Electron Microscopy of Chromatophores of
Rhodopseudomonas spheroides

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Received for publication 17 May 1965

ABSTRACT

GIBSON, K. D. (St. Mary's Hospital Medical School, London, England). Electron microscopy of Rhodopseudomonas spheroides. J. Bacteriol. 90:1059–1072. 1965.—Fixed and stained chromatophores and whole cells of anaerobically grown Rhodopseudomonas spheroides were examined in thin sections in the electron microscope. Both purified chromatophores and intracellular membrane-bound vesicles had exactly the same appearance, namely that of spheres or ellipsoids with a thin, electron-dense shell surrounding an electron-lucent interior. The distribution of diameters in the two types of structure was also found to be the same, and was compatible with a normal distribution, with a mean of 570 A and a standard deviation 40 A. Negatively stained chromatophores appeared like discs or collapsed spheres. The presence of invaginations of the cytoplasmic membrane in this species was confirmed, and a new structure resembling a twin chromatophore was observed. The bearing of these results on theories of the origin of chromatophores is discussed, and it is concluded that they offer some support for each one of the three main theories about the origin of particulate organelles.

There are two alternative views about the structure of the insoluble organelle which contains the photosynthetic pigments in Rhodopseudomonas spheroides and related organisms. One is that it consists of more or less separate and independent particles of similar sizes; this was the original view of Schachman, Pardee, and Stanier (1952). The other is that it is a reticular type of structure consisting essentially of a series of membranes surrounding empty vesicles, which may or may not be attached to one another (Cohen-Bazire and Kunisawa, 1963). In other reports in this series, it was shown, by moving boundary and zone centrifugation, that in R. spheroides the evidence is strongly in favor of the original hypothesis of Schachman et al. (Gibson, 1964a, b). This report deals with an electron microscopic investigation into the chromatophores of this species.

MATERIALS AND METHODS

The organism used in this study was a streptomycin-resistant mutant of R. spheroides strain S. The method of growth and the preparation of extracts with a French press (French and Milner, 1955) have been described (Gibson, Biochemistry, in press). Chromatophores were isolated from extracts by zone centrifugation through linear gradients of CsCl (Gibson, 1964b and in press). The material isolated by this method is at least 90% pure by chemical criteria.

Electron microscopy. Preparations of chromatophores and of whole cells were fixed in buffered 1% osmium tetroxide, dehydrated, and embedded in Epoxy resin according to Boatman and Douglas (1961). Sections, about 60 to 100 mg thick, were cut on a Porter-Blum (Servall) microtome. The sections were stained over a 45-min period with lead hydroxide by inserting the grid, with the exclusion of air, in stain prepared by method A of Karnovsky (1961) and diluted 1:30; the stained sections were washed for about 15 sec in NaOH, followed by distilled water (twice).

Chromatophores were also examined by use of the negative-staining technique with a 1% solution of phosphotungstic acid adjusted to pH 7.5 with KOH. The chromatophores were layered onto the surface of this solution from a needle, as described by Parsons (1963), and were transferred to the grids when these were touched on the surface of the solution.

Grids were examined in a Siemens Elmiskop I electron microscope operated at 80 kv, and photographed with Eastman Kodak lanternslide plates (high contrast). Original magnifications ranged from 20,000 to 40,000.

Measurement of diameters. The lengths of the images of chromatophores and membrane-bound vesicles were measured with a micrometer gauge in two directions at right angles to the nearest 0.01 cm, by use of prints whose overall magnifications were 70,000 to 140,000. The experimental error of this procedure was not greater than ±0.03

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Only chromatophores or vesicle images with sharp outlines were measured, and only negatives whose magnifications were known to be comparable were used. The actual magnifications are probably only accurate to about 5%, but the relative differences between the various prints which were used for the measurements were less than 2%.

**Results**

Preparations of chromatophores isolated on a CsCl gradient had a characteristic appearance in the electron microscope after negative staining with phosphotungstate (Fig. 1 and 2). Such preparations appeared to consist entirely of particles of very similar sizes, most of which presented a circular profile, although some resembled discs on edge. These photographs are very much like some of the published pictures of chromatophores from *Rhodospirillum rubrum* (Cohen-Bazire, 1963), and are consistent with the idea that chromatophores are really fragments of a membrane. However, thin sections of preparations fixed in osmium tetroxide and stained with lead hydroxide had an appearance which is quite incompatible with this hypothesis, and can only be interpreted in terms of discrete spherical or ellipsoidal particles (Fig. 3 to 5). With few exceptions, the objects in these photographs had exactly the same appearance as the “membrane-bound vesicles” that are visible in sections of whole cells (Fig. 6, 7, 11, 12). There is abundant evidence to show that the vesicles in whole cells contain or are associated with the photosynthetic apparatus (Vatter and Wolfe, 1958; Hickman and Frenkel, 1959; Cohen-Bazire and Kunisawa, 1963). The thickness of the outer shell in isolated chromatophores and intracellular vesicles was the same, and was estimated to lie between 30 and 70 Å. The number of objects which did not resemble membrane-bound vesicles was quite small; in the sample which was used for measuring diameters (see below), which comprised 496 chromatophore images, only 10 objects were seen which had a different shape. Thus, by this criterion the preparation of chromatophores was 98% pure.

**Distribution of diameters.** Further evidence for the identity of isolated chromatophores with the intracellular membrane-bound vesicles comes from a study of the distribution of their diameters. Examination of photographs showed that
most sections had been flattened in one direction and stretched at right angles by the procedure for cutting sections; this is clearly visible in Fig. 3 to 7. The lengths of the apparent major and minor axes were measured, and their geometric mean was taken as the diameter of the particle. This assumes that the distortion of the section has occurred in such a way as to preserve areas on the surface of the section. Other assumptions are possible, e.g., that the volume of the section rather than its area is maintained or that the lengths of the fibers of resin in two different directions remain the same; but provided that no length in any direction is altered by more than 15%, all these assumptions lead to values for the diameter which lie within 3% of each other. Six photographs were used for these measurements, three being of chromatophores and three of whole cells (including Fig. 4 to 7). By calculating the mean length of the particle images in one direction and their mean length in the direction at right angles, it was possible to determine the approximate amounts by which the sections had been stretched and flattened. The results lay between 12 and 15%; also, all sections which were cut from the same block were distorted to the same extent. When the possible error due to distortion is added to the experimental error of the measurements, the overall error in the estimates of the diameters is less than 8%.

The relative frequencies of the diameters of the samples of 496 isolated chromatophores and of 307 intracellular membrane-bound vesicles are shown in Fig. 8. Clearly, the diameters of isolated particles have virtually the same distribution as those of the intracellular vesicles. The hypothesis that the distributions are different was tested by dividing them into 10 classes (Table 1), and calculating $\chi^2$ from the resulting $2 \times 10$ contingency table. This gave $\chi^2(10) = 12.65$, with probability between 0.10 and 0.20. This is compatible with the view that the two samples come from the same population.

The samples were combined, and the resulting population was found to have a mean diameter of 571.1 A, with standard deviation 67.7 A. As there may well have been a systematic error in estimating the overall magnifications, these figures can only be taken to be accurate to about 5%. Figure 9 shows the frequencies of the combined population together with a normal curve of mean 571 A and standard deviation 67.7 A, and
layer of material is removed from the surface of
the particle (Gibson, 1964c). This layer consists
mainly of phospholipid with some protein and
 carbohydrate and is about 30 A thick; its removal
does a drop in the sedimentation coefficient
from 160 to 120 S. Virtually all the photosynthetic
pigment is found in the 120 S particles, which
have been referred to as core particles. Unlike
the chromatophores from which they arise, these
particles are very unstable and disintegrate or
aggregate rapidly in the cold.

Figure 10 shows core particles negatively
stained with phosphotungstate. The core
particles were prepared by adding sodium cholate
and deoxycholate (1 mg of each per mg of pro-
tin) to a suspension of chromatophores at 5 C,
and layering them on a solution of phospho-
tungstate after 10 min. The appearance of core
particles which were layered on the phospho-
tungstate 5 or 15 min after the bile salts were
added was much the same. The sharp outlines
shown by untreated chromatophores (Fig. 2) had
largely disappeared in the core particles (Fig. 10),
which give a strong impression of having been
fixed while actually in the course of disintegrat-
ing. This is in keeping with the observations re-
ferred to above which suggest instability on other
grounds.

Other membranelike structures. The most obvi-
ous membranelike structures in anaerobically
grown R. spheroides are the cytoplasmic mem-
brane and the almost circular membrane-bound
vesicles. However, occasional structures can be
seen which do not obviously belong to either of
these categories. One structure of this type is the
invagination from the cytoplasmic membrane
observed in R. rubrum and R. spheroides by
Cohen-Bazire (1963) and Cohen-Bazire and
Kunisawa (1963), examples of which are shown
in Fig. 6 and 11. There are also occasional mem-
brane-bound vesicles which appear much larger
than chromatophores (Fig. 7); these may be
granules of storage materials, as observed in R.
rubrum. Finally, in the dividing cells (Fig. 12)
there are several dumb-bell shaped objects which
resemble twin chromatophores, as well as what
appears to be a rather complicated invagination
of the cytoplasmic membrane reminiscent of
structures which have been observed by Cohen-
Bazire in R. rubrum and other species. In all
preparations of whole cells there was clearly some
distortion of the cytoplasm introduced during
fixation and dehydration, and it is possible that
some of the structures observed here are arti-
facts produced by distorting chromatophores.
However, the results with purified chromatop-
hores indicate that these particles are not dis-

Fig. 5. Thin section of fixed chromatophores;
poststained with lead hydroxide. X 96,500.
torted to any significant extent by fixation, and, indeed, this is to be expected in view of their known stability to extremes of tonicity and to mechanical stresses (Gibson, in press). It therefore seems unlikely that the structures observed in whole cells are produced by distortion of chromatophores during the preparations for electron microscopy.

**Fig. 6.** Thin section of fixed, anaerobically grown cells; poststained with lead hydroxide. M, membrane-bound vesicle; C, invagination of cytoplasmic membrane; L, large vesicle. X 71,000.
Fig. 7. Thin section of fixed, anaerobically grown cells; poststained with lead hydroxide. M, membrane-bound vesicle; C, invagination of cytoplasmic membrane; L, large vesicle. × 109,000.
CHROMATOPHORES OF RHODOPSEUDOMONAS SPHEROIDES

**FIG. 8.** Relative frequencies of diameters of purified chromatophores (solid line) and intracellular membrane-bound vesicles (dotted line).

**TABLE 1.** Frequencies of observed diameters

<table>
<thead>
<tr>
<th>Interval</th>
<th>No. of intracellular membrane-bound vesicles</th>
<th>No. of isolated chromatophores</th>
<th>Total no. in combined sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;520 A</td>
<td>43</td>
<td>61</td>
<td>104 (92)*</td>
</tr>
<tr>
<td>520-540 A</td>
<td>26</td>
<td>48</td>
<td>74</td>
</tr>
<tr>
<td>540-550 A</td>
<td>23</td>
<td>43</td>
<td>66</td>
</tr>
<tr>
<td>550-560 A</td>
<td>22</td>
<td>43</td>
<td>65</td>
</tr>
<tr>
<td>560-570 A</td>
<td>22</td>
<td>50</td>
<td>72</td>
</tr>
<tr>
<td>570-580 A</td>
<td>28</td>
<td>59</td>
<td>87</td>
</tr>
<tr>
<td>580-590 A</td>
<td>27</td>
<td>53</td>
<td>80</td>
</tr>
<tr>
<td>590-600 A</td>
<td>20</td>
<td>34</td>
<td>54</td>
</tr>
<tr>
<td>600-620 A</td>
<td>39</td>
<td>45</td>
<td>84</td>
</tr>
<tr>
<td>&gt;620 A</td>
<td>57</td>
<td>60</td>
<td>117 (92)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the numbers observed when all diameters below 440 A or above 700 A were ignored.

**DISCUSSION**

Chromatophores and membrane-bound vesicles. The identity of the chromatophores isolated in this work with the membrane-bound vesicles observed in whole cells can be inferred from the experiments with fixed material presented here. In electron micrographs, both structures are circular or slightly elliptical objects with rather sharp electron-dense edges and electron-sparse centers, presenting the same appearance in the photographs. However, the most convincing evidence for the identity of the two types of structures is the fact that their diameters have very similar,

**FIG. 9.** Relative frequencies of diameters of combined sample of chromatophores and intracellular vesicles. The curves represent normal curves with a mean of 571 A and a standard deviation of 67.7 A (dotted line) or 49 A (dashed line).

**FIG. 10.** Core particles from chromatophores, negatively stained with phosphotungstate. X 80,000.
if not the same, distributions. The differences between the two samples observed in this study corresponded to a value of $\chi^2$ with probability between 0.10 and 0.20. Although this is not a very large probability, it is certainly not small enough to be significant, and, when allowance is made for the approximations and errors introduced during the experimental procedure, this result becomes absolutely compatible with the view that the two samples have the same distribution of diameters. Since the isolated chromatophores are undoubtedly particles, it seems very probable that the intracellular vesicles, or at least the majority of these structures, are also particles, and that any resemblance they may have to a reticular structure is due to their non-random distribution through the cytoplasm of the cell.

**Size and shape of chromatophores.** The distribution of diameters in Fig. 2 and Table 1 refer to the diameters obtained by taking the geometrical mean of two perpendicular diameters of the particle images. To obtain some information about the actual distribution of particle size, it is necessary to make some assumption about their shape. Unfortunately the distortion of the electron micrographs, although slight, was sufficient to make it impossible to decide by inspection alone whether the particles were truly spherical or were ellipsoids with low axial ratios. However, the following considerations make it probable that one of these alternatives is true, and that the axial ratio is less than 1.4. The ratio of the lengths of the apparent longest and shortest diameter of each particle image was corrected for the distortion introduced during sectioning by means of the factor calculated from the average length of each image in the two perpendicular directions, as described in Results. The corrected ratio is, of course, not the axial ratio of the image but merely the ratio of two perpendicular diameters chosen at random. The largest value that can be attained by this ratio is equal to the maximal value of the axial ratio over all plane projections of the chromatophores. In a sample comprising 410 of the intracellular and purified chromatophores, none of these ratios exceeded 1.4 and only 30 exceeded 1.2. The distribution of this ratio of diameter lengths is expected to be heavily biased in favor of values near 1.0, but even so the results provide good grounds for concluding that the chromatophores have axial ratios less than 1.4.

The thickness of the sections examined in the electron microscope was between 600 and 1,000 A, or about one to two times the mean diameter of the particle images. Thus, if any part of a chromatophore is included in the section, it is rather probable that an equatorial plane of the particle will also be included. The chromatophores clearly consist of a thin shell of electron-dense material surrounding an electron-sparse center. A section through a structure of this type will only have a sharp outline if it contains an equatorial plane; this has been demonstrated very elegantly by Boatman (1964), theoretically and in practice. Hence, it is probable that any image with a sharp outline comes from a chromatophore whose center is included in the section. Since only images with sharp outlines were measured, it is safe to conclude that the observed distribution of diameters is approximately the same as the distribution of the diameters of equatorial planes of the chromatophores, at least over most of the range of sizes observed. The observed diameter of any chromatophore whose center was not included in the section would be smaller than the diameter of the corresponding equatorial plane section, and if
Fig. 12. Thin section of fixed dividing cells, showing invagination of cytoplasmic membrane (C) and dumbbell-shaped twin vesicles (T). Poststained with lead hydroxide. X 105,000.
any images of such particles were included in the measurements their diameters would tend to lie at the lower end of the range. Undoubtedly some images of this type were measured; in particular, it seems quite likely that the "tail" of the distribution in Fig. 9, for diameters less than 440 A, might be derived primarily from such images. Hence, in attempting to assess the distribution of the diameters of the equatorial planes, it seems reasonable to attach little significance to this tail. The number of diameters which were actually ignored in this way was 13 of 803, or fewer than 2%. 

The most useful measure of the size of an ellipsoidal particle is the geometrical mean $\rho$ of the lengths of its three axes, since the volumes and surface areas can both be expressed in terms of this quantity. If $r$ is the geometric mean of the lengths of the major and minor axes of an equatorial plane section of the particle, Wicksell (1926) has shown that, for ellipsoids with axial ratios lower than 1.4, the difference between the distributions of $\rho$ and $r$ is of the same order of magnitude as the sampling error likely to be met in estimates based on samples of several thousand particles. In the present work, the observed diameters were not the same as $r$, but would be approximately the geometric mean of two perpendicular diameters chosen at random. A slight extension of Wicksell's calculations shows that, provided the axial ratio is less than 1.4, the first four moments of this distribution differ from those of the distribution of $r$ by less than 2%. These arguments show that the error which is incurred by taking the distribution of the observed diameters to be the same as the distribution of $\rho$ is not likely to be any greater than the experimental error involved in the measurements, so that whatever distribution is found experimentally for the observed diameters will apply approximately to the quantity $\rho$, which is a direct measure of the particle size.

The actual results were found to be compatible with a normal distribution, provided that diameters less than 440 A or more than 700 A were ignored. Reasons for ignoring the lower tail of the distribution were advanced above. The argument does not apply to the upper tail, which must be considered to represent either a different type of structure or a very small fraction of large chromatophores. It is shown elsewhere (Gibson, 1964e) that the "empty" electron-lucent interior of the chromatophore probably contains all the pigment and the phosphorylating system, the outer electron-dense layer being apparently only a layer of lipid which holds the structure together. Thus, the properties of the chromatophore which distinguish it chemically are associated with a part of the structure which does not show in the electron micrographs. There is, therefore, no absolute criterion, apart perhaps from size, by which to judge whether a particle seen in the electron microscope is a chromatophore or some other type of particle, such as a granule of storage material, especially since the latter can sometimes have an appearance which is not too unlike that of an intracellular membrane-bound vesicle (Boatman, 1964). Thus, it is possible that the large diameters in the distributions in Fig. 9 were due to particles of a different kind. The actual frequency of diameters greater than 700 A was 23 of 803, or less than 3%.

These considerations suggest that it is probably reasonable to ignore the very large and the very small diameters and to take the distribution of chromatophore diameters as being approximately normal, with the mean near 570 A and a standard deviation of about 40 A, or 7% of the mean. As the values of the mean diameter may be subject to a systematic error of perhaps 5%, this estimate of 570 A is quite compatible with Worden and Sistrom's (1964) value of 600 A, which was based on X-ray scattering data.

Behavior of chromatophores on drying. It was shown elsewhere that the observations reported here considered together with the data from experiments with moving boundary sedimentation lead to a value for the hydration of chromatophores of $R$. sphaeroides which amounts to 55% by volume (Gibson, in press). This large proportion of water accounts for the disc-shaped appearance which the particles assume when they are dried out on electron-microscope grids. This rather characteristic shape has been observed in negatively stained preparations (Fig. 1 and 2) and in preparations which were dried and shadowed (unpublished data). Clearly, the change from spheres to discs is due to the fact that the particles must contract to less than one-half their original volume on drying. The appearance of core particles dried down with phosphotungstate can be explained in terms of the high proportion of water in the chromatophore coupled with the loss of the stabilizing influence of the outer layer of lipid. Although phosphotungstate preserves much smaller hydrated structures (Lucy and Glauber, 1964), it does not seem to be able to prevent the collapse of these fairly large ones. It is of interest that isolated chromatophores of $R$. rubrum, which are probably hydrated to about the same degree as those of $R$. sphaeroides, also collapse to dislike structures when dried down for observation under the electron microscope; this has been elegantly demonstrated by Frenkel and Hickman (1959).
Size and shape of other chromatophores. There is considerable evidence to show that all photosynthetic bacteria contain pigmented particles, or structures which can give rise to such particles, and that these structures are responsible for the essential photosynthetic reactions upon which the cell relies for its metabolism under anaerobic conditions (Geller, 1961). It seems that these photosynthetic reactions do not differ much from species to species, and that the gross chemical composition of the pigmented structure is also much the same in different species (Lascelles, 1962). It is therefore of some interest to compare the physical characteristics of the structures which contain the pigment in different organisms as far as this is possible from published data. Most of these data refer to purified particulate preparations, and there is no guarantee that the structures are the same in vivo; but in view of the discussion here and in other papers in this series, which make it seem rather probable that the pigmented structure of *R. spheroides* is not significantly altered when it is released from the cell, the data obtained with isolated preparations can be taken to represent the structures found in vivo.

The shapes, sizes, and degrees of hydration of chromatophores from *R. spheroides, R. rubrum, Chromatium* spp., *Chlorobium* thiosulfatophilum, and *R. molischianum*, as obtained from the literature, are shown in Table 2. The hydration of chromatophores of *R. rubrum* was calculated roughly from the approximate value of 400 Å for the diameter of the equivalent hydrodynamic sphere given by Schachman et al. (1952). Newton and Newton (1957) found the space permeable to inorganic phosphate in chromatophores of *Chromatium* to be about 50% but the diameters calculated by Bergeron (1958) for the hydrated and unhydrated particle indicate a value nearer 25%. A similar calculation by Bergeron and Fuller (1961) suggests that the water space of the small pigmented particle which they obtained from *Chlorobium* is around zero; however, the relation of Bergeron and Fuller’s particles to the elongated vesicles isolated by Cohen-Bazire, Pfennig, and Kunisawa (1964) has not been established, nor are there any data available from which the hydration of the latter particles can be estimated. There is also no information about the hydration of the disc-shaped chromatophores of *R. molischianum*.

It is clear that the pigment-bearing structures of *Chlorobium* and *R. molischianum* have a very different shape from those of the other three species. On the other hand, *R. rubrum* has chromatophores which are rather similar in size, shape, and even hydration to those of *R. spheroides* and species of *Chromatium*, even though it is not as closely related to these species as it is to *R. molischianum*. It must be concluded that there are several possible ways in which a pigmented photophosphorylating system can be constructed; although there are many properties that such systems have in common, structure at the level of the chromatophore is, generally speaking, not one of them.

### Table 2. Size and shape of chromatophores from some photosynthetic bacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>Shape</th>
<th>Axial ratio</th>
<th>Longest diameter (Å)</th>
<th>Hydration (per cent by volume)</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodopseudomonas spheroides</em></td>
<td>Almost spherical</td>
<td>≤1.4</td>
<td>570 ± 40</td>
<td>55</td>
<td>A</td>
</tr>
<tr>
<td><em>Rhodospirillum rubrum</em></td>
<td>Almost spherical</td>
<td>1–1.5</td>
<td>600–800</td>
<td>60–70</td>
<td>B–E</td>
</tr>
<tr>
<td><em>Chromatium</em> spp.</td>
<td>Almost spherical</td>
<td>1–1.5</td>
<td>300–500</td>
<td>50</td>
<td>B, F, G</td>
</tr>
<tr>
<td><em>Chlorobium</em> thiosulfatophilum</td>
<td>Prolate ellipsoid</td>
<td>2–3</td>
<td>1,000–1,500</td>
<td>—</td>
<td>H</td>
</tr>
<tr>
<td><em>Rhodospirillum molischianum</em></td>
<td>Oblate ellipsoid</td>
<td>—</td>
<td>1,200–2,000</td>
<td>—</td>
<td>I, J</td>
</tr>
<tr>
<td></td>
<td>(circular disc)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* (A) Gibson, Biochemistry, *in press*; this paper; (B) Vatter and Wolfe, 1958; (C) Hickman and Frenkel, 1959; (D) Cohen-Bazire and Kunisawa, 1963; (E) Schachman et al., 1952; (F) Newton and Newton, 1957; (G) Bergeron, 1958; (H) Cohen-Bazire, Pfennig, and Kunisawa, 1964; (I) Drews, 1900; (J) Hickman, Frenkel, and Cost, 1963.

† Insufficient data to support an estimate.
spheroides by Cohen-Bazire (1963) and in the present work. These invaginations roughly resemble the membrane-bound vesicles in size and shape, and like the vesicles they consist of electron-lucent interior regions surrounded by a layer of electron-dense material; in fact they have exactly the appearance that would be expected of nascent chromatophores caught in the act of budding from the cytoplasmic membrane.

However, there are two other possible explanations of these structures, neither of which has been excluded and for each of which it is possible to adduce some evidence. The first explanation is that the structures do not arise by budding from the membrane but by the reverse process of fusion. Evidence advanced elsewhere (Gibson, 1964c) and referred to above shows that chromatophores of R. spheroides have a surrounding layer of unpigmented lipid which corresponds to the electron-dense layer observed in electron micrographs. There is evidence suggesting that the lipid of the chromatophore of R. spheroides is rather similar to the lipid of the cell wall or cytoplasmic membrane (Gorchein, 1964). If chromatophores were synthesized by a mechanism in which the lipid layer was added on to the outside of a preformed core, some of those formed in the vicinity of the membrane might well have their outer layers added in such a way as to become continuous with the membrane. This would give rise to budlike structures and would account for the fact that, although apparently fully formed buds have been observed frequently, there has so far been no indication of a structure resembling any of the early stages in bud formation.

The second explanation of the invaginations is that they have nothing to do with chromatophores but are concerned with the aerobic processes of metabolism of the cell. From the work already referred to (Gibson, 1964c), it is clear that the part of the chromatophore which contains the photosynthetic pigments and is responsible for its particular biological activity is the interior region, which appears empty and featureless in electron micrographs. Thus, it seems that in this instance the electron microscope emphasizes a part of the structure which plays a nonspecific role in its physiological function. The same could also be true of any other type of organelle. Thus, the mere possession of an appearance similar to that of a chromatophore is no guarantee that another organelle is physiologically related to it. It is therefore perfectly possible that the invaginations of the cytoplasmic membrane represent totally different subcellular structures whose appearance in sections of fixed and stained cells fortuitously resembles that of chromatophores. In fact, extremely similar invaginations have been seen in four species of nonphotosynthetic bacteria; these are Staphylococcus aureus (Suganuma, 1961), Azotobacter vinelandii (Wyss, Neumann, and Soefolosky, 1960), Spirillum serpens (Chapman and Kroll, 1957), and Brucella abortis (de Petris, Karlsbad, and Kessel, 1964). It has been suggested that in Brucella the invaginations are the bacterial equivalent of mitochondria.

The hypothesis is therefore put forward that at least some of the membrane invaginations observed in R. spheroides and R. rubrum correspond to structures involved in oxidative reactions and bear no relation to chromatophores, other than a chance resemblance in certain fixed and stained preparations. This view explains one fact which is otherwise somewhat puzzling, namely, the much greater number of invaginations that can be seen in sections of aerobic or semiaerobic cells as compared with anaerobic ones. This is well illustrated by Boatman's (1964) pictures of R. rubrum and is also shown by those of Cohen-Bazire and Kunisawa (1965). Even in strictly aerobic R. spheroides, which completely lack any trace of bacteriochlorophyll (Laseelles, 1959), many membrane invaginations are visible (Cohen-Bazire, 1963). These observations become simpler to understand if the invaginations are concerned not with photosynthesis but with oxidation.

Origin of chromatophores. One of the outstanding problems of molecular biology concerns the mechanism of biosynthesis of organized subcellular particulate structures such as mitochondria and chloroplasts. Probably more work has been done with mitochondria than with any other organelle, and the theories that have been advanced fall into three groups (Lehninger, 1964). (i) Mitochondria are synthesized de novo, (ii) they originate from other membranous structures in the cell, and (iii) they grow and divide semiautonomously. Evidence can be adduced to support each view, but the most convincing evidence favors the third mechanism in Neurospora crassa (Ephrussi, 1950; Luck, 1963; Luck and Reich, 1964). The question arises whether all subcellular organelles are formed by a similar mechanism. If it is accepted that chromatophores are particles, then they are among the smallest and simplest of all particulate organelles, and it is therefore of some importance to determine their mode of origin.

The most widely accepted view concerning the formation of chromatophores is probably that of Cohen-Bazire and Kunisawa (1963) mentioned already. The main evidence for this view, which comes from electron micrographs and from the behavior of the pigmented structure in lysates of...
spheroplasts, is capable of alternate explanations as discussed here and elsewhere (Gibson, in press). On the other hand, at least two of the observations based on electron microscopy which are reported here favor a different view of the origin of chromatophores. One of these is the observation in the dividing cell (Fig. 12) of dumb-bell-shaped objects which strongly resemble chromatophores undergoing binary fission and suggest that these particles might arise in the same way as mitochondria. The other is the distribution of chromatophore diameters in Fig. 9, which shows a standard deviation that is only about 7% of the mean, a smaller figure than the corresponding one for rat-liver mitochondria (Bahr and Zeitler, 1962). When considered together with the extreme rarity of chromatophores whose shape is not spherical, this suggests that straightforward binary fission is not the mechanism of biosynthesis. The distribution of sizes also seems much narrower than might be expected if the particles are budded off from a membrane, and is much more compatible with de novo synthesis. It appears from this discussion that it is possible to adduce at least one piece of evidence from electron microscopic examinations of chromatophores to support each of the three main theories about the origin of organelles. Clearly, the question of the origin of these particles is by no means settled, and it will not be settled by electron microscopic techniques alone but requires a more dynamic approach.

Acknowledgments

I wish to acknowledge the fine technical assistance of Chris Stolinski and Naomi Brent. I also wish to thank F. Goldby, F.R.C.P., and The Department of Anatomy, St. Mary's Hospital Medical School, for making their electron microscope available to me; and J. Pettitt of the Department of Zoology and Comparative Anatomy, St. Bartholomew's Medical College, London, for assisting me with photographic techniques.

Addendum

After this paper was submitted for publication, a series of papers appeared concerning the physical nature of the pigmented structures in extracts of Rhodospirillum rubrum prepared by grinding with abrasives, shaking with glass beads, or by osmotic shock (Holt and Marr, J. Bacteriol. 89:1402, 1965; Holt and Marr, J. Bacteriol. 89: 1413, 1965). The results are in direct opposition to those presented here and apparently support conclusively the view that the pigmented structure is a continuous membrane. The methods that Holt and Marr used for breaking the cells are undoubtedly milder than those used in the present work and, thus, it might seem that the structures they describe are closer to the real ones than the independent particles which were found here. However, it should be noted that Holt and Marr washed their cells extensively with ion-free water and that during subsequent manipulations MgSO₄ was added and maintained at a concentration of 10⁻⁴ M. Since purified chromatophores from Rhodopseudomonas spheroides aggregate readily at very low ionic strengths and in the presence of 10⁻⁴ M Mg⁺⁺ (Gibson, in press), it is possible that at least some of the interconnections between chromatophores observed by Holt and Marr were artifacts resulting from aggregation.

Nevertheless, it does appear that there may be a genuine difference between the structures of the pigmented organelles of R. rubrum and R. spheroides. The intracellular vesicles in published electron micrographs of R. rubrum appear on superficial examination to be less homogeneous in both shape and size than those of R. spheroides or Chromatium; this is true even when the different species were examined by the same worker (e.g., Cohen-Bazire, 1963). There is also evidence suggesting that the chromatophores of R. spheroides are enzymatically simpler than those of R. rubrum (Gibson, in press). In view of the very different manner in which the photosynthetic apparatus of R. molischianum is put together, there may well be more subtle differences between the structures in R. rubrum and R. spheroides than are revealed by electron microscopy. Thus, the divergence between the results of Holt and Marr and the present work could represent a genuine difference in the molecular structure of the photosynthetic organelles of these two species.

Literature Cited


Cohen-Bazire, G., and R. Kunisawa. 1963. The


