The surface coat of chylomicrons: lipid chemistry

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ABSTRACT Chylomicrons from the thoracic duct lymph of dogs fed corn oil were isolated by centrifugation and disrupted by either freezing and thawing or rotary evaporation and rehydration. A pellet, representing the surface coat, was isolated by centrifugation. Pellets isolated by freezing and thawing contained a higher percentage of saturated triglycerides than pellets isolated by rotary evaporation; the presence of saturated triglyceride in the pellet was probably an artifact of the preparation of the surface coat material at low temperature.

Exchange of free cholesterol between surface and core lipid of chylomicrons was complete within 1 hr. The percentage of cholesterol in pellets of surface material isolated by freezing and thawing was about twice that found for pellets after rotary evaporation at 25–40°C. Cholesteryl ester was not present in the surface lipid and that present in the core lipid did not exchange with serum lipoprotein cholesteryl ester.

For phosphatidyl choline, the percentage of linoleic acid in lymph chylomicrons was markedly higher than that in clear lymph or plasma, while the percentage of arachidonic acid was lower. Sphingomyelin of lymph chylomicrons was characterized by very high levels of 16:0 and relatively small percentages of very long-chain fatty acids as compared with clear lymph or plasma.

The data are consistent with the view that in lymph chylomicrons: (*a*) cholesteryl esters are dissolved in a core of triglycerides which contain fatty acids derived primarily from dietary fatty acids, (*b*) free cholesterol is partitioned between core and surface and is freely exchangeable between the two, (*c*) the phospholipid fractions are present on the surface and are intracellular in origin.

KEY WORDS chylomicrons · core and surface lipid composition · fatty acids · dog · lymph · plasma

In a previous study (1) it was shown that when lymph chylomicrons are repeatedly frozen and thawed, a lipid pellet and an oil layer can be separated by centrifugation. The oil layer, which contains mostly triglyceride and small amounts of free and esterified cholesterol, probably represents the "core lipid" of the chylomicron. The pellet, on the other hand, consists primarily of phospholipids and some free cholesterol, triglyceride, and protein. Since this material probably represents interfacial material, it was referred to as "chylomicron membrane." The structure of this "membrane" was unknown and the term "membrane" was not used to imply a morphology similar to that of cellular membranes. In the present and accompanying papers, the chemical and electron-microscopic characteristics of these two chylomicron fractions have been studied in greater detail. In order to discuss the structural aspects of the two chylomicron fractions without suggesting a particular morphology, we shall refer to them as "core" and "surface" fractions.

In the earlier study, the triglycerides of the surface fraction were found to be considerably more saturated than those of the core lipid (1). We considered the possibility that surface triglycerides were derived from fatty acids synthesized de novo in the intestinal wall, whereas the core triglycerides represented primarily dietary fat. An alternative explanation existed, namely, that saturated triglycerides were preferentially adsorbed at the oil–water interface at the low temperatures employed in the isolation of the surface fraction. In the present paper, we will examine this question.

It was also noted that after dogs had been fed corn oil or cream, the chylomicron phospholipids did not differ as radically in fatty acid composition as did the chylomicron triglycerides (1). This was also observed by Whyte, Karmen, and Goodman (2) in rats fed different fatty acid mixtures. Studies on phospholipid turnover in rat intestine during fat absorption have shown an increase in phosphatidyl choline synthesis from phosphate-^{32}P (3). This might suggest that the chylomicron phospholipids are made during the formation of triglyceride droplets in the intestinal cell or that the phospholipids of

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the chylomicrons represent fragments of plasma membrane which coat the lipid droplets as they leave the intestinal epithelial cell. Alternatively, the lipid droplet might develop inside a cellular vacuole which then fuses with the plasma membrane as the droplets are extruded into the extracellular spaces. In this instance, the polar constituents at the chylomicron surface could be derived from adsorbed lymph or interstitial fluid lipoprotein. The studies to be reported here were aimed at localizing the origin of various chylomicron lipids.

METHODS

Preparation of Chylomicrons, Lymph Infranatant Fraction, and Plasma

Thoracic duct lymph was collected from anesthetized dogs[1] immediately after cannulation, or from dogs after recovery from anesthesia, usually on the next day. Before lymph collection, the animals were fed 100 ml of 36% (v/v) corn oil emulsified in skim milk. Samples were collected in glass containers at 0°C, room temperature (about 25°C), or 36–40°C.

The lymph was defibrinated by gentle stirring with a wooden applicator stick and filtered through a small piece of gauze at the bottom of a syringe barrel. The filtrate was centrifuged in a swinging bucket rotor (Spinco SW25.1 at 25,000 rev/min, average g = 64,000 or Spinco SW25.2, average g = 75,000) for 0.5–1 hr either with refrigeration (10°C) or without refrigeration (slightly above room temperature). The packed layer of chylomicrons was removed with a spatula, resuspended in 0.9% sodium chloride, and recentrifuged as before. After another saline wash, the resuspended chylomicrons were washed once with distilled water. During processing (defibrination, centrifugation, and washing), the sample was maintained at the temperature used for collection, unless otherwise indicated. No samples were kept for more than 2 days before extraction, and in those kept at room temperature or 40°C, streptomycin and penicillin (0.5 mg/100 ml each) were present.

Lymph infranate, from which most of the chylomicrons had been removed, and plasma were centrifuged at 25,000 rev/min for 1 hr and the sample, except for the turbid top layer, was extracted with chloroform–methanol.

Preparation of Core Lipid and Surface Material

Two different techniques were used for the preparation of core lipid and surface material from washed chylomicrons. In one method, previously described, the washed chylomicrons were frozen (−40°C) and thawed through several cycles until a layer of oil floated on the top of the aqueous phase (1). A second method, hereafter referred to as "rotary evaporation," consisted of drying the washed chylomicron suspension in a flash evaporator with the sample rotating in a water bath at 2°C, room temperature, or 40°C. The residue was rehydrated by the addition of water and the evaporation repeated. This cycling of dehydration and hydration was continued until the preparation had visibly broken up into an oil phase and a somewhat turbid aqueous layer.

The oil obtained in both procedures was removed from the aqueous phase by low-speed centrifugation with an excess of water added to the system. A pellet, representing mostly surface material, was isolated from the aqueous phase by centrifugation (105,000 g average, Spinco 40 rotor) for 1 hr or more. The pellet was resuspended in water and centrifuged once more under the same conditions.

Chemical Procedures

Chylomicrons, surface material, and lymph and plasma infranatant fractions were extracted with chloroform–methanol 2:1 or with ethanol–diethyl ether 3:1 brought to a boil. Protein was removed by filtration or centrifugation. The precipitate was washed several times with diethyl ether and the washings were added to the original extract. The extract was evaporated under vacuum at room temperature. The lipid residue was dissolved in chloroform and an aliquot was weighed for the determination of total lipid. Lipid classes were separated on silicic acid columns as previously described (4). Column fractions were checked for purity by thin-layer chromatography of small samples on Silica Gel G (E. Merck A.G., Darmstadt, Germany) in Skellysolve B[2]–diethyl ether–acetic acid 80:20:1 for neutral lipids or chloroform–methanol–water 140:50:9 for phospholipids. Lipid spots were detected by charring with sulfuric acid and subsequent illumination with ultraviolet light.

Esterified and unesterified cholesterol were determined in the appropriate lipid fractions after saponification with 4% alcoholic KOH at 65°C for 1 hr and extraction of the cholesterol with Skellysolve B. After removal of the solvent, the cholesterol was redissolved in glacial acetic acid and color developed with ferric chloride–sulfuric acid (5). Phospholipid phosphorus was determined, after digestion of the sample with sulfuric acid, by the method of Bartlett (6). Glycerides were determined by saponification and determination of glycerol by a modification of the procedures previously described (1, 7).

Individual phospholipid fractions were separated on thin-layer plates of Silica Gel G or Silica Gel H contain-

[1] Mongrel dogs or purebred beagles (Cornell University Dog Farm).

[2] A crude fraction of petroleum ether bp 60–70°C (Skelly Oil Co., Kansas City, Mo.).

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ing the fluorescent agent Ultraphor (8). Plates were developed with chloroform–methanol–water 140:50:9 or with chloroform–methanol–acetic acid–water 25:15:4:2 as described by Skipski, Peterson, and Barclay (9). For the preparation of methyl esters, scrapings from thin-layer plates were heated with 2% sulfuric acid in methanol at 65°C overnight. In the case of sphingomyelins, two modifications were found to be necessary. Methylation was performed with 5% sulfuric acid. In addition, it was found necessary to remove contaminants from the lipid that migrates with the R_f of sphingomyelin by a mild alkaline hydrolysis (10). This was especially important when Silica Gel G was used since without alkali treatment excessive amounts of the fatty acids[3] 18:1, 18:2, and 20:4 were present (Table 1). The fatty acid compositions of sphingomyelin reported subsequently were determined on fractions treated with alkali.

Methyl esters of various lipid fractions were separated by gas–liquid chromatography on Chromosorb W columns coated with 14–15% polyethylene glycol adipate polyester. Columns were operated at 200°C in an F & M 810 gas chromatograph with a flame ionization detector. Standard mixtures of equal amounts of 16:0, 18:0, 18:1, 18:2, and 18:3 were analyzed routinely and the percentage deviation from the known composition varied from 1 to 7%. All results are expressed as percentages of the total fatty acid methyl esters.

RESULTS

In a previous study (1), surface material isolated from dog lymph chylomicrons by freezing and thawing showed a much higher concentration of saturated triglyceride than the core lipid. In Table 2, the triglyceride fatty acids of chylomicron surface fractions prepared by different methods are shown. Again, the disruption of the chylomicrons by freezing and thawing resulted in surface material containing highly saturated triglycerides. Surface material prepared by rotary evaporation at room temperature contained triglycerides that were more similar to those found in the core lipid. On the other hand, the temperature at which the chyle was collected appears to be less important in determining the fatty acid composition of the surface material (Table 2).

In a similar study (1), surface material from chylomicrons of rats fed corn oil was prepared by the freezing and thawing method and the triglycerides contained 38–55% saturated fatty acids (compared to 13% in the core lipid). In the course of the present study, one rat was again fed corn oil, and lymph was collected at room temperature. Chylomicron surface material prepared by rotary evaporation at 37°C contained only 20% saturated fatty acids (not shown in Table 2). It appears that the temperature at which the surface material is prepared has a significant effect on its triglyceride component.

A shift of triglycerides between core lipid and surface lipid of an artificial emulsion was illustrated by the following experiment. Labeled tripalmitin and triolein, both purified on thin layers of Silica Gel G, were dissolved in 5 ml of corn oil and emulsified in dog serum. The emulsion was centrifuged and fat particles were washed as described for chylomicrons. One portion of the washed fat particles was frozen and thawed repeatedly and the other was dried, rehydrated, and dried again in a rotary evaporator at 39°C. Core lipid and surface material were prepared as described for chylomicrons. After extraction, the lipids were rechromatographed on Silica Gel G and the triglyceride spots were removed and counted in a liquid scintillation mixture (11). $^{3}H/^{14}C$ ratios were used to calculate the relative proportions of added tripalmitin and triolein in core lipid and surface material. Results in Table 3 show that in core and surface material prepared by rotary evaporation at elevated temperature, the relative concentrations of tripalmitin and triolein were similar to each other and to that found in the original corn oil. In the surface material prepared by freezing and thawing, a considerable enrichment of the tripalmitin had taken place. The same shift in triglycerides was observed in a second, similar experiment.

Lipid Classes of Surface Material

Insofar as the method of preparing the surface material appears to have an effect on the fatty acid composition of its triglycerides, it might be reasonable to assume that the method of preparation might affect the distribution of other lipids between core and surface material. As a test of this possibility, chylomicron surface material was prepared by freezing and thawing or by dehydration in a rotary evaporator at 40, 25, or 2°C. Table 4 shows that in most instances relatively more triglyceride and less phospholipid was present in surface material prepared by freezing and thawing than in that prepared at higher temperatures. The percentage of cholesterol in surface material prepared by freezing and thawing was consistently higher than in that prepared at room or elevated temperatures. The shift between core lipid and surface material occurred only in the unesterified cholesterol fraction. Under no circumstances was a significant amount of cholesteryl ester detected in the surface material. Rotary evaporation of chylomicrons at 25 or 40°C gave rise to surface material with similar cholesterol content, which was lower than that from frozen-thawed chylomicrons (cf. Table 4, dog 46, fraction Nos. 1 and 2), but when the rotary evaporation took place at 2°C the cholesterol content was high, like that found in

[3] Designated by chain length: no. of double bonds.

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TABLE 1 Fatty Acid Composition of "Sphingomyelin" after Alkali Treatment and (or) Thin-Layer Chromatography

Silica Gel	Alkali-Treated	Sample*	16:0	18 0	18 1	18:2	18:2–20:4†	20 4	>20.4‡
						% of total methyl esters			
G	–	F–T	38	30	4.9	8.3	2.2	8.0	6.9
G	–	RE	39	27	4.2	7.7	2.6	8.0	9.6
G	+	F–T	63	17	0.5	0	4.9	0	12.3
G	+	RE	61	14	0.5	0	4.8	0	17.0
H	–	F–T	68	16	0.9	0.8	3.3	0.3	8.9
H	–	RE	63	14	0.8	2.0	3.5	0.3	13.0

* Surface material was prepared from one batch of chylomicrons subjected to freezing and thawing (F–T) or rotary evaporation (RE). Three different methods were used to isolate sphingomyelin: thin-layer chromatography (TLC) on Silica Gel H with chloroform–methanol–acetic acid–water 25:15:4:2; TLC on Silica Gel G with chloroform–methanol–water 140:50:9; or alkali treatment (10) followed by TLC on Silica Gel G as before.
† Constituents with retention times between those of 18:2 and 20:4.
‡ Retention times longer than that of 20:4.

TABLE 2 Composition of Triglyceride Fatty Acids of Lymph Chylomicron Core and Surface Material in Two Dogs Fed Corn Oil

	Dietary Fat	Chylomicron Core Lipid	Surface Material from					
			Chyle Collected at Room Temperature				Chyle Collected on Ice	
			RE		F–T		RE	F–T
					% of total methyl esters			
16:0	11	11	22*	16†	51*	33†	14*	33*
18:0	2.0	1.8	3.7	3.0	13	8.3	3.1	15
18:1	28	28	31	28	14	22	29	19
18:2	59	59	41	50	19	34	53	32
Total saturated		13	29‡	19	64	42	17	49

RE, rotary evaporation (25–28°C); F–T, freezing and thawing.
* Dog 45. Lymph was collected for several periods of 15–30 min, alternating between room temperature (about 25°C) and 0°C. Alternate fractions were pooled, washed chylomicrons were prepared, and portions of each were subjected to freezing and thawing or to rotary evaporation.
† Dog 46. Lymph was collected at room temperature. Core and surface material were prepared as for dog 45 except that all steps were done at room temperature.
‡ Includes 3% 14:0.

TABLE 3 Effect of Temperature on the Triglyceride Distribution in an Artificially Prepared Fat Emulsion*

Sample	Added Tripalmitin† / Added Triolein	
Original Corn Oil	1.4	
"Core lipid" from emulsion	1.3 (F–T)	1.3 (RE)
"Surface material" from emulsion	62 (F–T)	1.1 (RE)

F–T, freezing and thawing; RE, rotary evaporation.
* 1.16 μc of glycerol-2-^{3}H tripalmitate (417 μc/mg) and 0.3 μc of glycerol trioleate-1-^{14}C (155 μc/mg) were added to 1 ml of corn oil. The labeled corn oil was sonicated at 0°C for 1 min with 10 ml of dog serum (Branson sonifier model LS 75).
† Ratio expressed as w/w.

fractions prepared by freezing and thawing (dog 49). Hence the cholesterol content in surface material was related to the temperature rather than the method of preparation; it was not influenced by keeping part of the chylomicrons in the refrigerator overnight before rotary evaporation.

Exchange of Cholesterol Between Core and Surface

It has been observed previously that free cholesterol of chylomicrons exchanges with serum cholesterol (13, 14) but it is not known whether the free cholesterol on the surface is in rapid equilibrium with that dissolved in the core lipid. In a study of this question, a dog was fed 100 μc of cholesterol-4-^{14}C incorporated into two dog pellets, and kept without further food until the next day. Then 55 ml of the labeled serum was mixed with 5 ml of washed lymph chylomicrons obtained from a different dog fed corn oil. After 1 hr of incubation at 37°C, the chylomicrons were separated by centrifugation and washed, and surface material was prepared by freezing and thawing. The results are presented in Table 5.

In 1 hr, the chylomicron unesterified cholesterol reached a specific activity of about 60% of that in the corresponding serum fraction. This can be accounted for to a large extent by the chylomicrons' uptake of extra cholesterol, which has been demonstrated previously

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TABLE 4 Effect of Temperature on Lipid Composition of Lymph Chylomicron and Surface Material*

	Cholesterol	Phospholipid	Triglyceride
	% of total lipid		
Dog 46, No. 1*			
Chylomicron	1.37	4.6	94
Surface material (F–T)	9.4	63	28
" " (RE 40°C)	3.9	81	15
Dog 46, No. 2†			
Chylomicron	0.86	4.4	95
Surface material (F–T)	7.0	74	19
" " (RE 25°C)	3.4	92	5.0
Dog 49			
Chylomicron	0.63	3.1	96
Surface material (F–T)	6.7	82	10.9
" " (RE 2°C)	5.9	82	12.3
" " (RE 25°C)	3.2	88	9.1
" " (RE 37°C)‡	3.0	81	16.5
Dog 50			
Chylomicron	0.66	4.2	95
Surface material (F–T)	4.9	78	16.8
" " (RE 25°C)	3.0	85	11.8
Dog 3§			
Chylomicron	—	1.4	98
Surface material (F–T)	3.7	60	32
" " (F–T)	2.5	45	49
" " (RE 37°C)	1.8	71	23

RE, rotary evaporation; F–T, freezing and thawing.

* A portion of fraction No. 1 was collected at 39–44°C and centrifuged, and chylomicrons were washed and disrupted at 40°C. A different portion was collected at room temperature, stored in the refrigerator overnight and centrifuged, etc., in the cold; chylomicrons were disrupted by freezing–thawing.

† Fraction No. 2 was collected at room temperature from the same anesthetized animal 4–8 hr later than No. 1 and split in two portions. One portion was centrifuged and washed in the cold before freezing–thawing; the other portion was treated at room temperature.

‡ This sample was kept at room temperature before rotary evaporation at 37°C. The three preceding samples were kept at 4°C before disruption of chylomicrons.

§ Beagle. Lower phospholipid content than observed in other dogs may be related to unusually high chylomicron content of lymph. Protein, expressed as a percentage of the phospholipid (12), was 8.1% in washed chylomicrons. In surface material prepared at high and low temperatures it was 5.4 and 7.0%, respectively.

(4). Exchange of unesterified cholesterol within the chylomicron appears to be complete inasmuch as the specific activities of the unesterified cholesterol present in the core lipid and surface material were equal. The cholesteryl ester, in agreement with data obtained on other lipoproteins (13, 14), shows a negligible degree of equilibration but the data are too inaccurate to be conclusive.

Phospholipid Fatty Acids

Two phospholipid fractions from chylomicrons and from infranatant solutions of centrifuged lymph and plasma were selected for fatty acid analysis. Phosphatidyl choline was chosen because it represents the major phospholipid fraction, and sphingomyelin because its

TABLE 5 Equilibration of Serum and Chylomicron Cholesterol

	Specific Activity	
	Unesterified	Esterified
	$cpm/mg \times 10^{-3}$	
Serum, initial	28.3	11.3
Serum, after incubation	29.3	12.0
Chylomicron		
Intact	17.4	1.48
Core lipid	18.5	1.03
Surface material	19.0	—

Lymph chylomicrons were incubated with serum for 1 hr at 37°C. Initial serum phospholipid/chylomicron phospholipid = 11.6. Initial serum unesterified cholesterol/chylomicron unesterified cholesterol = 7.

fatty acid pattern appeared to differ significantly in chylomicrons and plasma (1). The results are shown in Tables 6 and 7.

The fatty acid patterns of the various chylomicron phosphatidyl choline samples were similar (Table 6). They differed markedly, however, from those in the infranatant fraction of lymph, in which 25% of the fatty acid was present as 18:2 and 17% as 20:4 whereas in chylomicrons the percentages were 42 and 4%, respectively. The similarity in fatty acid composition of lymph and plasma infranatant fractions is consistent with the view that lymph phospholipids primarily represent a filtrate of plasma rather than a product of intestinal synthesis.

Table 7 furnishes information on sphingomyelin fatty acids. Again, the sphingomyelin fractions of lymph and plasma infranate show a great similarity in fatty acid composition but that of chylomicrons exhibits quite a different pattern. The latter fraction has an average of 57% 16:0 compared with 27% for lymph and plasma sphingomyelin; the difference is made up primarily by the much higher percentage of fatty acids with more than 20 carbons. These results demonstrate that under the conditions of these experiments phospholipids of chylomicrons fail to equilibrate with those of the lymph lipoproteins.

DISCUSSION

In a previous publication, we reported the isolation of surface material from thoracic duct lymph chylomicrons. Electron micrographs of this material fixed with glutaraldehyde and osmium tetroxide and sectioned showed a network of electron-opaque lines which suggested the presence of deformed globular structures (1). The surface material was found to contain primarily phospholipid, of which phosphatidyl choline was the most prominent representative. In addition, a small amount of free cholesterol and variable small amounts of protein and

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triglyceride were present. No esterified cholesterol was found in this fraction. The studies reported here extend these findings and were designed in particular to elucidate the origin of the surface lipid components.

We observed previously that the triglyceride of the isolated surface material contained a high proportion of saturated fatty acids, even in chylomicrons in which the core lipid contained little saturated triglyceride (1). The hypothesis that the saturated triglyceride of the surface lipid was derived from fatty acids synthesized by the intestinal wall was rejected on the basis of preliminary (unpublished) experiments in which acetate-^{14}C was administered during fat absorption to a dog with a thoracic duct fistula. The specific activities of triglyceride in core and surface lipid were found to be equal. This meant that either the surface triglyceride was derived primarily from the diet, as the core triglyceride was, or that exchange between surface and core triglyceride takes place. In neither case would this support the presence, in vivo, of a surface triglyceride fraction consisting of highly saturated fatty acids. This conclusion was strengthened when we found that the degree of saturation of triglyceride isolated in the pellet, representing primarily surface material, depends to a large extent on the method whereby the surface coat is isolated. In artificial emulsions of corn oil in serum, the isolation of surface material by freezing and thawing also led to enrichment of the surface with saturated triglyceride. It seems reasonable, therefore, to assume that the saturated triglyceride found in the isolated surface material of chylomicrons resulted from the freezing employed in the isolation procedure. A similar enrichment of saturated triglycerides in milk globule membranes prepared at low temperatures has been postulated by Vasić and deMan (15).

The relative amount of free cholesterol present in the isolated surface lipid of chylomicrons was also found to depend on the temperature of isolation and varied between 5 and 10% of the phospholipid present in the fraction. Exchange of free cholesterol between that present on the surface and that found in the core appeared to be complete within 1 hr. This is consistent with the findings of Roheim, Haft, Gidez, White, and Eder (14), who observed that labeled free cholesterol of rat chylomicrons equilibrated completely with that of plasma. It throws no light, however, on the observation of Goodman (13), who was unable to exchange all the free cholesterol of chylomicrons by repeated equilibrations with whole blood.

Although it has been shown previously that the phospholipids of chylomicrons exchange with those of serum lipoproteins (4), such exchange, although it probably takes place in lymph, is far from complete under the conditions employed in this study. In freshly collected lymph the fatty acid composition of the chylomicron phospholipid differs greatly from that of the clear lymph fluid, which in turn resembles plasma. Sphingomyelin of lymph chylomicrons is characterized by a very high percentage of palmitic acid. The percentage of fatty acids

TABLE 6 Phosphatidyl Choline Fatty Acids

	Dog*	16:0	18:0	18:1	18 2	20 4
		% of total methyl esters				
Lymph chylomicrons	1	15	24	10	45	3.7
	2	17	22	14	42	2.6
	3	16	26	11	37	5.9
	4	17	23	12	42	3.9
	10†	13	25	12	43	4.4
Lymph infranate‡	1	13	24	8.5	18	29
	3	16	28	11	29	10
	4	15	24	12	28	14
	10†	13	30	11	27	16
Plasma infranate‡	1	14	31	9.1	14	25
	3	20	26	11	24	12
	4	16	29	11	15	19
	10	13	33	9.9	16	24

* In Tables 6 and 7, dogs 1, 2, 3, and 10 are purebred beagles; dog 4 is part beagle, part collie.

† This sample was collected at 0°C and in the presence of EDTA to minimize oxidative losses of polyunsaturated fatty acids.

‡ Lymph and plasma infranatant fractions were prepared by centrifugation at 64,000–75,000 *g* (average) for 60–90 min to remove chylomicrons.

TABLE 7 Sphingomyelin Fatty Acids

	Dog	16 0	18:0	18:1	18 2	20:0	22:0	23:0	24:0	24:1
					% of total methyl esters					
Lymph chylomicrons	1	54	14	6.0	0.6	3.8	3.4	4.3	—	—
	2	70	13	0.4	0.3	5.0	3.2	2.5	2.5	—
	3	61	14	0.5	—	4.1	3.9	4.7	3.6	3.9
	4	42	9	2.6	4.5	6.3	2.6	2.3	3.0	6.0
Lymph infranate*	3	26	14	0.5	0.8	4.3	6.4	11	9.6	18
	4	22	12	1.7	1.5	4.4	6.6	8.2	7.9	18
Plasma infranate*	2	33	16	0.9	0.8	4.0	5.4	9.4	7.6	14
	4	25	15	5.6	2.5	1.5	5.1	7.3	4.4	15

* Lymph and plasma infranatant fractions were centrifuged at 64,000–75,000 *g* (average) for 60–90 min to remove chylomicrons.

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longer than 18 carbons in the chylomicron sphingomyelin fraction was found to vary from 14 to 23% whereas the corresponding lymph and plasma fractions contained 36–53% of these acids. In a previous study (1), we found the same type of difference between the sphingomyelin fatty acid spectrum of lymph chylomicrons and blood plasma but the absolute values for each showed relatively more palmitate and less long-chain fatty acids.[4] In a personal communication, P. D. S. Wood reports that in human thoracic duct lymph chylomicrons 16–32% of the sphingomyelin fatty acids are longer than C_{18}. When these values are compared with 47% for human plasma sphingomyelin (16), a similar difference in sphingomyelin fatty acids appears to exist in man.

A second difference in chylomicron phospholipid fatty acid pattern and that of clear lymph or plasma pertains to the phosphatidyl choline. In nearly each instance the chylomicron lecithin fraction contained about twice as high a percentage of 18:2 as lymph or plasma, whereas the relative amounts of 20:4 differed in the opposite direction.

These experiments strengthen the evidence concerning the origin of the surface coat of chylomicrons. In principle, the chylomicron could acquire its coat from (*a*) intracellular components, (*b*) pinched-off plasma membrane, or (*c*) extracellular components. Electron-microscopic evidence presented in the accompanying paper (17) lends no credence to the view that the surface coat represents plasma membrane. The difference in fatty acid composition of chylomicron and lymph infranatant phospholipids indicates that at least a substantial portion of the surface lipid is not acquired by random adsorption of extracellular phospholipid. By default one may, therefore, assume that the surface coat of chylomicrons is derived from intracellular constituents. This is supported by the finding of increased incorporation of ^{32}P in mucosal lecithin of fat-fed rats (3, 18), and by the finding of very high percentages of 16:0 in sphingomyelin of intestinal mucosa of rats fed a fat high in this fatty acid (19).

In view of the very high phospholipid content of the chylomicron surface coat, one might assume that the coat is derived from endoplasmic reticulum, which is known to synthesize phospholipids actively. Whether the phospholipid of the chylomicron coat is part of a cellular lipoprotein complex is not known. A lipoprotein adsorbed at an oil–water interface does release its less polar lipids to the oil phase (20), which could account for the observation that all of the esterified cholesterol and some of the free cholesterol is present in the core lipid of the chylomicron. On the other hand, to account for the low protein-to-phospholipid ratio in the washed chylomicron, one would have to postulate that the intracellular lipoprotein contained only 10% protein or that washing the chylomicrons removed most of the adsorbed protein. The latter is not supported by electron micrographs of chylomicrons before and after washing (17). Recent work by Hatch, Aso, Hagopian, and Rubenstein (21) also supports the view that at least part of the protein originates in the intestinal mucosal cell.

[4] It is probable that the discrepancy is due, in part, to our failure to purify the sphingolipid by mild alkaline hydrolysis.

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