Phase Transitions in Binary Mixtures of Cholesteryl Esters

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Abstract □ Phase transitions in mixtures of mesomorphic (liquid crystalline) cholesteryl esters were studied by visual thermal microscopy. Binary mixtures of saturated cholesteryl esters (cholesteryl nonanoate, myristate, and palmitate) and binary mixtures of these saturated cholesteryl esters and an aromatic cholesteryl ester (cholesteryl benzoate) show transitions from the solid phase to the mesomorphic phase at temperatures that are depressed well below the phase transition temperature of either pure compound. However, the mesomorphic to isotropic phase transition temperatures are virtually linear functions of the composition of the mixtures. The result is that the temperature range over which the binary mixtures are mesomorphic is greatly expanded when compared to the narrow range observed for the individual components. The solid to mesomorphic phase transitions of these mixtures occur above body temperature. However, binary mixtures of the saturated cholesteryl esters with unsaturated cholesteryl esters (cholesteryl oleate and linoleate) are mesomorphic at body temperature except at low concentrations of the unsaturated ester. The unsaturated cholesteryl esters along with small amounts of other cholesteryl esters are major components of lipid droplets found in atheromatous lesions. The mesomorphic behavior of mixtures of the cholesteryl esters may be related to their interassociations in these lesions.

Keyphrases □ Phase transitions—binary mixtures of cholesteryl esters □ Cholesteryl esters—phase transitions in binary mixtures □ Thermal microscopy, visual—determination, phase transitions in mixtures of cholesteryl esters

Cholesteryl esters belong to a class of compounds that do not melt directly from a crystalline solid to an isotropic liquid but proceed from the solid state to the isotropic liquid through an intermediate mesomorphic phase or liquid crystalline phase (1–4). The latter term refers to the liquid mobility of the phase while the molecules remain in domains arranged in an anisotropic ordered array similar to a solid (1–4). Two phase transitions are observed: the first from crystalline solid to the mesomorphic phase and the second, at a higher temperature, from the mesomorphic phase to an isotropic liquid.

Liquid crystals can be categorized into three structural arrangements. The simplest and least-ordered molecular arrangement is the nematic type, consisting of parallel-oriented rodlike molecules arranged in bundles but not in layers (1, 4). The smectic mesomorphic phase has rodlike molecules arranged parallel to one another in stratified layers. Within each layer, the molecules may be in a regular or random arrangement (1).

The cholesteric mesomorphic phase is the third type of liquid crystal and is exhibited primarily by cholesterol esters but not by cholesterol itself. The cholesteric mesomorphic phase resembles the smectic type in that the molecules are arranged in stratified layers; it is also similar to the nematic type in that the molecules are in a random parallel arrangement. However, the layers are thin and the long axes of the molecules are parallel to the plane of the layer. The projection of a methyl group from the otherwise reasonably flat molecule gives a slight displacement of the long axes of the molecules in each layer. This gives rise to a screw-axis that runs through the layers, and it is this helical pattern that gives the very high optical activity characteristic of this class of liquid crystals (1, 2).

Most cholesteric compounds are white solids at temperatures below the transition temperature into the mesomorphic phase, and they are colorless liquids at temperatures above which they can exist in the mesomorphic phase. However, they exhibit a spectrum of colors as a function of temperature as they pass through the mesomorphic phase (3, 5). Lower temperatures, which correspond to higher degrees of molecular order, result in the scattering of colors of longer wavelength, while higher temperatures create lower degrees of molecular order and cause the scatter of lower wavelength light (5). In addition, cholesteric liquid crystals have an iridescence, due to circular dichroism, when they are illuminated by white light (3).

Not only is the basic physical chemistry of the cholesteryl esters of interest, but many of these compounds are found *in vivo* and are known to be of importance in disease states such as atherosclerosis. Their wide distribution in the body suggests that they are important in many normal or pathological functions (1, 6).

Although the physical state of individual saturated, monounsaturated, and polyunsaturated cholesteryl esters has been studied (7, 8), the properties and behavior of mixtures of these esters have received only limited attention. Small (8) studied the physical state of mixtures of cholesteryl oleate and cholesteryl linoleate. However, there are no reports of the state of binary mixtures of saturated cholesteryl esters, of saturated and

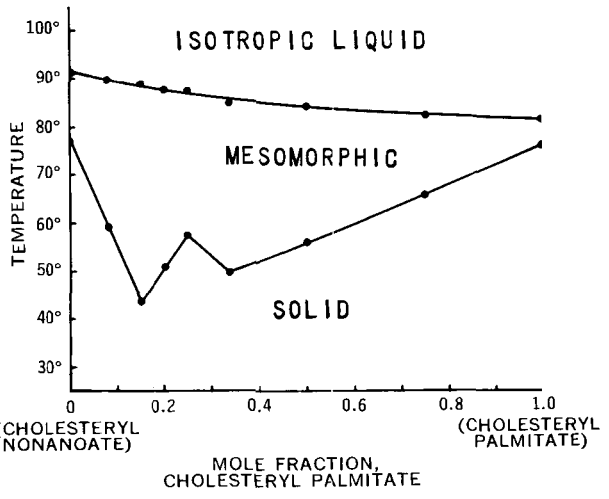

Figure 1—*Phase diagram for mixtures of cholesteryl nonanoate and cholesteryl palmitate.*

unsaturated cholesteryl esters, and of various combinations of saturated or unsaturated cholesteryl esters with an aromatic cholesteryl ester. The object of this report is to discuss the physical state of representative mixtures in each category with reference to their possible relationships to biological systems.

EXPERIMENTAL

Materials—Cholesteryl nonanoate[1], myristate[1], palmitate[1], stearate[1], oleate[1], and linoleate[1] were recrystallized three times from boiling ethanol (7) and dried under vacuum at 30° for 48 hr. Cholesteryl benzoate[2] was recrystallized three times from boiling *n*-pentanol (9) and dried under vacuum at 30° for 48 hr. The mesomorphic phase transition temperatures of these esters as observed in the polarizing microscope were in good agreement with previously reported values (2, 8).

Instrumentation—All samples were observed between crossed polarizers in a microscope[3]. A microscope hot stage[4] was used to maintain isothermal conditions when necessary and to heat the samples at linear programmed rates. The initial heating rate for scanning purposes was an increase of 2°/min., while most experimental determinations were made at 0.2°/min.

Preparation of Mixtures and Samples for Microscopy—Mixtures of the cholesteryl esters were prepared by fusing the two components in small Pyrex culture tubes with Teflon-lined screw caps. The mixtures were melted and stirred with a fine glass rod before sealing. The mixtures were slowly cooled to room temperature and allowed to equilibrate at ambient temperature for 72 hr. before use.

A sample of the powdered mixture was placed on a glass slide, and a coverslip was placed over the sample to produce a thin wedge-like sample with one edge of the coverslip resting on the slide.

Microscopic Examination of Samples—All samples were heated from 30° to their respective isotropic transition temperatures. The anisotropic solid to anisotropic mesomorphic phase transition temperature was that at which the first melting of the sample was observed. Depending on the sample, this took the form of a small liquid ring on the slide or a slight darkening or blurring of the sample. The mesomorphic phase to isotropic liquid transition temperature was the temperature at which the sample became completely isotropic. Three samples for microscopy were taken from different portions of each mixture. Agreement between these runs was good (±0.1°), indicating good mixture homogeneity. Duplicate mixtures agreed within ±1°.

RESULTS AND DISCUSSION

As seen in Fig. 1, the solid to mesomorphic phase transition temperature for mixtures of cholesteryl nonanoate and cholesteryl palmitate is depressed below that of the phase transition temperature of either pure compound. However, the mesomorphic to isotropic phase transition temperatures change linearly with the changing composition of the mixture. When two pure crystalline compounds are mixed, the melting point of the mixture probably will be depressed below that of either of the pure compounds. When two compounds that give mesomorphic phases are mixed, it is reasonable to assume that the mesomorphic transition temperatures will be depressed (2). If both components are capable of giving a mesomorphic phase, then the mixture can be expected to be mesomorphic. The result (Fig. 1) is that for mixtures of cholesteryl nonanoate and cholesteryl palmitate, the mesomorphic phase region is greatly enlarged when compared to the mesomorphic region of the individual pure compounds. For particular mixtures of the two components, the lower limit of the mesomorphic region extends to rather low temperatures. This is not unlike a eutectic phenomenon, and indeed there are two minima on the solid to mesomorphic phase transition temperature curve (Fig. 1) at molar ratios of cholesteryl palmitate to cholesteryl nonanoate of approximately 1:6 and 1:2. The overall pattern of phase transitions in the mixtures of mesomorphic compounds differs from that of mixtures of nonmesomorphic eutectic compounds (10, 11); however, certain behavioral aspects may be similar in nature. Mixtures of the cholesteryl esters are miscible in the mesomorphic phase and in the isotropic liquid phase. The two minima on the solid to mesomorphic phase transition curve each appear to represent the precipitation of two mutually saturated liquid crystalline solutions to form an interstitial or a substitutional (10) solid solution. The latter structural arrangement is more probable since the components are virtually identical in molecular size and shape. The lower transition temperature of the 1:6 mixture indicates that the solid solution crystal lattice energy of this mixture is lower than that of the 1:2 mixture. It is of interest to note that at 0.25 mole fraction of cholesteryl palmitate, the solid to mesomorphic phase transition curve shows a peak, indicative of a favorable composition having a small thermal stabilization effect on the solid solution.

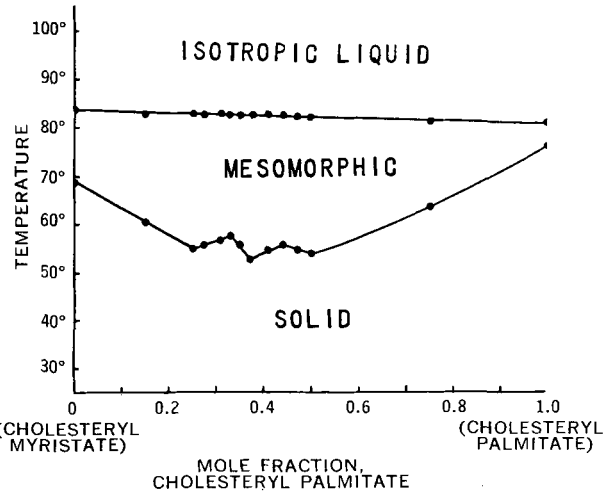

Figure 2—*Phase diagram for mixtures of cholesteryl myristate and cholesteryl palmitate.*

The virtual linearity of the mesomorphic to isotropic transition temperature curve with changing composition of the mixture was reported by Gray (2). This behavior suggests that the two mesomorphic components have a low degree of mutual interaction at this transition temperature, consistent with the low degree of molecular order expected at this transition. This is in general agreement with calorimetric studies of aliphatic esters of cholesterol in which it was found that less than 1 cal./g. was required for the mesomorphic to isotropic phase transition (4, 12, 13). Approximately 2% of the total entropy gain on heating these esters from the solid to the isotropic phase is at the mesomorphic to isotropic phase transition temperature (4, 12, 13).

The detailed binary phase diagram can be expected to be considerably more complex than the relatively simple diagrams in Fig. 1 and in subsequent figures in this report. Aside from the major phase transitions, two or more phases can be expected to exist in equilibrium at a number of locations on the phase diagram (8). Such phase equilibria do exist; however, this report is concerned with the major phase changes in these systems. Work is currently underway to identify and characterize these equilibrium phases. These studies are facilitated by the use of depolarized light intensity analysis (14, 15) in conjunction with visual thermal microscopy.

The effect of an increase in the chain length of one of the cholesteryl esters in a binary mixture is illustrated in Fig. 2, which shows the thermal behavior of mixtures of cholesteryl myristate and cholesteryl palmitate. Increasing the chain length of one component has the effect of increasing the temperature at which the entire solid to mesomorphic phase transition occurs, apparently due to the stronger chain interactions of the long chain cholesteryl esters in the solid solution of this mixture compared to those in the previous mixture (Fig. 1) of short and long chain cholesteryl esters. The solid to mesomorphic phase transition temperature curve for these cholesteryl ester mixtures has three minima and two peaks, the interpretation of which was already discussed for the similar behavior of the cholesteryl nonanoate–cholesteryl palmitate systems.

The mesomorphic behavior of mixtures of an aromatic cholesteryl ester (cholesteryl benzoate) and a saturated cholesteryl ester (choles-

[1] Eastman Organic Chemicals.
[2] Aldrich Chemical Co.
[3] Leitz Laborlux.
[4] Mettler FP-2.

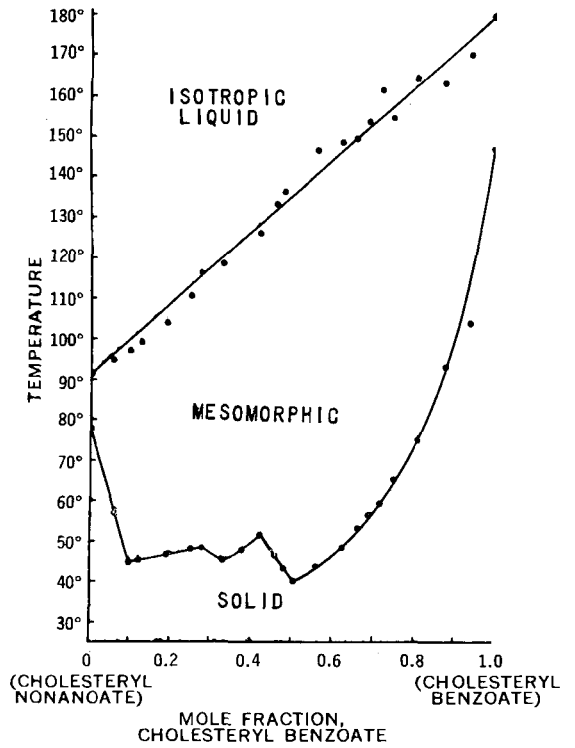

Figure 3—*Phase diagram for mixtures of cholesteryl nonanoate and cholesteryl benzoate.*

teryl nonanoate) can be seen in Fig. 3. The general pattern of the thermal behavior of these systems is similar to that of the mixtures of saturated cholesteryl esters, except that the mesomorphic region in the cholesteryl benzoate–cholesteryl nonanoate mixtures is larger and the solid to mesomorphic phase transition temperature curve occurs at slightly lower minimum temperatures. This is of interest in view of the rather high mesomorphic phase transition temperature limits for cholesteryl benzoate. However, an increase

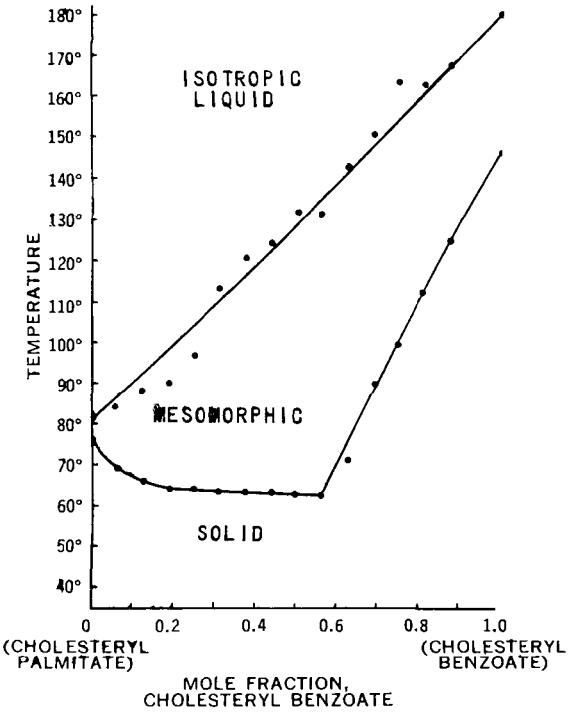

Figure 4—*Phase diagram for mixtures of cholesteryl palmitate and cholesteryl benzoate.*

in the chain length of the saturated cholesteryl ester to cholesteryl palmitate (Fig. 4) produces a reduction in the size of the mesomorphic phase region over the entire concentration range and a significant rise in the temperature at the lower limits of this phase when compared to that of the cholesteryl benzoate–cholesteryl nonanoate mixtures (Fig. 3). A single minimum in the solid to mesomorphic phase transition temperature curve is seen in Fig. 4 at 0.57 mole fraction of cholesteryl benzoate.

The mesomorphic to isotropic liquid phase transition temperature curve remains a linear function of concentration for both mixtures (Figs. 3 and 4), similar to the behavior of the binary mixtures of the saturated cholesteryl esters (Figs. 1 and 2).

The study of mixtures that are mesomorphic at body temperature may have relevance to an understanding of the physical state of some lipids of biological importance, particularly as related to atherosclerosis. It has been well established that the α- and β-lipoproteins in the plasma (16–18) and the lesions of atherosclerosis (17, 18) contain a large variety of cholesteryl esters. The mechanism by which the cholesteryl esters accumulate in the lesions has been the object of much controversy. Abnormal filtration of low density plasma lipoprotein, metabolic faults affecting the outgoing flux of lipoprotein from the arterial wall, and enzymatic synthesis from cholesterol within the arterial wall are but a few of the possible mechanisms (17–20). The lipid accumulations have been found to exist in droplet form within cells and in the extracellular spaces (18).

Approximately 70–80% of the cholesteryl esters in α- and β-lipoproteins and in atheromatous lesions are cholesteryl oleate and cholesteryl linoleate. There are also small amounts of cholesteryl palmitate, cholesteryl palmitoleate, cholesteryl arachidonate, cholesteryl stearate, and cholesteryl myristate, and lesser amounts of a number of other cholesteryl esters (18, 21).

Preliminary experiments conducted in this laboratory indicate that binary mixtures of a saturated and an unsaturated cholesteryl ester show thermal behavior similar to that of the mixtures of cholesteryl esters reported here. Mixtures of cholesteryl oleate (mesomorphic range 17–50°) and cholesteryl nonanoate are mesomorphic in the body temperature range at mole fractions of cholesteryl oleate of 0.1 or greater. However, for mixtures of cholesteryl palmitate and cholesteryl oleate, the mesomorphic phase at body temperature is found at mole fractions of cholesteryl oleate greater than 0.25. Similar values are obtained for mixtures of cholesteryl linoleate (mesomorphic range 38–46°) and cholesteryl nonanoate or cholesteryl palmitate.

All mixtures of cholesteryl oleate and cholesteryl linoleate are mesomorphic at body temperature. This is consistent with the work of Small (8).

The chain length of the cholesteryl esters in binary mixtures appears to have a significant role in the mesomorphic properties and thermal behavior of the mixtures. The depression of the solid to mesomorphic phase transition temperature is of sufficient magnitude in most binary mixtures of an unsaturated and a saturated cholesteryl ester to bring the mesomorphic phase of the mixture into the body temperature range. This may be a possible mechanism by which the high melting cholesteryl esters are prevented from precipitating from the cholesteryl ester droplets found in atheromatous lesions. The cholesteryl ester droplet is believed to be mesomorphic (8) and, as indicated earlier, analysis of the droplets revealed a wide variety of cholesteryl esters, many of which are solid at body temperature.

Considering the biological and pathological importance of these cholesteryl esters, studies such as this may prove helpful in understanding the function and mutual dependency of these lipids.

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Toxogonin and Pralidoxime: Kinetic Comparison after Intravenous Administration to Man

FREDERICK R. SIDELL▲, WILLIAM A. GROFF, and ANDRIS KAMINSKIS

Abstract □ After intravenous administration to humans, the chloride and methanesulfonate salts of pralidoxime were found to have identical pharmacokinetic characteristics. Toxogonin had a much smaller volume of distribution and a lower renal clearance rate. These findings explain the fivefold difference in plasma concentrations after similar doses of toxogonin and pralidoxime.

Keyphrases □ Toxogonin–pralidoxime—kinetic comparison after intravenous administration, man □ Pralidoxime–toxogonin—kinetic comparison after intravenous administration, man □ Volume of distribution—review of definitions and interrelationships of various terms □ Anticholinesterase intoxication therapy—kinetic comparison of toxogonin and pralidoxime, intravenous administration, man □ Pyridinium oximes—kinetic comparison of toxogonin and pralidoxime, intravenous administration, man □ Pharmacokinetics—comparison of toxogonin–pralidoxime, intravenous administration, man, volume of distribution terms reviewed and compared

The pyridinium oximes are widely accepted as valuable adjuncts to atropine in the therapy of anticholinesterase intoxication. Pralidoxime chloride (2-pyridine aldoxime methochloride) is the preparation used in this country, and toxogonin [*N,N'*-oxydimethylene bis(pyridinium-4-aldoxime)dichloride] is preferred in Europe. The two structures are basically similar, but toxogonin consists of two pyridinium rings linked by an oxygen molecule. Thus, it is about twice the size and weight of pralidoxime chloride.

Previous studies with pralidoxime chloride and toxogonin showed a marked difference in the relationship between apparent dose and plasma concentration between these two closely related materials. After intramuscular administration of equal doses (milligrams per kilogram), plasma levels of the oxime produced by toxogonin were four times higher than those produced by pralidoxime chloride (1, 2). Urinary excretion of both oximes was quite high: 84% of the dose for toxogonin and 91% of the dose for pralidoxime chloride.

These findings strongly suggest a difference in the volume of distribution for these compounds. To investigate this further, the two drugs, along with pralidoxime methanesulfonate, were given intravenously to volunteer subjects.

Although in principle its meaning should be clear, the term "volume of distribution" has been defined in various ways (3). A brief review of these definitions and their interrelationships is also given in this report.

EXPERIMENTAL

Subjects—The subjects were U. S. Army enlisted men who volunteered to participate after the test was discussed with them. Each had a complete medical evaluation including a physical examination, chest X-ray, ECG, and laboratory tests [hematocrit, total and differential white blood cell count, urinalysis, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, bilirubin, creatinine, and red blood cell and plasma cholinesterase] and were found to be free from abnormality before being accepted into the study.

Methods—Ideally, this study should have had a crossover design; however, the subjects were available for only a short time, and multiple venipunctures twice in this period did not seem warranted.

The subjects were admitted to the test ward on the evening before the test. On the morning of the test, they were given a light breakfast and thereafter they were urged to drink large amounts of