THE FINE STRUCTURE OF *CHONDROCOCCUS COLUMNARIS*

III. The Surface Layers of *Chondrococcus columnaris*

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ABSTRACT

An electron microscope study of the myxobacterium *Chondrococcus columnaris* has revealed the following structures in the peripheral layers of the cells: (1) a plasma membrane, (2) a single dense layer (probably the mucopeptide component of the cell wall), (3) peripheral fibrils, (4) an outer membrane, and (5) a material coating the surfaces of the cells which could be stained with the dye ruthenium red.The ruthenium red–positive material is probably an acid mucopolysaccharide and may be involved in the adhesive properties of the cells. The outer membrane and plasma membrane both have the appearance of unit membranes: an electron-translucent layer sandwiched between two electron-opaque layers. The peripheral fibrils span the gap between the outer membrane and the mucopeptide layer, a distance of about 100 A, and run parallel to each other along the length of the cell. The fibrils appear to be continuous across the ends of the cells. The location of these fibrillar structures suggests that they may play a role in the gliding motility of these bacteria.

INTRODUCTION

Questions which might be answered by studies of the fine structure of gliding bacteria are the following: Are there any basic differences between gliding microbes and the eubacteria? Is there any basis for the often repeated statement that gliding bacteria lack a rigid cell wall, as opposed to the cell walls of eubacteria (Stanier, 1942)? What is meant by the "flexibility" which the cells of gliding microbes are said to possess; how (morphologically) do flexible cells differ from nonflexible ones? Do gliding bacteria have any peculiar surface properties demonstrable by electron microscopic techniques that might explain their tendencies to aggregate or that might be involved in motility?

Answers to some of the above questions have been found for a few organisms. Voelz and Dworkin (1962) described the cells of *Myxococcus xanthus* as being surrounded by two limiting membranes and noted the absence of slime in any organized form. Dworkin (1966) has expressed the opinion that flexibility is not a peculiar property of the myxobacterial cells and is probably only a reflection of their large length-to-width ratio. Lautrop et al. (1964), in an examination of the fine structure of the cell walls of 27 gram-negative bacteria, found the cell wall profiles of myxobacteria to resemble those of other gram-negative bacteria. The surface structures of many gram-negative bacteria have been shown by

electron microscopy to be composed of a single dense layer sandwiched between two unit membranes (Lautrop, Reyn, and Birch-Andersen, 1964; Clark and Lilly, 1962; Pate and Ordal, 1965; and others). Evidence has been presented to indicate that the dense intermediate layer of *Veillonella* (Bladen and Mergenhagen, 1964) and of *Escherichia coli* and *Spirillum serpens* (Murray et al., 1965) is the lysozyme-sensitive, rigid mucopeptide layer of the gram-negative cell wall. This dense layer corresponds to a dense layer seen in the surface structures of the gliding bacteria. Mason and Powelson (1958) purified and analyzed the cell walls of *M. xanthus* and reported the presence of diaminopimelic acid—a component of the "basal" cell wall structure (Work, 1960).

Costerton, Murray, and Robinow (1961) examined the surface of *Vitreoscilla* and other gliding bacteria by observing India ink particles in contact with the cells during movement. They concluded that there was no elongation or contraction of the cells during movement, that motility was probably not due to extrusion of slime or to the release of a surface tension-reducing substance, and that motility was not confined to one area of the trichome. They reported that the slime layer gave no reaction with potassium permanganate, iodine, alcian blue, ninhydrin-Schiff reagent, periodic acid Sudan black B, bromphenol blue, and was not dissolved by ethyl alcohol or ether. They found that specific antibodies to the cells would halt motility, which suggested to them the involvement of superficial structures in motility. They hypothesized that motility was due to orderly waves of contraction in an elastic outer layer of the cell walls. Thus far, no contractile elements have been reported in the walls of gliding bacteria. Nor is it clear how such contractile elements might function to bring about the smooth gliding type of motility under discussion. Gräf (1965) has suggested that the gliding type of locomotion is brought about by a contractile tube of fibrils which surrounds the cells. Fibrillar structures have been reported in membranes of broken cells of *C. columnaris* (Pate, Johnson, and Ordal, 1967). These structures were seen only when the cells were treated with a fixative immediately after lysis.

In the present study, the fine structure of the myxobacterium *C. columnaris* is examined with the use of the electron microscope. Special attention is given to surface structures in an attempt to discover any variation from the structures occurring in the eubacteria, with the idea that any organized structures responsible for the unusual type of motility of the gliding microbes or for their ability to aggregate should be located at or near the surface of the cells and would be absent from the cells of the eubacteria.

MATERIALS AND METHODS

Organisms

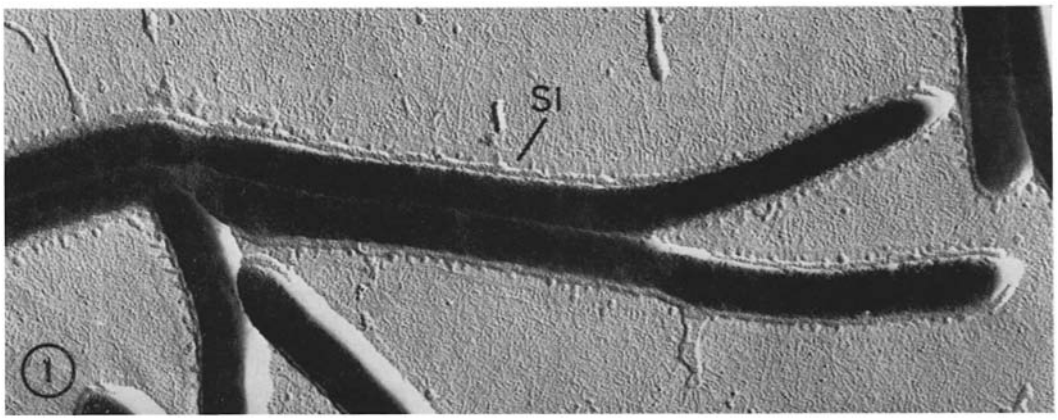
The principal organism used in this study was *C. columnaris* strain 1-R43. Other organisms used for comparative purposes were *Bacillus subtilis*, *Myxococcus xanthus* FB, *Chondrococcus coralloides*, *Spirillum serpens*, *Cytophaga* AL-1, and *Escherichia coli*. The growth medium used was the cytophaga medium described elsewhere (Pate and Ordal, 1967).

Preparations for Electron Microscopy

SHADOW-CAST PREPARATIONS: After 12 hr of growth in cytophaga broth at 25°C on a rotary shaker, formaldehyde was added to the culture to a concentration of 3.7%. The cells were left in the fixative for 3 hr. The cells were then washed three times by centrifugation in 0.067 M phosphate buffer. The final pellet was resuspended in the buffer to give a slightly turbid suspension. This suspension was placed on electron microscope grids, and excess fluid was drawn off with filter paper. The grids were placed in a Mikros (Mikros, Inc., Portland, Ore.) vacuum evaporator model VE-10, and a carbon rod wound with 8 cm of platinum wire was evaporated over them at a vertical distance of 2 cm and a horizontal distance of 4 cm.

PHOSPHOTUNGSTIC ACID (PTA) PREPARATIONS: The specimens to be examined were diluted with distilled water until just barely turbid, placed on an electron microscope grid with a carbon supporting film, and the excess fluid was removed with filter paper. A solution of 2% PTA in distilled water, adjusted to a pH of 7.1 with NaOH, was then placed on the grid. The excess PTA was drawn off with filter paper, and the grid was ready for examination.

FIXATION OF CELLS PRIOR TO LYSIS: Cells of *C. columnaris* were grown in cytophaga broth on a rotary shaker at 25°C and harvested by centrifugation after 12 hr of growth. The cells were resuspended in 0.067 M phosphate buffer with a pH of 7.4 and separated into four aliquots. Glutaraldehyde was added to one aliquot to a concentration of 2.4%, the second aliquot received formaldehyde to a concentration of 3.7%, the third aliquot received OsO_4 to a concentration of 1.0%, and the fourth aliquot received no fixative. The cells were broken open by sonication or by grinding with dry ice with a mortar and pestle. Sonication was carried out by a 60-sec



Abbreviations

DL, dense layer
LS, lamellar structure
N, Nuclear material
OM, outer membrane
PF, peripheral fibrils
PM, plasma membrane
R, ribosome
RR, Ruthenium red–stained
Sl, slime layer

FIGURE 1 Shadow-cast cells of *C columnaris*. No external appendages are visible; there is a suggestion of a slime layer surrounding the cells (*Sl*), and some of the cells appear to adhere together. × 22,500.

exposure to a 75-watt sonifier (Bronsen Instruments, Inc.) with a 0.5-in. probe tuned to an output of 5 amps.

Thin Sections

Two methods of fixation were used to prepare the cells for sectioning:

1. The double fixation employing glutaraldehyde and OsO_4 (Sabatini et al., 1963). The method used for the fixation of cells of *C. columnaris* was described elsewhere (Pate and Ordal, 1967).
2. Fixation in the presence of ruthenium red (Luft, 1965; Luft, J. H. 1966. Personal communication.). The fixation procedure is one worked out by Luft and is described below.

Cells were harvested from 30 ml of an actively growing culture by centrifugation, and the pellets were divided into small clumps and fixed for 1 hr in the cold (cracked ice) in the following solution:

a. 3.6% glutaraldehyde	0.5 ml
b. 0.2 M cacodylate buffer, pH 7.3	0.5 ml
c. Ruthenium red stock solution, 1500 ppm in distilled water	0.5 ml.

The clumps of cells were then rinsed with three changes of 0.15 M cacodylate buffer over a period of 10 min and fixed for 3 hr at room temperature in the following solution:

a. 4% OsO_4 in distilled water	0.5 ml
b. 0.2 M cacodylate buffer, pH 7.3	0.5 ml
c. Ruthenium red stock solution	0.5 ml.

The cells were then rinsed briefly with the buffer, dehydrated in a graded series of alcohols, and embedded in Epon 812 (Luft, 1961). The source of ruthenium red was Alfa Inorganics, Inc., Beverly, Massachusetts.

Sections were made with a diamond knife on an LKB Ultratrome. The sections were stained with lead citrate alone or were stained for 15 min with 2% uranyl acetate followed by a 10-min treatment with lead citrate (Reynolds, 1963). All of the micrographs were made with an RCA EMU 3G electron microscope.

RESULTS

Whole Cells

Fig. 1 shows cells of *C. columnaris* that have been shadowed with carbon and platinum. Whole cells are between 0.3 and 0.5 μ in diameter and are 4–6 μ in length. There is a suggestion of an extracellular slime surrounding the cells, and some of the cells appear to adhere together. There

is no evidence for external organelles of locomotion.

Fig. 2 shows a PTA preparation of a cell of *C. columnaris* harvested from a cytophaga broth culture after 12 hr of growth at 25°C on a rotary shaker. Cells in these preparations are quite opaque to the electron beam so that internal structures are not visible. No surface structures such as flagella or fimbriae can be seen. There appears to be a slime layer surrounding the cells.

Broken Cells

The sonicates of cells fixed with either glutaraldehyde or formaldehyde contain what appear to be fragments of membranes with networks of fibrils. These fibrils run parallel to each other in wide bands; in some regions two bands converge upon each other, and one seems to pass over the other, resulting in a criss-cross pattern (Fig. 3). Where the fibrils are aligned parallel to each other, there is a center-to-center distance of 150–160 A from fibril to fibril.

The same fibrillar structures were found when the cells were fixed with formaldehyde, as above, and broken open by grinding with dry ice (Figs. 4 and 5). When unfixed cells or cells fixed in 2% OsO_4 were broken open by sonication or by grinding with dry ice, no membranes with fibrils were found.

Sectioned Cells

The peripheral fibrils have a characteristic appearance in sections cut perpendicular to the long axis of the cell (Figs. 6 and 7). In these sections, the peripheral fibrils extend from the inner surface of the outer membrane to the dense layer (a distance of 90–100 A), apparently connecting the two. The peripheral fibrils appear triangular, with the base of the triangle adjoining the outer membrane and the apex abutting upon the dense layer. The distance from the center of one fibril to the center of the next is approximately 160 A.

A few sections were taken at, or very near the tips of the cells (Figs. 8–10). The peripheral fibrils give the appearance of spiraling around the cell in groups, passing over the tip of the cell and down the other side. The cell shown in Fig. 8 is assumed to be sectioned very near the tip because of its small diameter. The fibrils appear to be arranged in groups. In one group, the fibrils are all lined up parallel to each other; then the direction of incidence upon the cell changes, and there is another group of fibrils all parallel to each other but not with those in the first group.

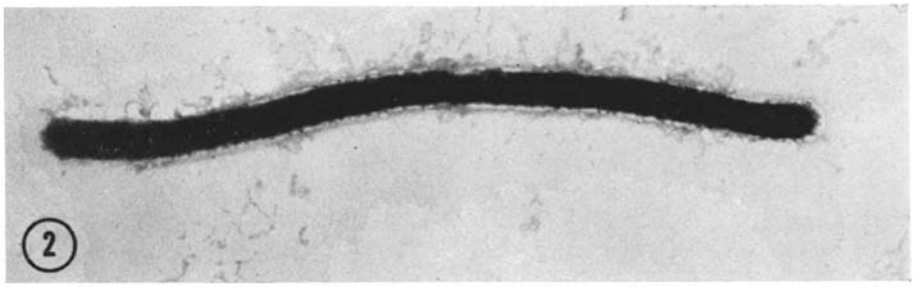


FIGURE 2 PTA preparation of a cell of *C. columnaris*. Notice the thin layer of material surrounding the cell. × 16,500.

Fig. 9 shows a section taken right at the tip of the cell. Again, groups of fibrils can be seen converging upon the tip from different directions. At one edge, continuity of one group of fibrils across the tip of the cell can be seen. Toward the center of the cell, another group of fibrils crosses the tip from a different direction. This second group of fibrils is out of the plane of section in the center of the cell, but its continuity across the tip can be seen (arrows). A third group of fibrils comes in from another direction, leaves the plane of section, and must pass over or under the first two groups or swirl around to join the fibrils on the other side. In Fig. 10, two groups of fibrils can be seen running in different directions and passing over the tip of the cell. It is not clear

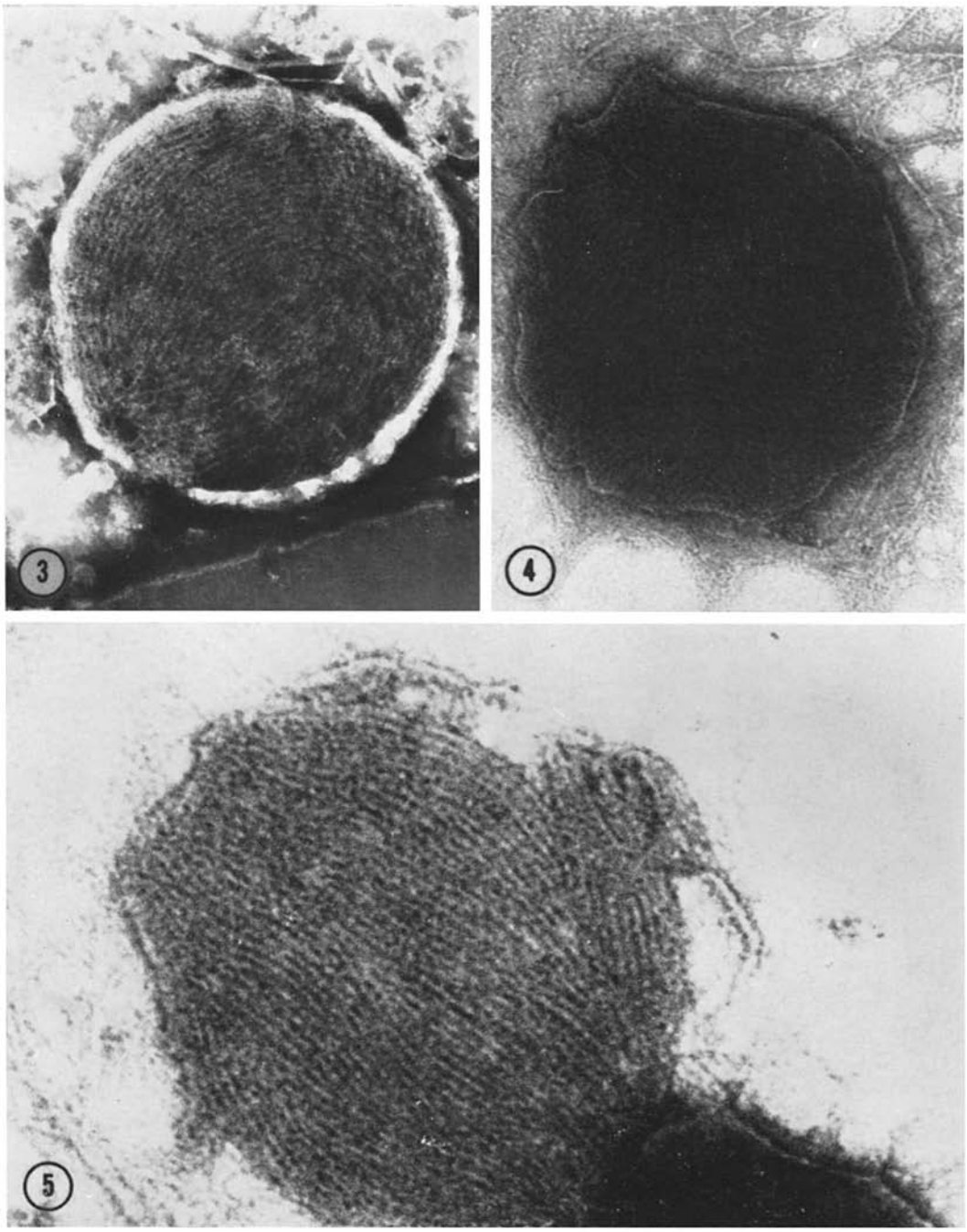
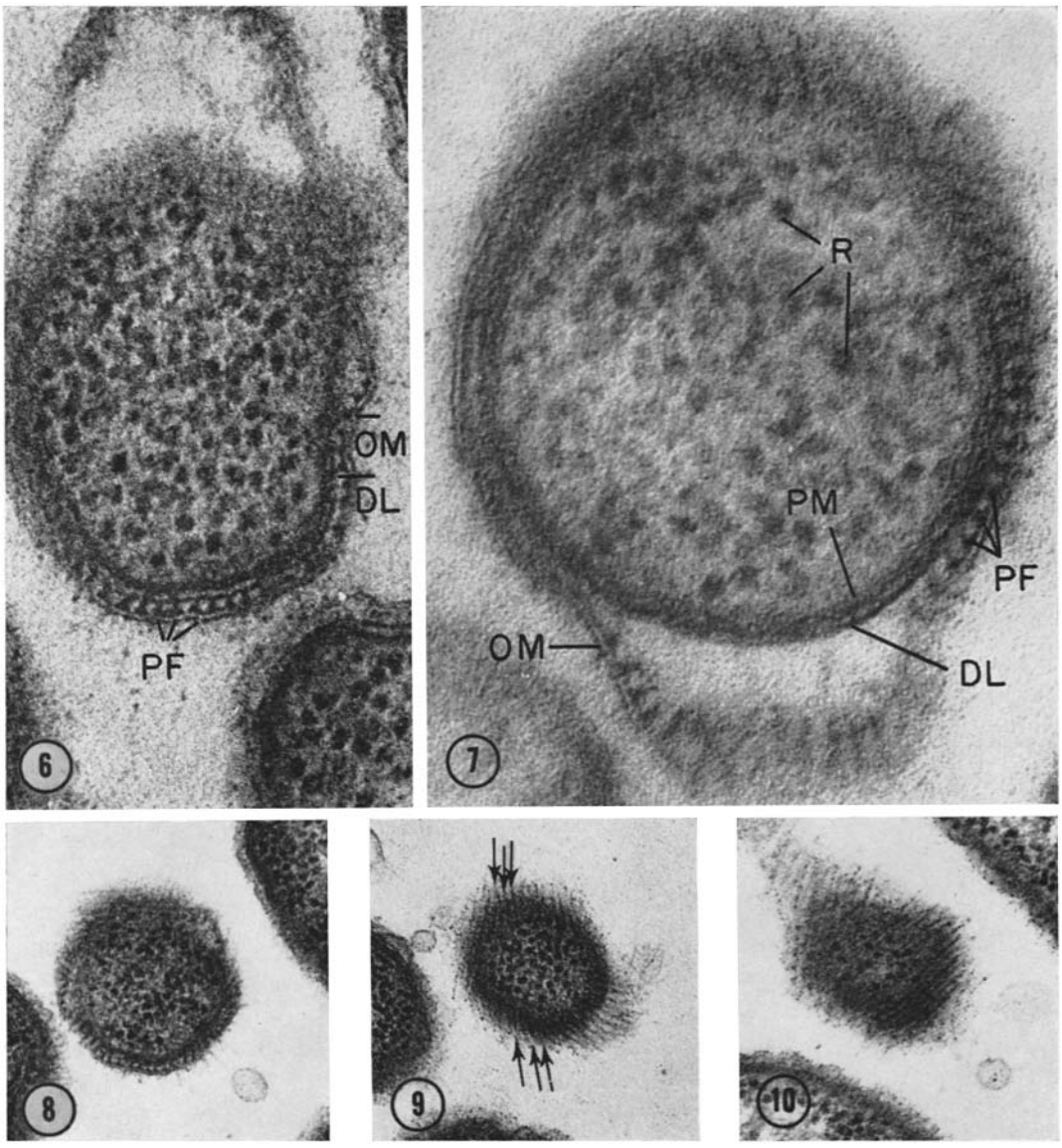


FIGURE 3 PTA preparation of fibrillar structure from lysate of cells of *C. columnaris* which had been fixed in formaldehyde and then sonicated for 3 min. All of these lysates contained structures which appear to be fragments of membranes with a network of fibrils running through them. The fibrils appear to run parallel to each other with a center-to-center distance of about 160 A. × 97,000.

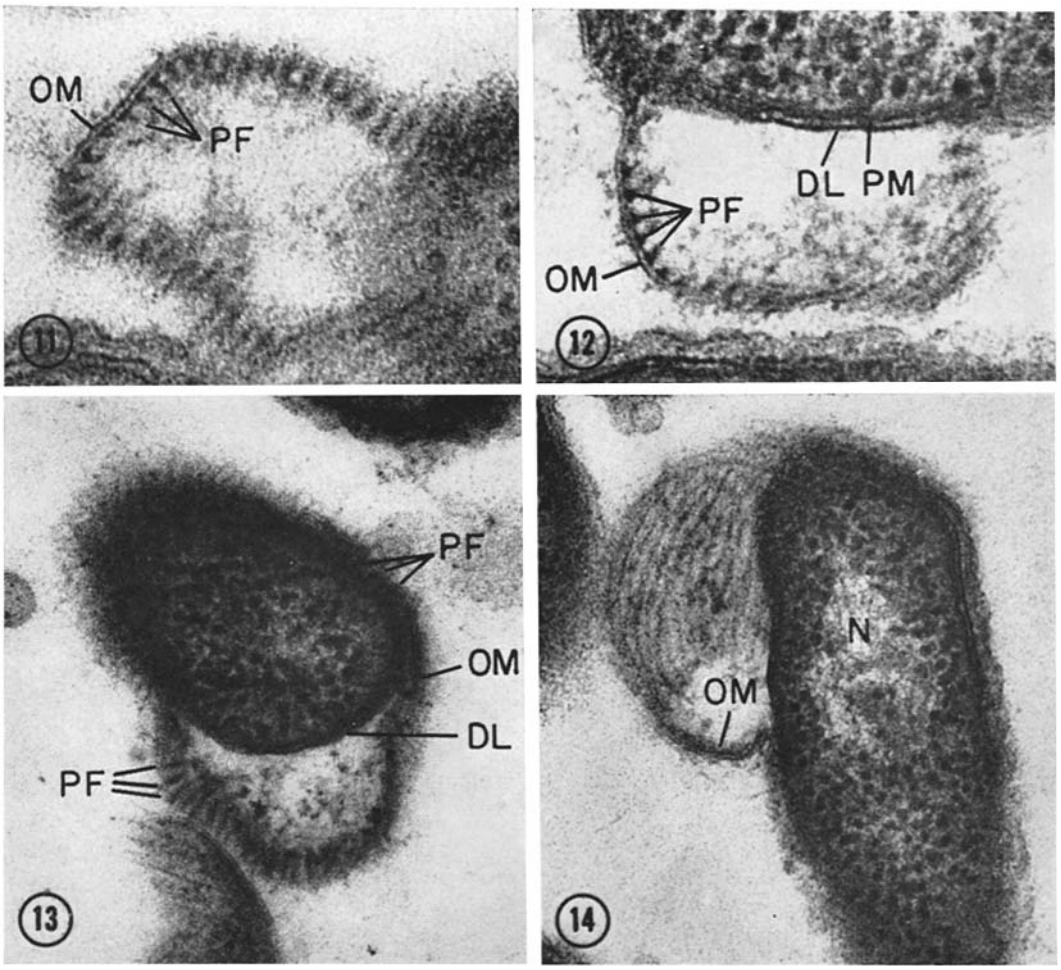
FIGURES 4 and 5 PTA preparations of fibrillar structures from cells of *C. columnaris* which had been fixed with formaldehyde and ground with dry ice. Fig. 4 shows one of the fibrillar structures in which the fibrils are arranged in a criss-cross pattern, as though one layer of fibrils were lying over another layer. In Fig. 5, a sheet of parallel fibrils is shown which appears to be peeled back from the tip of a cell. Fig. 4, × 100,000; Fig. 5, × 194,000.



FIGURES 6–10 Sections through cells of *C. columnaris* showing peripheral fibrils. Glutaraldehyde and OsO_4 fixation. The fibrils are located between the outer membrane and the dense layer. Figs. 8–10 show sections through fibrils near the tips of the cells. The arrows in Fig. 9 point to fibrils which give the appearance of continuing across the end of the cell. In Fig. 10 the fibrils can be seen to continue across the tip of the cell. Fig. 6, × 157,000; Fig. 7, × 216,000; Figs. 8–10, × 69,000.

what happens in the region in which the two groups of fibrils come together, but it seems likely that one group passes over the other.

Occasionally, electron micrographs are obtained which show cells with the outer membrane separated from the dense layer in a region in which the peripheral fibers are present (Figs. 7, 11–14). Whenever this occurs, the fibrils remain attached to the outer membrane, rather than to the dense layer. In Fig. 7, the outer membrane has folded over slightly into the plane of section, so that the peripheral fibrils can be seen to run



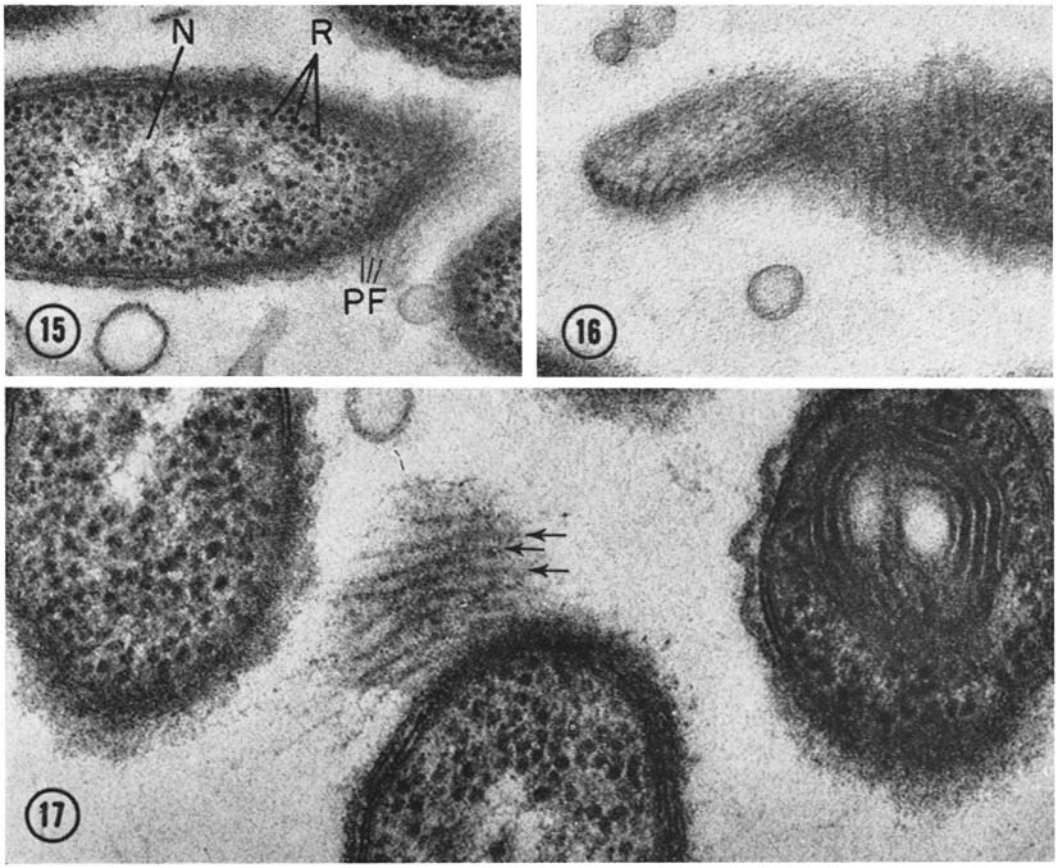
FIGURES 11–14 Sections through cells of *C. columnaris* showing the attachment of the peripheral fibrils to the outer membrane. Glutaraldehyde and OsO_4 fixation. In these micrographs, the outer membranes have separated from the cells, and the peripheral fibrils are always asociated with the outer membrane rather than with the dense layer. Figs. 11 and 12, × 172,000; Fig. 13, × 97,000; Fig. 14, × 70,000.

longitudinally along the cell. Fig. 14 shows a longitudinal section through one end of a cell, the outer membrane of which has flared out. In this section, the peripheral fibrils can be seen to pass along one side of the cell and over the tip. In all of these sections, the fibrils appear as an integral part of the inner layer of the outer membrane.

The peripheral fibrils are shown in oblique sections in Figs. 15–17. The fibrils shown in Fig. 17 are probably from a cell just out of the plane of section. The appearance is very similar to that of the fibrillar structures seen in the PTA preparations of cell membranes. Fig. 15 shows a cell which curves in and out of the plane of section; and, as it does, the peripheral fibrils are seen in oblique section. In this case, the fibrils are curving across the cell, rather than running along its length. The same kind of section is shown in Fig. 16, in which a cell is seen with the peripheral fibrils traversing its diameter.

Fixation in the Presence of Ruthenium Red

Cells of strain 1-R43 fixed in the presence of ruthenium red showed very good general preserva-



FIGURES 15–17 Oblique sections through peripheral fibrils of *C. columnaris*. Glutaraldehyde and OsO_4 fixation. Fig. 15, × 70 000; Fig. 16, × 97,000; Fig. 17, × 125,000.

tion. All of the structural components of the cells which had been demonstrated with other fixations were present in the cells treated with ruthenium red. In addition to the structural components normally seen, two components appeared which had not been apparent in cells fixed by the conventional methods.

The most striking of the two components is a material which coats the surface of the cells and which frequently extends outward from the cells, connecting adjacent bacteria. Fig. 18 shows an unstained section of cells fixed in the presence of ruthenium red. The cytoplasm, plasma membrane, and dense layer of the cells are all very low in contrast compared to the electron-opaque layer covering the surfaces of the cells. Adjacent sections were stained with lead citrate to improve the general contrast of the cells. Figs. 19 and 20 are of sections of cells stained with lead citrate. In these micrographs, the ruthenium red–positive material is extremely opaque to the electron beam and the processes connecting one cell to another are clearly demonstrated. Fig. 21, a highly magnified micrograph of a very thin section, shows the density due to the ruthenium red–positive material to be restricted to the surface of the outer unit membrane.

Some of the electron micrographs show sections through cells whose outer unit membranes have been damaged (Figs. 22 and 23). These damaged cells are seen to possess a large amount of the ruthenium red–positive material located in the space between the plasma membrane and the outer unit membrane. The outer unit membrane shown in Fig. 23 exhibits increased density on both sides rather than on just the outer surface

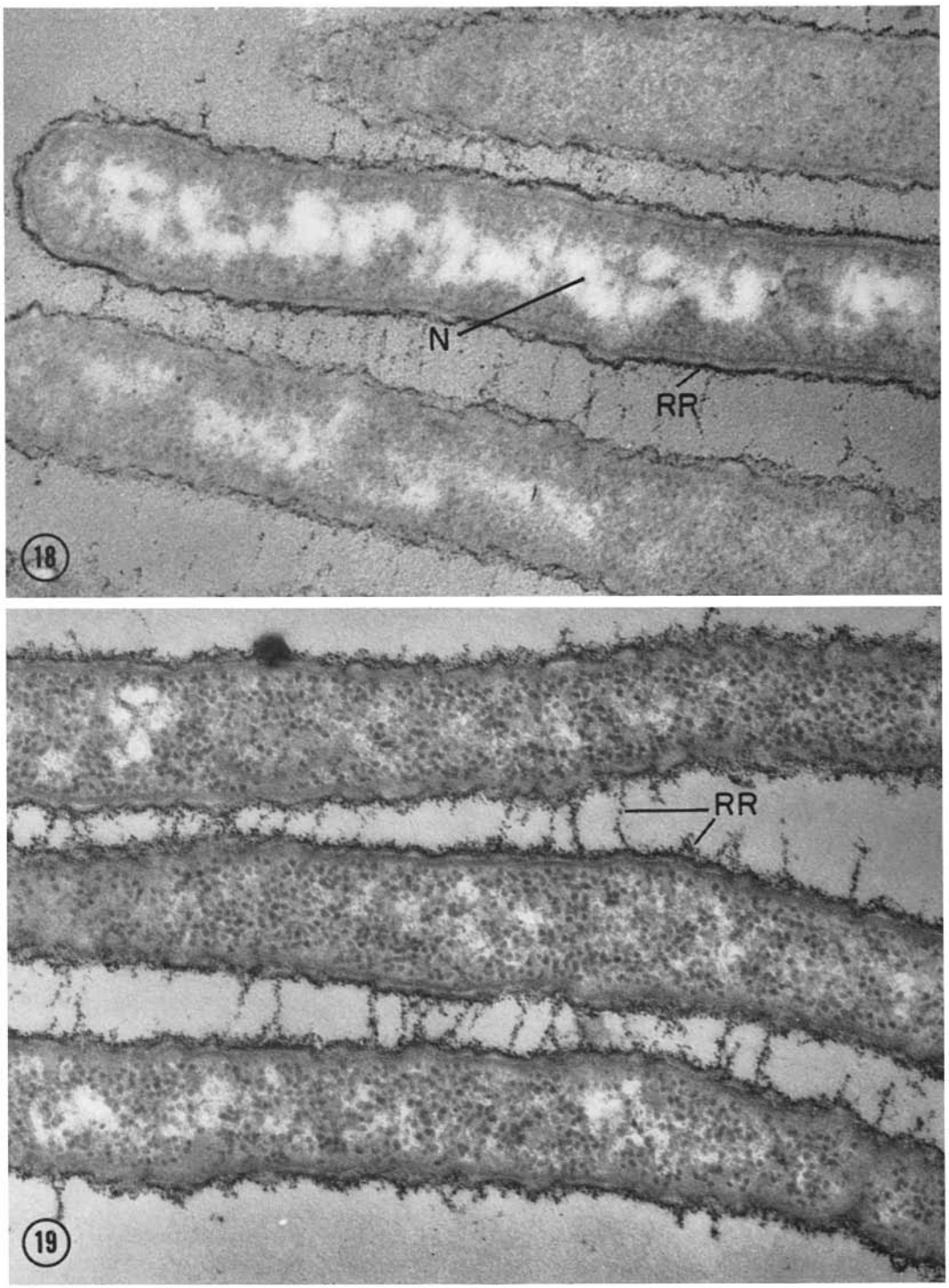
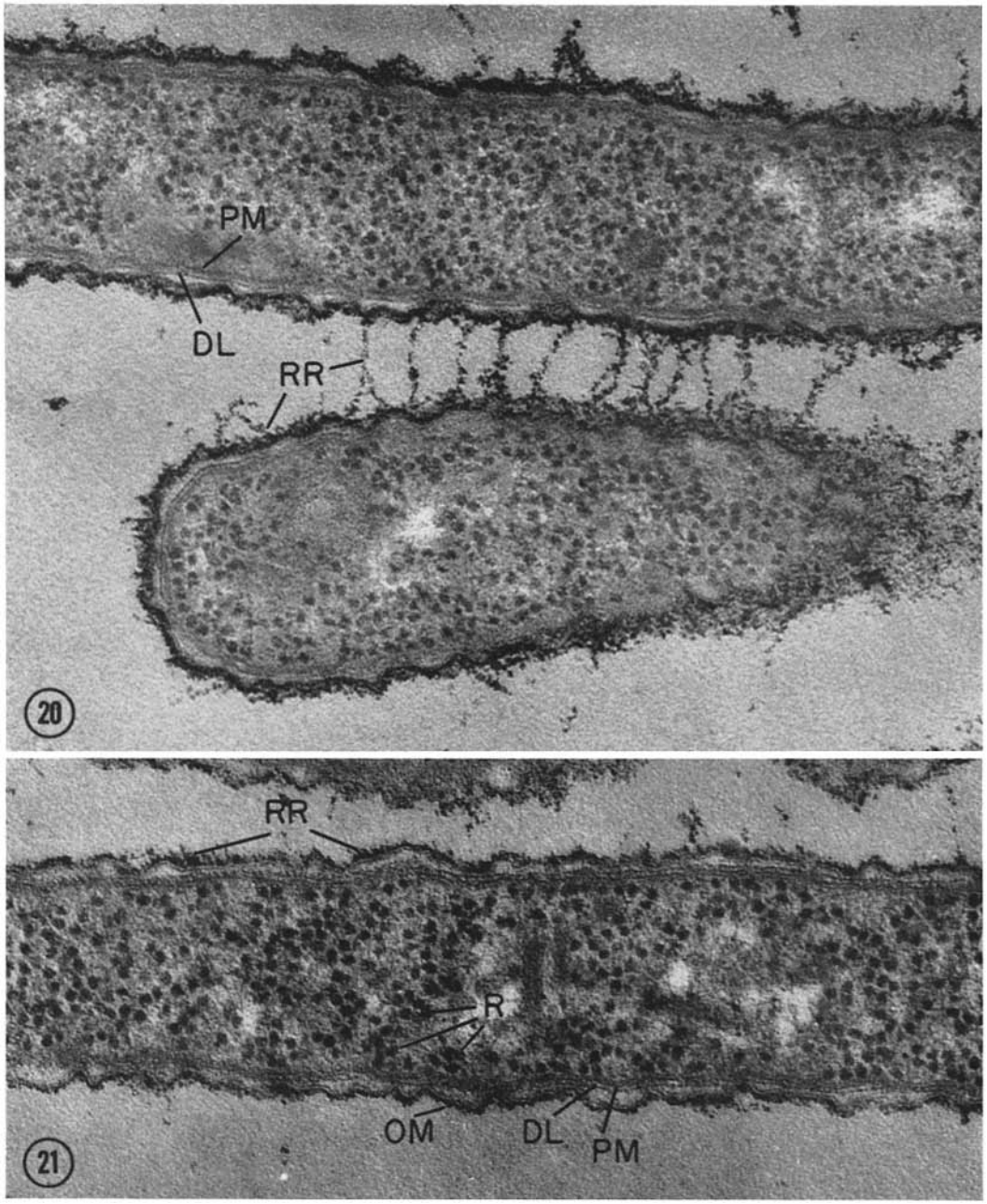


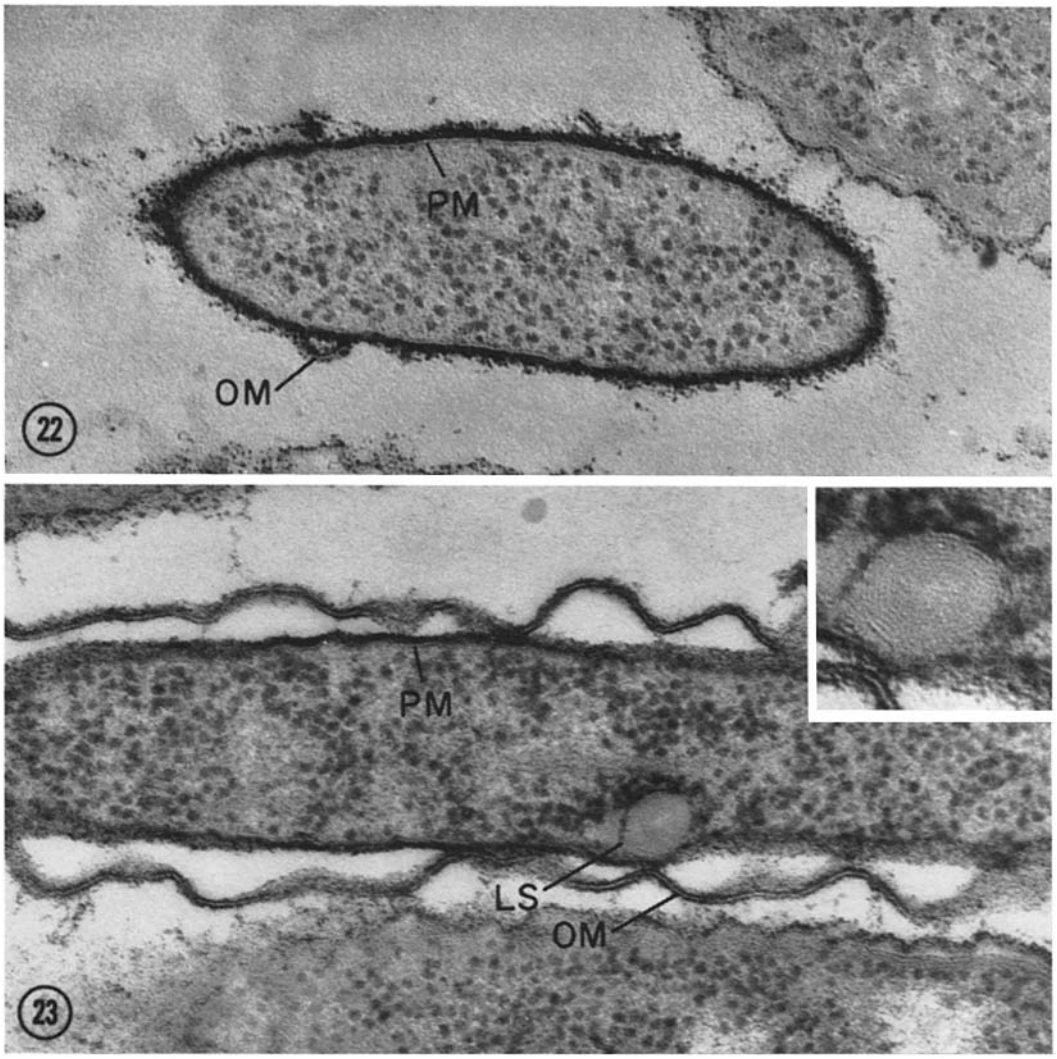
FIGURE 18 Section through cells of *C. columnaris* fixed in the presence of ruthenium red. Unstained. Notice the increased density at the surfaces of the cells (*RR*). × 64,600.

FIGURE 19 Section through cells of *C. columnaris* fixed in the presence of ruthenium red. Poststained with lead citrate. Notice the darkly stained material (*RR*) coating the surfaces of the cells and extending between the surfaces of adjacent cells. × 64,600.



FIGURES 20 and 21 Sections through cells of *C. columnaris* fixed in the presence of ruthenium red. Post-stained with lead citrate. Notice that the material stained with ruthenium red is on the outside surface of the outer membrane. × 97,000.

as is the case with membranes of intact cells. These observations on cells with damaged membranes indicate that the ruthenium red–positive material is present on both sides of the outer membrane and in the space which separates the outer membrane from the plasma membrane in intact bacteria, and that the ruthenium red is unable to pass through the membrane of the intact cell



FIGURES 22 and 23 Sections through damaged cells of *C. columnaris* fixed in the presence of ruthenium red. Poststained with lead citrate. Notice the lamellar structure (*LS*) in the cell shown in Fig. 23. This structure is shown at higher magnification in the inset. Both figures, × 97,000; inset, × 200,000.

to react with the material inside. These observations are in agreement with those of Luft on the impermeability of the membranes of cells of higher organisms to ruthenium red.

The second of the two components which have been demonstrated with the ruthenium red technique is a cytoplasmic structure located just inside the plasma membrane (Fig. 23). The structure in section is round, about 90–100 mμ in diameter, and is made up of concentric circles with a periodicity of about 37 A. In the intact cell, this structure is probably spherical and composed of layer upon layer of material arranged somewhat like the layers of an onion. This structure has been seen in two different sections. Although this latter structure has not been demonstrated in cells fixed in the absence of ruthenium red, its appearance cannot be stated to be due to the action of ruthenium red. Its occurrence within the cells may be extremely rare, and its presence in these micrographs may be due to fortuitous sections rather than to the

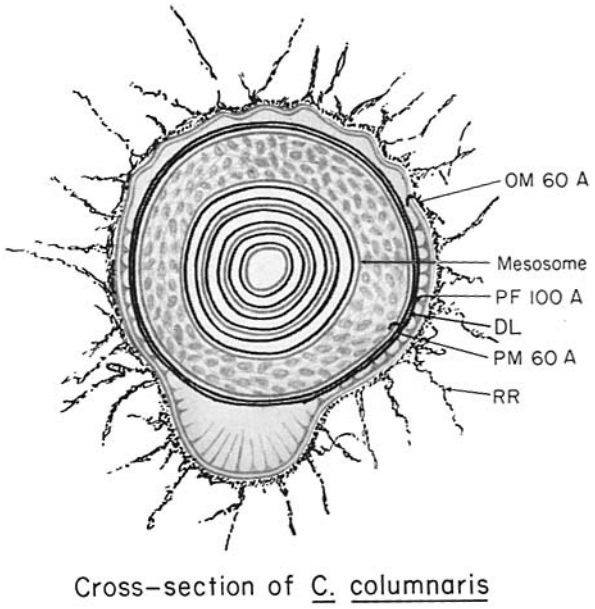


FIGURE 24 Diagram of a cross-section of *C. columnaris* summarizing the structures found in many different cells examined during this study. The peripheral fibrils appear to be intimately associated with the outer membrane. The outer membrane is folded away from the cell at the bottom of the diagram to illustrate that the peripheral fibrils run parallel to the long axis of the cell. The distance from the dense layer to the outer membrane is about 100 A. The center-to-center distance between peripheral fibrils is about 160 A. It is not known whether the peripheral fibrils completely girdle the cell or if they extend only part way around, as shown in the diagram.

ruthenium red technique. Also, the ruthenium red appears not to cross the plasma membrane, so that any cytoplasmic structures would be inaccessible for reaction with it. It must be noted, however, that in both cases in which the laminated structure was seen the outer membranes were damaged and ruthenium red had penetrated to the plasma membrane. Since the laminated structure appears to be in contact with the plasma membrane, the ruthenium red may possibly be responsible for its appearance.

With the same conditions of fixation, ruthenium red-positive material has been looked for in *Bacillus subtilis*, *Myxococcus xanthus*, *Chondrococcus coralloides*, *Spirillum serpens*, *Cytophage* (AL-1), and *E. coli*. The surfaces of *M. xanthus*, *M. fulvus*, and *S. serpens* were ruthenium red-positive while those of *B. subtilis*, *Cytophaga* (AL-1), and *E. coli* were negative.

DISCUSSION

Generalized Cell Structure

The structural components of cells of *C. columnaris* as determined by electron microscopy are summarized in diagrammatic form in Fig. 24. In the direction from the surface of the cell toward the center, the structures in their order of appearance are: (1) material stained with ruthenium red, (2) outer membrane, (3) peripheral fibrils, (4) dense layer (mucopeptide layer), (5) plasma membrane, (6) cytoplasm, and (7) mesosome. The nuclear material is not shown in this diagram, nor is the lamellar structure. The structure of the mesosome was reported earlier (Pate and Ordal, 1967). The ruthenium red-positive material and the peripheral fibrils have not been reported in cells of other bacteria, and the possible significance of their occurrence in cells of *C. columnaris* is discussed below.

Peripheral Fibrils

It has been shown that cells of *C. columnaris* possess fibrillar elements intimately associated with the inner layer of the outer unit membrane of the cell. These fibrils run parallel to each other in wide bands with a center-to-center distance of approximately 160 A. In several micrographs these peripheral fibrils appeared to be continuous across the ends of the cells, with one band of fibrils overlapping another. Owing to the apparent instability of these fibrils, fixation with glutaraldehyde or formaldehyde was necessary to prevent their distintegration during the preparative techniques normally employed for the examination of cells in the electron microscope.

The presence of such structures in gliding organisms immediately suggests a morphological basis for the gliding type of motility. Many theories have been put forth to explain this

peculiar mode of locomotion. These theories have included a directed production of slime (Jahn, 1924), theories of cellular contraction and expansion (Mayer-Pietschmann, 1951), waves of contraction along the surface of the cell (Costerton et al., 1961), and torsion pressure within protein helices wound around the cell (Jarosch, 1964). All of these theories, with the exception of a directed production of slime, would require the presence of some specialized structure to put them into effect. Jahn and Bovee (1965), in a review of movement in microorganisms, stated that the physical basis of protoplasmic movements, wherever found, probably consists of actomyosinlike proteins in fibrillar associations. Roth (1964) has pointed out that several types of motile systems present in many different cells possess continuous filaments with a diameter of 150–210 A. He suggests that all of these filaments may be closely related in composition.

The involvement of the peripheral fibrils in gliding motility can be established if similar structures can be demonstrated in all other gliding microbes. If such structures do exist in other gliding bacteria, it is likely that their extreme fragility might be responsible for their having gone undetected until now. Therefore, it is recommended that electron microscopic investigations of the surface structures of gliding bacteria be carried out after fixation with glutaraldehyde or formaldehyde, rather than with OsO_4 alone.

Ruthenium Red

Ruthenium red (ammoniated ruthenium oxychloride) has the empirical formula, $Ru_3O_2(NH_3)_{14}Cl_6 \cdot H_2O$, M.W. 858.5, and has been given the structural formula, $(NH_3)_5Ru{-}O{-}Ru(NH_3)_4{-}O{-}Ru(NH_3)_5^{6+}$ (Fletcher et al., 1961). Luft (1965; Luft, J. H., 1966. Personal communication) has developed a technique for the use of ruthenium red for electron microscopy. Ruthenium red must be added to the tissue at the same time as the fixative, and Luft believes the resulting increased electron opacity to be due to a catalytic reduction of osmium tetroxide to lower insoluble oxides. Experiments carried out in Dr. Luft's laboratory indicate that the ruthenium red dye molecule has a high affinity for acid mucopolysaccharides. One molecule of ruthenium red may catalyze the reduction of several molecules of osmium tetroxide and build up the mass in the region of the bound ruthenium red.

Possible Significance of Extracellular Polysaccharides

Kalckar (1965), in an interesting paper on galactose metabolism and cell "sociology," has pointed out the involvement of polysaccharides in the "social patterns" of cells. In microbial systems, mutations resulting in changes in polysaccharides on the exterior of cells have changed the morphology of colonies, altered the immunological response of cells, and obliterated phage receptor sites in certain bacteria. He also gives several instances of the effect of surface polysaccharides on social properties of cells from higher organisms. L-fucose and *N*-acetylgalactosamine seem to be important for the existence of recognition sites on red blood cells. Loss of organ-specific antigens has been correlated with defective galactose metabolism. A number of tumor cells have been found to be deficient in galactose metabolism. He notes that surface patterns may be crucial for normal cell contact and growth control.

In a study of the attachment areas of cells in the epidermis of developing newts, Kelly (1966) showed that acid mucopolysaccharides, demonstrated by ruthenium red staining, were heavily concentrated in areas of intercellular attachment (desmosomes). Also, the external leaflet of the plasma membranes was coated with a mucopolysaccharide. He suggests that this might be morphological evidence for the "general selective, weakly adhesive properties which cells are known to possess."

In view of the foregoing discussion on the characters controlled by surface polysaccharides in general and the adhesive properties attributed to acid mucopolysaccharides in particular, the possible significance of the ruthenium red–stainable material on the surface of cells of *C. columnaris* becomes apparent. That the cells of *C. columnaris* do possess adhesive properties is evidenced by their ability to aggregate to form fruiting bodies and by their affinity for tissues of salmonoid fishes (Ordal and Rucker, 1944). Also, electron microscopic evidence suggests that the material stained with ruthenium red functions as an adhesive, linking one cell to another by the narrow processes extending out from the surfaces of the cells. In respect to the formation of fruiting

bodies, the presence of the ruthenium red–stainable material on the surfaces of the other two fruiting myxobacteria examined in this study, and its absence in the nonfruiting Cytophaga should be recalled. However, it is not possible to make any final judgment until many more gliding bacteria have been examined for the presence of acid mucopolysaccharides.

Johnson and Chilton (1966) have purified a polysaccharide which is produced in large amounts by cells of *C. columnaris* when grown in a defined amino acid medium or a synthetic medium containing casein hydrolysate (Chase, 1965). The polysaccharide was a partially acetylated polygalactosamine. As expected, ruthenium red gave no reaction with this purified basic polysaccharide. Whether the polygalactosamine is layered on the surface of the cells along with the acid mucopolysaccharide or simply excreted into the medium is not known. No function, either physiological or physical (adhesion, motility), has yet been suggested for the polygalactosamine.

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BIBLIOGRAPHY

BLADEN, H. A., and S. E. MERGENHAGEN. 1964. Ultrastructure of *Veillonella* and morphological correlation of an outer membrane with particles associated with endotoxic activity. *J. Bacteriol.* **88**:1482.

CHASE, J. A. 1965. Nutrition of some aquatic myxobacteria. M.S. Thesis, University of Washington.

CLARK, P. H., and M. D. LILLY. 1962. A general structure for cell walls of gram-negative bacteria. *Nature.* **195**:516.

COSTERTON, J. W. F., R. G. E. MURRAY, and C. F. ROBINOW. 1961. Observations on the motility and structure of *Vitreoscilla*. *Can. J. Microbiol.* **7**:329.

DWORKIN, M. 1966. *Ann. Rev. Microbiol.* **20**:75.

FLETCHER, J., B. GREENFIELD, J. HARDY, D. SCARGILL, and . WOODHEAD. 1961. Ruthenium red. *J. Am. Chem. Soc.* **1961**:2000.

GRÄF, W. 1965. Bewegungsorganellen bei myxobakterien. *Arch. Hyg. Bakteriol.* 518.

JAHN, E. 1924. Beiträge zür botanischer protistologie. I. Die Polanziden. Gerbrüder Borntraeger, Leipzig. 107 pp.

JAHN, T. L., and E. C. BOVEE. 1965. Movement and locomotion of microorganisms. *Ann. Rev. Microbiol.* **19**:21.

JAROSCH, R. 1964. *In* Primitive Motile Systems in Cell Biology, R. O. Allen and N. Kamiza, editors. Academic Press Inc., New York. 599.

JOHNSON, J. L., and W. S. CHILTON. 1966. Galactosamine glycan of *Chondrococcus columnaris*. *Science.* **152**:1247.

KALCKAR, H. M. 1965. Galactose metabolism and cell "sociology." *Science.* **150**: 305.

KELLY, D. E. 1966. Fine structure of desmosomes, hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. *J. Cell Biol.* **28**:51.

LAUTROP, H., A. REYN, and A. BIRCH-ANDERSEN. 1964. A comparative electron microscope study of the cell walls of gram-negative bacteria. *Proc. 14th Scand. Congr. Pathol. and Microbiol.* Universitets ferlaget, Oslo, 25–27 June 1964, 157–160.

LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:407.

LUFT, J. H. 1965. The fine structure of hyaline cartilage matrix following ruthenium red fixative and staining. *J. Cell Biol.* **27**:61A (Abstr.)

MASON, D. J., and D. POWELSON. 1958. The cell wall of *Myxococcus xanthus*. *Biochim. Biophys. Acta.* **29**:1.

MAYER-PIETSCHMANN, K. 1951. Lebendbeobachtungen an *Myxococcus rubescens*. *Arch. Mikrobiol.* **16**:163.

MURRAY, R. G. E., P. STEED, and H. E. ELSON. 1965. The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. *Can. J. Microbiol.* 11:547.

ORDAL, E. J., and R. R. RUCKER. 1944. Pathogenic myxobacteria. *Proc. Soc. Exptl. Biol. Med.* **56**:15.

PATE, J. L., J. L. JOHNSON, and E. J. ORDAL. 1967. The fine structure of *Chondrococcus columnaris*. II. Structure and formation of rhapidosomes. *J. Cell Biol.* **35**:15.

PATE, J. L., and E. J. ORDAL. 1965. The fine structure of two unusual stalked bacteria. *J. Cell Biol.* **27**:133.

PATE, J. L., and E. J. ORDAL. 1967. The fine structure of *Chondrococcus columnaris*. I. Structure and formation of mesosomes. *J. Cell Biol.* **35**:1.

REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.

ROTH, L. E. 1964. *In* Primitive Motile Systems in Cell Biology. R. O. Allen and N. Kamiza, editors. Academic Press Inc., New York, 642 pp.

SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. *J. Cell Biol.* **17**:19.

STANIER, R. Y. 1942. The cytophaga group: a contribution to the biology of myxobacteria. *Bacteriol. Rev.* **6**:143.

VOELZ, H., and DWORKIN, M. 1962. Fine structure of *Myxococcus xanthus* during morphogenesis. *J. Bacteriol.* **84**:943.

WORK, E. 1960. Biochemistry of the bacterial cell wall. *Nature.* **179**:841.