Cholesteryl esters of saturated fatty acids: cosolubility and fractionation of binary mixtures

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Abstract Factors affecting the solid state miscibility of saturated chain cholesteryl esters were determined from electron diffraction and differential scanning calorimetric measurements on a homologous series which included two types of crystal packing. Electron diffraction patterns from solution- and epitaxially crystallized microcrystals gave measured unit cell constants consistent with the bilayer crystal form for myristate, pentadecanoate, palmitate, and stearate esters. Cholesteryl undecanoate crystallized as the monolayer I structure and cholesteryl laurate was polymorphic, packing in either monolayer I or bilayer forms. No evidence was found for the monolayer II form of the laurate claimed in earlier work. It is clear that solid solution formation follows general rules formulated earlier by Kitaigorodskii (23) for molecular crystals. A symmetry criterion must be satisfied first of all, i.e., two compounds that solidify in greatly different crystal structures will not form continuous solid solutions (e.g., cholesteryl undecanoate/cholesteryl myristate). Within a given crystal structure type, solid solution is permitted when the molecular volumes are similar. (For example, cholesteryl myristate forms an ideal solid solution with cholesteryl pentadecanoate, a nonideal solution with cholesteryl palmitate, and a eutectic of solid solutions with cholesteryl stearate.) For the polymorphic cholesteryl laurate, solid solutions of either the monolayer I structure (e.g., with cholesteryl undecanoate) or bilayer structure (e.g., with cholesteryl myristate) are permitted.—**Dorset, D. L.** Cholesteryl esters of saturated fatty acids: cosolubility and fractionation of binary mixtures. *J. Lipid Res.* 1987. **28:** 993—1005.

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The accumulation of lipid deposits in arterial walls in diseases such as atherosclerosis is a phenomenon which has been investigated on several levels. Considerable effort has been spent to uncover aspects of neutral lipid metabolism, especially factors of the receptor-mediated cellular uptake of transport proteins (such as LDL) that may create a high serum cholesterol concentration and thus encourage the nonspecific phagocytosis of lipoproteins by arterial macrophages to form foam cells (1, 2). The deposition of lipids has also received considerable attention, both in terms of the progression of a fatty lesion on its way to becoming a gruel plaque (3–5) and also what factors may favor the reversal of this deposition (5). It is clear that lipid deposition can be described in physical chemical terms, i.e., the progression of a lesion is characterized by the three-component phase diagram of cholesterol–cholesteryl ester–phospholipid in excess water (6, 7). Since mostly cholesteryl ester deposition is important for the progression of the lesion, it is also clear that the physical state (fluidity, comiscibility of components) of the lesion is dependent upon the chemical type of esters incorporated, since the phase behavior of these materials is largely determined by the fatty acid chain moiety (8).

Many of the physical properties of pure cholesteryl esters have been determined by Small (8, 9) and Ginsburg, Atkinson, and Small (10) including a description of their thermotropic mesomorphic behavior. Phase behavior is ultimately related to molecular aggregation and, thus, Craven (11) has determined the X-ray crystal structures of a wide variety of cholesteryl esters. With very few exceptions, it has been shown (12) that these crystal structures can be generalized as three basic packing modes that include a bilayer and two types of monolayer motif.

Since cholesteryl ester deposits in vivo are polydisperse, the understanding of mixed component phase behavior can be achieved via models systems that are initially based on binary mixtures. In addition to the characterization of cholesteryl ester mixtures with other lipid species (8, 13–15), phase diagrams have been published for a number of cholesteryl ester chain pairs (9, 14, 16–18), including the physiologically most significant oleate/linoleate system (9). It is apparent from these studies that the interpretation of the phase diagrams can be very difficult, particularly in the absence of supportive diffraction or other structural data. Moreover, a survey of existing data from, for example, saturated cholesteryl ester binary systems (9, 16, 17), does not easily allow one to formulate what structural features of the components are important for the comiscibility or fractionation of cooled binary melts, e. g.,

Abbreviation: DSC, Differential scanning calorimetry.

as discussed by Kitaigorodskii (19) for other binary molecular solids.

Given the recent success of electron diffraction techniques for the characterization of epitaxially oriented binary paraffin microcrystals (20, 21), and also the use of the technique to follow the thermotropic mesomorphic behavior of a pure cholesteryl ester (22), it is felt that a more complete understanding of the structural basis for phase behavior in binary cholesteryl ester mixtures can be gained via this microcrystallographic technique. The physical behavior of a number of binary mixtures from a homologous series of saturated chain cholesteryl esters is described in this report in an initial attempt to determine structural rules for comiscibility that are analogous to those already established for linear paraffins (23).

MATERIALS AND METHODS

Cholesteryl esters

Physical data for the cholesteryl esters used in this study are reviewed in **Table 1.** Included with purity and thermal data are the crystal forms for various members of the homologous series determined by X-ray diffraction in other laboratories compared to the results of electron diffraction determination (see below).

Crystal growth

Microcrystals for electron diffraction measurements were prepared in either of two ways. Solution grown crystals were formed on carbon film-covered electron microscope grids as a dilute solution of the cholesteryl ester in n-pentanol was evaporated on this surface. Epitaxially crystallized samples of pure cholesteryl esters and their binary mixtures were prepared on benzoic acid following a procedure described by Wittmann, Hodge, and Lotz (24). Briefly, a dilute solution in hot acetone was evaporated on a freshly cleaved mica surface. Carbon film-covered electron microscope grids were placed over regions with thin layers of cholesteryl ester crystals and excess benzoic acid was then sprinkled over the whole area. The other half of the cleaved mica sheet was placed over this physical mixture to form a sandwich and this was moved along a thermal gradient to form a dilute solution of the lipid in molten benzoic acid. When this was cooled, the benzoic acid surface directed the epitaxial growth of the ester by lattice matching. As stated earlier (22), crystal packing in the bilayer polymorph (12) has a (010) side to side packing of steroid nuclei or acyl chains (respectively, 9.9 and 4.9 Å) that matches the 5.14 Å lattice spacing of the major benzoic acid crystal face. For the monolayer I polymorph crystal structure (12), it can also be shown that the spacing of molecules along the [50$\bar{1}$] unit cell vector (which is perpendicular to the molecular axes (25)) is 4.78 Å, i.e., only a 7.1% mismatch with the 5.14 Å benzoic acid lattice repeat.

Bulk samples of cholesteryl esters used for differential scanning calorimetry (DSC) were weighed in aluminum pans on a five-place Mettler AE163 balance. These samples were then repeatedly fused and cooled and then remixed, refused, and cooled before sealing a small amount (ca. 10 mg) in an aluminum crucible for the DSC measurement.

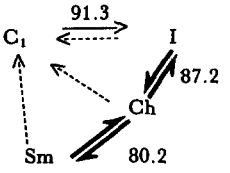
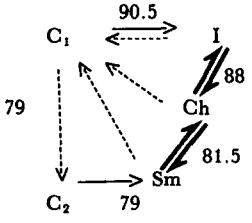
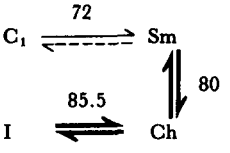
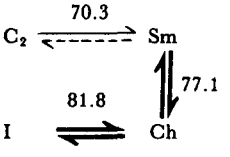
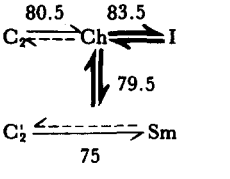
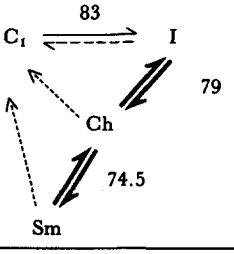
Electron diffraction

Electron diffraction patterns were obtained from the microcrystalline preparations at 100 kV using a JEOL JEM-100B electron microscope in the selected area diffraction mode (selected area diameter ca. 10 μm), taking usual precautions that the specimens were exposed to a minimum radiation dose (26). (This is governed by the use of low incident beam current density and very fast photographic films to record the diffraction pattern, e.g., Kodak DEF5 X-ray film). The camera length for electron diffraction was calibrated with a gold Debye-Scherrer diagram taken at the same lens settings used before for the cholesteryl ester diffraction study. In some of the experiments reported here, it was necessary to obtain diffraction patterns at reduced or elevated temperatures. This was possible with a GATAN model 626 specimen holder for the electron microscope which allows any temperature between −160°C to +150°C to be maintained during the experiment. For model calculations used to interpret some of the diffraction data, Doyle-Turner electron scattering factors (27) were used in the kinematical structure factor equation.

Differential scanning calorimetry

Qualitative differential scanning calorimetric measurements were made on thoroughly mixed, melt-crystallized mixtures of cholesteryl esters using a Mettler FP800 thermosystem with specimens sealed in aluminum crucibles. Scans at either 2°C/min. or 5°C/min. were made on specimens after they had been equilibrated at room temperature for at least 1 week. An initial heating scan followed by a cooling scan and then a second heating scan, respectively, to determine the mesomorphic phase transitions in compounds that undergo monotropic phase transitions and to assess the reproducibility of the phase behavior from freshly cooled material (i.e., to detect metastable phase behavior). The instrumental thermal lag found for cooled specimens can be corrected by observing mesomorphic phase behavior for enantiotropic transitions in compounds such as cholesteryl myristate (16). For binary mixtures that clearly formed continuous solid solutions, the onset and return temperatures of the phase transition were plotted to indicate two phase regions in the phase diagram. Otherwise, peak values were used, since it is often difficult to separate the multiple peaks found in a thermal

TABLE 1. Physical Data for Saturated Cholesteryl Esters

Compound (Source, Purity)	Phase Behavior[a]	Crystal Structure (X-ray)	Crystal Structure (Electron) This Study
Cholesteryl undecanoate (Nu Chek Prep, > 99%)		C_1:monolayer I (30)[b] a = 12.99, b = 9.00 c = 31.03 Å, β = 90.58°	C_1:monolayer I d_{100} = 13.33 ± 0.12 Å d_{010} = 9.24 ± 0.07 Å d_{001} = 31.43 ± 0.80 Å
Cholesteryl laurate (Nu Chek Prep, > 99%)		C_1:monolayer I (31-33) a = 12.989, b = 9.008 c = 32.02 Å, β = 91.36° C_2:monolayer II (?) (9)	C_1:monolayer I d_{100} = 13.20 ± 0.15 Å d_{010} = 9.14 ± 0.06 Å d_{001} = 31.57 ± 0.99 Å C_2:bilayer d_{100} = 10.47 ± 0.14 Å d_{010} = 7.83 ± 0.12 Å d_{001} = 46.99 ± 0.67 Å
Cholesteryl myristate (Sigma, unknown purity)		C_2:bilayer (29) a = 10.260, b = 7.596 c = 101.43 Å, β = 94.41°	C_2:bilayer d_{100} = 10.36 ± 0.10 Å d_{010} = 7.68 ± 0.06 Å d_{001} = 51.22 ± 0.63 Å
Cholesteryl pentadecanoate (Nu Chek Prep, > 99%)		C_2:undetermined, presumed bilayer (9)	C_2:bilayer d_{100} = 10.47 ± 0.11 Å d_{010} = 7.94 ± 0.16 Å d_{001} = 54.90 ± 0.54 Å
Cholesteryl palmitate (Serdary, unknown purity)		C_2:bilayer (43) a = 10.15, b = 7.55 c = 105.5 Å, β = 95.6°	C_2:bilayer d_{100} = 10.39 ± 0.14 Å d_{010} = 7.71 ± 0.12 Å d_{001} = 53.53 ± 0.57 Å
Cholesteryl stearate (Nu Chek Prep, > 99%)		C_1:bilayer (11) a = 10.20, b = 7.55 c = 57.5 Å, β = 96°	C_2:bilayer d_{100} = 10.54 ± 0.25 Å d_{010} = 7.90 ± 0.25 Å d_{001} = 56.87 ± 0.75 Å

[a]Legend: C_n, crystal form; Ch, cholesteric phase; Sm, smectic phase; I, isotropic phase.
[b]Numbers in parentheses are reference numbers.

scan. Protocols for the construction of phase diagrams have been discussed by several workers (8, 16-18).

Significance of the crystallographic study

In this study, crystallographic data in the form of measured lattice constants (mostly lamellar spacings) are used to identify the existence of solid solutions and to assess the deviation of a mixed component crystal packing from Vegard's law (19). The former data were obtained at ambient as well as elevated temperatures, whereas the latter data, which are compared to an idealized Vegard's law relationship, were obtained only at room temperature.

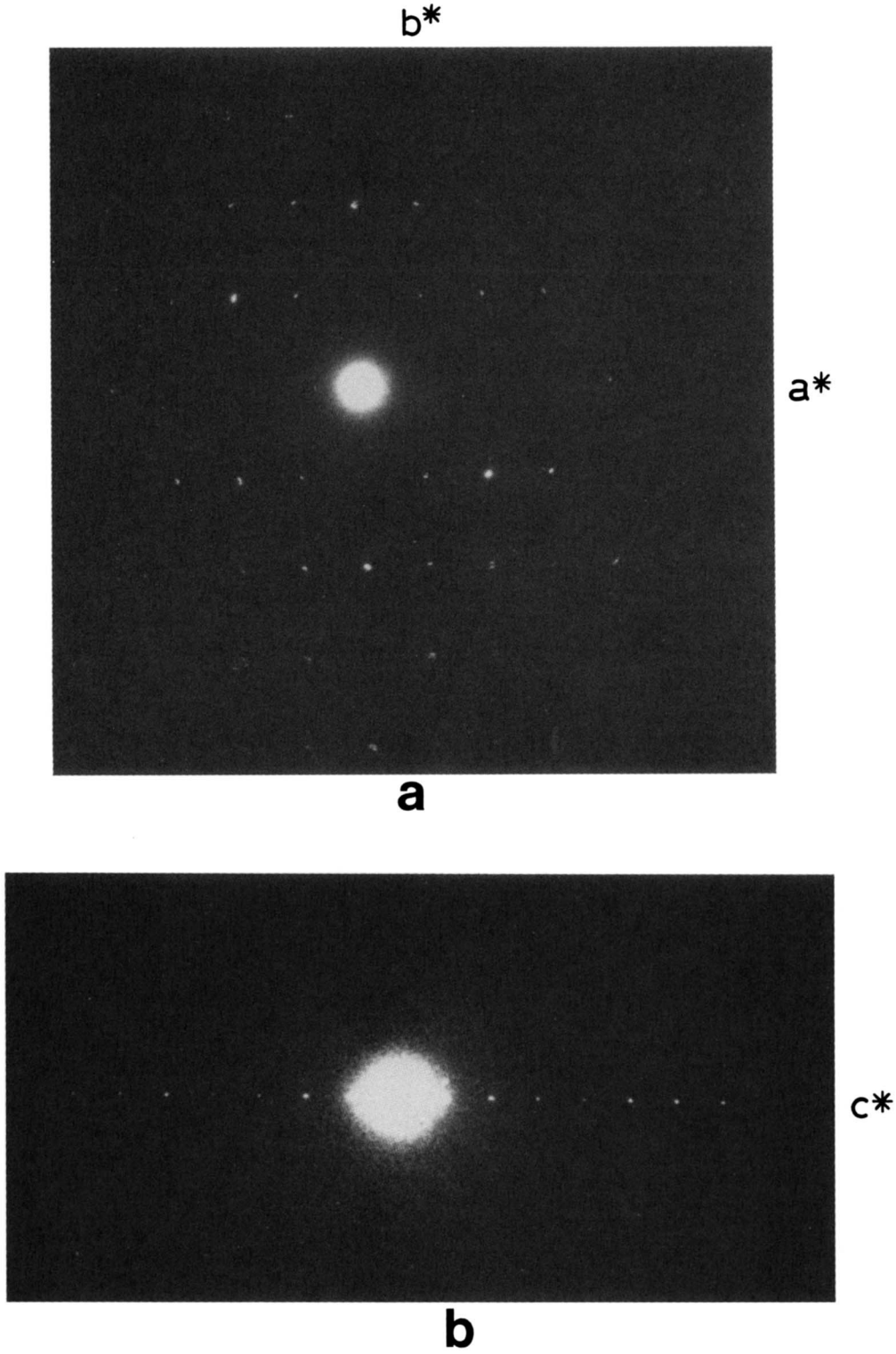
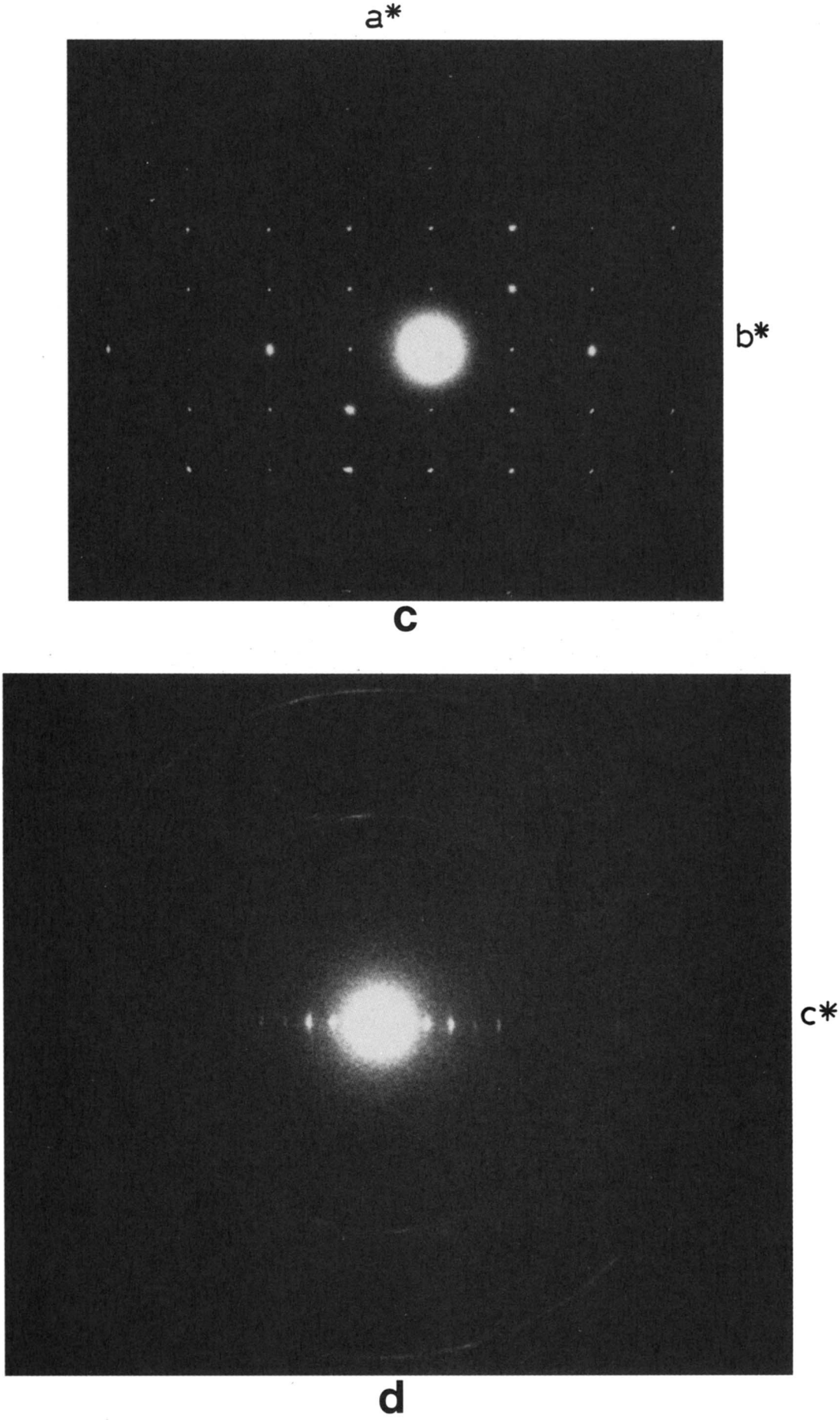


Fig. 1. Representative electron diffraction patterns from cholesteryl esters. Monolayer I structure: (a) hk0 pattern from crystals grown fron n-pentanol; (b) 00ℓ pattern from samples epitaxially crystallized on benzoic acid. Bilayer structure: (c) hk0 pattern-solution growth; (d) 00ℓ pattern-epitaxial growth. Lamellar (00ℓ) from binary solid solutions are similar to those from the pure compounds with no appreciable loss of diffraction resolution for either crystal form. As shown earlier, electron diffraction data from solution crystallized samples are unsuitable for quantitative structure analysis due to a diffraction incoherence caused by elastic bend deformation of the crystal plate (42). Thus, the intensities in (a) and (c) are not simply related to the unit cell transform, nor are the space group forbidden reflections necessarily absent. The measured lattice spacings, however, are correct.



Since the use of epitaxially crystallized linear chain microcrystals to investigate the structure of binary solids is a very new technique, it is necesary to emphasize the prominent characteristics of the method to appreciate both its strengths and weaknesses. Electron diffraction measurements probe an array of microstates, i.e., of a number of individual microcrystalline samples that are distributed on a carbon-film surface of a supporting copper mesh. In aggregate, these measurements are anticipated to resemble low angle X-ray diffraction measurements on a bulk polycrystalline sample, when enough samples are used to compute an average. Thus, some relationship can be established between the average diffraction data and the sample composition of the bulk binary solid used for differential scanning calorimetry. However, significant deviations of individual microcrystals from this bulk average can occur, as is illustrated by the standard deviation of lamellar spacings shown in figures below which are not necessarily due to measurement errors. As is shown in our most extensive work on n-paraffins (20, 21 and Dorset, D. L., unpublished data) these local variations in crystal form can be quite significant. For example, as will be shown in a future work, such measurements on individual microcrystals of paraffin binary solids have shown that Vegard's law is not necessarily a correct description of linear chain solid solutions; moreover, the crystal symmetries within discrete composition domains can violate rules proposed by Kitaigorodskii (19, 23) so that the concept of continuity may not be meaningful when considered solely on symmetry grounds.

For the diffraction data measured here on cholesteryl esters grown essentially from a melt which is oriented by a nucleating substrate on cooling, the electron diffraction data are mostly limited to lamellar reflections (see Fig. 1) which, although less complete than the zonal diffraction data from epitaxially crystallized n-paraffins (20, 21), are nevertheless characteristic for any given polymorphic form. These data are compared to melt crystallized samples used for DSC measurements but, in some cases, due to either polymorphism and monotropic phase behavior, there may be individual variations due also to local specificity of a crystal form or the non-attainment of thermodynamic equilibrium (which, for some paraffin samples, has required as much as a year's standing).

RESULTS

Pure cholesteryl esters

Transition temperatures for the pure cholesteryl esters used in this study are listed in **Table 2.** Although their values many differ from those included in Table 1 by one or two degrees, they all fall within the range of measurements reviewed by Davis, Porter, and Barrall (28). Omitted from this table, but indicated in the phase transition scheme of Table 1, is the crystal to smectic transition of the second crystalline form of cholesteryl laurate which had been recrystallized from the melt.

Electron diffraction data from both solution- and epitaxially crystallized samples (which give orthogonal projections of the same crystal structure) are in accord with previous X-ray measurements. The values reported in Table 1 were measured at room temperature (i.e., between 19 and 21°C). For the bilayer structure, the d_{001} spacing may, in fact, be d_{002} since the crystal structure can be centered (see ref. 11). The homologous series from cholesteryl myristate to cholesteryl stearate all crystallize in the bilayer structure found for cholesteryl myristate (29), including the odd chain pentadecanoate for which no X-ray data are available. Representative electron diffraction patterns are shown in **Fig. 1.** Cholesteryl undecanoate crystallized as the monolayer I structure described by Sawzik and Craven (30). (Typical patterns are shown in Fig. 1). Although the published X-ray crystal structure of cholesteryl laurate (31–33) is the monolayer I crystal packing, our electron diffraction data from both solution- and epitaxially-crystallized samples demonstrate that the bilayer structure, not the monolayer II, is the second polymorph. (This form was also postulated by Craven (11) to exist for the laurate and is also the polymorph found for the cholestanyl ester (34)). As also shown for the other linear chain compounds (35), the abundance of a polymorph can depend on the type of solvent used for crystallization, e.g., solution-grown crystals of cholesteryl

TABLE 2. *Peak transition temperatures (°C) for pure cholesteryl esters used in this investigation*

	Transition		
Ester	Crystal → Smectic	Smectic-Cholesteric	Cholesteric-Isotropic
Undecanoate	92.5	80.9	89.1
Laurate	91.3	81.2	88.2
Myristate	70.7	78.1	84.0
Pentadecanoate	70.6	77.3	82.0
Palmitate	78.2	77.6	82.2
Stearate	82.8	76.2	80.2

laurate were mostly the bilayer polymorph, while more of the epitaxial samples were of the monolayer I type.

The linearity of bilayer lamellar spacing with increasing chain length is demonstrated for the even chain series from laurate to stearate in **Fig. 2.** The lamellar spacing for the pentadecanoate is found to lie above this line. Lamellar spacing data from X-ray measurements on crystals (11) are also plotted in this figure and the value for the tridecanoate taken with the pentadecanoate indicates a possible odd-even effect in crystal packing density.

Finally, in her study of the low temperature form of cholesteryl esters, Sawzik (25) noted that the monolayer I polymorph crystals of cholesteryl laurate shattered when the temperature was lowered below $-81°C$. Preliminary studies in this laboratory on thin epitaxially crystallized microcrystals indicate that the samples can be cooled at least to $-127°C$ where there is a marked decrease in diffraction resolution. As the specimen is cooled to $-101°C$, there is a slight decrease in lamellar spacing (ca. 2%), followed by a sudden increase to the room temperature value when cooled to $-127°C$. Reheating the specimen to room temperature restores the original diffraction resolution.

Binary mixtures

In the study of binary mixtures described below, the major variables that could possibly influence the behavior of these mixtures include the following: *i*) relative fatty acid chain lengths, *ii*) crystal structures of the pure components, and *iii*) the enantiotropic or monotropic behavior of individual components.

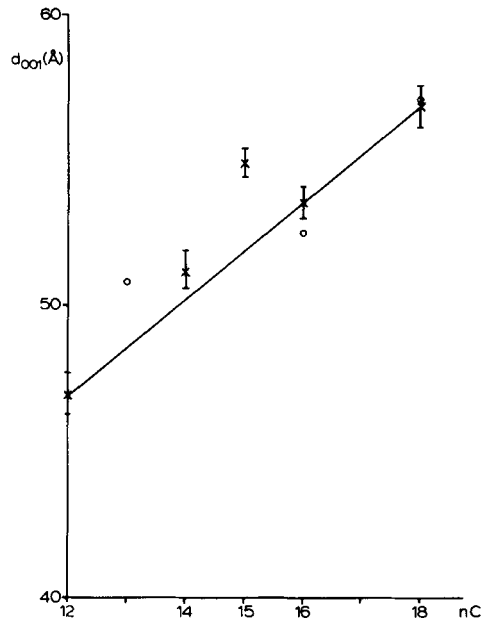


Fig. 2. Lamellar spacings for several cholesteryl esters packing in the bilayer crystal structure (29) as a function of fatty acid carbon chain length nC (x, value from electron diffraction measurement with standard deviation; O, literature X-ray lamellar spacing (9, 11)). Note that the values for two odd chain esters lie somewhat above the line for even chain esters indicating a possible odd-even effect. (The line drawn here connects the mean lamellar spacing values for the laurate and stearate found in electron diffraction patterns taken at room temperature).

In the following description, the phase diagram is interpreted in terms of the electron diffraction pattern from epitaxially crystallized mole ratios, both at room temperature, where either a stable solid solution or a fractionated mixture exists, and also at elevated temperatures, to define the limits of the continuous or mixed crystal domain in the phase diagram. (The existence of a crystalline phase, as shown by electron diffraction, is marked by the use of the sign ⊕ in the phase diagram). Diagrams of lamellar spacing versus composition were obtained for equilibrated samples at room temperature. As found also for paraffin solid solutions (20), the "error bars" may represent local concentration variations for rapidly grown microcrystals or, alternatively, slight changes in preferred crystal packing (see Discussion).

Cholesteryl undecanoate/cholesteryl laurate. The two esters, which differ by one methylene unit in the acyl chain, can crystallize as the monolayer I structure, and moreover, both exhibit monotropic phase behavior (Table 1). The phase diagram in **Fig. 3a** indicates that the two compounds form continuous solid solutions over all compositions, as is verified by the variation of lamellar spacing with composition shown in Fig. 3b. All diffraction patterns of the binary crystalline solids are of the monolayer I type.

Cholesteryl undecanoate/cholesteryl myristate. In the original study of this binary mixture (16), it was proposed that at high concentrations (>40 wt %) of cholesteryl myristate, a continuous solid solution could be formed with cholesteryl undecanoate, whereas the mixture was stated to fractionate at lower myristate concentrations. After repeated studies of this system, it has not been possible to reproduce the phase diagram shown in the earlier study. Rather, the phase diagram for mixtures of the two cholesteryl esters, which individually crystallize as different polymorphs, demonstrates the anticipated eutectic behavior, since neither pure component has a second polymorphic form which would accommodate the other. Measurements of lamellar spacings at different mole fractions **(Fig. 4b)** indicate the presence of either one pure component or the other, packing in respective polymorph structures. Therefore, the components seem to be totally immiscible in the crystalline phase. Hysteresis of phase behavior for just-cooled samples indicates the long period required for the recrystallization of the components.

Cholesteryl laurate/cholesteryl myristate. Since cholesteryl laurate was found to pack in either of two crystal forms, it is anticipated that at least metastable continuous solid solutions can crystallize as the bilayer polymorph. The partial phase diagram **(Fig. 5a)** was obtained after pains were taken to ensure that the fused components were well

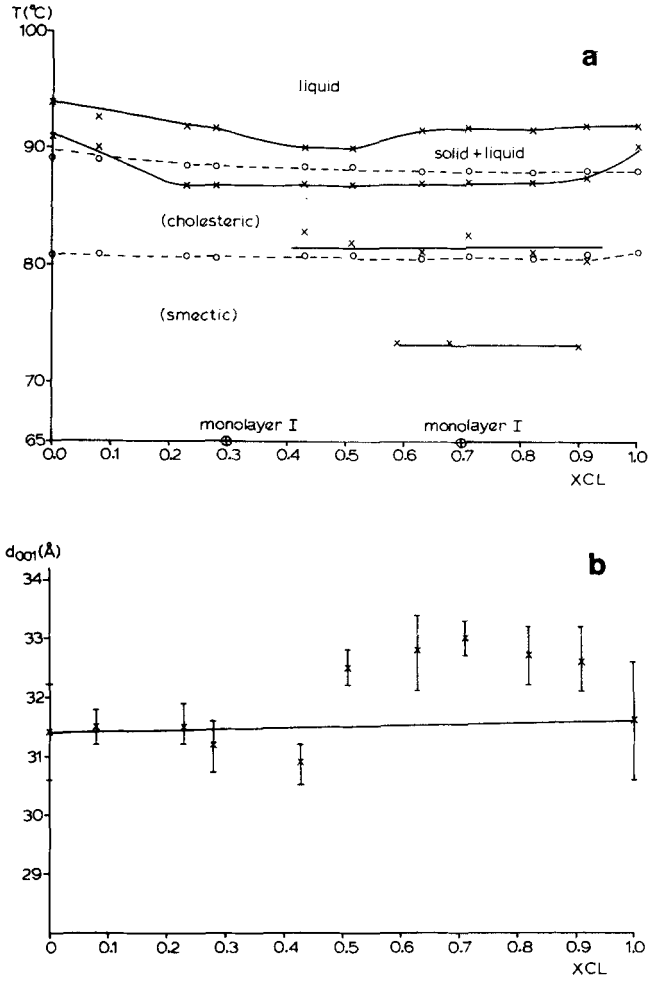


Fig. 3. Binary mixtures of cholesteryl undecanoate/cholesteryl laurate (CL). The undecanoate crystallizes only in the monolayer I form, whereas the laurate can crystallize as either the monolayer I or bilayer forms. However, electron diffraction patterns indicate that the solid solutions are only monolayer I types. (a) Phase diagram determined from DSC measurements. The solid lines denote liquidus and solidus lines of the continuous solid solutions (and also a lower temperature crystal-crystal transformation); the dotted lines denote the mesophases which are found when the specimens are cooled. Correspondingly, experimental transition points measured from heating scans are represented by x in all phase diagrams, while those measured from cooling scans are represented by O. The symbol ⊕ in all phase diagrams represents a temperature where crystalline phase was found by electron diffraction measurement. (b) Lamellar spacings for various compositions (obtained at room temperature) indicating continuous solution behavior. Note positive (lower density) deviation from the Vegard's law line.

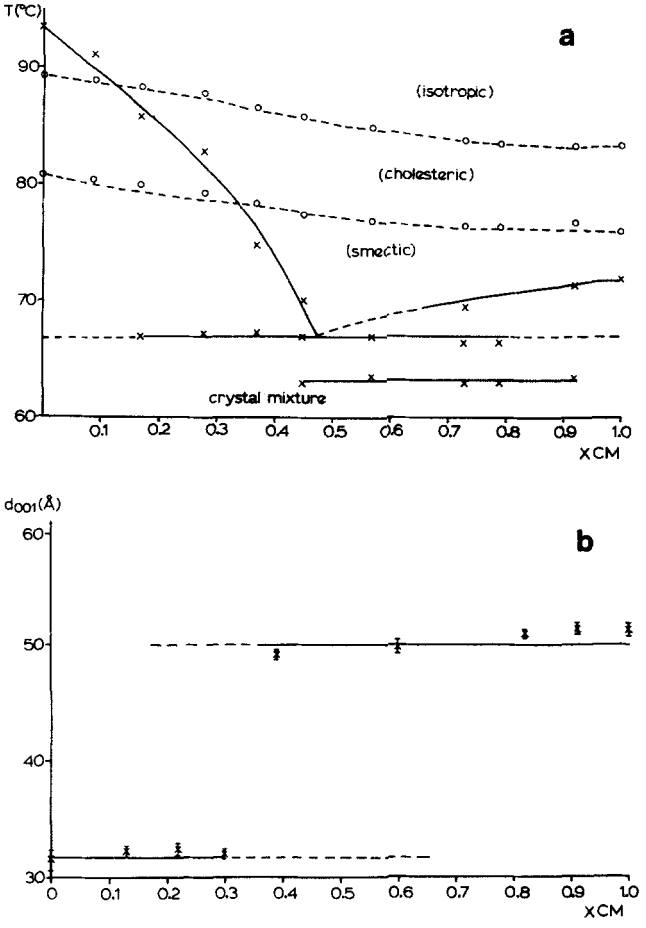


Fig. 4. Binary mixtures of cholesteryl undecanoate/cholesteryl myristate (CM). Two different crystal structures are represented, i.e., the monolayer I for the undecanoate and the bilayer for the myristate Hence, as indicated by the phase diagram (a) and the lamellar spacing dependence on composition (b), the two compounds are immiscible and form a eutectic in the crystalline phase. It must be emphasized here that the lamellar spacings were obtained from the most commonly encountered crystal form on the grid for any given concentration and therefore it does not necessarily exclude the presence of the second component which might be found with enough searching. Extension of the lamellar spacing lines for pure components (dotted regions) illustrates this possibility and also shows that no transition of crystal forms, say at x = 0.35, is implied by the experimental data. The mesophases (dotted lines in (a)), are miscible. These results are different from a previous report (16).

mixed. It is, nevertheless, incomplete, showing mainly what is presumed to be the continuous transition line for the bilayer co-crystals. However, there is also a eutectic relationship between the monolayer I polymorph of the laurate with the myristate bilayer form which is difficult to find in samples crystallized from the melt. (They have not yet reached total equilibrium.) The eutectic diagram should resemble Fig. 4a. Electron diffraction patterns formed from fused samples indicate that a continuous solid solution may indeed be possible for the bilayer structure since it can be detected at all compositions (Fig. 5b) *including* the pure laurate crystals. On the other hand, ample diffraction evidence exists for an eutectic between the laurate monolayer I crystals and the myristate bilayer form both at room temperature (compare to Fig. 4b) and with heated crystals. Below x = 0.49, the crystals are mainly monolayer. Those above this value are largely bilayer. Thus, one arm of the eutectic may be defined by the domain for the monolayer I crystal form (Fig. 5b). Electron diffraction measurements on heated specimens indicate that some of the apparent isotherms in the phase

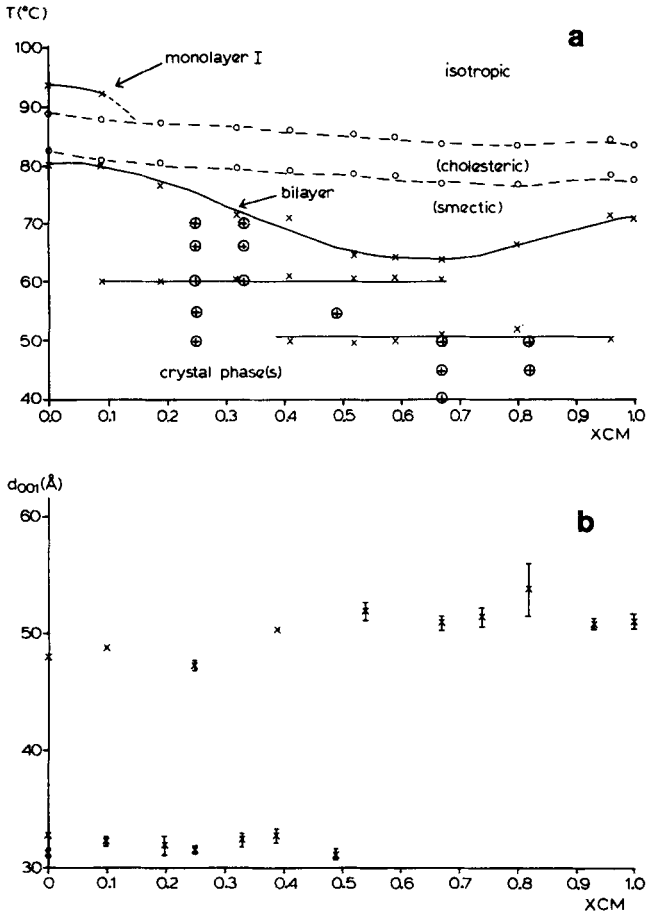


Fig. 5. Binary mixtures of cholesteryl laurate and cholesteryl myristate (CM). Both compounds can crystallize as the bilayer form. Hence, the partial phase diagram from peak values of DSC measurements in (a) indicates continuous solubility as does a portion of the lamellar spacing versus composition diagram in (b). However, some portion of the cholesteryl laurate component also crystallizes as the monolayer I form which cannot form a solid solution with the bilayer. The domain of this eutectic cannot be accurately defined for unequilibrated samples crystallized from the melt. (see (a) and (b)). The mesophases (dotted curves in (a)) are miscible and solid–solid transitions are also observed.

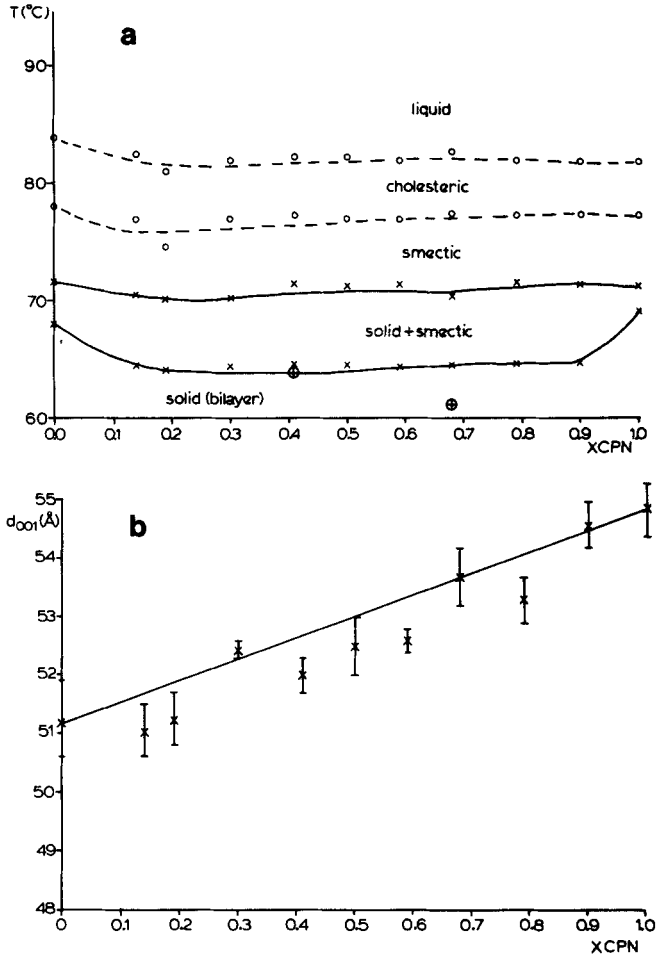


Fig. 6. Binary mixtures of cholesteryl myristate and cholesteryl pentadecanoate (CPN). Both compounds crystallize as the bilayer form and are enantiotropic in their phase behavior. A continuous solid solution is indicated in the phase diagram (a) for which the solid lines denote liquidus and solidus curves. This behavior is also expressed by the lamellar spacing dependence on composition (b). A negative deviation from the Vegard's law line (higher density) could indicate a more stable crystal structure for the solid solutions than e.g., the pentadecanoate (see also Fig. 2).

diagram (e.g., near 50 and 60°C) may not be due to eutectic behavior, but denote the presence of slight conformational changes in the crystal structure (so-called solid–solid transitions (17, 18)). The hysteresis in phase behavior of these mixtures again indicates a long nucleation time for recrystallization from the melt.

Cholesteryl myristate/cholesteryl pentadecanoate. Both components in the mixtures crystallize as the bilayer polymorph and are enantiotropic in phase behavior. There is only one methylene unit difference in the molecular composition and, just like the cholesteryl undecanoate/cholesteryl laurate system, this component pair can form continuous solid solutions **(Fig. 6a).** This behavior is also indicated by the lamellar spacings found at intermediate compositions (Fig. 6b), which are near the line drawn from Vegard's rule (19) (assuming the major volume change to be expressed by the lamellar spacing).

Cholesteryl myristate/cholesteryl palmitate. Both components crystallize as the bilayer polymorph but only one undergoes enantiotropic phase transitions. The phase diagram **(Fig. 7a)** resembles one obtained by Small (9). The lamellar spacing–composition diagram (Fig. 7b) reveals a continuous relationship between these two variables. The positive deviation of these spacings from the Vegard's law line for mole fractions near x = 0.5 could indicate that the crystal structure has a lower density than the pure components (19). An inflection in the crystal-mesophase transition in the phase diagram may indicate nonideal behavior (36).

Cholesteryl myristate/cholesteryl stearate. The properties of the two components are similar to the previous example ex-

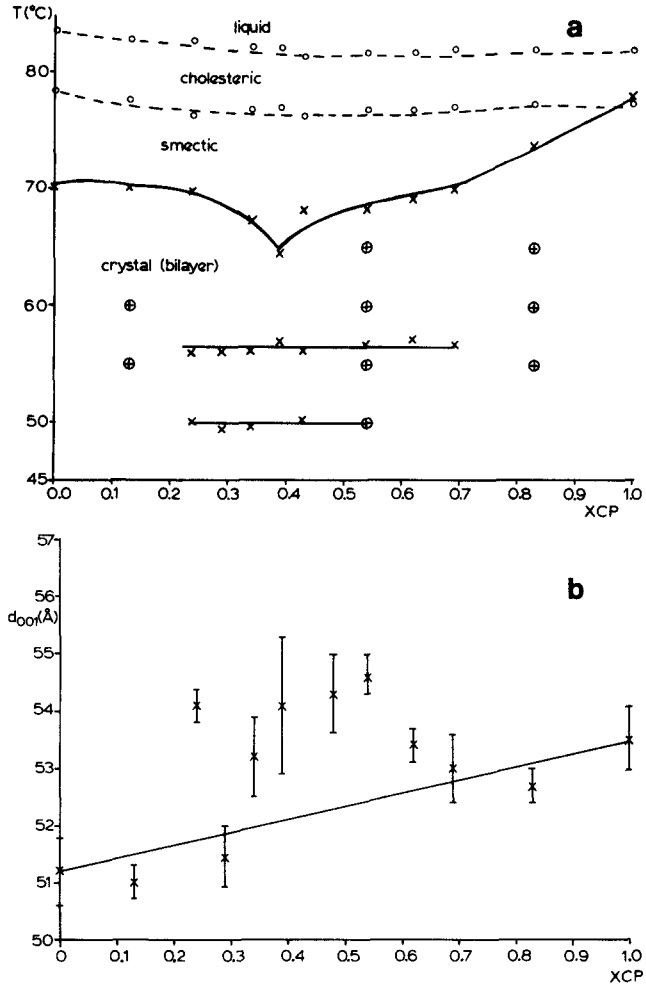


Fig. 7. Binary mixtures of cholesteryl myristate/cholesteryl palmitate (CP), both of which form bilayer crystals. In the phase diagram (a) only peak values are plotted. Comparison to the lamellar spacing versus composition curve (b) indicates that these two compounds form a continuous solid solution which, however, may be nonideal. (Note the positive deviation from the Vegard's law line in (b) near the inflection point of the crystal-smectic transition curve). The mesophases are co-soluble. Also note the existence of crystal-crystal transitions in (a) near 55°C which are shown by electron diffraction measurements (⊕) not to be a eutectic isotherm.

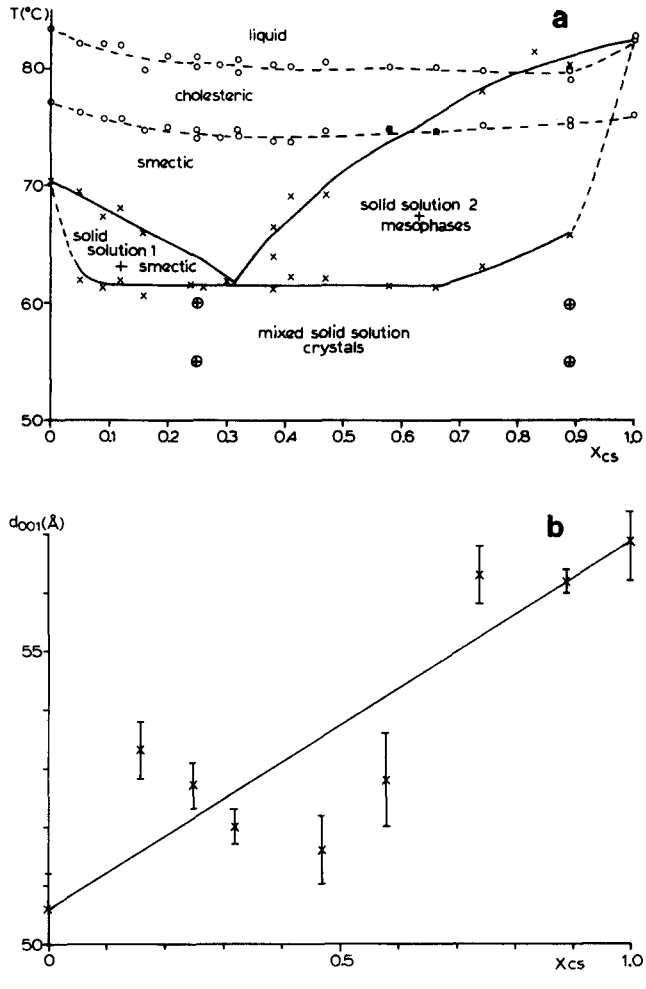


Fig. 8. Binary mixtures of cholesteryl myristate/cholesteryl stearate (CS). The phase diagram (a) is similar to one published previously (16), except that the eutectic isotherm does not extend over the whole concentration range. The lamellar spacing dependence on composition (b) shows positive deviations from Vegard's law in the regions indicated in (a) for the solid solutions. The mesophases (dotted curves) are again miscible.

cept that there is a four methylene unit difference in the molecular composition. Galanti and Porter (16) interpreted their phase diagram to indicate eutectic behavior between two immiscible components. Our phase diagram **(Fig. 8a)** is similar to theirs except that the isotherm does not extend over the total composition range. Thus, the eutectic more likely exists between two solid solutions. Continuity of the lamellar spacing relationships with composition is shown in Fig. 8b. Two regions are found where the lamellar spacings appear to deviate from the Vegard's law line in a positive direction, roughly corresponding to the regions where the separate solid solutions are defined by the phase diagram.

DISCUSSION

From the data given above it is possible to predict the existence of stable continuous solid solutions in binary cholesteryl ester mixtures using rules that are consistent with those given by Kitaigorodskii (23). First of all, the crystal structures of the two esters must be similar enough to allow the occupancy of a lattice site in a "host" crystal by a "guest" molecule. Here we see that an ester (the laurate) which can assume either of two polymorphic crystalline forms can be induced to cocrystallize in the symmetry form preferred by the solvent (the host crystal) in the solid solution. Given the similarity of the structures, a second factor is that of comparable molecular volumes. That is, even when two members of a homolo-

gous series have the same crystal structure, they will not form continuous solid solutions when the parameter of geometrical similarity, defined by Kitaigorodskii (19) to compare the molecular shapes, is too small. Obviously, the application of these rules is less strict for comesophases than for mixed crystalline phases, as evidenced by the phase diagrams shown above. Nevertheless, Ginsburg et al. (10) have discussed possible geometric factors that may affect the molecular packing in the smectic phase.

Within the symmetry criterion for the two components of a potential solid solution, conditions favorable for cosolubility are illustrated by the examples above. Using the known crystal structures (30–33), the two structures that can pack in the monolayer I crystal form can be shown to occupy nearly the same molecular volume and, thus, the introduction of an additional methylene group causes no significant strain to the crystal packing in the solid solution. Recently, a further example of similar molecular volumes in monolayer I crystal structures was found for cholesteryl *cis*- and *trans*-9-hexadecenoates (37). It will be interesting to test chain length dependence on cosolubility for this polymorph in future work. Although all esters from nonanoate to laurate assume this crystal form, there is less of a unit cell expansion with increasing chain length than is found in the bilayer polymorph (10).

Effects of chain length on cosolubility are demonstrated here for the bilayer crystalline forms. With a chain length difference of one methylene unit, an ideal continuous solid solution is formed. A difference of two methylene units on either side of a myristate ester reference still permits solid solution. In the case of the myristate/palmitate system (Fig. 7a), there may be non-ideal behavior (as indicated by the inflection point (36)) and thus a miscibility gap might be expected at true equilibrium at lower temperature. Partial fractionation is observed when the chain length difference is four carbons.

Of the two crystal packings seen in this study, the behavior of the binary solutions packing in the bilayer polymorph is most like the n-paraffins, since lattice vacancies are concentrated in a particular lamellar region (20, 21) as would be seen by examination of the cholesteryl myristate crystal structure (29). That paraffin solid solutions can accommodate a four-carbon chain difference and maintain ideal solid solution behavior (38) probably illustrates an entropic contribution to the total free energy of the mixed system (i.e., the translational freedom of a shorter paraffin chain in a mixed chain lamellae (20, 39)) which cannot be expressed for the cholesteryl ester since these must also satisfy the internal energy requirements for packing of the steroid nuclei.

One deviation from paraffin-like behavior for the cholesteryl esters is that no evidence for superlattices is found in the diffraction from the one fractionated system examined here. In paraffin mixtures where components have different crystal structures, e.g., in even and odd chain orthorhombic crystal structures, a fractionated mixture might be found which contains a random stacking of pure lamellae of either component in crystallographic register across similar methyl end planes (40) to form a superlattice. This is implied by Mazee's (41) study of the $nC_{30}H_{62}/nC_{35}H_{72}$ mixtures. Such a lamellar epitaxy has been characterized for fractionated components that have identical crystal structures (21). It is obvious from the diffraction data (or also from comparison of monolayer and bilayer crystal structures and/or unit cell data in Table 1) that no epitaxial relationship can exist for stacking monolayer I lamellae with a bilayer crystal form, since there is no similarity of lattice spacings. The eutectic in this case must be a "mechanical mixture" in the absence of specific crystallographic interactions. It remains to be seen whether interlamellar epitaxy can be found in fractionated esters when both have a bilayer crystal structure.

Another problem to be examined in more detail is the actual relationship between lamellar spacing and mole ratio for a cholesteryl ester binary solid. Although reference is made to a Vegard's law relationship, as is usual for such linear chain materials (19), recent unpublished work on n-paraffin solid solutions from this laboratory indicate that this behavior is actually more complicated. For example, in solid solutions of $nC_{32}H_{66}$ with $nC_{36}H_{74}$, indices of Okℓ electron diffraction patterns for compositions with increasing $nC_{36}H_{74}$, indicate that the apparent crystal structure of successive concentration domains mimic $nC_{32}H_{66}$, $nC_{33}H_{68}$, $nC_{34}H_{70}$, $nC_{35}H_{72}$, $nC_{36}H_{74}$, where particular regions are governed by one or two prominent crystal structures. This results in a steplike increase in lamellar spacing which fits the observed data far better than the Vegard's law line. In addition, the change in unit cell symmetry represents only a very small energetic difference and it can be shown that the chain end void volume is also minimized by this alternating structural sequence. Similar trends may be indicated by some of our lamellar spacing data from cholesteryl esters. For example, cholesteryl undecanoate/cholesteryl laurate (Fig. 3b) seems to be dominated by a undecanoate structure at low laurate concentrations and a laurate structure at higher laurate concentrations. The myristate/palmitate solutions (Fig. 7b) might have sequences with quasi-myristate, pentadecanoate, and palmitate structures. (Remember that the odd chain spacings lie somewhat above the even chain line (Fig. 2.) A sequence of steps may also be indicated in Fig. 8b, even though this represents a eutectic of solid solutions. These relationships would be more easily established if good zonal diffraction patterns were available to clarify the lamellar data used in this paper. Perhaps these better crystals can be realized by annealing the samples in the presence of the nucleating substrate for epitaxial growth as is possible with linear polymers (Dr. J. C. Wittmann, personal communication).

In summary, the above studies show that the miscibility of cholesteryl esters is determined both by symmetry and size considerations while enantiotropic versus monotropic phase behavior seems to be less important. Such factors will undoubtedly be important in determining the physical state and phase behavior for cholesteryl esters found in fatty lesions and thus will contribute to their fluidity or immobilization.

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