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Comparison, by Freeze-Fracture Electron Microscopy, of Chromatophores, Spheroplast-Derived Membrane Vesicles, and Whole Cells of *Rhodopseudomonas sphaeroides*

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By using freeze-fracture electron microscopy, chromatophores and spheroplast-derived membrane vesicles from photosynthetically grown *Rhodopseudomonas sphaeroides* were compared with cytoplasmic membrane and intracellular vesicles of whole cells. In whole cells, the extracellular fracture faces of both cytoplasmic membrane and vesicles contained particles of 11-nm diameter at a density of about 5 particles per 10^4 nm^2. The protoplasmic fracture faces contained particles of 11 to 12-nm diameter at a density of 14.6 particles per 10^4 nm^2 on the cytoplasmic membrane and a density of 31.3 particles per 10^4 nm^2 on the vesicle membranes. The spheroplast-derived membrane fraction consisted of large vesicles of irregular shape and varied size, often enclosing other vesicles. Sixty-six percent of the spheroplast-derived vesicles were oriented in the opposite way from the intracellular vesicle membranes of whole cells. Eighty percent of the total vesicle surface area that was exposed to the external medium (unenclosed vesicles) showed this opposite orientation. The chromatophore fractions contained spherical vesicles of uniform size approximately equal to the size of the vesicles in whole cells. The majority (79%) of the chromatophores purified on sucrose gradients were oriented in the same way as vesicles in whole cells, whereas after agarose filtration almost all (97%) were oriented in this way. Thus, on the basis of morphological criteria, most spheroplast-derived vesicles were oriented oppositely from most chromatophores.

Several photosynthetic bacteria possess an extensive internal vesicular membrane system, which houses all or part of the components of the photosynthetic apparatus (14). The intracellular vesicles are believed to be part of a continuous membrane system which includes the cell surface cytoplasmic membrane (7, 9). Thus, it appears that the intracellular vesicles are formed as a consequence of invagination of the cytoplasmic membrane.

Two principal methods of isolating this membrane system are used. The first, involving French-press or sonic disruption of cells, produces discrete, closed vesicles, termed "chromatophores," which are capable of catalyzing several reactions related to photosynthetic metabolism (6, 18). Recently, a second method involving osmotic lysis of spheroplasts has also been used (8, 13).

In *Rhodopseudomonas sphaeroides*, accumulating biochemical evidence indicates that chromatophores are oriented in the same way as the intracellular vesicles and opposite to the cytoplasmic membrane, whereas spheroplast-derived vesicles are oriented in the reverse manner, that is, in the same way as the cytoplasmic membrane and opposite to the intracellular vesicles. For example, Hellingwerf et al. (8) obtained membrane vesicles of *R. sphaeroides* after osmotic lysis which were capable of light-energized transport of amino acids in the same direction as whole cells. Chromatophores, however, demonstrate an opposite polarity to whole cells in the translocation of protons and ions (11, 19). Matsuura and Nishimura (13) recently demonstrated that the shift in the carotenoid absorption band induced by potassium ions in chromatophores is opposite to that induced in spheroplast-derived vesicles; this evidence indicates possible opposite membrane orientation in the two preparations, since there is evidence that the carotenoid spectral change depends on the polarity of the membrane potential (10). Finally, Prince et al. (16) demonstrated that cytochrome c_2 molecules are localized both within the internal space of isolated chromatophores and in the intracellular vesicle compartments of whole cells, which are continuous with the periplasmic space. Thus, on the basis of cytochrome c_2 lo-

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cation, it appears that chromatophores are oriented in the same direction as the intracellular vesicles in vivo.

The technique of freeze-fracture coupled to electron microscopy has proven invaluable for evaluating the orientation of membrane vesicles isolated from erythrocytes (20) and *Escherichia coli* (1). The value of the technique lies in the fact that freeze-fracturing cleaves the central, hydrophobic plane of biological membranes, resulting in the exposure of inner half-membrane faces (3). The exposed faces are characterized by particles believed to be protein complexes embedded in the membrane, and the asymmetric distribution of the particles observed on the half-faces serves as markers for the respective membrane halves. By correlating the particle distribution with the convexity or concavity of the membrane halves, conclusions about the orientation of the vesicles can be formulated.

In view of the recent biochemical evidence on the chromatophores and spheroplast-derived vesicles of *R. sphaeroides*, we sought to independently investigate and quantitate the orientation of these membrane preparations by using the technique of freeze-fracture electron microscopy. Our results indicate that, on the basis of morphological criteria, most chromatophores are oriented similarly to most intracellular vesicles and oppositely from the majority of spheroplast-derived vesicles.

MATERIALS AND METHODS

Organism, growth conditions, and harvesting procedures. *R. sphaeroides* (NCIB 8253) was grown photosynthetically under semi-anaerobic conditions. One-liter-capacity serum bottles were filled to capacity with MG medium (12) and inoculated with 2 ml of an active culture. The bottles were capped and incubated at 30°C with an incident light intensity of approximately 20,000 lx. During midlogarithmic growth, when the cell cultures attained an optical density of approximately 1.0 measured at 680 nm (1-cm path length), the cells were harvested by centrifugation at 3,000 × *g* for 10 min. These cells contained about 8 nmol of bacteriochlorophyll per mg (dry weight) of cells. The cells were suspended in either 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5) or 20 mM potassium phosphate (pH 7.5) to an optical density of 40 measured at 680 nm. Harvesting procedures were performed between 0 and 5°C.

Preparation of spheroplast-derived membrane vesicles. Ten milliliters of fresh cell suspension was diluted with 5.5 ml of distilled water. With slow stirring at 37°C, the following were added in order: 5 ml of 1 M Tris-hydrochloride (pH 8.0), 25 ml of 40% (wt/vol) sucrose, 2.5 ml of lysozyme (10 mg/ml of water, Sigma, grade I), and 2 ml of 0.05 M ethylenediaminetetraacetic acid. After 30 min of incubation, spheroplasts were evident by examination with a light microscope; then 75 ml of 10 mM Tris-hydrochloride (pH 8.0) was added with stirring at room temperature. Brij-58 was then added to a final concentration of 0.1% (wt/vol), and the mixture was incubated for an additional 30 min at room temperature.

Two milliliters of 0.1 M $MgCl_2$ and 1 to 5 mg of deoxyribonuclease I (Sigma, crude) were added to the crude extract. The mixture was incubated for 30 min at 37°C with gentle stirring. The crude extract was then centrifuged at 3,000 × *g* for 5 min, and the pigmented supernatant was recovered and centrifuged at 48,000 × *g* for 30 min. The pellet was suspended in 10 mM Tris-hydrochloride (pH 7.5) and gently homogenized with a Dounce homogenizer. The suspension was then layered onto 30 to 55% (wt/vol) linear sucrose gradients made up in the same buffer and centrifuged for about 10 h in a Beckman SW41 rotor at 150,000 × *g*. The pigmented material sedimenting at approximately 38% (wt/vol) sucrose was recovered and washed by suspension in 10 mM Tris-hydrochloride (pH 7.5) and centrifugation at 48,000 × *g* for 30 min.

Preparation of chromatophores. Chromatophores were prepared by two different methods. Method 1 (sucrose gradient): Suspended cells were disrupted by one passage through a French press cell at 1,265 kg/cm^2. Approximately 200 μg of deoxyribonuclease I was added per ml, and the crude extract was centrifuged at 10,000 × *g* for 20 min. The supernatant was recovered and centrifuged at 150,000 × *g* for 1 h. The crude membrane pellet was suspended in 10 mM Tris-hydrochloride (pH 7.5) and gently homogenized in a Dounce homogenizer. The suspension was then layered onto 30 to 55% (wt/vol) linear sucrose gradients prepared in 10 mM Tris-hydrochloride (pH 7.5) and centrifuged for 10 h at 150,000 × *g*. The pigmented chromatophore fraction sedimenting at approximately 38% (wt/vol) sucrose was recovered, suspended in buffer, and centrifuged at 150,000 × *g* for 1 h.

Method 2 (gel filtration): Chromatophores were also purified by a modification of the method described by Fraker and Kaplan (5). The suspended and homogenized crude membrane fraction obtained as described above for method 1 of chromatophore preparation was layered onto a column bed (0.9 by 60 cm) of Bio-Gel A-150M (100 to 200-mesh, Bio-Rad) and eluted with 10 mM Tris-hydrochloride (pH 7.5). The peak fractions of pigmented material were pooled and concentrated by centrifugation at 150,000 × *g* for 1 h.

The procedures for preparing spheroplast-derived vesicles and chromatophores were performed between 5 and 10°C. In some experiments, all Tris-hydrochloride buffers used in the procedures for membrane preparations were replaced with potassium phosphate buffers of equivalent ionic strength and pH.

Freeze-fracture. Cells or vesicles were suspended in 20 to 100 mM potassium phosphate buffer (pH 7.5), and glutaraldehyde was added to give a concentration of 4% (vol/vol). After 1.5 to 2.5 h of fixation at about 10°C, sufficient glycerol in unbuffered glutaraldehyde was added slowly, over 20 to 30 min, with mixing to give a final concentration of 4% (vol/vol) glutaraldehyde and 30% (vol/vol) glycerol in 10 mM potassium phosphate buffer. Fixed material was centrifuged, and small samples of the pellet were rapidly frozen in

aluminum cups by plunging into liquid Freon 22 at −160°C. Frozen samples were transferred rapidly with a prechilled specimen wrench to the specimen mounting post, maintained at −140°C, of a Denton freeze-fracture apparatus. During pumping to high vacuum (15 to 20 min), the specimen temperature was raised to −105°C, and the blade was held firmly against the liquid nitrogen-cooled shroud. From one to five specimens, maintained at −105 to −115°C, were then rapidly fractured with the cold blade and replicated immediately after the last fracture. Replicas were cleaned with commercial sodium hypochlorite (Clorox) and distilled and filtered water.

Electron microscopy. Specimens were examined with either a JEM 100B electron microscope at an accelerating voltage of 80 kV or a Zeiss EM-9S at 50 kV. Microscope magnification was calibrated using a carbon grating replica (E. F. Fullam) and did not vary more than 3%.

Measurements. Vesicle dimensions were measured on micrographs enlarged to between ×40,000 and ×55,000. Measurements of vesicle dimension on freeze-fractured material produce an underestimate of true dimension but were performed here for comparative purposes. Particle population density was counted on micrographs enlarged to between ×100,000 and ×130,000. Particle dimensions were measured perpendicular to the direction of shadowing on micrographs enlarged to ×200,000; a Camrex ×5 magnifier was used. To compensate partially for different amounts of etching and shadowing, measurements of particles from different membrane halves from any one given preparatory method were made, as far as possible, on the same micrographs. However, the varying angles of fracture within any one micrograph and the corresponding differences in accumulation of shadow introduce an error in particle measurement for which it was not possible to compensate.

RESULTS

Whole cells. The main structural features seen in freeze-fractured whole cells of *R. sphaeroides* correspond to those described by previous workers (4, 21) in thin sections. The outer membrane of the bacterium was usually cross-fractured (Fig. 1 and 2), and its cleaved surfaces were rarely seen. However, the cleavage plane frequently exposed the extracellular fracture (EF) face and the protopasmic fracture (PF) face (terminology of Branton et al. [2]) of the cytoplasmic membrane.

The EF face of the cytoplasmic membrane is a concave face with a population of sparsely (4.8 particles per 10^4 nm^2) and randomly distributed small particles with a mean diameter of 10.7 nm (Fig. 1; Tables 1 and 2). This face is marked by several raised areas, which represent indentations of the membrane into the cell cytoplasm; the raised areas also often exhibit the small particles which are characteristic of the other regions of this membrane half.

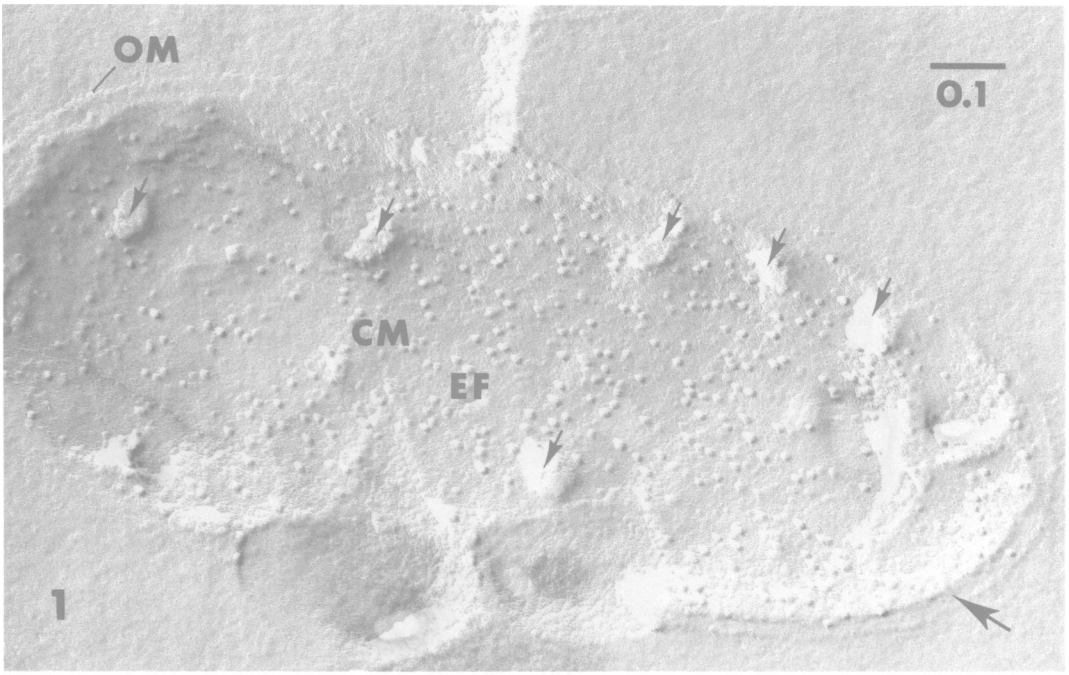
The PF face of the cytoplasmic membrane is a convex face with a population of densely (14.6 particles per 10^4 nm^2) and randomly distributed small particles with a mean diameter of 11.4 nm (Fig. 2; Tables 1 and 2). This face is marked by several depressions of about the same size as the raised areas on the EF face. These depressions sometimes appear to be devoid of particles, but when the depressions are viewed in a cross-fractured profile, they are seen to be lined with densely packed particles (Fig. 2). The depressions on the PF face and the raised areas on the EF face are interpreted to be areas where intracellular vesicles are forming or have formed by invagination of the cytoplasmic membrane.

The cytoplasm contains small scattered single particles, occasional smooth-surfaced spherical bodies of diameters varying from 50 to 180 nm found in the central or the peripheral cytoplasm, and membrane-bound vesicles largely confined to the peripheral cytoplasm (Fig. 2 to 4). The vesicles are roughly spherical, with an average length of 74 nm and an average width of 59 nm (Table 3).

The membrane fracture faces of these intracellular vesicles resembled very closely the fracture faces of the cytoplasmic membranes, except that their concavity and convexity were reversed (Fig. 3 and 4). The convex fracture face, that is, the fracture face of the interior half of the vesicle membrane or the EF face, usually had a sparse population of particles (5.2 particles per 10^4 nm^2) with an average diameter of 10.5 nm. The concave or PF face usually had a dense population of particles with an average diameter 12.2 nm; these particles were more densely packed (31.3 particles per 10^4 nm^2) than those on the cytoplasmic membrane (Tables 1 and 2).

From an analysis of 209 spherical vesicles including the smooth-surfaced bodies from whole cell preparations, 72% were found to have either a convex (EF) face with sparse particles (convex-sparse) or a concave (PF) face with densely distributed particles (concave-dense) (Table 4). The smooth-surfaced bodies, which were often larger and more centrally located than typical photosynthetic vesicles (Fig. 4), may represent structural elements in the cell not related to the photosynthetic membrane system, for example, poly-β-hydroxybutyrate granules or gas vacuoles. If these were eliminated from the analysis, then 88% of the remaining vesicles showed an orientation of either convex-sparse or concave-dense.

Spheroplast-derived vesicles. The pigmented membrane fraction isolated after osmotic lysis of spheroplasts and subsequent density gradient centrifugation was characterized by vesicles of irregular shape and varied size, which often appeared to enclose other vesicles (Fig. 5,



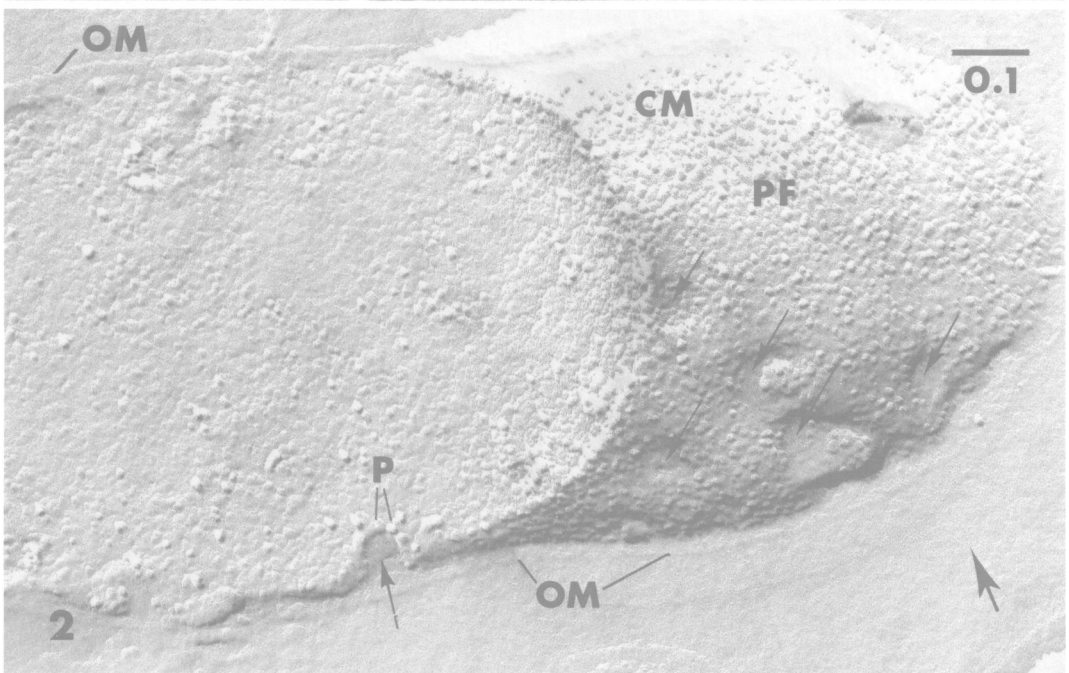


FIG. 1. *Freeze-fracture replica of whole cell. Fracturing has exposed the EF face (outer leaflet) of the cytoplasmic membrane (CM). Raised areas of the cytoplasmic membrane are marked by arrows. The outer membrane (OM) is seen in profile. 97,000×. The bar in the upper right of all figures equals 0.1 or 0.2 μm, as designated. The arrow in the lower right indicates the shadowing direction.*

FIG. 2. *Replica of whole cell showing the PF face (inner leaflet) of the cytoplasmic membrane (CM) as well as fractured cytoplasm. Depressions (arrows) occur in the cytoplasmic membrane, and particles (P) are sometimes seen to line the depressions. Outer membrane (OM). 97,000×.*

Tables 3 and 4). The enclosed vesicles were sometimes nested one inside another. Some of the apparently enclosed vesicles must be the result of infoldings of the surface of autonomous vesicles; such infoldings were occasionally seen in profile (Fig. 5). The average length of all the vesicles, enclosed and unenclosed, was 140 nm, and their average width was 100 nm (Table 3).

Analysis of about 400 vesicles of this fraction (Table 4) revealed that the majority have the reverse orientation from vesicles observed in whole cells, that is, 66% were either convex with densely distributed particles (convex-dense) or concave with sparse particles (concave-sparse).

To estimate the orientation of only the membrane surface area which was exposed to the external medium, only unenclosed vesicles were considered next. Estimation of the membrane surface area of these vesicles combined with scoring of their orientation showed that about 80% of this exposed membrane surface area was oriented in the convex-dense or concave-sparse manner.

Sparse particles were distributed with a density equal to that in the whole cell membranes. However, on densely populated surfaces, particles were present at a density of 27.9 particles per 10^4 nm^2 (Table 1), a value between those observed for cytoplasmic and vesicle membranes in whole cells. As in the whole cells, the sparse particles were slightly smaller in diameter than the densely distributed particles (Table 2). Vesicle preparations isolated in Tris-hydrochloride or in phosphate buffers were not observed to differ from each other in morphology or vesicle orientation.

Chromatophores. The chromatophore fraction isolated from sucrose gradients after French-press cell disruption contained nearly spherical individual vesicles of slightly variable size (Fig. 6; Table 3). Their average dimensions were 79 by 65 nm, and only 1% were enclosed in other vesicles (Table 4). Their orientation was similar to that found in the whole cells, that is, 79% were either convex-sparse or concave-dense (Table 4). Often very large vesicles, fragments of membranes, and occasional fragments of cells enclosing cytoplasm were seen in this fraction.

The chromatophore fraction purified by agarose filtration after French-press disruption of cells was characterized by spherical individual vesicles of more uniform size (Fig. 7; Table 3). Their average length was 67 nm, slightly smaller than that in whole cells, and their average width was 61 nm. No vesicles were enclosed in other vesicles, and 97% were oriented in the convex-sparse or concave-dense manner, that is, similarly to vesicles in whole cells (Table 4). The fraction contained only rare small membrane fragments.

In both chromatophore preparations, the sparse particles were slightly smaller than the densely distributed particles, and the population densities of particles were similar to those of spheroplast-derived vesicles (Tables 1 and 2). As in the case with spheroplast-derived vesicles, chromatophores prepared with potassium phosphate buffers did not differ from those prepared in Tris-hydrochloride buffers.

TABLE 1. *Population density of particles on fractured faces of whole cell membranes, spheroplast-derived vesicles, and chromatophores*

Membrane source	No. of particles per 10^4 nm^2 of half-membrane surface area[a]	
	Sparsely populated surfaces	Densely populated surfaces
Whole cells		
Cytoplasmic membrane	4.8 ± 0.3	14.6 ± 0.7
Vesicles	5.2 ± 1.3	31.3 ± 1.2
Spheroplast-derived vesicles	4.8 ± 0.4	27.9 ± 1.4
Chromatophores		
Sucrose gradient	5.5 ± 1.4	26.7 ± 1.0
Gel filtration	5.4 ± 0.8	27.2 ± 1.5

[a] ±Standard error.

TABLE 2. *Diameters of particles on fractured faces of whole cell membranes, spheroplast-derived vesicles, and chromatophores*

Membrane source	Sparsely populated surfaces		Densely populated surfaces	
	Diameter[a] (nm)	N[b]	Diameter (nm)	N
Whole cells				
Cytoplasmic membrane	10.7 ± 0.1	98	11.4 ± 0.2	97
Vesicles	10.5 ± 0.6	18	12.2 ± 0.3	76
Spheroplast-derived vesicles	11.5 ± 0.2	50	12.4 ± 0.3	50
Chromatophores				
Sucrose gradient	11.9 ± 0.2	50	12.3 ± 0.4	56
Gel filtration	10.7 ± 0.2	50	12.3 ± 0.3	50

[a] Mean ± standard error.
[b] N, Number of particles measured.

DISCUSSION

Whole cells. The only significant difference observed between the cytoplasmic membranes and vesicle membranes of whole cells was that the PF face particles of the vesicles were twice as densely packed as those of the cytoplasmic

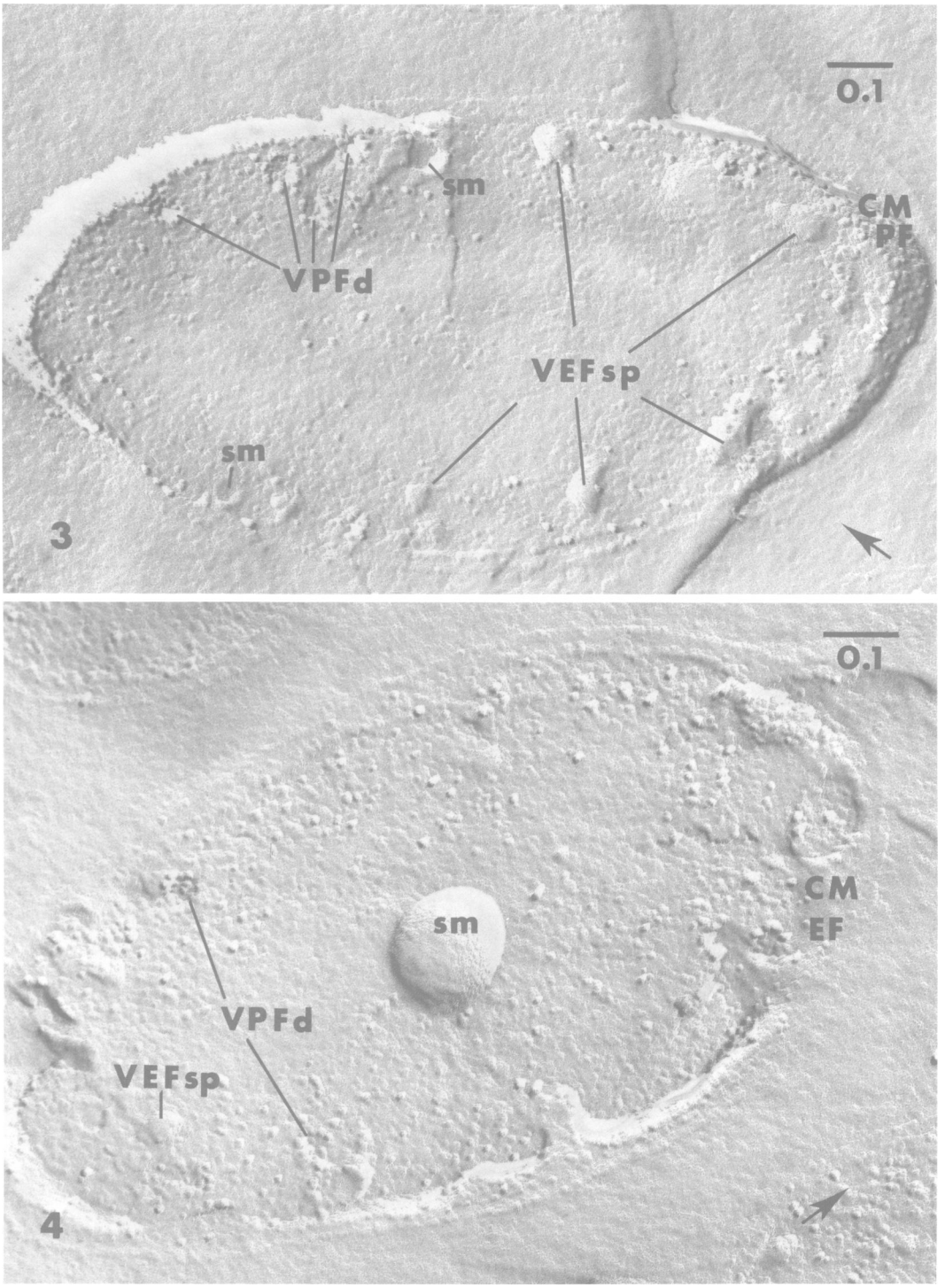


FIG. 3 and 4. *Replicas of whole cells showing several vesicles (V). Fracturing has exposed the concave (PF) face of some vesicles and the convex (EF) face of others. The fracture faces are usually either densely (d) or sparsely (sp) populated with particles. Several smooth-surfaced bodies (sm) are seen. Figure 4 shows the kind of large and centrally located smooth-surfaced body that is frequently seen in cells and is clearly distinct from the photosynthetic vesicles. In Fig. 3, the smooth-surfaced bodies might be considered photosynthetic vesicles, but as concave surfaces they would then usually exhibit a dense particle distribution (see Table 4). PF and EF faces of the cytoplasmic membrane (CM) are shown. Figure 3, 95,000×; Fig. 4, 106,000×.*

membrane. This suggests that condensation of these particles in the vesicle membranes may occur during formation of photosynthetic vesicles from cytoplasmic membrane. In contrast, the EF particles in both kinds of membranes were equally sparsely distributed. All the particles averaged 11 to 12 nm in diameter. (For every membrane type and preparation examined, the densely packed particles were slightly larger than the sparse particles; however, given the errors in particle measurement discussed under Materials and Methods, it is difficult to ascribe any significance to this difference.)

Membrane vesicles. The results presented indicate that by morphological criteria the majority of membrane vesicles derived from spheroplasts are oriented oppositely from the majority of chromatophores. Furthermore, the chromatophores are oriented similarly to intracellular vesicles occurring in whole cells. These findings are consistent with previous biochemical evidence on membrane preparations of *R. sphaeroides* as cited in the introduction. Virtually all of the chromatophores (97%) purified by agarose filtration were oriented in the same direction as the intracellular vesicles, and 66% of the spheroplast-derived vesicles were oriented in the opposite direction. Moreover, about 80% of the membrane area exposed to the medium in spheroplast-derived vesicle preparations was oriented in the opposite direction.

In a previous study, Reed and Raveed (17) observed, by freeze-fracture electron microscopy, chromatophores obtained from strain R-26 of *R. sphaeroides*, a mutant incapable of synthesizing carotenoids. Fractures of chromatophores of this strain produced rough faces, covered with 42 particles per 10^4 nm^2 with diameters of about 13 nm, and smooth faces with very few particles. From their micrographs, the rough faces appear to be largely concave and the smooth faces appear to be largely convex. These results are similar to observations made in the present study on chromatophores from a wild-type strain. Similar observations have been made with membrane preparations derived from French-press cell extracts of a related organism, *Rhodospirillum rubrum* (15).

The chromatophore preparations isolated on sucrose density gradients were more heterogeneous in vesicle size and orientation than the preparations isolated after agarose filtration. The ability to recover more homogeneous chromatophores after agarose filtration suggests a relationship between vesicle size and orientation in the chromatophore preparations. For spheroplast-derived vesicles, however, preliminary analysis of the size distribution and orientation did not reveal a similar relationship (data not

TABLE 3. *Vesicle dimensions in whole cells and in spheroplast-derived and chromatophore preparations*

Vesicle source	Length[a] (nm)	Width[a] (nm)	No. of vesicles measured	Avg of length and width (nm)
Whole cells	74 ± 2	59 ± 1	196	67
Spheroplast-derived vesicles	140 ± 6	100 ± 3	199	120
Chromatophores				
Sucrose gradient	79 ± 2	65 ± 1	201	72
Gel filtration	67 ± 1	61 ± 1	200	64

[a] Mean ± standard error.

TABLE 4. *Orientation of vesicles in whole cells and in spheroplast-derived and chromatophore preparations*[a]

Vesicle source	Orientation									
	Convex			Concave			Convex-dense + Concave-sparse (%)	Convex-sparse + Concave-dense (%)	Enclosed (%)	No. of vesicles analyzed
	Dense (%)	Sparse (%)	Smooth (%)	Dense (%)	Sparse (%)	Smooth (%)				
Whole cells	1	26	10	46	9	7	10	72	0	209
Corrected[b]	2	32		56	11		13	88	0	174
Spheroplast-derived vesicles	46	9	1	21	20	3	66	30	32	399
Chromatophores										
Sucrose gradient	12	22	1	57	6	3	18	79	1	200
Gel filtration	2	34	0	63	1	1	3	97	0	200

[a] The number of vesicles of each orientation is expressed as a percentage of the total number of vesicles analyzed from each source.

[b] Results after removing smooth-surfaced bodies from whole cell vesicle counts.

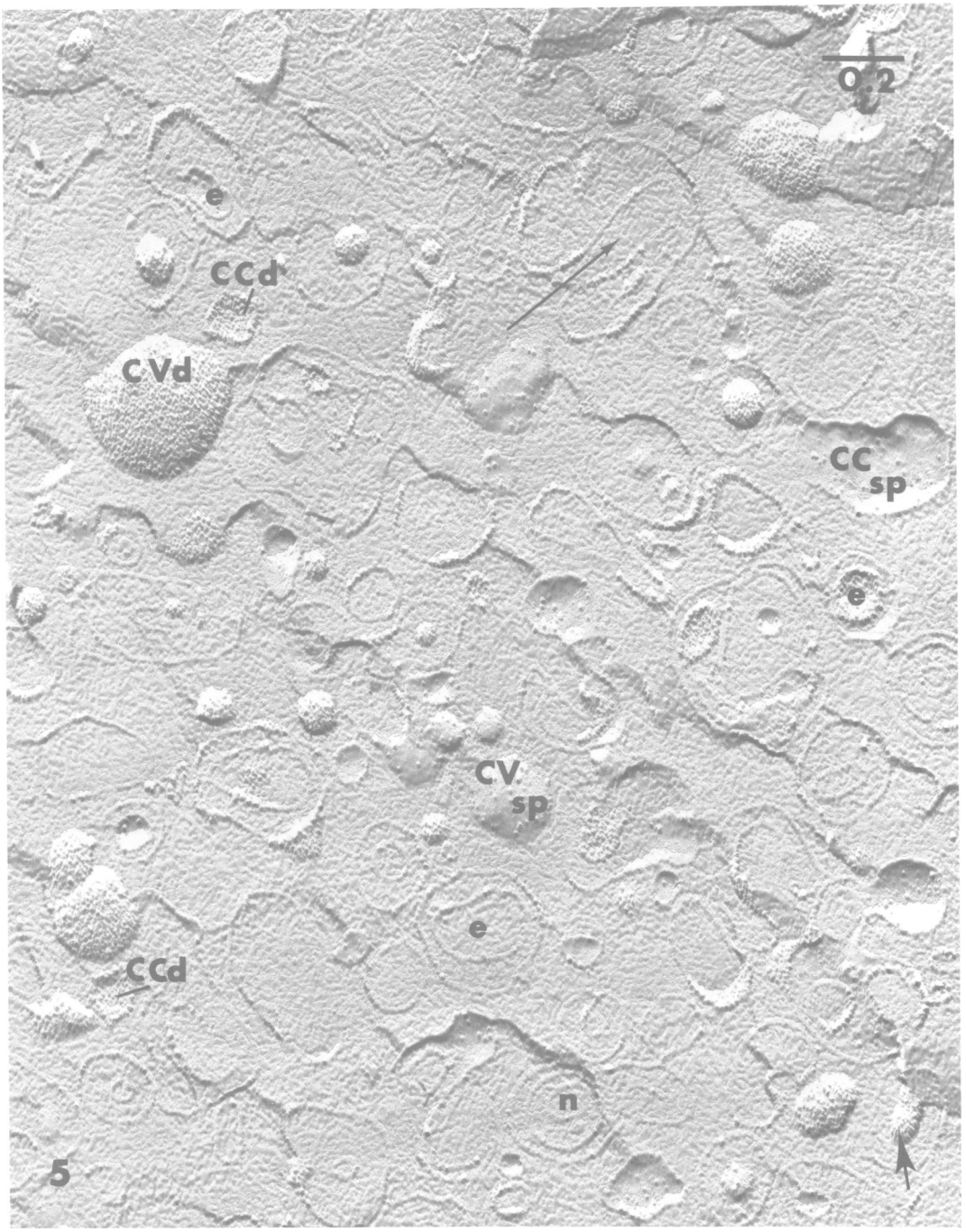


FIG. 5. *Spheroplast-derived vesicles. Concave (CC) and convex (CV) fracture faces are densely (d) and sparsely (sp) populated with particles. Smooth-surfaced bodies are rarely seen. Many vesicles are enclosed (e) in others; sometimes several vesicles are nested (n) inside one another. Small arrow marks an infolded autonomous vesicle. 57,000×.*

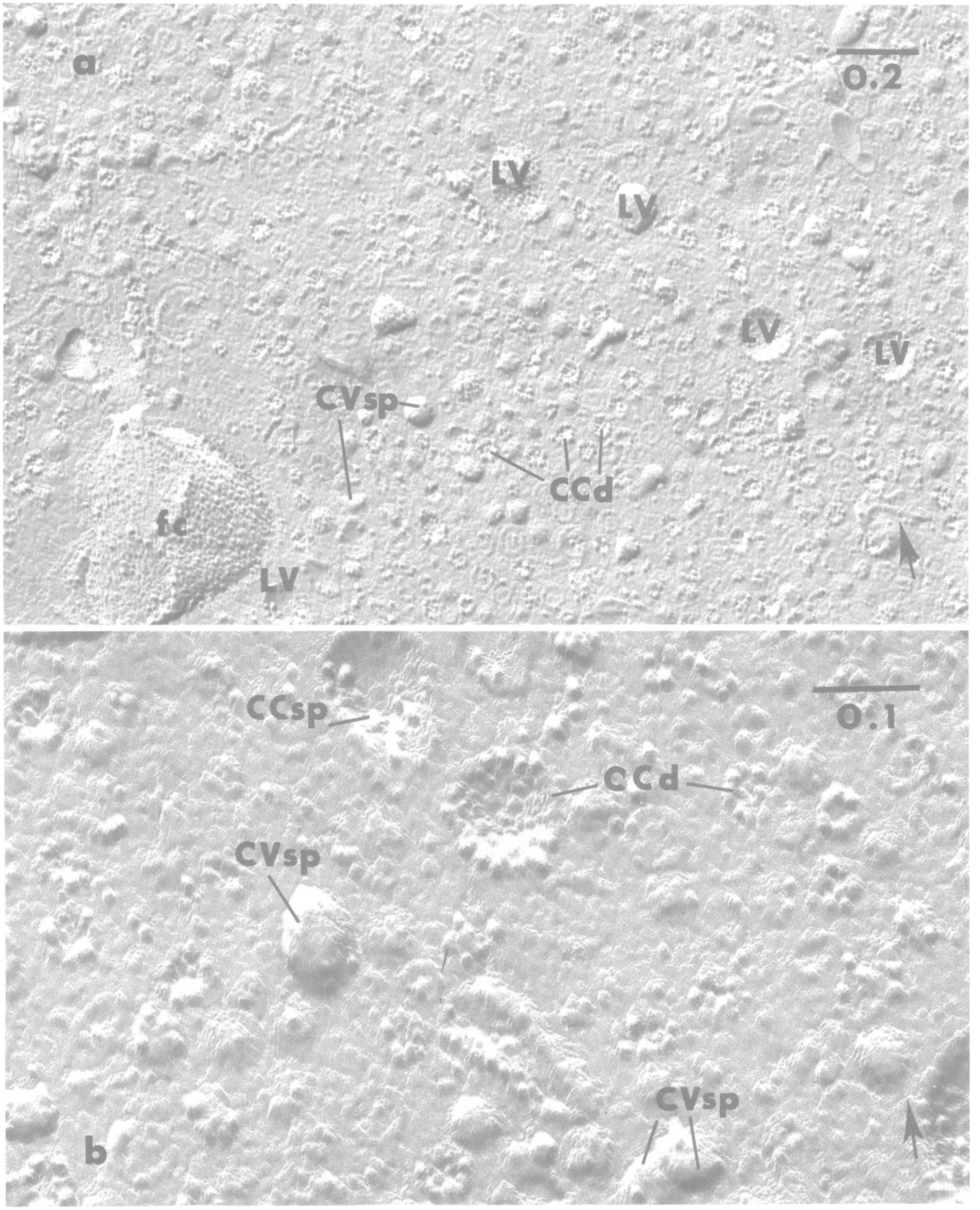


FIG. 6. *Chromatophores prepared by French-press disruption followed by sucrose gradient centrifugation. Most of the concave (CC) faces are densely (d) populated with particles, and most of the convex (CV) faces are sparsely (sp) populated, although the reverse orientations are also seen. Smooth-surfaced bodies are rare. Several large vesicles (LV) (100 to 180 nm in diameter) are present, although most vesicles are smaller. What appears to be a fragment of a cell (fc) including outer membrane, cytoplasmic membrane, and cytoplasm is present. (A) 57,000×; (B) 150,000×.*

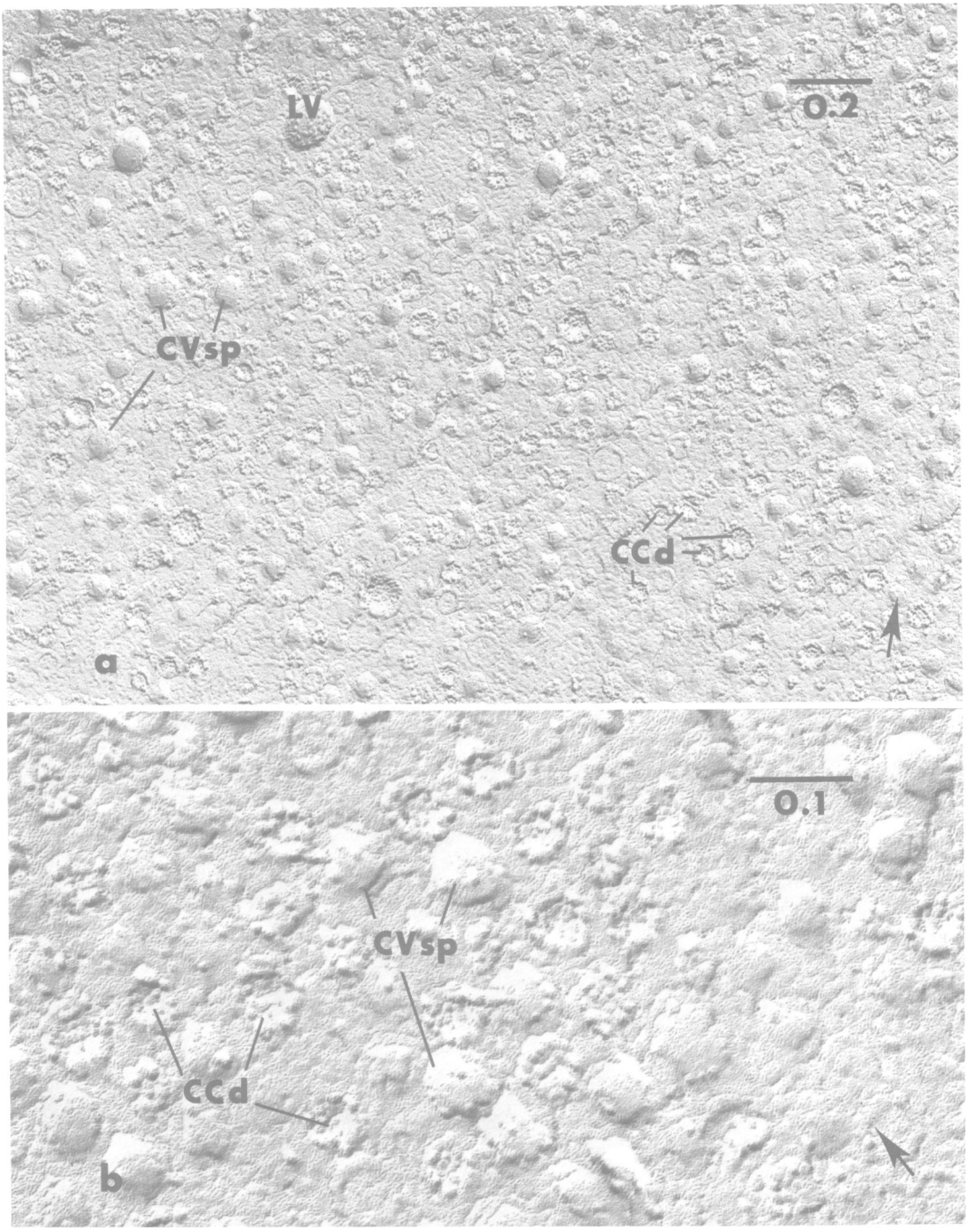


FIG. 7. *Chromatophores prepared by French-press disruption followed by agarose filtration. Almost all the concave (CC) faces are densely (d) populated with particles, and almost all the convex (CV) faces are sparsely (sp) populated. Only one vesicle larger than 100 nm in diameter is present (LV). There are no large cell fragments. (A) 57,000×; (B) 150,000×.*

shown). In addition, efforts to recover more homogeneous preparations of spheroplast-derived vesicles by agarose filtration were not successful.

Speculations on how spheroplast-derived vesicles and chromatophores may be formed during cell disruption can be considered in view of these results. The spheroplasts swell in hypotonic solution, and the intracellular vesicles that are

continuous with the cytoplasmic membrane may become part of the stretched limiting surface of the spheroplast. With lysis, the surface membranes fragment and reseal in an orientation similar to the cytoplasmic membrane in situ. With French-press disruption, the intracellular membranes apparently reseal in the original orientation. Fragmentation and resealing in this case perhaps occur at points of constriction in the continuous membrane system, since the physical dimensions of the chromatophores resemble those of the whole cell vesicles.

The light intensity used in growing cells for membrane isolation may have significant bearing on the orientation of vesicles obtained. A relatively high light intensity was chosen in these experiments (20,000 lx) since under such conditions the intracellular membrane system is largely confined to the cell periphery (Fig. 2 to 4) (4). Thus, during osmotic lysis, the resealing of membrane fragments into spheroplast-derived vesicles with an orientation of the in situ cytoplasmic membrane might be facilitated since the extent of membrane invagination is minimized. A similar approach was used by Hellingwerf et al. (8) to obtain a preparation of membrane vesicles from *R. sphaeroides* capable of actively transporting L-alanine.

The three kinds of isolated vesicle preparations were all observed to have densely packed particle populations with densities intermediate between those of the cytoplasmic and vesicle membranes in whole cells (Table 1). This suggests that both cytoplasmic and intracellular membranes contribute to the preparations producing the observed intermediate population densities. However, the density differences are too small to allow individual vesicles or parts of vesicles to be assigned to one or the other source. Thus, the extent of contribution by the cytoplasmic membrane in the formation of these vesicle preparations is difficult to assess. With the high light intensity used for growth, the amount of cytoplasmic membrane relative to intracellular membrane is higher than with growth under lower light intensities. Therefore, the contribution of cytoplasmic membrane to the preparations may possibly be higher than for preparations obtained from low-light-grown cells. Chromatophore preparations from cells grown with relatively low light intensities have been reported to be essentially free of cytoplasmic membrane material (5). Interestingly, cytoplasmic membrane vesicles obtained from *E. coli* after French-press disruption show inside-out orientations as revealed by freeze-fracture electron microscopy (1), and these vesicles resemble the chromatophores observed in the present study in size and particle distribution.

The ability to prepare membrane vesicles of the photosynthetic apparatus of *R. sphaeroides* with opposite and known orientation should allow further studies on the topographical distribution of components which comprise this system.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

A recent report (P. A. M. Michels and W. N. Konings, Biochim. Biophys. Acta **507**:353–368, 1978) describes observations on freeze-fractured preparations of spheroplast-derived vesicles and chromatophores of *R. sphaeroides* similar to those reported here.

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