Differential scanning calorimetry and fluorescence probe investigations of very low density lipoprotein from the isolated perfused rat liver

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Abstract Isolated rat livers were perfused at 37°C with a blood-free, defined medium containing delipidized bovine serum albumin (BSA), BSA-oleate, or BSA-palmitate. Very low density lipoproteins (VLDL) were isolated from the perfusate at 12°C and lipid components were extracted and purified. Differential scanning calorimetry indicated multiple phase alterations in intact VLDL. The extracted triglycerides exhibited phase alterations at similar temperatures as the intact VLDL. The small quantities of cholesteryl esters present in the VLDL generally did not greatly affect the VLDL triglyceride transitions. The extracted phospholipids showed detectable transitions that were abolished by cholesterol at mole ratios found in the respective VLDL. The phase behavior of VLDL and its component triglycerides was associated with the degree of unsaturation of the infusate fatty acid, and by variation of fatty acid chain length. The structural differences between VLDL lipid fractions noted by DSC were also monitored by fluorescence probes. The data indicated that in the intact VLDL the physical properties of the 'interior core' lipids can affect the properties of the 'surface monolayer'. In addition, Arrhenius plots of corrected fluorescence indicated that *trans*-parinarate and diphenylhexatriene detected different characteristic breakpoint temperatures in the phospholipids. The breakpoints of triglycerides were highly dependent on the type of fatty acid in the infusate, but were similar to those noted in intact VLDL. Finally, the breakpoints in Arrhenius plots of fluorescence probe parameters did not necessarily coincide with onset or end temperatures of DSC transitions.—**Hale, J. E., and F. Schroeder.** Differential scanning calorimetry and fluorescence probe investigations of very low density lipoprotein from the isolated perfused rat liver. *J. Lipid Res.* 1981. **22:** 838–851.

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Very low density lipoproteins (VLDL) are the primary triglyceride-carrying particles secreted by the liver. These particles are subsequently broken down enzymically to form low density lipoproteins. The activities of the degradative enzymes lipoprotein lipase, lecithin:cholesterol acyltransferase, and phospholipase A are dependent on the physical state of the substrate lipid (1, 2). However, as pointed out in several recent review articles, very little is known of the structure and physical state of the lipids or their regulation in VLDL (3, 4). Using fluorescence and differential scanning calorimetry (DSC), other investigators have not detected phase alterations in intact VLDL isolated from plasma (5–9). In contrast, triglyceride transitions have been determined in human VLDL by DSC (10) while phase changes in human VLDL have been described with pyrene and tempo probes (11). VLDL isolated from plasma represents a heterogeneous family of particles with regard to size, density, composition, and degree of metabolism (12). In contrast, using an isolated perfused liver system, we have purified VLDL secreted by rat livers infused with oleate or palmitate (12–14). Fluorescence probe analysis of these VLDL indicated that the triglyceride-cholesteryl ester core was surrounded by a monolayer of phospholipid, cholesterol, and protein (13–15) consistent with earlier predictions made from compositional analysis (16, 17). In addition, breakpoints indicative of phase alterations were noted in Arrhenius plots of fluorescence parameters. The temperatures at which these breakpoints occurred were dependent upon the type of fatty acid (oleate or palmitate) with which the rat liver was perfused.

Fluorescence probe molecules and differential scanning calorimetry (DSC) have both been successfully used to determine phase alterations in model membrane systems with good correlation between the two methods. However, fluorescence methodology intrinsically differs from DSC in that the probe molecules report only on the limiting microenvironment in which they reside, while DSC measures bulk lipid phase alterations. Recently the two methods have

Abbreviations: DSC, differential scanning calorimetry; VLDL, very low density lipoprotein; LDL, low density lipoprotein; BSA, bovine serum albumin.

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been utilized to investigate the structure of VLDL and LDL (5–9, 13–15). Comparisons between the two methods have been few and are hampered by the heterogeneity of VLDL isolated from human or animal plasma. A recent report (9) indicated that phase alterations in LDL determined by DSC were not detected with the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (6, 8, 9, 12). Therefore, the experiments presented herein were performed to determine: *1*) if the VLDL from the perfused rat liver has phase alterations by DSC and fluorescence; *2*) if the onset and end temperatures of the phase alterations noted by DSC correspond to breakpoints in Arrhenius plots determined fluorimetrically; and *3*) if these phase alterations are dependent upon the type of fatty acid present in the infusate of the perfused rat liver. These results should help in correlating DSC bulk and fluorescence probe microenvironment phase alterations such that the structure of the VLDL may more thoroughly be understood.

MATERIALS AND METHODS

Oleic and palmitic acid were obtained from the Sigma Chemical Co., St. Louis, MO and from the Hormel Institute, respectively. Bovine serum albumin (BSA) was purchased from Miles Labs., Elkhart, IN and was delipidized according to the method of Goodman (18), and modified by Wilcox, Dunn and Heimberg (12). BSA-fatty acid complexes were prepared as described previously (12). *Trans*-parinaric acid was from Molecular Probes Inc., Plano, TX while 1,6-diphenylhexatriene was from Eastman Chemical Co., Rochester, NY.

Liver perfusion and VLDL isolation

Livers were removed from male Sprague-Dawley rats (200–250 g body weight) and perfused in vitro at 37°C with a blood-free Krebs buffer solution. VLDL was enriched with oleic or palmitic acid by infusion of the fatty acid bound to bovine serum albumin (BSA) (12–15). The VLDL was isolated from the buffer medium by ultracentrifugation at 39,000 RPM in a Ti-60 rotor (Beckman Inst., Palo Alto, CA), for 18 hr at 12°C as described earlier (13).

Lipid extraction, purification, and analysis

Lipids were extracted from the VLDL by the method of Bligh and Dyer (19). The phospholipid and neutral lipid fractions were separated by silicic acid chromatography. Triglyceride, cholesterol, and cholesteryl esters were separated by thin-layer chromatography on silica gel G plates (Analtech, Newark, DE) in petroleum ether–ethyl ether–acetic acid 84:15:1. Triglycerides were quantitated enzymatically as previously described (20, 21). Cholesterol and cholesteryl esters were determined by gas–liquid chromatography as described earlier (22). Phospholipid content was quantitated by the method of Ames (23).

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed with a Perkin-Elmer DSC-2 (Perkin-Elmer Corp., Norwalk, CT). Intact VLDL was suspended in ethylene glycol–water 1:1 in order to avoid the water fusion peak (25, 26), and then concentrated by ultracentrifugation at 180,000 *g* for 1 hr on a Beckman Airfuge (Beckman Instruments Co., Fullerton, CA). Ethylene glycol–water 1:1 did not affect the phase transitions described herein. The phospholipid and neutral lipid fractions (0.5 to 2.0 mg) were solubilized in 50 μl of chloroform–methanol 1:2 or chloroform, respectively, transferred to 10-μl aluminum sample pans, and warmed to about 50°C to promote solvent evaporation. The pans containing the samples were then lyophilized for 12 hr to remove any residual solvent. A 10-μl aliquot of distilled water was then added to the phospholipid sample and reference pans and the pans were sealed (24). No solvent was added to triglyceride or cholesteryl ester samples or reference pans. All lipids and lipid mixtures were weighed into the DSC pans. Weights ranged from 0.5 to 2.0 mg. Intact VLDL was quantitated in the DSC pans by an enzymatic triglyceride assay (19, 20). Samples were placed in the DSC pans at room temperature, 24°C, sealed, and cooled to −50°C at a rate of 1.25°C/min. The samples were equilibrated about 5 min at the lower temperature limit before a scan was initiated. The samples were then reheated to 65°C at a rate of 1.25°C/min. This cycle was repeated four times. The recorded traces in the figures were taken during the third cycle on heating and the fourth cycle on cooling. Sensitivities were either 0.2 or 0.5 mcal/sec as stated in the figure legends. This procedure assured that the thermal history of the samples was the same and that none of the peaks was irreversible, as in protein denaturation. The baseline was determined by extrapolating the terminal baseline back to the origin. Overlapping individual peaks were resolved by extrapolating the peaks back to the baseline such that the peaks were symmetrical (27–30). This method has been shown to give good estimates of onset and final temperatures. Although enthalpies accurate to within 10% have been calculated by this method in model systems of phospholipids (10), in the present work only total enthalpies are given. The areas under the

TABLE 1. Very low density lipoprotein lipid composition[a]

Lipid Class	Infusate		
	BSA	BSA-oleate	BSA-palmitate
Triglyceride (TG)	100	100	100
Cholesterol (C)	11.6 ± 1.5	16.2 ± 2.5	12.5 ± 0.9
Cholesteryl ester (CE)	2.5 ± 0.3	4.4 ± 0.8	2.7 ± 0.3
Phospholipid (PL)	24.9 ± 4.3	16.3 ± 3.1	30.3 ± 3.0
(C + PL)/(TG + CE)	0.36	0.31	0.42
C/PL	0.47	0.99	0.41

[a] Triglyceride was set to 100 and all other lipid species were expressed in relation to the triglyceride value on a molar ratio basis. Values represent the mean ± S.E.M. (n = 4 to 9).

peaks of the phase transition were found by weighing the paper; the weights were compared to the weight of a standard area of known enthalpy (Indium).

Fluorescence probe incorporation and instrumentation

Trans-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene were incorporated into lipid fractions at 1:100 mole ratios unless otherwise specified as described earlier (22, 31). The computer-centered spectrofluorimeter first developed by Holland and coworkers (32–34) was used to determine all fluorescence parameters as described earlier (13–15, 22, 31). This instrument corrects fluorescence for both instrumental artifacts of the excitation and emission system as well as for the inner filter effect up to absorbances of 2.0 (33, 34). Light scattering was corrected by cut-off filters. Absorbance, absorption-corrected fluorescence, and relative quantum efficiency are determined simultaneously as a function of wavelength or temperature. Fluorescence polarization was measured as $(I_{11} - I_1)/(I_{11} + I_1)$ corrected for grating anomalies as described elsewhere (13–15). *Trans*-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene were excited at 313 and 362 nm, respectively; fluorescence emission was measured at 415 and 424 nm, respectively. Sample temperature was varied with an Exocal 100, Endocal 850, and ETP-3 Temperature Programmer system (Neslab Inst., Portsmouth, NH). Sample temperature was monitored with a thermocouple directly in the cuvette and a Kernco WR-700 Digital Thermometer (Kernco Inst., El Paso, TX). The thermal histories of these samples were the same as for those used to obtain the DSC results except that each sample was cooled only to 4°C, equilibrated for 5 min, and then subjected to the four heating and cooling regimens as described in the DSC procedures. All determinations represent the average of 40 values taken in several milliseconds for each sample at each temperature. All values presented represent the mean of three samples. All samples were studied in aqueous phosphate-buffered saline (50 μg lipid/ml). All fluorescence Arrhenius plots are heating runs, although cooling scans were performed and similar data were obtained.

RESULTS

Effect of palmitate or oleate infusion on lipid composition of VLDL isolated from the perfused rat liver

Table 1 shows the lipid composition of VLDL from livers perfused with BSA, BSA-palmitate, or BSA-oleate. Triglycerides were arbitrarily assigned a value of 100, and the other lipid concentrations were expressed on a molar basis as a ratio to the triglyceride. The major alteration noted was in phospholipid content which was two times greater in VLDL from palmitate-enriched VLDL as compared to oleate-enriched VLDL. Control values were intermediate. This observation was also reflected in the 'surface' to 'interior core' lipid (C + PL/TG + CE) and cholesterol to phospholipid (C/PL) ratios. This may indicate secretion of different size VLDL (13–15). The major fatty acid composition of the primary lipid, the triglycerides, of the three types of VLDL is shown in **Table 2.** The VLDL triglycerides, were enriched with the respective fatty acid present in the infusate. In addition, the unsaturated/saturated fatty acid ratio was 3.29 times higher in the triglycerides of VLDL isolated from livers perfused with BSA-oleate than with BSA-palmitate. These results suggested that the various VLDL particles might have very different phase transitions which could be detected by differential scanning calorimetry (DSC).

TABLE 2. Major fatty acid composition of triglycerides from VLDL enriched with oleate or palmitate[a]

Fatty Acid	Infusate		
	BSA	BSA-oleate	BSA-palmitate
	% composition		
14:0	27.52 ± 3.22	13.63 ± 0.53	1.37 ± .12
16:0	3.05 ± .72	2.38 ± .06	39.41 ± 2.72
16:1	3.44 ± .73	1.52 ± .25	5.82 ± .71
18:0	12.74 ± 2.61	8.22 ± 2.18	10.00 ± 1.28
18:1	32.79 ± 2.92	57.50 ± 2.29	23.45 ± 1.36
Unsaturated/ saturated	0.95	2.14	0.65

[a] Major fatty acid composition (weight percent) was determined as described in Methods. Values represent the mean ± S.E.M. (n = 4 to 9). Unsaturated/saturated values calculated using all fatty acids.

Phase behavior of intact VLDL

Temperature scans of the intact VLDL are presented in **Fig. 1.** Upon heating, the oleic acid-enriched VLDL exhibited three endothermic, thermotropic peaks centered at −19, −12, and −1°C. Since the extrapolated peaks are only estimates, values determined from these extrapolations are denoted by a (†) in Tables 4 and 5. The peaks were 15, 12, and 8°C wide respectively. Between the second and third endotherms was a small exotherm centered at about −6°C. These peaks were reproducible on successive heating scans although no peaks were obtained on cooling scans at the same scan rates. The total enthalpy (sum of the three peaks) was 74.1 cal/g of VLDL triglyceride. The thermotropic behavior of palmitic acid-enriched VLDL was quite different from that of the oleic acid-enriched VLDL. The broad endothermic peaks were centered at 1, 19, 27, 38, and 48°C. A small exotherm at 30°C, present in this VLDL suspended in ethylene glycol–water was more evident when the VLDL was suspended in water (data not shown). A sharp exothermic peak was also present at 5°C in cooling scans (10°/min) following which the baseline veered offscale exothermically. All the peaks were reproducible on successive scans. The total enthalpy of these heating transitions was considerably smaller than that noted in the oleate enriched VLDL transitions. The cooling peak enthalpy was 11.0 cal/g. The control VLDL exhibited two broad endotherms with peaks at −12 and 1°C upon heating. No peaks were observed in cooling scans of the control VLDL, but the baseline gradually veered in the exothermic direction, as it did in cooling scans of oleic acid and palmitic acid enriched VLDL. The heating transitions were reproducible on successive scans of the same sample. The total enthalpy of these peaks was 8.3 cal/g VLDL triglyceride. Thus the control VLDL had transitions with the lowest enthalpy of all three groups. Finally, one transition centered near −1 to 1°C appeared in all three types of VLDL. Both transitions found in the control VLDL also appeared in the oleate enriched VLDL. However, the major peaks in the palmitate-enriched VLDL were separated by 50–60°C.

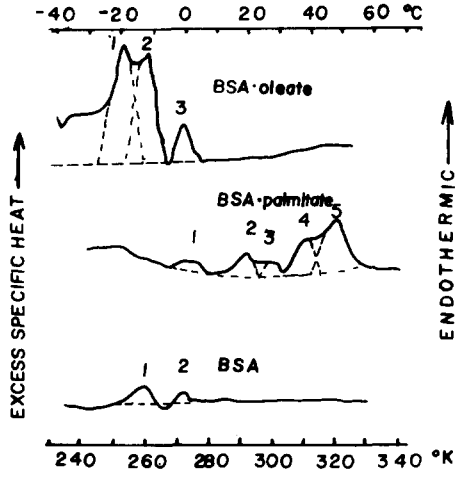

Fig. 1. Differential scanning calorimetry of intact very low density lipoproteins. VLDL was isolated from the perfusate of rat livers infused with BSA-oleate, BSA-palmitate, or BSA and resuspended in ethylene glycol–water 1:1 as described in Methods. Heating and cooling curves were obtained as described in Methods except that scanning rates of 10°C/min were used. (a) Heating curve of VLDL (0.18 mg of triglyceride) from livers perfused with BSA-oleate; sensitivity 0.5 mcal/sec, 230–330°K. (b) Heating curve of VLDL (0.21 mg of triglyceride) from livers perfused with BSA-palmitate; sensitivity 0.2 mcal/sec, 230°–340°K. (c) Heating curves of VLDL (0.26 mg of triglyceride) perfused with BSA; sensitivity 0.5 mcal/sec, 230°–330°K. The extrapolated individual peaks and baseline are shown in dashed lines.

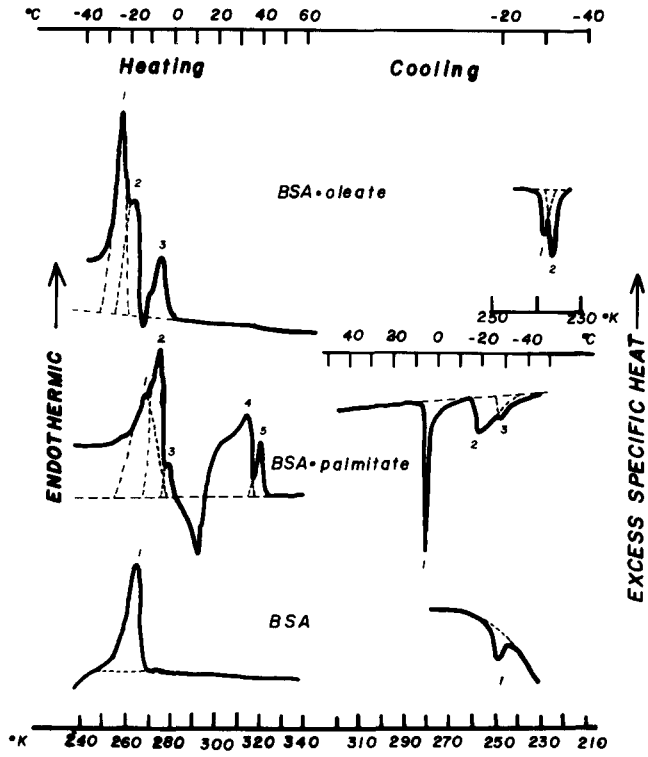

Fig. 2. DSC of triglycerides extracted from VLDL of livers perfused with different fatty acids. All methods were as described in the legend of Fig. 1, except that extracted purified triglycerides were used without solvent and triglycerides from livers perfused with BSA-palmitate (1.59 mg) were determined at a sensitivity of 0.5 mcal/sec, at 10°C/min. Cooling scans for each perfusate are shown. The scan rate for BSA-oleate triglyceride (1.41 mg) was 1.25°C/min at 0.5 mcal/sec. BSA triglyceride (0.92 mg) was scanned at 10°C/min at 0.2 mcal/sec. The extrapolated individual peaks and baseline are shown in dashed lines.

Phase behavior of isolated lipid fractions

Triglycerides represent the single largest class of lipids in the VLDL (Table 1). As shown in **Fig. 2,**

the heating scans of triglycerides extracted from VLDL isolated from the perfusate generally resembled the corresponding scans of intact VLDL scans, with regard to the temperature at which the transitions occurred. In addition cooling peaks were obtained. The isolated triglyceride peaks had different individual transition enthalpies and were more sharply defined than those in the intact VLDL. In some cases individual peaks overlapped, causing some peaks to appear as shoulders on other peaks.

Triglycerides from oleic acid-enriched VLDL displayed three endothermic peaks centered at −20.7 ± 0.7, 14.4 ± 0.6, and 0.8 ± 0.3°C when scanned at 10°C/min. An exotherm appeared at −9°C between the two higher temperature and the first lower temperature peaks. The peaks were reproducible and reversible, since two exothermic peaks centered at −31.3 ± 1.3 and −35.5 ± 0.8°C were obtained when the sample was cooled at 1.25°C/min. The thermotropic behavior of palmitic acid-enriched triglyceride also resembled that of the intact palmitate-enriched VLDL scans (Fig. 2). Upon heating at 10°C/min, two groups of endothermic peaks appeared separated by an exotherm of similar magnitude to the endotherms. The first group of peaks consisted of three individual peaks. The first centered at −7.6 ± 0.5°C was a shoulder on the second peak at −1.6 ± 0.7°C. The third was a small shoulder on the end of the second peak centered around 4.2 ± 0.6°C. The large exotherm was centered at about 20°C. The second group of peaks was comprised of a broad shoulder leading into the fourth peak at 37.3 ± 1.8°C. The fifth peak was located at 46.7 ± 0.7°C. Upon cooling at 10°C/min, a large sharp peak was observed centered at 20.9 ± 0.8°C. Following this was a shorter, broader peak (possibly two peaks) centered at −3.5 ± 1.2°C with the possible second peak centered at −11.0 ± 0.4°C. All the peaks were reversible and reproducible on successive heating or cooling scans. The control triglyceride (Fig. 2) differed from the control intact VLDL in that only one peak was noted upon heating at 10°C/min. This peak was centered at −13.8 ± 1.7°C and was reversible and reproducible, with a cooling peak centered at 1.3°C when scanned at 10°C/min.

The total enthalpies for each group of peaks in both heating and cooling scans, noted in Fig. 2 are shown in **Table 3.** The enthalpy is reported as cal/g total triglyceride present. The total enthalpies of triglycerides extracted from the VLDL of livers infused with BSA-oleate, BSA-palmitate, or BSA were 16.38, 19.97, and 8.95 cal/g on heating and 1.13, 7.6, and 1.3 cal/g on cooling, respectively. Thus, the enthalpies of VLDL triglyceride taken from heating scans of VLDL obtained from livers infused with fatty acids were about twice as high as control values. The corresponding total enthalpies in the intact VLDL heating scans were 74.1, 24.7, and 8.3 cal/g intact VLDL triglyceride, respectively. Only the palmitate-enriched VLDL and control VLDL had total enthalpies similar to those of the extracted triglycerides. The intact oleate-enriched VLDL had 4–5-fold higher enthal-

TABLE 3. Total enthalpies of triglycerides and triglyceride-cholesteryl ester transitions[a]

	Total Enthalpy (cal/g)			
	Heating Scans		Cooling Scans	
Infusate	Group 1 Transitions[b,e]	Group 2 Transitions	Group 1 Transitions	Group 2 Transitions
	Triglycerides			
BSA-oleate	13.08 ± 0.76	3.30 ± 0.23	1.13 ± .20	
BSA-palmitate	11.25 ± 1.31	8.22 ± 0.77	4.4 ± 0.50	3.20 ± 0.64
BSA	8.95 ± 0.38		1.3[d]	
	Triglycerides + Cholesteryl Esters[c]			
BSA-oleate	13.00 ± 1.33	2.40 ± 0.30	1.08 ± 0.27	
BSA-palmitate	12.27 ± 0.62	8.4 ± 0.61	4.4 ± 0.60	3.99 ± 0.39
BSA	6.28 ± 0.65			

[a] VLDL was isolated from perfused rat liver; triglyceride and cholesteryl ester fractions were obtained as described in Methods. Total enthalpies are expressed as cal/g total triglyceride present. Values represent the mean ± S.E.M. (n = 4 to 9).

[b] Group 1 and Group 2 transitions refer to peaks below and above the exotherm.

[c] Cholesteryl esters were mixed with triglycerides in the ratios found in the intact VLDL; for VLDL from BSA-oleate infused livers this ratio was 0.034, while for VLDL from livers infused with BSA-palmitate or BSA the ratio was 0.023 and 0.018, respectively.

[d] n = 1.

[e] Group 1 and Group 2 transitions refer to non-overlapping peaks.

pies than the corresponding extracted triglycerides. Individual transition enthalpies were not reported for the following reasons: *1*) the individual peak enthalpies were considerably variable; *2*) detailed decomposition of the multiple overlapping peaks was difficult; and *3*) it is not known if the lipid composition of each peak is the same as the calculated area composition.

The individual peaks were also extrapolated to the baseline by previously reported methods (27–30). The onset, peak, and final temperature as well as transition width are summarized in **Table 4.** BSA-palmitate infusion resulted in a shift of onset, maximal, and final temperatures of the heating transitions to higher temperatures, many of which were in the physiological temperature range near 37°C (peaks 4 and 5). Neither the control (BSA) nor the BSA-oleate triglycerides had onset, maximal, or final temperatures in the physiological range; although some temperatures were above 0°C (1 to 8°C). The width of the transition (ΔT) generally decreased at higher temperatures.

Effect of cholesteryl esters on triglyceride transitions

Cholesteryl esters are readily soluble in triglyceride and they are believed to partition into the triglyceride core of lipoproteins (8, 24). Triglycerides present in low percentages abolished the cholesteryl ester phase transition noted by DSC (8, 24). Conversely, it seems possible that small amounts of cholesteryl esters may also abolish triglyceride transitions. Therefore, mixtures of triglycerides and cholesteryl esters at mole ratios found in the lipid composition of VLDL were prepared and scanned by DSC. The results are shown in **Fig. 3** and the thermal data are summarized in Table 3. As noted in Fig. 3, the cholesteryl esters did not abolish any of the triglyceride transitions nor were the onset, maximal, or final temperatures shifted significantly (data not shown). In addition, no new cholesteryl ester transitions were noted, as expected from the high amount of triglyceride present (8, 24). The scans of the oleic acid-enriched mixture were very similar in appearance to the scans of the oleic acid-enriched triglycerides. The peak temperatures were shifted only slightly, typically less than 1°C for both heating and cooling scans. The total enthalpies of heating peaks and cooling peaks were not changed significantly by addition of cholesteryl esters. Scans of palmitic acid-enriched triglycerides mixed with cholesteryl esters were also similar to the pure triglycerides and peak temperatures differed by no more than 3°C for heating and cooling scans. The control mixture exhibited the only striking effect of cholesteryl esters on the triglyceride transitions. Only one peak was noted in the triglyceride scan while in the mixture a possible second peak was noted at −37°C, as well as a more clearly defined shoulder preceeding the main peak between −30 to −20°C. The main peak was shifted about 0.7°C and was sharper. The enthalpy was decreased 1.9 cal/g. No cooling peak was obtained. However, Table 3 indicated that the total enthalpy (sum of all peaks) of the triglycerides was not altered significantly by addi-

TABLE 4. Effect of infusate fatty acid on thermal properties of extracted triglyceride transitions

Infusate	Peak[a]	Onset Temperature	Peak Temperature	Final Temperature	ΔT
			°C		
BSA-oleate	Heating 1	−31.2 ± 1.2†	−20.7 ± 0.7	−13.2 ± 0.5†	18.0 ± 1.1†
	Heating 2	−23.9 ± 1.0†	−14.4 ± 0.6	−7.5 ± 1.2	16.4 ± 1.3†
	Heating 3	−6.5 ± 0.05	0.8 ± 0.3	4.4 ± 0.5	10.9 ± 0.9
	Cooling 1	−29.0 ± 0.7	−31.3 ± 1.3	−34.4 ± 1.5†	5.4 ± 1.1†
	Cooling 2	−32.3 ± 0.8†	−35.5 ± 0.8	−38.8 ± 0.8	6.8 ± 0.3†
BSA-palmitate	Heating 1	−18.8 ± 0.9†	−7.6 ± 0.5	−1.6 ± 0.3†	17.2 ± 1.0†
	Heating 2	−12.5 ± 1.3†	−1.6 ± 0.7	5.1 ± 0.7†	10.4 ± 1.1†
	Heating 3	−1.6 ± 1.0†	4.2 ± 0.6	8.8 ± 0.6	17.0 ± 2.5†
	Heating 4	25.3 ± 0.9	37.3 ± 1.8	42.3 ± 1.6†	17.0 ± 2.5†
	Heating 5	38.3 ± 0.8†	46.7 ± 1.7	50.7 ± 0.6	12.4 ± 1.1†
	Cooling 1	23.0 ± 1.1	20.9 ± 0.8	7.4 ± 1.0	15.6 ± 1.6
	Cooling 2	−0.6 ± 1.3	−3.5 ± 1.2	−19.8 ± 1.6†	17.5 ± 2.0†
	Cooling 3	−9.6 ± 0.2†	−11.0 ± 0.4	−22.4 ± 2.1	13.3 ± 2.7†
BSA[b]	Heating 1	−27.6 ± 1.6	−13.8 ± 1.7	−5.5 ± 1.8	22.1 ± 0.5
	Cooling 1	−19.0	−23.0	−30.0	11.0

[a] Peaks were numbered from right to left. Values represent the mean ± SEM (n = 4). A dagger (†) represents values obtained from extrapolation as described in Materials and Methods.

[b] n = 1.

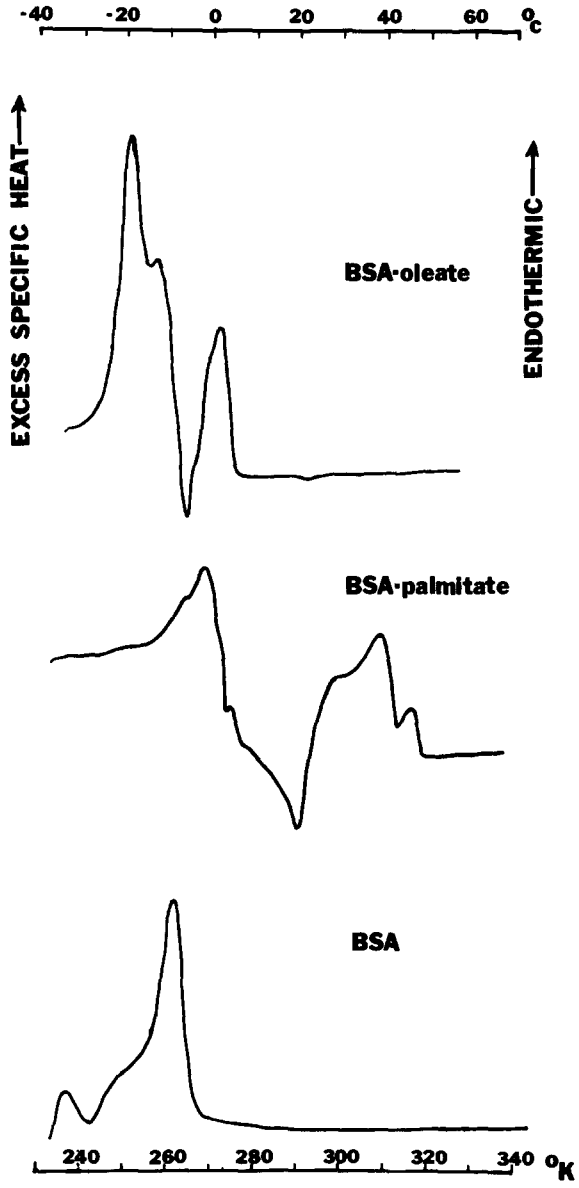

Fig. 3. Effect of cholesteryl esters on triglyceride phase behavior. All methods and sample sizes were the same as described in the legend of Fig. 2, except that cholesteryl esters isolated from the VLDL were added to the respective triglycerides in the same molar ratios as found in the intact VLDL (see text for details).

tion of the cholesteryl ester. Thus, no systematic pattern of cholesteryl ester effects was apparent.

Effect of fatty acid infusion on thermal properties of VLDL phospholipids

It was previously shown that the VLDL triglyceride-cholesteryl ester core appears to be surrounded by a phospholipid-cholesterol monolayer (12–14). Thus, the VLDL surface lipid may have different thermal properties than the 'interior core' lipids. DSC heating scans for total phospholipid are illustrated in **Fig. 4.** The thermal data are listed in **Table 5.** The phospholipids from VLDL of livers perfused with BSA-oleate exhibited a reversible transition centered at 45.6 ± 0.6°C when scanned at 10°C/min and a small cooling peak at 30.4°C at the same scanning rate. The phospholipid of VLDL from livers perfused with BSA-palmitate exhibited three peaks centered at 35.3°, 43.0°, and 50.5°C, and a cooling peak at 14.8 ± 4.0°C. The total enthalpies of the transitions were low (less than 2.18 cal/g of phospholipid) when compared to triglyceride transitions. Phospholipid from VLDL of livers perfused with BSA exhibited no phase alterations when scanned at 10°C/min from 2 to 63°C on either heating or cooling scans. The widths of the phospholipid transitions were generally narrower than those of the triglycerides and decreased for peaks occurring at higher temperatures.

Thermal properties of phospholipid-cholesterol mixtures

Cholesterol and phospholipids are both 'surface' lipids of the VLDL. Therefore, the effect of cholesterol on phospholipid phase behavior was determined. Cholesterol was added to the phospholipid of VLDL from livers perfused with BSA-oleate, BSA-palmitate, or BSA in the same mole ratio as found in the respective VLDL (0.99, 0.41, and 0.47, respectively, see Table 1). None of the cholesterol-phospholipid mixtures exhibited transitions when heated or cooled at 10°C/min (data not shown). Thus, cholesterol ef-

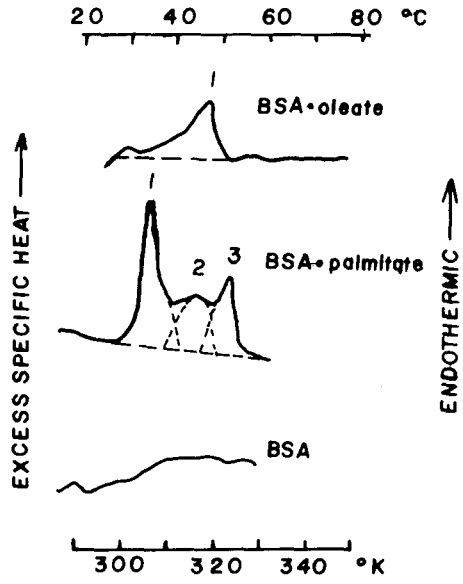

Fig. 4. Phase alterations in purified phospholipids from VLDL of perfused liver. Extracted phospholipids were resuspended in 10 μl of distilled H_2O and scanned from 270 to 350°C at 10°K/min, sensitivity 0.2 mcal/sec: BSA-oleate, 0.56 mg; BSA-palmitate, 0.49 mg; BSA, 0.61 mg of phospholipid, respectively.

TABLE 5. Thermal properties of phospholipids of VLDL isolated from rat livers perfused with BSA-oleate or BSA-palmitate[a]

Infusate	Peak	Onset Temperature	Peak Temperature	Final Temperature	ΔT	Total ΔH
			(°C)			(cal/g)
BSA-oleate	Heating 1	38.6 ± 1.3	45.6 ± 0.6	48.8 ± 0.6	10.2 ± 1.3	0.45 ± 0.19
	Cooling 1	33.9 ± 1.9	30.4 ± 1.9	26.3 ± 1.9	7.6 ± 0.2	0.30 ± 0.10
BSA-palmitate	Heating 1	29.4 ± 1.4	35.3 ± 0.8	40.9 ± 1.1†	11.5 ± 1.7†	2.18 ± 0.8
	Heating 2	37.0 ± 1.3†	43.0 ± 1.8	46.2 ± 1.9†	9.2 ± 0.8†	
	Heating 3	46.0 ± 3.0†	50.5 ± 1.0	53.0 ± 0.5	7.0 ± 2.0†	
	Cooling 1	18.6 ± 3.9	14.8 ± 4.0	10.3 ± 4.8	8.4 ± 3.5	0.70 ± 0.70
BSA		n.d.	n.d.	n.d.	n.d.	n.d.

[a] Enthalpy expressed as cal/g total phospholipid present. Values represent the mean ± S.E.M. (n = 3 to 9). n.d. means not detectable. A dagger (†) represents values obtained from extrapolation as described in Materials and Methods.

fectively eliminated the phospholipid transitions noted in the previous section. Similar effects of cholesterol on phospholipid transitions have been noted elsewhere (35). However, the effect of cholesterol on abolishing phase transitions of mixtures of phosphatidylcholine species was highly dependent on the fatty acid chain length (35).

Thermal properties of the cholesteryl esters

The cholesteryl ester fraction of each VLDL type constituted only a very small fraction of the lipid, usually less than 3% on a mole basis of the total lipids. The cholesteryl esters did not show a separate transition in the intact VLDL or triglyceride-cholesteryl ester mixtures. We were also unable to detect a distinct phase transition in the isolated cholesteryl ester fractions from VLDL obtained from livers perfused with BSA-oleate, BSA-palmitate, or BSA.

Effect of palmitate or oleate infusion on fluorescence parameters of probe molecules in lipid fractions extracted from VLDL

The structure of the VLDL appears to be that of a spherical particle with an 'interior core' composed of triglyceride and small amounts of cholesteryl esters (13–15, 36, 37). The core is surrounded by a monolayer of phospholipid, cholesterol, and protein. The effect of interior core lipids on the surface monolayer and vice-versa is not known. **Table 6** illustrates the fluorescence polarization of *trans*-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene in intact VLDL and in isolated lipid fractions. The location of *trans*-

TABLE 6. Fluorescence polarization and corrected fluorescence of *trans*-parinaric acid and 1,6-diphenyl-1,3,5-hexatriene in lipid fractions isolated from VLDL secreted by the perfused rat liver[a]

Fatty Acid Infused	Lipid Fraction	Fluorescence Probe	Polarization	Corrected Fluorescence
Palmitic (16:0)	Intact VLDL	*trans*-parinarate	0.387 ± 0.010	205 ± 20
	Phospholipids	*trans*-parinarate	0.310 ± 0.010	73 ± 16
	Phospholipids + cholesterol[b]	*trans*-parinarate	1.332 ± 0.007	146 ± 12
	Intact VLDL	1,6-diphenyl-1,3,5-hexatriene	0.352 ± 0.012	237 ± 16
	Phospholipids	1,6-diphenyl-1,3,5-hexatriene	0.299 ± 0.020	72 ± 13
	Phospholipids + cholesterol[b]	1,6-diphenyl-1,3,5-hexatriene	0.327 ± 0.014	197 ± 18
	Triglycerides	1,6-diphenyl-1,3,5-hexatriene	0.377 ± 0.012	15 ± 2
Oleic (18:1)	Intact VLDL	*trans*-parinarate	0.215 ± 0.008	95 ± 10
	Phospholipids	*trans*-parinarate	0.283 ± 0.011	40 ± 5
	Phospholipids + cholesterol[b]	*trans*-parinarate	0.309 ± 0.004	193 ± 27
	Intact VLDL	1,6-diphenyl-1,3,5-hexatriene	0.230 ± 0.012	106 ± 4
	Phospholipid	1,6-diphenyl-1,3,5-hexatriene	0.300 ± 0.015	108 ± 10
	Phospholipids + cholesterol[b]	1,6-diphenyl-1,3,5-hexatriene	0.371 ± 0.006	174 ± 9
	Triglycerides	1,6-diphenyl-1,3,5-hexatriene	0.155 ± 0.014	16 ± 3

[a] Fluorescence probe to lipid ratios were 0.2 μg *trans*-parinarate acid/50 μg lipid and 0.1 μg diphenyl hexatriene/50 μg lipid. However, for triglycerides 1.1 μg diphenylhexatriene/50 μg lipid was used. All corrected fluorescence and polarization values were determined at 24°C as described in Methods. Each value represents the mean ± S.E.M. of three samples.
[b] Phospholipid was mixed with cholesterol in the same molar ratio as found in the intact VLDL.

parinarate in the VLDL appears to be in the surface monolayer while the diphenylhexatriene partitions primarily into the interior core lipids, about 4:1 (13, 15). The fluorescence polarization of the *trans*-parinarate in the phospholipids from palmitate-enriched VLDL was 0.310 ± 0.010, considerably lower than in the intact VLDL, 0.387 ± 0.010. The higher fluidity of the phospholipids was thought to be due to the absence of cholesterol that is found in the surface monolayer of the VLDL. However, the fluorescence polarization of the *trans*-parinarate in a molar mixture of phospholipids and cholesterol equivalent to that found in the intact VLDL was only 0.332 ± 0.007, still less than in the intact VLDL. Possibly, the higher polarization in the intact VLDL was due to protein or interaction of the surface monolayer with the triglyceride core that was enriched with saturated fatty acids after palmitate infusion. If this were true, then the opposite effect should be noted with the highly unsaturated triglyceride core produced after oleate infusion. Indeed the polarization of the extracted phospholipids or phospholipids + cholesterol was much higher (0.283 ± 0.01 and 0.309 ± 0.004, respectively) than in the intact VLDL, 0.215 ± 0.008. This observation is supported by the fact that the fluorescence polarization of diphenylhexatriene in the triglycerides from VLDL of livers perfused with palmitate was 0.377 ± 0.012 as compared to triglycerides from VLDL of livers perfused with oleate, 0.155 ± 0.014. Also, since about 20% of the diphenylhexatriene in the intact particle is in the surface lipids, a higher total polarization of diphenylhexatriene may be expected in the intact VLDL as compared to the extracted triglycerides in the oleate enriched VLDL (0.230 versus 0.155); while a lower polarization in the intact VLDL as compared to the extracted triglycerides would be expected in the palmitate-enriched VLDL (0.352 versus 0.377) as shown in Table 6. Finally, the data indicate that generally phospholipids, phospholipids + cholesterol, and triglycerides had lower polarization values in oleate-enriched VLDL than in palmitate-enriched VLDL with both probe molecules.

The fluorescence polarization of probe molecules is a parameter that is independent of probe concentration. In contrast, corrected fluorescence of a probe molecule is a concentration-dependent parameter that is sensitive to probe uptake (or concentration) as well as to environmental constraint. Table 6 also illustrates the corrected fluorescence of *trans*-parinarate and diphenylhexatriene in the VLDL and extracted lipid fractions. The corrected fluorescence of *trans*-parinarate in intact VLDL isolated from palmitate-infused livers was higher than in the phospholipids (205 versus 73). Addition of cholesterol to phospholipids increases environmental restraint and the corrected fluorescence increases to 146. However, this was still less than in the intact particle. The higher corrected fluorescence of *trans*-parinarate in the intact particle may possibly be due to interaction of the surface monolayer of phospholipid and cholesterol with the highly saturated (palmitate) rigid interior core triglycerides. If so, the opposite behavior would be expected in the unsaturated fatty acid (oleate)-enriched VLDL. Indeed, as shown in Table 6 this was the case; the corrected fluorescence of *trans*-parinarate was lower in the intact particle than in the phospholipid-cholesterol mixture. Similar data were obtained with diphenylhexatriene. A major difference between intact VLDL and triglycerides was also noted with diphenylhexatriene. In the intact VLDL the probe is quickly taken up (13–15). However, the extracted triglycerides took up only small amounts of the probe even at 5-fold higher probe to lipid ratios. Small amounts of cholesteryl esters had no effect on probe uptake.

Arrhenius plots of *trans*-parinarate and 1,6-diphenyl-1,3,5-hexatriene corrected fluorescence in VLDL lipid fractions

We have previously shown that both *trans*-parinarate and diphenylhexatriene displayed characteristic break temperatures in intact VLDL (13–15). In **Fig. 5A**, *trans*-parinarate exhibited characteristic temperatures at 24, 32, and 40°C. Addition of cholesterol to the phospholipids in molar ratios as found in the intact VLDL did not abolish these characteristic temperatures noted with *trans*-parinaric acid (Fig. 5B). The characteristic temperatures were shifted only slightly if at all.

Since diphenylhexatriene also partitions into the surface monolayer of the VLDL, Arrhenius plots were performed as described in Fig. 5. A breakpoint appeared at 28°C for phospholipids from palmitate- as well as oleate-enriched VLDL (Fig. 5C). Addition of cholesterol in molar ratios found in the intact VLDL did not abolish these breakpoints and shifted them only slightly (Fig. 5D). Fig. 5E illustrates Arrhenius plots of diphenylhexatriene in the triglyceride fraction. The triglycerides from oleate-enriched VLDL exhibited breakpoints at 19 and 30°C while those from palmitate-enriched VLDL showed breakpoints at 26 and 38°C. Thus, the characteristic temperatures determined by diphenylhexatriene were different in the triglycerides as compared to the surface lipids (phospholipids + cholesterol); the latter exhibited only one breakpoint while the former showed

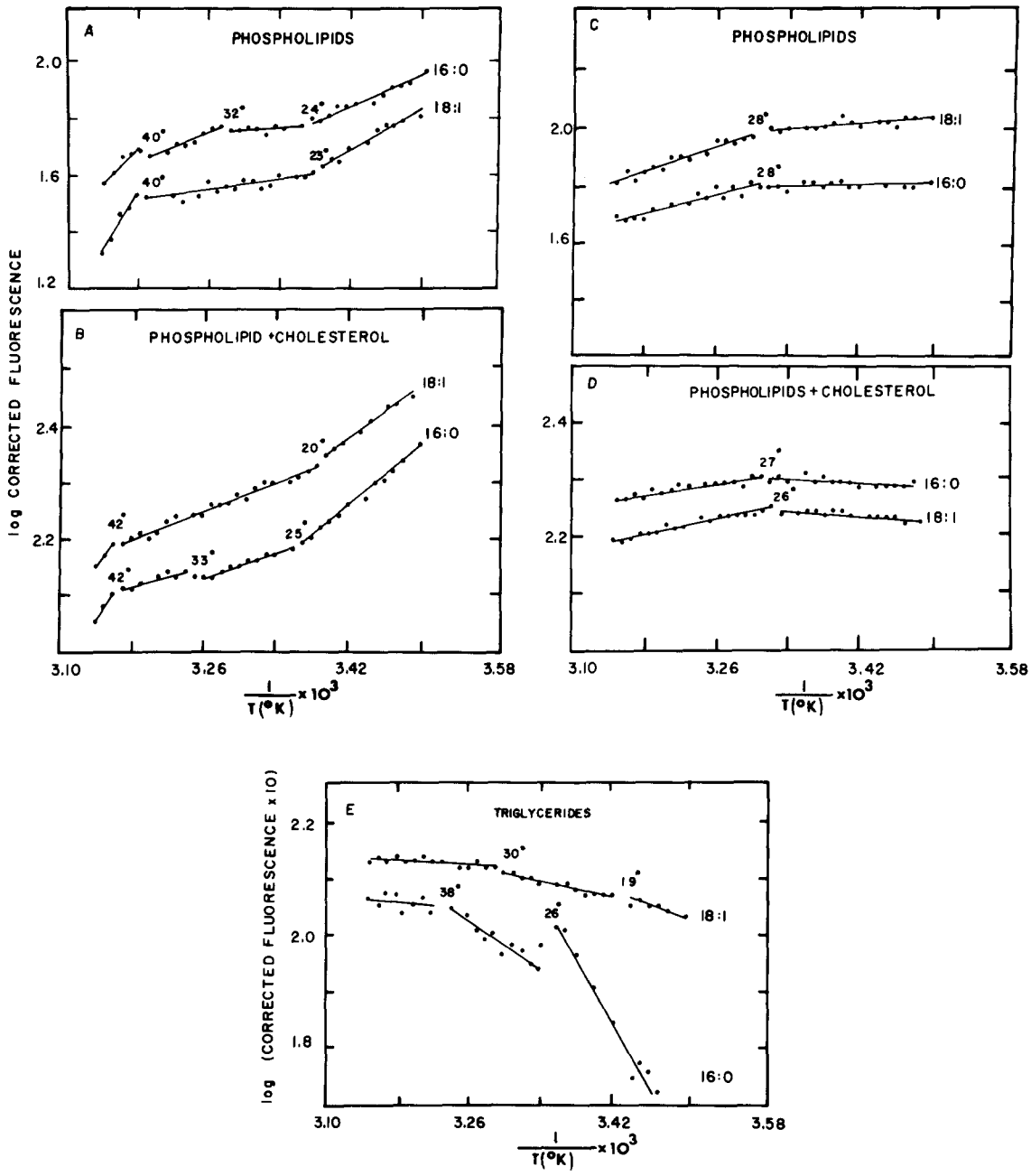

Fig. 5. Arrhenius plots of absorption corrected fluorescence for *trans*-parinarate and 1,6-diphenyl-1,3,5-hexatriene lipids extracted from very low density lipoproteins. In A, the absorption corrected fluorescence of *trans*-parinarate (1:100 mole ratio) in VLDL phospholipids secreted by the liver perfused with palmitate (16:0) or oleate (18:1) was measured as a function of temperature as described in Material and Methods. In B, cholesterol was added to the phospholipids in molar ratios as found in the intact VLDL in the lower panel. In C and D, 1,6-diphenyl-1,3,5-hexatriene (1:100 mole ratio) was used instead of *trans*-parinarate. In E, 1,6-diphenyl-1,3,5-hexatriene was incorporated into triglycerides (1:100 mole ratios) at 50 μg of triglyceride/ml phosphate-buffered saline.

two. It should be noted that the single breakpoint in the phospholipid + cholesterol was in each case close to one of the two breakpoints evident in the respective triglycerides. In summary, the fluorescence probe molecule detected characteristic temperatures in the triglycerides that were 7 to 8 degrees higher in the palmitate-enriched triglycerides than in the oleate-enriched triglycerides. If these breakpoints are interpreted as the onset and end of phase alterations, the midpoints of the phase transition would be at 24.5°C and 32.0°C in triglycerides from oleate- and palmitate-enriched VLDL, respectively.

DISCUSSION

The currently accepted structure of VLDL consists of a triglyceride core to which cholesteryl esters partition (13–17). Surrounding the core is a phospholipid monolayer containing cholesterol and protein. Our investigations utilizing DSC have shown that the triglyceride core is the major contributor to the phase alterations of the intact VLDL particle. The intact VLDL exhibited similar multiple peaks by differential scanning calorimetry as the purified triglyceride. A similar finding was reported recently for human VLDL (10). The multiplicity of the peaks may be partially explained as follows. *1*) The triglyceride exhibited polymorphic behavior. The exotherm noted with palmitrate- and oleate-enriched triglycerides and VLDL supports this explanation. An exotherm of this type is considered strong evidence of a conversion from a metastable to a stable state (38). Exotherms appear between endotherms on DSC and DTA scans of pure samples of single acid triglyceride as they convert from α to β' and β crystalline forms (39, 40). *2*) There is more than one species of triglyceride (present as separate phases). The compositional data of Table 1 and 2 suggest that even though we have enriched the VLDL triglycerides with oleate or palmitate there are still significant quantities of triglyceride present with other esterified fatty acids. It has been noted previously that, in triglycerides containing both saturated and unsaturated fatty acids, there is a greater tendency for saturated fatty acids to partition into a different set of layers than unsaturated fatty acids (41). Indeed if only polymorphic forms were present, some peaks would be expected to dissappear on successive heatings (38–42). On the other hand, if only different triglyceride species were present, no exotherm would be expected since exotherms are indicative of metastable or polymorphic forms.

Each DSC peak of the triglycerides from VLDL of livers perfused with BSA-oleate, BSA-palmitate, or BSA had characteristic onset, maximal, and final temperatures as well as transition width and enthalphy that were determined by the type of fatty acid in the perfusate. Only livers perfused with palmitate secreted VLDL that had phase transitions in the physiological temperature range. Transitions in the other VLDL occurred at low temperature ranges and demonstrated considerable supercooling. In addition, the transitions were similar to those of the triglyceride extracted from the VLDL. These data are in agreement with those reported elsewhere for human VLDL (10, 11). It is apparent that the transitions from human and rat VLDL were similar and that perhaps the type of fatty acid infused or fatty acid ingested may not only determine the microviscosity of the VLDL (11, 13–15) but also the phase behavior (10, 11, 13–15). The thermal behavior of other lipid components (phospholipids, cholesteryl esters, and cholesterol) of the VLDL was also determined and found not to be contributory to the intact VLDL thermotropic behavior except in the case of oleate-enriched VLDL. Although cholesterol abolished the DSC transitions of the phospholipids, with fluorescence probe molecules fatty acid dependent breakpoints in Arrhenius plots were noted by fluorescence of *trans*-parinarate (13–15). At least two explanations for this difference are evident: *1*) the fluorescence techniques detect crystalline behavior not sensitive to DSC as discussed above, or perhaps, *2*) the underlying triglyceride core affects the motional properties of acyl chains in the surface monolayer as previously suggested (13–15, 31).

The data obtained herein on possible phase behavior detected by fluorescence probes in lipid fractions extracted from the VLDL may be compared to DSC of the same fractions. Diphenylhexatriene illustrated only one characteristic breakpoint independent of the type of fatty acid enrichment in the VLDL extracted surface lipids (Fig. 2) while *trans*-parinarate showed two to three characteristic breakpoints some of which were sensitive to the type of fatty acid enrichment (Fig. 1). Breakpoints at 23 and 40°C for phospholipid from oleate-enriched VLDL and 23, 32, and 40°C for phospholipids from palmitate-enriched VLDL were noted with *trans*-parinarate. DSC results with the same fractions showed three phase transitions in the phospholipids from palmitate-enriched VLDL with onset and end temperatures at 29.4 and 40.9°C, 37 and 46.2°C, and 45 and 53°C, respectively. Only the temperatures of the first DSC transition at 29.4 and 40.9°C correlate reasonably well with those noted with *trans*-parinarate at 32 and 40° and with diphenylhexatriene at 28°C. The higher temperature DSC transitions were not detected by fluorescence since the fluorescence Arrhenius plots only went as high as 45°C. No DSC transitions were noted below 29°C; while fluorescence of *trans*-parinarate showed a breakpoint at 24°C. No DSC transitions were noted below 38.6°C in phospholipids from oleate-enriched VLDL. *Trans*-parinarate showed a characteristic break temperature at 40°C and an additional one at 23°C not detected by DSC. Addition of cholesterol to the phospholipids in molar ratios found in the intact VLDL abolished all the DSC phase alterations but did not change the characteristic break temperatures noted with *trans*-parinarate or diphenylhexatriene significantly. Thus, the DSC and fluorescence probe behavior of VLDL surface phospholipids and cholesterol did not correlate well. This may be due to the

presence of quasi-crystalline structures or clusters in the liquid crystalline state (43–45) or to differential partitioning of diphenylhexatriene and *trans*-parinaric acid. The *trans*-parinaric acid in contrast to *cis*-parinaric acid has been reported to be very sensitive to cluster formation in bovine retinal rod outer segment membranes and phospholipids (46). Differential scanning calorimetry may not be sensitive to cluster formation. Thus, fluorescence techniques may provide information on microdomain or cluster formation, while differential scanning calorimetry provides data on bulk lipid phase dependencies. Lastly, the triglycerides had characteristic breakpoints (Fig. 3) at 19 and 30°C and at 26 and 38°C for triglycerides from oleate- and palmitate-enriched VLDL, respectively. No phase transitions were noted by DSC above 4.4°C in triglyceride from oleate-enriched VLDL. In contrast, transitions with onset and endpoints at 25.3 to 42.3°C and 38.3 to 50.7°C were noted in triglycerides from palmitate-enriched VLDL. Two of these onset temperatures correlate well with breakpoints shown by *trans*-parinarate fluorescence (26 and 38°C). Thus, the fluorescence probe diphenylhexatriene reported similar breakpoints as DSC for the triglycerides from palmitate- but not oleate-enriched VLDL.

The data presented here indicate that the interior core lipids of the VLDL may affect the physicochemical properties of the surface monolayer lipids (Table 1). An alternate, less likely possibility must also be considered: the properties of phospholipids and cholesterol in a monolayer may be different than in bilayer depending on their symmetric or asymmetric distribution (47, 48). These properties may also depend on the type of fatty acid infused and its effects on phospholipid synthesis. The effect of inner monolayer phospholipids on the motional properties of outer monolayer phospholipids in model or biological bilayer membranes is not known. Other investigations suggest that the two monolayers are not coupled with respect to motional properties (47, 49, 50). In contrast, monolayer coupling may occur in sphingomyelin systems (51). Also, interior core lipids such as cholesteryl esters may increase the microviscosity of liver plasma membranes (52). Thus, the data presented here are also consistent with the possibility that the rigidity of the surface monolayer of the VLDL, as determined by the polarization of *trans*-parinarate, is affected by the degree of unsaturation and/or rigidity of the interior core triglycerides. Finally, it must be recognized that VLDL apoproteins can also affect the thermal behavior of the VLDL lipids. Rosseneu et al. (53, 54) have demonstrated that interactions of apoC-I, apoC-II, apoA-I, or apoA-II with phospholipids are highly exothermal processes. Structural alterations in apoprotein C-III from human VLDL upon interaction with phospholipids have also been reported (55). Herein, we have also demonstrated that the total ethalpies of the intact VLDL differ significantly from the extracted lipids.

In summary, both DSC and fluorescence probe molecules detect differences in physical properties of VLDL from rat livers perfused with oleate as compared with palmitate. However, because of differences in environments detected by these methodologies (bulk versus microenvironment) the results are not simply comparable and should be considered complementary in structural characterization of very low density lipoproteins.

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REFERENCES

1. Soutar, A. K., C. W. Garner, N. H. Baker, J. T. Sparrow, R. O. Jackson, A. M. Gotto, and L. C. Smith. 1975. Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin:cholesterol acyltransferase. *Biochemistry.* **14:** 3057–3064.
2. Verger, R., M. C. E. Mieras, and G. H. de Haas. 1973. Action of phospholipase A at interfaces. *J. Biol. Chem.* **248:** 4023–4034.
3. Smith, L. C., H. J. Pownall, and A. M. Gotto. 1978. The plasma lipoproteins: structure and metabolism. *Ann. Rev. Biochem.* **47:** 751–777.
4. Bradley, W. A., and A. M. Gotto. 1978. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, and J. A. Ontko, editors. Williams and Wilkins, Baltimore, MD. 111–138.
5. Jonas, A. 1977. Microviscosity of lipid domains in human serum lipoproteins. *Biochim. Biophys. Acta.* **486:** 10–22.
6. Menz, V. H. 1975. Polymorphism of diacid triglycerides of stearic acid and behenoic acid series. *Fette Seifen Anstrichm.* **77:** 170–173.
7. Castellino, F. J., J. K. Thomas, and V. A. Ploplis. 1977. Microviscosity of lipid domains of normal and hypercholesterolemic very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **75:** 857–862.
8. Deckelbaum, R. J., A. R. Tall, and D. M. Small. 1977. Interactions of cholesterol-ester and triglyceride in human plasma very low density lipoprotein. *J. Lipid Res.* **18:** 164–168.
9. Deckelbaum, R. J., S. Eisenberg, M. Fainaru, Y. Barenholz, and T. Olivecrona. 1979. In vitro production of human plasma low density lipoprotein-like particles. *J. Biol. Chem.* **254:** 6079–6087.
10. Deckelbaum R. J., G. G. Shipley, A. R. Tall, and D. M. Small. 1978. Lipid distribution and interaction in human plasma LDL and VLDL. Lipid core fluidity

and surface properties. *In* Protides of the Biological Fluids. H. Peeters, editor. Vol. 25. Pergamon Press, New York. 91–98.
11. Morrisett, J. D., H. J. Pownall, R. L. Jackson, R. Segura, A. M. Gotto, Jr., and O. D. Taunton. 1977. Effect of polyunsaturated and saturated fat diets on the chemical composition and thermotropic properties of human plasma lipoproteins. *In* Polyunsaturated Fatty Acids. W. H. Kunau and R. T. Holman, editors. *American Oil Chemists' Soc.*, Champaign, IL. 139–161.
12. Wilcox, H. G., G. D. Dunn, and M. Heimberg. 1975. Effects of several common long chain fatty acids on the properties and lipid composition of the very low density lipoprotein secreted by the perfused rat liver. *Biochim. Biophys. Acta.* **398:** 39–54.
13. Schroeder, F., E. H. Goh, and M. Heimberg. 1979. Regulation of the surface physical properties of the very low density lipoprotein. *J. Biol. Chem.* **254:** 2456–2463.
14. Schroeder, F., and E. H. Goh. 1979. Regulation of very low density lipoprotein interior core lipid physicochemical properties. *J. Biol. Chem.* **254:** 2464–2470.
15. Schroeder, F., E. H. Goh, and M. Heimberg. 1979. Investigation of the surface structure of the very low density lipoprotein using fluorescence probes. *FEBS Lett.* **97:** 233–236.
16. Gustafson, A., P. Alaupovic, and R. H. Furman. 1966. Studies of composition and structure of serum lipoproteins, separation and characterization of phospholipid-protein residues obtained by partial delipidization of very low density lipoproteins of human serum. *Biochemistry.* **5:** 632–640.
17. Gustafson, A. 1966. Studies on human serum very low density lipoproteins. *Acta Med. Scand.* **179:** 1–44.
18. Goodman, D. 1957. Preparation of human serum albumin free of long-chain fatty acids. *Science.* **125:** 1296–1297.
19. Bligh, E. G., and W. N. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
20. Pinter, J. K., J. A. Hayashi, and J. A. Watson. 1967. Enzymic assay of glycerol, dihydroxyacetone, and glyceraldehyde. *Arch. Biochem. Biophys.* **313:** 404–414.
21. Garland, P. B., and P. J. Randal. 1962. A rapid enzymatic assay for glycerol. *Nature.* **196:** 987–988.
22. Schroeder, F., J. F. Perlmutter, M. Glaser, and P. R. Vagelos. 1976. Isolation and characterization of subcellular membranes with altered phospholipid composition from cultured fibroblasts. *J. Biol. Chem.* **251:** 5015–5026.
23. Ames, B. N. 1968. Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol.* **8:** 115–118.
24. Deckelbaum, R. J., G. G. Shipley, and D. M. Small. 1977. Structure and interactions of lipids in human plasma low density lipoproteins. *J. Biol. Chem.* **252:** 744–754.
25. Blazyk, J. F., and J. M. Steim. 1972. Phase transitions in mammalian membranes. *Biochim. Biophys. Acta.* **266:** 737–741.
26. van Dijck, F. W. M., E. J. J. van Zoelen, R. Seldenrijk, L. L. M. van Deenen, and J. deGier. 1976. Calorimetric behavior in individual phospholipid classes from human and bovine erythrocyte membranes. *Chem. Phys. Lipids.* **17:** 336–343.
27. Hinz, H-J., and J. M. Sturtevant. 1972. Calorimetric studies of dilute aqueous suspensions of bilayers formed from synthetic L-α-lecithins. *J. Biol. Chem.* **247:** 6071–6075.
28. Tsong, T. Y., R. P. Hearn, D. P. Wrathall, and J. M. Sturtevant. 1970. A calorimetric study of thermally induced conformational transitions of ribonuclease A and certain of its derivatives. *Biochemistry.* **9:** 2666–2676.
29. Hinz, H. J., and J. M. Sturtevant. 1972. Calorimetric investigation of influence of cholesterol on the transition properties of bilayers formed from synthetic L-alpha-lectins in aqueous suspension. *J. Biol. Chem.* **247:** 3697–3700.
30. Suurkuusk, J., B. R. Lentz, Y. Barenholz, R. L. Biltonen, and T. E. Thompson. 1976. Calorimetric and fluorescent probe study of gel-liquid crystalline phase-transition in small, single-lamellar dipalmitoylphosphatidylcholine vesicles. *Biochemistry.* **15:** 1393–1401.
31. Schroeder, F., and E. H. Goh. 1980. Effect of fatty acid in physical properties of microsomes from isolated perfused rat liver. *Chem. Phys. Lipids.* **26:** 207–224.
32. Holland, J. F., R. E. Teets, and A. Timarick. 1973. A unique computer centered instrument for simultaneous absorbance and fluorescence measurements. *Anal. Chem.* **45:** 145–153.
33. Holland, J. F., R. E. Teets, P. M. Kelly, and A. Timnick. 1977. Correction of right angle fluorescence measurements for absorbtion of excited radiation. *Anal. Chem.* **49:** 706–710.
34. Christman, D. R., S. R. Crouch, J. F. Holland, and A. Timnick. 1980. Correction of right-angle molecular fluorescence measurements for absorption of fluorescence radiation. *Anal. Chem.* **52:** 291–295.
35. de Kruijff, B., P. W. M. van Dijck, R. A. Demel, A. Schuijff, F. Brants, and L. L. M. van Deenen. 1974. Nonrandom distribution of cholesterol in phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* **356:** 1–7.
36. Gustafson, A., P. Alaupovic, and R. H. Furman. 1966. Studies of composition and structure of serum lipoproteins. Separation and characterization of phospholipid-protein residues obtained by partial dilipidization of very low density lipoproteins of human serum. *Biochemistry.* **5:** 632–640.
37. Gustafson, A. 1966. Studies on human serum very low density lipoproteins. *Acta Med. Scand.* **179:** 1–44.
38. Gray, A. P. 1975. The detection and characterization of polymorphic forms by differential scanning calorimetry. *Instrum. News* **17:** 9–10.
39. Hagemann, J. W., W. H. Tallent, and K. E. Kolb. 1972. Differential scanning calorimetry of single acid triglycerides: effect of chain length and unsaturation. *J. Am. Oil Chem. Soc.* **49:** 118–123.
40. Lutton, E. S., and A. J. Fehl. 1969. The polymorphism of odd and even saturated single acid triglycerides, C8-22. *Lipids.* **5:** 90–99.
41. Larsen, K. 1966. Classification of glyceride crystal forms. *Acta Chem. Scand.* **20:** 2255–2260.
42. Lutton, E. S., C. B. Stewart, and A. J. Fehl. 1972. Polymorphism of mixed triglycerides containing odd fatty acids. *J. Am. Oil Chem. Soc.* **49:** 333–335.
43. Baldassarre, J. J., K. B. Rhinehart, and D. B. Silbert. 1976. Modification of membrane lipid: physical properties in relation to fatty acid structure. *Biochemistry.* **15:** 2986–2994.

44. Lee, A. G., N. J. M. Birdsall, J. C. Metcalfe, P. A. Toon, and G. B. Warren. 1974. Clusters in lipid bilayers and the interpretation of thermal effects in biological membranes. *Biochemistry.* **13:** 3699–3705.
45. Wunderlich, F., A. Ronai, V. Speth, J. Seelig, and A. Blume. 1975. Thermotropic lipid clustering in tetrahymena membranes. *Biochemistry.* **14:** 3730–3735.
46. Sklar, L. A., G. P. Miljanich, S. L. Bursten, and L. A. Dratz. 1979. Thermal lateral phase separations in bovine retinal rod outer segment membranes and phospholipids as evidenced by parinaric acid fluorescence polarization and energy transfer. *J. Biol. Chem.* **254:** 9583–9591.
47. Schroeder, F. 1978. Differences in fluidity between bilayer halves of tumour cell membranes. *Nature.* **275:** 528–530.
48. Tanaka, K-I., and S-I. Ohnishi. 1976. Heterogeneity in fluidity of intact erythrocyte membrane and its homogenization upon hemolysis. *Biochim. Biophys. Acta.* **426:** 218–231.
49. Bystrov, V. F., N. I. Dubrovina, L. I. Barsukov, and L. D. Bergelson. 1971. Differentiation of internal and external phospholipid membrane surfaces using paramagnetic Mn^{2+} and EU^{3+} ions. *Chem. Phys. Lipids.* **6:** 343.
50. Schroeder, F. 1980. Fluorescent probes as monitor of surface membrane fluidity gradients in murine fibroblasts. *Eur. J. Biochem.* **112:** 293–307.
51. Schmidt, C. F., Y. Barenholz, C. Huang, and T. E. Thompson. 1978. Monolayer coupling in sphingomyelin bilayer systems. *Nature.* **271:** 775–777.
52. Davis, R. A., F. Kern, R. Showalter, E. Sutherland, M. Sinensky, and F. R. Simon. 1978. Alterations of hepatic Na^+K^+ATPase and bile flow by estrogen: effects on liver surface-membrane lipid structure and function. *Proc. Natl. Acad. Sci. U.S.A.* **75:** 4130–4134.
53. Rosseneu, M., F. Soetewey, H. Peeters, L. L. Bausserman, and P. N. Herbert. 1976. Interaction of apoproteins of the VLDL and HDL with synthetic phospholipids. *Eur. J. Biochem.* **70:** 285–289.
54. Rosseneu, M., F. Soetewey, G. Middelhof, H. Peeters, and W. V. Brown. 1976. Studies of the lipid binding characteristics of the apolipoproteins from human HDL: II. Calorimetry of the binding of apo A-I and apo A-II with phospholipids. *Biochim. Biophys. Acta.* **441:** 68–80.
55. Morrisett, J. D., H. J. Pownall, and A. M. Gotto. 1977. Interaction of apo C-III with phosphatidylcholine vesicles. Dependence of apoprotein-phospholipid complex formation on vesicle structure. *Biochim. Biophys. Acta.* **486:** 36–46.