Size and lipid composition of chylomicrons of different Svedberg units of flotation

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ABSTRACT Chylomicrons from thoracic duct lymph of rabbits which were fed corn oil were separated in a preparative ultracentrifuge into subfractions of different S_f values in order to compare their size, as determined by electron microscopy, with that expected from ultracentrifugation data. The lipid composition of the chylomicrons of different S_f values was also correlated with their morphology in order to elucidate more about their structure.

Although the diameter distribution of chylomicrons from subfractions of lower S_f ranges corresponded approximately to the expected size distribution, that of the higher S_f ranges contained many small particles. The TG:PL ratio showed a highly significant correlation with the V:SA ratio of chylomicrons from all subfractions. The findings were consistent with the hypothesis that, irrespective of the S_f range of chylomicrons, the core is comprised of TG, while PL is spread as a monomolecular layer on the surface of the particles.

SUPPLEMENTARY KEY WORDS triglyceride . phospholipid . electron microscopy . ultracentrifugation . volume . surface area . monomolecular layer

THE SIZES of lipid particles in thoracic duct lymph of rabbits fed corn oil can be measured by electron microscopy. A close correlation has been shown between the ratios of the volumes to the surface areas of chylomicrons ($S_{f>400}$) and the ratios of their TG content to PL content (1). The results were consistent with the hypothesis that TG makes up the core, while PL, if evenly spread, is a monomolecular layer on the surface of chylomicrons.

It was decided to separate chylomicrons into further subfractions by means of the preparative ultracentrifuge to see if there was a correlation between morphology and lipid composition, irrespective of size distribution. At the same time a comparison could be made between the diameter of chylomicrons, as determined by electron microscopy, and the diameters expected from ultracentrifugation data, based on nomograms prepared from physical laws (2, 3).

MATERIALS AND METHODS

Experimental Animals and Collection of Thoracic Duct Lymph

Four young New Zealand white rabbits (2 kg) were fed daily for 1 wk a mixture of 15 g of corn oil and 35 g of powdered rabbit chow. By the end of the week, the diet was consumed within 2 hr. 4–8 hr after the last feeding, the rabbits were anesthetized with intravenous sodium pentobarbital; respiration was maintained with intermittent oxygen at positive pressure. Lymph was collected from a vinyl tube inserted into the thoracic duct in the superior mediastinum in proximity to the aortic arch (4). Lymph flowed at about 8 ml/hr and was kept at room temperature (about 25°C) to facilitate clotting and defibrination.

Ultracentrifugation

5 ml of lymph from rabbits 1 and 2 were placed in a Spinco SW 50L swinging bucket rotor (average radius of 7.3 cm) and centrifuged at 20°C. The first centrifugation was for 10 min at 15,000 rpm (180,000 *g*-min).

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Abbreviations: TG, triglyceride(s); PL, phospholipid(s); C, cholesterol; V, volume; SA, surface area.

The centrifuge tube was sliced, and the creamy top layer, which consisted of chylomicrons, was removed through a small syringe fitted with a 22 gauge needle and was thoroughly dispersed in 0.5 ml of a NaCl–NaBr solution (d 1.35) by repeated passages through the needle. The infranate, containing lipid particles of $S_{f\,<10,000}$, was saved for further centrifugation. The resuspended chylomicrons were then placed in the bottom of a new centrifuge tube over which a 4 cm layer of saline (d 1.006) was carefully pipetted. The chylomicrons were again centrifuged for 180,000 *g*-min, were removed from the top of the tube as above, and resuspended in 5 ml of saline. The infranatant solution was discarded. The washed chylomicrons thus collected consisted of a subfraction of approximately $S_{f\,>10,000}$ (2, 3).

The infranatant solution from the first centrifugation was centrifuged for 12.5 min at 40,000 rpm (1,562,000 *g*-min). This resulted in an infranatant fraction containing chylomicrons of $S_{f\,<1000}$ and a creamy top layer which, after thorough resuspension and further washing by centrifugation (1,562,000 *g*-min) through a 4 cm layer of saline, resulted in chylomicrons of approximately $S_{f\,1000-10,000}$ (2, 3).

The infranatant solution from the second procedure was centrifuged a third time for 35 min at 40,000 rpm (4,376,000 *g*-min), and the resulting creamy top layer was washed by centrifugation through a 4 cm layer of saline. This yielded chylomicrons of approximately $S_{f\,400-1000}$ (2, 3).

30 ml of lymph from the third rabbit were centrifuged in a Spinco SW 25.1 swinging bucket rotor (average radius of 9.1 cm). Except for the time and speed of centrifugation, the subfractions of chylomicrons were separated and washed through 6 cm of saline (d 1.006) in a manner similar to that described above for the smaller rotor. The first subfraction was centrifuged at 25,000 rpm for 10 min (636,000 *g*-min) resulting in chylomicrons of approximately $S_{f>6300}$; the second sub fraction was centrifuged for 38 min at 25,000 rpm (2,416,000 *g*-min) resulting in chylomicrons of approximately $S_{f\,1000-6300}$; and the third subfraction was centrifuged for 79 min at 25,000 rpm (5,023,000 *g*-min) resulting in chylomicrons of approximately $S_{f\,400-1000}$ (2, 3).

Thoracic duct lymph from the fourth rabbit was centrifuged only once through a layer of saline, thus omitting the step of packing and resuspending the chylomicrons. Into the bottom of a centrifuge tube 0.5 ml of whole lymph and 0.5 ml of saline (d 1.35) were placed and thoroughly mixed. Over this a 4 cm layer of saline (d 1.006) was pipetted, and the specimen was then centrifuged for 10 min at 15,000 rpm (180,000 *g*-min) to yield a creamy layer of chylomicrons of approximately $S_{f\,10,000}$ at the top of the tube. The chylomicrons were removed from the surface after the tube had been sliced, and they were resuspended in normal saline.

Measurement of Size of Chylomicrons by Electron Microscopy

1 drop from each of the reconstituted chylomicron subfractions was added to 1 ml of 1% osmium tetroxide to make a final TG concentration of less than 1 mg/ml. The suspensions were fixed for 1 hr at room temperature, and after thorough mixing, a drop was placed on a grid with a collodion membrane. The grids with the fixed chylomicrons were, in some cases, shadowed with chromium at an angle of 15°. Random fields were photographed in a Siemens Elmiskop 1 electron microscope at an instrumental magnification of 10,000. The shadowed plates were examined to confirm the spherical shape of chylomicrons. The unshadowed plates were examined under a binocular microscope with a micrometer in one eyepiece to measure the diameters of chylomicrons. The scale had been calibrated into units equivalent to 120 A by means of measuring electron microscope plates of polystyrene spheres of known diameter. In each subfraction the diameters of 200 chylomicrons selected at random were measured, and their total surface area (Σ SA) and total volume (Σ V) were calculated. From these values the mean surface area ($\overline{SA}$) and mean volume ($\overline{V}$) of chylomicrons in each subfraction were determined. This method of measurement by electron microscopy has been described previously in more detail (1).

Lipid Analysis

The reconstituted subfractions of chylomicrons were extracted with a minimum of 20 volumes chloroform–methanol 2:1 and were washed by equilibration with an excess of water (5). After digestion of an aliquot of the lipid extract, inorganic phosphorus was measured and the value was multiplied by a factor of 25 to determine the PL content (6). Total cholesterol (C), after saponification of the lipid extract (7), was estimated colorimetrically (8), and the results expressed as free C. A portion of the sample was chromatographed on a column of silicic acid-Supercel; the TG were eluted with chloroform and were measured colorimetrically (9). All results were expressed as mg/100 ml of lymph.

RESULTS

Size of Chylomicrons

Electron micrographs of chylomicrons from the three subfractions of thoracic duct lymph from rabbit 1 are shown in Fig. 1. The distributions of diameters of a random selection of 200 chylomicrons from each sub-

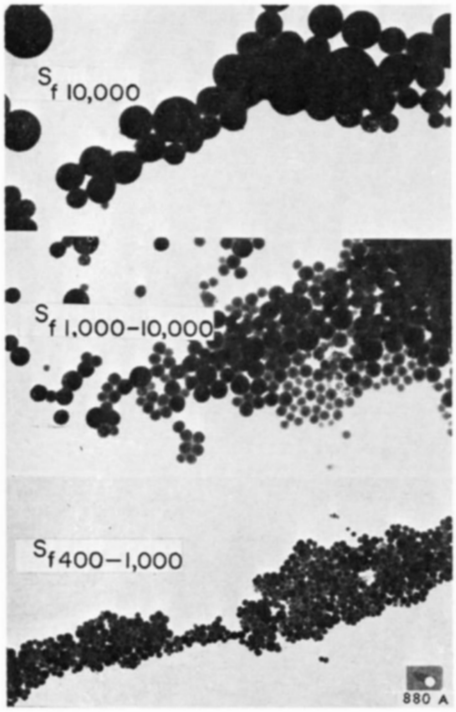

FIG. 1. Electron micrographs, at the same magnification, of unshadowed chylomicrons of three different S_f values from subfractions of thoracic duct lymph of rabbit No. 1. The polystyrene marker indicates the dimensions.

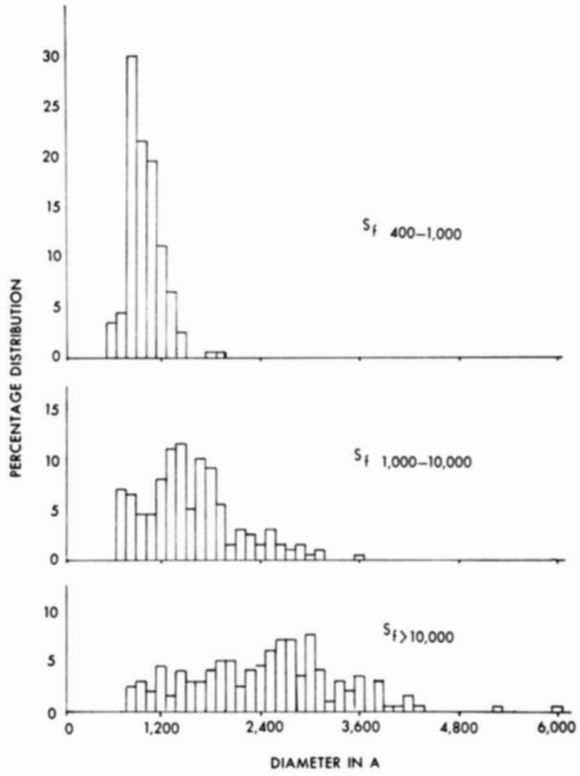

FIG. 2. The diameter distribution of a random selection of 200 chylomicrons of three different S_f values from subfractions of thoracic duct lymph of rabbit No. 1.

fraction from the lymph of rabbit 1 are depicted in Fig. 2. It can be seen that chylomicrons of $S_{f>10,000}$ are, on the whole, larger than those of $S_{f\ 1000-10,000}$, which in turn are larger than those of $S_{f\ 400-1000}$. However, as seen in Fig. 2, there is considerable overlap in size, especially in the subfractions of $S_{f>10,000}$ in which many small chylomicrons are present. Small chylomicrons are present also in the subfraction of $S_{f>10,000}$ prepared from lymph from rabbit 4 (Fig. 3).

The small chylomicrons of $S_{f>10,000}$, however, account for only a small proportion of the chylomicron mass, since mass is proportional to volume or to the third power of the diameter. The mean diameters and the diameter at median volume of chylomicrons from each subfraction have been calculated and are shown in Table 1. Also shown in Table 1 are the mean volume ($\bar{V}$), the mean surface area ($\overline{SA}$), and the ratio of $\bar{V}:\overline{SA}$ of a random selection of 200 chylomicrons from each subfraction.

Lipid Composition

Table 2 shows the TG, PL, and C contents of various subfractions of chylomicrons. It can be seen that TG and PL are the major lipid components. These lipids make up 86–95% and 4–13%, respectively, of the total lipid, while C accounts for only 0.7–1.7% of the lipids.

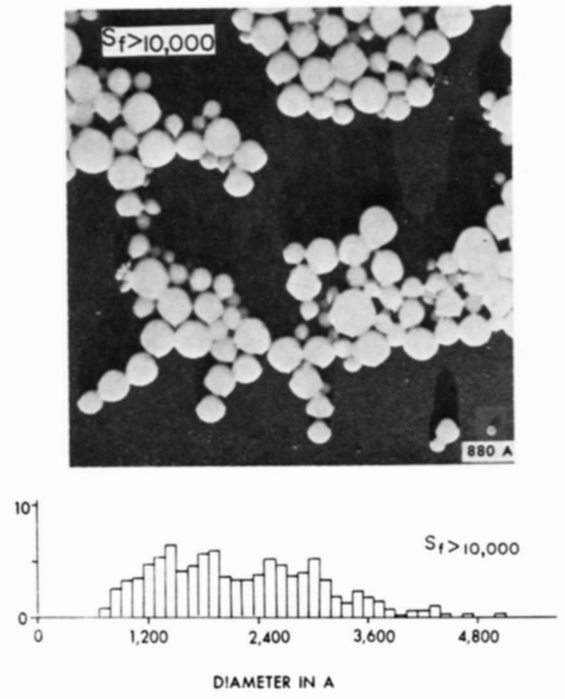

FIG. 3. An electron micrograph of shadowed chylomicrons (above) and the diameter distribution of a random 500 particles (below) of a subfraction of $S_{f>10,000}$ prepared from thoracic duct lymph of rabbit No. 4.

TABLE 1 DIMENSIONS OF A RANDOM SAMPLE OF 200 CHYLOMICRONS OF DIFFERENT S_f VALUES FROM VARIOUS SUBFRACTIONS OF THORACIC DUCT LYMPH

Rabbit No.	Subfraction	Mean Diameter	Diameter at Median Volume	Mean Volume ($\bar{V}$)	Mean Surface Area Area ($\bar{SA}$)	$\frac{\bar{V}}{\bar{SA}}$ (y)
	S_f	A	A	A^3	A^2	A
1	>10,000	2477	3240	1125×10^7	217×10^5	5.18×10^2
	1000–10,000	1556	2160	283×10^7	86×10^5	3.29×10^2
	400–1000	995	1080	58×10^7	32×10^5	1.81×10^2
2	>10,000	2740	3480	1575×10^7	276×10^5	5.71×10^2
	1000–10,000	1452	1920	227×10^7	74×10^5	3.07×10^2
	400–1000	736	840	24×10^7	13×10^5	1.85×10^2
3	>6300	2017	2640	600×10^7	143×10^5	4.20×10^2
	1000–6300	1077	1200	75×10^7	38×10^5	1.97×10^2
	400–1000	782	840	28×10^7	20×10^5	1.40×10^2

TABLE 2 LIPID CONTENT OF CHYLOMICRONS OF DIFFERENT S_f VALUES FROM VARIOUS SUBFRACTIONS OF THORACIC DUCT LYMPH

Rabbit No.	Sub-fraction	TG	PL	C	$\frac{TG}{PL}$ (x)
	S_f	*mg/100 ml lymph*			
1	>10,000	762	29.3	5.5	24.8
	1000–10,000	1172	74.0	11.4	15.8
	400–1000	121	16.4	2.4	6.6
2	>10,000	828	35.8	—	23.1
	1000–10,000	896	82.0	—	10.9
	400–1000	148	21.6	—	6.8
3	>6300	1365	70.5	—	19.4
	1000–6300	256	28.5	—	9.0
	400–1000	135	19.5	—	7.1

The TG:PL ratios of the various subfractions are also shown in Table 2.

Relationship of Size Distribution to TG and PL

Fig. 4 shows the TG:PL ratio (x) plotted against the V:SA ratio (expressed as $A \times 10^{-2}$) (y) for the various subfractions of chylomicrons. The regression of y against x

$$y = 0.211\ x + 0.27 \quad (s_b = 0.0186,\ d_f = 7)$$

was found to be highly significant ($P < 0.001$).

Area Covered by PL Molecules on the Surface of Chylomicrons

The calculations (explained fully in reference 10) were based on data from Tables 1 and 2, assuming that PL is evenly distributed in a thin layer over the surface of chylomicrons while TG constitutes the core of the particles.

The area occupied by one PL molecule $= x/y \times 1409.8\ A^2 =$

$$\frac{\bar{SA} \times 1297 \times TG}{\bar{V} \times 0.92 \times PL}\ A^2$$

where:

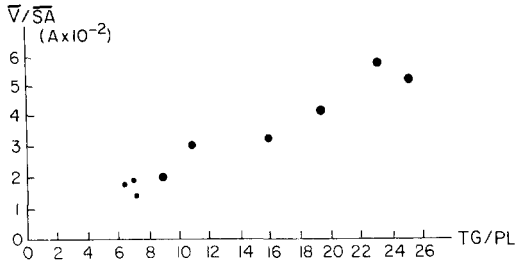

FIG. 4. The ratio TG:PL (x) plotted against the ratio $\bar{V}:\bar{SA}$ (y) of chylomicrons of different S_f values from various subfractions of thoracic duct lymph (r = 0.95). The three large dots on the right represent data from chylomicrons of high S_f values; the three intermediate dots represent data from chylomicrons from the middle range of S_f values, while the three small dots on the left represent data from chylomicrons of the lowest S_f range.

$\bar{V}$ = mean volume of a random selection of chylomicrons (A^3);

$\bar{SA}$ = mean surface area of a random selection of chlomicrons (A^2);

TG = concentration of TG of the sample (mg/100 ml);

PL = concentration of PL of the sample (mg/100 ml);

0.92 = density of corn oil (g/ml);

1297×10^{-24} = mass (g) of one molecule of PL, mol. wt. = 775.

The mean area covered by one PL molecule on the surface of each of the nine chylomicron subfractions of varying S_f values was 61 ± 3 A^2. As will be pointed out in the discussion, however, protein and some free C are also present on the surface, thus probably decreasing the actual area occupied by each PL molecule.

DISCUSSION

Diameter Distribution of Chylomicrons

The expected sizes of chylomicrons in the different subfractions separated by ultracentrifugation can be calcu-

lated by referring to a nomogram (3). For example, chylomicrons of $S_{f>10,000}$ have a theoretical diameter greater than about 3600 A; those of $S_{f\ 1000-10,000}$ have diameters of 1200–3600 A, and those of $S_{f\ 400-1000}$, 750–1,200 A. The size distribution, as measured by electron microscopy, is seen in Fig. 2 and Table 1. The distribution of diameters of subfractions $S_{f\ 400-1000}$ and $S_{f\ 1000-10,000}$ roughly correspond to the expected distribution. In the subfraction of $S_{f>10,000}$, however, the majority of chylomicrons have a diameter of less than 3600 A. A possible explanation is that some smaller chylomicrons tend to adhere to larger chylomicrons, thus being centrifuged as larger particles.

The above findings may have importance when ultracentrifugation techniques are used to separate chylomicrons of different sizes, as in the method described by Pinter and Zilversmit (11). The technique used by these authors, however, did not include a preliminary separation and resuspension of chylomicrons. During this step chylomicrons might tend to adhere to each other unless they are thoroughly resuspended. Nevertheless, as seen in Fig. 3, the subfraction of $S_{f>10,000}$, centrifuged only once from a small volume of lymph over which saline was layered, also contained a large number of small chylomicrons.

Chemical Composition

The lipid composition of chylomicrons within the various subfractions is seen in Table 2. TG make up the bulk of lipid, especially in the subfractions containing the largest chylomicrons. It has been shown that TG in thoracic duct lymph of animals absorbing dietary TG have a fatty acid composition resembling that of the diet (12).

The amount of PL present was found to be related inversely to the amount of TG present. The PL of rat thoracic duct lymph have been shown to contain more than 70% lecithin, with cephalin and, to a lesser extent, sphingomyelin making up the remainder (13, 14). In this paper, the factor of 25 used for converting inorganic phosphorus to phospholipid (6) assumes that the mean molecular weight of the PL is 775.

Comparison of Composition to Morphology

Only the TG:PL ratios were considered for comparison with the $\bar{V}:\bar{SA}$ ratios, since C makes up less than 2% of the lipid of chylomicrons and is distributed both in the core of the particles as cholesteryl esters and on the surface as free C (15, 16). A highly significant relationship was shown between the TG:PL ratio and the $\bar{V}:\bar{SA}$ ratio (Fig. 4), which further confirms the hypothesis that TG makes up the core, while PL is a thin layer on the surface of chylomicrons (1). This hypothesis has been suggested by a number of workers. First of all, the stability of an emulsion of chylomicrons is thought to be due to a surface coating of PL since the emulsion can be broken by the addition of phospholipases (17, 18). Secondly, electron microscopic studies of sectioned chylomicrons showed that the bulk of the particles consisted of a homogenous core surrounded by a thin layer or membrane of different electron opacity (19–21). Thirdly, the comparative lipid content of various lipoprotein fractions indicated that large lipoproteins have a higher TG:PL ratio than do small lipoproteins (22–25). However, as pointed out by Dole and Hamlin (3), the "chemical analysis of a particle mixture, even apart from problems of contamination and loss, gives little information about the structure of the surface layer. The snag lies in the heterogeneity of particle size."

The measurement of a random selection of chylomicrons by electron microscopy, however, takes into account the heterogeneity of lipid particles and results in a good correlation between TG:PL (x) and $\bar{V}:\bar{SA}$ (y). The regression of y against x is similar to that derived from data previously obtained from chylomicrons and very low-density lipoproteins of thoracic duct lymph from both rabbits and rats (1), and it was calculated by Courtice and Fraser (26) as: $y = 0.202\ x + 0.169$ ($s_b = 0.0114$, $d_f = 19$).

PL Layer on the Surface of Chylomicrons

PL probably covers the surface of chylomicrons as a thin membrane (20, 21). The average area occupied by each PL molecule on the surface was calculated to be 61 ± 3 A^2, as compared with an average of 65 ± 5 A^2 found previously (1). Protein, small amounts of free C, and TG are also present in the surface membrane of chylomicrons (15, 16). Lossow et al. (27) calculated that protein covers about 20% of the chylomicron surface area. Gustafson (28) estimated the surface of chylomicrons and lipoproteins in serum to contain a monomolecular mixed film of PL, free C, and protein. PL molecules in a monomolecular film at an air-to-water interface have been shown to have an average area of approximately 40 A^2 when densely packed (29). Slight variations in area have been shown to occur depending on the type of PL present, the fatty acid composition, and whether the film is mixed with other compounds such as C (30). The results in this paper are consistent with the hypothesis that PL is arranged as a monomolecular layer in a mixed film on the surface of chylomicrons, irrespective of their S_f values.

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