Size and shape determination of fixed chylomicrons and emulsions with fluid or solid surfaces by three-dimensional analysis of shadows

Donald Gantz, Susanne Bennett Clark, Arie Derksen, and Donald M. Small

Departments of Biophysics, Medicine, Biochemistry, and Physiology, Boston University School of Medicine, Housman Medical Research Center, 80 East Concord Street, Boston, MA 02118-2394

Summary Chylomicrons and chylomicron-sized emulsions are spherical particles in suspension but their shape and apparent size may be distorted by electron microscopy processing. To assess adsorption to grids, flattening, and shrinkage, chylomicrons and emulsions were fixed with osmium tetroxide and together with polystyrene beads were shadowed with platinum. Vertical profiles projected from particle shadows indicated that the chylomicrons and emulsions were slightly shrunken, slightly truncated, oblate spheroids while the polystyrene beads were spheres. Particle diameters were corrected by assuming that volumes of oblate spheroids on the grid surface were equal to volumes of spheres in the original lipid suspension. Because of the compensating effects of shrinkage (decreases diameter) and flattening (increases diameter) the differences between the means of measured diameters and corrected diameters were ≤5%.— **Gantz, D., S. Bennett Clark, A. Derksen, and D. M. Small.** Size and shape determination of fixed chylomicrons and emulsions with fluid or solid surfaces by three-dimensional analysis of shadows. *J. Lipid Res.* 1990. **31:** 163–171.

Supplementary key words electron microscopy • triolein • unesterified cholesterol • osmium tetroxide fixation • unidirectional platinum shadowing • phosphatidylcholines

Electron microscopic (EM) techniques have been used for more than 30 years to determine the sizes of chylomicrons (1–5), smaller lipoproteins (6–8), and synthetic emulsions of chylomicron size (2, 9–11). A comprehensive review of early work involving various EM techniques has been prepared by Forte and Nichols (12). One of the frequently used techniques has been fixation with osmium tetroxide and subsequent shadowing with metals such as chromium or platinum (13–17). In general, conclusions about the sphericity of particles have been based on the assumption that an osmium-fixed particle is rigid. Particle diameters have been obtained by direct measurement of the fixed or fixed and shadowed particle.

Some workers have expressed concern about particle collapse/flattening and the resulant effect on apparent particle diameter (5–7, 14, 18). It is possible that the particle surface adsorbs to and spreads on the grid surface causing an asymmetric flattening of the bottom of the particle. Also, irradiation of the particle by the electron beam may alter particle size. In the present study, we have quantitated particle flattening and corrected the apparent diameter by using a three-dimensional analysis of shadowed chylomicrons and chylomicron-sized emulsions with fluid or solid surfaces.

MATERIALS AND METHODS

Preparation of emulsions

The two emulsions prepared for this study differed only in phosphatidylcholine (PC) species. At ambient temperatures, emulsions made with egg yolk PC (EYPC) have a fluid surface while those made with distearoyl PC (DSPC) have a solid surface (19). Stock solutions of trioleoylglycerol (melting point 4°C) and unesterified cholesterol in chloroform, and EYPC or DSPC in chloroform–methanol 2:1 (v/v) were mixed in a glass vial, yielding lipid mixtures, after solvent evaporation, of 80% triolein, 1.5% cholesterol and 18.5% (w/w) EYPC or DSPC (20). Emulsification of the lipid mixtures and isolation of the particles were performed as described previously (21). The isolated emulsions contained 88 wt% triolein, 1.5 wt% cholesterol, and 10.5 wt% EYPC or DSPC.

Preparation of chylomicrons

Cannulas for gastric infusion and mesenteric lymph collection were surgically implanted into male Sprague-Dawley rats weighing 280–300 g under pentobarbital anaesthesia as previously described (22). A gastric saline infusion (0.15 M NaCl) was interrupted after 12 h to administer a gastric bolus of 300 μl triolein. Chylous lymph was collected for 10 h at room temperature in the presence of 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride to inhibit proteolysis. Chylomicrons were separated from the lymph by ultracentrifugation in a swinging bucket rotor (SW 41, 30,000 rpm, 30 min. at 10°C) and were harvested from the tops of the tubes by tube slicing.

Polystyrene beads

Three polystyrene bead samples were used to confirm the geometric arrangement of shadowing. Polystyrene samples of diameters 109 nm and 204 nm were obtained from Seragen Diagnostics, Inc., Indianapolis, IN (23) and a 364-nm sample composed of styrene-butadiene 95:5 (wt%) was furnished by Duke Scientific Corp., Palo Alto, CA (24).

Fixation

Emulsions and chylomicrons were diluted to 1 mg/ml (total lipids) with HEPES-NaCl buffer or 0.15 M NaCl, pH 7.4, respectively, and then fixed as described by Forte and Nordhausen (6). A 0.5-μl aliquot was dialyzed against 0.15 M cacodylate, pH 7.4, buffer containing 2% OsO_4 for at least 4 h at room temperature (12–14 kDa molecular weight cut off; Spectrum Medical Industries, Inc., Los Angeles, CA) and then dialyzed overnight against distilled water.

Abbreviations: EM, electron microscopy; EYPC, egg yolk phosphatidylcholine; DSPC, distearoyl phosphatidylcholine.

Grid and sample preparation

Copper grids of 300 mesh were coated with Formvar and carbon and the surface was rendered hydrophilic by a 5-min glow discharge (25, 26). A 1-μl droplet of fixed emulsions or chylomicrons was placed on a coated grid and allowed to air-dry.

Shadowing

Prepared grids of chylomicrons, emulsions, and polystyrene beads were placed in a Denton 502A vacuum evaporator (Denton Vacuum, Inc., Cherry Hill, NJ) for unidirectional platinum shadowing, using a modification of the method of Hall (27). Grids and platinum wire were precisely arranged at a geometric shadow ratio (28) of 2:1 (distance/height) or a shadowing angle of 30°. The grids were placed in an arc so that the horizontal distances from the grid centers to the metal source were equal. Care was taken to ensure that the grids lay flat.

Viewing

Images were examined and recorded in a Hitachi HU-11C Electron Microscope with a 50-μ objective aperture. A carbon grating replica with 2160 crossed lines/mm (Ernest F. Fullam, Inc., Latham, NY) was used for calibration.

Measurements

Particle and shadow measurements were taken directly from electron image film using a Peak 7 × measuring magnifier fitted with a graticule with 0.1-mm divisions (Polyscience, Inc., Warrington, PA). Particles from at least four different fields near a grid center were measured. All particles were measured except those that were clumped or had overlapping shadows. The total number of EYPC emulsion, DSPC emulsion, and chylomicron particles measured were 88, 268, and 213, respectively. Particles with identical shadow diameters were grouped; groups contained between 1 to 36 particles depending upon the lipid preparation.

We assumed that shadow diameter is the most accurate representation of original particle diameter because particle dimensions can be altered by irradiation. Excessive metal deposition can alter the particle diameter and change the apparent particle center (29). The best measure of the total shadow length of a particle is the distance from shadow tip to the edge of the particle plus one-half of the shadow diameter (**Fig. 1**). Particle height was obtained by dividing the total shadow length by 2.

Vertical profiles

The vertical profile of a particle was constructed from its shadow (refer to Fig. 1). An enlarged image of a sha-

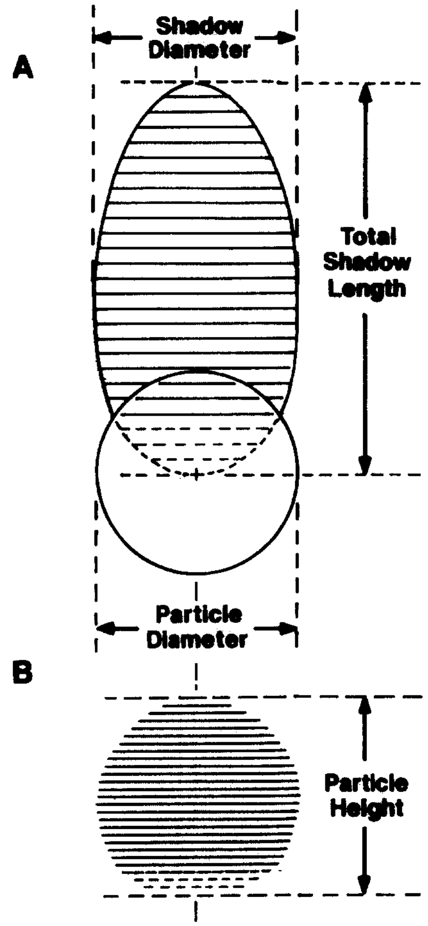

Fig. 1. Tracing of an enlarged image of a rigid particle shadowed at a distance:height ratio of 2:1 or an angle of 30° (A) and its vertical profile constructed by projection (B). A horizontal segment on the shadow is 2 × a segment on the projection. The dashed lines represent an interpolation of unseen shadow. See Methods for details on vertical profile construction.

dowed particle was overlayered with thin graph paper and positioned so that the particle and its shadow were bisected by a single grid mark. The outline of the particle and its shadow was traced. The shadow outline was divided into equal horizontal segments. The unseen portion of the shadow was interpolated and is represented by dashed lines. As a result of 2:1 geometry, one segment of shadow length was twice that of a corresponding segment of height on the projection. Proceeding from the shadow tip to center of particle, a vertical profile of the particle was obtained.

The typical vertical profile of an emulsion or chylomicron particle on a grid surface is a slightly truncated oblate spheroid. Assuming that the volume of the oblate spheroid on a grid is equal to the volume of the spherical particle in suspension, then a corrected diameter may be calculated in the following way.

Volume of oblate spheroid = volume of sphere

$$4/3\pi a^2 b = 4/3\pi r^3$$
$$a^2 b = r^3$$
$$\sqrt[3]{a^2 b} = r \text{ (radius)}$$
$$2\left(\sqrt[3]{a^2 b}\right) = 2r \text{ (sphere diameter)}$$

where a = shadow diameter and b = particle height ÷ 2.

RESULTS AND DISCUSSION

Electron micrographs of shadowed 109-nm polystyrene beads, EYPC and DSPC emulsions, and chylomicrons are shown in **Fig. 2**. Shadow margins are sharp and distinct indicating particle stability during platinum shadowing. Particle clumping occurred in each of the four preparations, and was more prevalent in the DSPC emulsion and chylomicrons (up to 30% of particles) than in the EYPC emulsion and polystyrene beads (up to 15%). Size distribution histograms, composed of all particles analyzed in this study, are presented in **Fig. 3**. Each polystyrene bead sample is quite homogeneous. The size ranges and modal diameters of the three lipid preparations are similar. The skewed chylomicron population is similar to that obtained by Fraser, Cliff, and Courtice (17), whereas the pop-

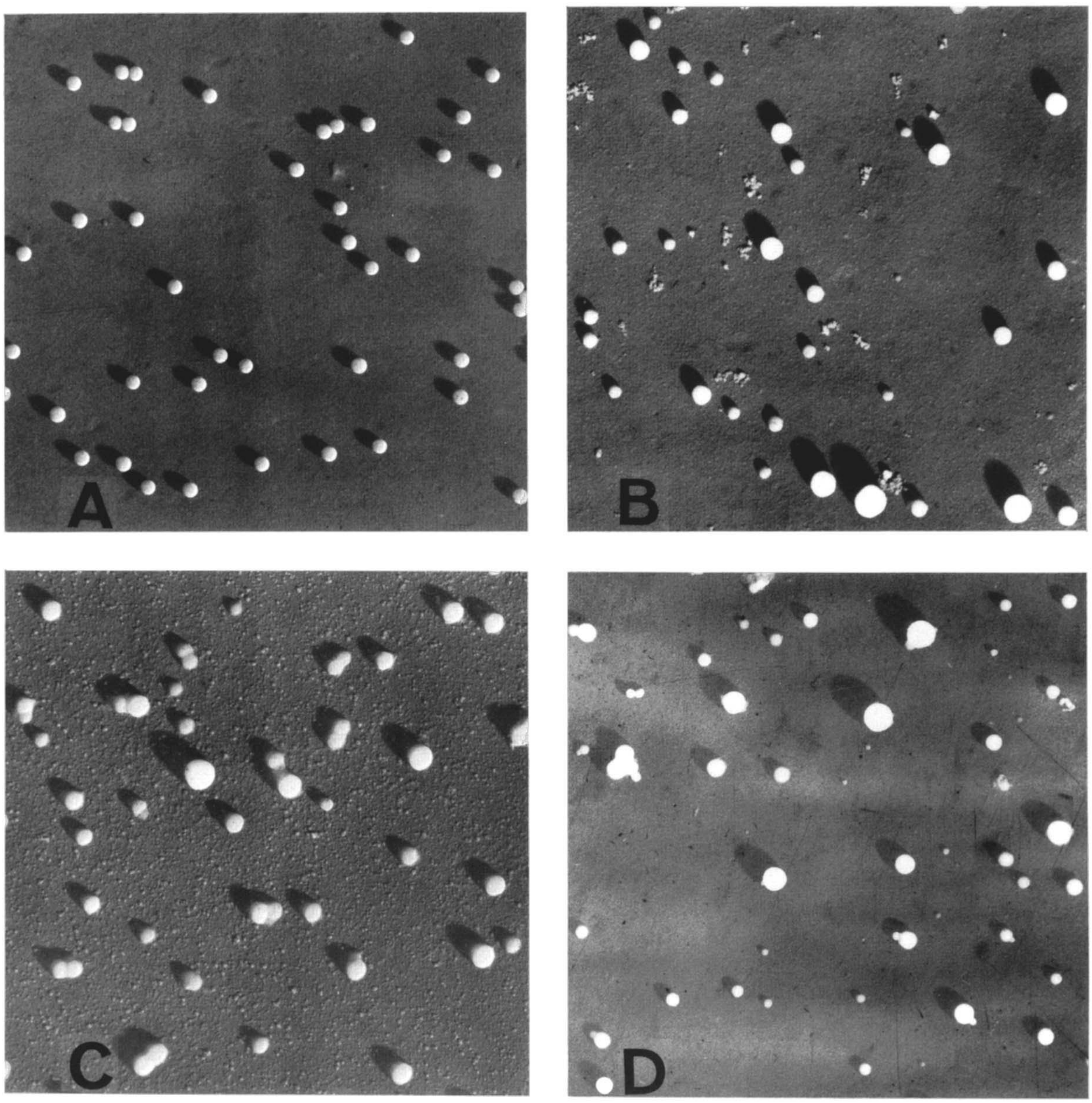

Fig. 2. Electron micrographs of platinum shadowed A) polystyrene beads (109 nm), B) EYPC emulsion, C) DSPC emulsion, and D) chylomicrons. Emulsions and chylomicrons were fixed with osmium and dialyzed against water before shadowing. Histograms in Fig. 3 include particles shown here (× 21,400).

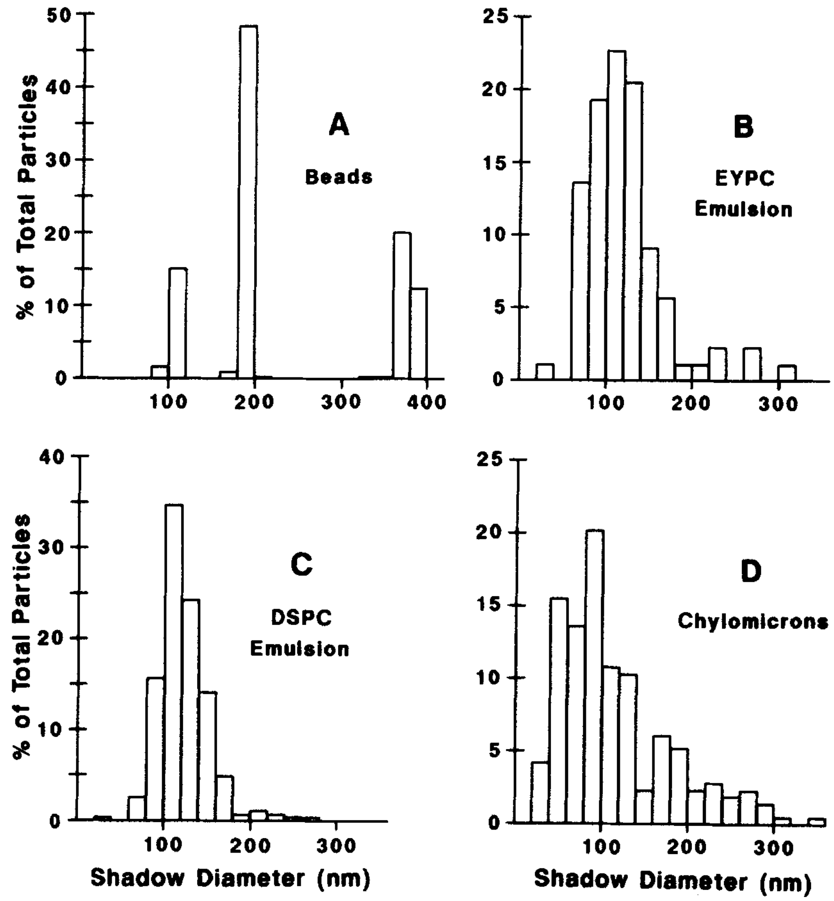

Fig. 3. Size distribution histograms containing all particles analyzed in this study; A) polystyrene beads (includes three samples), B) EYPC emulsion, C) DSPC emulsion, and D) chylomicrons. Electron micrographs of some of these particles appear in Fig. 2. See Methods for details on measurements.

ulations of EYPC and DSPC emulsions are more symmetrical.

The relationships between shadow diameter and particle height for polystyrene beads, emulsions, and chylomicrons are illustrated in **Fig. 4**. For polystyrene beads, shadow diameter was the same as particle height (Fig. 4A) indicating that the beads, which are rigid, did not flatten. However, the heights of the emulsion and chylomicron particles were reduced by approximately 16–24 nm ($P < 0.05$) (Fig. 4B-D). Since the slopes of these lines did not differ significantly from 1.00, the observed flattening appears to be independent both of particle size and of surface phospholipid chain saturation (physical state). It is likely that core fluidity is a determining factor in the flattening of these particles.

Vertical profiles of selected polystyrene and EYPC emulsion particles shown in **Fig. 5** illustrate the nature of the flattening. The rigid polystyrene bead (Fig. 5A) has minimal surface contact and is spherical. The emulsion particle in Fig. 5B shows typical flattening; particles in Fig. 5C and 5D represent extremes in surface contact and shape. All three EYPC particles are oblate spheroids slightly truncated where they contact the carbon surface. We have quantitated differences in surface contact and expressed them as a percentage of total particle surface area (**Table 1**). A comparison of two of the emulsion particles (B and C of Fig. 5; 1 and 2 of Table 1) is particularly interesting. According to projections, these particles have unequal apparent diameters and unequal areas of surface to grid contact because of different degrees of flattening. However, their corrected diameters (spherical diameters) are almost the same. Since these two particles are presumably identical in composition (30) and fixation, it is probable that the extent of particle flattening is related to differences in adhesion to the grid surface.

In previous work, no attention was directed to possible irradiational effects of the electron beam on fixed particles; stability was assumed. Our data suggest that emulsion and chylomicron particles are reduced in size during electron microscopy (**Fig. 6**). In the absence of shrinkage the particle diameter should be equal to the shadow diameter as shown for the polystyrene beads (Fig. 6A). However,

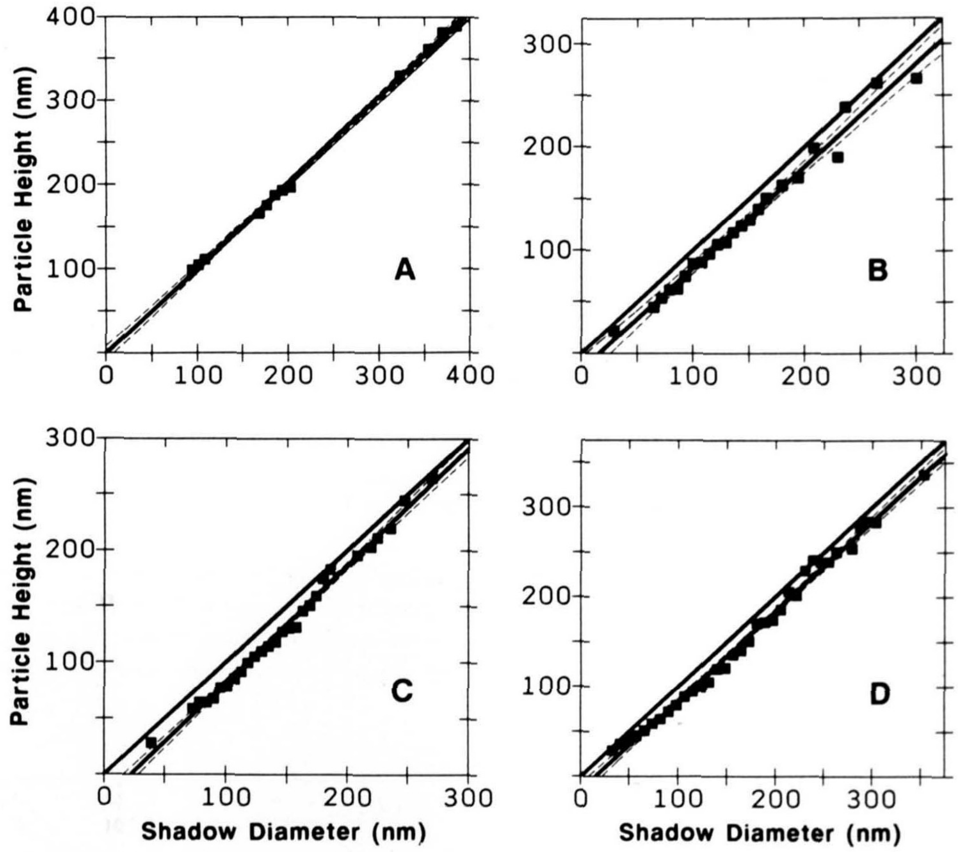

Fig. 4. Relationships between shadow diameters and particle heights (■) of A) polystyrene beads, B) EYPC emulsion, C) DSPC emulsion, and D) chylomicrons. The linear regression equation SD = PH · X + B gives the relation between shadow diameter (SD) and particle height (PH). Equations are as follows: polystyrene beads: SD = 1.02 PH − 0.89; EYPC emulsion: SD = 0.98 PH − 15.71; DSPC emulsion: SD = 1.05 PH − 23.71; chylomicrons: SD = 1.00 PH − 15.67. The dashed lines represent the 95% confidence limits ($P < 0.05$).

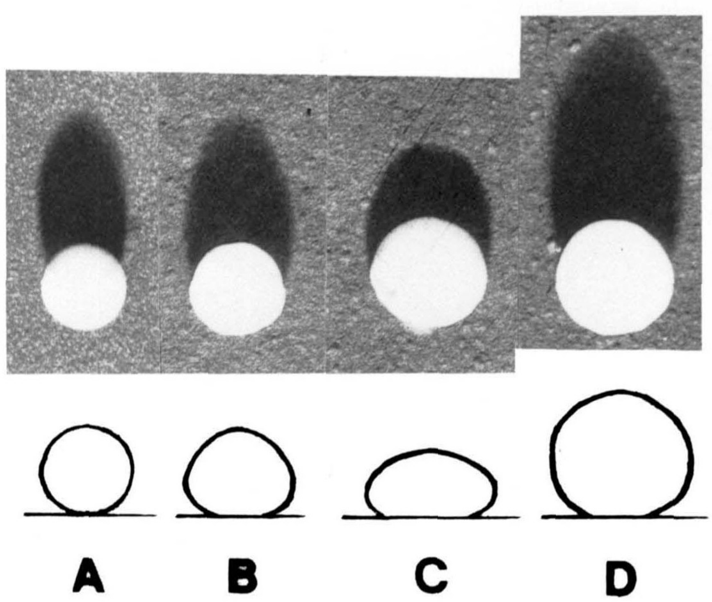

Fig. 5. Vertical profiles of four particles and the shadowed particle from which each was projected; A) polystyrene bead and B–D) EYPC emulsion particles. B is an average case and C and D are extreme cases. An analysis of surface area contact between each particle and the support film appears in Table 1 (× 28,900).

TABLE 1. Comparison of surface area contact between particles and grid support film

Particles[a]	Radii of Oblate Spheroid[b] a	Radii of Oblate Spheroid[b] b	Radius of Sphere[c]	Volume of Sphere	Surface Area of Sphere[d]	Radius of Contact Circle[e]	Area of Contact	Surface Contact as % of Total Particle Surface[f]
	nm		*nm*	$\times 10^6$ nm^2	$\times 10^5$ nm^2	*nm*	$\times 10^3$ nm^2	
Polystyrene bead	n/a	n/a	99.5	4.13	1.24	10.8	0.37	0.3
EYPC emulsion								
1	111.6	93.6	105.3	4.89	1.39	64.8	13.2	9.5
2	129.6	68.4	104.7	4.81	1.38	97.2	29.7	21.5
3	140.4	131.5	137.3	10.80	2.37	64.8	13.2	5.6

[a]These four particles are shown in Fig. 5 as follows: polystyrene bead (from 5A); EYPC emulsion particles 1-3 (5B-5D).
[b]Radius "a" equals one-half shadow diameter; radius "b" equals one-half particle height.
[c]Values for sphere radii of emulsions were obtained by assuming that volumes of oblate spheroids ($4/3\pi a^2 b$) and spheres ($4/3\pi r^3$) were equal; see Methods.
[d]Surface area equals $4\pi r^2$.
[e]These values are estimates because the projections of particle bases in Fig. 5 have been derived from interpolated shadow contours.
[f]Values were obtained by dividing the area of contact (πr^2) by the total particle surface area ($4\pi r^2$) and multiplying by 100.

the particle diameters of emulsions and chylomicrons larger than approximately 150 nm are systematically smaller than the shadow diamters (Fig. 6B, C, D). We explain this phenomenon as follows. When a fixed particle is loaded into the vacuum evaporator it is subjected to a vacuum of the same order of magnitude as in the electron microscope (10^{-6} torr). Hence any volume changes caused by drying or reduced pressure should occur prior to metal

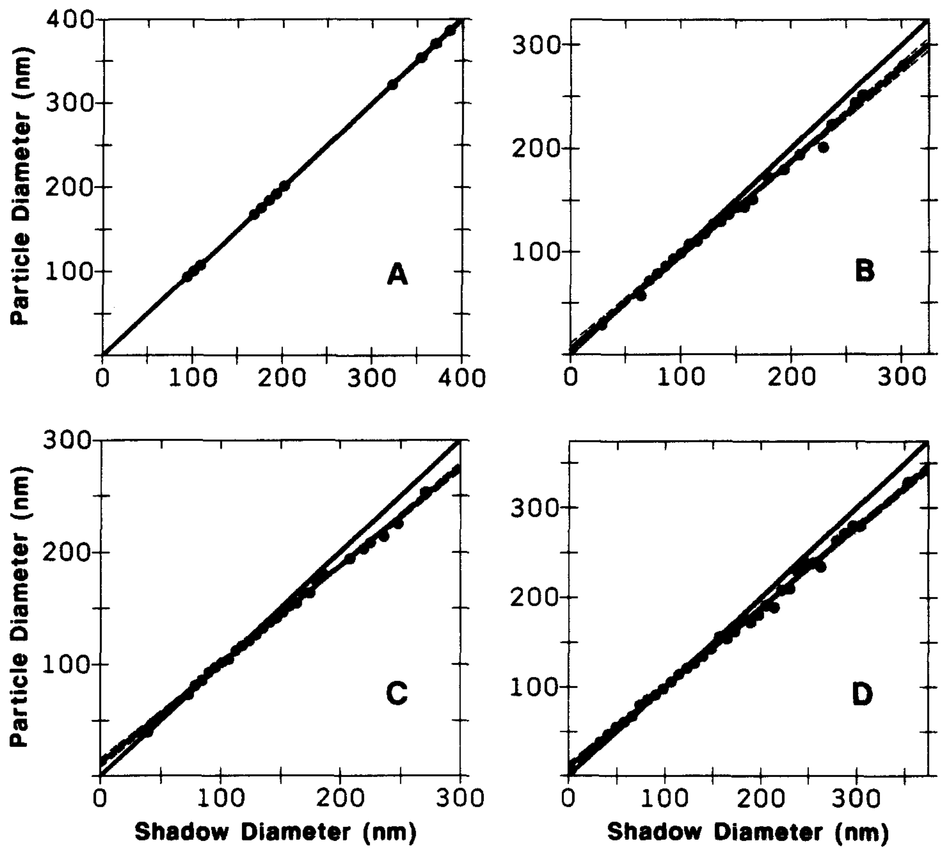

Fig. 6. Relationships between shadow diameters and particle diameters (●) of A) polystyrene beads, B) EYPC emulsion, C) DSPC emulsion, and D) chylomicrons. The linear regression equation SD = PD · X + B gives the relation between shadow diameter (SD) and particle diameter (PD). Equations are as follows: polystyrene beads: SD = 1.01 PD − 0.38; EYPC emulsion: SD = 0.91 + 5.52; DSPC emulsion: SD = 0.88 PD + 11.40; chylomicrons: SD = 0.89 PD + 10.09. The dashed lines represent the 95% confidence limits ($P < 0.05$).

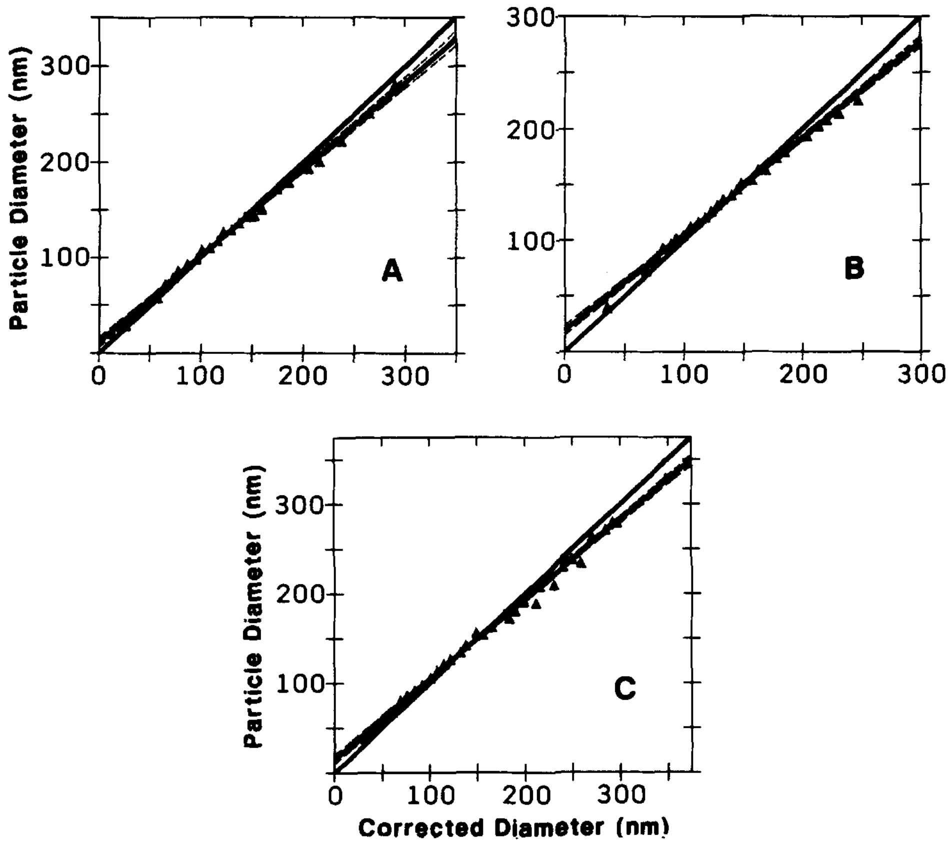

Fig. 7. Relationships between particle diameters and corrected diameters (▲) of A) EYPC emulsion, B) DSPC emulsion, and C) chylomicrons. The linear regression equation CD = PD·X + B gives the relation between corrected diameter (CD) and particle diameter (PD). Equations are as follows: EYPC emulsion: CD = 0.91 PD + 11.28; DSPC emulsion: CD = 0.86 PD + 19.01; chylomicrons: CD = 0.89 PD + 15.23. The dashed lines represent the 95% confidence limits ($P < 0.05$).

shadowing. Further, if any distortion, deformation, or shrinkage occurs during the evaporation of platinum, the resultant shadows should be indistinct and difficult to measure; this was not the case. We conclude, therefore, that the reduction in particle size occurred subsequent to shadowing and was probably due to an effect of irradiation on the triolein-rich core. This effect (shrinkage) would be expected to be most apparent in larger particles, which have larger core:surface ratios.

Overall, the relationships between measured particle diameters and the corrected diameters were similar for both emulsions and chylomicrons (**Fig. 7**). Measured diameters of particles < 100 nm overestimate the corrected diameters, because the particles have flattened and widened. However, the measured diameters of particles > 175 nm underestimate corrected diameters, probably because the decrease in diameter caused by particle shrinkage during irradiation is greater than the increase in diameter due to

TABLE 2. Comparison of mean shadow, particle and corrected diameters among chylomicrons, EYPC and DSPC triolein emulsions

Diameter	Chylomicrons (n = 213)	EYPC Emulsion (n = 88)	DSPC Emulsion (n = 268)
		nm	
Shadow	114.6 ± 64.9	120.6 ± 40.1	121.6 ± 29.2
Particle	112.3 ± 57.3	116.0 ± 41.7	119.4 ± 25.7
Corrected	109.8 ± 64.4	114.2 ± 38.7	114.5 ± 29.7

[a]Mean ± SD. Electron micrographs and size distribution histograms of these particles appear in Fig. 2 and 3, respectively. Comparisons of mean differences between particle and corrected diameters, particle and shadow diameters, or shadow and corrected diameters, by paired *t*-tests indicated significant differences ($P < 0.001$).

flattening. Mean differences between particle diameters and corrected diameters of EYPC and DSPC emulsions and chylomicrons were statistically significant ($P < 0.001$) as determined by paired *t*-tests (**Table 2**). However, comparison of the mean particle and mean corrected diameters within each preparation indicates that the opposing effects of shrinkage and flattening over the whole population are approximately equal (Table 2).

Relatively small errors in radius measurements represent larger errors in calculated particle areas (proportional to r^2) and particle volumes (proportional to r^3). For example, four chylomicron particles had particle diameters of 191 nm and corrected diameters of 198 nm, a difference of 3.5%. Differences in surface areas and volumes would be 6.9% and 10.2%, respectively.

In summary, osmium-fixed EYPC and DSPC emulsions and chylomicons are not rigid spheres when resting on grid surfaces. Instead, these particles flatten and widen to become slightly truncated oblate spheroids. The particle flattening is independent of particle size and of surface phospholipid physical state. Differences in the amount of flattening of two emulsion particles of identical composition and similar size can be explained by variation in the adhesion of these particles to the grid. Shrinkage of chylomicrons and EYPC and DSPC emulsions is attributed to irradiational effects on the triolein cores. Direct particle measurement of a population of fixed chylomicrons or DSPC or EYPC emulsions is a reasonably accurate method to determine the mean of that population. However, measurements of subpopulations of very small or very large particles are less accurate.

We thank Dr. T. E. Felker for critical discussion of the manuscript. The assistance with statistical analysis by Suzette Levenson, Data Coordinating Center, School of Public Health, BUMC, was appreciated. Excellent technical help with preparation of figures was kindly provided by John Steiner, Dept. of Biophysics, BUSM. We also thank Irene L. Miller for assistance with preparation of the manuscript. This work was supported by National Institutes of Health research grant HL-26335 and training grant HL-07291.

Manuscript received 9 June 1989 and in revised form 19 September 1989.

REFERENCES

1. Salpeter, M. M., and D. B. Zilversmit. 1968. The surface coat of chylomicrons: electron microscopy. *J. Lipid Res.* **9:** 187-192.
2. Schoefl, G. I. 1968. The ultrastructure of chylomicra and of the particles in an artificial fat emulsion. *Proc. R. Soc. Lond. B. Biol. Sci.* **169:** 147-152.
3. Fraser, R. 1970. Size and lipid composition of chylomicrons of different Svedberg units of flotation. *J. Lipid Res.* **11:** 60-65.
4. Blanchette-Mackie, E. J., and R. O. Scow. 1976. Retention of lipolytic products in chylomicrons incubated with lipoprotein lipase: electron microscope study. *J. Lipid Res.* **17:** 57-67.
5. Bhattacharya, S., and T. G. Redgrave. 1981. The content of apolipoprotein B in chylomicron particles. *J. Lipid Res.* **22:** 820-828.
6. Forte, T. M., and R. W. Nordhausen. 1986. Electron microscopy of negatively stained lipoproteins. *Methods Enzymol.* **128:** 442-457.
7. Glomset, J. A., K. Applegate, T. Forte, W. C. King, C. D. Mitchell, K. R. Norum, and K. Gjone. 1980. Abnormalities in lipoproteins of d < 1.006 g/ml in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **21:** 1116-1127.
8. Forte, T. M., and L. A. Carlson. 1984. Electron microscopic structure of serum lipoproteins from patients with fish eye disease. *Arteriosclerosis.* **4:** 130-137.
9. Du Plessis, J., L. R. Tiedt, C. J. van Wyk, and C. Ackermann. 1986. A new transmission electron microscope method for the determination of particle size in parenteral fat emulsions. *Int. J. Pharmaceut.* **34:** 173-174.
10. Hamilton-Attwell, V. L., J. du Plessis, and C. J. van Wyk. 1987. A new scanning electrron microscope (SEM) method for the determination of particle size in parenteral fat emulsions. *J. Microsc.* **145:** 347-349.
11. Connelly, P. W., and A. Kuksis. 1981. Effect of core composition and particle size of lipid emulsions on apolipoprotein transfer of plasma lipoproteins in vivo. *Biochim. Biophys. Acta.* **666:** 80-89.
12. Forte, T., and A. V. Nichols. 1972. Application of electron microscopy to the study of plasma lipoprotein structure. *Adv. Lipid Res.* **10:** 1-41.
13. Jones, A. L., and J. M. Price. 1968. Some methods of electron microscopic visualization of lipoproteins in plasma and chyle. *J. Histochem. Cytochem.* **15:** 366-370.
14. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757-768.
15. Hayes, T. L., and J. E. Hewitt. 1957. Visualization of individual lipoprotein macromolecules in the electron micriscope. *J. Appl. Physiol.* **11:** 425-428.
16. Bierman, E. L., T. L. Hayes, J. N. Hawkins, A. M. Ewing, and F. T. Lindgren. 1966. Particle-size distribution of very low density plasma lipoproteins during fat absorption in man. *J. Lipid Res.* **7:** 65-72.
17. Fraser, R., W. J. Cliff, and F. C. Courtice. 1968. The effect of dietary fat load on the size and composition of chylomicrons in thoracic duct lymph. *J. Exp. Physiol.* **53:** 390-398.
18. Ockner, R. K., and A. L. Jones. 1970. An electron microscopic and functional study of very low density lipoproteins in intestinal lymph. *J. Lipid Res.* **11:** 284-292.
19. Small, D. M., J. W. Steiner, A. Derksen, and S. Bennett Clark. 1988. Thermal transitions of phosphatidylcholines on the surface of lipoprotein-like emulsion particles. *Biophys. J.* **53:** 211a.
20. Derksen, A., and D. M. Small. 1989. Interaction of apoA-1 and apoE-3 with triglyceride-phospholipid emulsions containing increasing cholesterol concentrations. Model of triglyceride-rich nascent and remnant lipoproteins. *Biochemistry.* **28:** 900-906.
21. Derksen, A., S. Ekman, and D. M. Small. 1989. Oleic acid allows more apoprotein A-I to bind with higher affinity to large emulsion particles saturated with cholesterol. *J. Biol. Chem.* **264:** 6935-6940.

22. Bennett Clark, S. 1978. Chylomicron composition during duodenal triglyceride and lecithin infusion. *Am. J. Physiol.* **235:** E185–E190.
23. Bangs, L. B. 1985. Uniform Latex Particles. 2nd ed. Seragen Diagnostics, Inc., Indianapolis, IN. 11–13.
24. Analytical Reference Particles. Duke Scientific Corp., Palo Alto, CA. Bulletin 83. 2.
25. Dubochet, J., and M. Groom. 1982. The mounting of macromolecules for electron microscopy with particular reference to surface phenomena and the treatment of support films by glow discharge. *Adv. Opt. Electron Microsc.* **8:** 107–135.
26. Reissig, M., and S. A. Orrell. 1970. A technique for the electron microscopy of protein-free particle suspensions by the negative staining method. *J. Ultrastruct. Res.* **32:** 107–117.
27. Hall, C. E. 1960. Measurement of globular protein molecules by electron microscopy. *J. Biophys. Biochem. Cytol.* **7:** 613–618.
28. Slayter, E. M. 1969. Electron microscopy of globular proteins. *In* Physical Principles and Techniques of Protein Chemistry. S. G. Leach, editor. Academic Press, New York. 1–58.
29. Misra, D. N., and N. N. Das Gupta. 1965. Distortion in dimensions produced by shadowing for electron microscopy. *J. R. Microsc. Soc.* **84:** 373–384.
30. Miller, K. W., and D. M. Small. 1987. Structure of triglyceride-rich lipoproteins: an analysis of core and surface phases. *In* Plasma Lipoproteins. A. M. Gotto, Jr. editor. New Comprehensive Biochemistry, Vol. 14. Elsevier, Amsterdam, The Netherlands. 1–75.