

FINE STRUCTURE OF THE PHOTOSYNTHETIC BACTERIUM *RHODOMICROBIUM VANNIELII*

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ABSTRACT

The fine structure of the photosynthetic bacterium *Rhodomicrobium vannielii* was studied by the ultra thin sectioning technique. Cells were fixed in buffered osmium tetroxide and embedded in Epoxy resin. The feature most common to nearly all cells was an array of intracellular membranes situated in a concentric manner at the periphery of the cell. The membranes were mostly paired and quite often five pairs were seen aligned together. Calculations from densitometric tracings showed the average width of a "unit" membrane to be 65 Å. Sections of material from disrupted cells after passage through a sucrose gradient revealed vesicular forms composed of membranes similar in width to those in the intact cell. Absorption spectra of both intact cells and isolated membranes were very similar in the bacteriochlorophyll regions. Septa and membranes were demonstrated in the filaments that join mature cells. No evidence for chromatophores was obtained although the methods used were adequate for their demonstration in *Rhodospirillum rubrum*.

INTRODUCTION

The occurrence of specialized cytoplasmic organelles in photosynthetic bacteria was first demonstrated in cell extracts of *Rhodospirillum rubrum* by Pardee, Schachman, and Stanier (1). These workers found that the pigment complex of *R. rubrum* was bound to spherical structures about 60 m μ in diameter which they termed bacterial chromatophores. It was estimated that several thousand chromatophores were contained in a single cell. The direct demonstration of chromatophores *in situ* was made by Vatter and Wolfe (2), who examined thin sections of *R. rubrum*, *Chlorobium limicola*, *Rhodopseudomonas spheroides*, and *Chromatium* sp.

A different type of cytoplasmic organelle was observed in the photosynthetic budding bacterium *Rhodomicrobium vannielii* (3). Although this organism has a typical bacterial pigment complex (4) and carries out a bacterial type of photo-

synthesis (5), it contains a system of closely packed membranes rather than chromatophores (3). A similar membrane system has also been described in certain of the blue-green algae (6-8).

The present work provides additional observations on the nature of the membrane system and other structural features of *R. vannielii* as seen in sectioned material with the electron microscope.

MATERIAL AND METHODS

a) Cultures: The culture of *R. vannielii* used was strain 450 from the departmental collection, and the culture of *Rhodospirillum rubrum* was supplied by Dr. R. S. Wolfe. A single medium (5) was adequate for the growth of both organisms, but the yield of *R. rubrum* was greatly improved by a fourfold increase in the concentration of yeast extract. The bulk medium was inoculated in a ratio of 1 ml to 150 ml of medium and transferred to screw-capped tubes 6

inches long and $\frac{5}{8}$ inch in diameter which were filled to the brim to ensure anaerobic conditions. Illumination was supplied by a 5000 volt neon discharge tube which emitted diffuse red light between 342 and 966 $m\mu$. The neon source, enclosed by a larger glass tube, was centrally located in a circular water bath. The culture tubes were placed radially around the light source and submerged up to their necks in water held at 33°C. The cells were harvested by centrifugation at the early log phase, mid-log phase, and early stationary phase on the basis of optical density readings.

b) *Fixation and Embedding*: Osmium tetroxide in a concentration of 1 per cent was used as a fixative and was either incorporated in *s*-collidine buffer of pH 7.5 (9) or used in conjunction with the Kellenberger, Ryter, and Séchaud fixation technique (10). Fixation was carried out in the dark at room temperature or at 0°C, and extended over periods varying from 3 to 17 hours. The fixed cells were centrifuged, washed once in the appropriate buffer, and then spread over the surface of a glass slide coated with a 0.5 mm thick layer of 2.5 per cent agar. When the moisture in the bacterial film had been absorbed the cells were covered with a thin layer of agar, thus forming an agar "sandwich" with the bacteria in the middle. Agar blocks measuring 5 by 15 mm were cut out and dehydrated with solutions of ethanol ranging from 30 per cent to absolute in gradations of 10 per cent. The blocks were held in each solution for 10 minutes and finally treated with a second change of absolute alcohol for an additional 5 minutes. This was followed by exposure to two changes of propylene oxide, each of 20 minutes duration, and then by infiltration with a solution of equal parts of propylene oxide and Epoxy resin (11) for 3 hours at room temperature. Each agar block was then transferred to a gelatin capsule containing undiluted Epoxy resin and polymerized for 18 hours at 35°C, 24 hours at 45°C, and 24 hours at 60°C.

Sections were cut with a Porter-Blum microtome using glass knives. The sections were mounted on 150 mesh carbon coated copper grids and stained with 2 per cent uranyl acetate for 3 hours. The specimens were examined with an RCA EMU-2C electron microscope equipped with a 400 μ condenser aperture and a specially stabilized power supply.

c) *Densitometric Tracing of Cell Membranes*: High resolution negatives with a magnification of about 12,000 times were enlarged on photographic film or plate to about 150,000 times. These were surveyed with a densitometer (Kipp and Company, Delft, Netherlands) to obtain measurements of membrane width.

d) *Isolation of Membranes*: Cells from 225 ml of a stationary phase culture of *R. vannielii* were harvested by centrifugation, washed and suspended in 4 ml of

veronal-acetate buffer, pH 6.1, and broken by shaking with highway marking beads (Minnesota Mining and Manufacturing Company, St. Paul) in a Mickle disintegrator for 1½ hours at 4°C. The beads and debris were allowed to settle and the dark red supernatant fluid was removed. A linear sucrose gradient was prepared from solutions of 4 per cent and 40 per cent strength, and the supernatant fluid from the disrupted cell suspension was layered on its surface. The material was then centrifuged at 2000 *g* at 2°C for 4 hours, or until a concentrated band of pigmented material had been formed beneath the surface. This band was removed, mixed with buffer, and centrifuged at 6590 *g* for 30 minutes. The clear supernatant fluid was discarded and the red sediment resuspended in buffered fixative. After 1 hour at room temperature the suspension was centrifuged and the fixative poured off. The sediment was stained for 9 hours at 4°C with 0.5 per cent solution of uranyl acetate prepared in the buffer system of Kellenberger *et al.* (10) and then processed and sectioned as described for intact cells.

OBSERVATIONS

For purposes of orientation the salient features of the morphology and mode of division of *R. vannielii* are illustrated in Fig. 1. Multiplication is by budding, a daughter cell being born on the end of a thin filament which grows out from the pole of a mature terminal cell, or from some point on a connecting filament between two mature cells. The nucleus, which occupies a large part of the central portion of a mature cell, divides and migrates through the filament to the daughter cell after the daughter cell has attained about half its maximal size (12). The cytoplasmic continuity between mother and daughter cell is finally broken by the deposition of an electron opaque material in a small section of the bore of the filament. Most of the daughter cells remain attached, and this results in the formation of large aggregates of cells held together by filaments. However, the daughter cells are flagellated (13) and some of them eventually tear themselves free and swim away.

Fig. 2 shows that the chromatophores of *R. rubrum* can be demonstrated by the processing methods used. The cells are packed with these spherical to ellipsoidal structures, which are surrounded by membranes and display no discernible internal structure. The cytoplasmic membrane and the cell wall of the spirilla are also evident.

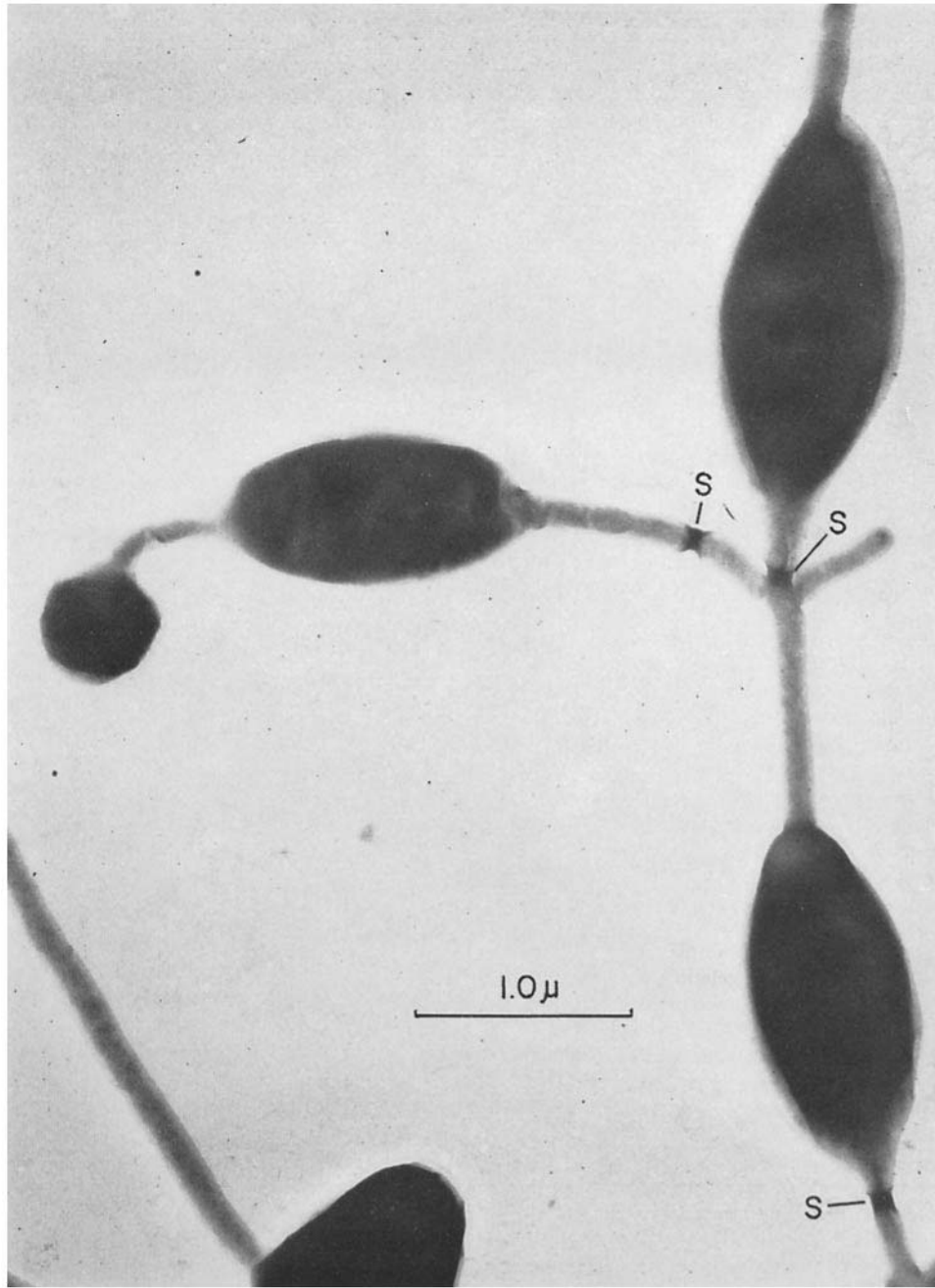


FIGURE 1

Electron micrograph of unsectioned *Rhodomicrobium vannielii* cells showing mature cells connected by filaments. Dense septa (*s*) are present in some filaments, and a daughter cell is seen to arise from the filament of a mother cell. $\times 30,000$.

Fig. 3 is a section of a mixture of *R. rubrum* and *R. vannielii* cells; Figs. 4 to 6 are *R. vannielii* cells alone, harvested at the early log, mid-log, and early stationary phases of growth. It is clear from an examination of these preparations that nothing resembling the chromatophores of *R. rubrum* is apparent in the cells of *R. vannielii*, and, conversely, that the membrane system which is so prominent in *R. vannielii* does not occur in *R. rubrum*.

dinal plane. Such cells are oval in shape and can be positively identified if a filament is present at one or both poles. Cells sectioned in the longitudinal plane are shown in Figs. 6 to 8. The membranes in these cells are not continuous as they are in cross-sections, but are open at one or both ends. Paired membranes can be seen which have separated, and the two unit membranes, which when folded together make up the paired or



FIGURE 2

Section of *Rhodospirillum rubrum* showing the circular profiles of the chromatophores (*Ch*); the cell wall (*CW*) and the cytoplasmic membrane (*CM*) are also apparent. $\times 89,000$.

Cross-sections of *R. vannielii* cells, such as those shown in Figs. 3 to 5, indicate that the membranes are concentrically arranged at the periphery of the cell and are often found paired.¹ Four or five pairs are often seen closely packed together.

A different membrane configuration is seen in cells that have been sectioned close to the longitu-

¹ A pair of membranes appears as three electron opaque lines separated by two less dense spaces.

compound membrane, can be resolved. The configuration of the membranes in the cell in Fig. 7 suggests that the membrane system in this cell consists of a number of sacs bounded by unit membranes, which are compressed and folded in such a way as to form a hollow laminated ellipsoid that is open at one end. A cell in which the membranes appear to be open at both ends is shown in Fig. 8. It would seem that an opening

of some sort in the membrane system would be necessary to enable the daughter nucleus to migrate from mother to daughter cell. This question could be decided by electron microscopy if adequate preservation of the nuclear material could be obtained. Unfortunately, none of the processing methods provided this, even though the

average width of a pair was 130 Å and of a single membrane 65 Å. The width of the cell wall was determined from the same negatives and found to be 150 Å, or 80 Å peak to peak. An example of such a tracing is shown in Fig. 10.

To assess the significance of the membranes of *R. vannielii* to its photosynthetic metabolism, a

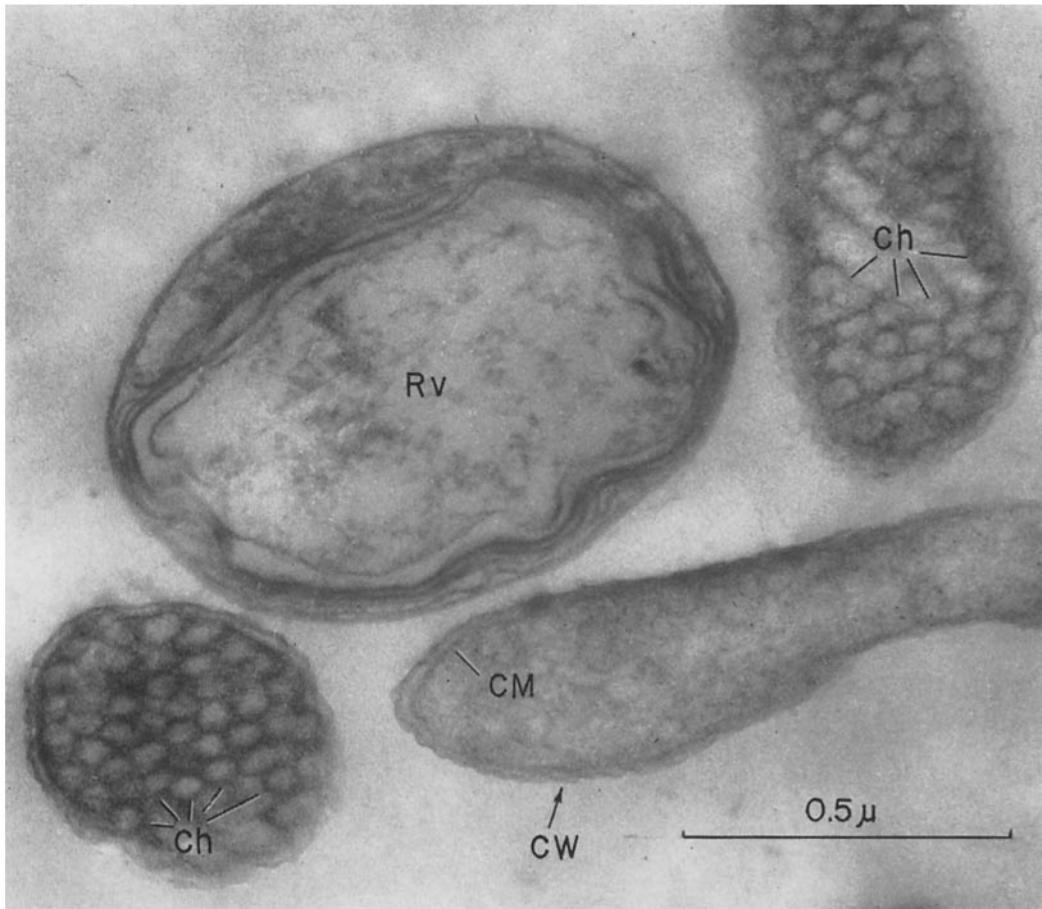


FIGURE 3

Rhodomicrobium vannielii and *Rhodospirillum rubrum* cells processed together. Section shows typical chromatophores (*Ch*) in *R. rubrum*; the cell wall (*CW*) and cytoplasmic membrane (*CM*) of the spirillum are also evident. The single large *R. vannielii* cell (*Rv*) shows only peripheral membranes. $\times 89,000$.

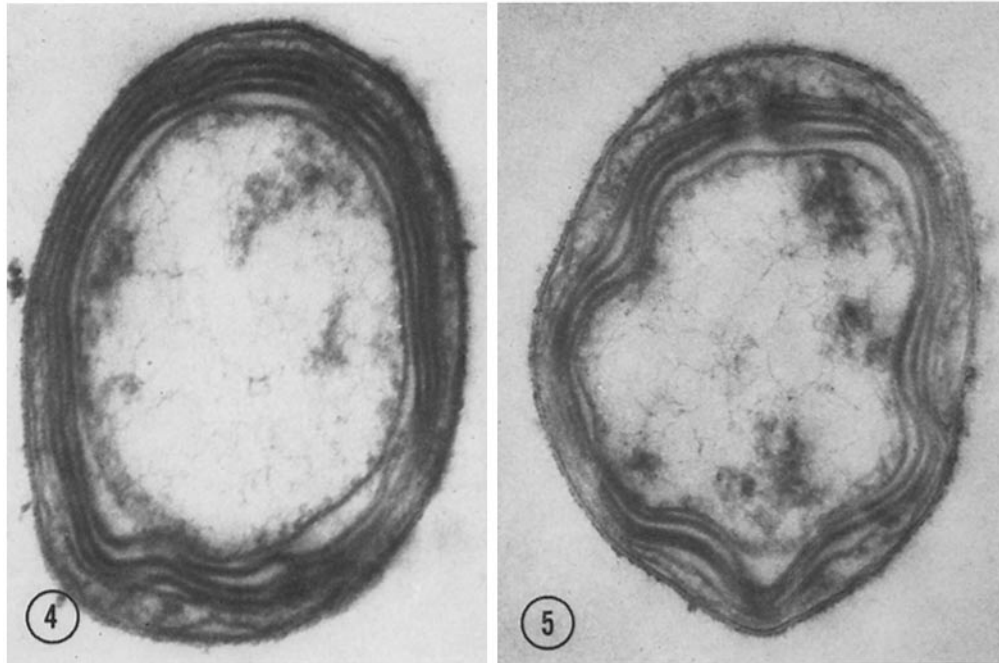
methods have given satisfactory nuclear preservation with eubacterial cells.

The dimensions of paired and individual membranes were ascertained from high resolution negatives like the one shown in Fig. 9. The average width of a pair of membranes was found to be 174 Å and that of a single membrane 87 Å. However, if peak to peak measurements were made, the

subcellular fraction was obtained by centrifuging a cell homogenate in a sucrose gradient. This material was examined for the infrared absorption spectrum of bacteriochlorophyll and was sectioned and examined in the electron microscope. The absorption spectra of a suspension of intact cells and the cell fraction are given in Fig. 11. The two types of material displayed very similar spectra

with peaks in the regions of 800 and 865 $m\mu$ such as are characteristic of bacteriochlorophyll (14). When sectioned, the cell fraction was found to contain vesicles which were surrounded by membranes the width of which closely resembled that of the unit membranes observed in sectioned cells (Fig. 12). Most of the vesicles were 0.2 to 0.3 μ in diameter, but some approached 1.0 μ in diameter. The larger vesicles may represent profiles of intact membranes which survived the treatment used in breaking the cells, while the

membranes along the length of the filament. An exception to this has been found in a single filament tip which is pictured in Fig. 16. A number of fine membranes can be seen lining the bulbous tip and extending back into the filament for a short distance. The appearance of this preparation suggests that it may be an early stage in daughter cell formation and that the fine membranes may be the precursors of the photosynthetic membranes of the daughter cell. However, sections which include filament tips have been rare, and it would



FIGURES 4 AND 5

Cross-sections of *R. vannielii* cells showing four pairs of closely aligned peripheral membranes. $\times 89,000$.

smaller ones may have been produced as a consequence of membrane breakage followed by healing (15-17, 7).

Only a single membrane can be observed in longitudinal sections of the filaments. This membrane extends into the cell proper (Figs. 13 and 14) and can be shown to be an extension of the cytoplasmic membrane. A curious feature of the membrane in the filament is that it appears in some preparations to be periodically constricted and bridged by septa (Fig. 15).

Except for the instance noted below, we have no evidence for the occurrence of the photosynthetic

be premature to speculate at this time on the origin of the photosynthetic membranes in young cells.

The structure of the material which blocks the bore of the filament between two mature cells is illustrated in Figs. 13 and 14. This structure lies within the confines of the filament wall, and in Fig. 14 a fine line which is continuous with the inner side of the filament cell wall can be seen to traverse the material itself. The cytoplasmic membrane of the cell on the left can be seen to follow the contour of this material. It seems probable that this structure is formed by the

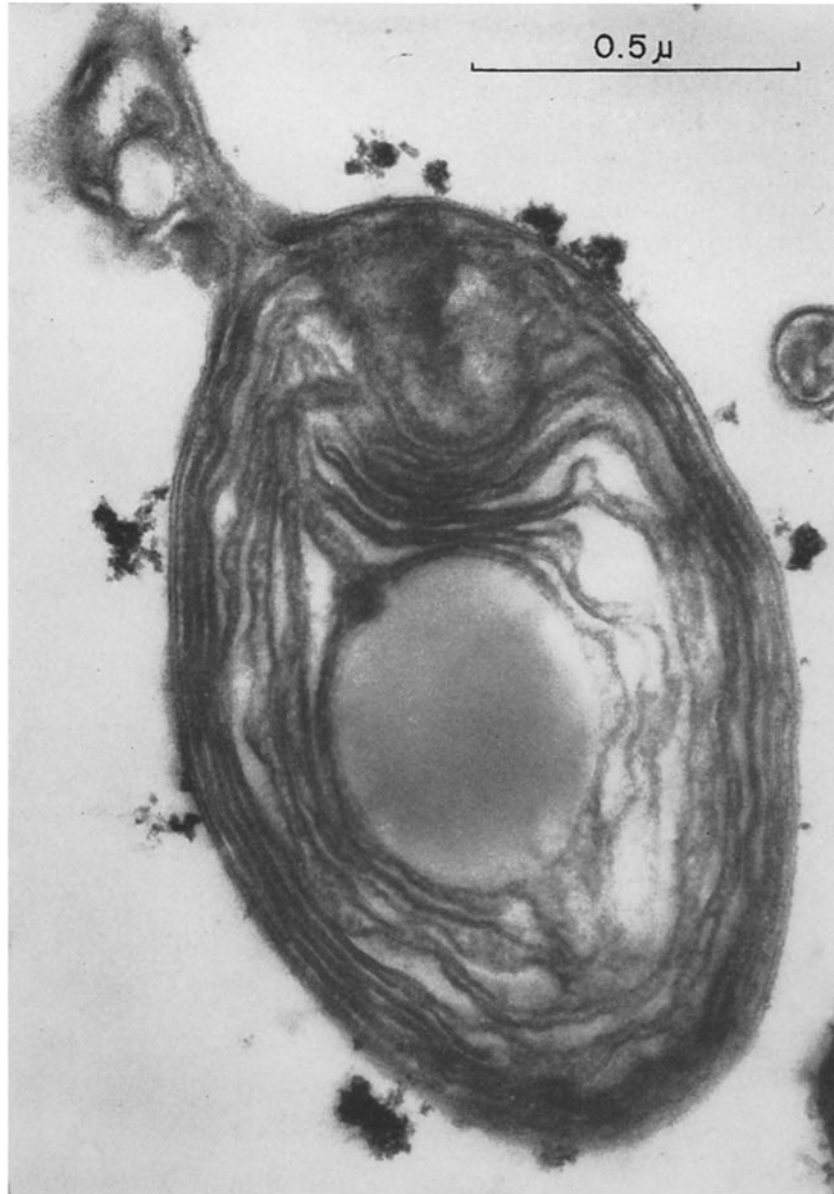


FIGURE 6

Longitudinal section of an *R. vannielii* cell showing extension of a filament at one pole. A number of well delineated paired membranes are seen lying below the cell wall. $\times 89,000$.

deposition of cell wall substance and that it seals the opening between the daughter cell and the filament. It probably also serves as a fracture point which permits the escape of the motile daughter cells from the filament. A somewhat similar structure has been observed between

mother and daughter cells in thin sections of yeast (18).

A feature often noted in cells cut in cross-section (Fig. 17) has been the compounding of two unit membranes in the form of a loop arising from a centrally located unit membrane. In this cell

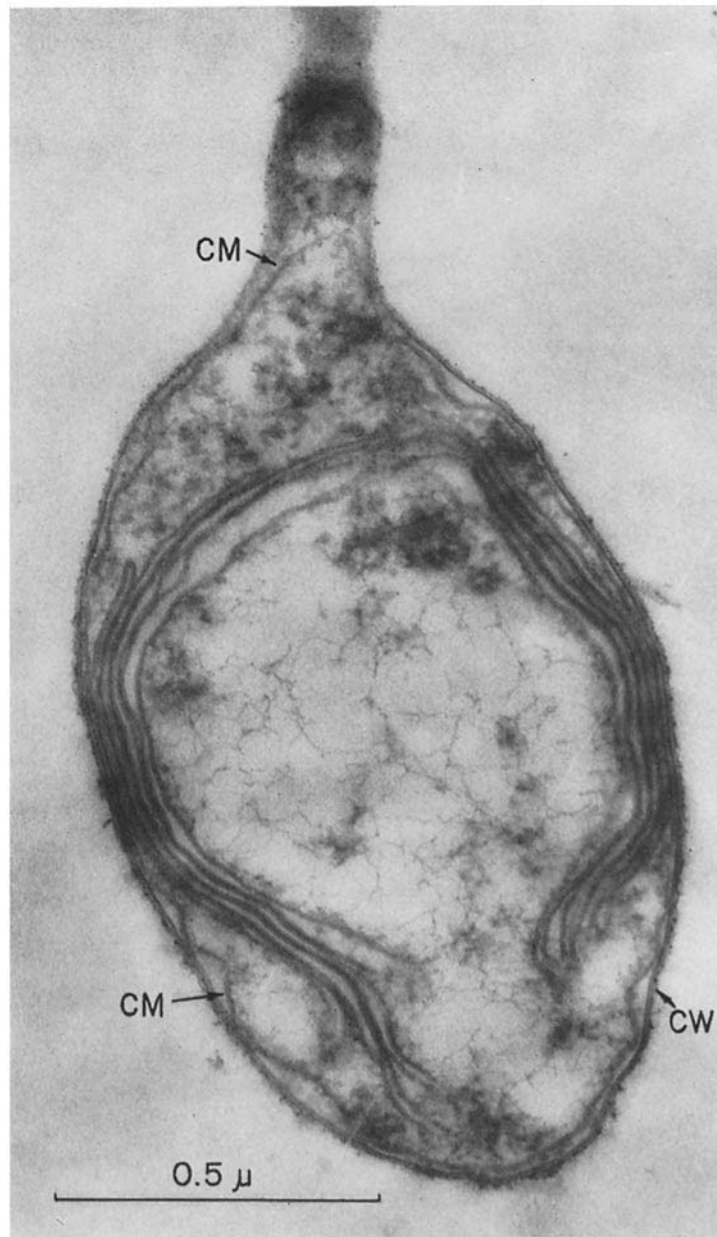


FIGURE 7

Longitudinal section of an *R. vannielii* cell indicating configuration of photosynthetic membranes which appear open at one end. *CM*, cytoplasmic membrane; *CW*, cell wall. $\times 89,000$.

additional paired membranes are seen lying above and concentric to the central area. In Fig. 18, pairing has occurred at four points, forming two distinct and separate closed sacs. No other compound membranes are present, and this cell may

represent an early stage in the formation of the peripheral membranes.

Variation in fixation time or in the temperature at which fixation occurred produced no evident differences in preservation of the cells. However,



FIGURE 8

Longitudinal section of an *R. vamielii* cell. Photosynthetic membranes are seen closely aligned and with their ends looped. The configuration of the membranes is interrupted at both poles of the cell. $\times 89,000$.

FIGURE 9

High resolution electron micrograph of a portion of the photosynthetic membranes shown in Fig. 6. Note pairing of the unit membranes. $\times 170,000$.

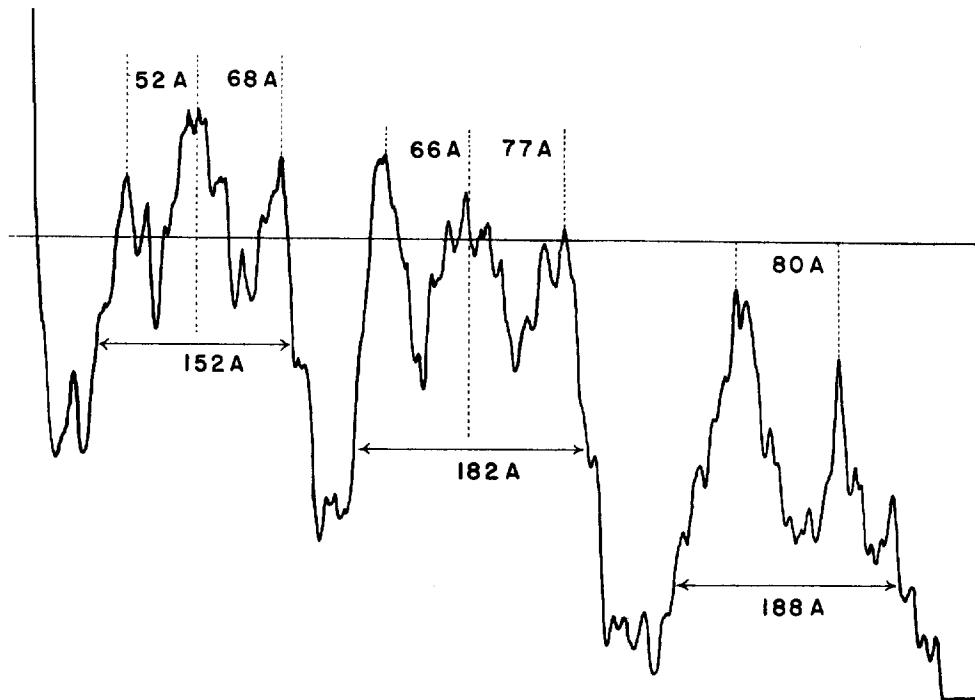


FIGURE 10

Densitometric tracing of membrane area denoted by rectangle in Fig. 9. The lower numbers indicate the width of a pair of unit membranes, and those above indicate the peak to peak measurements. Equivalent dimensions for the cell wall are shown at the extreme right.

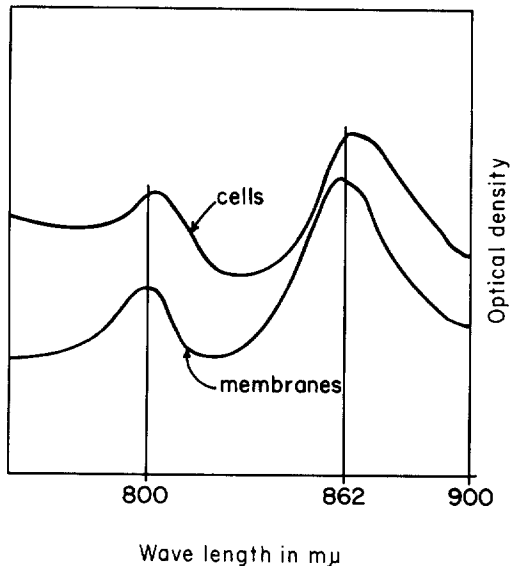


FIGURE 11

Infrared absorption spectra of intact cells and isolated membranes of *R. vannielii* showing peaks in the bacteriochlorophyll regions.

the use of *s*-collidine-buffered osmium was more suitable for the preservation of connecting filaments and septa than for internal membranes, whereas the method of Kellenberger markedly improved the preservation of membranes. Since the characterization of internal membranes was the main concern of this study, the Kellenberger method was used in most cases.

DISCUSSION

Rhodospirillum vannielii is distinguished by its unique mode of reproduction and its internal system of membranes. Although membranous systems are, under certain conditions, to be found in other photosynthetic bacteria, they are hardly comparable to the well developed membrane system which is a constant feature of the cells of *R. vannielii*. For example, Niklowitz and Drews (19) could not find chromatophores in *Rhodospirillum rubrum* but observed paired lamellae distributed randomly in the cytoplasm. Later, Drews (20) observed typical chromatophores

with a lamellar substructure in *R. molischianum*. These conflicting observations were resolved when Hickman and Frenkel (21) showed that very young cells of *R. rubrum* had few chromatophores, but older cells had chromatophores and laminated structures as well. However, it seems doubtful whether these laminated structures play any specific role in photosynthesis, since similar structures have been demonstrated in several non-photosynthetic bacteria (22-25). Of greater interest is the similarity in structure of the membrane system of *R. vannielii* and the blue-green algae. A number of algal species have been examined and without exception an extensive system of internal membranes has been found. For example, Shatkin (7) studied *Anabaena variabilis* and found chlorophyll-bearing membranes evenly spaced parallel to one another and arranged concentrically at the periphery of the cell. The membranes were paired and looped at each end, forming closed discs about 200 A wide. Ris and Singh (8) investigated twelve species of blue-green algae including *A. variabilis* and found in each case an extensive lamellar system of one configuration or another. The width of a unit mem-

brane was about 70 A. Thus, the blue-green algae and *R. vannielii* appear to have comparable membrane systems for photosynthesis.

The mean width of 87 A or 65 A peak to peak for the unit membrane of *R. vannielii* is in good agreement with values reported for membranes in blue-green algae and in the grana of seed plants. The association of membranes with photosynthetic reactions is well documented, and it has been shown that the development of the membranes in some instances occurs only as a response to growth in the presence of light (17, 26, 27). Unfortunately, the obligatory photosynthetic nature of *R. vannielii* does not permit such an experiment with this organism.

Elucidation of the mechanism of membrane formation in *R. vannielii* must await investigation of the fine structure of filament tips which are in the process of maturation into daughter cells.

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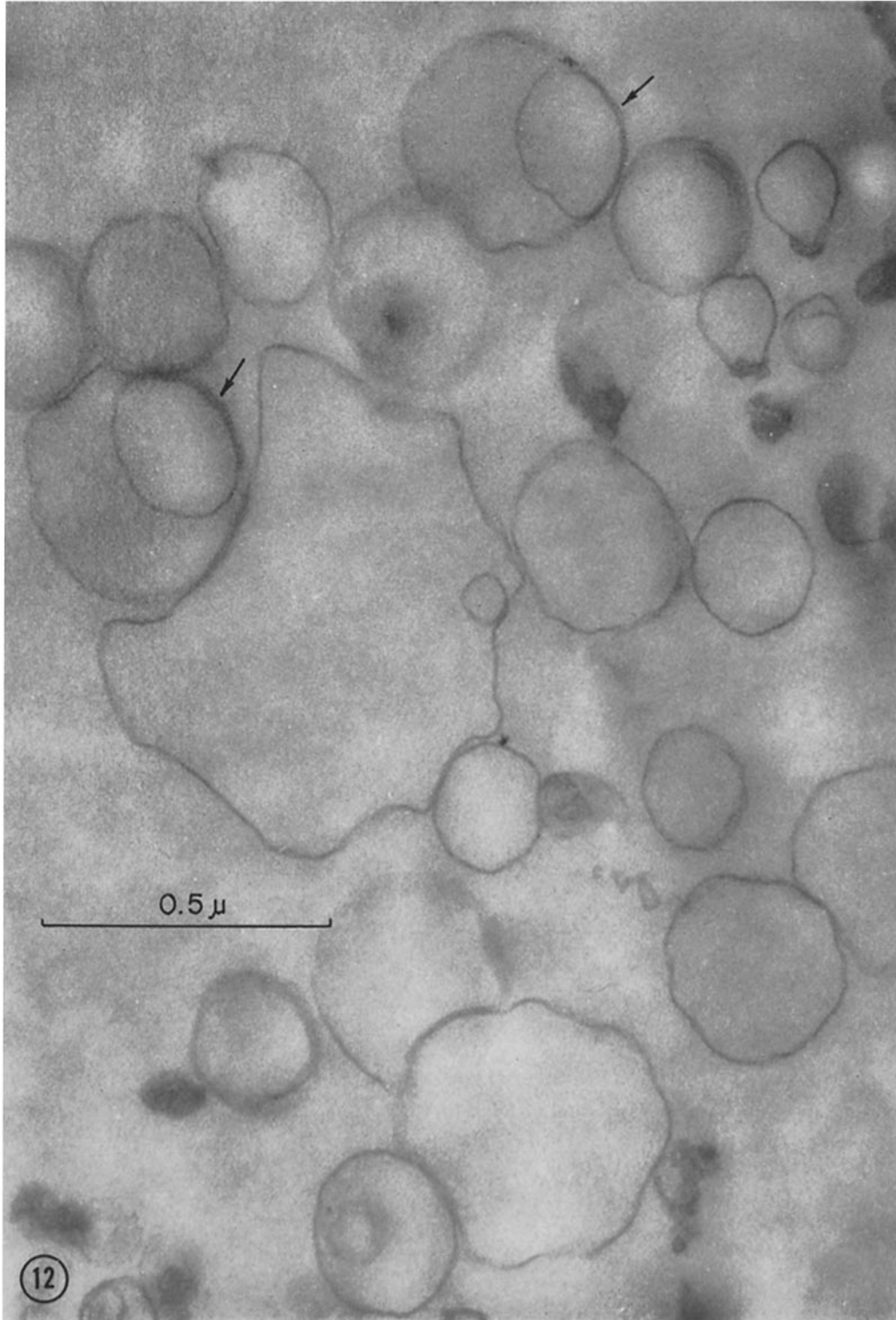
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FIGURE 12

Section of membranous material isolated from *R. vannielii* cells after shaking with glass beads. In some areas the membranes appear to retain evidence of pairing (arrows). $\times 89,000$.



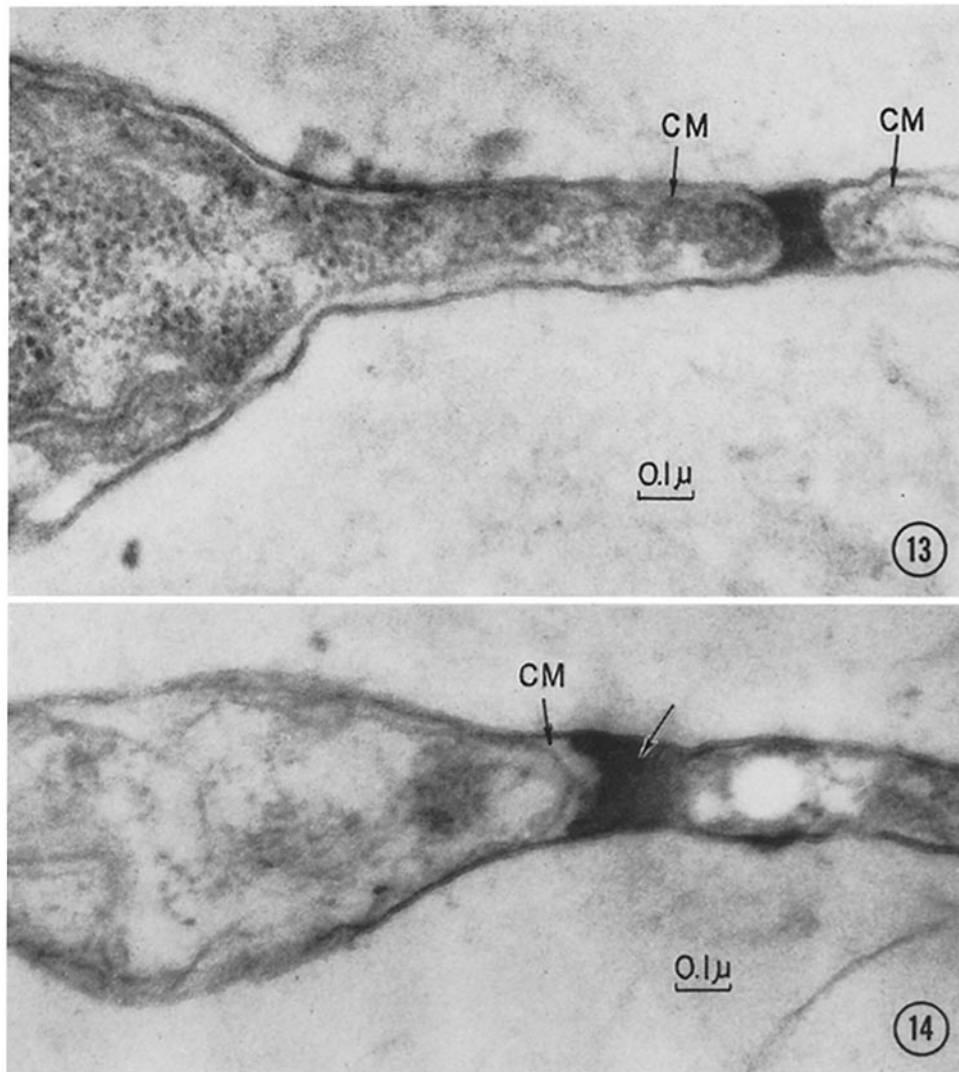


FIGURE 13

Longitudinal section of an *R. vannielii* cell showing extending filament and an electron opaque septum. Note continuation of cytoplasmic membrane (CM) into the filament and up to, and on either side of, the septum. Cell fixed in *s*-Collidine-buffered osmium tetroxide. $\times 89,000$.

FIGURE 14

Longitudinal section of an *R. vannielii* cell showing an electron opaque septum traversed by what appears to be a membrane (arrow). The cytoplasmic membrane (CM) of the cell is seen to follow the contour of the septum. *s*-Collidine buffered. $\times 89,000$.

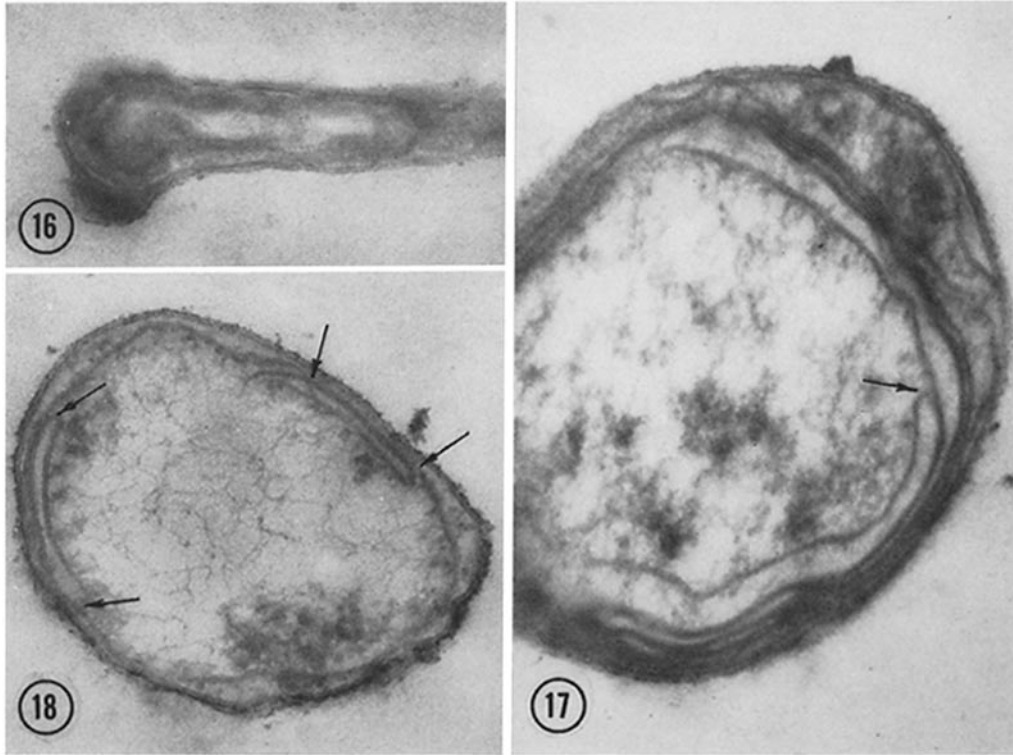
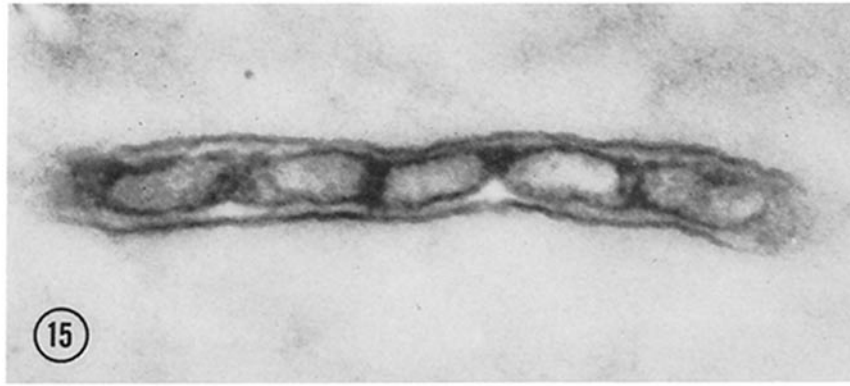


FIGURE 15

Longitudinal section of a filament of *R. vannielii* indicating the internal membranes bridged at intervals by electron opaque material. *s*-Collidine buffered. $\times 89,000$.

FIGURE 16

Longitudinal section of a filament of *R. vannielii* showing bulbous end in the vicinity of which is a series of fine, apparently continuous membranes. $\times 89,000$.

FIGURE 17

Cross-section of an *R. vannielii* cell showing a loop arising from a central unit membrane (arrow). Three other paired membranes have already been formed and lie close to the periphery of the cell. $\times 89,000$.

FIGURE 18

Cross-section of an *R. vannielii* cell showing a single peripheral unit membrane giving rise in two positions to loops of paired membranes (arrows). $\times 89,000$.