# The content of apolipoprotein B in chylomicron particles

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**Abstract** The number of particles and the mass of apoprotein B were measured in samples of rat lymph chylomicrons in order to assign the mass of apoprotein B per particle. Chylomicron numbers were estimated by electron microscopy after negative staining with 2% potassium phosphotungstate at pH 4.8. The distribution of particle numbers was plotted against particle volume. An exponential relationship was found. Integration of the function gave an estimate of the total number of particles for the total chylomicron volume, which was calculated from the chemical composition and the partial specific volumes of the constituents. Apoprotein B was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Two procedures were employed to allow for the influence of artefactual flattening of the chylomicrons on the electron microscope grid. First, it was reasoned that the flattening artefact would be greatest for the largest particles, so measurements of subfractions of smaller chylomicrons would be less affected by flattening. The mass of apoprotein B per particle was $0.48 \times 10^6$ daltons in the smallest subfraction. Second, a correction was applied such that the apparent volume of the oblate spheroid produced by flattening was converted to the true volume of the native spherical particle. The flattening artefact increased exponentially with particle size and was an inverse power function of the surface tension. At an assumed value of chylomicron surface tension of 2 mN/m, the apoprotein B per particle was $0.47 \times 10^6$ daltons.—**Bhattacharya, S., and T. G. Redgrave.** The content of apolipoprotein B in chylomicron particles. *J. Lipid Res.* 1981. **22:** 820–828.

**Supplementary key words** electron microscopy · emulsion · surface tension

In rats and in humans the intestinal lymph chylomicrons contain large molecular weight hydrophobic apoprotein that is similar to, but not identical with, the apoprotein B of plasma VLDL and LDL (1, 2). The apoprotein B content of VLDL is constant at about $0.7 \times 10^6$ daltons per particle (3–6). The content is maintained when VLDL is catabolized to LDL (7), despite removal of triacylglycerols from the particle core and phospholipids and other non-B apoproteins from the particle surface (8, 9).

Only about 0.2% or less of chylomicron (CM) mass is due to apoprotein B. CM particles are spherical (10) and their mass varies over a range of about 200-fold, because particle diameter ranges from about 75 to 500 nm. For these reasons it is difficult to assign a mass of apoprotein B per particle, independent of particle size. Mjøs et al. (4) calculated that CM contain about $1.2 \times 10^6$ daltons per particle, but that figure has not been confirmed. In the present study quantitative electron microscopy of CM populations has been used to measure CM numbers, and hence to calculate the mass of apoprotein B per particle.

## MATERIALS AND METHODS

### Preparation of CM

Male Wistar albino rats weighing 180–200 g were prepared with a thoracic duct cannula (11) and maintained postoperatively as previously described (12). Lymph was collected for 2 to 3 days while the rats consumed their usual pellet diet.

CM were isolated and purified by centrifugation in a discontinuous salt gradient in the JS-13 rotor of the Beckman J-21B centrifuge. The gradient was prepared from 8 ml of NaCl solutions of density 1.065, 1.041, 1.020, and 1.006 g/ml. After a preliminary low speed centrifugation to remove cells, lymph was adjusted to density 1.10 g/ml with solid KBr, degassed, and then 13 ml was layered under the gradients. Particles with diameters >75 nm ($S_f > 400$) were recovered from the top 0.5 cm of the gradient after centrifugation at 13,000 rev/min (18,000 *g*-max) for 5 hr at 20°C (13). Subfractions of the CM population were obtained by sequential centrifugation as previously described (8), by centrifuging first at 13,000 rev/min for 30 min, then for 50 min, and last for 4 hr and 20 min. These three sequential centrifugations were calculated to give particles with diameters >250 nm, 150–250 nm, and 75–150 nm, respectively.

Abbreviations: CM, chylomicron; LDL, low density lipoprotein; VLDL, very low density lipoprotein.



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### Electron microscopy

To 20 $\mu$l of CM suspension containing about 30 $\mu$g CM protein, 2 ml of 2% potassium phosphotungstate (pH 4.8) and 1 ml of 0.05% albumin were added. After mixing, a drop of the mixture was placed on the carbon-coated formvar film on a 400 mesh copper grid. After 15–30 sec, excess fluid was removed with filter paper and the particles were viewed immediately with a Siemens Elmiskop 102 electron microscope at 40 kV. Fields containing 10–20 particles were photographed at an instrument magnification of 20,000×. The diameters of 200 particles from 10–20 micrographs of each sample were measured from prints enlarged 2.5-fold. Using a calibrated magnifier, CM diameters were measured as the mean of two estimates perpendicular to each other. The measured diameters were converted to apparent volumes using the formula for a sphere, $V = \pi D^3/6$, and a histogram showing numbers of particles of specific volume was constructed for each sample.

### Calculations

The histograms of the calculated volumes of the CM particles showed that the number of particles decreased in an exponential manner with increasing particle volumes. By the method of least-squares, a curve was fitted to the data points with the form:

$$y = ae^{-bx} \qquad \text{Eq. } 1)$$

where y is the number of CM of a particular volume, x is particle volume, and a and b are constants.

We wished to use this data to obtain the relationship between N, the total number of CM in the sample, and V, the total volume of CM material in the sample, i.e.,

$$N = \int dy \qquad \text{Eq. } 2)$$

and

$$V = \left(\int dy\right)\cdot dx \qquad \text{Eq. } 3)$$

Differentiating (*3*)

$$dV = y\cdot dx$$

Substituting (*1*)

$$dV = ae^{-bx}\cdot dx \qquad \text{Eq. } 4)$$

Differentiating (*1*)

$$dy = -abe^{-bx}\cdot dx$$

Dividing by (*4*)

$$\frac{dy}{dV} = -b$$

i.e.,

$$dV = \frac{1}{-b}\cdot dy \qquad \text{Eq. } 5)$$

Integrating (*5*)

$$V = \frac{-1}{b}\int dy$$

Substituting (*2*) and re-arranging

$$N = -bV.$$

Hence, the total number of CM in the sample was the product of −b, obtained from the fitted exponential, multiplied by the volume of CM material in the sample, which was derived from the chemical analysis and the partial specific volumes of the constituents, viz. 1.093 for triacylglycerol, 1.044 for cholesteryl ester, 0.968 for cholesterol, 0.970 for phospholipids and 0.7765 for protein (6).

A correction for artefactual flattening of CM on the electron-microscope grid was applied as follows. Assuming the effect of gravity (1 *g*) on spherical particles of density 0.92 g/cm$^3$, at equilibrium the initially spherical CM will become oblate spheroids, with an increased surface area. The change in surface energy will equal the change in potential energy due to the decrease in height of the centre of mass of the particle, i.e.,

$$x\rho g(r - r_1) = T(S_{os} - S_{sp})$$

where x is particle volume, $\rho$ is density, g the acceleration due to gravity, r the radius of the sphere, $r_1$ the minor axis of the spheroid, T the surface tension, $S_{os}$ and $S_{sp}$ the surface areas of the spheroid and sphere, respectively.

The surface tension of CM is unknown. That of a lecithin monolayer as studied by Shah and Schulman (14) is about 20 mN/m, and mixed monolayers of phospholipid and cholesterol have a lower surface tension (15). The CM surface is very complex containing phospholipids, cholesterol, other minor lipids, and at least six proteins. The surface tension is probably less than 5 mN/m, and perhaps less than 1 mN/m. If 2 mN/m is assumed, then the above reduces to:

$$r_1 = r - 0.22 \times 10^5(S_{os} - S_{sp})1/x$$

with dimensions in nm. We also assume that surface tension is independent of particle size.

The major axis, $r_2$ of an oblate spheroid, is given by

$$r_2 = \left(\frac{3x}{4\pi} \times \frac{1}{r_1}\right)^{1/2}.$$

This dimension would represent the apparent radius of the CM measured from the electron micrographs. According to the relationship $4/3\ \pi r_2^3$, the effect of particle flattening on the apparent volume was ascertained for CM of sizes encountered



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TABLE 1. Chemical composition of chylomicrons with $S_f > 400$

| Constituents | Percent of Total Mass |
|---|---|
| Lipids | |
| Total | 98.3 ± 0.27 |
| Triacylglycerol | 85.5 ± 0.82 |
| Cholesteryl ester | 2.6 ± 0.45 |
| Phospholipid | 9.1 ± 0.43 |
| Cholesterol | 1.1 ± 0.11 |
| Proteins | |
| Total | 1.7 ± 0.27 |
| Apoprotein B | 0.091 ± 0.017 |
| Apoprotein A-I | 0.573 ± 0.039 |
| Apoprotein A-IV | 0.218 ± 0.029 |
| Apoprotein E | 0.294 ± 0.026 |
| Apoprotein C | 0.538 ± 0.045 |

Results are the mean ± SEM of analyses of ten samples.

in this study. The predicted relationship between apparent and true volume is given in Results, where the probable effect of flattening on the derivation of particle number is ascertained.

### Chemical analysis

The lipids of CM samples were extracted and purified by the procedure of Folch, Lees, and Sloane Stanley (16). Lipid classes were separated on columns of 1 g of silicic acid according to Goodman and Shiratori (17). Assays for cholesterol (18), triacylglycerol (19), and lipid phosphorus (20) were as previously described (21).

Total protein in CM samples was assayed by the procedure of Lowry et al. (22) after extracting turbidity due to lipids with chloroform (6). Polyacrylamide gel electrophoresis was in 10% gel with 0.1% SDS according to Weber and Osborn (23). Samples were delipidated by adding CM with 1–2 mg protein to 40 volumes of ethanol–diethyl ether 3:2 (v/v) at 4°C. After standing overnight at 4°C, the extract was centrifuged at 300 *g* for 45 min. The supernatant was removed and the precipitate was washed once with diethyl ether. Residual ether was evaporated under a stream of nitrogen. The pellet was dissolved in a small volume of buffer, pH 7.0, containing 0.25 M sodium phosphate, 5% SDS, 5% $\beta$-mercaptoethanol, and bromophenol blue, and heated at 100°C for 5 min before application to the gels. Optimum load was 50 $\mu$g of protein in 10–25 $\mu$l. Electrophoresis was at 8 mA per gel for 2.5 hr. Staining was for 3 hr in 1.5% Coomassie blue in 50% methanol and 10% acetic acid, then overnight with 0.05% Coomassie blue in 20% propan-2-ol and 1% acetic acid. Destaining was in 10% acetic acid and 5% methanol. Gels were scanned at 554 nm wavelength in a Varian Techtron model 635D spectrophotometer with scanning width of 0.4 mm at a speed of 25 mm/min. Peaks were recorded, cut out, and weighed for quantitation.

## RESULTS

The CM population with $S_f > 400$ under the feeding conditions used in this study had the chemical composition given in **Table 1.** Apoprotein B comprised only about 0.1% of total particle mass, and only 4–5% of the particle protein was accounted for by apoprotein B. As expected, the majority of the CM mass was made up of triacylglycerol and phospholipid, which are the predominant constituents of particle core and surface, respectively. The fatty acid composition of the CM lipids under these conditions was 20.5% palmitate, 7.9% stearate, 32.1% oleate, 34.7% linoleate, 3.3% linolenate, and 1.4% arachidonate.

Preliminary experiments showed that except at pH < 5, negative stains of CM were unsatisfactory either because of aggregation of the lipoprotein particles, or because particles failed to adhere to the surface of the carbon-coated film on the grid. Both considerations were important for this study. Aggregation obscured small CM, which were wholly or

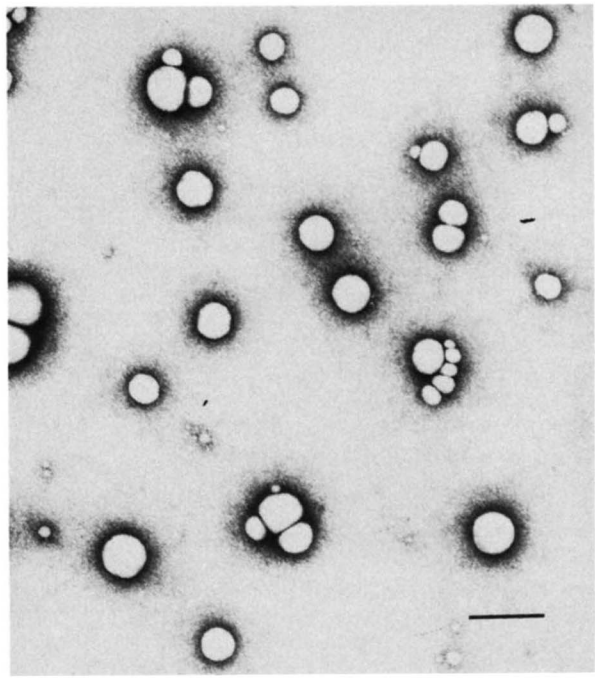

**Fig. 1.** An electron micrograph of rat lymph chylomicrons $S_f > 400$, negatively stained with 2% potassium phosphotungstate at pH 4.8. The length of the bar is 200 nm. Particle diameters were measured as the mean of two estimates perpendicular to each other from prints with overall magnification 50,000×.



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partially overlapped by large CM. Failure to retain a representative sample of the CM population on the grid rendered meaningless extrapolation from the sample examined. The isoelectric point of CM is 5.0–5.5 (24). Below pH 5 CM adhered to the film and were not aggregated.

A typical electron micrograph of the negatively-stained CM is shown in **Fig. 1**. The curve, plotted from the means of CM volumes computed from the measured diameters of six samples, is shown in **Fig. 2,** which clearly illustrates the exponential relationship between particle numbers and apparent particle volume. The coefficient of determination for the fitted exponential was 0.98 (a value of 1.00 is a perfect fit). The slope, b, of the exponential was −0.0114.

To check on the accuracy of our measurements, uniform latex particles of diameters 85 nm, 234 nm, and 500 nm were obtained from Ernest F. Fullam, Inc. (Schenectady, NY). Measured diameters agreed with stated diameters with an overall accuracy of 93.1 ± 2.6%. At the magnifications used in this study, the average error of measurement of diameter was <2% for the largest latex particles, and about 10% for the smallest particles, with the greatest absolute error not more than 9 nm in all cases. Errors of this size, when converted to apparent particle volumes and assigned to the histogram intervals, produced changes in the slope of the fitted exponential that were less than the variations between individual CM samples.

The same latex particles were mixed together in known proportions to check on the possibility that the method of sample preparation discriminated against large or small particles. At least 500 particles were counted. The distribution of particles was found to agree closely with the expected distribution, calculated from the data of particle concentrations supplied by the manufacturer (**Table 2**).

As described in Methods, the number of particles in a CM sample was derived as the product of −b, the slope of the exponential, and V, the volume of CM material in the sample. Then the mass of apoprotein B (in daltons) per particle was calculated as:

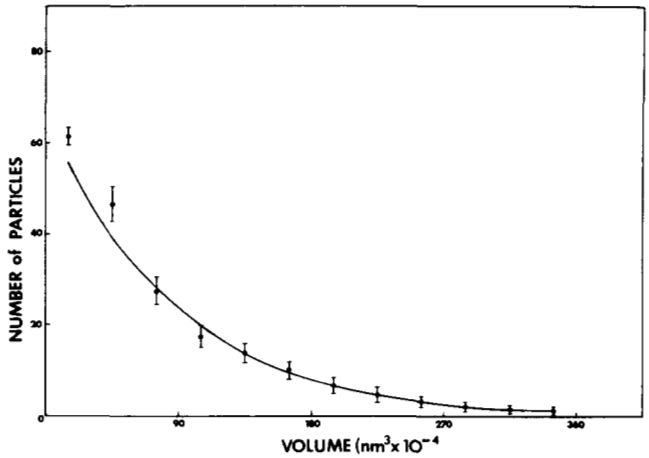


**Fig. 2.** The relationship between particle numbers and particle volumes, of rat lymph chylomicrons $S_f > 400$. Each point is the mean of six observations, with the brackets indicating standard errors. The curve fitted is exponential, and its equation is $y = 65.8\text{-}e^{-0.0114x}$. The coefficient of determination $r^2 = 0.98$, but it is clear that the curve deviates from the data points at small particle volumes.

$$\frac{\text{total apoprotein B mass}}{\text{total CM number}} \times 6.023 \times 10^{23}.$$

For seven independent CM samples, a mass of 0.64 ± 0.18 × $10^6$ daltons of apoprotein B was obtained.

Two procedures were used to assess the influence of artefactual flattening of CM on our measurements. First, it was reasoned that the flattening artefact would be greatest for the largest particles, so measurements of smaller subfractions of CM should be asymptotic to a value that was not affected by artefact. Three subfractions of CM, of decreasing size, were isolated as described in Methods and exponentials were fitted to the histograms of particle numbers plotted against particle volumes as above. **Fig. 3** shows typical electron micrographs of each subfraction. **Table 3** shows that the slope of the exponential decreased substantially as particle size increased. Table 3 also shows that the apparent mass of apoprotein B in daltons per particle decreased as predicted for smaller

TABLE 2. Physical properties of uniform latex spheres[a]

| Material | Mean Diameter | Std. Dev. | Mean Vol/ Particle | Standard Deviation | Particles/ml (10 wt % solid) |
|---|---|---|---|---|---|
| | *nm* | *nm* | $nm^3$ | $nm^3$ | |
| Polystyrene | 85 | 5.5 | $3.28 \times 10^5$ | $6.1 \times 10^4$ | $2.91 \times 10^{14}$ |
| Polystyrene | 234 | 2.6 | $6.70 \times 10^6$ | $2.2 \times 10^5$ | $1.42 \times 10^{13}$ |
| Polystyrene | 500 | 2.7 | $6.54 \times 10^7$ | $1.0 \times 10^6$ | $1.46 \times 10^{12}$ |

[a] This information was furnished by the supplier of the Uniform Latex Spheres, Ernest Fullam Inc. (Schenectady, NY). These particles were used to check on the accuracy of the electron microscopic measurements and their distribution.



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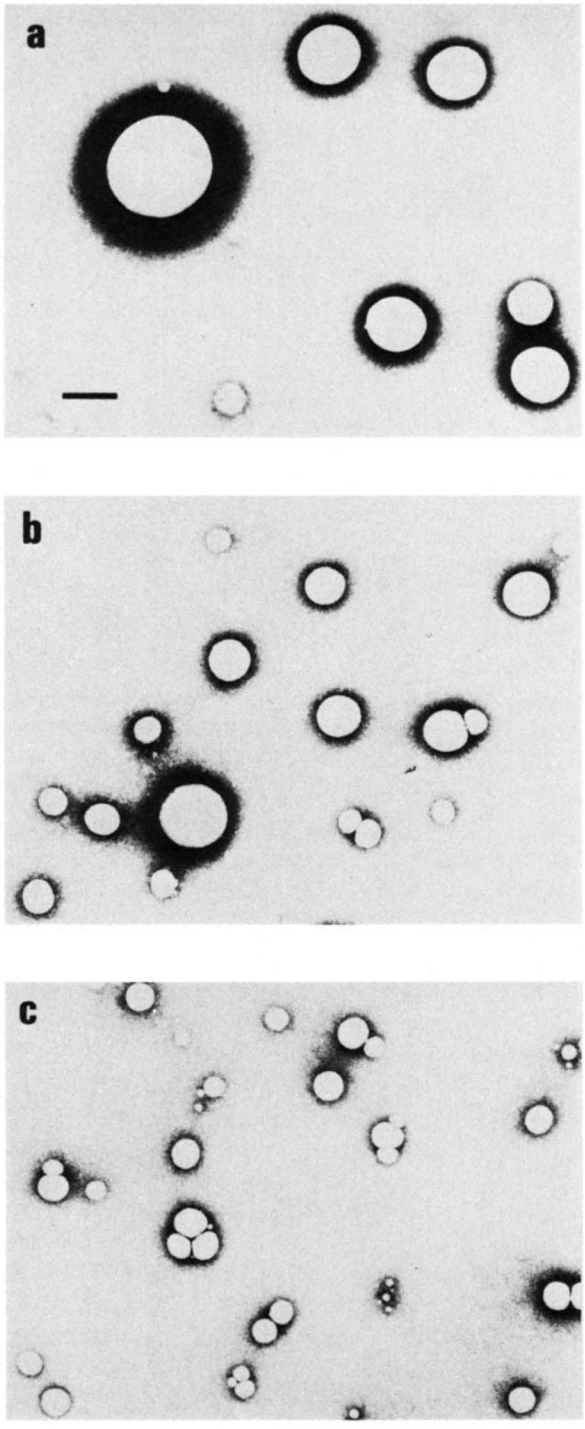


**Fig. 3.** Electron micrographs of subfractions of rat lymph chylomicrons, separated by density gradient centrifugation calculated to yield particles of diameters > 250 nm (fraction a), 150–250 nm (fraction b), and 75–150 nm (fraction c). The micrographs are of samples negatively stained with 2% phosphotungstate at pH 4.8. The length of the bar is 200 nm.

particles, to a value of 0.48 ± 0.21 × $10^6$ daltons for the smallest subfraction studied.

The second procedure for assessing the influence of flattening was to introduce a correction into our measurements, such that the apparent volume of an oblate spheroid was converted to the true volume of the native spherical CM particle. The calculations relating the true volume to the apparent volume are given in Methods. **Table 4** shows how true volume decreases when assumed values of surface tension of the CM particles ranged from 2 to 20 mN/m. The flattening artefact increased exponentially with particle size, and was inversely related to the surface tension by a power function.

From the original data, the exponential $y = ae^{-bx}$ was substituted algebraically so that x, the apparent volume, became the corrected true volume. **Fig. 4** shows the new exponential plotted from the data of Fig. 2. A new value for the slope, b′ was thereby obtained. When the new value was used to calculate CM numbers, and hence the mass of apoprotein B per particle, a new value of 0.50 × $10^6$ daltons was obtained for an assumed surface tension of 10 mN/m, or 0.47 × $10^6$ daltons for an assumed surface tension of 2 mN/m. These values are clearly in good agreement with the value obtained for the smallest subfraction of CM. Furthermore, if a similar correction is applied to the data of Table 3, at a surface tension of about 2 mN/m, the apoprotein B content of the largest particles (Fraction 1) is also about 0.49 × $10^6$ daltons and not 4-fold greater, which appears to be the case without applying a correction for flattening.

## DISCUSSION

Apoprotein B has been localized by immunofluorescence in the intestinal mucosa of rats (25) and

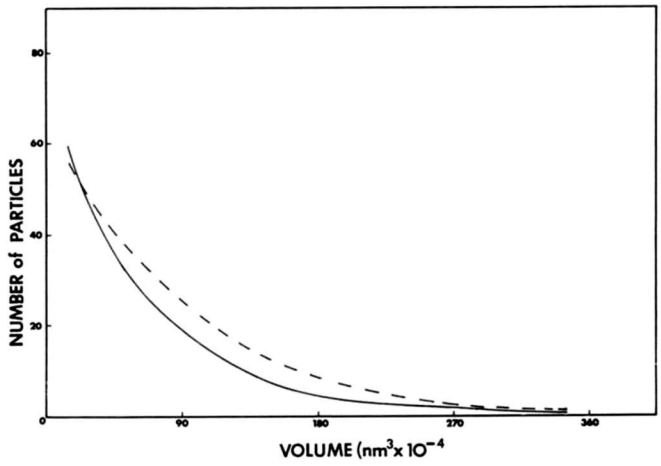


**Fig. 4.** The relationship between particle numbers and particle volumes, with a correction for artefactual flattening calculated for an assumed surface tension of 2 mN/m (solid line). In comparison with the original curve (dashed line), the fit to the data points is improved for particles of small volume. The equation of the new exponential is $y = 75.4e^{-0.0158x}$ and $r^2 = 0.99$.



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TABLE 3. Chemical composition and characteristics of the chylomicron subfractions[a]

| | Fraction 1 | Fraction 2 | Fraction 3 |
|---|---|---|---|
| Nominal diameter | >250 nm | 150–250 nm | 75–150 nm |
| Composition (%) | | | |
| Triacylglycerol | 89.3 ± 0.65 | 87.2 ± 1.36 | 79.8 ± 2.17 |
| Cholesteryl ester | 1.7 ± 0.88 | 2.4 ± 0.86 | 3.5 ± 1.21 |
| Phospholipid | 6.6 ± 0.51 | 7.8 ± 0.75 | 13.0 ± 0.68 |
| Cholesterol | 0.9 ± 0.13 | 0.3 ± 0.13 | 1.3 ± 0.27 |
| Total Protein | 0.9 ± 0.27 | 1.9 ± 0.49 | 2.5 ± 0.63 |
| Apoprotein B (% of total protein) | 4.1 ± 0.50 | 4.1 ± 0.44 | 3.7 ± 0.28 |
| Slope of fitted exponential ($-b \times 10^2$) | 0.110 ± 0.015 | 0.782 ± 0.290 | 1.840 ± 0.378 |
| Coefficient of determination for fitted exponential | 0.94 ± 0.02 | 0.80 ± 0.08 | 0.95 ± 0.01 |
| Apoprotein B content daltons $\times 10^{-6}$/particle | 1.88 ± 0.58 | 1.07 ± 0.30 | 0.48 ± 0.21 |

[a] Results are means ± SEM for three subfractionated chylomicron samples.

humans (26) and its quantity varies with lipid absorption. Apoprotein B is synthesized by the mucosa during active lipid absorption (27, 28) and apparently becomes incorporated into CM during their biosynthesis, to become an integral part of the lipoprotein. Although apoprotein B from CM is similar to that of VLDL and LDL, some apoprotein from CM is unique with smaller molecular weight (1, 2). Probably because of common subunits however, there is immunological cross-reactivity with antisera between CM apoprotein B and LDL apoprotein B (2, 25, 29).

Compared with CM, LDL is relatively uniform in size, molecular weight and chemical composition (3) even allowing for polydispersity in some individuals (30). For VLDL there is greater heterogeneity, but this has not been sufficient to prevent a characterization of the particles with reasonable precision (6, 7, 31). The lack of a more precise description of the molecular characteristics of CM has impeded a full appreciation of the biosynthesis of CM by the intestinal mucosa, and of the events accompanying their degradation and catabolism to remnant particles (32).

The large range of diameter of CM populations is accompanied by changes in chemical composition dependent on particle size (10). The procedure of Sata, Havel, and Jones (31) assumes that all particles have identical size and composition and hence cannot be applied to CM populations without introducing appreciable errors. The present method avoids both these assumptions, and it is also unaffected by uncertainties about the distributions of constituents between the CM surface and core. Errors that can reduce the accuracy of the present determination are of three types. First, the determination can be no more precise than the measurements from the micrographs or the chemical estimation of sample apoprotein B mass. Second, the fitting of the exponential curves to the experimental data might not adequately describe the CM volume distribution. Third, flattening of the CM on the microscope grid distorts the measurements and therefore introduces error into the fitted exponential curves.

Most previous electron microscopic studies of CM populations have used either osmium tetroxide for fixation before direct examination, or the particles have been fixed, embedded, and sectioned. Jones and Price (33) observed that the first procedure did not

TABLE 4. The effect of particle flattening on CM volume at surface tensions of 2 to 20 mN/m.

| True Diameter | True Volume | Apparent Volume ($nm^3$) | | | |
|---|---|---|---|---|---|
| *nm* | $nm^3$ | *2 mN/m* | *5 mN/m* | *10 mN/m* | *20 mN/m* |
| 50 | $6.54 \times 10^4$ | $6.78 \times 10^4$ | $6.62 \times 10^4$ | $6.58 \times 10^4$ | $6.57 \times 10^4$ |
| 100 | $5.24 \times 10^5$ | $5.99 \times 10^5$ | $5.52 \times 10^5$ | $5.39 \times 10^5$ | $5.31 \times 10^5$ |
| 200 | $4.19 \times 10^6$ | $6.60 \times 10^6$ | $5.12 \times 10^6$ | $4.67 \times 10^6$ | $4.43 \times 10^6$ |
| 300 | $1.41 \times 10^7$ | $3.28 \times 10^7$ | $2.14 \times 10^7$ | $1.77 \times 10^7$ | $1.59 \times 10^7$ |

As described in Methods, the increase in apparent volume was calculated as the effect of gravity converting spherical particles to oblate spheroids. From the data of this table, functions of the form $l_n$ (true volume) = $ae^{b.1n^{(\text{apparent volume})}}$ were derived for the selected surface tensions. The original data (apparent volumes) of the CM samples were then corrected to true volumes by using these functions, as illustrated in Fig. 4.



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prevent flattening, and they recognized flattening as a problem especially for large particles. Similarly Sata et al. (31) noted by a shadowing technique that some particles were flattened on the grid leading to an increase in apparent diameter. These workers did not develop a procedure for estimating or correcting for flattening. From embedded particles to estimate population parameters such as mean volume, mean surface area, and size distributions from observations in a planar section of randomly distributed spheres is extremely complex. The statistical considerations are outlined in articles by Hilliard (34) or Jakeman and Anderssen (35). By experimentation we observed that section thickness was an important variable but one that could not be controlled with the precision necessary for computation.[1]

In our studies we used negative staining of CM at pH 4.8, under conditions which enabled the examination of a representative sample and avoided overlap of particles. We used defined latex particles to calibrate our measurement techniques, and to establish that our technique produced a representative sample of the particle population, without discrimination against large or small particles. The measurement of apoprotein B is technically difficult because of its small contribution to CM total mass, and also because of its small proportion of CM protein (Table 1). Our measurements are similar to those published by Imaizumi, Fainaru, and Havel (36) and Green et al. (37), but less than those of Kostner and Holasek (38).

The coefficient of determination of the exponential fitted to our data points was about 0.98 for whole populations, indicating that the mathematical model was a good representation of the relationship between the particle volume and number. This relationship could be peculiar to the conditions chosen in these experiments, and ought not to be extrapolated without testing to CM populations affected by feeding fats that influence particle size or number (39) or by feeding different quantities of fat (10, 40). The fitting of curves to the CM subfractions was not so precise, probably because of imperfect separations of particles in the ultracentrifuge. Consequently the distributions were not truncated for the small sizes of the selected ranges of particles.

Two methods were used to assess the influence of particle-flattening on our measurements and calculations. As described in Results, measurements of subpopulations of the CM showed a systematic change in the slope of the fitted exponential, suggesting that such an error was smallest for populations of small CM. For example, the data of Table 4 show that at a surface tension of 2 mN/m, 50-nm particles will have an apparent volume 4% greater than true volume; 100-nm particles are overestimated by 14%, and similarly, 200-nm particles by 58%, and 300-nm particles by 133%. Hence the numbers of CM with large diameter are seriously underestimated, leading to a falsely high assignment of apoprotein B mass per particle as shown in Table 3. Instead of large CM $> 250$ nm having apparently 4-fold greater apoprotein B content, the appropriate correction restores large CM to equivalence with small CM.

Mathematical modelling of the predicted flattening of a spherical particle has enabled a correction to be introduced into our calculations, depending on what value was assumed for particle surface tension. The physical state of CM is an emulsion, and the size distribution suggests that surface tension is less than 5 mN/m (41). As discussed by Phillips (42), lecithin at an oil–water interface forms a monolayer at much higher areas per molecule than at the air–water interface, and thus at lower surface tension. However, the CM surface is complex and multicomponent with both oil- and water-soluble surfactants, a combination known to give low surface tension and high stability (43). Our data were consistent with a value for CM surface tension of about 2 mN/m, and using this value we deduced that CM contain about $0.47 \times 10^6$ daltons of apoprotein B per particle. This figure was derived from the polyacrylamide gel scans and the accuracy of this assay clearly imposes a limit on its precision. CM contain at least one peptide $M_r \sim 250{,}000$ (1, 2) and speculation on the detailed subunit composition of the CM apolipoprotein seems premature in view of the well-documented complexity of apoprotein B (44, 45). Nevertheless our data are consistent with the presence of two polypeptide chains $M_r \sim 250{,}000$ of apolipoprotein B on each CM particle.

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