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PHYSICAL PROPERTIES OF CHOLESTERYL ESTERS

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CONTENTS

I. INTRODUCTION

Cholesteryl esters are structurally composed of a cyclopentaphenanthrene ring system linked via an ester bond at the steroid 3-position to an aliphatic hydrocarbon. The ability of these molecules to form liquid crystals has fascinated chemists for nearly a century. Studies of the physical properties of biologically important cholesteryl esters have suggested a role of the liquid crystalline state in disease processes.[111] Droplets of cholesteryl esters appear histologically or submicroscopically in a variety of normal and pathological cellular processes.[112] For example, cholesteryl ester droplets have been described in neural tissue prior to nerve myelination.[42] The presence of a cholesteryl ester-rich core characterizes the lipoprotein particles responsible for cholesterol transport in the blood to and from the tissues.[45,109] The adrenal gland accumulates cholesteryl esters for the genesis of steroid hormones. Pathologically, massive cellular cholesteryl ester accumulations occur in cholesteryl ester storage disease,[110] Wolman's disease,[4] Coat's disease,[51] Tangier disease[74] and cholesterolosis of the gallbladder.[69] Atherosclerosis is by far the most common and most devastating result of cholesteryl ester accumulation with both intra- and extracellular deposits forming a major part of the lesion.[68,73,113,116,118]

In systems such as cholesteryl ester-rich lipoproteins and atheromatous lesions, the cholesteryl esters (primarily cholesteryl linoleate and cholesteryl oleate) undergo a phase transition from a smectic liquid crystal→disordered liquid phase close to or above body temperature.[44,45,73,119] However, in isolated pure systems, these cholesteryl esters not only have slightly higher transition temperatures but also exhibit an additional intermediate cholesteric liquid crystal phase.[60,111] Elucidation of the factors which affect the phase behavior and transition temperature of pure cholesteryl esters is thus important in order to understand the phase behavior of cholesteryl esters in biological systems.

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A number of authors have studied the esters of saturated fatty acids of cholesteryl in some detail,[17-19,39-41,56,59,60,64,72,111] and we have studied the unsaturated esters.[7,14,26,44,45,60,61,71,83,107,111,116] This review will describe in detail the physical states of cholesteryl esters. Data is presented from polarizing microscopy, differential scanning calorimetry (DSC), ^{13}C nuclear magnetic resonance spectroscopy (NMR) and X-ray diffraction, as well as other physical techniques and will provide some unifying concepts which describe the transition from one phase to another as well as provide a structural basis for the phase organization.

Although this review concentrates primarily on esters formed between cholesterol and fatty acids, thio-esters, alkanoics[64] and ethers[43] also form liquid crystals. Further, the long-chain esters of the saturated analog of cholesterol, cholestanol, form thermotropic liquid crystals and their properties are similar to cholesteryl esters.[92,93] Finally, a variety of other sterol esters, including plant sterols, show mesomorphic behavior.[9-13,38a,53-55,75,76,78,91,95-98,124,127] Those interested in the effects of the structure of the sterol moiety on mesophase behavior are referred to the above articles.

II. CHEMICAL FORMULATION AND NOMENCLATURE

Cholesteryl esters are the condensation product of the polycyclic alcohol, cholesterol, and an aliphatic carboxylic acid. The basic chemical structure represented by cholesteryl linoleate is shown in Fig. 1; the steroid cyclopentaphenanthrene nucleus is rigid and internal rotation about C–C bonds is prohibited. Standard steroid nomenclature is used to describe ring carbons and there is a double bond at $\Delta^{5,6}$. An isocytyl aliphatic side chain is present at C–17 and the fatty acyl chain is esterified at C–3.

Unsaturation in the fatty acyl chain is described by two different terminologies: Δ and ω. The Δ terminology indicates the double bond position by denoting the carbonyl carbon as C–1 whereas, in the ω system, the double bond position is indicated relative to the terminal methyl carbon as C–1. Cholesteryl linoleate, for example, can thus be described as cholesteryl *cis*-$\Delta^{9,12}$ octadecadienoate or cholesteryl ω-9,6 octadecadienoate, or simply $C_{18:2}$, $\Delta^{9,12}$ or $C_{18:2}$ ω-9,6. The ω terminology is more useful in biological systems because unsaturation is limited to carbons beyond the sixth carbon from the terminal methyl, i.e. linoleic acid with an ω-6 double bond must be supplied exogenously. (See volume 9 of this series.)

This class of molecules is of particular interest because the linkage of the rigid steroid ring system to a highly mobile fatty acyl chain results in a product whose physical properties are unique and not readily determined *a priori* through a knowledge of the physical properties of cholesterol and fatty acids. In 1888 Reinitzer observed that, on heating, cholesteryl benzoate "melted" first to a viscous turbid liquid and then some degrees higher became optically clear. In 1889 Lehmann[78a] studied the intermediate turbid phase and called it "Fliessende Krystalle" or "Flüssige Krystalle" (flowing or fluid crystals). Friedel[57] called this the mesomorphic state, i.e. a state between solid and liquid.

III. PHYSICAL STATES OF CHOLESTERYL ESTERS

A. *Crystalline States*

The existence of more than one crystalline form for a given substance is known as polymorphism. The importance of polymorphism of cholesteryl esters is that not only might the properties such as solubility, density (volume) and specific heat be different for one form over another, but also the melting point and mesomorphic behavior may differ as well. In fact, crystal polymorphism and mesomorphism may be quite closely related. Crystallization from a mesomorphic state may result preferentially in one polymorphic form over another and this behavior may provide structural information relevant to the mesomorphic state.

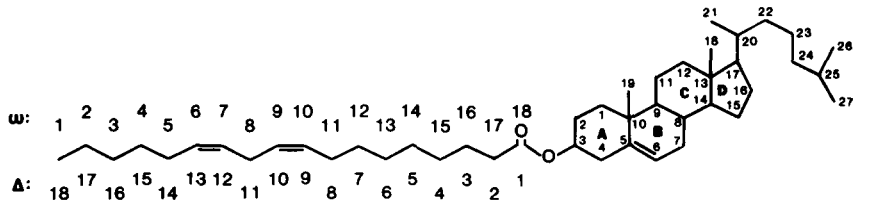


FIG. 1. Schematic representation of cholesteryl linoleate. Standard steroid nomenclature is used to denote ring carbons by number. The ω and Δ conventions are depicted along the fatty acyl chain.

The crystal structures of several cholesteryl esters have been solved by single crystal X-ray crystallography (Table 1). Three general molecular packing arrangements have been described by Craven for cholesteryl esters with six carbons or greater: *monolayer type I*, *monolayer type II* and *bilayer*.[32] For reasons which are unclear at this time, shorter chained esters do not appear to belong to any of these crystal systems. In cholesteryl esters, the relative importance of intermolecular chain–chain, ring–ring and ring–chain interactions will be a factor which determines crystallization to one crystal type or another. These interactions thus determine the relative stability of polymorphic forms at a particular temperature (and pressure). Cholesteryl esters which exhibit polymorphic behavior have one of two crystal forms—monolayer type I or monolayer type II. Three polymorphic crystal forms for a given cholesteryl ester have not been observed and cholesteryl esters of the bilayer crystal type apparently do not exhibit polymorphism.

1. *Monolayer Type I*

Cholesteryl nonanoate,[15,16,103] decanoate,[15,16,94] laurate,[3,15,16,104] palmitoleate[105] and nervonate (Sawzik, He and Craven, unpublished data) form monolayer type I crystals. Crystals of these esters are monoclinic with a $P2_1$ space group. Figure 2 shows the crystal structures of three of these esters viewed along the *b*-axis. The unit cell contains molecules of two types which are not related by crystal symmetry (denoted A and B). The molecules are packed to form layers with the alkanoate chains of the B molecules and the isooctyl C–17 side chain of the A molecules at the interfacial region. A molecules are related to A molecules (and B to B) by the two-fold screw axis through the center of the monolayer. Several types of interactions have been described by Craven[32] as characteristic of this crystal form. The predominant interaction is ring–chain with C- - -C internuclear interaction distances of less than 4.5 Å occurring between A-chains and A-rings, A-chains and B-rings and B-chains and B-rings. The acyl chains do not pack with one another; however, there is some overlap between the ends of B-chains from opposing monolayers. The change in monolayer thickness is not related to the number of atoms in the acyl chain but rather is associated with packing requirements of the C–17 isooctyl group of the A-molecules since, in all cases, overlap of B-chains from opposing monolayers occurs at the C–C bond seven carbons from the ester linkage. Thermal motions within the crystal structure (not shown in Fig. 2) indicate that the A-chain which is involved in interactions with both A and B rings is more restricted than the B chains. The most thermally mobile groups are the aliphatic groups at the interface. A dipole–dipole interaction between opposed carbonyl groups of adjacent A molecules may also stabilize the crystal.

2. *Monolayer Type II*

Cholesteryl esters with a known crystal structure of monolayer type II are cholesteryl octanoate[15,16,4] and cholesteryl oleate[35] (Fig. 3a,b). This crystal form is monoclinic, space group $P2_1$, with two molecules per unit cell. Molecules in the unit cell are of a single type related to one another by the two-fold screw axis parallel to the *b*-direction. The molecules pack in an antiparallel arrangement with significant ring–ring and chain–chain interactions; limited ring–chain interactions, however, are present in this crystal type between the isooctyl side chain and the C-ring and the fatty acyl chain terminus and the A-ring

TABLE 1. Single Crystal X-ray Data for n-Aliphatic Cholesteryl Esters[a]

Common name	n	Space group	Z	a (Å)	b (Å)	c (Å)	β (°)	Vol. (Å^3)	D_c	ρ (gm/cc)	m.p. (°C)	Ref.
(a) *Saturated*												
Chol. Formate	1	$P2_1$	2	15.65	6.06	13.53	96	1276	1.027	1.050	—	b
Chol. Acetate	2	$P2_1$	4	17.51	9.44	16.4	105.5	2612	1.023	1.056	—	b
(at −150°C)	4	$P2_1$	4	16.54	9.29	17.64	106.95	—			114–115	c
	2	$P2_1$	4	17.6	9.5	16.21	104.0	—			—	d
	2	$P2_1$	4	17.6	9.5	16.4	104.0	—			—	e
Chol. Butyrate	4	$P2_1$	8	25.36	9.55	23.6	95	5693	1.064	1.037	—	b,f
	4	$P2_1$	8	23.65	9.62	25.80	94.4					g
Chol. Valerate	5	$P2_12_12_1$	4	21.45	21.5	6.4	—	2951	1.014		—	b,f
Chol. Caproate	6	$P2_1$	2	13.67	9.30	12.19	92	1548	1.028	1.034	—	b,f
	6	$P2_1$	2	12.16	9.31	13.64	93.6					h
Chol. Heptanoate	7	$P2_1$	2	14.02	9.23	12.54	92	1622	1.062	1.032	—	b,f
Chol. Caprylate	8	$P2_1$	2	13.95	9.2	12.67	94	1622	1.034	1.042	—	b
	8	$P2_1$	2	12.80	9.20	14.12	93.81	—		1.035	—	i
Chol. Nonanoate	9	$P2_1$	2	14.44	9.33	12.81	95.4	1718	1.051		—	b
	9	$P2_1$	4	13.96	9.18	27.24	91.95	—		1.005	—	j
Chol. Caprate	10	$P2_1$	4	30.0	9.05	12.85	92	3486	1.001	1.020	—	b
	10	$P2_1$	4	12.93	9.07	30.22	91.14	—		1.010	83.8	k
Chol. Undecanoate	11	$P2_1$	4	12.99	9.00	31.03	90.58	—			—	f
	11	$P2_1$	4	13.01	9.01	31.06	90.61					e
Chol. Laurate	12	$P2_1$	4	31.8	8.92	12.92	93	3659	1.023		—	b
	12	$P2_1$	4	12.99	9.01	32.02	91.37	—		1.007	90.2	m
	12	$P2_1$	4	34.9	9.02	13.13	110.9	—			—	n
	12	$P2_1$	4	32.63	9.02	13.13	91.0	—			—	n
	12	$P2_1$	4	12.995	9.013	34.25	110.97	3745.4	1.009			o
Chol. Tridecanoate	13	A2 or $P2_1$	(8)	10.03	7.61	101.8	93.4	—			80	j
Chol. Myristate	14	$P2_1$	4	50.3	7.5	10.18	92.5	3851	1.061		—	b
	14	A2	8	10.26	7.60	101.44	94.42	—			—	p
	14	—	4	55.5	7.6	11.3	116.0	—			—	q
Chol. Palmitate	16	$P2_12_12_1$	8	25.53	34.86	9.00	—	7382	1.103	1.066	—	b,f
	16	A2	8	10.15	7.55	105.5	95.6	—			78	j
Chol. Stearate	18	$P2_1$	4	57.5	7.55	10.2	96	4400	1.086			b,f
	18	—	4	62.8	7.6	11.35	116.0					o
	18	$P2_1$	4	54.0	7.4	10.3	~100	—			—	e
(b) *Unsaturated*												
Chol. Palmitoleate	16:1	$P2_1$	4	12.87	9.17	35.43	93.47					j
Chol. Oleate	18:1	$P2_1$	2	12.65	9.13	18.80	93.3					r
Chol. Linolelaidate	18:2t	$P2_1$	2	12.86	8.98	18.73	92.30					s
(at −150°C)	18:2t	$P2_1$	2	13.03	8.76	17.90	89.72					s
Chol. Nervonate	24:1	$P2_1$	4	12.95	8.81	42.98	105.93					t

[a] n = number of carbons in acyl chain; Z = number of molecules in unit cell; D_c = calculated crystal density; ρ and m.p. measured on crystal used.
Refs: b, Barnard;[15] c, Sawzik and Craven;[103] d, Bernal *et al.*;[20] e, Lydon (1970) (unpublished); f, Craven;[32] g, Kinoshita and Craven (1979) (unpublished); h, Ruble, Park and Craven (unpublished); i, Craven and Guerina;[34] j, Sawzik and Craven;[105] k, Pattabhi and Craven;[94] l, Sawzik and Craven;[106] m, Sawzik and Craven;[104] n, Abrahamsson and Selin;[3] o, Dahlen;[38] p, Craven and DeTitta;[33] q, Wendorff[122] (unpublished); r, Craven and Guerina;[35] s, Craven and Sawzik;[36] t, Sawzik, He and Craven (unpublished).

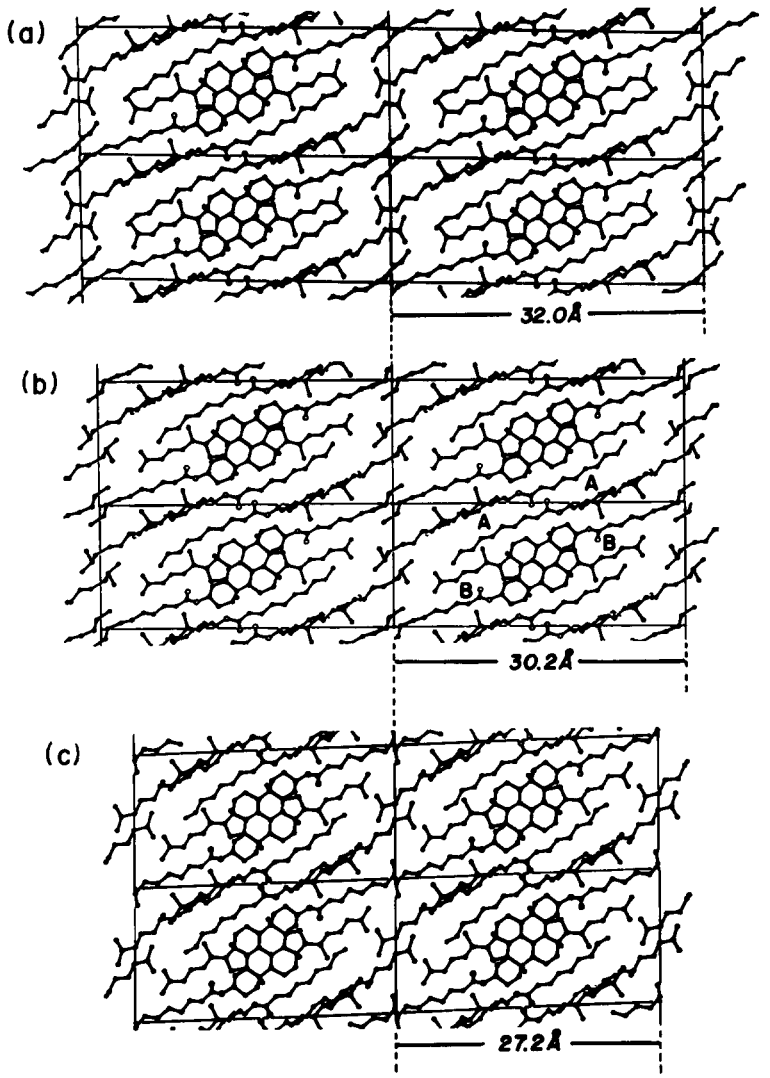


FIG. 2. Crystal structures with monolayers of type I in projection down the *b*-axis. (a) cholesteryl laurate, (b) cholesteryl decanoate, (c) cholesteryl nonanoate. The two molecules not related by crystal symmetry are A (ring system viewed on edge) and B.[94]

of neighboring molecules. The angular methyl groups (C–18, C–19) of adjacent rings interlock as do the C–21 methyls of adjacent pairs of molecules. An important feature of this crystal structure is that acyl chains from opposing monolayers interdigitate although they do not pack in a specific hydrocarbon subcell. In fact, the unsaturated acyl chain of cholesteryl oleate is kinked in such a manner so as to preserve the linearity of the chain. This is in marked contrast to the acyl chain of oleic acid (Fig. 4) which, in the crystal, is bent at the *cis* double bond.[2] Analysis of the anisotropic temperature factors for both cholesteryl oleate and octanoate indicates that the acyl chains are quite mobile. The mobility of the chain does not appear to be related to the double bond in the cholesteryl oleate since cholesteryl octanoate also has a large degree of thermal mobility (Fig. 3).

3. *Bilayer*

The bilayer crystal form was the first crystal structure solved for a cholesteryl ester, cholesteryl myristate (Fig. 5).[33] Cholesteryl 17-bromoheptadecanoate is isostructural.[1] Cholesteryl myristate crystals are monoclinic, belong to space group A2, with eight molecules per unit cell. Two molecules (A and B) form the asymmetric unit and are arranged in an antiparallel configuration to form the bilayer. This arrangement, in contrast

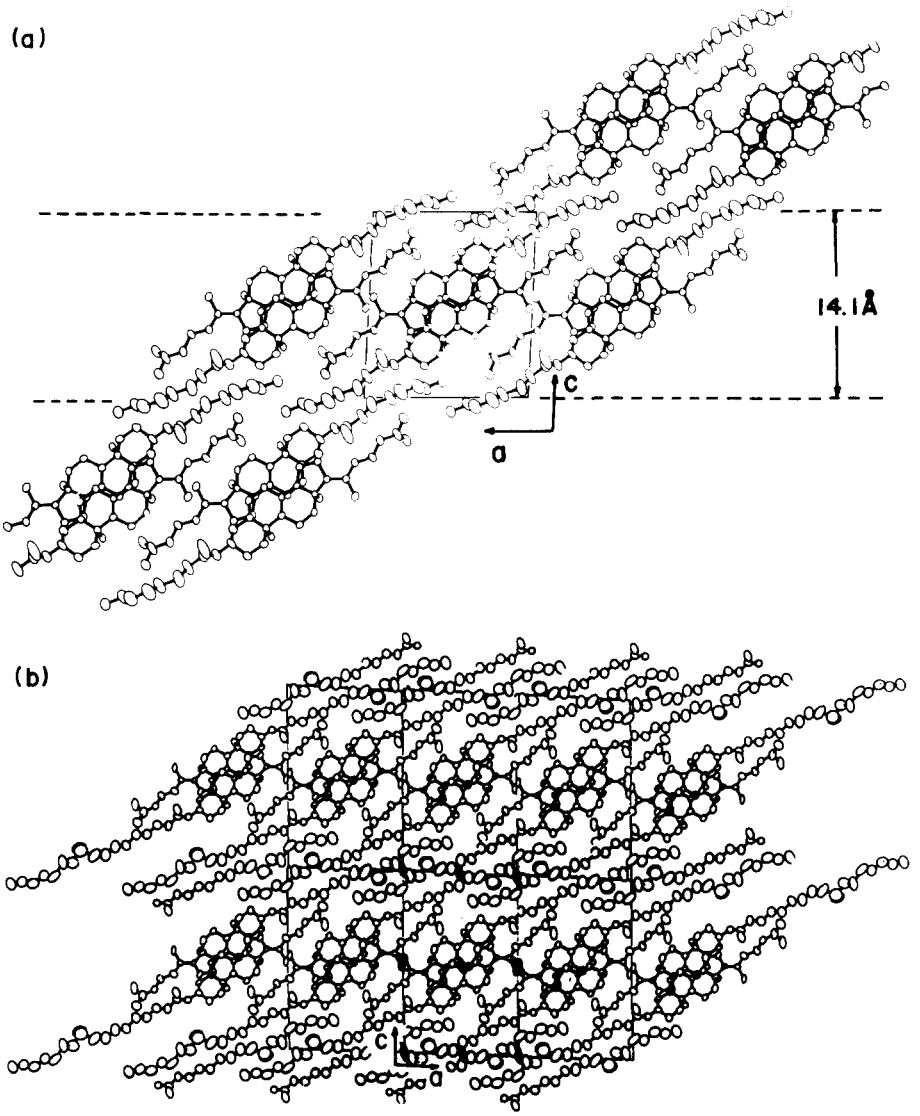


FIG. 3. Cholesteryl esters of monolayer Type II crystal structure. (a) The crystal structure of cholesteryl octanoate in projection down the *b*-axis. Dashed lines separate the monolayers of type II. The cholesterol groups have the packing arrangement of cholesteryl iodide.[30] The octanoate chains have been resolved into two randomly disordered conformers, but only the average structure is shown here. Atoms are represented as 25% ellipsoids,[34] (b) projection of the crystal structure of cholesteryl oleate along the $-b$-axis. The crystal type monolayer type II. The $+b$-axis is directed below the page so as to present the same view as for the isostructural cholesteryl octanoate. The oleate chains are almost straight except for a slight kink near the *cis*-double bond. Atoms shown as 25% probability ellipsoids.[35]

to the monolayer structures, permits the accommodation of cholesteryl esters with larger acyl chains by a simple increment of the bilayer thickness.[32] Cholesterol rings of adjacent molecules are roughly parallel to one another. The angular methyls (C–18, C–19) project between neighboring molecules but do not significantly interact. Ring–ring interactions are thus probably not a dominant stabilizing force in this crystal form. The hydrocarbon chain packing is specific and conforms to an orthorhombic subcell. Analysis of the anisotropic temperature factors indicates that the hydrocarbon chains are relatively restricted as a result of this specific packing. In marked contrast, the isooctyl groups forming the interface between the bilayer are highly mobile.

B. *The Mesomorphic State*

In general, the mesomorphic state has been observed in many cholesteryl esters whose stability or metastability is related to the degree of unsaturation and the chain length of

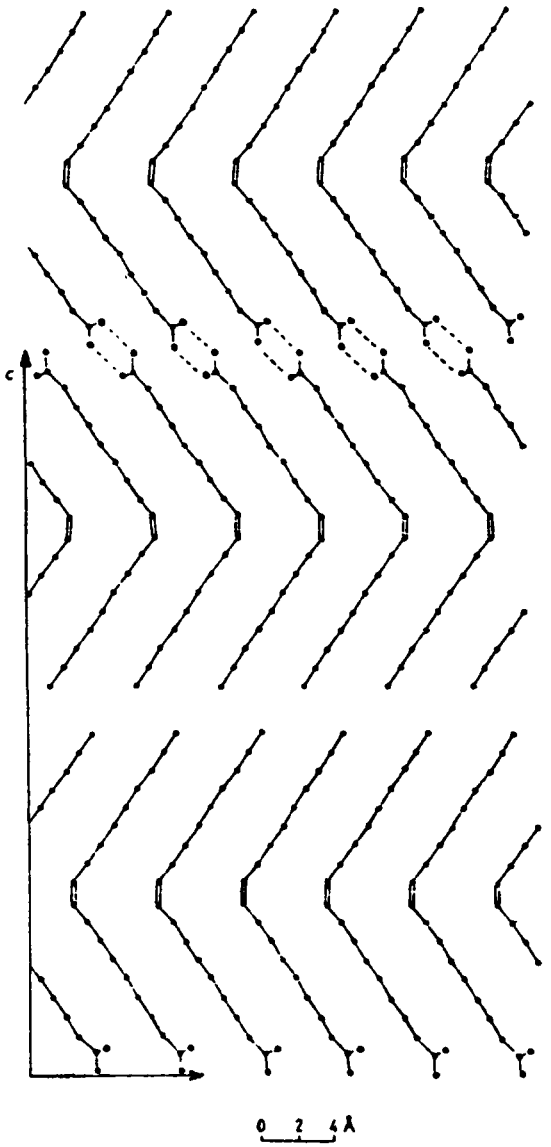


FIG. 4. Molecular arrangement of oleic acid seen along *b*-axis.[2]

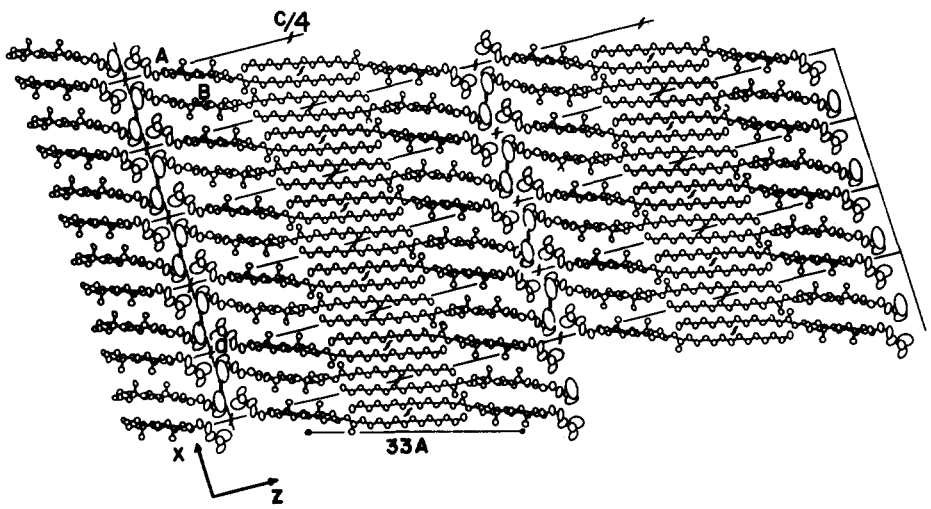


FIG. 5. Crystal structure of cholesteryl myristate in projection down the *b*-axis, showing the bilayers. Atoms are shown as 50% probability ellipsoids. The two molecules (A,B) which are not related by crystal symmetry are labelled in the unit cell at top left.[33]

the fatty acyl chain.[59,60,111,115] Some cholesteryl esters exhibit *no* liquid crystalline mesophases while others can exist in either one or two mesomorphic states—a smectic liquid crystalline phase or a cholesteric liquid crystalline phase. If a cholesteryl ester forms both a smectic and a cholesteric phase, the smectic mesophase is always the lower melting of the two, is more grossly opaque and colorless and transmits polarized light more intensely. The cholesteric phase often exhibits a wide variety of reflected colors. It is grossly translucent and more fluid than the smectic phase and gives a faint birefringence by polarizing microscopy.

The first evidence for the structure of the liquid crystalline mesophases was inferred by microscopy. Grandjean[62a] observed that liquid crystals form "goutte a gradin" or stepped drops when viewed on edge and focal conic textures under polarized light. A stratified structure for the smectic phase was hypothesized and later confirmed by Friedel[57] through a detailed optical analysis and by X-ray diffraction. More recent freeze fracture electron microscopic studies are consistent with this interpretation.[86] The main structural feature of the smectic mesophase is that the molecules are arranged in layers usually one molecule thick (two molecules thick in amphiphiles), with their long axes approximately normal to the planes of the layers (see Fig. 6). The layers are flexible and glide over one another and the intralayer molecular spacings are not uniform as they would be in a true crystal.

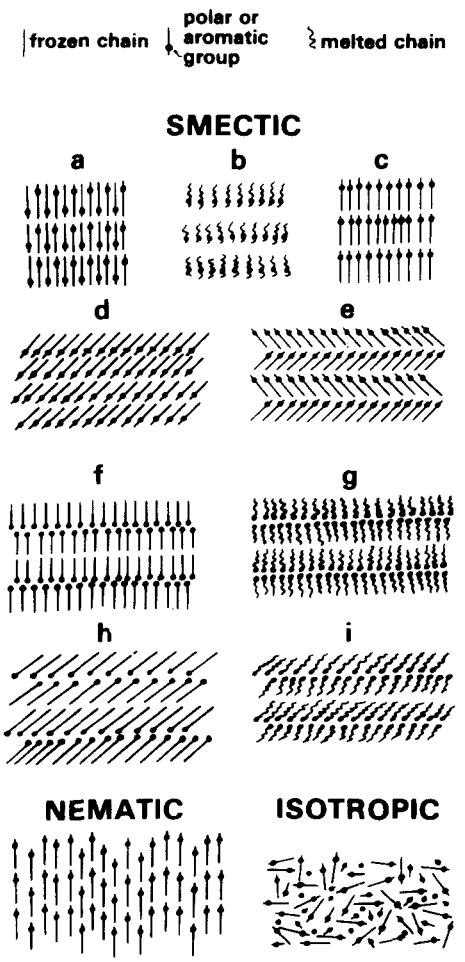


FIG. 6. Schematic representation of a few of the many thermotropic liquid crystalline phases. Straight line indicates immobile, stiff hydrocarbon aliphatic chain; wavy line indicates melted chain. Polar or aromatic groups may be at the end of the molecule (f–i) or more centrally located (a–e). Above, smectic or layered phases with chains normal to layer plane (a,b,c,f,g) or tilted (d,e,h,i). Note that letters a–i are not related to the common smectic A–H nomenclature.[65] Structures f–i are bilayered structures. Structure g would be equivalent to L_x and h to L_β of Tardieu *et al.*[120] (From Small[112].)

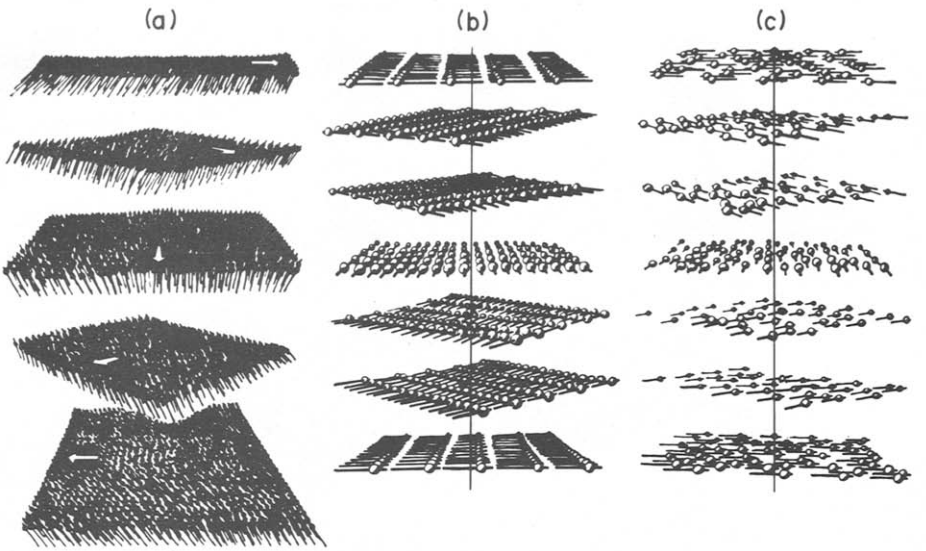


FIG. 7. Twisted liquid crystalline structures (a) twisted smectic, (b) and (c) twisted nematic (cholesteric) structure. (From Small[112].)

One-dimensional long range order is thus characteristic of the smectic mesophase. By polarizing microscopy, the smectic mesophase has a positive sign of birefringence similar to that of uniaxial crystals in which the long axes of the molecules are also the direction of maximal refractive index and directions normal to this have minimum refractive indices.

The cholesteric mesophase is strongly optically active and conoscopically displays a negative sign of birefringence. In this mesophase molecules are also arranged in layers; however, the long axes of the molecules at right angles to the optic axis and are parallel to each other over small elements of volume; the whole structure has an either right- or left-handed twist about the optic axis (Fig. 7). This twist results in a helicoidal type of structure whose periodicity is related to the wavelength of the transmitted light.[64] Some authors suggest that the broad flat sterol moiety of the molecule would be likely to set itself normal to the optic axis in the twisted structure—this would be most efficient in terms of molecular packing. Untwisting this structure results in the classical nematic phase from which no colors are reflected. These mesophases possess only short range molecular order.

X-ray diffraction of cholesteryl esters in the smectic mesophase yields a diffraction pattern exhibiting a sharp intense diffraction ring at low angles and a diffuse weak diffraction ring at wide angles. Representative diffraction data from the crystal, liquid, cholesteric and smectic phases of cholesteryl oleate are shown in Fig. 8. Long range order in the direction of the long axes of the molecules is reflected in the narrow angle region while the diffuse outer ring corresponds to the disordered packing normal to the long axes of the molecules. A truly layered liquid crystalline system, such as occurs with soaps and phospholipids or with cholesterol/H_2O and cholesterol/glycerol, often has several diffraction orders whose intensities differ.[84,115] Such data allow the electron density across the one-dimensional lattice to be calculated. However, smectic A* liquid crystals have been shown to have only a single spacing in the narrow angle range (Fig. 8).[48,49,56a,67,89,121] The optical properties (focal conic texture) of the smectic phase, as originally described by Friedel,[57] clearly indicate a layered structure. The organization of the molecules within such a structure, however, is not entirely clear. In the cholesteric phase, however, diffuse scattering maxima are seen at narrow and wide angles indicative of diminished long range order and consistent with the structural interpretation of the microscopic data.

*Sackmann and Demus[101] proposed a classification system for smectic phases with the main types denoted A, B and C. Smectic A is a layered structure with each layer essentially a two-dimensional liquid. Smectic C is similar to smectic A except that the molecules within the layers are tilted with respect to the normal to the layer plane. Smectic B is a quasi-crystalline structure and will not be discussed in this review.

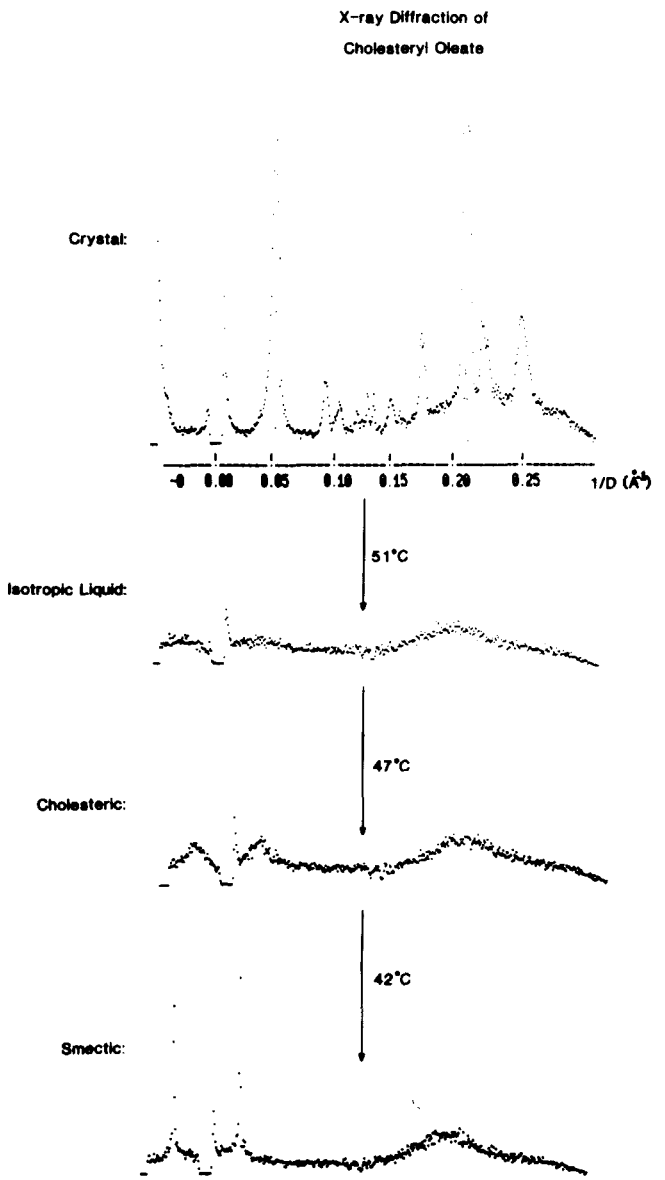


FIG. 8. X-ray diffraction of cholesteryl oleate. Characteristic wide angle powder diffraction pattern of cholesteryl oleate in the crystalline state is shown (top). The sample is heated directly to the isotropic liquid phase and cooled through its mesophase transitions. A diffraction pattern was recorded in each phase. Note the single sharp narrow angle reflection in the smectic phase at about 0.03 $1/DA^{-1}$. This gives a smectic plane periodicy of 35 Å

The liquid crystalline phases of cholesteryl esters can occur as either stable or metastable phases. A stable mesophase forms as the crystal melts and is called an "enantiotropic" transition, whereas a metastable mesophase forms at a temperature below the crystal melt and thus forms from an undercooled isotropic liquid (also known as a "monotropic" phase transition). These transitions may be represented diagramatically as shown in Table 2.

Nearly all the liquid crystal transitions are almost perfectly reversible (assuming the nucleation and crystallization do not occur prior to reheating). If, however, crystallization occurs to a crystal of higher melting point, no liquid crystalline transformations will occur on reheating, and the crystal will simply melt to an isotropic liquid. Stable mesophases can exist indefinitely in the temperature range above the crystal melt and below the isotropic liquid phase transition. Metastable mesophases will either crystallize rapidly or can remain for long periods but eventually will nucleate or can be nucleated with crystalline ester to form true crystals—the more thermodynamically stable state.

C. *The Liquid State*

The liquid state is characterized by a high degree of fluidity and relatively low viscosity. Molecular motions are quite rapid in this state, and order, in a gross molecular sense, is lost. For polar, hydrophilic, or aqueous molecules, the forces which maintain liquids are ionic and hydrogen bonds, but for nonpolar hydrocarbons Van der Waals forces predominate as the cohesive force. Liquids, under polarized light, display no birefringence and thus are called isotropic, or zero-dimensional order states. However, the important concept is that microdomains or cybotactic clusters with short range order must occur in molten liquids of cholesteryl esters. When one considers that the change in volume from the crystalline state to the isotropic liquid state of many long chained lipids is only 10 to 15%,[114] then some packing order must be maintained in liquids of these molecules. De Gennes,[46] McMillan[88,89] and McMillan and Myer[90] proposed that submicroscopic ordering must exist in the liquid state several degrees above the phase transition to the cholesteric or smectic phase. X-ray scattering studies of liquid alkanes[21 25] and triacylglycerols[77] indicate that there are ordered domains of lipids ~ 200 Å in size in which the chains are roughly parallel and the ends of the molecules are in register. X-ray scattering of cholestanol[92] and cholesteryl esters in the liquid state shows two broad maxima (similar to scattering from the cholesteric phase, but broader and lower in intensity; see Fig. 8). An analysis of X-ray diffraction data for cholesteryl oleate in the liquid state shows a measurable longitudinal and transverse coherence length based on the peak width at half height of the Bragg peaks at low and wide angles, respectively.[5] Diffuse scattering from cholesteryl esters in the liquid state has been observed for many cholesteryl esters regardless of their phase behavior.

IV. TRANSITION BETWEEN STATES

The changes in structure and physical properties of cholesteryl esters as they undergo a phase transition can be illustrated with cholesteryl myristate, perhaps the most thoroughly studied of all cholesteryl esters. Taken from Small[115] is a composite (Fig. 9) illustrating the calorimetric, densitimetric and X-ray diffraction changes as a function of temperature. The crystalline state is of the bilayer type which melts at 72°C with an enthalpy of 11.4 kcal/mol. At the crystal→smectic phase transition, a marked change in density occurs. Using the density calculated from the crystal and the change in density on heating,[47] the change in density associated with the crystal melting transition is indicated in Fig. 9. At this transition, the relevant spacing also decreases from 50.7 to 33 Å. Using ^{2}H labeled cholesteryl myristate, Burks and Engelman[28] showed by neutron scattering that the molecules in all the phases are in an extended conformation, as will be discussed in detail in a later section.

The changes occurring between smectic and cholesteric and cholesteric and isotropic phases are clearly first order changes, as an excess enthalpy and rather discrete changes in volume are observed. The estimated volume increments for the crystal–smectic, smectic–cholesteric and cholesteric–isotropic phase transitions are 84.4 Å^3, 6.3 Å^3 and ~ 1 Å^3, respectively. A minor change in the specific heat, indicating a second order phase change, occurs just below the isotropic–cholesteric phase transition. This transition is called the blue phase transition.[8,47,70] The exact nature of this second order transition is not known.

Yang,[127] using Raleigh light scattering, observed pretransitional ordering of cholesteryl 2-(2-ethoxyethoxy)ethyl carbonate on cooling from the isotropic liquid phase to the cholesteric liquid crystal. Short range order dynamical behavior that could be described by Landau–de Gennes theory and Bragg interference fringes were observed near the transition. Nematic materials also display some pretransitional effects in the isotropic liquid phase[82] but the correlation length in nematic pretransitional ordering remains so small that its effect on the intensity and width of the Raleigh scattered peak remains negligible. Thus, Yang's results would appear to be a consequence of ordering specific to

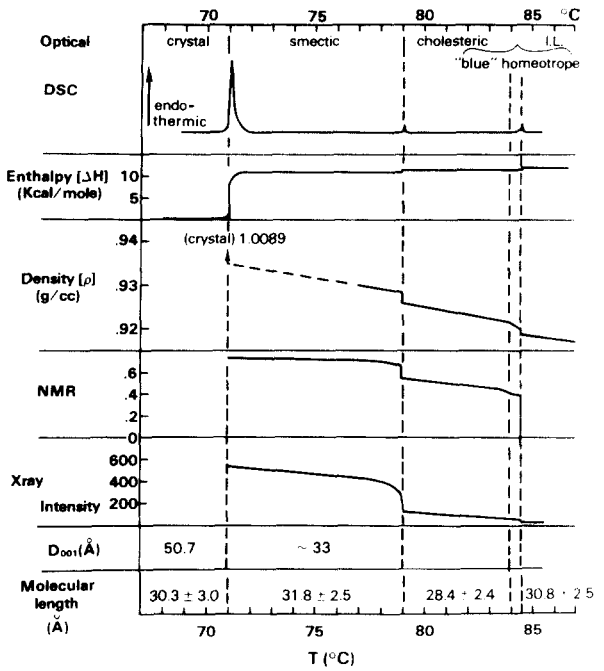


FIG. 9. Physical characteristics of cholesteryl myristate. Optical observations;[47,99] DSC trace (Small, unpublished results); enthalpy;[19] density;[47] NMR;[85] X-ray spacings ($d_{(001)}$);[33] X-ray intensity[89] and molecular length.[28] Curves have been adjusted for distance of reported transition temperatures. Note the abrupt changes which occur with transitions (from Small[115]).

the cholesteric phase and perhaps to a specific characteristic of the cholesterol ester molecule. Hamilton *et al.*[66] and Ginsburg *et al.*,[61] using ^{13}C NMR spectroscopy, examined cholesteryl oleate and linoleate as a function of temperature in the isotropic liquid phase. Their data suggested specifically that the motions of the sterol ring were anisotropic as shown by differential line broadening of methine carbons of the ring moiety as the liquid→cholesteric liquid crystal phase transition is approached. These data are consistent with the concept described earlier of ordered domains in the liquid phase but that interactions unique to the cholesteryl ester molecule induce the formation of a cholesteric phase and that the ring system is probably a major determinant of these interactions.

V. PHASE BEHAVIOR OF CHOLESTERYL ESTERS FROM POLARIZING MICROSCOPY AND DIFFERENTIAL SCANNING CALORIMETRY

A. *Effects of Chain Length in Saturated and Unsaturated Cholesteryl Esters*

The phase behavior for saturated and unsaturated cholesterol esters is shown diagrammatically in Tables 2a and b, respectively. Indicated are the transition temperatures for each phase and, if possible, the crystal type as determined by comparison of the X-ray powder diffraction pattern with those of cholesteryl ester of known crystal structure as determined by Craven.[32] The phase transition temperatures have been plotted as a function of chain length in Figs 10, 11 and 12. For the saturated series, plots of the transition temperatures vs. number of carbons in the fatty acyl chain have been divided into odd- and even-chained esters to eliminate odd–even variation. In general, the short chain esters have higher crystalline melting temperatures and extension of the chain shows a general trend to lower crystal melting temperatures. Discontinuities in crystal transition temperatures are correlated to changes in the crystal structure which occur at the same chain lengths. For example, cholesteryl octanoate ($C_{8:0}$) is a high melting C_1 crystal of monolayer type II whereas cholesteryl nonanoate ($C_{9:0}$) is a relatively low melting C_2 crystal of monolayer type I. A similar change in melting behavior and structural organization may be observed for $C_{12:0}$ and $C_{14:0}$ which have monolayer type I and bilayer crystal structures, respectively.

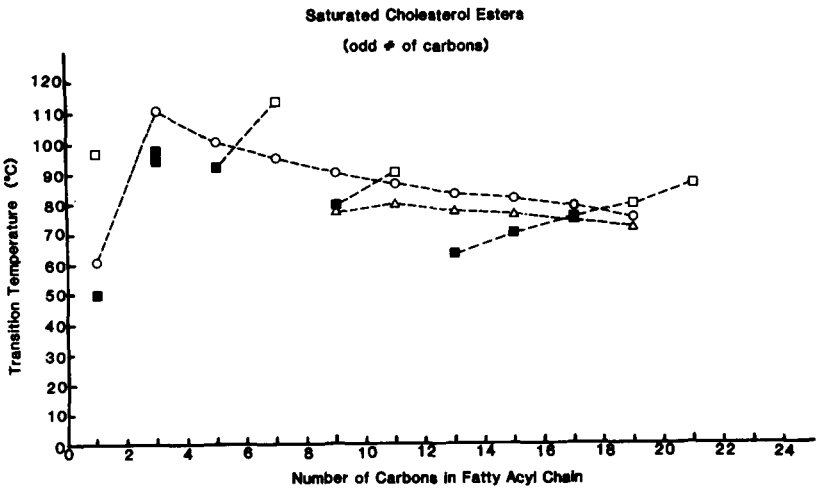


FIG. 10. Phase transition temperatures for saturated cholesteryl esters with an odd number of fatty acyl chain carbons. ■ = C_2 (crystal having a melting point lower than the highest liquid crystal transition); □ = C_1 (crystal having a melting point higher than the highest liquid crystal transition), ○ = cholesteric and △ = smectic melting temperatures. Dashed lines connect transition temperatures of like phases or, for crystals, from a known isostructural series. Data from Table 2.

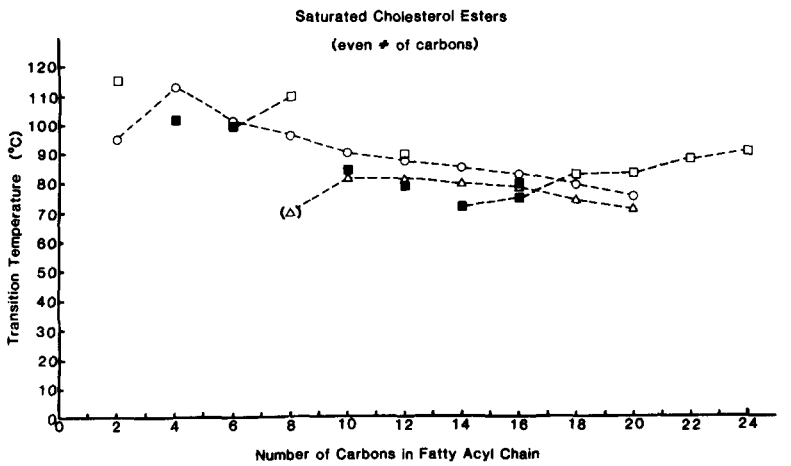


FIG. 11. Phase transition temperatures for saturated cholesteryl esters with even number of fatty acyl chain carbons. Parentheses at eight carbons indicated some doubt as to the existence of this phase in a pure sample of cholesteryl octanoate. Data from Table 2. Symbols and dashed lines as in Fig. 10.

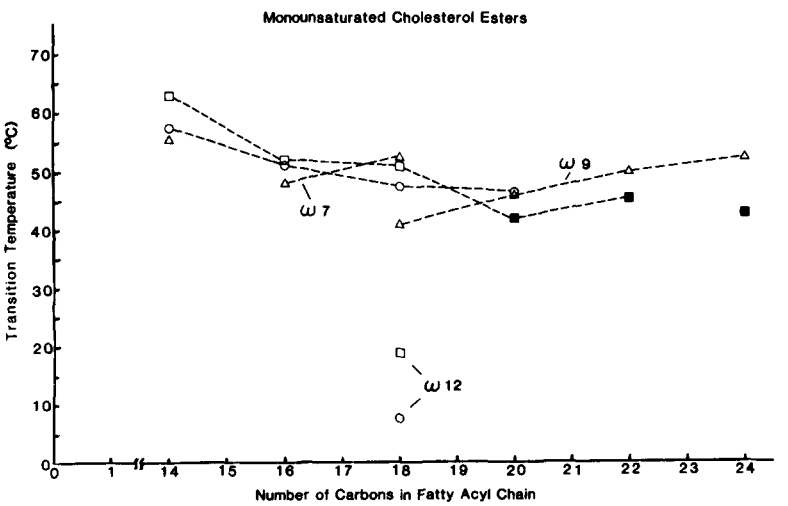
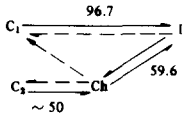


FIG. 12. Phase transition temperatures for monounsaturated cholesteryl esters. Symbols as in Fig. 10. For smectic phase, dashed lines connect only homologous series with respect to double bond position indicated by ω. Data from Table 2.

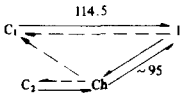
TABLE 2. Transition Temperatures and Phase Behavior of Cholesteryl Esters*

(a) *Saturated*

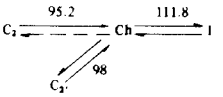
Chol. Formate $C_{1:0}$



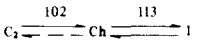
Chol. Acetate $C_{2:0}$



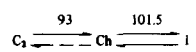
Chol. Propionate $C_{3:0}$



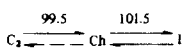
Chol. Butyrate $C_{4:0}$



Chol. Valerate $C_{5:0}$

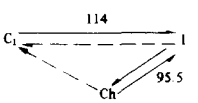


Chol. Caproate $C_{6:0}$



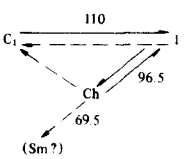
C2: Monolayer II

Chol. Heptanoate $C_{7:0}$



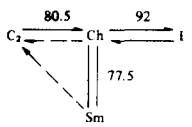
C_1: Monolayer II

Chol. Octanoate $C_{8:0}$



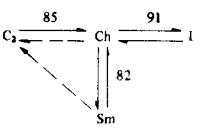
C_1: Monolayer II

Chol. Nonanoate $C_{9:0}$



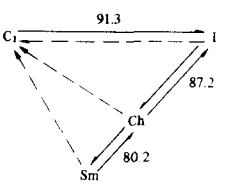
C_2: Monolayer I

Chol. Decanoate $C_{10:0}$



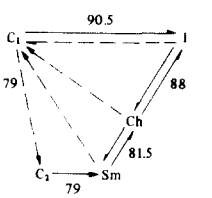
C_2: Monolayer I

Chol. Undecanoate $C_{11:0}$



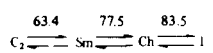
C_1: Monolayer I

Chol. Laurate $C_{12:0}$



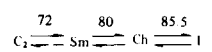
C_1: Monolayer I; C_2: II

Chol. Tridecanoate $C_{13:0}$



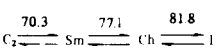
C_2: Bilayer

Chol. Myristate $C_{14:0}$



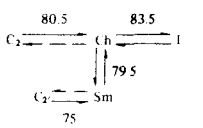
C_2: Bilayer

Chol. Pentdecanoate $C_{15:0}$



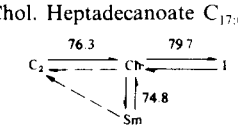
C_2: Bilayer (?)†

Chol. Palmitate $C_{16:0}$



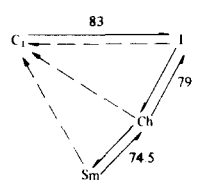
C_2: Bilayer

Chol. Heptadecanoate $C_{17:0}$



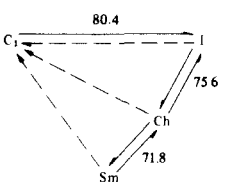
C_2: Bilayer‡

Chol. Stearate $C_{18:0}$



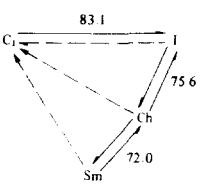
C_1: Bilayer

Chol. Nonadecanoate $C_{19:0}$



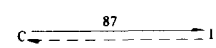
C_1: Bilayer (?)

Chol. Arachidate $C_{20:0}$



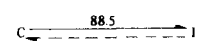
C_1: Bilayer

Chol. Heneicosanoate $C_{21:0}$



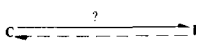
C: Bilayer

Chol. Behenate $C_{22:0}$



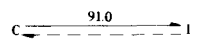
C: Bilayer

Chol. Tricosanoate $C_{23:0}$



C: Bilayer (?)

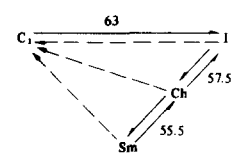
Chol. Lignocerate $C_{24:0}$



C: Bilayer

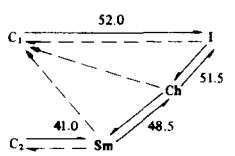
(b) *Unsaturated*

Chol. Myristoleate $C_{14:1}$ ω–5



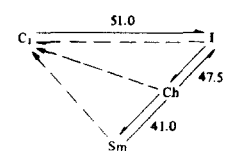
C_1: Monolayer II

Chol. Palmitoleate $C_{16:1}$ ω–7



C_1: Monolayer II; C_2: Monolayer I

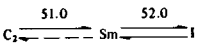
Chol. Oleate $C_{18:1}$ ω–9



C_1: Monolayer II

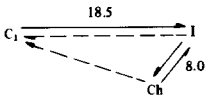
TABLE 2 (*continued*).

Chol. Vaccenate $C_{18:1}\,\omega-7$



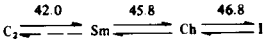
C_2: Monolayer II

Chol. Petroselinate $C_{18:1}\,\omega-12$



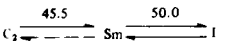
C_1: Monolayer II

Chol. 11-eicosenoate $C_{20:1}\,\omega-9$



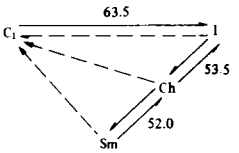
C_2: Monolayer II

Chol. Erucate $C_{22:1}\,\omega-9$

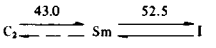


C_2: Monolayer II

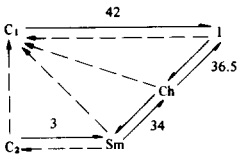
Chol. Brassidate $C_{22:1}$ (t) $\omega-9$



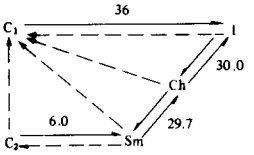
Chol. Nervonate $C_{24:1}\,\omega-9$



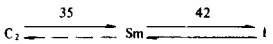
Chol. Linoleate $C_{18:2}\,\omega6,9$



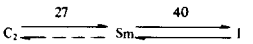
Chol. Linolenate $C_{18:3}\,\omega3,6,9$



Chol. 11,14-eicosadienoate $C_{20:2}\,\omega6,9$



Chol. 11,14,17-eicosatrienoate $\omega3,6,9$



*Common name, chain length and double bond position(s) are given if present. The data is presented diagrammatically utilizing the following symbols: C_1 = crystal having a melting point higher than the highest liquid crystal transition; C_2 = crystal having a melting point lower than the highest liquid crystal transition; Ch = cholesteric mesophase; Sm = smectic mesophase; I = isotropic liquid melt. If a crystal structure is known, its crystal type is given according to Craven.[32] Crystal types are given for several esters which were found by this work to be isostructural with esters whose crystal structure has been solved.

†$C_{15:0}$, $C_{19:0}$ and $C_{23:0}$ esters have not been studied by X-ray diffraction but are presumed to have a bilayer crystal structure.

‡Crystal structure from 17-bromo derivative.

Refs: C_1; C_2; C_3, Barrall and Johnson;[16a] C_4; C_5; C_6; C_7; C_8; C_9, Gray,[63] C_{10}; C_{12}; C_{14}; C_{16}; C_{18}, Small;[111] C_{11}; C_{13}; C_{15}; C_{17}; C_{19}, Davis *et al.*;[40] C_{20}; C_{22}; C_{24}, this work; C_{21}, this work; C_{23} (compound unavailable); $C_{14:1}\,\omega5$; $C_{16:1}\,\omega7$, this work; $C_{18:1}\,\omega9$; $C_{18:2}\,\omega6,9$; $C_{18:3}\,\omega3,6,9$, Small;[111] $C_{18:1}\,\omega7$; $C_{18:1}\,\omega12$, this work; $C_{20:1}\,\omega9$; $C_{22:1}\,\omega9$; $C_{11:1}$ (t) $\omega9$, this work; $C_{20:2}\,\omega6,9$; $C_{20:3}\,\omega3,6,9$, Small;[111] $C_{24:1}\,\omega9$ this work.

Apparently, within an isostructural series as the number of carbons is increased, the crystal becomes progressively more stable (increasing T_m) and more ordered (increasing ΔH, ΔS; see Figs 13 and 14); however, at a specific chain length, packing is no longer favored in that crystal form. The next crystal form adopted is less thermally stable (i.e. lower T_m) than the one preceding it but is generally more ordered (i.e. increased ΔH). Changes in molecular symmetry and intermolecular interactions resulting from the increase in fatty acyl carbon units force the molecules into a different molecular packing. Interestingly, the shortest chain length member of each new crystal form melts directly to a stable liquid crystalline phase. As the chain length increases and crystal stability supersedes that of the liquid crystalline phase, the liquid crystalline phases become metastable. The bilayer crystal form could accommodate any chain length and thus becomes the most stable crystal form for esters with long (>13 carbons) saturated fatty acyl chains. Thermal stability of this crystal precludes the formation of mesophases at chain length greater than 20 carbons (see below).

The cholesteric mesophase is characteristic of all cholesteryl esters from chain lengths 1 to 20. At shorter chain lengths, this phase is high melting, but as the chain length

increases the melting temperature progressively decreases. The smectic phase is not observed in short chain esters until the chain length is ~9 carbons (the literature is unclear as to whether or not cholesteryl octanoate forms a smectic phase but all reports agree that cholesteryl nonanoate does). The transition temperature for this phase remains relatively constant for C_9 to C_{17} and decreases from C_{18} to C_{20}.

The smectic temperature domain (that range of temperatures over which the smectic phase is stable) decreases with increasing chain length while the crystalline state becomes a more favorable thermodynamic state. Specifically, metastability is a characteristic of cholesteryl esters having a chain length greater than C_{16} and *no* mesophases are observed in saturated esters with greater than 20 fatty acyl carbons. These long chain esters have high crystal→isotropic transition temperatures and lack significant undercooling on crystallization. For example, a cholesteric↔isotropic transition for $C_{22:0}$ would be expected (if the cholesteric transition temperature line were extrapolated in Fig. 11) at around 72–73°C, but, on cooling, recrystallization takes place at ~83°C. Apparently, nucleation to the crystal occurs before the expected mesophase formation and thus no liquid crystalline transitions are observed. Similar observations have been made in long chain esters of cholestanol.[92]

TABLE 3. Selected Thermodynamic Data for Cholesteryl Esters[a]

	C→I.L.		C→L.C.			Sm↔Ch		Ch↔I.L.		
n	ΔH	ΔS	ΔH	(Sm/Ch)	ΔS	ΔH	ΔS	ΔH	ΔS	Ref.
1	5.29	14.4						0.09	0.26	b
2	5.35	12.4						0.14	0.37	b
3			5.75	(Ch)	15.5			0.12	0.30	b
4			5.52	(Ch)	14.8			0.13	0.33	c
5			6.04	(Ch)	16.5			0.13	0.35	c
6			7.27	(Ch)	18.5			0.15	0.40	c
7	8.48	21.8						~0.12	~0.46	c
8	9.69	25.1						0.23	0.63	c
9			5.37	(Ch)	15.2	0.06	0.17	0.12	0.36	d
10			7.30	(Ch)	20.3	0.10	0.28	0.15	0.40	d
11	8.88	24.3				0.21	0.58	0.18	0.50	e
12	10.07	23.7				0.34	0.94	0.31	0.86	b
13			9.56	(Sm)	28.4	0.31	0.90	0.19	0.52	e
14			11.40	(Sm)	33.2	0.34	0.96	0.27	0.75	b
15			11.85	(Sm)	32.6	0.38	0.95	0.23	0.69	e
16			14.60	(Ch)	41.7	0.39	1.14	0.32	0.90	b
17			14.56	(Sm)	41.6	0.44	1.26	0.31	0.89	e
18	16.84	47.4				0.39	1.14	0.39	1.13	e
19	17.54	49.6				0.45	1.28	0.40	1.15	e
20	16.43	46.1				0.87	2.52	0.68	1.95	f
21	19.17	53.2								f
22	19.33	53.4								f
24	22.80	62.6								f

			C→I.L.		C→Sm		Sm↔Ch		Ch↔I.L.		
n	Δ	ω	ΔH	ΔS	ΔH	ΔS	ΔH	ΔS	ΔH	ΔS	Ref.
(b) *Monounsaturated*											
14:1	9	5	6.37	18.97			0.43	1.30	0.20	0.59	f
16:1	9	7			5.62	17.90	0.41	1.27	0.25	0.77	f
18:1	6	12	2.79	9.60					0.11	0.39	f
18:1	9	9	5.60	17.27			0.36	1.16	0.16	0.51	e
18:1	11	7					0.83	2.56	*	*	f
20:1	11	9					0.63	1.98	0.19	0.60	f
22:1	13	9			5.16	16.2	1.06	3.28	*	*	f
24:1	15	9			7.50	23.7	1.25	3.84	*	*	f
(c) *Polyunsaturated*											
18:2	9,12	9,6	5.20	16.52			0.51	1.65	0.18	0.57	d
18:3	9,12,15	9,6,3	4.04	13.08			0.49	1.65	0.10	0.34	d
20:2	11,14	9,6			5.22	16.75	0.88	2.80	*	*	e
20:3	11,14,17	9,6,3			4.86	16.20	0.83	2.66	*	*	e

[a] C = crystal; Sm = smectic; Ch = cholesteric; I.L. = isotropic liquid.
Refs: b, Barrall and Johnson;[17] c, Barrall *et al.*[18] d, Johnson *et al.*[72] e, Davis *et al.*[39,40] f, this work.
*Smectic↔I.L. (no cholesteric mesophase).

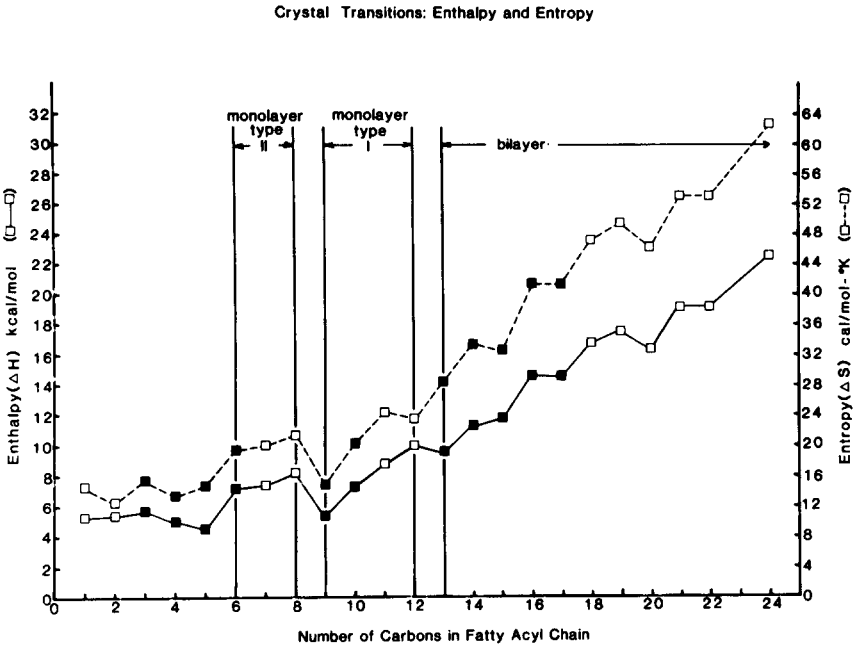


FIG. 13. Enthalpy (solid line) and entropy (dashed line) of crystal melting for saturated cholesteryl esters as a function of acyl chain length. ■ = C_2, □ = C_1 as in Fig. 10. Data from Table 3.

Table 3 summarizes the enthalpy (ΔH) and entropy (ΔS) changes for the crystal and liquid crystalline transitions of the saturated series and these values are plotted in Figs 13 and 14. For esters with less than five carbons in the acyl chain, the ΔH and ΔS are variable and show very little trend. This may suggest that the increase in ΔH or ΔS added by increased chain length is offset by some disordering of ring packing as the chain length increases. At chain lengths >5 carbons, both the enthalpy and entropy increase. The increase in enthalpy is ~1.2 kcal/–CH_2– group, rather similar to many of the other aliphatic compounds for a tightly packed crystal to liquid transition.[114,115] Thus, fatty acyl

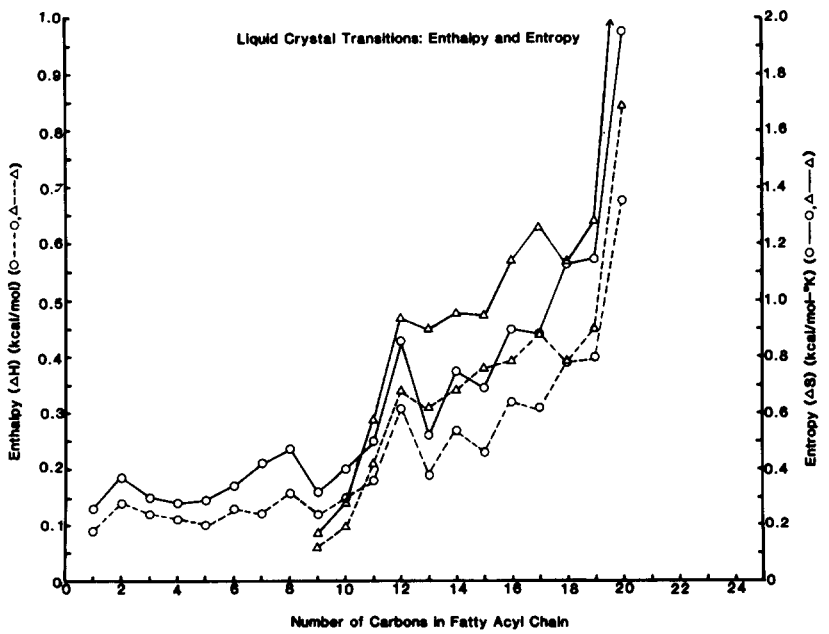


FIG. 14. Enthalpy (○---○, △---△) and entropy (○—○, △—△) of liquid crystal phase transitions for saturated cholesteryl esters as a function of acyl chain length. ○ = cholesteric melt, △ = smectic melt. Data from Table 3.

contributions to the enthalpy and entropy of melting appear when the chain length is greater than five carbons.

Small[115] has extrapolated the enthalpy vs. carbon number separately for monolayer II, monolayer I and bilayer packing (between C_{12} and C_{16}), and has shown that the intercepts are zero (monolayer type II) or less (monolayer type I and bilayer). This suggests that most of the enthalpy of melting is related to the chain packing and that steroid interactions do not contribute greatly to the melting phenomena. However, a plot of the enthalpy vs. the number of carbons from C_{16} to C_{20} (bilayer structure) gives an intercept at about 4.7 kcal/mol, which is not greatly different from some of the very short chain esters or from pure cholesterol, indicating that ring–ring interactions may contribute significantly to the enthalpy of melting of these long chain esters.[115] Note that ring–ring interactions are important in the bilayer structure (Fig. 5).

In general, the curves in Figs 13 and 14 show odd–even variation and apparent breaks occur at $C_{8,9}$ and possibly $C_{12,13}$, i.e. at the same fatty acyl chain lengths as occur changes in crystal structure and crystal transition temperature. The enthalpic and entropic breaks signify a chain dependent change in the order, symmetry and structure of the crystal phase. Interestingly, for the mesophase transitions (Fig. 14), breaks in the entropy and enthalpy curves occur at the same chain lengths as they do for the crystal. These data strongly suggest that similar interactions determine the order and packing of the crystal and liquid crystal phases for a given chain length. The properties, interactions and molecular organization of the liquid crystal may truly be an extrapolation of the crystal structure and its properties.

Studies of the saturated series of cholesteryl esters thus make it clear that the order and stability of the solid phase and of the mesophases of these molecules are dependent on the length of the alkyl chain. Cholesteryl ring systems readily form a cholesteric phase but an alkyl chain of at least 13 Å (nine carbons) is necessary for the formation of a smectic phase. The relative stability of the crystal form will determine whether or not a given cholesteryl ester will exhibit thermotropic mesomorphism.

B. *Influence of the Unsaturation in the Fatty Acyl Chain*

1. *Effect of Double Bond Position*

Transition temperatures of monounsaturated cholesteryl esters are quite depressed as compared with their saturated homologues.[111] In the limited series studied, there is no obvious relationship between changes in the crystal melting temperature and crystal structure (again determined by comparison of powder patterns with those of cholesteryl esters with known crystal structure). With the exception of $C_{16:1}$ ω–7 and $C_{24:1}$ ω–9 (which are monolayer Type I), all of the unsaturated cholesteryl esters studied are isostructural with cholesteryl oleate, i.e. monolayer Type II.

The monounsaturated ω–9 series of cholesteryl esters of even numbered chain length demonstrates a steadily increasing thermodynamic stability of the smectic mesophase with increasing chain length from 18 to 24 carbons. Cholesteryl 11-eicosenoate ($C_{20:1}$), cholesteryl erucate ($C_{22:1}$) and cholesteryl nervonate ($C_{24:1}$) all have smectic↔isotropic transitions far above their crystal↔smectic transitions. Note that no cholesteric mesophase is observed with chain lengths of >20 carbons in the monounsaturated series. By extrapolation of the cholesteric↔isotropic transition temperature curve (Fig. 12), it can be shown that between chain lengths of 20 and 21 carbons this transition falls below the increasing smectic↔isotropic transition temperature curve, thus precluding the cholesteric phase. A stable smectic phase results for monounsaturated cholesteryl esters of chain length greater than 20 carbons.

In the monounsaturated series $C_{14:1}$ Δ^9, $C_{16:1}$ Δ^9, $C_{18:1}$ Δ^9, the transition temperature of the smectic and cholesteric phases decreases linearly, suggesting that at a constant ring-to-double bond distance (nine carbons), extending the length of the terminal methyl portion destabilizes the liquid crystalline phases[115]. In fact, the decrease in T_m is directly

correlated with a decrease in the ΔH and ΔS for the smectic phases of these esters, suggesting that the segment of the acyl chain between the double bond and the chain terminus disorders the smectic phase. Spectroscopic studies, discussed later in this chapter, have confirmed that this segment of the chain is indeed disordered.

On the other hand, in the $C_{18:1}$ ω–9, $C_{20:1}$ ω–9, $C_{22:1}$ ω–9, $C_{24:1}$ ω–9 series, the length of the distal portion of the molecule is held constant (nine carbons) while the ring-to-double bond distance increases from 9 to 15 carbons. A linear increase in the smectic phase transition temperature is observed in this series[115]. In fact, the smectic phase becomes stable (higher melting than the crystal) beyond 20 carbons concomitant with the absence of the cholesteric phase. Thus, the smectic phase is sensitive to double bond position; both the ring-to-double bond distance and the double-bond-to-terminal methyl distances are important and have opposing effects on smectic phase stability.

For the smectic phase, defined uninterrupted segments of acyl chain are important, reinforcing the concepts derived from the saturated series in which a smectic phase was not found for esters with a chain length of less than nine carbons. In fact, the thermal behavior of isomers of $C_{18:1}$ in which the double bond is located either at Δ^6 (cholesteryl petroselinate), Δ^9 (cholesteryl oleate) or Δ^{11} (cholesteryl vaccenate) is quite revealing. Cholesteryl vaccenate exhibits a single stable smectic phase and *no* cholesteric phase, in contrast to cholesteryl oleate which has a cholesteric phase and a smectic phase both of which are metastable. The increment of the ring-to-double bond distance from 9 to 11 carbons is enough to change the phase behavior, stabilize the smectic phase and raise its transition temperature to a point at which the cholesteric phase can no longer be formed. In contrast, cholesteryl petroselinate, Δ^6 and ω–12, forms no smectic phase and has a single monotropic cholesteric phase with long-lived metastability. Its transition temperature is quite low consistent with its long double bond-to-terminal methyl distance, and the ΔH and ΔS of the cholesteric transition is similar to that of short chain saturated cholesteryl esters which form only the cholesteric mesophase (Table 3).

Figure 15 is a plot of the total enthalpy and entropy change between the smectic phase and isotropic liquid phase vs. the ring-to-double bond distance for a series of mono-

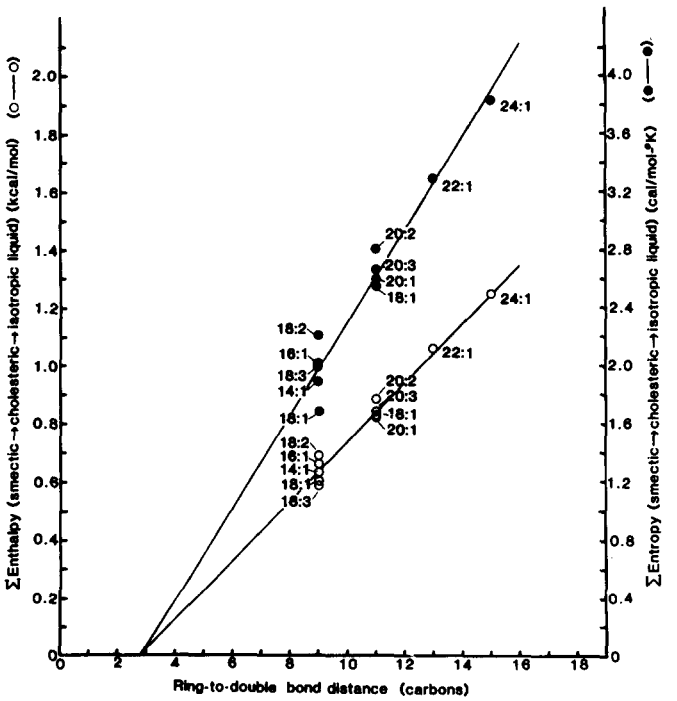


FIG. 15. Influence of double bond position on the total enthalpy and entropy for the smectic to isotropic liquid phase transition. The total enthalpy (○—○) to melt the smectic phase to isotropic liquid and total entropy (●—●) of the same transition(s) are plotted vs. ring-to-double bond distance for a series of Δ^9, Δ^{11}, Δ^{13} and Δ^{15} mono- , di-, and polyunsaturated cholesteryl esters.

unsaturated and polyunsaturated cholesteryl esters. The relationship is linear with correlation coefficients of 0.99 and 0.97 for enthalpy and entropy, respectively. This relationship is independent of the double bond–ω carbon distance, the overall chain length and the degree of polyunsaturation as exemplified in the Δ^9 series (14:1, 16:1, 18:1, 18:2 and 18:3) of cholesteryl esters and the Δ^{11} series (20:1, 20:2, 20:3 and 18:1) of cholesteryl esters. The conclusions may be as follows: (1) the formation of the smectic phase appears to be driven by the ring-to-double bond distance; (2) the presence of a double bond excludes the remainder of the chain from participating in the order of the phase and, in fact, may disorder it and (3) for a given ring-to-double bond distance increasing unsaturation toward the terminal methyl does not affect the order of the smectic phase (see below).

2. *Effect of Increasing Unsaturation*

The effect of increasing unsaturation will depend on the position of the additional double bonds. In order to assess the effect of increasing unsaturation independent of double bond position, two homologous series of esters (C_{18} and C_{20}) have been studied in which the ring-to-double bond distance was held constant (Δ^9 and Δ^{11}, respectively) but in which the unsaturation was increased toward the terminal methyl group.[60,111] The results show that both the melting points of the crystalline material and the liquid crystalline transitions of the unsaturated and polyunsaturated cholesteryl esters are considerably lower than those of their saturated homologues. To illustrate this graphically, the crystal melting and liquid crystalline transition temperatures were plotted as a function of the number of *cis* double bonds in the C_{20} fatty acids in Fig. 16a and in the C_{18} fatty acids in Fig. 16b.

In the C_{20} series (Fig. 16a), increasing unsaturation decreases the crystal melting temperature but *increases* the domain of the *smectic* mesophase (shaded). Cholesteryl

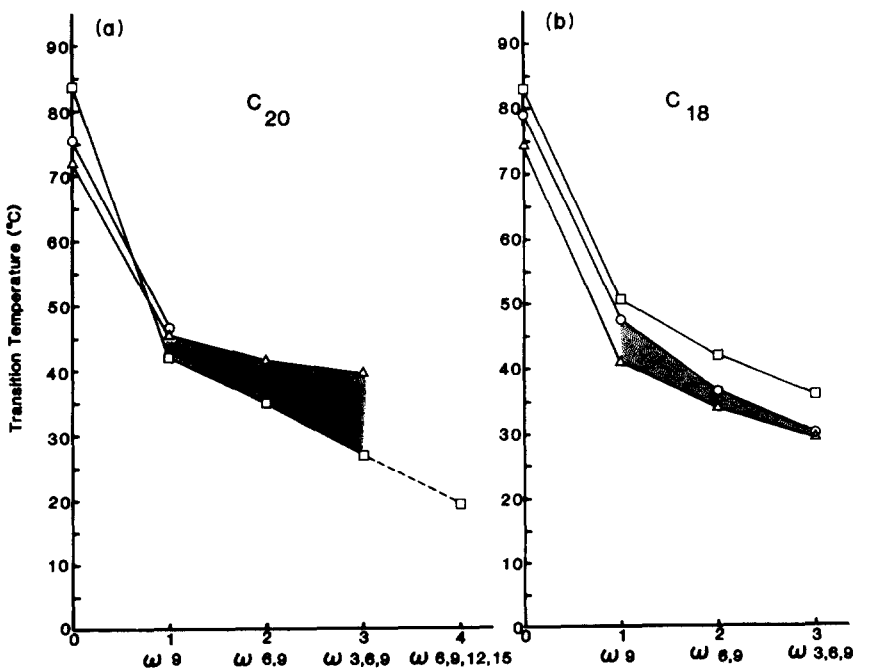


FIG. 16. Effect of increased unsaturation on crystal and mesophase transition temperatures in a series of C_{20} and C_{18} cholesteryl esters. Melting temperature of crystal (□), smectic (△) and cholesteric (○) phases plotted vs. number and position of double bonds in the acyl chain. (a) Data for the C_{20} series was plotted (homologous ω–9 esters with the exception of $C_{20:4}$) and shaded area represents the temperature domain of the stable smectic phase.[60] In (b), data for ω–9 C_{18} series was plotted and shaded area represents the temperature domain of the cholesteric phase although it is metastable.[111] Data from Table 2.

arachidonate ($C_{20:4}$ ω–6,9,12,15) also plotted in Fig. 16a is low melting with no smectic (see above) or cholesteric mesophases. The positions of the double bonds in this ester are not homologous with the rest of the series, demonstrating a continued dependence of crystal melting on the degree of unsaturation while reinforcing the notion of a necessary minimum uninterrupted chain length between ring and double bond required to form a stable liquid crystal.

Among the C_{18} unsaturated esters, the temperature domain of the *cholesteric* phase (shaded area, Fig. 16b) decreases sharply with the number of double bonds. Thus, for cholesteryl oleate, the cholesteric phase extends from 42 to 47.5° or a range of 5.5°; for cholesteryl linoleate, this domain is 2.5°; for cholesteryl linoleate, the domain is only 0.3°. These data suggest that, for cholesteric-phase-forming cholesteryl esters, increasing unsaturation destabilizes or disorders the cholesteric mesophase. While cholesteryl esters with a propensity to form stable smectic phases because of long ring-to-double bond distances, increasing unsaturation further stabilizes the smectic phase. Note that the smectic phases of the polyunsaturated C_{20} esters are stable for several days at room temperature (under-cooled).[59]

The summed enthalpies and entropies of the smectic→cholesteric→isotropic liquid phase transitions of 18:1, 18:2 and 18:3 esters and those of 20:1, 20:2 and 20:3 esters are approximately constant (for the C_{18} series ΔH ~0.6 kcal/mol, ΔS ~1.96 cal/mol–K; for the C_{20} series ΔH ~0.8 kcal/mol, ΔS ~2.71 cal/mol–K). Thus, the overall order of the smectic phase with respect to the isotropic liquid phase is independent of unsaturation in the homologous series in confirmation of the suggestion that, for a given chain length, that portion of the chain beyond the double bond does not participate in the *order* of the phase, while the ring-to-double bond segment does.

VI. VISCOSITY, FLUIDITY AND MOLECULAR MOTIONS

Viscosity measurements have been made in the liquid crystalline and the liquid phases of a few cholesteryl esters.[102,126] A relatively complete study of the viscosity of cholesteryl myristate at different shear rates using a column and plate viscometer was conducted by Sakamoto *et al.*[102] A plot of the log of viscosity vs. 1/temperature is given in Fig. 17. The inset shows the effect of increasing shear rate on the viscosity of the smectic, cholesteric and isotropic phase. Both the smectic and the cholesteric phases are clearly non-Newtonian and their viscosities decrease with increasing shear rate. The isotropic phase measured at one degree above the cholesteric–isotropic transition appears to be Newtonian. The nearly linear decrease in the log of viscosity vs. shear rate indicates that the viscosity of both the smectic and the cholesteric phases will reach a limiting value of viscosity similar to the isotropic phase at higher shear rates. The cholesteric phase is much more sensitive to shear rate than the smectic phase. The authors believe that the structures of the smectic and cholesteric phases were disrupted at these high shear rates. The energies of flow activation may be calculated from a plot of the log of viscosity vs. $1/T$. For cholesteryl myristate, the activation energies in kcal/mol are 11 to 16 for liquid crystalline states and about 8 for the isotropic liquid state.

A distinct maximum in viscosity occurs at the isotropic–cholesteric transition. Similar maxima have been reported for cholesteryl oleyl carbonate.[126] The maximum is shear rate dependent and at high shear rates it is reported to disappear[102] and is believed to be the result of flow turbulence created at the phase transition.

The suggestion that the ring systems play a large role in the organization of the cholesteric phase is supported by vibrational spectrographic studies of cholesteric and liquid states.[27] In these studies, the Raman spectra were interpreted to show that, at least in terms of the Raman time scale, the cholesteric phase was more "crystalline" in terms of the ring system. In contrast, infrared and far infrared spectra of cholesteryl laurate in the cholesteric phase were interpreted by Shivaprakash *et al.*[108] to show that the fatty acyl chain is extended in the cholesteric phase and that perhaps the extended chain stabilizes this phase.

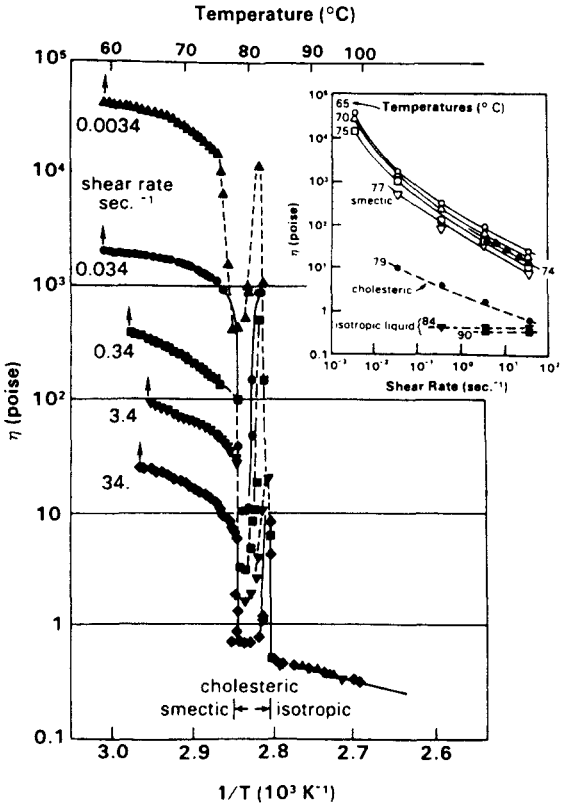


FIG. 17. The log of viscosity vs. 1/temperature for cholesteryl myristate at different shear rates. The inset shows the viscosity of each phase at different shear rates.[102]

^{13}C NMR studies of cholesteryl oleate, nervonate and erucate, which differ only in the number of carbons between the carboxyl and the first double bond, help to unravel questions about what kind of ordering occurs in the isotropic phase and what forces initiate the formation of either the cholesteric phase or the smectic phase.[61] Cholesteryl oleate first forms the cholesteric phase a few degrees above the smectic phase whereas both cholesteryl erucate and nervonate form stable smectic phases without forming a cholesteric phase.[60]

Previously, few studies examined neat cholesteryl esters by NMR. ^{1}H NMR[37,52,87,117] showed non-exponential spin-lattice relaxation in the liquid and liquid crystal phases and marked abrupt increases in linewidth on going from liquid to cholesteric and from cholesteric to smectic. The early data suggested that at least two types of molecular motions were responsible for the relaxation process. ApSimon *et al.*[6] used ^{13}C NMR to show that anisotropic motions characterized the cyclopentaphenanthrene ring system in various steroids and suggested that modes of rotation about the long molecular axis were preferred.

The high spectral resolution obtained by Ginsburg *et al.*[61] at 50.3 MHz in a natural abundance ^{13}C NMR study of liquid cholesteryl esters provided numerous well-resolved single carbon resonances from both steroid ring carbons and fatty acyl carbons which were used to probe the temperature-dependent dynamics of these two components of the cholesteryl ester molecule. The ^{13}C NMR spectrum of neat cholesteryl oleate and the peak assignments are given in Fig. 18. Protonated carbon resonances of the fused steroid ring system exhibited a large range of linewidth values which were temperature sensitive. In the isotropic liquid phase near the liquid→liquid crystal transition temperature, T_m, methine carbon resonances showed a nonlinear differential broadening with decreasing temperature; the increasing C-3/C-6 linewidth ratio reflects steroid ring motions which are increasingly anisotropic.[61,66,100] Analysis of the linewidth data in terms of temperatures

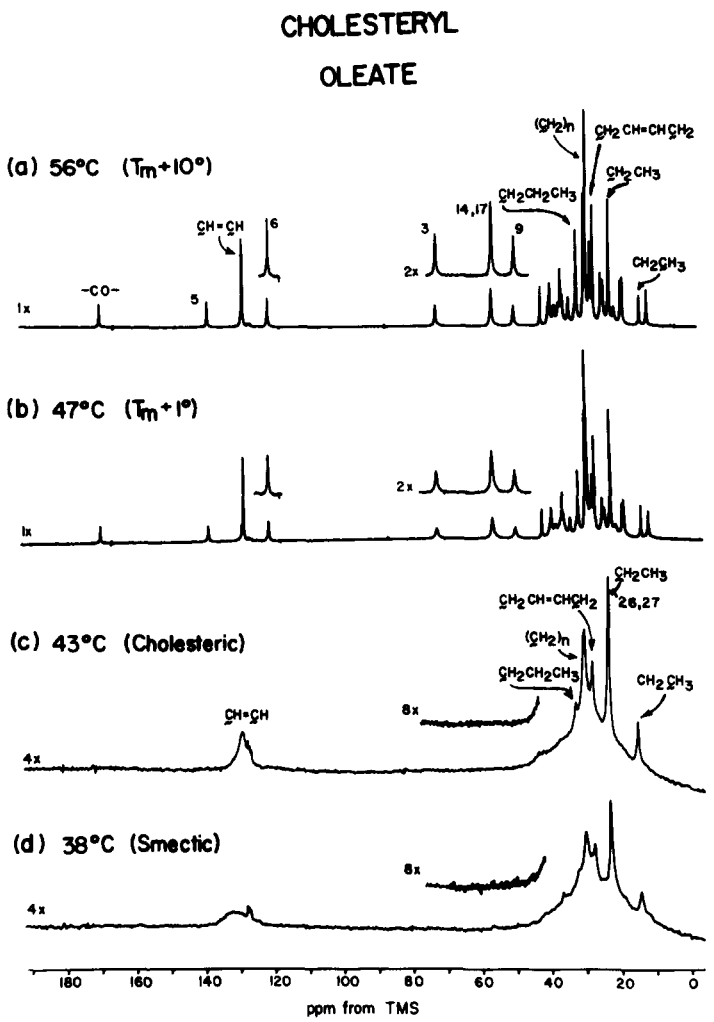


FIG. 18. Proton-decoupled natural abundance ^{13}C Fourier transform NMR spectra in the isotropic liquid phase (a) and (b), the cholesteric phase (c) and the smectic phase (d) of (neat) cholesteryl oleate. Spectra were recorded at 50.3 MHz after 250 accumulations with the use of a 10,000 Hz spectra width, 32,768 time-domain addresses and a recycle time of 1.62 s. Spectra (a) and (b) were recorded at 10° ($T_m + 10°C$) and at 1° ($T_m + 1°C$) relative to the isotropic liquid↔cholesteric phase transition temperature (T_m), and 1 Hz digital line broadening was applied to increase the signal-to-noise ratio. Spectra in the liquid crystalline phases (c) and (d) were processed with 3 Hz digital linebroadening. All spectra were normalized to spectrum (a), and thus a four-fold vertical expansion was used in printing spectra (c) and (d). All insets were printed with a two-fold vertical expansion of the main spectrum. The inset in spectrum (d) is from the same data set as the main spectrum except after 1500 accumulations. The assignments are indicated as follows: numbered peaks correspond to ring carbons as in Fig. 1; fatty acyl carbons are indicated by chemical formula with the symbol ~ beneath the specific carbon(s) represented by the peak. The triplet at ~128.0 ppm is from the C_6D_6 lock. Reproduced with permission of Ginsburg *et al.*[61]

relative to T_m (Fig. 19) showed that the esters with a stable smectic phase and no cholesteric phase (nervonate and erucate) had smaller linewidths for the C–3 and C–6 resonances and smaller linewidth ratios (C–3/C–6) at any temperature relative to T_m when compared with the esters exhibiting a cholesteric phase (oleate and linoleate). Thus, at any relative temperature above T_m, the steroid ring motions of the nervonate and erucate esters were more rapid and less anisotropic than those of cholesteryl oleate and linoleate.

Ring ordering is apparently an important feature of liquid crystalline phases of cholesteryl esters, and a higher degree of ring ordering is characteristic of the formation of a cholesteric phase. In fact, calorimetry studies on *di*cholesteryl esters have shown that these lipids undergo a cholesteric→isotropic liquid phase transition, with at least *twice* the expected entropy, indicating that the steroid ring interactions are important in ordering the cholesteric phase.[18]

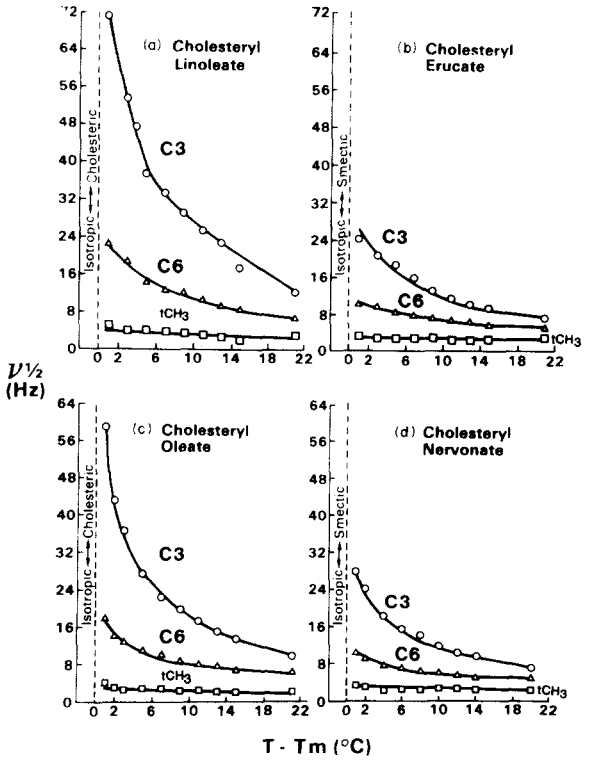


FIG. 19. Plots of linewidths of resonances for cholesteryl ester ring carbons C-3 (○), C-6 (△) and fatty acyl terminal methyl (□) resonances vs. temperature relative to isotropic liquid↔liquid crystal transition temperature (T_m) for (a) cholesteryl linoleate, (b) cholesteryl erucate, (c) cholesteryl oleate and (d) cholesteryl nervonate. Data was acquired under the same conditions as in Fig. 18 (a) and (b). In all cases, the digital linebroadening introduced to increase the signal-to-noise ratio was subtracted from the measured linewidth to give the values plotted. The temperature at which a liquid crystal phase forms from the isotropic liquid (T_m) is indicated by a vertical dashed line. The type of liquid crystal below this temperature is indicated to the left of this line (printed vertically) for each system.

The motions of a fatty acyl carbon can be approximated by assuming a single "effective" correlation time.[50] If motions are rapid and isotropic, then the increasing NT_1's reflect decreasing "effective" correlation times (increasingly rapid rotations). The relatively long correlation times of the FA–2 and FA–3 (Fig. 20) result from the close proximity of these carbons to the anchor point on the bulky ring system, and the relatively short correlation time of the terminal methyl results from the additional degrees of rotational freedom and the flexibility of the end of the long chain (Fig. 20).

T_1 gradients along hydrocarbon chains have been documented in numerous ^{13}C NMR studies. For n-alkanes, NT_1 values increase away from the center of mass of the chain,[80,115] while for long chain-fatty alcohols and acids[115], short chain phospholipids in micelles[29] and long chain phospholipids in vesicles[58,79,81] NT_1 values of fatty acyl carbons increase away from the anchor point at the polar bond. For neat cholesteryl esters, the most striking feature of NT_1 vs. carbon number profiles is the constancy of NT_1 in the region of the chain between the C–3 and the olefinic carbons. This result indicates that the effective correlation times are similar and the motions are restricted in this portion of the hydrocarbon chain relative to the terminal methyl end of the chain. A plateau in T_1 profiles appears to be a feature of fatty acyl T_1 values of phospholipids in vesicles[62] and in micelles[29] but is absent for monomeric solutions of short-chain phospholipids.[29] The plateau is much less pronounced for cholesteryl esters in $CDCl_3$ solution suggesting that *inter*molecular interactions are an important determinant of the acyl chain motions in neat esters. Although liquid cholesteryl esters do not have a defined orientation as do phospholipids in vesicles, strong hydrocarbon chain associations may take place perhaps within microdomains in the liquid phase.

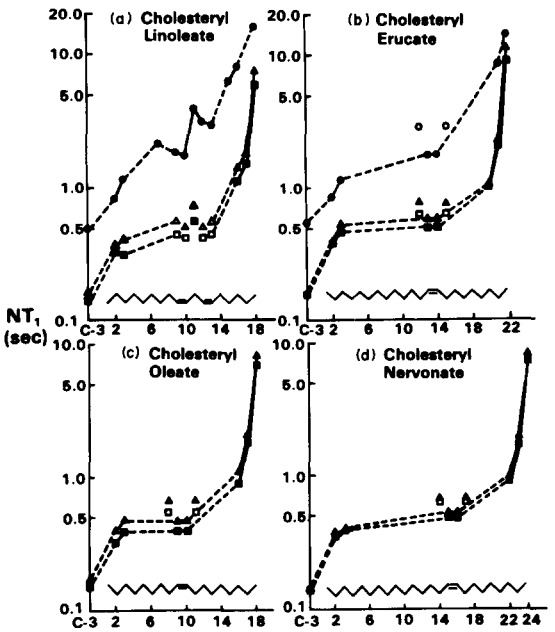


FIG. 20. Semilogarithmic plots of NT_1 (where N = number of directly bonded protons and T_1 = measured spin-lattice relaxation time) vs. carbons along the fatty acyl chain in deuterochloroform (0.2 M), ○, ●; and for the isotropic liquid at $T_m + 10°C$, △, ▲; at $T_m + 1°C$, □, ■; for cholesteryl linoleate (a), cholesteryl erucate (b), cholesteryl oleate (c) and cholesteryl nervonate (d). Abscissa represents the fatty acyl chain of each system; C − 3 is the point of attachment to the cholesterol ring carbon 3 and the acyl chain carbons are numbered consecutively from the carbonyl. *Closed* symbols are values measured for a well resolved peak assigned to a single carbon or to two adjacent carbons. *Open* symbols are values measured from a peak assigned to two non-adjacent carbons. *Solid* line connects values measured for *adjacent* carbons for which T_1 was measured. Dashed line connects values for *non-adjacent* carbons. It is important to note that although values are not plotted for unresolved methylene carbon resonances, T_1 values were obtained for partially resolved resonances for these carbons and none exceed the value of the dashed line between carbon 3 and the double bond. Note also that CH_2CH_3 resonance includes C–26,27 methyl carbons.

Thus, these results suggested specifically that, as a result of intermolecular interactions, fatty acyl chains of the four liquid esters are in an approximately extended configuration, at least up to the double bond region. An extended configuration of the fatty acyl chain had been previously suggested for cholesteryl esters in the liquid state near T_m from X-ray studies[123] and recently by neutron scattering studies of deuterated cholesteryl myristate.[28]

On the basis of spin-lattice relaxation time measurements for fatty acyl carbons, Ginsburg *et al.*[61] showed that the ring-to-double bond region of the chain had relatively restricted molecular motions in the isotropic liquid phase (Fig. 20). The two esters which form stable smectic phases, erucate and nervonate, had significantly longer regions of hydrocarbon chain interactions than the two esters which form cholesteric phases, linoleate and oleate. These data were consistent with the direct correlation between the ring-to-double bond distance (carbons) and the ΔH and ΔS of the smectic→liquid transformation. Order between the ring and double bond is illustrated in the crystal structure of cholesteryl palmitoleate at 298K (Craven and Sawzik, unpublished) in which the unit cell contains three different types of fatty acyl chain, each differing in conformation beyond the double bond but which maintain a relatively rigid conformation between the ring and the double bond. The earlier conclusion that the hydrocarbon chain region between the ω–9 double bond and the terminal methyl does not participate in the order of the phase is consistent with the T_1 results showing that carbons beyond the double bond(s) have relatively rapid and/or unrestricted motions, which may exclude these carbons from strong intermolecular interactions. The similarity between the NMR and crystal data suggests again the

structural similarity between the crystal and liquid crystal phases as well as the isotropic liquid phase near the phase transition.

A quantitative treatment relating the linewidth values of the steroid ring methine carbon C–6 and C–3 ring rotation based on the model of Woessner[125] has been recently developed.[61,100] In this model, the shape of the cholesteryl ester molecule is approximated by a prolate ellipsoid with the z-axis passing through the length of the molecule (see Fig. 21). The two shorter axes orthogonal to the z-axis, which describe the motions of the ring, are considered to be of equal length (a simplification of the true picture). This model was used to calculate the rotational correlation times (τ_{rz} and τ_{rx}) from the linewidth data.[61] The rotation about the long axis (τ_{rz}) is in the order of 10^{-9} s and rotation about the shorter axis (τ_{rx}) is about 50 to 100 times greater; that is, the motion is much slower. This is true for all the cholesteryl esters studied. As a function of decreasing temperature towards the transition temperature, correlation time τ_{rx} increases four-fold while τ_{rz} increases only two-fold. Thus, cooling towards the transition temperature restricts rotation about x more than about z. As stated previously, however, use of this rod-shaped model is an oversimplification. The two short axes are not the same (~5 and 7 Å) and the two faces of the ring are clearly not identical. Furthermore, the formation of a cholesteric phase implies ring–ring interactions that are relatively specific and, to some extent, dependent upon the chemical structure of the ring. Further work will be needed to sort out the specific interactions of the ring in the cholesteric phase.

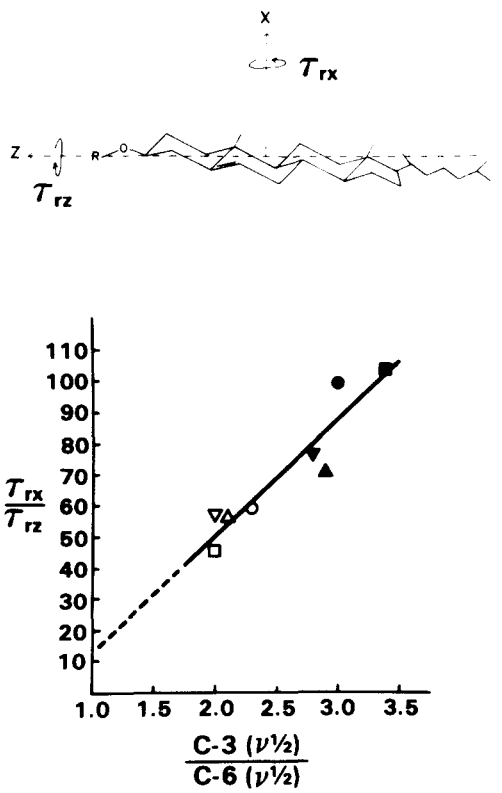


FIG. 21. Top. A schematic of the cholesteryl ester molecule which is assumed to be a prolate ellipsoid having a long molecular axis (z) and a short molecular axis (x). The z-axis, as defined by Quinn,[100] passes through the C–3 and C–13 carbon nuclei and the x-axis is non-unique and may assume any orientation perpendicular to the z-axis. τ_{rz} and τ_{rx} are the correlation times which describe motions about these axes as shown. Bottom. Plot of the ratio of calculated rotational diffusion correlation times τ_{rx} and τ_{rz} vs. the linewidth ratio of the C–3 and C–6 cholesterol ring carbon resonances at $T_m + 10$°C (closed symbols) and $T_m + 1$°C (open symbols) for four cholesteryl esters (values from Table 5). □,■ = cholesteryl oleate; ○,● = cholesteryl linoleate; △,▲ = cholesteryl erucate, ▽,▼ = cholesteryl nervonate. The relationship is linear with a correlation coefficient of 0.93. The dashed line indicates extrapolation of the relationship to a linewidth ratio of 1.0 (see text).

As a general summary, it is clear that some ordering in the liquid phase is present and that tumbling about the short axis is much less likely to occur than rotation around the long axis. Furthermore, interactions increase as the temperature decreases, and, if chain–chain interactions are weak, a cholesteric phase will be formed. On the other hand, if chain–chain interactions are strong, as in the case of esters with a long distance between the ester group and the first double bond, than a stable smectic phase will be formed before ring–ring interaction is strong enough to nucleate a cholesteric phase. Finally, if the chain is saturated and long, nucleation and crystallization will occur at temperatures above the temperature of potential formation of the liquid crystals and no liquid crystalline phases can be formed.

VII. CRYSTAL AND SMECTIC MESOPHASE STRUCTURE OF CHOLESTERYL ESTERS FROM X-RAY POWDER PATTERNS

The structure of the liquid crystalline phases of cholesteryl esters has been enigmatic for nearly three quarters of a century. Not much more is known beyond the original interpretations of Grandjean and Friedel in 1922.[57] As stated earlier, the smectic phase is characterized by a single order reflection at narrow angles and diffuse scattering at wide angles (Fig. 8). Smectic A liquid crystals have been shown to have only a single spacing in the narrow angle range.[48,67,89,121] This, together with the optical properties of the smectic phase, clearly indicates a layered structure. Several investigators have modeled the structural organization of this mesophase based on the crystal structure of cholesteryl myristate and assuming that an overall extended conformation of the molecule was maintained from phase to phase.[14,33] It was not until recently, however, that this assumption was proved correct. Neutron scattering from cholesteryl myristate, in which the ω and isooctyl carbon hydrogens were substituted with deuterium, showed that the molecule maintains its extended conformation in the crystal, smectic, cholesteric *and* isotropic liquid phases.[28] With this important contribution, the mesophase structure of cholesteryl esters can be approached by interpolation between the crystal structure and the interactions in the isotropic liquid phase, provided the thermodynamics and phase behavior between the two are known.

Table 4 presents data from X-ray powder patterns of saturated mono- and poly-unsaturated cholesteryl esters having a variety of chain lengths, double bonds and double bond positions. The d_{001} (or symmetry allowed primary diffraction) spacing for the crystalline material and the smectic phase (recorded in the stable temperature domain) are presented. A plot of the Bragg spacing represented by the d_{001} reflection from both the crystalline and smectic phases vs. the chain length of saturated and monounsaturated cholesteryl esters is shown in Figs 22 and 23, respectively.

Crystals of long chain C_{13} to C_{24} saturated cholesteryl esters are an isostructural series of the bimolecular layered type. The slope of the line of d_{001} vs. chain lengths for the even numbered members of this series is ~ 1.30 Å/carbon, indicative of a nearly perpendicular arrangement of the acyl chains to the bilayer interface (001 lattice plane). The intercept of 31.4 Å corresponds to the hypothetical spacing expected from a unit cell at zero chain length. This reduction would yield two antiparallel cholesterol ring systems with a long axis dimension of 15.7 Å per ring, consistent with the long axis dimension of cholesteryl monohydrate which, as determined from the crystal structure, yields a value of 17 Å per carbon ring including the isooctyl side chain.[31]

In general, the medium and long chain monounsaturated cholesteryl esters crystallize as monolayer Type II. A plot of the Bragg spacing from the d_{001} reflection in the crystalline state (Fig. 23) shows an increase in periodicity which is correlated with an increase in carbons along the alkyl chain ($r = 0.99$). The increased spacing is *not* correlated specifically with changes in the ring-to-double bond distance nor with the double bond-to-ω carbon distance as exemplified by the similar spacing for three $C_{18:1}$ isomers with ω–7, ω–9 or ω–12 double bonds. As a model for the packing of unsaturated esters in a monolayer, Type II packing cholesteryl oleate (Fig. 3) shows that, in spite of the double bond, a linear chain

TABLE 4. X-Ray Long Spacings for Cholesteryl Esters

n	Common name (Cholesteryl...)	IUPAC name (Cholesteryl...)	Crystal (d_{001})	Smectic (Å)	Cryst. Structure	Ref.
(a) *Saturated*						
8	Caprylate	Octanoate	14.1	c	MLII	a
10	Caprate	Decanoate	29.3	28.3	MLI	b
12	Laurate	Dodecanoate	30.3	30.5	MLI	b
14	Myristate	Tetradecanoate	50.7	33.0	BL	a
16	Palmitate	Hexadecanoate	52.8	35.5	BL	b
18	Stearate	Octadecanoate	54.5	37.5	BL	b
20	Arachidate	Eicosanoate	58.5	39.5	BL	a
22	Behenate	Docosanoate	61.4	c	BL	a
24	Lignocerate	Tetracosanoate	63.5	c	BL	a
(b) *Monounsaturated*						
14:1 (ω5)	Myristoleate	*cis*-9 tetradecenoate	17.0	30.3		a
16:1 (ω7)	Palmitoleate	*cis*-9 hexadecenoate	34.9	33.3		a
18:1 (ω7)	Vaccenate	*cis*-11 octadecenoate	18.5	35.6		a
18:1 (ω9)	Oleate	*cis*-9 octadecenoate	18.7	35.5	MLII	a
			18.8	36.0	MLII	a
18:1 (ω12)	Petroselinate	*cis*-7 octadecenoate	18.2	c	BL	a
20:1 (ω9)		*cis*-11 eicosenoate	19.8	38.1		a
22:1 (ω9)	Erucate	*cis*-13 docosenoate	20.2	40.3	MLII	a
22:1 (ω9) (t)	Brassidate	*trans*-13 docosenoate	29.7	d		a
24:1 (ω9)	Nervonate	*cis*-15 tetracosenoate	42.9	43.5	MLI	a
(c) *Polyunsaturated*						
18:2 (ω6,9)	Linoleate	*cis*-9,12 octadecadienoate	40.3	35.0		c
18:3 (ω3,6,9)	Linolenate	*cis*-9,12,15 octadecatrienoate	27.85			b
20:2 (ω6,9)	Gondoate	*cis*-11,14 eicosadienoate	39.9	38.1		b
20:3 (ω3,6,9)		*cis*-11,14,17 eicosatrienoate	19.0	37.8		b
20:4 (ω6,9,12,15)	Arachidonate	*cis*-5,8,11,14 eicosatetraenoate	19.7	*		b

Refs: a, this work; b, G. C. Shipley, M. Halks, and D. M. Small (unpublished results); c, no smectic present (see Table 2).
*Not attainable.

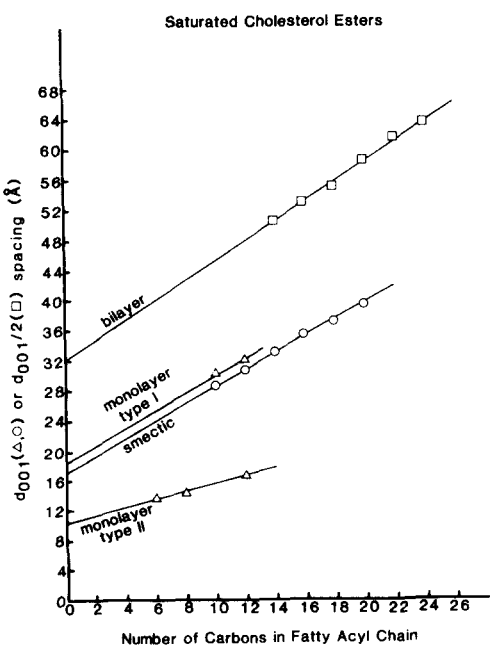


FIG. 22. d_{001} spacings vs. chain length for saturated cholesteryl esters. The Bragg spacing corresponding to d_{001} (or symmetry allowed primary diffraction) reflection for crystalline (□,△) and smectic phase (○) cholesteryl esters is plotted as a function of carbons in the fatty acyl chain. Isostructural series plotted on the same line and are labeled accordingly. Data from Table 4.

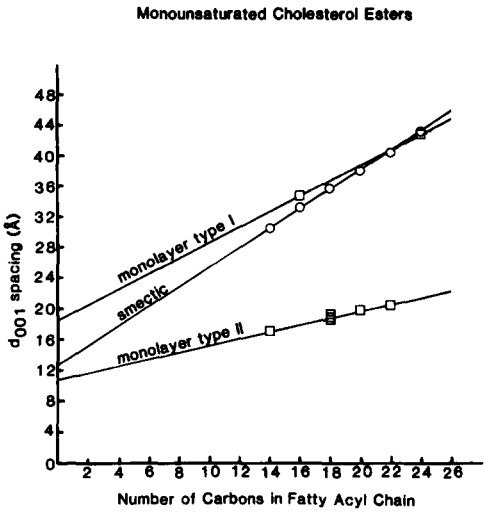


FIG. 23. d_{001} spacings vs. chain length for monounsaturated cholesteryl esters. The Bragg spacings corresponding to the d_{001} reflection for crystalline (□) and smectic phase (○) cholesteryl esters are plotted as a function of carbons in the fatty acyl chain. Isostructural series are plotted on the same line and are labeled accordingly. Isomeric C_{18} cholesteryl esters are ω–7, ω–9 and ω–12. Data from Table 4.

conformation is maintained. The slope of the line calculated from the d_{001} spacings from the isostructural series is 0.49 Å/carbon. The angle, calculated from this value, which the chain (and thus the long molecular axis) makes with the 001 lattice plane is approximately 23.0°—in reasonable agreement with the angle measured by Craven and Guerina[34,35] of 26.5° for cholesteryl oleate. This close agreement between the data from Fig. 23 and the crystal data further substantiates not only the accuracy of the powder pattern data but also the assignment of these esters as isostructural with cholesteryl oleate.

If the d spacing for the smectic phase of each cholesteryl ester is plotted against the number of carbons in the chain, a rather interesting difference emerges between the saturated and monounsaturated series.[59] The saturated esters have d spacings about 1.5 to 2.5 Å greater than the unsaturated series. Further, the lines are different. The slope for the saturated system is 1.14 Å/–CH_2–, while for the monounsaturated series, it is 1.21 Å/–CH_2–. Therefore, the chains must lie fairly normal to the plane of the putative smectic layers. Even if the chains were fully extended (1.27 Å/–CH_2–), the angles of tilt with respect to the layer plane would be 74° and 65°, respectively. However, the rheologic, spectrographic and X-ray data all indicate that the chains are not in a rigid, totally extended state, but are rather in a "liquid" state. Further, the estimated volume of the –CH_2– group in the liquid crystal phase is around 28 to 29 $Å^3$/–CH_2–,[115] a value consistent with nearly liquid chains. If the volume were 29 $Å^3$/–CH_2– group, then the surface area of the chains, normal to the layer plane, would be about 25 $Å^2$ for the saturated series and about 24 $Å^2$ for the unsaturated series.[115] The latter value clearly suggests that the double bond is "straightened out" in the smectic state, perhaps like it is in the crystal structure of cholesteryl oleate (see Fig. 3b).

The extrapolation of the d vs. n lines to zero indicate the thickness of the cholesterol region in the smectic phase. The estimated thickness of the sterol region of the saturated esters is 17 Å, quite close to the extended length of the cholesterol molecule (17.5 Å), indicating that the sterol axis lies nearly normal to the smectic planes. The thickness of the cholesterol region of the monounsaturated series is only 13.8 Å, and this suggests that the sterol axis is tilted about 54° with respect to the smectic phase. Thus, the saturated series appears to be a smectic A liquid crystal (molecular long axis normal to smectic planes), while the unsaturated series is a smectic C liquid crystal (molecular long axis tilted with respect to the smectic planes).

d_{001} spacings from monolayer Type I crystals fall on a line nearly identical to that in

slope and intercept as the smectic phase of saturated cholesteryl esters. This observation would suggest that an orientation of the cholesteryl ester molecules in this crystal type may be related to the molecular arrangement of the cholesteryl esters in the smectic phase. At zero chain length, the unit cell of monolayer Type I would consist of four sterol nuclei, two opposing "A" molecules and two adjacent antiparallel "B" molecules. A dependence of unit cell dimension on chain length is *not* obvious since chain overlap occurs at the interfacial region. Additionally, only two cholesteryl esters with smectic phases pack in this crystal form. Therefore, modelling of a chain length dependent structure for the smectic phase based on this structure is difficult. However, the "B" molecules are probably a structural element common to the crystal and smectic mesophase. Cholesteryl laurate can be used to illustrate this point. Cholesteryl laurate is polymorphous and packs in crystals of monolayer Types I and II. The smectic phase of this ester may be formed from the Type I monolayer crystal by first heating to the isotropic liquid and undercooling the liquid phase. Alternatively, the Type I crystal form may be allowed to undergo an isothermal polymorphic transition to a monolayer Type II crystal which, on further heating, melts *directly* to the smectic phase. The unit cell of the Type II monolayers actually contains components of the Type I crystal form, i.e. the "B" molecules. Thus, it is implied that the smectic phase of cholesteryl laurate cannot form directly from the melt of its Type I crystal due to constraints imposed by the "*A*" molecules packing in the unit cell. The "disordered" liquid state must be realized in order to remove them from the interaction, whereas monolayer Type II crystals of cholesteryl laurate need not be totally disordered to form the smectic phase.

A plot of the narrow angle reflection from the smectic phase of the monounsaturated esters vs. the fatty acyl chain length (carbons) is linear ($r = 0.99$). This linear relationship is distinct from that obtained for the smectic phase of homologous saturated cholesteryl esters. It might be expected that the d_{001} spacing from the smectic phase of monounsaturated cholesteryl esters differ by a constant amount from their saturated homologues due to the effective shortening of the chain by the double bond. This is not the case; not only is the intercept different but so is the slope. The intercept of ~ 13.5 A at zero chain length is similar to the intercept from the crystal d_{001} spacing of monolayer Type II esters at zero chain length. This would suggest a similar basic orientation in the smectic phase of unsaturated esters as in monolayer type II crystals.

The crystals of the monounsaturated esters are isostructural with short chain saturated cholesteryl esters which also pack in monolayer type II. The crystal structure of these esters suggests that ring–ring interactions prevail between antiparallel ester pairs whose overall monolayer structure is stabilized through the interlocking of C-21 methyl groups of adjacent pairs and van der Waals forces between double bonds and acyl chains from opposing monolayers. The ^{13}C NMR data further suggest that these fundamental interactions prevail as well in the isotropic liquid phase. Thus, it seems reasonable to interpolate that the liquid crystalline phases are stabilized by similar specific interactions. A likely model for the smectic phase would then be derived from the monolayer type II crystal structure. In fact, with the exception of one,* all do so directly from the monolayer type II crystal form. Furthermore, in the monounsaturated series, cholesteryl palmitoleate like cholesteryl laurate, is polymorphous with two stable crystalline forms: the monolayer Type I structure melts to an isotropic liquid with mesophase formation occurring from the undercooled liquid but the monolayer Type II crystal melts directly to the smectic phase.

To summarize, a general organization of the smectic mesophase is proposed and features an antiparallel arrangement of cholesteryl ester molecules in which adjacent cholesteryl ester molecules contribute fatty acyl chains to adjacent layers resulting in interdigitation of acyl chains. Features of all three crystal types are seen in this model with the predominant scheme being similar to the molecular arrangement monolayer Type II

*Cholesteryl nervonate ($C_{24:1}$) melts directly from a crystal with a monolayer type I structure to the smectic phase. Note that, because of its long chain, extensive overlap of the fatty acyl chain occurs at the layer interface. Thus, the extent of chain–chain interaction is significantly greater for this ester than for shorter cholesteryl esters which are monolayer type I.

crystals. At C_8, which has no smectic phase, ring–ring interactions are probably maximized and the structure would be similar to the crystal structure for cholesteryl octanoate (monolayer Type II). Furthermore, steric interactions between steroid rings and isooctyl side chains from opposing layers perhaps disorder the structure enough to give the cholesteric phase but no smectic phase in this as well as shorter acyl chain lengths. At C_{22}, for which *no* mesophases are observed, significant chain–chain interactions probably occur similar to those in the bilayer crystal type which esters of this chain length form. Finally, ring–chain interactions undoubtedly occur at all chain lengths C_{10} to C_{20} and perhaps impart enough disorder and root-mean-square displacement of the molecules in the smectic phase to explain the *single* order observed in the diffraction pattern.

VIII. SUMMARY

Cholesteryl esters, the intracellular storage form and intravascular transport form of cholesterol, can exist in crystal, liquid crystal and liquid states. The physical state of cholesteryl esters at physiologic temperatures may be a determinant of their pathogenicity.[111–113] This review has surveyed saturated aliphatic cholesteryl esters of chain length 1 to 24 carbons and a series of medium-chained unsaturated cholesteryl esters from chain lengths 14 to 24 carbons. A systematic study of transition temperatures by polarizing microscopy and enthalpies by differential scanning calorimetry has provided unifying concepts concerning the phase behavior as a function of chain length and unsaturation. Neat cholesteryl esters show chain-length dependence of transition temperature and enthalpy of both the crystal and liquid crystal transitions. Double bond position along the fatty acyl chain affected stability of the liquid crystal phases; a smectic phase was not observed for any cholesteryl ester with a double bond more proximal than Δ^9. ^{13}C NMR spectroscopy in the isotropic liquid phase has provided evidence suggesting a balance of ring–ring vs. chain–chain interactions as a determinant for isotropic liquid→cholesteric vs. isotropic liquid→smectic transitions. Specifically, anisotropic molecular motions of the steroid ring are greater for cholesteryl esters forming a cholesteric phase than a smectic phase from the melt. Chain–chain interactions apparently predominate in smectic phase formation. The X-ray diffraction patterns of cholesteryl esters as a function of chain length reveal several isostructural series and known single crystal data are presented. A chain length depending on the periodicity of the smectic phase is observed which may be different for saturated vs. unsaturated esters. In summary, the phase behavior of cholesteryl ester molecules is complex and cannot be determined *a priori* from the phase behavior of component cholesterol and fatty acid. The data presented here should provide insight into the biological behavior of this lipid class.

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REFERENCES

1. ABRAHAMSSON, S. and DAHLEN, B. *J.C.S. Chem. Commun.* **117** (1976).
2. ABRAHAMSSON, S. and RYDERSTEDT-NAHRINGBAUER, I. *Acta Cryst. allogr.* **15,** 1261–1268 (1962).
3. ABRAHAMSSON, S. and SELIN, K. *Acta Cryst allogr.* **16,** A58 (1963).
4. ABRAMOV, A., SCHORR, S. and WOLMAN, M. *Am. J. Dis. Child.* **91,** 281–286 (1956).
5. ALBERTINI, G., DUBIN, B., MELONE, S., PONZI-BOSSI, M. G. and RUSTICHELLI, F. *Mol. Cryst. Liq. Cryst.* **70,** 1447–1460 (1981).
6. APSIMON, J. W., BEIERBECK, H. and SAUNDERS, J. K. *Can. J. Chem.* **53,** 338–342 (1975).
7. ARMITAGE, D., DECKELBAUM, R. J., SHIPLEY, G. G. and SMALL, D. M. *Mol. Cryst. Liq. Cryst.* **42,** 203–214 (1977).
8. ARMITAGE, D. and PRICE, F. P. *J. Chem. Phys.* **66,** 3414–3417 (1977).
9. ATALLAH, A. M. and NICHOLAS, H. J. *Mol. Cryst. Liq. Cryst.* **17,** 1–17 (1972).
10. ATALLAH, A. M. and NICHOLAS, H. J. *Mol. Cryst. Liq. Cryst.* **18,** 339–344 (1972).
11. ATALLAH, A. M. and NICHOLAS, H. J. *Mol. Cryst. Liq. Cryst.* **19,** 217–222 (1973).

12. ATALLAH, A. M. and NICHOLAS, H. J. *Mol. Cryst. Liq. Cryst.* **24,** 213–222 (1973).
13. ATALLAH, A. and NICHOLAS, H. J. *Lipids* **9,** 613–622 (1974).
14. ATKINSON, D., DECKELBAUM, R. J., SMALL, D. M. and SHIPLEY, G. G. *Proc. natn. Acad. Sci. U.S.A.* **74,** 1042–1046 (1977).
15. BARNARD, J. A. W. Ph.D. Dissertation, University of Leeds, Yorkshire, England (1973).
16. BARNARD, J. A. W. and LYDON, J. E. *Mol. Cryst. Liq. Cryst.* **26,** 285–296 (1974).
17. BARRALL, E. M. and JOHNSON, J. F. In *Liquid Crystals and Plastic Crystals*, Vol. 2, p. 254–306 (GRAY, G. W. and WINSOR, P. A., eds), John Wiley & Sons, Inc., New York 1974.
18. BARRALL, E. M., JOHNSON, J. F. and PORTER, R. S. *Mol. Cryst. Liq. Cryst.* **8,** 27–44 (1969).
19. BARRALL, E. M., PORTER, R. S. and JOHNSON, J. F. *J. phys. Chem.* **71,** 895–900 (1967).
20. BERNAL, J. D., CROWFOOT, D. and FRANKUCHEN, I. *Phil. Trans. R. Soc.* **239A,** 135–182 (1940).
21. BRADY, G. W. *J. chem. Phys.* **57,** 91–95 (1972).
22. BRADY, G. W. *J. chem. Phys.* **58,** 3542–3546 (1973).
23. BRADY, G. W. *J. chem. Phys.* **60,** 3466–3473 (1974).
24. BRADY, G. W. *Accounts Chem. Res.* **7,** 174–180 (1974).
25. BRADY, G. W. and FEIN, D. B. *J. Appl. Cryst.* **8,** 261–265 (1975).
26. BRECHER, P. I., CHOBANIAN, J., SMALL, D. M. and CHOBANIAN, A. V. *J. Lipid Res.* **17,** 239–247 (1976).
27. BULKIN, B. J. and KRISHMAN, K. *J. Am. Chem. Soc.* **93** 5998–6004 (1971).
28. BURKS, C. and ENGELMAN, D. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6863–6867 (1981).
29. BURNS, R. A., JR. and ROBERTS, M. F. *Biochemistry* **19,** 3100–3106 (1980).
30. CARLISLE, C. M. and CROWFOOT, D. *Proc. R. Soc.* **A184,** 64–83 (1945).
31. CRAVEN, B. M. *Nature* **260,** 727–729 (1976).
32. CRAVEN, B. M. In SMALL D. M. *The Physical Chemistry of Lipids from Alkanes to Phosphalipids*, in press (1985).
33. CRAVEN, B. M. and DETITTA, G. T. *J. Chem. Soc., Perkin Trans.* **27,** 814–822 (1976).
34. CRAVEN, B. M. and GUERINA, N. G. *Chem. Phys. Lipids* **24** 157–166 (1979).
35. CRAVEN, B. M. and GUERINA, N. G. *Chem. Phys. Lipids* **29,** 91–98 (1979).
36. CRAVEN, B. M. and SAWZIK, P. *J. Lipid Res.* **24,** 784–789 (1983).
37. CUTLER, D. *Mol. Cryst. Liq. Cryst.* **8,** 85–92 (1969).
38. DAHLEN, B. *Chem. Phys. Lipids* **23,** 179–188 (1979).
38a. DAVE J. S. and VORA R. A. In *Liquid Crystals and Ordered Fluids*, Proc. Am. Chem. Soc. Symp. 2d. pp. 477–487 (JOHNSON, J. F., ed.) Plenum N.Y., 1970.
39. DAVIS, G. J., PORTER, R. S. and BARALL, E. M. *Mol. Cryst. Liq. Cryst.* **10,** 1–19 (1970).
40. DAVIS, G. J., PORTER, R. S. and BARRALL, E. M. *Mol. Cryst. Liq. Cryst.* **11** 319–330 (1970).
41. DAVIS, G. J., PORTER, R. S., STEINER, J. W. and SMALL, D. M. *Mol. Cryst. Liq. Cryst.* **10,** 331–336 (1970).
42. DAVISON, A. N. *Adv. Lipid Res.* **3,** 171–196 (1965).
43. DECKELBAUM, R. J., HALPERIN, G. and ATKINSON, D. *J. Lipid Res.* **24,** 657–661 (1983).
44. DECKELBAUM, R. J., SHIPLEY, G. G. and SMALL, D. M. *J. biol. Chem.* **252,** 744–754 (1977).
45. DECKELBAUM, R. J., SHIPLEY, G. G., SMALL, D. M., LEES, R. S. and GEORGE, P. K. *Science* **190,** 392–394 (1975).
46. DE GENNES, P. G. *The Physics of Liquid Crystals*, Clarendon Press, Oxford 1–347 (1974).
47. DEMUS, D., HAHN, G-G. and KUSCHEL, F. *Mol. Cryst. Liq. Cryst.* **44,** 61–70 (1978).
48. DE VRIES, A. *Mol. Cryst. Liq. Cryst.* **20,** 119–131 (1973).
49. DE VRIES, A. *Mol. Cryst. Liq. Cryst.* **63,** 215–230 (1981).
50. DODDRELL, D. and ALLERHAND, A. *J. Am. Chem. Soc.* **93,** 1558–1559 (1971).
51. DUKE, J. R. and WOODS, A. C. *Br. J. Opthalmol.* **47,** 413–434 (1963).
52. DYBOWSKI, C. R. and WADE, C. G. *J. chem. Phys.* **55,** 1576–1578 (1971).
53. ELSER, W. *Mol. Cryst. Liq. Cryst.* **2,** 1–13 (1966).
54. ELSER, W., POHLMANN, J. L. and BOYD, P. R. *Mol. Cryst. Liq. Cryst.* **15,** 175–182 (1971).
55. ELSER, W., POHLMANN, J. L. and BOYD, P. R. *Mol. Cryst. Liq. Cryst.* **20,** 77–86 (1973).
56. ENNULAT, R. D. *Mol. Cryst. Liq. Cryst.* **8,** 247–265 (1969).
56a. FRIEDEL, E. *C. R. Acad. Sci., Paris* **180,** 269–271 (1925).
57. FRIEDEL, G. *Ann. Phys.* **18,** 273–474 (1922).
58. GENT, M. P. N. and PRESTEGARD, J. H. *J. Mag. Res.* **25,** 243–262 (1977).
59. GINSBURG, G. S. Ph.D. Dissertation, Boston University (1984).
60. GINSBURG, G. S. and SMALL, D. M. *Biochim. biophys. Acta* **664,** 98–107 (1981).
61. GINSBURG, G. S., SMALL, D. M. and HAMILTON, J. A. *Biochemistry* **21,** 6857–6867 (1982).
62. GODICI, P. E. and LANDSBERGER, F. R. *Biochemistry* **13,** 362–368 (1974).
62a. GRANDJEAN F. *C. R. Acad. Sci., Paris* **172,** 71 (1921).
63. GRAY, G. W. *J. Chem. Soc.* 3733–3739 (1956).
64. GRAY, G. W. In *Molecular Structure and Properties of Liquid Crystals*, p. 1–314, Academic Press, New York (1962).
65. GUERINA, N. G. and CRAVEN B. M. *J. Chem. Soc.* 1414–1419 (1979).
66. HAMILTON, J. A., OPPENHEIMER, N. and CORDES, E. H. *J. biol. Chem.* **252,** 8071–8080 (1977).
67. HERRMANN, K. *Z. Crist.* **92,** 49–81 (1935).
68. HILLMAN, G. M. and ENGLEMAN, D. M. *J. Clin. Invest.* **58,** 1008–1018 (1976).
69. HOLZBACH, R. T., MARSH, M. and TANS, P. In *Advances Bile Acid Research III*, Bile Acid Meeting, Freiburg i. Br., June 13–15, 1974, pp. 339–341 (1975).
70. JABARIN, S. A. and STEIN, R. S. *J. phys. Chem.* **77,** 399–408 (1973).
71. JANIAK, M. J., LOOMIS, C. R., SHIPLEY, G. G. and SMALL, D. M. *J. molec. Biol.* **86,** 325–339 (1974).
72. JOHNSON, J. F., PORTER, R. S. and BARRALL, E. M. *Mol. Cryst. Liq. Cryst.* **8,** 1–7 (1969).
73. KATZ, S. S., SHIPLEY, G. G. and SMALL, D. M. *J. Clin. Invest.* **58,** 200–211 (1976).
74. KATZ, S. S., SMALL, D. M., BROOK, J. G. and LEES, R. S. *J. Clin. Invest.* **59,** 1045–1054 (1977).

75. KNAPP, F. F. and NICHOLAS, H. J. *Mol. Cryst. Liq. Cryst.* **10,** 173–186 (1970).
76. KODEN, M., TAKENAKA, S. and KUSABAYASHI, S. *Mol. Cryst. Liq. Cryst.* **88,** 137–150 (1982).
77. LARSSON, K. *Fette Seifen. Anstrichen.* **74,** 136–142 (1972).
78. LEDER, L. B. *J. chem. Phys.* **58,** 1118–1125 (1973).
78a. LEHMANN O. *Flüssige Kristalle*, pp. 1–264. Liepzig, Verlag von Wilhelm Engelmann, 1904.
79. LEVINE, Y. K., BIRDSALL, M. J. M., LEE, A. G. and METCALFE, J. C. *Biochemistry* **11,** 1416–1421 (1972).
80. LEVINE, Y. K., BIRDSALL, N. J. M., LEE, A. G., METCALFE, J. C., PARTINGTON, P. and ROBERTS, G. C. K. *J. chem. Phys.* **60,** 2890–2899 (1974).
81. LEVINE, Y. K., PARTINGTON, P., ROBERTS, G. C. K., BIRDSALL, N. J. M., LEE, A. G. and METCALFE, J. C. *FEBS Lett.* **23,** 203–207 (1972).
82. LITSTER, J. D. and STINSON, III T. W. *J. Appl. Phys.* **41,** 996–997 (1970).
83. LOOMIS, C. R., JANIAK, M. J., SMALL, D. M. and SHIPLEY, G. G. *J. molec. Biol.* **86,** 309–324 (1974).
84. LOOMIS, C. R., SHIPLEY, G. G. and SMALL, D. M. *J. Lipid Res.* **20,** 525–535 (1979).
85. LUZ, Z., POUPKO, R. and SAMULSKI, G. T. *J. chem. Phys.* **74,** 5825–5837 (1981).
86. LYDON, J. E. and ROBINSON, D. G. *Biochim. biophys. Acta* **260,** 298–311 (1972).
87. MATTHEWS, R. M. C. and WADE, C. G. *J. Mag. Res.* **19,** 166–172 (1975).
88. MCMILLAN, W. L. *Phys. Rev.* **A4,** 1238–1246 (1971).
89. MCMILLAN, W. L. *Phys. Rev.* **A6,** 936–947 (1972).
90. MCMILLAN, W. L. and MYER, R. J. *Phys. Rev.* **A7,** 1419–1422 (1974).
91. MERRITT, W. G., COLE, G. D. and WALKER, W. W. *Mol. Cryst. Liq. Cryst.* **15,** 105–120 (1971).
92. NORTH, B. E., SHIPLEY, G. G. and SMALL, D. M. *Biochim. biophys. Acta* **424,** 376–385 (1976).
93. NORTH, B. E. and SMALL, D. M. *J. phys. Chem.* **81,** 723–727 (1977).
94. PATTABHI, V. and CRAVEN, B. M. *J. Lipid Res.* **20,** 753–759 (1978).
95. POHLMANN, J. L. W. *Mol. Cryst. Liq. Cryst.* **2,** 15–26 (1966).
96. POHLMANN, J. L. W. *Mol. Cryst. Liq. Cryst.* **8,** 417–425 (1969).
97. POHLMANN, J. L. W., ELSER, W. and BOYD, P. R. *Mol. Cryst. Liq. Cryst.* **13,** 243–254 (1971).
98. POHLMANN, J. L. W., ELSER, W. and BOYD, P. R. *Mol. Cryst. Liq. Cryst.* **20,** 87–97 (1973).
99. PRICE, F. P. and WENDORFF, J. M. *J. phys. Chem.* **75,** 2839–2849 (1971).
100. QUINN, D. M. *Biochemistry* **21,** 3548–3555 (1982).
101. SACKMANN, H. and DEMUS, D. *Mol. Cryst. Liq. Cryst.* **2,** 81–102 (1966).
102. SAKAMOTO, K., PORTER, R. S. and JOHNSON, J. J. In *Liquid Crystals* 2, Part II, pp. 237–249 (G. H. BROWN, ed.), Gordon and Breach Science Publishers, New York 1969.
103. SAWZIK, P. and CRAVEN, B. M. *Acta Cryst.* **B35,** 895–901 (1979).
104. SAWZIK, P. and CRAVEN, B. M. *Acta Cryst.* **B35,** 789–791 (1979).
105. SAWZIK, P. and CRAVEN, B. M. In *Liquid Crystals* pp. 171–178 (CHANDRASRKHAR, S., ed.), Heyden & Son, Philadelphia (1980).
106. SAWZIK, P. and CRAVEN, B. M. *Acta Cryst.* **B36,** 215–218 (1980).
106a. SAWZIK, P. and CRAVEN, B. M. *J. Appl. Cryst.* **14,** 351–352 (1981).
107. SEARS, B., DECKELBAUM, R. J., JANIAK, M. J., SHIPLEY, G. G. and SMALL, D. M. *Biochemistry* **15,** 4151–4157 (1976).
108. SHIVAPRAKASH, N. C., MARASIMHAMURTHY, B. and PRASAD, J. S. *Mol. Cryst. Liq. Cryst.* **76,** 133–144 (1981).
109. SKIPSKI, V. P. In *Blood Lipids and Lipoproteins*, pp. 471–584 (NELSON, G. J., ed.), Robert E. Krieger Publishing Company, Huntington, New York (1979).
110. SLOAN, H. R. and FREDRICKSON D. S. In *Metabolic Basis for Inherited Disease*, Chap. 36, pp. 808–832 (STANBURY, J. B., WYNGARDEN, J. B. and FREDRICKSON, D. S., eds), McGraw-Hill, New York (1972).
111. SMALL, D. M. In *Surface Chemistry of Biological Systems*, p. 55–83 (BLANK, M., ed.), Plenum Publ. Co., New York (1970).
112. SMALL, D. M. *J. Colloid Interface Sci.* **58,** 581–602 (1977).
113. SMALL, D. M. *N.E.J.M.* (*Seminars in Physiology*) **297,** 873–877 and 924–929 (1977).
114. SMALL, D. M. *Pure Appl. Chem.* **53,** 2095–2103 (1981).
115. SMALL, D. M. *The Physical Chemistry of Lipids from Alkanes to Phospholipids, Handbook of Lipid Research*, Vol. 3 (HANAHAN, D., ed.) Plenum Publ. Co., New York, in press (1985).
116. SMALL, D. M., BOND, M. G., WAUGH, D., PRACK, M. and SAWYER, J. K. *J. Clin. Invest.* **73,** 1580–1605 (1984).
117. SMALL, D. M., LOOMIS, C. R., JANIAK, M. J. and SHIPLEY, G. G. In *Liquid Crystals and Ordered Fluids* Vol. 2, pp. 11–22 (JOHNSON, J. F. and PORTER, R. S., eds) Plenum Press, New York (1974).
118. SMALL, D. M. and SHIPLEY, G. G. *Science* **185,** 222–229 (1974).
119. TALL, A. R., SMALL, D. M., ATKINSON, D. and RUDEL, L. L. *J. Clin. Invest.* **62,** 1354–1363 (1978).
120. TARDIEU, A., LUZZATI, V. and REMAN, F. C. *J. Molec. Biol.* **75,** 711–733 (1973).
121. USOL'TSEVA, V. A. and CHISTYAKOV, I. G. *Russ. Chem. Rev.* **32,** 495–509 (1963).
122. WENDORF, J. H. and PRICE, F. P. *The Structure of the Mesophases of Cholesteryl Esters* (unpublished) (1972).
123. WENDORF, J. H. and PRICE, F. P. *Mol. Cryst. Liq. Cryst.* **24,** 129–144 (1973).
124. WIEGAND, C. *Z. Naturforsch.* **46,** 249–251 (1949).
125. WOESSNER, D. E. *J. chem. Phys.* **37,** 647–654 (1962).
126. YAMADA, T. and FUKADA, E. *Jap. J. appl. Phys.* **12,** 68–72 (1973).
127. YANG, C. C. *Phys. Rev. Lett.* **28,** 955–958 (1972).