THE FINE STRUCTURE OF *RHODOSPIRILLUM RUBRUM*

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ABSTRACT

The fine structure of *Rhodospirillum rubrum* grown under a series of defined conditions has been examined in thin sections prepared by the methods of Ryter and Kellenberger. In cells grown anaerobically at different light intensities, the abundance of 500 A membrane-bounded vesicles in the cytoplasm is inversely related to light intensity, and directly related to cellular chlorophyll content. When the chlorophyll content of the cell is low, the vesicles are exclusively peripheral in location; they extend more deeply into the cytoplasm when the chlorophyll content is high. Typical vesicles also occur, though rarely, in cells grown aerobically in the dark, which have a negligible chlorophyll content. When synthesis of the photosynthetic pigment system is induced in a population of aerobically grown cells by incubation under semianaerobic conditions in the dark, the vesicles become increasingly abundant with increasing cellular chlorophyll content, and the cells eventually acquire the cytoplasmic structure that is characteristic of cells growing anaerobically at a high light intensity. Poststaining with lead hydroxide reveals that the membranes surrounding the 500 A vesicles are indistinguishable in structure from the cytoplasmic membrane, and continuous with it in some areas of the sections. The bearing of these observations on current notions concerning the organization of the bacterial photosynthetic apparatus is discussed.

INTRODUCTION

The photosynthetic apparatus of purple bacteria cannot be resolved as a structural element of the cell by light microscopy, and its characterization was first attempted by physicochemical rather than cytological methods. French (1–3) observed that the photosynthetic pigment system (bacteriochlorophyll and carotenoids) of several species of purple bacteria is released from broken cells in the form of a seemingly soluble pigment-protein complex, which retains the absorption spectrum characteristic of the intact cell. Schachman, Pardee, and Stanier (4, 5) found that in extracts prepared by mechanical abrasion or sonic oscillation of *R. rubrum*, the photosynthetic pigment system is readily sedimentable by ultracentrifugation. By differential centrifugation, they succeeded in isolating a relatively homogeneous pigmented fraction consisting of particles some 600 A in diameter, which they designated as *chromatophores*. Aerobically grown cells of *R. rubrum* do not contain appreciable quantities of bacteriochlorophyll and carotenoids; in extracts of these cells, no material that sedimented at the rate characteristic of chromatophores could be detected (4, 5). Analysis of purified chromatophores showed that they contain a large amount of protein, no DNA, and only traces of RNA (4).

Frenkel's discovery that purified chromatophores can perform a light-dependent anaerobic synthesis of ATP (6, 7) demonstrated that they

retain photosynthetic function, and suggested that the bacterial photosynthetic apparatus had indeed been isolated in cell-free form. Although chromatophore preparations have since been extensively used for studies on the mechanism of bacterial photosynthesis, there is little information about their chemical structure. The most detailed analyses have been performed by Newton and Newton (8), who showed that chromatophore material contains a large number of different chemical substances. On a weight basis, the major constituents are protein (*ca.* 60 per cent) and phospholipid (*ca.* 20 per cent); in addition to bacteriochlorophyll and carotenoids, minor constituents include several catalysts of electron transport. At least one enzyme system not directly involved in photophosphorylation, namely succinoxidase, is present at fairly high levels in chromatophore preparations (9, 10).

Only after chromatophores had been characterized in terms of their physicochemical and functional properties were cytological studies on purple bacteria initiated. Vatter and Wolfe (11) studied the structure of *Rhodospirillum rubrum*, *Rhodopseudomonas spheroides*, and a *Chromatium* sp. by electron microscopy of thin sections. In photosynthetically grown cells, membrane-bounded vesicles of relatively low electron opacity occupied much of the cytoplasm, but these structures could not be detected in cells of *R. rubrum* grown aerobically in the dark. The diameter of the vesicles in *R. rubrum* was 500 to 1000 A, in reasonably good agreement with the estimated diameter of the chromatophores isolated from this species. Vatter and Wolfe accordingly concluded that the vesicles observed in thin sections are the intracellular structures which correspond to isolated chromatophores, a view that has been generally accepted by others (*e.g.* Bergeron and Fuller, 12).

Subsequent work has shown that not all purple bacteria contain these vesicular structures. In *Rhodomicrobium vannielii* (13, 14) and *Rhodospirillum molischianum* (15), thin sections reveal a system of paired cytoplasmic lamellae, the arrangement of which is characteristic for each species. Niklowitz and Drews (16) reported the presence of lamellae in a purple bacterium which they assumed to be *R. rubrum*, but Drews (15) later concluded that the organism in question was *R. molischianum*.

The general findings of Vatter and Wolfe (11) on the cellular structure of *R. rubrum* have been confirmed by Drews (15) and by Boatman and Douglas (14). However, Hickman and Frenkel (17) have given a different account of the fine structure of this bacterium. They were unable to detect vesicular elements in the cytoplasm of "very young" photosynthetically grown cells, those structures becoming evident only in cells from cultures 18 to 24 hours old. In cells from cultures more than 11 days old, typical vesicles were rare, their place being taken by larger lamellar elements. Since chronological age does not define the physiological state of a bacterial population, the observations of Hickman and Frenkel cannot be readily evaluated or compared with those of other workers.

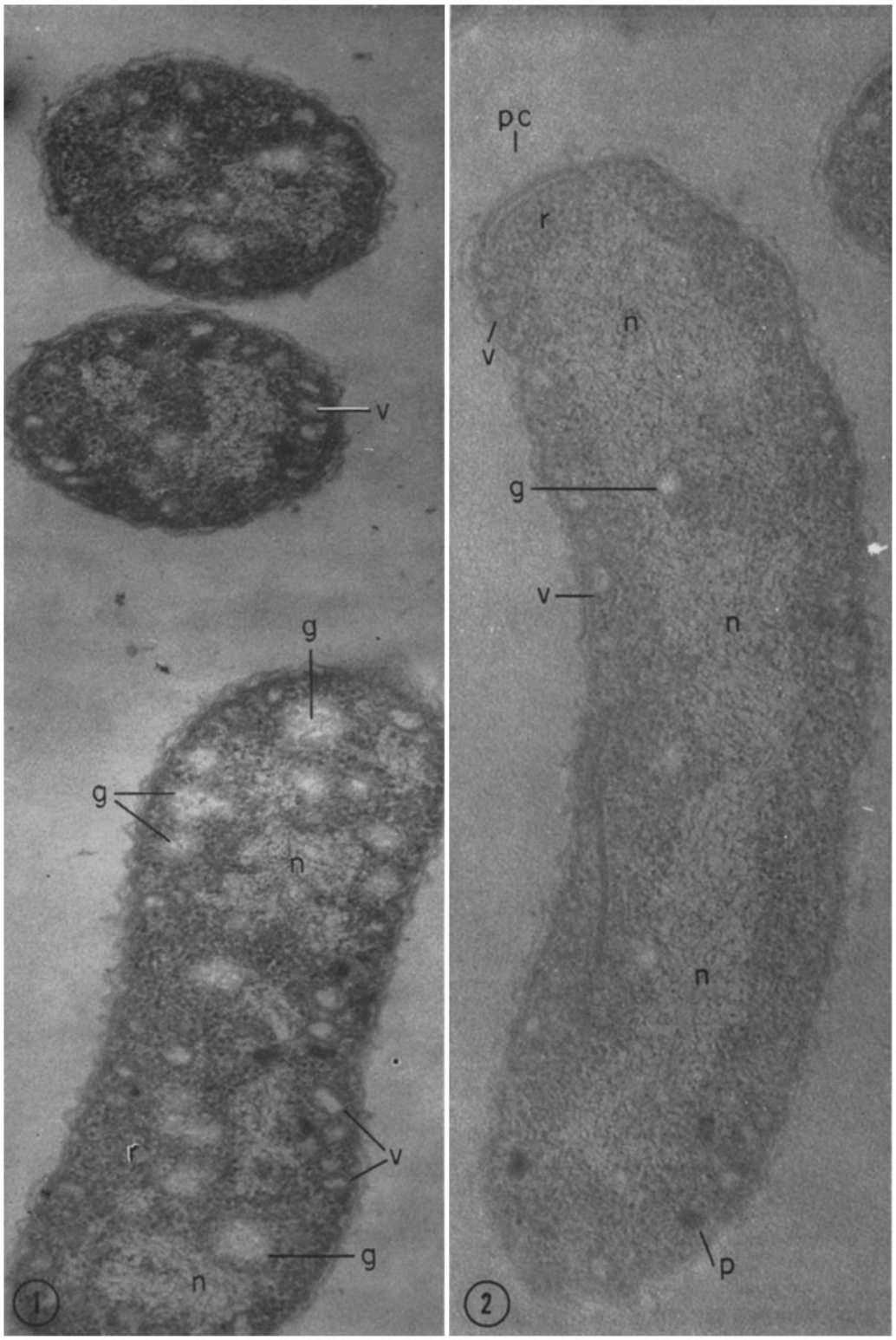
Physiological studies on *R. rubrum* have shown

FIGURE 1

Sections of *R. rubrum* growing exponentially with malate at a light intensity of 2000 foot-candles. In the longitudinal section, numerous glycogen granules (*g*) can be seen scattered among the ribosomes (*r*); the nucleoplasm (*n*) is visible in several regions. Characteristic for cells grown at this light intensity is the peripheral distribution of the 500 A membrane-bounded vesicles (*v*) particularly clearly evident in one of the transverse sections. Main fixation for 18 hours. × 85,500.

FIGURE 2

Median section of a cell from a culture grown to a malate limit at a light intensity of 2000 foot-candles, and then further incubated until the glycogen reserve was almost completely depleted. A few granules of glycogen (*g*) and polyphosphate (*p*) are interspersed among the ribosomes (*r*). The nucleoplasm (*n*) is particularly well preserved. The 500 A membrane-bounded vesicles (*v*) have the same peripheral distribution as in Fig. 1. The polar cap (*pc*) can be seen at the upper end of the cell. Main fixation for 18 hours. × 96,000.


v
g
g
n
r
v
g
n
1
pc
r
n
v
g
v
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n
p
2

that the chemical composition of the cells changes substantially in response to certain environmental factors. Under conditions of photosynthetic growth (anaerobiosis and light) both light intensity (10, 18) and temperature markedly affect the photosynthetic pigment content. *R. rubrum* can also accumulate large amounts of two organic reserve materials. The nature of the reserve material formed by the cells is determined by the chemical nature of the exogenous carbon source, and its concentration in the cell by the phase of growth (19, 20). In the cytological studies which have been discussed above, these environmental factors have not been strictly controlled, and in most cases their possible effects on structure have not even been considered. In the present paper, we report the results of a study of the fine structure of *R. rubrum*, performed on cells grown under a series of well defined conditions, the influence of which on cellular composition is known from previous work.

MATERIALS AND METHODS

All observations were made on *Rhodospirillum rubrum* strain 1.1.1. from the collection of Professor C. B. van Niel. Cultures were grown in the modified medium of Hutner (18), containing malate, succinate, or acetate as the major carbon source. The conditions employed for photosynthetic, aerobic, and semi-anaerobic cultivation have been described elsewhere (10, 18). Cells with identical chlorophyll content but different contents of endogenous carbon reserves were obtained by anaerobic cultivation at a light intensity of 2000 foot-candles, with either malate or acetate as the major carbon source. Cells with a normal level of reserve materials were harvested during exponential growth in the presence of an excess of the specific carbon source. Cells depleted of reserve materials were grown in media with a low initial concentration of the carbon source (malate, 0.0025 M; acetate, 0.005 M) and harvested some hours after entry into the stationary phase, which was induced by exhaustion of the carbon source. Since the final optical densities of these cultures were low, overshadowing with a consequent rise in chlorophyll content did not occur, and the chlorophyll content of the depleted cells remained at the level characteristic of exponentially growing cells.

Fixation, dehydration, and embedding of cells for electron microscopic examination were performed as described by Ryter and Kellenberger (21), except that the period of main fixation "*fixation principale*" was sometimes reduced from 18 to 2 hours. The embedding material was Vestopal. Sections were cut with a Porter-Blum microtome, using either a diamond or a glass knife. The sections were mounted on uncoated 300 or 400 mesh grids, and examined with a Bendix TRS 50 or a Siemens Elmiskop I electron microscope.

Poststaining with lead hydroxide was performed by the procedure of Millonig (22). In some experiments, the fixed cells were treated with ribonuclease. After 2 hours of fixation in osmium tetroxide, such cells were washed twice either with tryptone or with Veronal-acetate buffer (21) and resuspended in an aqueous solution containing 0.25 mg/ml of crystalline ribonuclease.[1] This suspension was incubated at 37°C for 30 minutes, then washed, and resuspended in Veronal-acetate buffer (21) prior to dehydration and embedding.

Determinations of bacteriochlorophyll were made by the method previously described (18), and determinations of protein by the Folin-Lowry method (23).

RESULTS

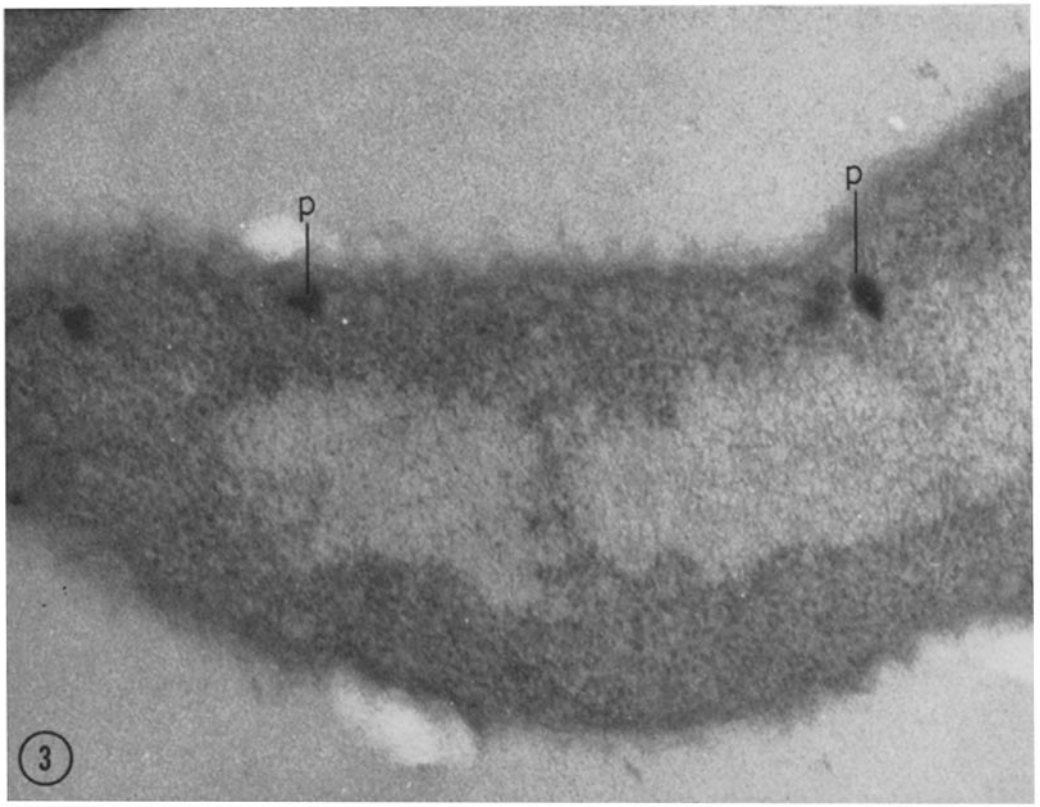
General Features of the Cell

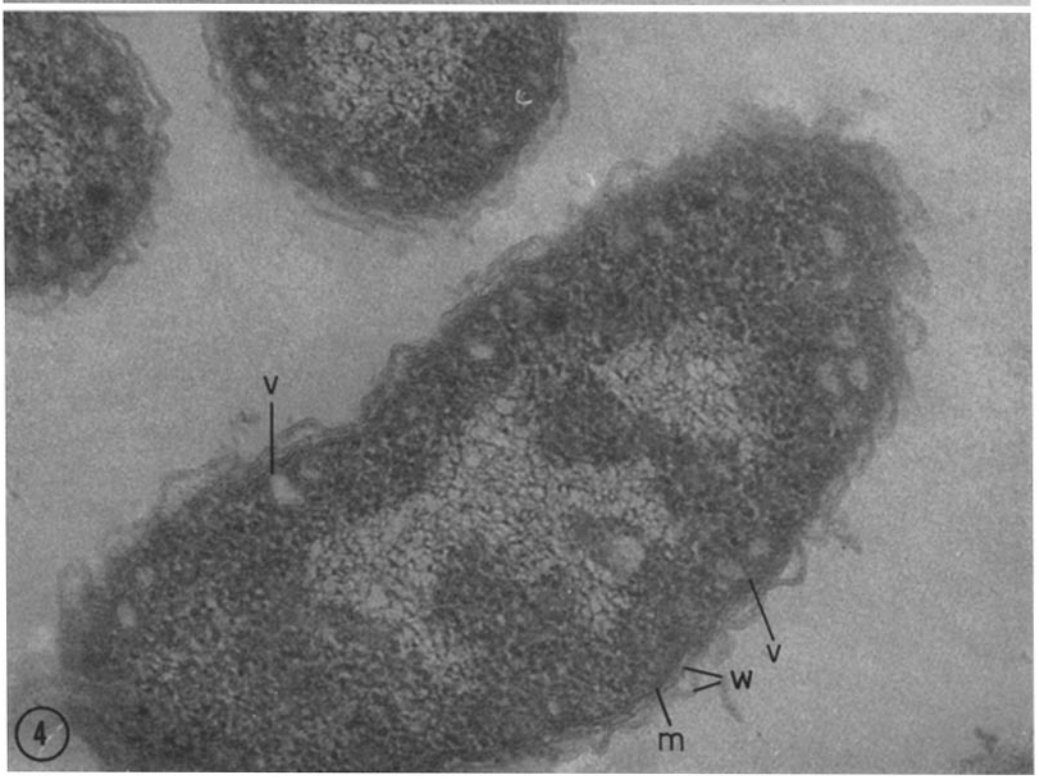
When applied to *Rhodospirillum rubrum*, the techniques developed by Ryter and Kellenberger (21) yield an electron microscopic image of cellular structure quite comparable in its general features

[1] Calbiochem, bovine pancreas, 5 times recrystallized, specific activity approximately 40 eu/mg protein.

FIGURES 3 AND 4

Relatively thick sections of depleted, malate-grown cells similar to the cell shown in Fig. 2. The section in Fig. 4 has been poststained with lead hydroxide, whereas that in Fig. 3 has not; otherwise, the techniques of preparation were identical. Note how poststaining sharpens the structure of the two layers of the cell wall (*w*), the cytoplasmic membrane (*m*), and the membrane-bounded vesicles (*v*). In Fig. 3, the vesicles are barely detectable, and the outer structures of the cell appear hazy. This type of preparation may have led Hickman and Frenkel (17) to conclude that vesicles are absent from very young, photosynthetically grown cells of *R. rubrum*. Polyphosphate granules (*p*) are evident in Fig. 3. Fig. 3, × 90,000; Fig. 4, × 96,000.


p
p
3


v
v
w
m
4

to that obtained with *Escherichia coli*. The nuclear region is well filled with fine fibrils 30 to 60 A in diameter, and is not separated from the cytoplasm by a membrane (*e.g.* Figs. 2 and 3). The cytoplasm is packed with ribosomes (showing as granules 100 to 150 A in diameter), except where membrane-bounded vesicular elements or deposits of reserve materials occur. As illustrated by Figs. 3 and 4, the structure of the cell wall and cytoplasmic membrane shows most clearly in sections poststained with lead hydroxide. In favorable areas of poststained sections, the cytoplasmic membrane can be resolved as a structure bounded by two dense lines, with a total thickness of 75 to 100 A. The wall consists of two layers. The inner layer, about 40 A thick, for the most part closely follows the contours of the membrane. The outer layer consists of two dense parallel lines with a total thickness of approximately 100 to 150 A and has a more irregular, ridged profile (see Fig. 4).

In occasional sections that pass through one pole of the cell, a distinctive cytoplasmic structure which lies in direct contact with the inner side of the membrane can be detected (Figs. 9 and 10). It appears to consist of an array of very fine fibrils, oriented perpendicularly to the surface of the membrane. Its total thickness varies from 140 to 190 A, and it extends from the pole for a distance of some 1500 A in each direction, spanning about half the total width of the cell. We shall term this structure the *polar cap*. The cell membrane and sometimes the wall overlying the polar cap have an unusually smooth and regular profile, suggesting that the whole region may be more rigid than other surface areas of the cell. Significantly, the underlying cytoplasm is free of membrane-bounded vesicles. R. G. E. Murray (personal communication) has observed the same structure in *Spirillum serpens*, and interprets it as the basal apparatus of the flagellar tuft.

Characterization of Reserve Materials

In *Rhodospirillum rubrum*, the chemical nature of the exogenous carbon source determines the nature of the organic reserve material that is formed within the cell. The C_4 dicarboxylic acids and propionate give rise to the deposition of glycogen, while acetate gives rise to the deposition of poly-β-hydroxybutyric acid (20). Figs. 1 to 4 show sections of cells grown anaerobically in the light with malate as carbon source. The cells in Fig. 1 were fixed during exponential growth, those in Figs. 2 to 4 after depletion of the endogenous organic reserves. In Fig. 1, the glycogen deposits are represented by many light areas, scattered irregularly through the cytoplasm. The smallest glycogen granules are about 500 A in diameter, similar in dimensions to the membrane-bounded vesicles characteristic of photosynthetically grown cells. However, the glycogen granules can be readily distinguished by their diffuse periphery, which reflects the absence of a limiting membrane. The largest glycogen deposits have a diameter that approaches 0.15 μ. As is evident from Fig. 1, glycogen is not laid down in close association with the membrane-bounded vesicles.

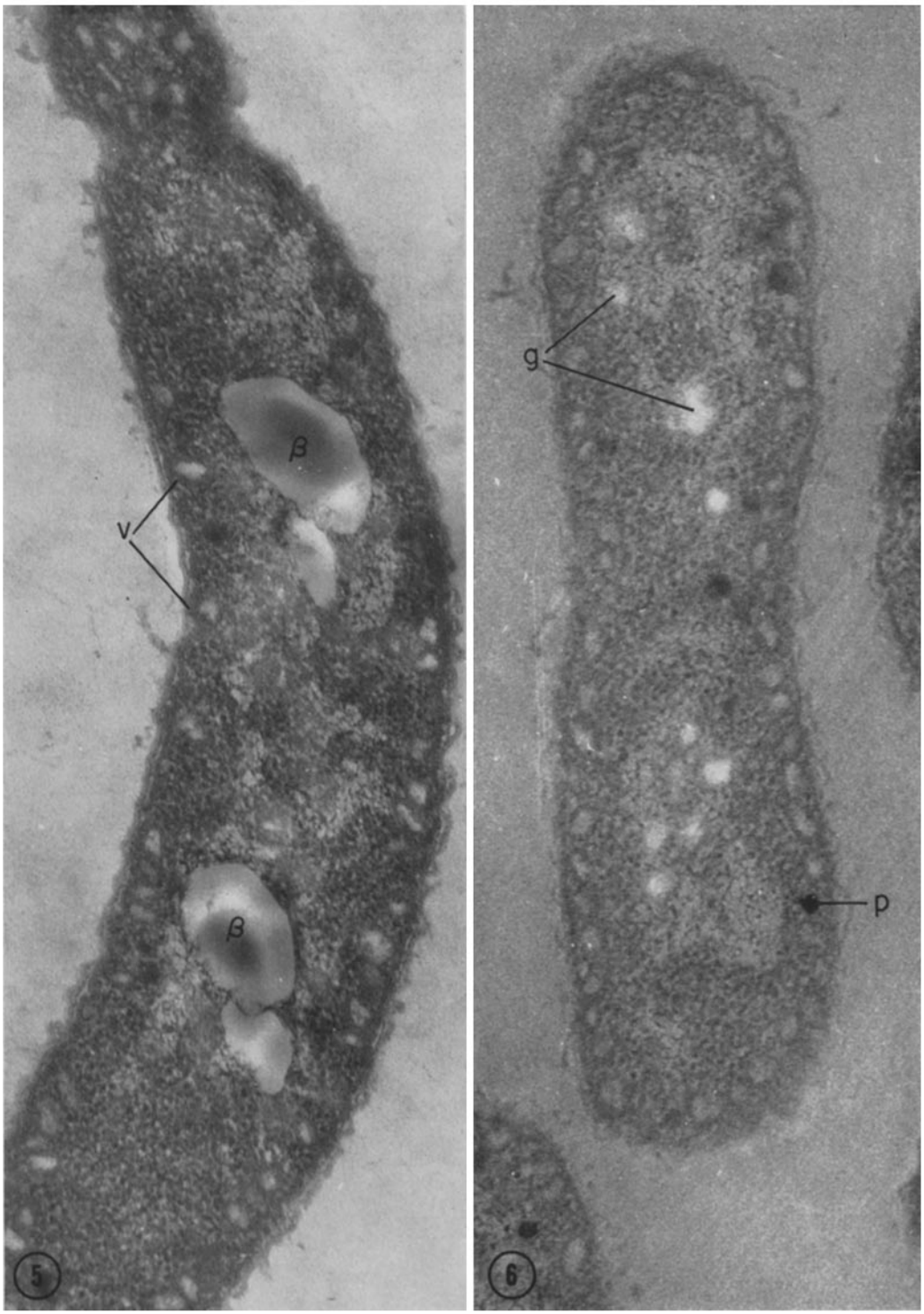
Acetate-grown cells, fixed during exponential growth and after depletion of endogenous carbon reserves, are shown in Figs. 5 and 6, respectively. In Fig. 5, the intracellular sites of poly-β-hydroxybutyric acid deposition are represented by large empty areas, since this cell material is dissolved and removed during embedding. These areas correspond in size and number to the refractile,

FIGURE 5

Median section of a cell fixed during exponential growth with acetate as principal carbon source at a light intensity of 2000 foot-candles. The areas originally occupied by poly-β-hydroxybutyric acid (β) are empty, since this substance is soluble in Vestopal. Note the typical peripheral distribution of vesicles (v), characteristic of cells grown at this light intensity. Main fixation 18 hours, poststained with lead hydroxide. × 88,000.

FIGURE 6

A cell grown with acetate under the same conditions as the cell shown in Fig. 5, but subsequently depleted of poly-β-hydroxybutyric acid. Some glycogen (g) and polyphosphate (p) have been formed. Main fixation, 18 hours, no poststaining. × 96,000.


β
V
β
5
g
p
6

sudanophilic granules visible by light microscopy in acetate-grown cells, which have been shown to consist almost entirely of poly-β-hydroxybutyric acid (19). The same areas were observed in sections of *R. rubrum* by Vatter and Wolfe (11) who interpreted them as "fat vacuoles." However, they are not vacuolar bodies, since no membrane separates them from the surrounding region of the cytoplasm (see Fig. 7). After depletion (Fig. 6), acetate-grown cells contain some glycogen. This is in accord with the earlier observation (20) that depletion of the poly-β-hydroxybutyric acid reserve in the presence of CO_2 is accompanied by glycogen synthesis. As shown in Figs. 2, 3, and 6, depleted cells characteristically contain volutin, which appears in sections as small, very electron-opaque bodies.

Structure of the Photosynthetic Apparatus

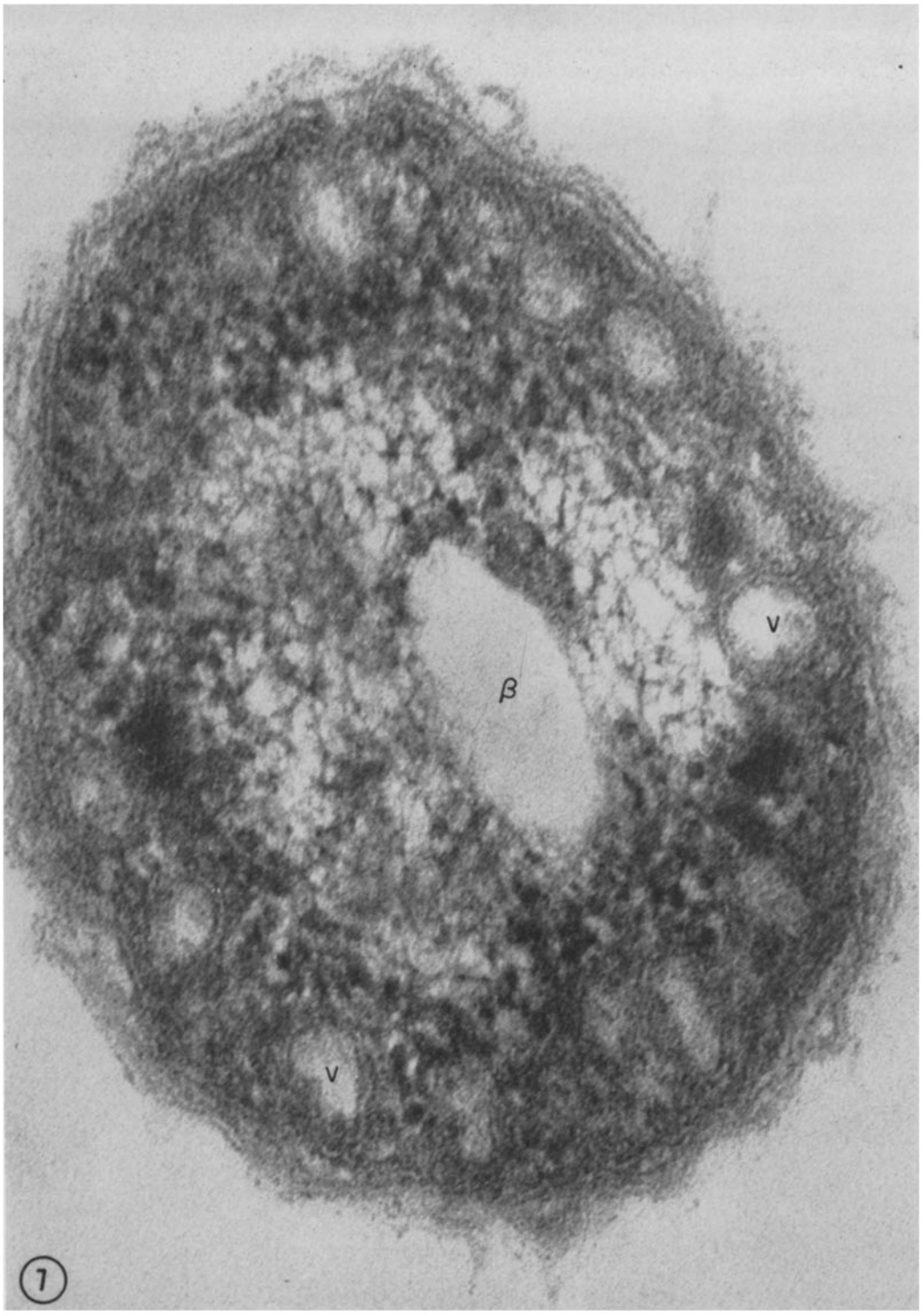
The membrane-bounded vesicles which have been interpreted as the intracellular site of the photosynthetic apparatus of *R. rubrum* are well evidenced in micrographs of thin sections of photosynthetically grown cells. Their frequency and intracellular distribution are markedly influenced by the light intensity employed for photosynthetic growth as shown by Figs. 1, 5, 8, 9, and 12, all of which represent sections of cells, growing exponentially under anaerobic conditions in the light with malate as carbon source. Figs. 11 and 12 represent thin sections of cells growing at a light intensity of 50 foot-candles, far below the level necessary for growth at maximal rate. Vesicles about 500 A in diameter occur throughout the cytoplasm, although careful examination of median sections shows that they are most abundant in the peripheral region of the cell. This cytological picture corresponds well to the structure of photosynthetically grown *R. rubrum* as presented by Vatter and Wolfe (11) and Boatman and Douglas (14). Characteristically, some cells grown in very dim light contain considerably larger vesicles, ranging in diameter up to 1500 A (Fig. 12).

At light intensities above 1500 foot-candles, which is approximately the saturating light intensity for growth, the vesicles are quite constant in size, with a diameter of approximately 500 A, and are entirely confined to the peripheral region of the cytoplasm. In median sections of cells growing at 2000 foot-candles (*e.g.* Figs. 1, 5, and 6) the vesicles assume the form of a regular single layer around the periphery of the cell. This cytological picture corresponds to the structure of photosynthetically grown *R. rubrum* as described by Drews (15). The highest light intensity used in our experiments was 6000 foot-candles; median sections of cells growing at this light intensity show a very sparse and irregular distribution of vesicles in the peripheral cytoplasm, most of the cytoplasmic region being filled with a dense array of ribosomes (Fig. 8). These changes in vesicular structure as a function of increasing light intensity parallel a decline in the specific chlorophyll content of the cells, as shown by the data presented in Table I. Accordingly, there is a close correlation between chlorophyll content and the extent of internal vesicle formation in cells growing photosynthetically under conditions comparable in all respects save for the factor of light intensity.

In succinate-grown cells fixed during exponential growth aerobically in the dark, vesicles apparently identical with those found in photosynthetically grown cells do occur, but they are excessively rare, and cannot be seen in every cell examined (Fig. 13). These cells have a negligible specific chlorophyll content (Table I). If such cells are placed under conditions of limited oxygen access (slow agitation) in the dark, the growth rate falls as a result of the diminished oxygen supply, and at the same time chlorophyll synthesis begins; even though not exposed to light, the cells are immediately capable of performing photo-

FIGURE 7

Transverse section (highly magnified) of a cell fixed during exponential growth with acetate at a light intensity of 2000 foot-candles. The structure of the membrane, bounded by two dense lines, which encloses the vesicles (v) is very clearly shown. The absence of a limiting membrane surrounding the deposit of poly-β-hydroxybutyric acid (β) is likewise evident. Main fixation 18 hours, poststained with lead hydroxide. × 258,000.


β
V
V
7

synthesis once they have acquired a photosynthetic pigment system (10). As shown in Table I, the specific chlorophyll content of the cells rises rapidly under these semianaerobic conditions, and after 5 hours of incubation reaches the level characteristic of cells grown anaerobically at a light intensity of 6000 foot-candles. Sections of aerobically grown cells fixed after 3 and after 5 hours of semianaerobic incubation are shown in Figs. 14 and 15, respectively. The abundance of vesicles increases with the increase in chlorophyll content; after 5 hours of semianaerobic growth in the dark, the cells cannot be distinguished cytologically from cells grown anaerobically at a high light intensity (*e.g.* Fig. 8).

In cells fixed by the procedure of Ryter and Kellenberger (21) but not otherwise treated, the membranous investment of the vesicles can just be perceived, but it is not very distinct (see Figs. 1, 2, 6, 11, and 12). This structural feature becomes much sharper in sections that have been poststained with lead hydroxide (see Figs. 4, 5, and 7); and in these preparations, the dimensions and structure of the cytoplasmic membrane and of the membranes that surround the vesicles appear identical. Both are defined by two dense parallel lines when observed in sufficiently thin section, and have a total thickness of 75 to 85 A. The structure and disposition of the intracellular membrane system stand out most clearly in sections of cells that have been treated with ribonuclease after fixation, and poststained with lead hydroxide (Figs. 16 and 17). In many cells, the enzymatic treatment causes an almost complete removal of the ribosomes, with a consequent clearing of the cytoplasmic background. Particularly in these preparations, some of the vesicles lying close to the surface of the cell can be seen to represent invaginations of the cytoplasmic membrane: the membrane which defines the vesicle is continuous with adjacent parts of the cytoplasmic membrane, and the central transparent area of the vesicle opens into the space between the cytoplasmic membrane and the innermost layer of the wall. The opening is a narrow one approximately 100 to 200 A in width. The clearest examples are indicated by arrows in Figs. 8, 16, and 17. In the region of the polar cap, which lies in direct contact with the cytoplasmic membrane, peripheral vesicles have not been observed, even in cells where such vesicles are abundant in adjacent areas. It would seem, therefore, that invagination of the membrane cannot occur through the polar cap.

DISCUSSION

Possible Interpretations of the Structure of the Photosynthetic Apparatus in R. rubrum

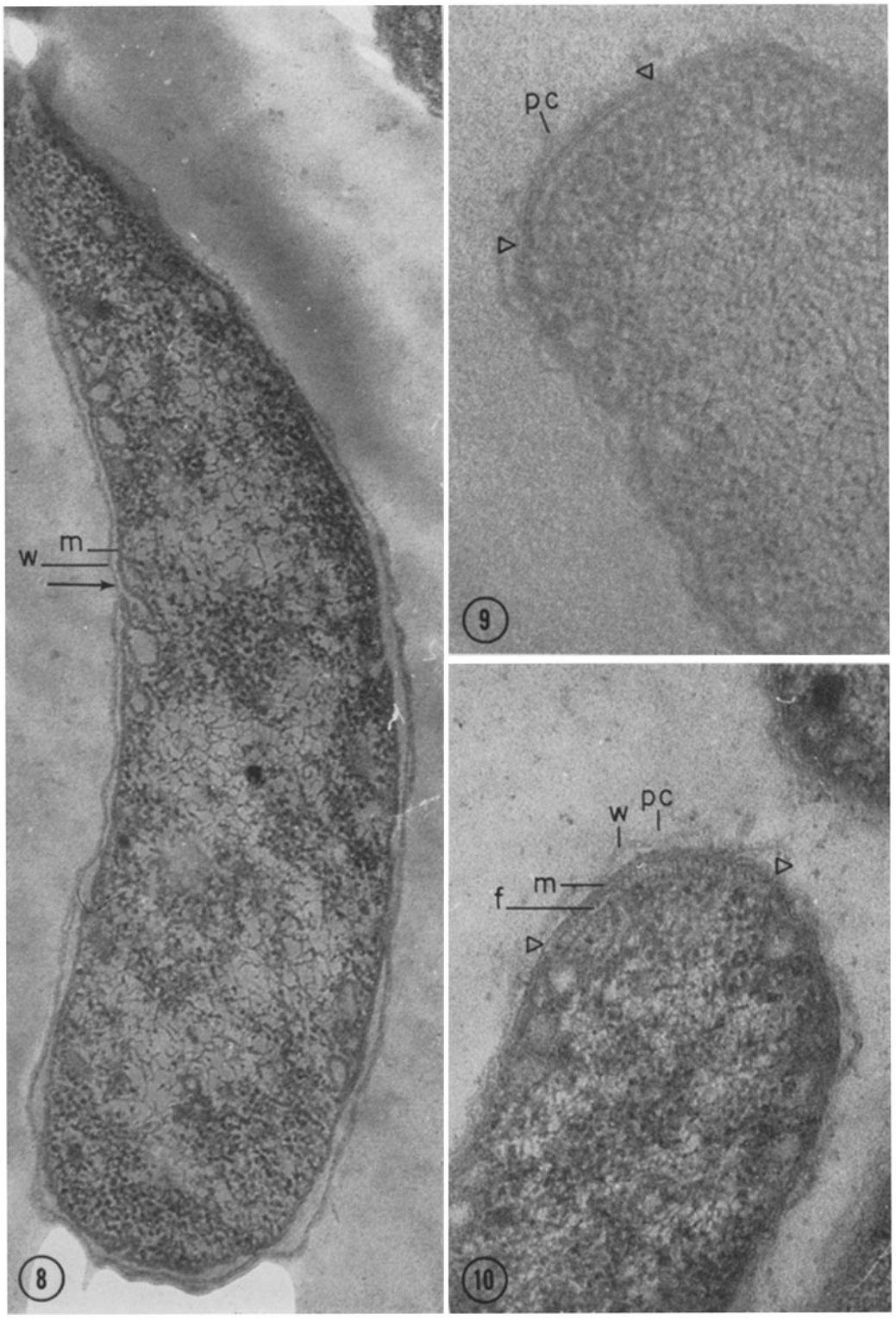
We have shown that in both light- and dark-grown cells of *R. rubrum* the extent of the membrane-bounded vesicular system in the cytoplasm is closely correlated with the chlorophyll content of the cells. This strengthens the assumption, made by previous workers (11, 12, 15), that the vesicles constitute the intracellular site of the photosynthetic apparatus, and are the cellular elements

FIGURE 8

Section of a cell growing exponentially with malate at a light intensity of 6000 foot-candles. A small number of irregularly distributed peripheral vesicles occur in place of the regular peripheral array characteristic of cells grown at 2000 foot-candles (see Figs. 1, 2, 5, and 6). At the point indicated by the arrow, the vesicular membrane is continuous with the cytoplasmic membrane, and the light central area of the vesicle opens into the space that lies between the wall (*w*) and the membrane (*m*). Main fixation 2 hours, poststained with lead hydroxide. × 96,000.

FIGURES 9 AND 10

Transverse sections through the poles of two cells, to illustrate the structure of the polar cap (*pc*). The triangles define the extent of this organelle. Its fibrillar structure (*f*), and its relation to the cytoplasmic membrane (*m*) and wall (*w*) are particularly clear in Fig. 10. Note the absence of membrane-bounded vesicles in the cytoplasm that underlies the polar cap. Fig. 9 is an enlargement (× 165,000) of a portion of Fig. 2. Fig. 10, main fixation 2 hours, poststained with lead hydroxide. × 128,000.


w
m
8
pc
9
w
pc
f
m
10

from which chromatophores arise following mechanical breakage of the cell. We have also observed that the membrane which bounds these vesicles is identical in structure and dimensions with the cytoplasmic membrane, and can in favorable cases be seen to be physically continuous with it. When such continuity exists, the transparent central area of the vesicle opens into the space between the membrane and the wall; in other words, it is external to the cytoplasm. These observations throw doubt on the interpretation (12) of the vesicles as hollow spheres, identical in their structure with the chromatophores present in extracts of broken cells. The apparently closed vesicles which are most frequently seen in thin sections might, like the rarer open peripheral vesicles, represent invaginations of the cytoplasmic membrane, sectioned in a plane that does not reveal the structural continuity. If this interpretation is correct, the photosynthetic apparatus of *R. rubrum* is incorporated in a continuous membrane which also serves as the cytoplasmic membrane, and shows more or less deep infolding into the cytoplasm, depending on the pigment content of the cell. The transparent central area of the vesicle must then be regarded as a region that lies outside the cytoplasm but is enclosed by the cell wall.

The alternative interpretation is that most of the vesicles are in fact hollow, membrane-bounded spheres embedded in the ribosomal matrix, and that they arise from invaginations of the cytoplasmic membrane but eventually achieve physical separation from it. Clearly, a final decision between these two possible interpretations must await the study of serial sections.

There is, however, other evidence in support of the view that the photosynthetic apparatus of *R. rubrum* is built into a continuous matrix within the cell. As reported by Karunairatnam, Spizizen, and Gest (24), cells of *R. rubrum* can be converted to osmotically sensitive spheroplasts by treatment with lysozyme and versene; and if they are then subjected to osmotic lysis, essentially no pigmented particulate material with the dimensions of chromatophores is released. Instead, the entire photosynthetic pigment system remains associated with the cytoskeleton. We have observed that under such conditions the granules of poly-β-hydroxybutyric acid present in acetate-grown cells are liberated from the cytoskeleton, which demonstrates that particulate material large enough to be visible in the light microscope can leave the lysed cell. If part of the photosynthetic apparatus were present in the cells as isolated spheres with the dimensions of chromatophores, at least this fraction should be free to leave the cell when osmotic lysis occurs.

When Schachman, Pardee, and Stanier (4, 5) isolated and defined the chromatophores of *R. rubrum*, there was little or no information about the bacterial cytoplasmic membrane. This structure was first physically characterized in *Bacillus megaterium* by Weibull (25, 26), who isolated it as an empty "ghost" after osmotic lysis of cells which had been completely stripped of their walls by treatment with lysozyme. Subsequent work has shown that sonic breakage of non-photosynthetic bacterial cells comminutes the cytoplasmic membrane into particles of heterogeneous size (27, 28). It is, accordingly, quite reasonable to assume that the "chromatophores" isolated from cells of purple bacteria that have been broken by these methods may also represent fragments derived from an initially continuous cytoplasmic membrane. Their gross chemical composition (8) is typical of bacterial membrane systems. In support of this interpretation, it may be noted that when extracts of *R. rubrum* are prepared by very brief sonic oscillation, the pigment-bearing particulate material is far from homogeneous in size. A minor part of the pigment system sediments at the rate characteristic of chromatophores as defined by Schachman *et al.* (4); most of it is carried on much

FIGURES 11 AND 12

Sections of cells growing exponentially with malate at a light intensity of 50 foot-candles. Membrane-bounded vesicles extend deep into the cytoplasm, some impinging on the nucleoplasm. However, as the median section in Fig. 11 shows, the vesicles are most closely packed at the periphery of the cell. In Fig. 12, one cell contains several very large vesicles, which are not uncommon in cells growing at very low light intensities. Main fixation 18 hours, no poststaining. × 84,000.

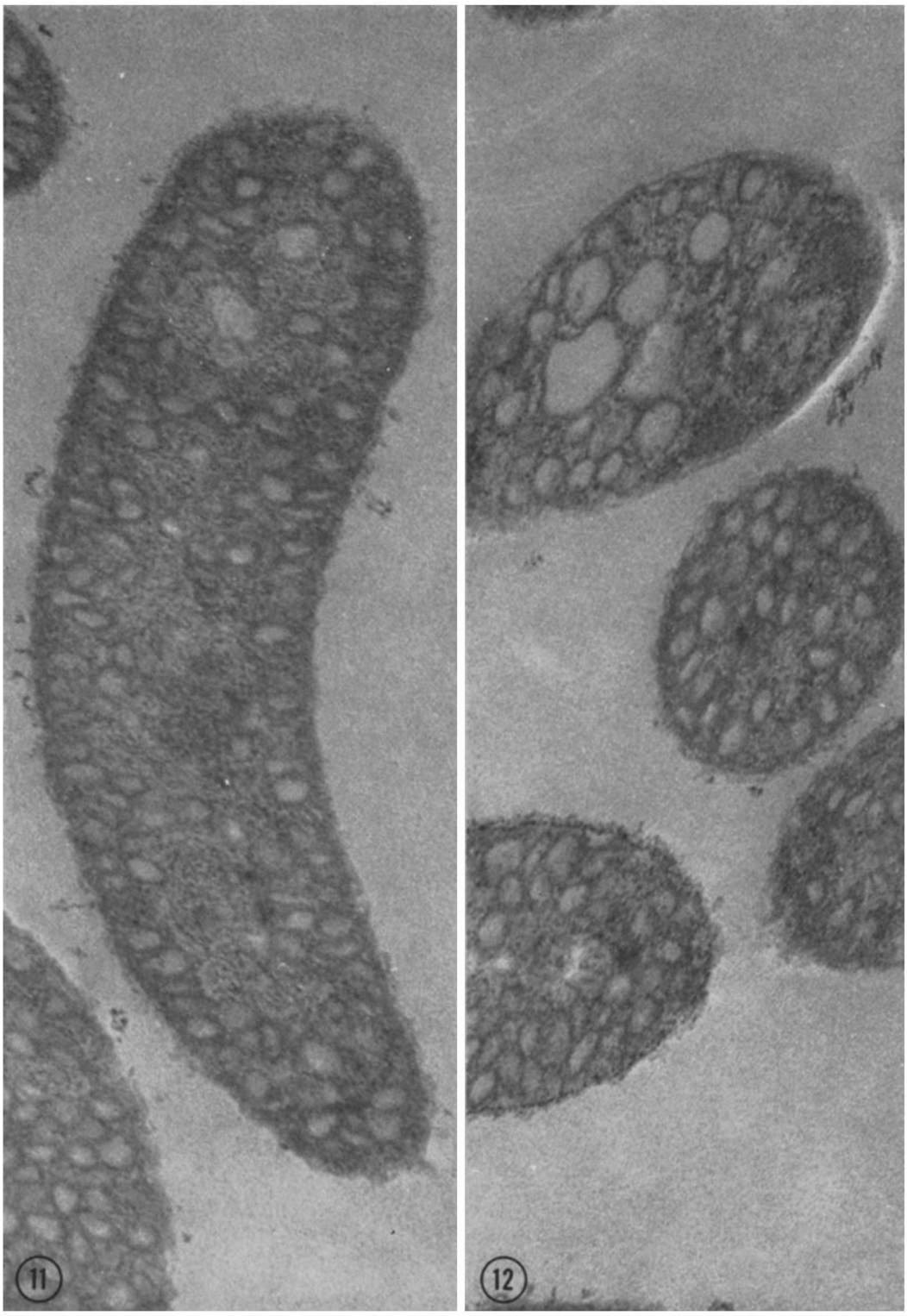

11
12

TABLE I

The Bacteriochlorophyll Content of Rhodospirillum rubrum Growing under Different Environmental Conditions

	Chlorophyll content of cells, μg/mg cellular protein
Photosynthetic growth conditions*	
at light intensity of	
50 foot-candles	25.0
2000 foot-candles	10.2
6000 foot-candles	5.6
Respiratory growth conditions‡	
Full aeration	0.2
Full aeration, then limiting oxygen for 3 hrs.	3.3
Full aeration, then limiting oxygen for 5 hrs.	6.0

* Malate as carbon source; gas phase: 95 per cent N_2–5 per cent CO_2; illumination.
‡ Succinate as carbon source; gas phase: air; no illumination.

larger cellular fragments with a relatively low specific chlorophyll content (10). Such fragments are probably analogous to the "hulls" obtainable by brief sonic oscillation of *Azotobacter*, which were shown by Marr and Cota-Robles (27) to consist of relatively large pieces of the wall and membrane. In this connection, it should be recalled that Newton (29) found antisera prepared against chromatophores of *R. rubrum* to be capable of reacting with and agglutinating intact cells. His observation suggests that the supposedly pure chromatophore fraction used for immunization still carried antigenic components derived from the wall of the cell. We are thus led to believe that chromatophores, despite their retention of photosynthetic function, are structural artifacts produced by the disruption of the bacterial cytoplasmic membrane. It must still be admitted, however, that the chromatophore fraction in extracts of purple bacteria shows far greater homogeneity of size than do the wall and membrane fragments produced from non-photosynthetic bacteria by similar methods of cell breakage. This could reflect the fact that a bacterial membrane system which contains a photosynthetic apparatus has regularly disposed "weak points" at which preferential breakage occurs. Since Schachman *et al.* (4) could not find a well defined particulate fraction with the size range of chromatophores in extracts from aerobically grown cells of *R. rubrum*, the pigment-free cytoplasmic membrane of this purple bacterium evidently undergoes random fragmentation, like that of a non-photosynthetic bacterium.

Functional Implications of the Proposed Model

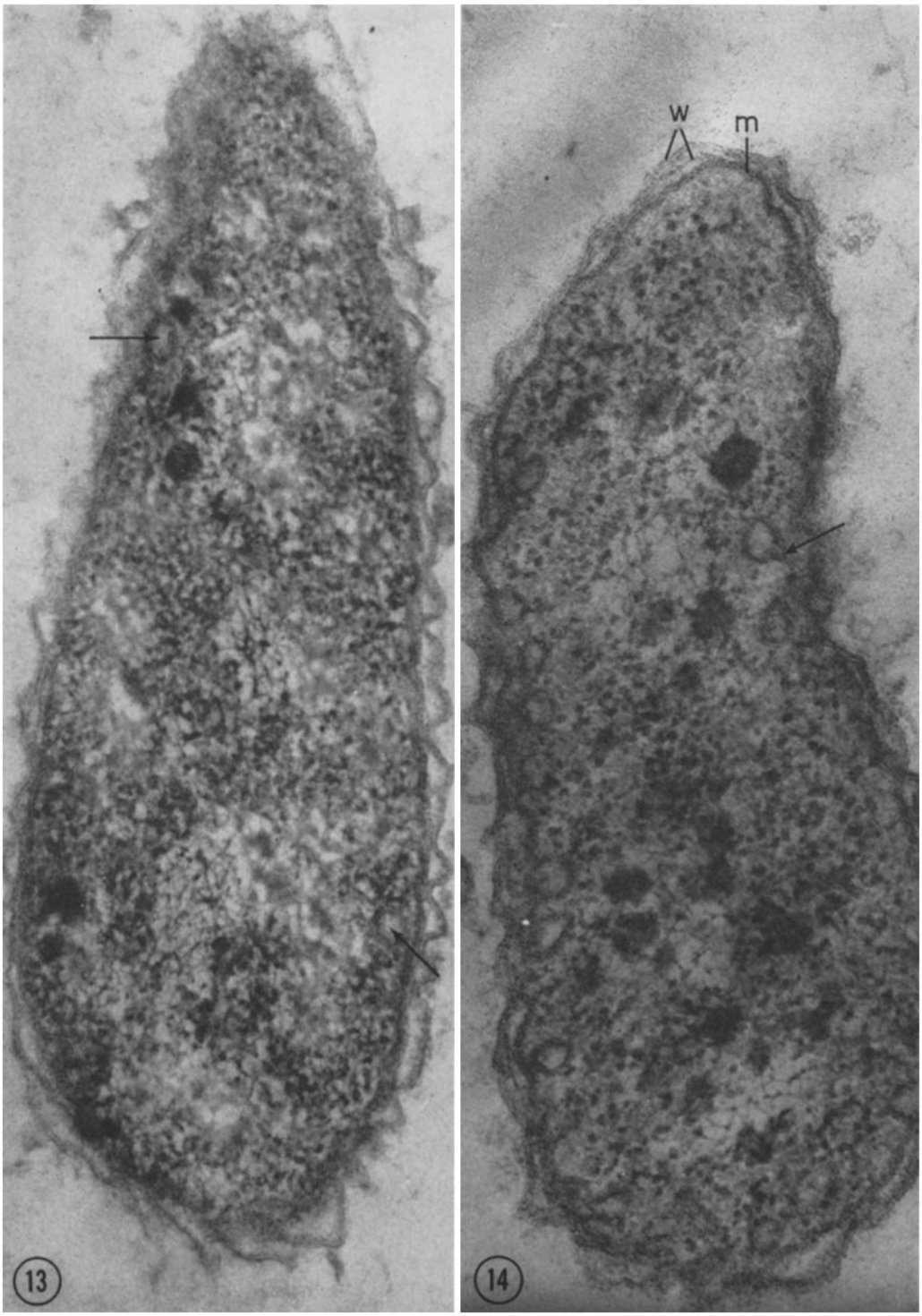
There is now considerable evidence, summarized by Marr (30), to show that in non-photosynthetic bacteria the enzymatic machinery of respiratory electron transport, together with succinoxidase, is localized in the cytoplasmic membrane. The bacterial cytoplasmic membrane therefore assumes functions that are displayed by the inner membrane system of the mitochondrion in the eucaryotic cell, as well as the functions in

FIGURE 13

Section of a cell growing exponentially with succinate as a carbon source under strictly aerobic conditions in the dark, and containing only traces (see Table I) of photosynthetic pigments. Membrane-bounded vesicles are either very rare or absent in this type of cell. The arrows indicate two such vesicles in the cell that is illustrated. Main fixation 2 hours, followed by short RNase treatment; poststained with lead hydroxide. × 120,000.

FIGURE 14

Section of a cell from a culture grown aerobically in the dark with succinate and then placed for 3 hours under semianaerobic conditions in the dark. Wall, *w*; cytoplasmic membrane, *m*. Peripheral membrane-bounded vesicles (arrow) are much more numerous than in aerobically grown cells; compare with Fig. 13. Treatment as described in Fig. 13. × 120,000.


13
w
m
14

regulating permeability that are universal attributes of cytoplasmic membranes. In the case of photosynthetic bacteria, we suggest that the pigments and special enzymes required for photosynthetic electron transport are likewise built into the fabric of the cytoplasmic membrane. Generally speaking, therefore, the bacterial cytoplasmic membrane plays (at least) two major roles in the economy of the cell: it regulates entry and exit of materials, and serves as the site of respiratory and photosynthetic ATP synthesis. The commonly observed capacity of bacteria to vary the level of their respiratory or photosynthetic function in response to environmental changes should, according to this hypothesis, reflect changes in the differential rate of synthesis of specific membrane components. Since the membrane is of uniform thickness, the total cellular content of membrane material can be varied only by a change in its area. Within limits, levels of respiratory or photosynthetic activity can no doubt be increased by a high rate of differential synthesis of the special enzyme systems concerned, and their incorporation into a membrane of fixed area and simple structure. Beyond a certain point, however, a further increase in the specific level of these activities can occur only if the area of the membrane is increased, by intrusion into the cytoplasm.

Among non-photosynthetic bacteria, a variety of membranous intrusions into the cytoplasmic region have been observed in organisms which have a respiratory metabolism; these results are summarized and interpreted by R. G. E. Murray (31). Significantly enough, the most elaborate internal membrane system so far observed in an aerobic bacterium occurs in *Azotobacter* (32), which has the highest respiratory rate recorded for any living organism.

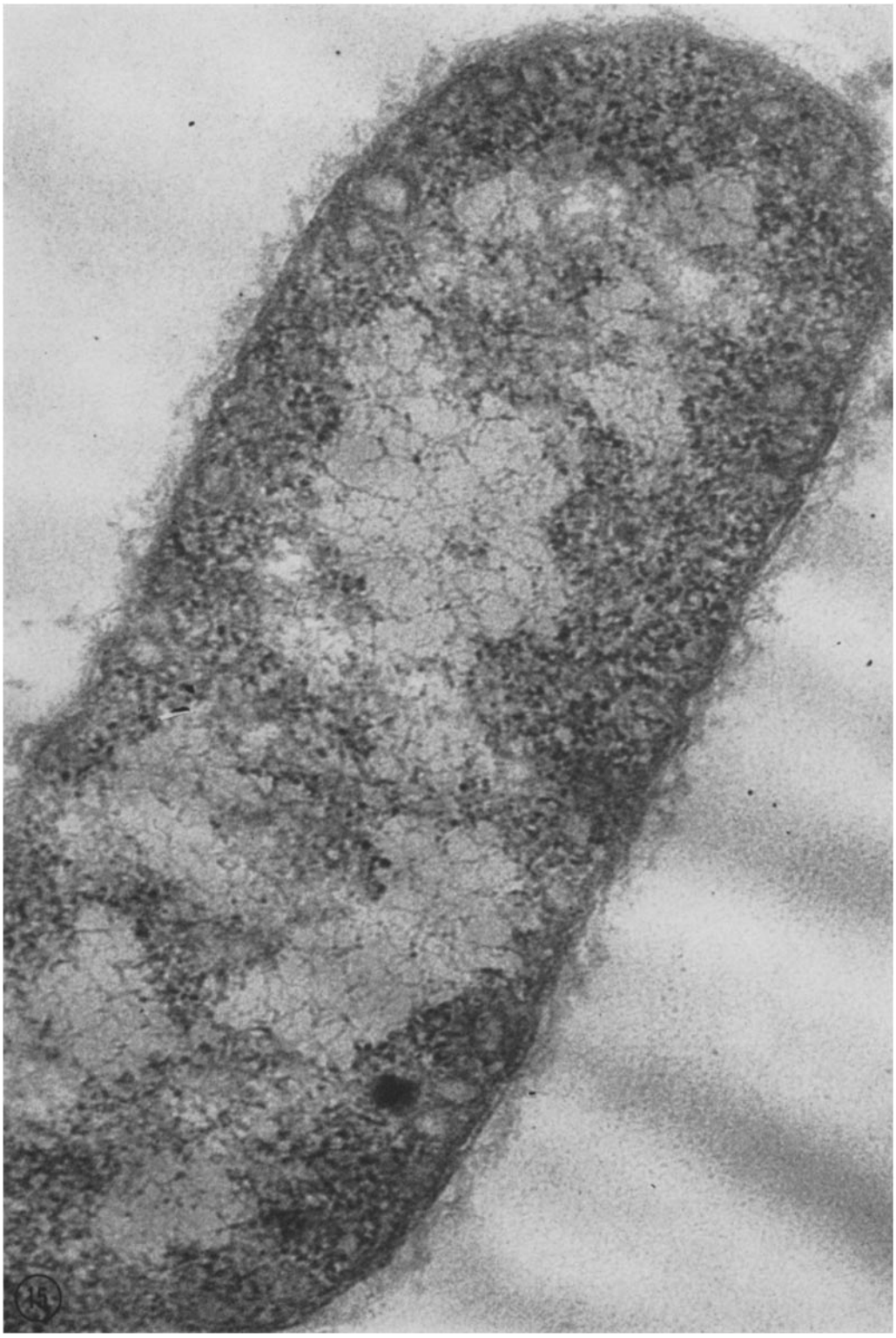
Rhodospirillum rubrum is physiologically transitional between the non-photosynthetic, aerobic bacteria and the purple bacteria which are strict anaerobes and obligate phototrophs. Grown aerobically, it does not synthesize photosynthetic pigments, and is dependent on respiration as a source of energy. We assume that the membrane in such aerobic cells is essentially similar in structure and function to the membrane of a non-photosynthetic bacterium. In thin sections, it appears simple, apart from very rare vesicular intrusions. These vesicles are comparable in abundance and fairly similar in appearance to the "simple intrusives" discovered by Murray (31) in the non-photosynthetic bacterium, *Spirillum serpens*. If such aerobic cells of *R. rubrum* are now exposed to a limited oxygen supply in the dark, chlorophyll, carotenoids, and other components of a functional photosynthetic apparatus start to be synthesized and incorporated into the growing cytoplasmic membranes (10); this modification of membrane function is accompanied by a steady increase in the frequency of membranous intrusions until, after some hours, the typical sparse peripheral distribution of vesicular elements also characteristic of cells grown anaerobically at high light intensity makes its appearance. The maximal extension of the area of the membrane, which gives the appearance in thin sections of a vesicle-filled cytoplasm, is characteristic of cells which have built up their photosynthetic pigment content to an extremely high level, as a result of anaerobic growth at low light intensity.

Note added in proof: Giesbrecht and Drews (*Arch. Mikrobiol.*, 1962, **43**, 152) have recently published a study of the structure and origin of the internal lamellae of *Rhodospirillum molischianum*. In their electron micrographs of thin sections prepared from osmotically lysed cells, the continuity between the internal lamellae and the cytoplasmic membrane is very clearly shown. Their interpretation of the organization of the lamellar photosynthetic apparatus in *R. molischianum* accordingly corresponds very closely to our interpretation of the vesicular photosynthetic apparatus of *R. rubrum*.

This study could not have been undertaken without the generous help and advice given to us by Dr. J. H. McAlear and by his associates on the staff of the Electron Microscope Laboratory. We are particularly indebted to Mr. Lloyd Thibodeau for his assistance

FIGURE 15

Section of a cell grown aerobically with succinate and then placed for 5 hours under semianaerobic conditions in the dark. Compare with Figs. 13 and 14. Treatment as described in Fig. 13. × 150,000.



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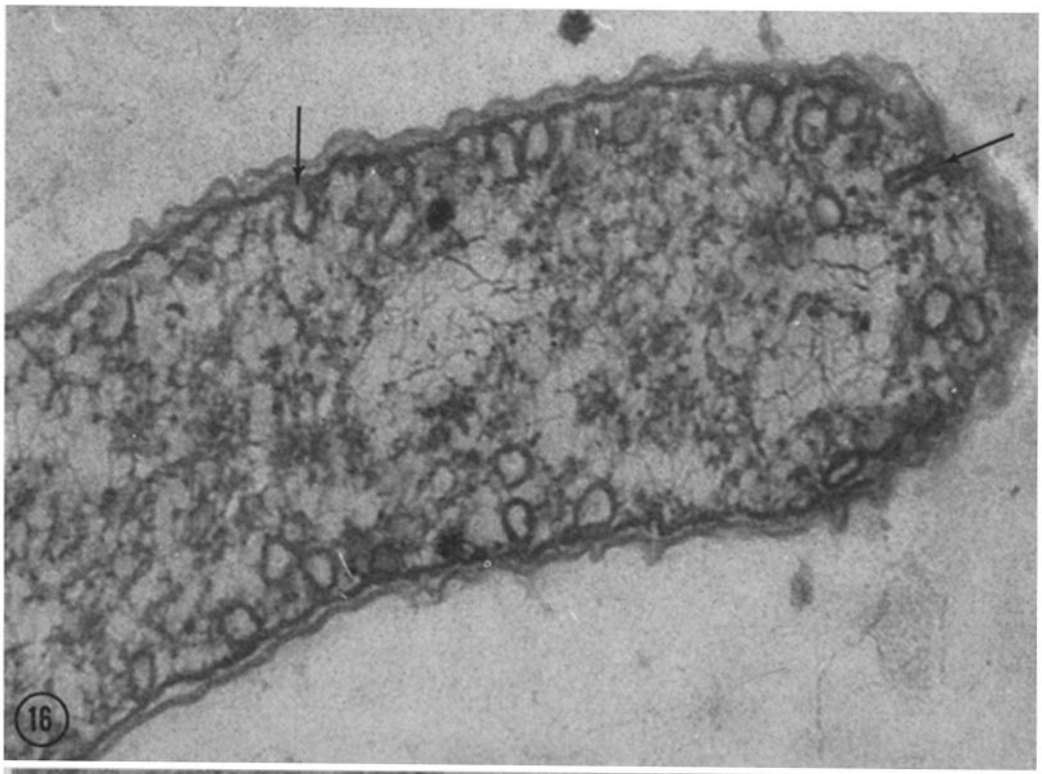
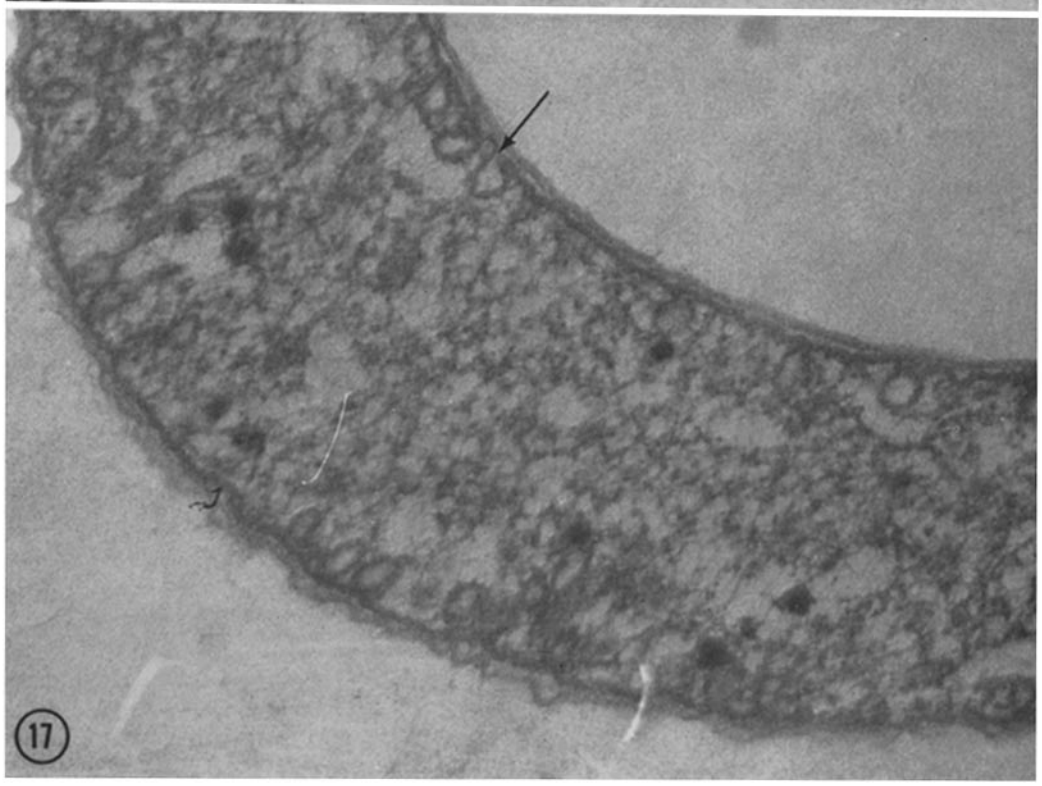
FIGURES 16 AND 17

Sections of cells growing with malate at a light intensity of 2000 foot-candles, treated with RNase after fixation, and poststained with lead hydroxide. As a result of the enzymatic destruction of the ribosomes, membrane structures show very sharply. The arrows indicate three vesicles bounded by membranes that are continuous with the cytoplasmic membrane. × 100,000.


16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