unit is thus very suitable for this evaluation. The metastable polymorph can be generally prepared by crystallization from highly supercooled melts. In the case of cholesteryl oleate it can be achieved by rapid cooling the isotropic liquid to − 30 °C. When slow cooling is used, the supercooling at the crystallization outset is not sufficient to prevent simultaneous nucleation of the stable form. Prolonged standing of solid produces complete recrystallization in the stable form.

Data published by Perkin–Elmer using the DSC–1B on cholesteryl oleate show also two well-separated endotherms at 47 and 50 °C and additional third endotherm followed by exotherm at 36 and 37 °C respectively.(18) Davis and Porter(19) observed only a single sharp endotherm at 51.8 °C with sample crystallized from *n*-pentanol. The explained this difference by the presence of impurity removable by crystallization from *n*-pentanol. The endotherm at 36 °C was also not detectable in any experiment with the pure oleate in this study. As will be shown later, the binary mixtures of oleate and stearate exhibited a well-expressed endotherm at 32–35 °C. This indicates that this endotherm in the DSC trace published by Perkin–Elmer Corporation could indeed be really due to impurity removable by crystallization from *n*-pentanol. This would also be consistent with a slight lowering of temperatures of melting (47, 50 °C) in comparison with new values here (49.1 and 50.6 °C). The presence of small quantity of impurity is also suggested by complete separation of both peaks which as will be shown later is enhanced by the presence of a second compound. In contrast, present results show clearly that the two endotherms observable at higher temperatures are as a consequence of the cholesteryl oleate polymorphism in the solid state.

Since different heating curves are obtained with solid oleate prepared by rapid or slow cooling, it is necessary to pay extra attention to this fact in purity determinations. It is necessary to have the stable solid form in order to obtain reliable curves. If certain precautions are not maintained, erroneous results can also be obtained in heat and entropy changes.

The temperature and heat for the solid–isotropic liquid transition found here for the stable form of the oleate is in good agreement with values previously reported by this laboratory.(5) The mesophase transition temperatures are lower than the corresponding temperatures found earlier (see Table 1).

Monotropic polymorphism with slow transformation of the metastable form into the stable form is a common phenomenon observed in long chain molecules like normal alkanes,(20) fatty acids or their esters.(21) As an example are the corresponding C_{18} hydrocarbon and oleic acid itself. Pure *n*-octadecane, mp 28.20 °C,(22) has a monotropic metastable melting point at 27.5 °C.(20) Oleic acid is known to crystallize on cooling first into a metastable form, mp 13.4 °C, which slowly changes into the stable form, mp 16.26 °C.(23) Correspondingly, fatty acids transform in several days; complete transformation for fatty acid methyl esters was not accomplished after several months.(24)

Cholesteryl Linoleate

All three reported transition temperatures were found at 0.8–1.6 °C lower than former values (see Table 1). The heat for the solid–isotropic liquid transition is the same as determined earlier here.(5) An additional very small endotherm at 32 °C was found on heating solid prepared by rapid cooling of melt to − 30 °C. This solid–solid transition has not been previously reported. The smectic mesophase can be supercooled without crystallization to a metastable glass. On heating spontaneous solidification occurs. There can be several reasons for observation of only very small solid–solid transition. It can be the formation of the metastable form itself; namely, that the supercooling at the crystallization outset is not sufficient to prevent simultaneous formation of the stable form. Also the different kinetic of nucleation can be important. The stable form with significantly higher nucleation rate can still preferably crystallize even from highly supercooled melts. It can also be due to the rapid transformation of the unstable form into the stable one.

Solid obtained from the melt by slow cooling did not exhibit additional endotherms on heating prior to the solid–isotropic transition.

It can be difficult to study polymorphism in the pure compounds. Polymorphism often shows up more clearly in binary systems. This has been demonstrated here for both oleate and linoleate in their blends and in their binary systems with stearate. It has also been shown that these effects are not due to imperfect crystals formed at rapid cooling.

4. Binary Systems

Cholesteryl Stearate–Cholesteryl Linoleate

The conditions for phase existence in this binary system are given in Fig. 1. Different designations are used for changes occurring upon

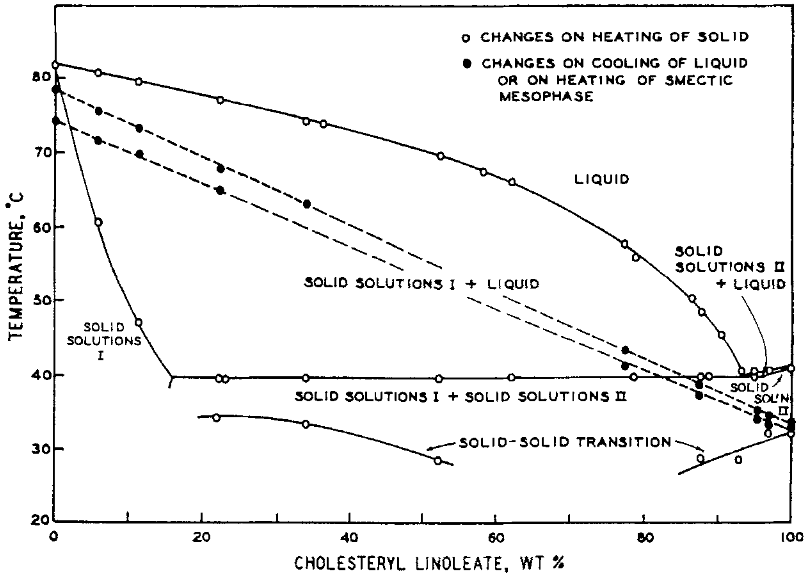

Figure 1. Phase diagram—cholesteryl stearate + cholesteryl linoleate.

heating from the solid state and upon either cooling from isotropic liquid or reheating from smectic mesophase. The data reveal a eutectic point at 39.9 ± 0.2 °C for a composition of 6% stearate and 94% linoleate. Two solid solutions containing 84% and ~3.5% stearate represent two solid phases which can exist in equilibrium with the eutectic melt. Experimental definition of the solidus and liquidus curves were obtained on heating solids prepared by slow cooling (0.6–1.2 °C/min) of melts to −30 °C. DSC curves for blends with composition within the miscibility gap have generally two peaks with the exception of the eutectic composition. The first sharp peak corresponds to the melting of the eutectic portion and the subsequent peak to the melting of solid solution portion of the blend. At compositions approaching the eutectic, the second peak diminishes becoming only a tail of the first peak. In this region, temperatures for the first peak and to the base line return after the second peak have been taken as eutectic and liquidus temperatures, respectively.

The limit of solid solubility of linoleate in stearate is difficult to determine unambiguously from only the heating curves. It was thus found independently by plotting composition versus the fusion heat for the eutectic portion of the blends, which is directly proportional to the amount of eutectic. The line drawn through the experimental points in Fig. 2 shows that no eutectic is detectable at $<16\%$ linoleate. The limit of solubility of stearate in linoleate cannot be defined because of limited calorimeter sensitivity. The endotherm of a blend containing 4.49% stearate can still be separated in two peaks at slow heating, 0.6 °C/min, and at high sensitivity. Since the area under the first peak, corresponding to eutectic melting, is smaller

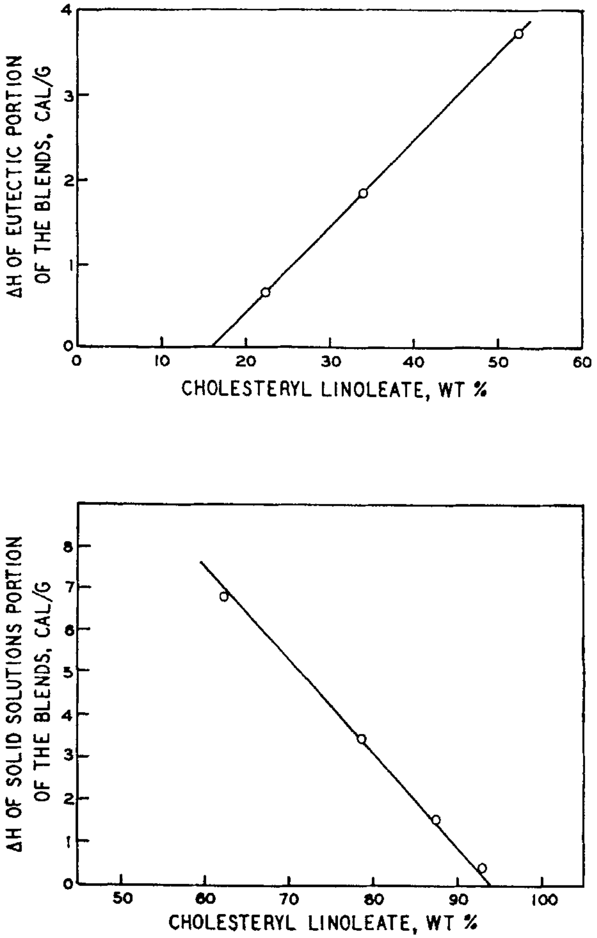

Figure 2. Limit of solubility of cholesteryl linoleate in cholesteryl stearate (top) and determination of liquid composition at eutectic (bottom).

than the area under the second peak, due to solid solution melting, the concentration is already closer to that of terminal solid solubility than to that of the eutectic. Only a single peak, without an indication of eutectic portion, is obtained with the blend containing 2.29% stearate. This composition is therefore already in the region of linoleate solid solutions. The terminal solubility in linoleate can be thus estimated at ~3.5% stearate.

Additional evidence on the eutectic composition can be found by plotting against composition the fusion heat for the solid solution portion of the blends (established as a difference between the total fusion heat and the eutectic portion). The line drawn through the points in Fig. 2 shows that no solid solution is detectable at ~6% stearate. The same value must provide the intersection of the curve

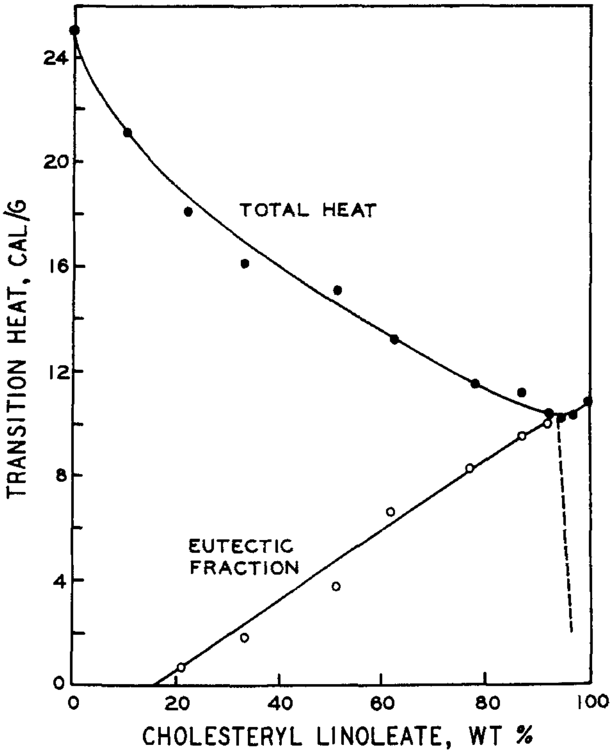

Figure 3. Crystalline melting transition heat cholesteryl stearate + cholestery linoleate.

representing the total heat and the line for the eutectic portion of heat in Fig. 3. This is essentially only a matter of different plotting. The dashed and steep line illustrates the sharp decline of the eutectic portion of the heat for blends between the eutectic and terminal linoleate solid solution.

Solubility of stearate in linoleate can be fairly well expressed by the modified equation for ideal solubility $\log x = A - B/T$ where x is mole fraction of stearate, T is absolute temperature and A and B are constants. The line drawn through points obtained by plotting the measured values for liquidus as $\log x$ against reciprocal temperature in Fig. 4 also indicates eutectic composition 6% stearate and 94% linoleate.

On heating solids after rapid cooling to −30 °C, an additional, small endotherm was observed prior to melting indicating a solid–solid transition. In binary mixtures with stearate, this endotherm becomes more distinct and larger than in the case of pure linoleate. This suggests that in mixtures either the formation of the metastable form is easier or the rate of transformation of the metastable form into the stable one is lower than in pure linoleate.

On cooling isotropic liquids at each end of the concentration scale, cholesteric and smectic mesophases were exhibited; on further cooling, stable crystals were formed from the smectic mesophase. It was also possible to determine mesophase transitions on reheating from the smectic mesophase. With increasing concentration of the second compound (with exception of the narrow concentration range close to the pure linoleate) increases the difference between the liquidus and the isotropic liquid–cholesteric transition temperature. It means that alignment of molecules into the cholesteric mesophase takes place from an ever more supercooled isotropic liquid. It, therefore, becomes a question of whether an isotropic mixture can be sufficiently supercooled to give rise to the liquid crystalline phases. From the phase diagram (see Fig. 1), it is clear that in the midconcentration range, a marked supercooling of the melt must take place. However, even in the mixture containing 34% linoleate, spontaneous crystallization took place immediately after the isotropic–cholesteric transition and no cholesteric–smectic transition was observable. For mixtures containing 52–63% linoleate it was not possible to sufficiently supercool to observe the mesophases. Therefore, no experi-

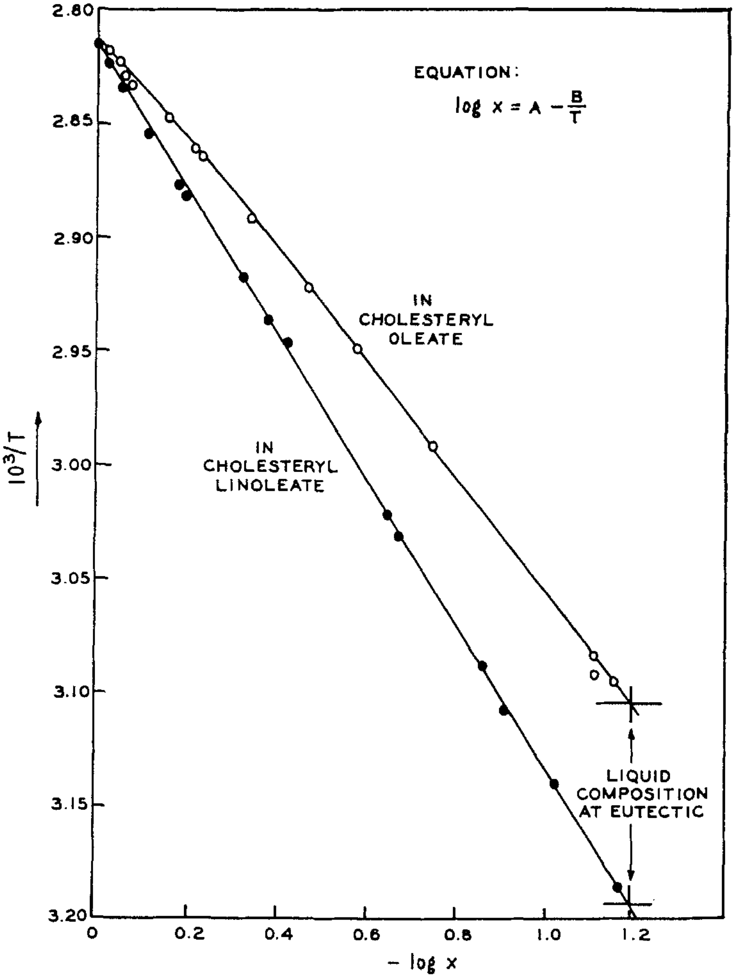

Figure 4. Solubility of cholesteryl stearate.

mental points are in Figure 1 for mesophase transitions in the mid-concentration range. The exact extent of this gap was not investigated.

Cholesteryl Stearate–Cholesteryl Oleate

The transitions on heating for this system illustrates Fig. 5. Since the mesophases for all compositions are monotropic, the figure represents the complex behavior of only the crystalline transitions which involves regions of metastability. Studies in the metastable regions permit investigation of the rate at which equilibrium is reached. Metastability which is normally enhanced in mixtures; may present practically-important and interesting situations than many cases when equilibrium does occur.

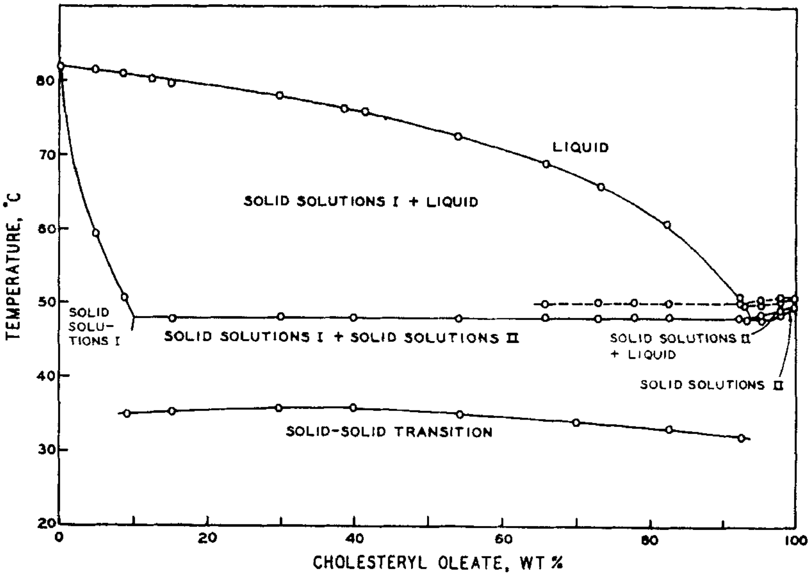

Figure 5. Transitions on heating from solid state—cholesteryl stearate + cholesteryl oleate.

This system shows a eutectic with a limited solid miscibility for each of two polymorphs of the oleate ester. The phase diagram for the metastable polymorph of oleate with stearate is given by the solid line. The dashed line depicts part of the phase diagram for stearate with the stable modification of oleate. The liquidus line for solid solutions I + liquid was found to be the same for both within the precision.

The mesophase transitions are reported in Table 2 and are not graphically presented. They are observable either upon cooling the isotropic liquid or upon reheating from the mesophases. Continuous and linear behavior of both mesophase transitions is observed, implying that both mesophases act as ideal mixtures over the entire composition range. Temperatures for the smectic–solid transitions vary with cooling rate.

TABLE 2 Mesophase transitions
Cholesteryl stearate + Cholesteryl oleate

	Transition temperature, °C (Reheating from smectic mesophase)	
Oleate, Wt %	Smectic–cholesteric	Cholesteric–isotropic
0.0	74.3	78.3
4.91	73.1	76.5
8.82	70.5	74.6
15.28	69.5	73.6
39.02	61.0	65.6
54.24	54.85	60.1
66.02	51.6	56.1
82.19	45.6	52.1
92.42	42.1	47.85
100.0	39.25	45.1

Cholesteryl oleate + Cholesteryl linoleate

	Transition temperature, °C (Reheating from smectic mesophase)	
Linoleate, Wt %	Smectic–cholesteric	Cholesteric–isotropic
0.0	39.25	45.1
7.5	39.15	44.35
31.79	37.25	41.35
42.42	36.7	40.2
53.42	35.85	38.55
67.56	34.85	37.55
84.94	34.05	35.55
90.23	33.45	35.05
100.0	32.6	33.5

Solidus and liquidus lines for the lower melting metastable form were determined on heating solids prepared by rapid cooling of melts to − 30 °C. The DSC curves were analagous to those for the system stearate–linoleate. Sharp peaks on heating indicate melting of the eutectic portion at 48.1 ± 0.2 °C. The limit of solid solubility of oleate in stearate (10%) and stearate in oleate (~3.5%), as well as the eutectic composition (6% stearate) were determined by using the same methods described for the stearate–linoleate system. Both systems exhibit very similar behavior. The same concentration of stearate (6%) was determined for their eutectic compositions. The estimated limit of solid solubility in stearate was found to be higher for linoleate (16%) than for oleate (10%).

All compositions within the miscibility gap show additional well expressed endotherm on heating at 32–35 °C. This suggests a solid–solid transition of a metastable crystal modification formed upon rapid cooling. It cannot be detected in pure esters and it does not appear on heating solids obtained by slow cooling. At high heating rates the endotherm is followed by an exotherm. It is known that transition rates for solids are often slow and that on heating they commonly superheat. At high heating rates metastable melting can thus occur before the transition is completed. The melt is unstable and spontaneous crystallization ensues, accompanied by an exothermic evolution of heat. Such an event is clearly a demonstration of a conversion from a metastable to a more stable state.(25) Introduction of second compound (impurity) helps to form the metastable modification.

The higher stable eutectic temperature 49.9 ± 2 °C was found on heating solids obtained either by crystallization on standing at ambient temperature for several days or by cooling to -30 °C followed by storage for weeks at ambient temperature.

Commonly on heating the solids, two eutectics appear. The amount of each varies with prior cooling conditions. The amount of the lower eutectic decreased at lower cooling rates, i.e., the closer thermodynamic equilibrium is approached. This is illustrated by the DSC traces shown in Fig . 6–8 for mixture containing 17.81% oleate. Figure 6 shows approximately equal portions of both eutectics obtained at higher cooling rates. For the measurable range, the eutectic composition and solid solubility of the esters is only slightly different from the corresponding values obtained for metastable state. Results also suggest (see Figs. 6–8), the general conditions for crystallization of stable and unstable modifications and transition rates.

The region in Fig. 5 close to the pure oleate was established by a combination of DSC and light microscopy. Slow DSC heating rates and high sensitivity settings were used to obtain resolution of thermal

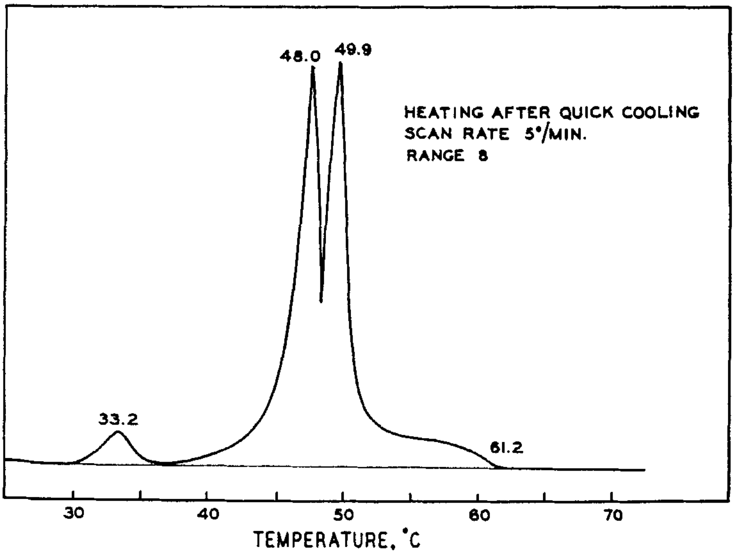

Figure 6. DSC trace—82.19% cholesteryl oleate + 17.81% cholesteryl stearate.

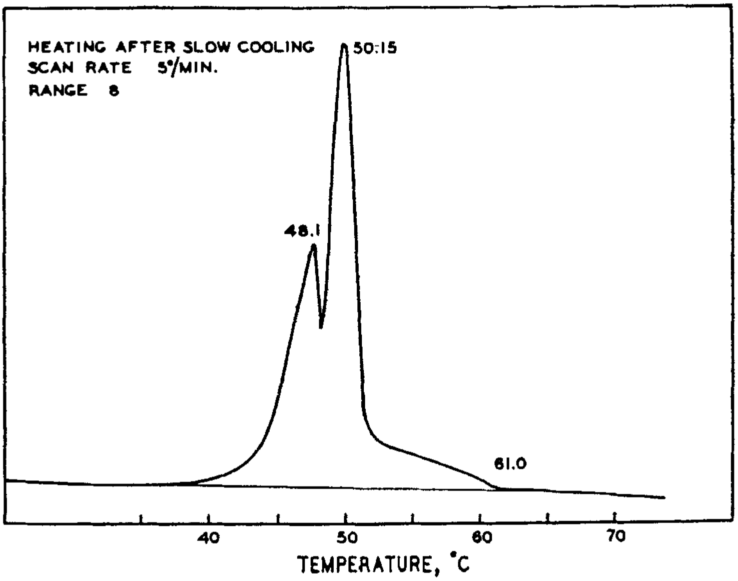

Figure 7. DSC trace—82.19% cholesteryl oleate + 17.81% cholesteryl stearate.

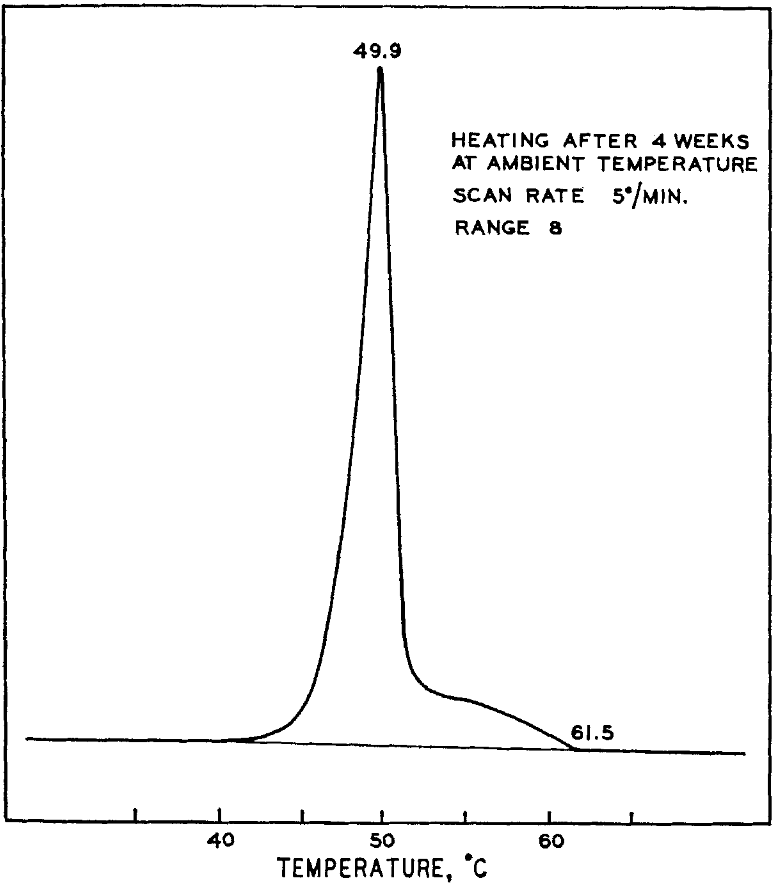

Figure 8. DSC trace—82.19% cholesteryl oleate + 17.81% cholesteryl stearate.

events which occur close together on the temperature scale. Melting was also observed in a polarizing microscope using Mettler hot stage with samples between slides and covers at heating rates 0.2 and 2 °C/min. Even by these combined methods, some uncertainty persists in the region of high oleate concentration in Fig. 5.

Cholesteryl Oleate–Cholesteryl Linoleate

The phase diagram in Fig. 9 for the solid transition shows complete miscibility of each ester in the other in both the liquid and solid phases and for the solid–solid transformation as well. The solid–liquid heterogenous region is narrow with behavior approaching an

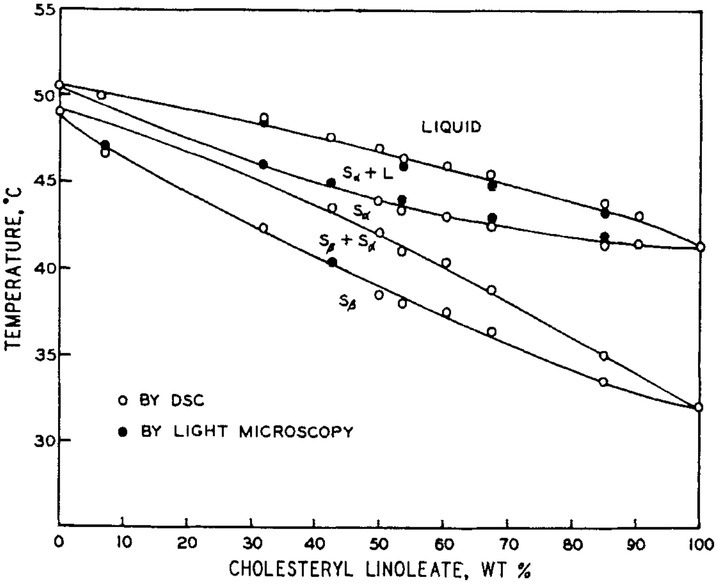

Figure 9. Changes on heating from solid cholesteryl oleate–cholesteryl linoleate.

ideal system. These features are consistent with only a 9 °C difference in melting points and almost equal heats of fusion for the pure esters. The temperatures given in Fig. 9 are only for changes occurring on heating from the solid state.

The mesophase transitions for this system are given in Table 2 and are not graphed. Significantly, continuous linear behavior of both mesophase transitions was found over the entire composition range. Likewise, all mesophase transitions were monotropic.

The heating curves obtained for solids immediately after cooling, or even a couple of days after cooling, were quite different from the curves obtained on solids which were considered to be in equilibrium after storage for several days to weeks.

The solid–isotropic transitions for the stable form of the solid solutions were established on heating (1.2 °C/min) solids prepared by crystallization plus annealing at ambient temperature for 3–4 weeks. Only one sharp melting endotherm is observed for these samples. Solidus and liquidus temperatures were taken as values corresponding to the intersection of the extrapolated leading edge and baseline and

TABLE 3 Cholesteryl Oleate–Cholesteryl Linoleate.
Crystalline melting transition heats.

Oleate, Wt %	ΔH, Cal/g
100.0	11.2
92.5	10.8
68.2	10.6
57.6	10.5
50.1	10.3
46.6	10.5
39.2	10.5
32.4	10.3
15.1	10.6
9.8	10.6
0.0	11.0

to the return of DSC trace to the base line, respectively. Fair agreement was found on checking melting under the polarizing microscope with a Mettler hot stage and with samples between slide and cover at heating rates 0.2 and 2 °C/min.

The crystal–isotropic liquid transition heats are given in Table 3. Only ~8 rel. % deviation from the linear interpolation of total heat was found in the middle of concentration range where the heterogenous region extends over 3 ° interval.

Two endotherms appear on heating solids prepared by quick cooling melts to −30 °C. The first for transition of the metastable form of mixed crystals to the stable form and the second for the stable solid–liquid transition. The beginning and the end of the solid–solid transition were taken as temperatures for the peak and for the return of DSC trace to the base line in experiments carried out at slow heating 1.2 °C/min. The beginning of transformation determined in this way is likely a little higher. It was found that it corresponds to the temperatures at which on heating under the polarizing microscope the first detectable changes in crystal color occured associated with the polymorphic transformation. With increasing oleate concentration, the endotherm for the solid–solid transition increased. This is in accordance with the behavior found with pure linoleate and with blends of linoleate with stearate. The addition of a second compound (stearate or oleate) helps to form on cooling the metastable modifica-

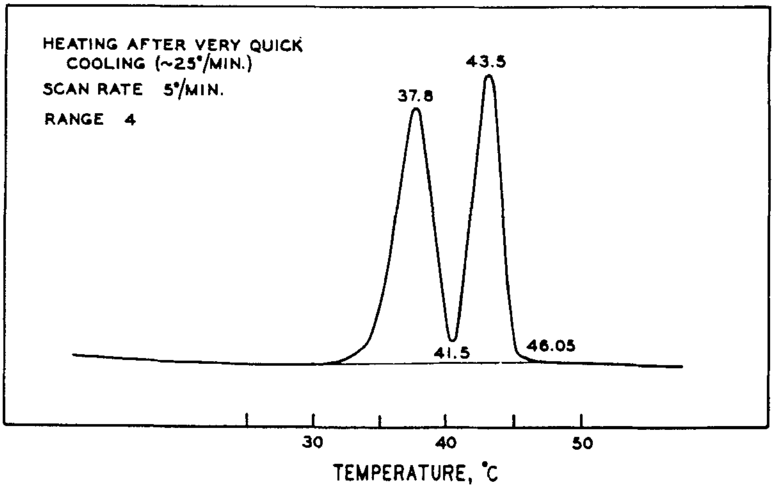

Figure 10. DSC trace—53.42% cholesteryl linoleate + 46.58% cholesteryl oleate.

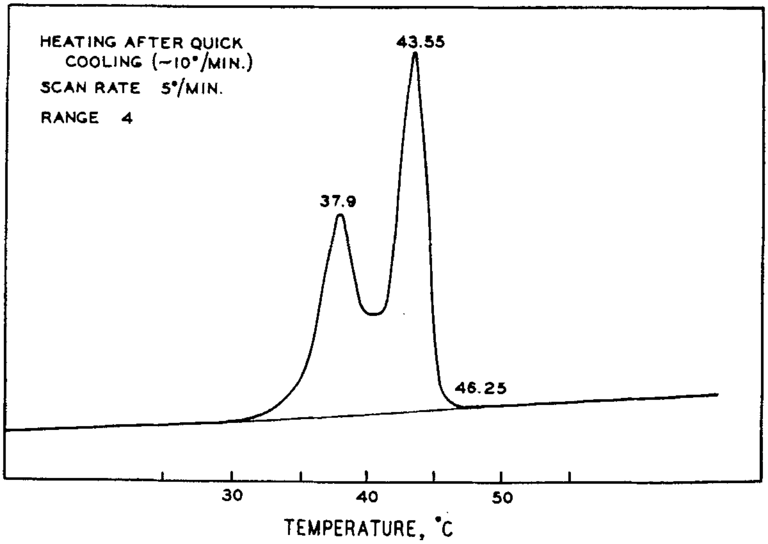

Figure 11. DSC trace—53.42% cholesteryl linoleate + 46.58% cholesteryl oleate.

tion or at least to slow down its transformation into the stable form. Calorimetric measurements prove that only a portion of the solid solutions was in the metastable form and that this amount increases with increasing oleate concentration and with increasing cooling rate used for solid formation.

With increasing oleate concentration the solid–solid and solid–liquid transition temperatures approach one another. At higher heating rates, therefore, metastable melting occurs before the transition into the stable form can be accomplished. Figures 10 and 11 illustrate this effect with DSC traces obtained on heating blend

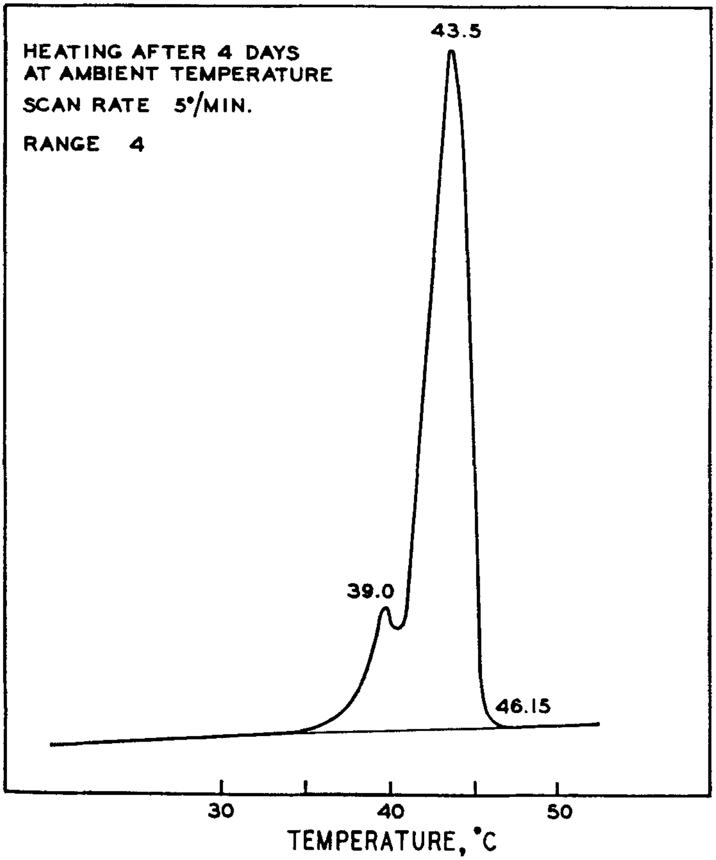

Figure 12. DSC trace—53.42% cholesteryl linoleate + 46.58% cholesteryl oleate.

containing 46.58% oleate and 53.42% linoleate as the solid was prepared by different cooling rates.

The transformation rate of the metastable modification into the stable form decreases with increasing oleate concentration. While annealing at ambient temperature for a few hours was sufficient to get the stable form for blends of high linoleate content, for solids of high oleate content small amounts of the metastable form were still detectable after storage for one month. Figure 12 shows the annealing effect for the same blend as in Figs. 10 and 11.

5. Discussion

All the principal types of solid–liquid phase diagrams have been observed in binary systems for the fatty acid esters of cholesterol. Systems have been defined with complete solid phase immiscibility (myristate–acetate,(12) myristate–nonanoate,(12) myristate–stearate),(11) system with complete miscibility in both liquid and solid phases (oleate–linoleate), as well as the intermediate types of limited solid solubility (stearate–oleate, stearate–linoleate), and system with appreciable solid solution formation at one side of phase diagram and with negligible solid solubility at the opposite side (myristate–undecanoate.(11)

There is a substantial difference between systems exhibiting complete solid phase immiscibility and complete solid miscibility. Crystal structures of the pure esters in the first case must be different; in the second case they must be the same. It has been previously observed experimentally that if two end members possess preciously the same crystal structures but exhibit lattice constants that differ by 10–15% or more, continuous solid solutions are not likely to occur. If the crystal structures are the same, and in addition, the lattice constants do not differ appreciably, the interaction may be such that a continuous series of solid solutions can be generate over the entire composition interval.(25)

The formation of a continuous series of solid solutions, over the entire composition interval, in the system oleate–linoleate indicates very close structure and lattice constants. Thermodynamically, both esters have very close melting points and almost identical heats of fusion.

On the other hand both systems linoleate–stearate and oleate–stearate show limited solid solubility indicating that the parameters for the saturated ester differ from those for both the equivalent length unsaturated esters. The structures are sufficiently similar, however, to permit limited formation of solid solutions.

The same limit of solid solubility of stearate in oleate and in linoleate with only a slightly different limit of solid solubility of oleate and linoleate in stearate serves as further evidence for a very close crystal structure and lattice constants of both unsaturated esters.

Both individual unsaturated esters exhibit a polymorphism in the solid state. The metastable modifications are formed from highly supercooled melts. Prolonged standing produces recrystallization in the stable form. The formation and stability of metastable modification are enhanced in investigated binary systems and result in complex behavior with solid–solid transitions and regions of metastability.

The mesophases in binary blends are monotropic with respect to the crystalline state. They can be observed only on cooling when the transition to the solid state is depressed or supercooled. Due to the substantial supercooling of the smectic mesophase prior to the formation of the solid phase they can be observed also upon reheating from the smectic mesophase.

Mesophase transitions are generally reversible without significant depression on cooling. Likewise, no evidence was found for superheating of mesophase transitions.

The cholesteric and smectic mesophases exist over the entire composition interval in both oleate systems. No sufficient supercooling was achieved to observe mesophases in the midconcentration range for the stearate–linoleate system.

Only single homogenous cholesteric and smectic mesophases were observed in binary mixtures. Similarly, only one isotropic liquid is formed at the cholesteric–isotropic transition. The mesophase transition temperatures are linear functions of composition. This continuous linear behavior implies that both mesophases act as ideal mixtures with uniformity of cohesive forces between like or unlike molecules.

There are concentration regions in all three binary systems where mesophases can exist at body temperatures. The largest concentration interval was found in the oleate–linoleate system. Only for mixtures with highest linoleate contents are the mesophase transitions slightly below body temperatures. In the stearate–linoleate system is this region approximately between 75 and 95% linoleate. In the stearate–linoleate system only the mixtures with lowest concentrations of saturated ester can exhibit mesophases at body temperatures.

The unsaturated and saturated cholesterol esters belong to the major components of arteriosclerotic deposits. Both unsaturated esters form a substantial part of cholesteryl ester content in β-lipoprotein blood serum. From this standpoint, the mesomorphic behavior of mixtures of cholesteryl esters can be very important.

The results suggest also methods of predicting phase diagrams for ther binary mesophase systems.

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