OBSERVATIONS ON THE STRUCTURE OF *RHODOSPIRILLUM MOLISCHIANUM*

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ABSTRACT

The lamellae of the bacterium *Rhodospirillum molischianum* originate as extensions of the cytoplasmic membrane into the cytoplasm of the cell. Initially, these extensions are narrow folds and occur independently of one another. The first lamellae to appear average about 80 A in width, representing one side of the infolded cytoplasmic membrane, or 160 A when the two sides of the fold are closely appressed. The 160-A lamellae increase in number and may associate to form larger lamellae, which represent varying degrees of association between adjacent folds. Later, the space within each fold increases; the two appressed regions of the cytoplasmic membrane in each fold separate to form distinct invaginations, and the lamellae observed at this stage are formed by an association of the sides of adjacent invaginations.

INTRODUCTION

Original observations on the existence of lamellae in *Rhodospirillum molischianum* date from 1955, when Niklowitz and Drews (13) reported these structures in an organism they considered to be *Rhodospirillum rubrum*. However, this organism was later identified as *R. molischianum* (4, 7). In a more detailed study, Giesbrecht and Drews (7) reported that five to fifteen lamellae formed a chromatophore, and that the observed stacking of lamellae was necessary for biochemical activity. It was further observed that these lamellae were continuous with the cytoplasmic membrane in cells treated with lysozyme. More recently, it has been shown that cells of *R. molischianum* which show lamellar structures will yield photochemically active particles upon disruption of the cells ultrasonically (8). These isolated particles are capable of carrying out photosynthetic phosphorylation and are structurally similar to the chromatophores isolated from *R. rubrum*.

In view of the differences in internal organization of *R. rubrum* and *R. molischianum*, it appeared worthwhile to obtain more detailed information on the formation and structure of the lamellar structures in the latter organism.

MATERIALS AND METHODS

Cultures were grown in 1-liter Erlenmeyer flasks containing 250 ml of culture medium, which was modified after Lindstrom (personal communication) and consisted of 0.5 per cent yeast extract, 0.5 per cent Difco bacto-casitone, 0.2 per cent sodium acetate and 0.1 per cent di-potassium phosphate in glass-distilled water. The pH of this medium is about 7.2 and was not adjusted.

The flasks were gassed with 1.5 per cent carbon dioxide in helium at a rate of about 3.5 cc per minute for 6 hours prior to inoculation, as well as during growth of the culture, and were maintained at 29°C in a water bath illuminated with incandescent lamps, which provided an incident illumination of about 350 foot candles as measured with a Weston model 756 illumination meter.

Cultures were transferred serially every 6 to 8 hours for 4 days to produce series of cultures of different ages. The ages of these cultures should be considered approximate and are used only to establish the relative ages of the cultures.

TABLE I

Comparisons between Observed and Expected Widths of Lamellae in Rhodospirillum molischianum

Fig. No.	Average width: Observed average width of lamellae	Average width: Multiples of the 90-A cytoplasmic membrane included in lamella	Average width: Expected average width of lamellae	Range in width: Observed range in width of lamellae	Range in width: Expected width of lamellae based on 70–105 A range in width of cytoplasmic membrane
	A		*A*	*A*	*A*
1, 10	Type "A"—80	1	90*	65–100	70–105*
1–12	Type "B"—160	2	180‡	140–190	140–210‡
12	Type "C"—300	4	360‡	270–325	280–420‡
13, 19	Folded extremity—80	1	90‡	75–90	70–105‡
13–22	Adjacent lamellae—160	2	180‡	140–185	140–210‡

* Observed average or range.

‡ Calculated from observed average or range.

Samples were fixed in 1 per cent osmium tetroxide in veronal-acetate buffer (14). Following fixation, the samples were stained with 0.5 per cent uranyl acetate in veronal-acetate (10), dehydrated, and embedded in Epon 812 (11).

Sections were prepared with a Porter-Blum MT-1 microtome using a diamond knife. The sections were stained with solutions of lead salts (12, 16), or with aqueous uranyl acetate (17) when indicated, and examined with an RCA EMU-3F microscope.

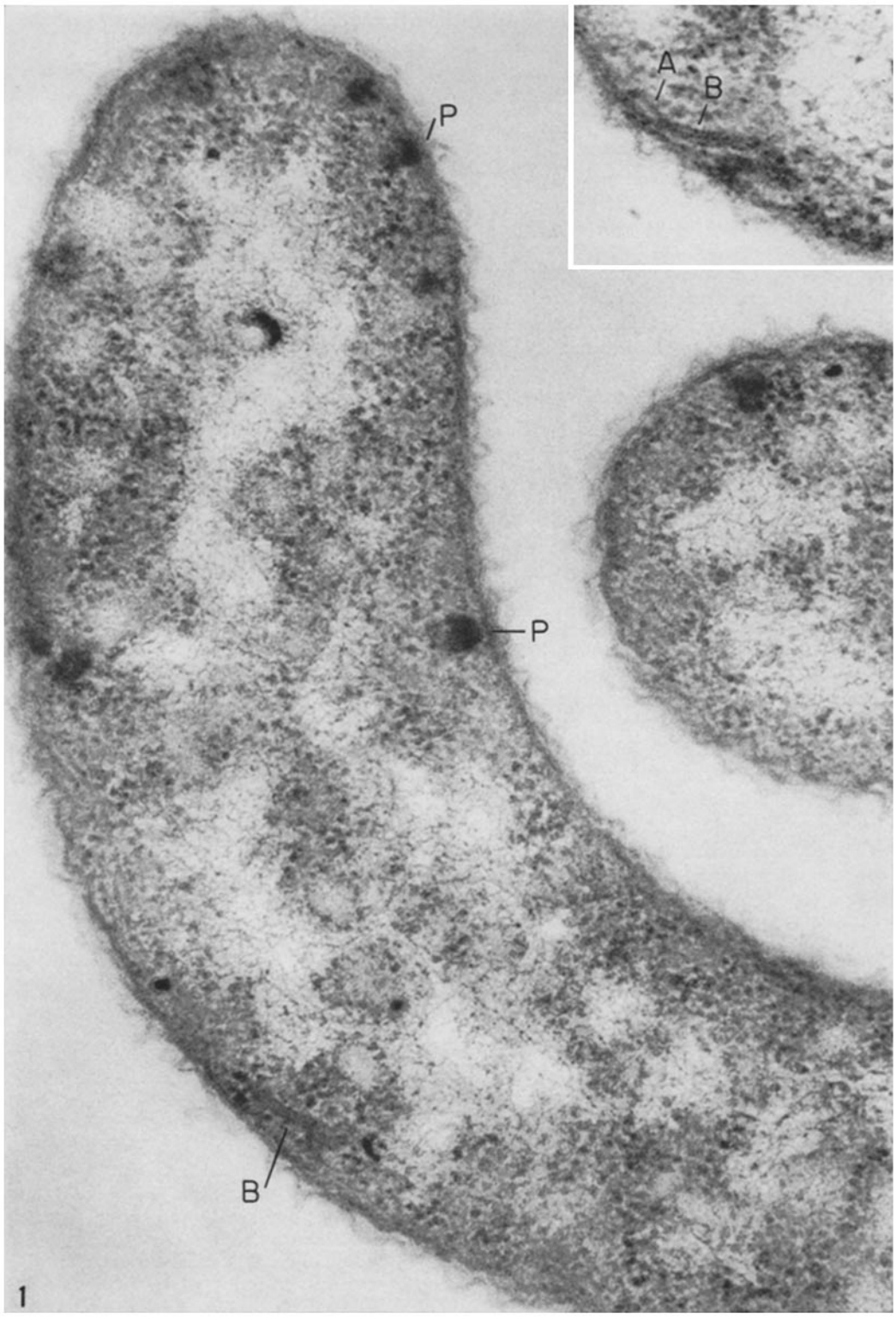
RESULTS

In the following results and discussion, the term "lamella" is used in a generic sense, and the actual structure of the lamellae observed in *R. molischianum* will be shown to depend, at least, upon the age of culture studied and upon the presence and degree of association of adjacent lamellae. The terms "fold" and 'invagination" are used to describe extensions of the cytoplasmic membrane into the cytoplasm of the cell. In a "fold," adjacent sides of the cytoplasmic membrane are appressed so there is little or no detectable space within the extension, and the two parallel regions of the cytoplasmic membrane forming the extension may be undetectable. In an "invagination," the extension of the cytoplasmic membrane encloses a visible space, so that the two sides of the extension are separate and distinct.

When the cytoplasmic membrane of *R. molischianum* is distinct, as in Fig. 22, it consists of two dense layers separated by a less dense interspace, with a total average width of about 90 A. This

FIGURE 1 Cells from a 16-hour culture of *Rhodospirillum molischianum*. In such cells the lamellae, as indicated at *B*, occur singly and peripherally, are relatively few in number, and average about 160 A in width. The dense peripheral bodies (*P*) are numerous in cells from young cultures (see also Figs. 2 through 9), but decrease in number in cells from older cultures. × 105,000.

Insert, A portion of a cell from a 16-hour culture, showing two lamellae at *A* about 80 A wide attached to the cytoplasmic membrane. Each of these 80-A lamellae represents a region of the infolded cytoplasmic membrane. These two separate 80-A lamellae associate to form the lamella at *B*, which is about 160 A wide and contains a dense center layer which is just visible (*cf.* Fig. 34). This dense center layer, which is also present at *O* in Fig. 12, represents two appressed regions of the dense outer layer of the cytoplasmic membrane. × 160,000.


A
B
P
P
B
1

average width, as well as the averages given below, is based upon individual measurements of a number of electron micrographs in addition to those presented here. The total number of individual measurements, however, is too small to be treated statistically. The observed range in widths of these structures is given in Table I.

Two types of cytoplasmic vacuoles are distinguishable in *R. molischianum*. The large vacuoles shown at *V* in Figs. 2 and 5 range between about 250 and 400 mμ in diameter and occur irrespective of the age of the culture. The consecutive sections of Figs. 2 through 9 indicate the variations in size and distinctness of the same vacuole in consecutive sections. The sharp boundary between these vacuoles and the cytoplasm suggests a limiting membrane. Even when sharply defined, however, the structure of this boundary is variable and may represent a peripheral condensation of vacuolar material, most of which is probably lost in processing the cells for sectioning; or it may represent a localized rearrangement of cytoplasmic components which occurs when the vacuole is formed.

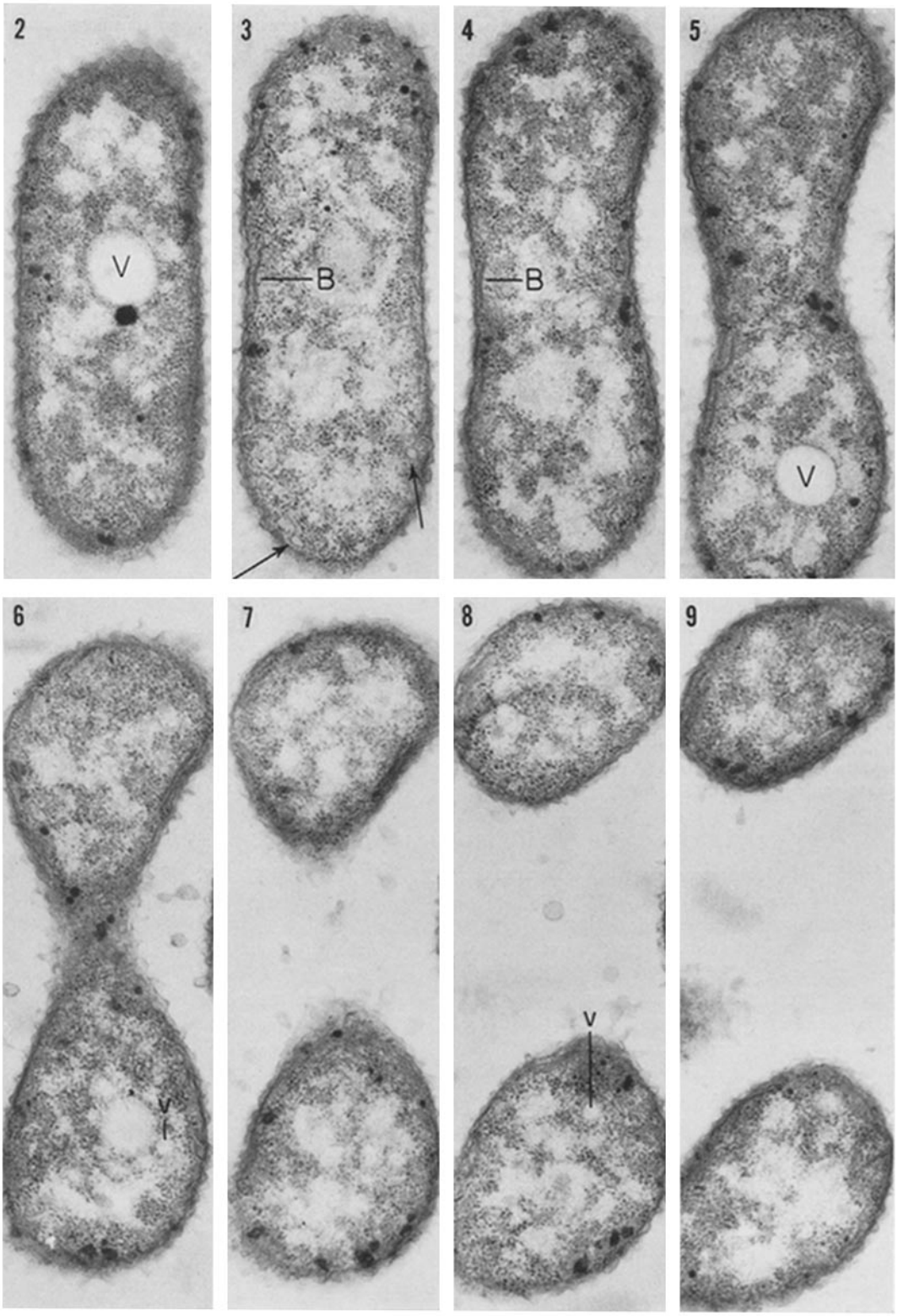
The smaller cytoplasmic vacuoles at *v* in Figs. 6 and 8 are a more prominent feature of *R. molischianum* than is indicated by the micrographs presented here. They are most numerous in cells from cultures about 24 hours old, but are always present in some cells regardless of the age of the culture. They range between about 70 and 100 mμ and are not sharply defined from the surrounding cytoplasm.

Cells of *R. molischianum* from 16-hour cultures show several distinct features, but do not contain the "stacks" of closely packed lamellae previously considered characteristic of this organism (4, 7, 8). In cells from these cultures (Fig. 1) the lamellae are peripheral, relatively few in number, occur singly rather than in closely packed arrays, and are relatively short and of low density. Two sizes of lamellae are present in such cells. The narrow lamellae indicated at *A* in the insert of Fig. 1 are attached to the cytoplasmic membrane and average about 80 A in width. Two such lamellae associate to form the wider lamella at *B* in Fig. 1, which is about 160 A wide and contains a dense center line. Fig. 10 shows a similar association of two narrow lamellae to form a wider lamella, but here the association is less complete than in Fig. 1.

The dense peripheral bodies visible in Fig. 1 (*P*) are often in contact with the cytoplasmic membrane, and are sometimes associated with the lamellae. They are quite numerous in cells from young cultures, but decrease in number in cells from older cultures. Also frequently present in such cells are small, peripheral, low-density vesicles enclosed by a membrane, as indicated by arrows in Fig. 3. These vesicles also decrease in number in cells from older cultures, and are rare or absent in cells containing parallel groups of lamellae, as in Fig. 13.

Figs. 2 through 9, which represent consecutive sections from the same cell, show that in cells from 22-hour cultures there is an increase in the number of lamellae per cell, but that the lamellae are not closely associated with one another. These lamellae are similar to those shown at *B* in Fig. 1. Individual lamellae can be traced through only one to three consecutive sections here, indicating that they do not extend so far perpendicular to the plane of sectioning as do the closely associated lamellae in the cells from older cultures shown in Figs. 14 through 18. However, if a single lamella in Figs. 2 through 9 turns at an angle to the plane of sectioning, it would be more likely to become undetectable in the section than would the close groupings of lamellae in Figs. 14 through 18.

FIGURES 2 to 9 Consecutive sections from a cell in a 22-hour culture. The lamellae, as indicated at *B*, are peripheral and occur singly or in loose association with one or two other lamellae. These lamellae are similar in width to the lamellae at *B* in Figs. 1, 10, and 12. The large vacuoles (*V*) range between about 250 and 400 mμ in diameter when distinct, and occur in cultures of all ages studied. The smaller vacuoles indicated at *v* in Figs. 6 and 8 are 70 to 100 mμ in diameter, are not sharply defined from the surrounding cytoplasm, and are most numerous in cultures about 24 hours old. The small vesicles surrounded by a membrane, indicated by arrows in Fig. 3, have been observed only in the youngest cultures studied. Figs. 7 through 9 of this series show that, in the case of spiral organisms, what appears as two cells may actually represent different portions of a single cell. × 45,000.


2
V
3
B
4
B
5
V
6
V
7
8
V
9

The consecutive sections in Figs. 2 through 9 also illustrate that in sections of spiral organisms, what may appear as two separate cells, as in Figs. 7 through 9, may actually represent portions of a single cell. The presence of a distinct cell wall and cytoplasmic membrane in such sections does not necessarily indicate that the section is through the polar region of such an organism. In subsequent sections of this cell, the upper portion of the cell in Fig. 9 disappeared, while the lower portion of the cell in Fig. 9 continued into another portion of the spiral which extended downward and to the left of the area included in Fig. 9.

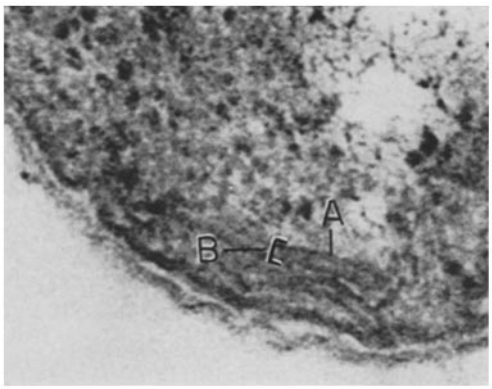


FIGURE 10 A portion of a cell from a 22-hour culture showing two pairs of lamellae, each about 80 A wide at *A*, loosely associated to form two lamellae about 160 A wide, one of which is indicated at *B*. The lower pair of 80-A lamellae is continuous with the cytoplasmic membrane and represents a fold of the cytoplasmic membrane into the cytoplasm of the cell. × 150,000.

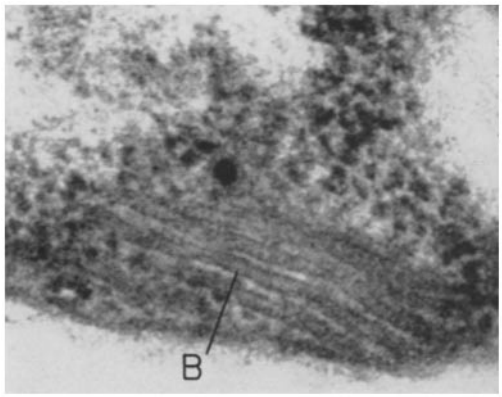


FIGURE 11 A portion of a cell from a 22-hour culture showing several parallel, separate lamellae about 160 A wide, as at *B*, which are attached at one end to the cytoplasmic membrane. Each of these lamellae presumably contains two of the 80-A lamellae seen at *A* in Figs. 1 insert, 10, and 12, but which are indistinct here. Each of these 160-A lamellae represents a single fold of the cytoplasmic membrane. The lamellae in this figure are shown diagrammatically in Fig. 34. × 160,000.

Although the single lamellae shown in Figs. 1 through 10 are typical of these cultures, selected sections shown in Figs. 11 and 12 indicate more extensive development of lamellae in some cells. The lamellae in Fig. 11 are in contact with the cytoplasmic membrane and are similar to the 160-A lamellae shown at *B* in Fig. 1 and in Figs. 2 through 9. These lamellae have developed parallel to one another but have remained discrete, and adjacent lamellae are not associated. In Fig. 12, lamellae of three different sizes may be distinguished. Lamellae of the type indicated at *A* are about 80 A wide, those at *B* are about 160 A wide, while that at *C* is about 300 A wide. The two lamellae at *A* in Fig. 12 associate to form the wider lamella at *B*. The lamella at *C* is about twice as wide as the lamella at *B* and contains a dense center line.

The peripheral groups of parallel lamellae previously considered characteristic of this organism (4, 7, 8) become visible in cells from 2- to 3-day-old cultures. A typical cell is shown in Fig. 13.

With regard to location and extent of lamellae within such cells, Figs. 14 through 18 show that the lamellae are associated into groups which are peripheral in the cell and extend for a greater distance perpendicular to the plane of sectioning than in the cells from younger cultures shown in Figs. 2 through 9. Occasional groups of lamellae may appear free in the cytoplasm in some sections in Figs. 14 through 18, but in adjacent sections they approach the periphery of the cell.

In cells from these older cultures, the distance between adjacent parallel lamellae increases (Figs. 13 through 20). Unlike the lamellae in Figs. 11 and 12, the extremities of the lamellae, indicated at *E* in Figs. 19 and 22, are folded back and contact adjacent lamellae at *D*. Therefore, the visible structure of these lamellae depends upon their association with adjacent lamellae. The folded extremities free from adjacent lamellae have

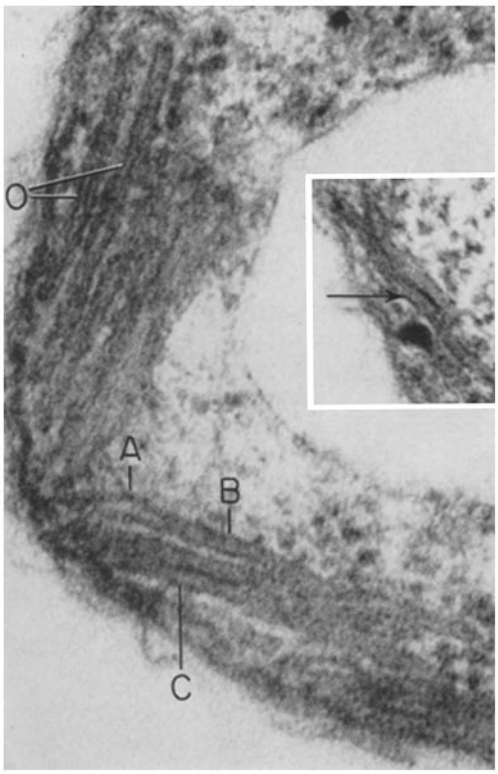


FIGURE 12 A portion of a cell from a 22-hour culture. The lamellae at *A* average 80 A in width. Two of these 80-A lamellae associate to form the 160-A wide lamella at *B*. The lamella at *C* is about 300 A wide and results from an association of two of the 160-A wide lamellae. These lamellae are shown diagrammatically in Fig. 35. Each 80-A lamella represents one region of the infolded cytoplasmic membrane. The 160-A lamellae represent two appressed regions of the cytoplasmic membrane in a single fold. The dense outer layer of the cytoplasmic membrane is often difficult to detect within these 160-A lamellae, but is visible at *O* where the 80-A lamellae are loosely associated. The 300-A lamella is formed by an association of two adjacent folds of the cytoplasmic membrane. The dense central layer in this 300-A lamella represents two appressed regions of the dense inner layer of the cytoplasmic membrane, one from each of the two folds, and is also visible in the lamellae in Figs. 13 through 19. × 185,000. *Insert*, A 300-A lamella showing faint dense layer (arrow) formed by the two appressed regions of the dense outer layer of the cytoplasmic membrane in one of the two folds forming this lamella. × 160,000.

a total average width of 80 A and consist of two dense layers. These folded extremities at *E* come into contact with one another at *D* to form lamellae which average about 160 A in width and consist of three dense layers, with the central layer more dense than the outer two layers.

Figs. 20 through 22 show that the lamellae described above actually represent invaginations of the cytoplasmic membrane, and it becomes evident that the structure of the lamellae formed by these invaginations depends upon an association of adjacent invaginations of the cytoplasmic membrane.

The beaded appearance of the lamella at *D* in Fig. 21 is observed occasionally and suggests that the invaginated cytoplasmic membrane contains a substructure which may be related to the surface pattern seen in the isolated chromatophores in Fig. 26. However, the relation between lamellae and chromatophores remains to be investigated in detail.

In older cultures, enlargement of the area within the invaginations becomes even more pronounced (Fig. 23), and here several invaginations of the cytoplasmic membrane can be seen.

The structure described in *R. rubrum* as a "polar cap" (3) is also present in *R. molischianum* (Figs. 24 and 25). Observations on this structure in *R. molischianum* are incidental, but it has been observed in cells from cultures between 1 and 4 days old, and in Fig. 24 it is associated with lamellae.

The consecutive sections in Figs. 27 through 32 represent lysed cells commonly found in older cultures of *R. molischianum*. Such cells are visible by phase microscopy, where they appear similar to the rounded, low-density spheroplasts produced by lysozyme treatment of other bacterial species. In these lysed cells, the lamellae formerly present have separated from one another and formed discrete vesicles, which may or may not be in contact with the cytoplasmic membrane. Fig. 33, which is an enlargement from Fig. 31, shows continuity between a vesicle and the cytoplasmic membrane, but this obviously cannot be considered characteristic of such cells. Few of these vesicles can be traced through more than two consecutive sections with any certainty, indicating that their depth perpendicular to the plane of sectioning is considerably less than that of the lamellae from which they were formed.

Chromatophore preparations from *R. molischia-*

num have been shown to be photochemically active (5, 8); a negatively stained preparation of these chromatophores is shown in Fig. 26. The chromatophores here are larger in diameter than those obtained from *R. rubrum*, and the membranes contain discrete light areas which have not been detected in negatively stained preparations of *R. rubrum* chromatophores (8).

DISCUSSION

With regard to the origin of the first detectable lamellae in *R. molischianum*, it is currently necessary to begin the discussion with the first structures recognizable as lamellae in cells from young cultures. It is possible, however, that further investigation can provide information on earlier structural changes associated with the appearance of the first detectable lamellae in the cells. For instance, the space between the two narrow lamellae at *A* in the insert of Fig. 1 suggests that these narrow lamellae may originate from the cytoplasmic membrane as a small vesicle, similar to those indicated in Fig. 3, which may elongate and narrow so that the sides are appressed to form lamellae like the one seen at *B* in the insert of Fig. 1. Another possibility is that the dense peripheral bodies seen in Figs. 1 through 9 represent the sites at which the vesicles originate. These dense peripheral bodies are sometimes seen in contact with the cytoplasmic membrane and with lamellae of the type seen in Figs. 2 through 9, and some micrographs suggest that these dense bodies contain a structure which has not been resolved in the present study. These possibilities remain speculative, however, and must await more complete investigation. Use of the term "peripheral bodies" here is purely descriptive, and any possible relation of these structures to the peripheral bodies present in other organisms (2, 6, 15) has not been investigated.

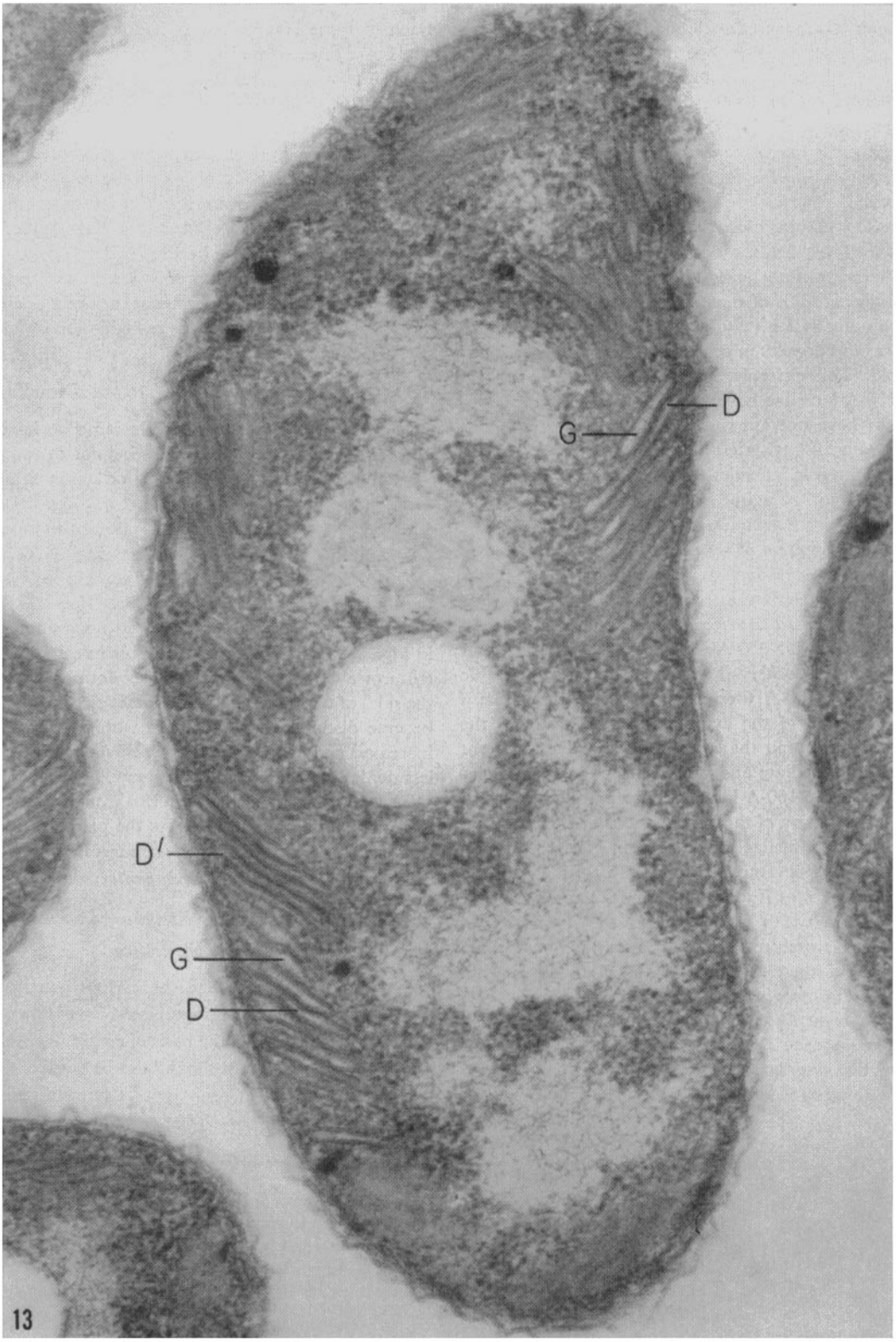
Lamellae Formed by Single Folds of the Cytoplasmic Membrane

In cells from young cultures, the narrow lamellae indicated at *A* in Figs. 1, 10, and 12 average about 80 A in width and are in contact with the cytoplasmic membrane. These 80-A lamellae are believed to represent one side of the folded cytoplasmic membrane. A closer association of two of these 80-A lamellae produces the wider lamellae, seen at *B* in Figs. 1 through 12, which average 160 A in width. These 160-A lamellae, shown diagrammatically in Fig. 34, would represent a fold of the cytoplasmic membrane in which adjacent regions of the membrane within the fold are usually closely appressed. Occasionally, as in Figs. 10 and 12, an interspace is observed within the folded membrane. The inner dense layer of the cytoplasmic membrane forms the outer dense boundary of the 160-A lamellae. The outer dense layer of the cytoplasmic membrane is folded in the center of these lamellae, in which it may be detectable, as at *B* in the insert of Fig. 1 and at *O* in Fig. 12, or not visible, as in Fig. 11.

Lamellae Formed by Multiple Folds and Invaginations of the Cytoplasmic Membrane

Two of the 160-A lamellae at *B* in Fig. 12 may also associate, forming the lamella at *C* which averages about 300 A in width. This 300-A lamella would contain four parallel, closely associated regions of the cytoplasmic membrane formed by two adjacent folds in the membrane, which should have a total width of about 280 to 400 A. The dense line in the center of this lamella is formed by one dense inner layer of the cytoplasmic membrane from each of the two adjacent folds, shown diagrammatically in Fig. 35. This 300-A lamella should also contain two intermediate dense lines representing the folded dense outer

FIGURE 13 In cultures 2 to 3 days old, the lamellae increase in number and become closely associated into groups (at *D'*). The space (*G*) within the folds then increases and separates adjacent sides of the folds. The distinct lamellae seen at *D* average about 160 A in width and contain two parallel regions of the invaginated cytoplasmic membrane, with one region originating from each of two adjacent invaginations. Lamellae similar to those at *D* in this figure are shown at higher magnifications in Figs. 19 through 22, and are distinct from the 160-A lamellae at *B* in Figs. 1 through 12, which originate from a *single* fold of the cytoplasmic membrane. Section stained with uranyl acetate. × 90,000.


D
G
D′
G
D
13

layer of the cytoplasmic membrane in each of the two adjacent folds, but these intermediate dense lines are only occasionally visible. This 300-A lamella at *C* in Fig. 12 is structurally similar to the lamellae at *D* in Fig. 13 through 22 to be described below, except that there is no detectable space *within* the folds of the cytoplasmic membrane.

The classification of lamellae as 300 A wide is somewhat arbitrary because the formation of these lamellae depends upon the association of adjacent folds of the cytoplasmic membrane, and this association may be either absent or present in varying degrees in cells from cultures ½ to 2 days old. For example, in Fig. 11 several parallel 160-A lamellae have formed close to one another, but have not associated to form larger lamellae. In Fig. 12, the left-hand portion of the 300-A lamella is independent of the 160-A lamella above it, but the right-hand portion of this 300-A lamella is associated with the 160-A lamella. There is no indication of what determines association or lack of association of adjacent folds of the cytoplasmic membrane, and additional multiples of the 160-A lamellae could be described. However, the 160- and 300-A-wide lamellae are the most frequently observed widths. It seems probable that the 160-A wide lamella, which represents a single fold of the cytoplasmic membrane, is the basic unit, and that the 300-A lamellae represent an intermediate and variable stage in the association of the 160-A lamellae into parallel groups in the formation of the lamellae seen at *D'* in Fig. 13.

In the lamellae shown at *D* in Figs. 13 through 22, and diagrammed in Figs. 36 through 38, the extremities at *E* in Figs. 19 and 22 average about 80 A in width and represent the normally observed structure of the cytoplasmic membrane. Back of these extremities, the membranes from adjacent invaginations come into contact with one another at *D* to form lamellae about 160 A wide. The lamellae at *D*, therefore, contain two regions of the cytoplasmic membrane, with one region originating in each of the adjacent invaginations. Each of these lamellae at *D* in Figs. 13 and 19 consists of three dense layers separated by two less dense interspaces, with the central layer being the most dense. This is shown diagrammatically in Fig. 38. The central dense layer of these lamellae averages about 45 A in width and represents an unresolvable association of one dense inner layer of the cytoplasmic membrane from each of the adjacent invaginations. Drews (4) has also reported this dense layer to be 45 A wide. The outer dense layer and less dense interspace on each side of the central dense layer represent the outer dense layer and interspace from one invagination of the cytoplasmic membrane.

Comparison of Two Types of 160-A Lamellae

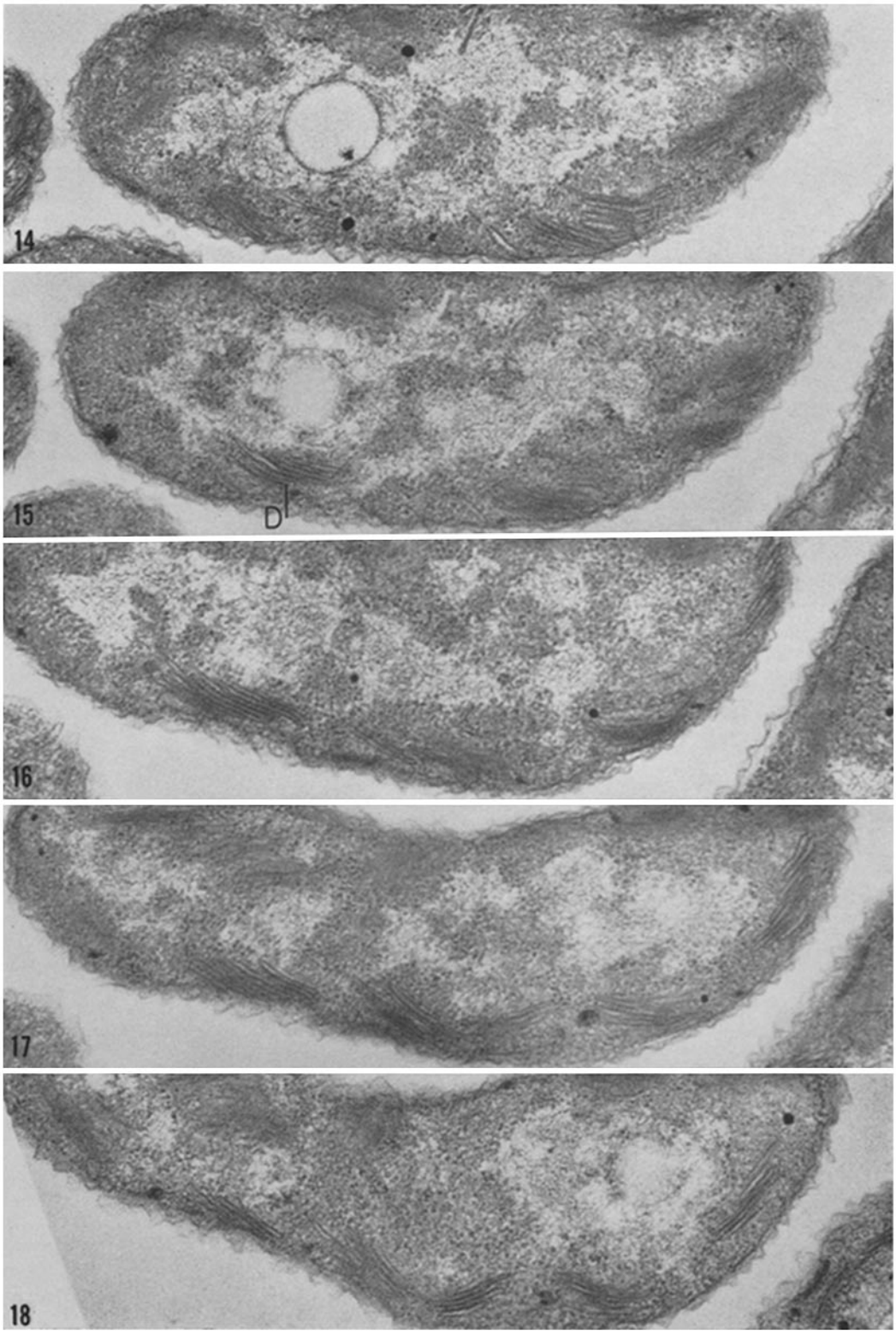
Since two types of 160-A wide lamellae have been described above, it may be helpful to compare them briefly. The 160-A lamellae at *B* in Figs. 1 through 12 (diagrammed in Fig. 34), which are observed in cells from cultures between about ½ to 2 days old, represent a single fold of the cytoplasmic membrane, and contain only a faintly visible central dense layer. The 160-A lamellae at *D* in Figs. 13 through 22 (diagrammed in Figs. 36 through 38), which occur in cells from cultures about 2 to 4 days old, are formed when the sides of two adjacent folds contact one another, become distinct when the space within the folds increases, and consist of a dense central layer, two less dense interspaces, and two dense outer layers.

The lamellae at *D* in Figs. 13 through 22 are similar in structure and width to the paired membranes reported in *Rhodomicrobium vannielii* (1), but differ from those found in *Rhodospirillum rubrum* (9).

Relationship of Lamellar Structure to Cytoplasmic Membrane Structure

The relationship between the various lamellae described above and the cytoplasmic membrane has been established on the basis of continuity and similarity of structure. It should also be possible to correlate the width of each type of lamella with

FIGURES 14 to 18 Consecutive sections of a cell from a 3-day culture. The appearance and extent of the lamellae here differ from that shown in consecutive sections of a cell from a 22-hour culture shown in Figs. 2 through 9. Several groups of lamellae can be traced through all of the sections. The group of lamellae at *D* in Fig. 15 is apparently free in the cytoplasm, but can be traced to Figs. 17 and 18 where it is in contact with the cytoplasmic membrane. × 50,000.


14
15
D
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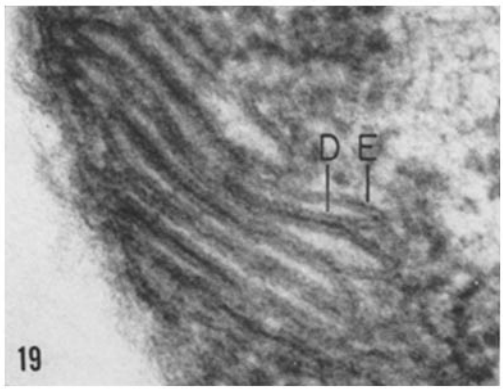


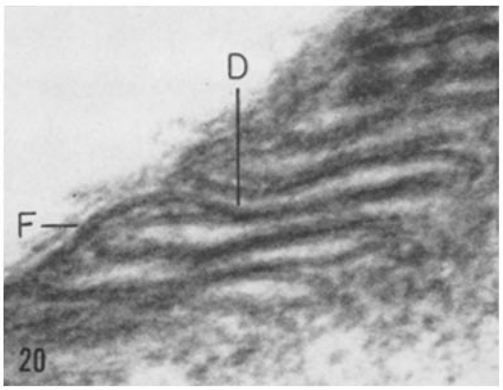
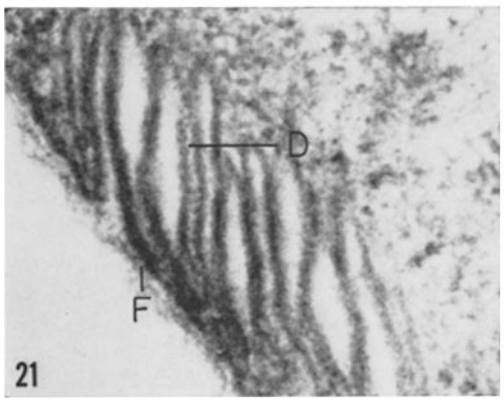


FIGURE 19 The type of lamella in Figs. 13 to 18 is shown here in more detail in a portion of a cell from a 3-day culture, and is diagrammed in Figs. 36 through 38. The extremities of the invaginations are unassociated at *E* and show the characteristic structure of the cytoplasmic membrane. Adjacent invaginations contact one another back of these extremities to form the lamellae at *D*. In these lamellae, each of the two dense outer layers is from an adjacent invagination and represents the dense outer layer of the cytoplasmic membrane. The dense central layer of the lamellae represents two appressed regions of the dense inner layer of the cytoplasmic membrane, with one region contributed by each of the adjacent invaginations. The two less dense areas between the three dense layers of this lamella represent the interspace of the cytoplasmic membrane. × 200,000.

FIGURE 20 At *F* in this figure the cytoplasmic membrane leaves its normal peripheral location and turns inward to form one side of the lamella indicated at *D*, as shown diagrammatically in Fig. 36. × 210,000.



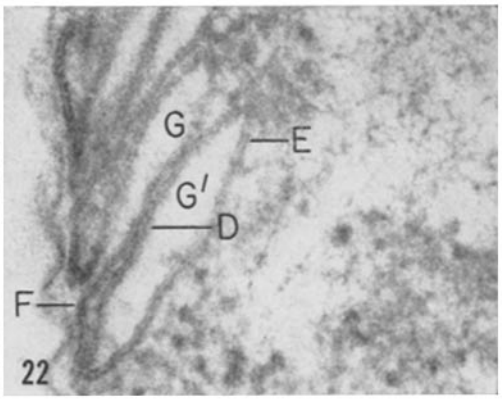


FIGURE 21 Here also, the cytoplasmic membrane at *F* can be seen to be continuous with one lamella. The beaded appearance of the lamella at *D* is seen only occasionally. × 145,000.

FIGURE 22 The cytoplasmic membrane turns into the cell at *F*. An unassociated region of the invaginated cytoplasmic membrane is shown at *E*, and a lamella formed from two adjacent invaginations is shown at *D*. The space enclosed by one invagination is shown at *G*, and *G'* indicates the space enclosed by an adjacent invagination. The invaginations of this figure are diagrammed in Fig. 37. × 170,000.

the number of layers of the cytoplasmic membrane contained in each lamella. This comparison is given in Table I. The average widths of the lamellae fall slightly below the widths expected from the number of layers of the cytoplasmic membrane contained in each lamella. The observed ranges in width of the lamellae fall within the expected ranges, although the upper limits of the ob-

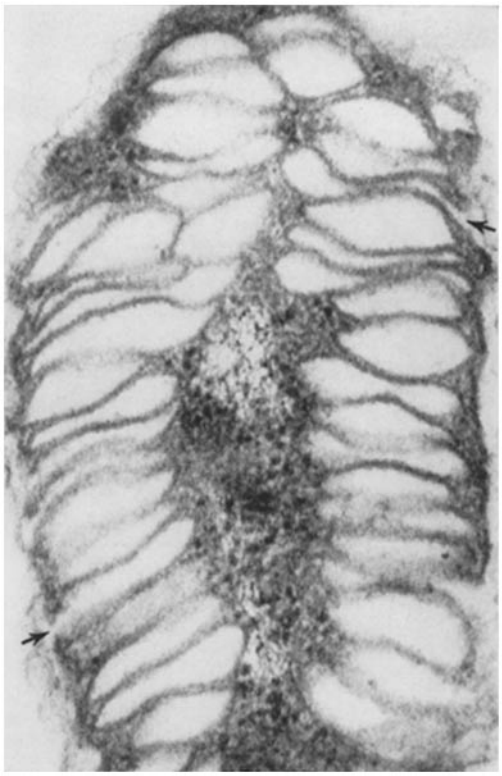


FIGURE 23 The space within invaginations of the cytoplasmic membrane increases progressively in cells from older cultures, as in Figs. 13 through 22. The cell in this figure represents an extreme condition, and is from a 4-day culture, but is more frequently observed in older cultures. Arrows indicate several points at which the invaginations of the cytoplasmic membrane are distinct. × 100,000.

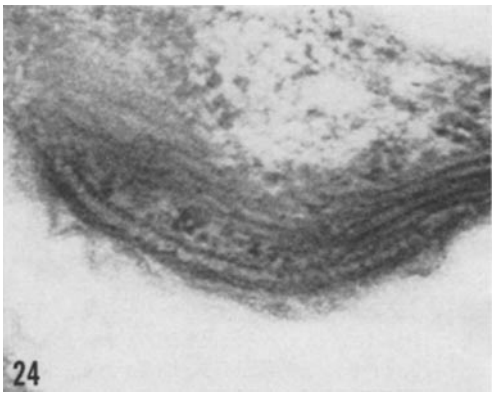
served ranges are slightly below the upper limits of the expected ranges. The greatest discrepancy occurs between the observed and expected calculated values of the 300-A lamella shown in Fig. 12. It should be noted, however, that any error in the average width of the cytoplasmic membrane would be quadrupled in calculating the expected width of the 300-A lamella. For example, if the average width of the cytoplasmic membrane is taken as 85 A instead of 90 A, the observed average width of the 300-A lamella would be within about 12 per cent of the expected value.

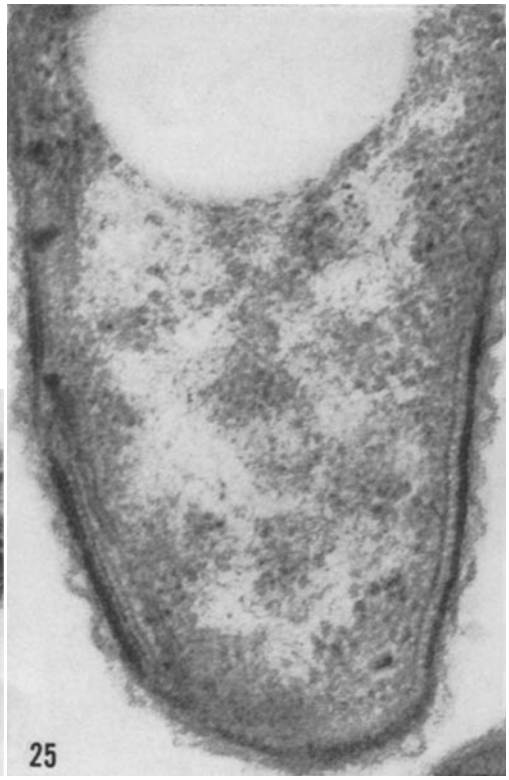
Aside from errors in the measurements, however, the comparisons between observed and expected widths of the lamellae given in Table I may be subject to several additional errors. It is, of course, only an assumption that the cytoplasmic membrane has, or should have, exactly the same dimensions in the invaginations as in its normal peripheral location. It is possible that in its peripheral location the cytoplasmic membrane may be more subject to variations in width than when involved in a fold or invagination, where the surrounding cytoplasm might provide some protection against changes. Some variation in the width of the lamellae is introduced by variations in the closeness of association between adjacent layers of the cytoplasmic membrane which form the lamellae, as is evident from Figs. 1 through 12.

In the usual peripheral location of the cytoplasmic membrane, between the cell wall and the cytoplasm, the two dense layers of this membrane are of the same density and can be distinguished from one another only on the basis of their location. When involved in the formation of lamellae, however, these two dense layers behave differently. When the dense outer layer of the cytoplasmic membrane is folded upon itself, as in the 160-A lamellae at *B* in Figs. 1 through 12 and in the 300-A lamella of Fig. 12, this layer is only faintly visible, as at *B* in the insert of Fig. 1 and at *O* in Fig. 12, or not detectable, as in the lamellae of Fig. 11. The density and width of this folded layer is certainly less than would be expected. This reduction in contrast of the folded dense outer layer is apparently temporary and occurs only when this layer is folded upon itself. When the closed folds of the lamellae in Figs. 1 through 12 open up to form the lamellae at *D* in Figs. 13 through 19, the folded regions of this layer separate, and the dense outer layer of the cytoplasmic membrane again becomes distinct and forms the outer dense layer of these lamellae (*D* and *E* in Fig. 19). In contrast to this, a similar association of two regions of the dense *inner* layer of the cytoplasmic membrane produces a layer which is wider and more dense than a single dense layer, as shown by the dense layer in the 300-A lamella at *C* in Fig. 12 and by the dense central line in the lamellae at *D'* in Fig. 13. When the closed folds of these lamellae open up to form the lamellae at *D* in Figs. 13 through 19, the relation of the two associated regions of the dense inner layer remains unchanged, as does the contrast and width of the layer formed by the two associated regions.

These observations on the lamellar structures present in *R. molischianum* indicate that all of the lamellae in this organism are formed by folds or

FIGURES 24 and 25 Located beneath the cytoplasmic membrane in these figures is an accessory dense layer which is separated from the cytoplasmic membrane by a striated area. A similar structure occurs in *Rhodospirillum rubrum* (3, 9). Fig. 24 is from a 4-day culture. × 145,000. Fig. 25 is from a 32-hour culture. × 115,000.





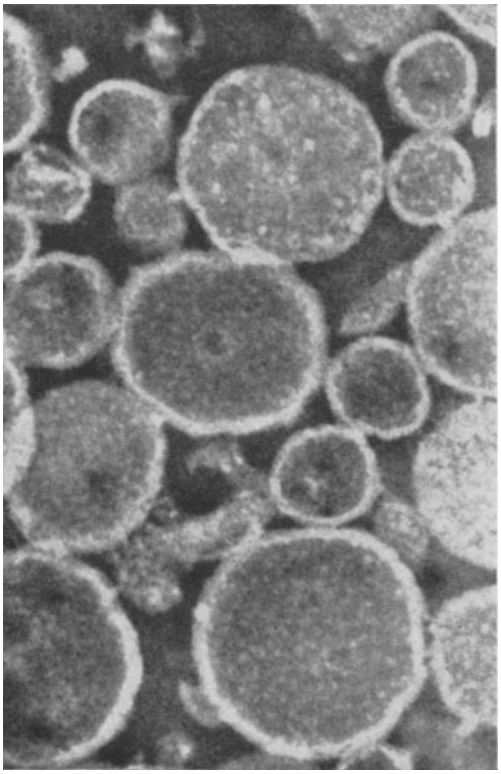
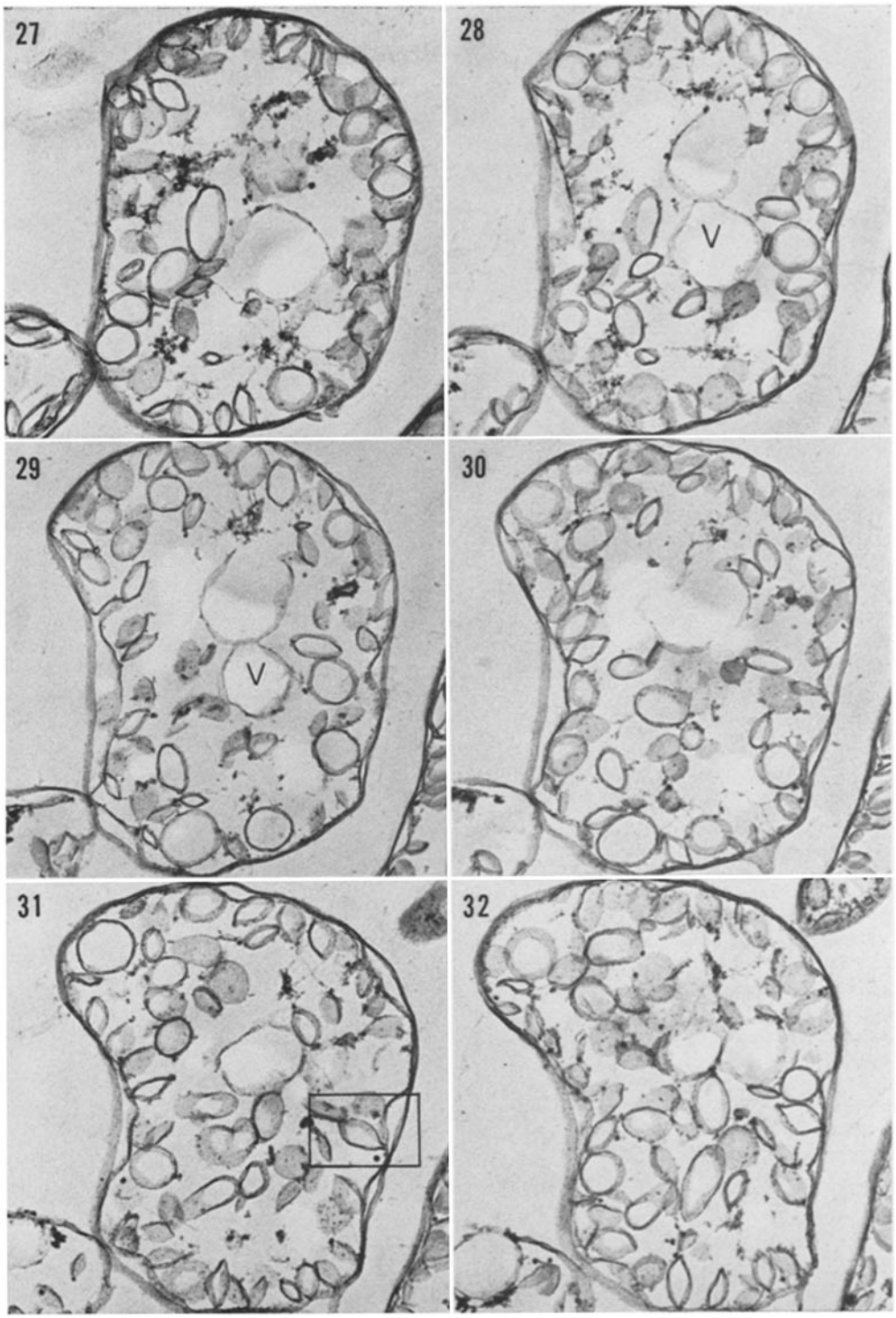


FIGURE 26 A negatively stained preparation of "chromatophores" isolated from cells similar to that in Fig. 13. These structures are photochemically active and show a differentiation of the surface into small, distinct light areas. × 130,000.



FIGURES 27 to 32 Consecutive sections of lysed cells commonly found in older cultures. The lamellae present in younger cultures have separated and formed rounded vesicles which may or may not be in contact with the cytoplasmic membrane. The larger vesicles at *V* are remnants of the vacuoles seen at *V* in Figs. 2, 5, and elsewhere. The area included within the square in Fig. 31 is enlarged in Fig. 33 to show the continuity between this vesicle and the cytoplasmic membrane. However, it is obvious that these lysed cells do not provide reliable information on the structure of unlysed cells. × 30,000.

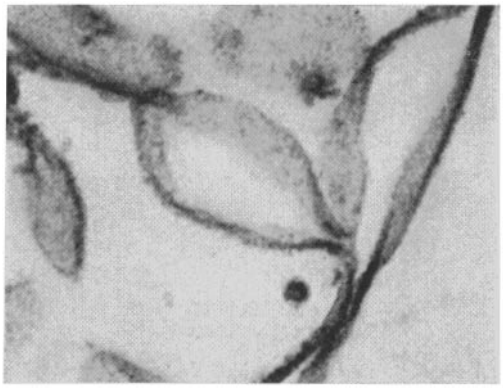


FIGURE 33 An enlargement of the area included within the square in Fig. 31. Continuity of a vesicle with the cytoplasmic membrane is shown, but the more extensive areas in Figs. 27 to 32 show that this cannot be considered typical of the vesicles occurring in lysed cells. × 115,000.

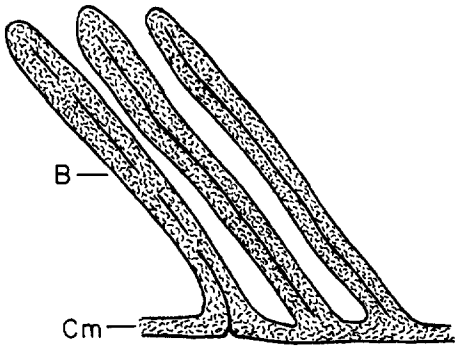


FIGURE 34 Semidiagrammatic drawing of the 160-A wide lamellae seen in Fig. 11. These lamellae represent folds of the cytoplasmic membrane (*Cm*) and are also seen at *B* in Figs. 1 through 12. The dense center line in these lamellae represents appressed regions of the dense outer layer of the folded cytoplasmic membrane and is seen in the insert of Fig. 1 and at *O* in Fig. 12.

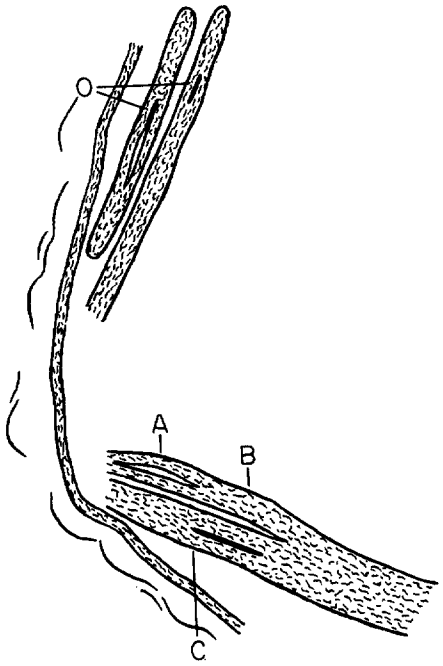


FIGURE 35 Semidiagrammatic drawing of the lamellae seen in Fig. 12. The 80-A wide lamellae at *A*, representing one region of the infolded cytoplasmic membrane, associate to form the 160-A wide lamella at *B*, which represents a fold of the cytoplasmic membrane. Two 160-A lamellae are associated at *C* to form a 300-A lamella. The dense central layer in the lamella at *C* represents two appressed regions of the dense inner layer of the cytoplasmic membrane, as diagrammed in Figs. 36 through 38. The dense layers in the 160-A wide lamellae at *O* represent the dense outer layer of the cytoplasmic membrane in the lamellae diagrammed in Fig. 34.

invaginations of the cytoplasmic membrane. If these lamellae serve as sites for photochemical reactions, these sites would be located in a structurally definable continuum within the cell. Some thoughts have been expressed already about possible relationships of the lamellar structures in photosynthetic bacteria to those of higher photosynthetic organisms and of non-photosynthetic microorganisms (15). Currently, however, there is no direct evidence that the lamellae visible in *R. molischianum* bear the photosynthetic pigments of this organism. The only pigment-bearing structures isolated from *R. molischianum* are the chromatophores, shown in Fig. 26, which were obtained by disruption of cells similar to those shown in Fig. 13. To relate these chromatophores to the lamellae seen in sectioned cells, it must be assumed that each chromatophore represents an invagination of the cytoplasmic membrane which has been separated from both the cytoplasmic membrane and the adjacent invaginations with which it is normally intimately associated within the cell. While this may be feasible, experimental evidence for such an assumption is lacking.

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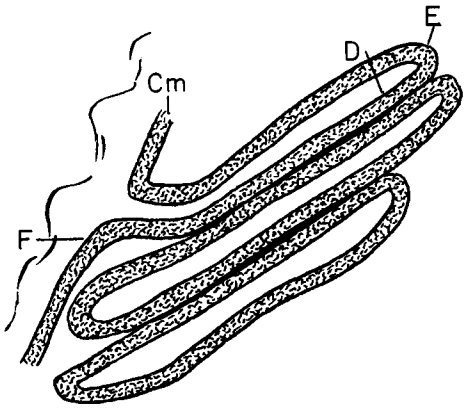

FIGURE 36 Semidiagrammatic drawing of the lamellae seen in Figs. 19 and 20. The invagination of the cytoplasmic membrane (*Cm*) indicated at *F* is seen in Fig. 20. The regions indicated at *D* and *E* are seen in Fig. 19. The structure of the region at *D* is diagrammed in more detail in Fig. 38.

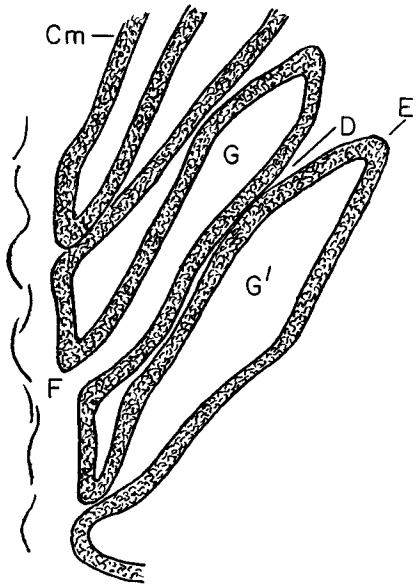

FIGURE 37 Semidiagrammatic drawing of Fig. 22, showing invagination of the cytoplasmic membrane (*Cm*) at *F*, spaces enclosed by adjacent invaginations at *G* and *G'*, unassociated regions of the invagination at *E*, and the association of adjacent invaginations at *D* to form a lamella whose structure is diagrammed in Fig. 38.

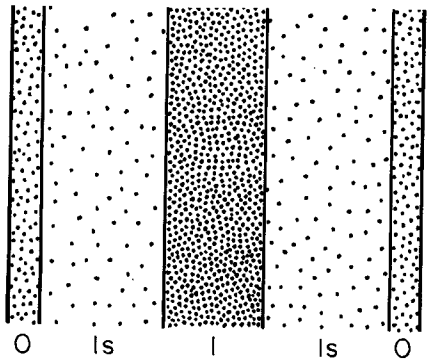

FIGURE 38 Semidiagrammatic drawing showing the structure of the lamellae seen at *D* in Figs. 13 through 22 and diagrammed in Figs. 36 and 37. The horizontal bars beneath the diagram indicate the structures contributed by each of the two adjacent invaginations forming this lamella. The outer dense layer (*O*) and less dense interspace (*Is*) of each invagination retain their identity, but the two regions of the dense inner layer of the cytoplasmic membrane associate to form a single dense layer (*I*).

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