THE FINE STRUCTURE OF TWO UNUSUAL STALKED BACTERIA

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ABSTRACT

Two strains of bacteria that produce slender appendages (pseudostalks) from their lateral surfaces were studied using the electron microscope. The pseudostalks were shown to be extensions of the cytoplasm and peripheral membranes of the cell proper. Both strains of bacteria produce holdfasts at the poles of the cells by the means of which attachment can take place. The pseudostalks are not involved in the attachment of cells. No specialized intracytoplasmic structures are present at the point of juncture of pseudostalk and cell. A discussion of the possible functions of the pseudostalks, based on the electron microscope findings, is presented.

INTRODUCTION

Bacteria that produce slender extensions from one pole of the cell during a stage of their life cycle were described and given the generic name, *Caulobacter*, by Henrici and Johnson (7). At that time the outgrowths of the cell were thought to be composed of gum secreted from one particular portion of the cell. Henrici and Johnson obtained these bacteria for study by allowing them to attach to the surface of submerged glass slides in natural bodies of water. The term "stalk" was used to describe the thin extensions of the cell because attachment always occurred at the tips of these structures. Since that time these organisms have been examined more closely with the use of the electron microscope. Houwink (9, 10) and Houwink and van Iterson (11) have shown that the stalks are extensions of the cytoplasm and wall of the bacterial cell. They have also demonstrated the presence of a "secreted holdfast" at the tips of the stalks by the means of which attachment takes place. The caulobacters exist in two different forms (23). During one stage of the life cycle, the cells are motile by means of a single polar flagellum. The stalks are produced at the flagellated pole, the flagellum is lost, and the immotile-stalked cells divide to give rise to motile cells. Since the stalks are always produced at the flagellated pole, Zavarzin (30) suggested that the flagellum becomes "ensheathed" and is thus converted into the stalk.

Houwink suggested that the stalks were used by the caulobacters to parasitize other bacteria to which they attached. Poindexter (23) has shown that attachment of the caulobacters to other bacteria is neither advantageous to the caulobacters nor harmful to the host cells, thereby disproving Houwink's hypothesis concerning the function of the stalks. Poindexter's electron micrographs of sections through cells of the *Caulobacter* group showed that some membranous intracytoplasmic structures are present in these organisms and that these structures are continuous with the plasma membrane. She suggested that the core of the stalk is composed entirely of membranes derived from the intracytoplasmic membrane structure. Poindexter concluded that

"the core of the stalk is membranous rather than cytoplasmic, as proposed by Bowers *et al.* (3), or flagellar, as proposed by Zavarzin (30)."

Poindexter has proposed the establishment of a new genus, *Asticcacaulis*, to accomodate bacteria which produce a structure identical in appearance with the *Caulobacter* stalk at right angles to the long axis of the cell. These bacteria secrete the adhesive substance at one pole of the cell, as do the caulobacters, but the stalk-like structures are produced from a different site on the cell surface so that, when attachment occurs, it is at one pole of the cell and never at the tips of the stalk-like structures.

In this paper, the slender appendages produced by members of the genus, *Asticcacaulis*, will be referred to as "pseudostalks." This term is introduced in order to accentuate the fact that the appendages in question, although they do not function as stalks (a supportive function), nevertheless have the same organization as the stalks of the caulobacters.

Two strains of bacteria have been isolated in our laboratory, one of which appears identical with Poindexter's *A. excentricus* (Fig. 1). Cells of the other strain each produce two pseudostalks from their lateral surfaces, one on the opposite side of the cell from the other (Fig. 2). This strain is tentatively considered to be a member of the genus, *Asticcacaulis*, although it differs widely from *A. excentricus* in nutrient requirements. In this report, the strain with cells producing two pseudostalks is referred to as strain C-19.

The present study was undertaken to determine the fine structure of the pseudostalks produced by these two organisms and the relationship of the pseudostalks to the main body of the cells. It was desired to compare the fine structure of the two organisms with that of the caulobacters as described by Poindexter and Cohen-Bazire (24). It was hoped also that a study of the fine structure of the pseudostalks would give some clues concerning the function of these appendages.

MATERIALS AND METHODS

Both species of *Asticcacaulis* were isolated from fresh water (Pate and Ordal, in preparation). The medium used for growing the cells contained 0.02 per cent beef extract, 0.05 per cent Bacto tryptone, 0.05 per cent yeast extract, and 0.02 per cent sodium acetate in tap water. The pH was adjusted to 6.8 before autoclaving. When a solid medium was desired, agar was added to a concentration of 1.5 per cent. Cultures were incubated at 30°C.

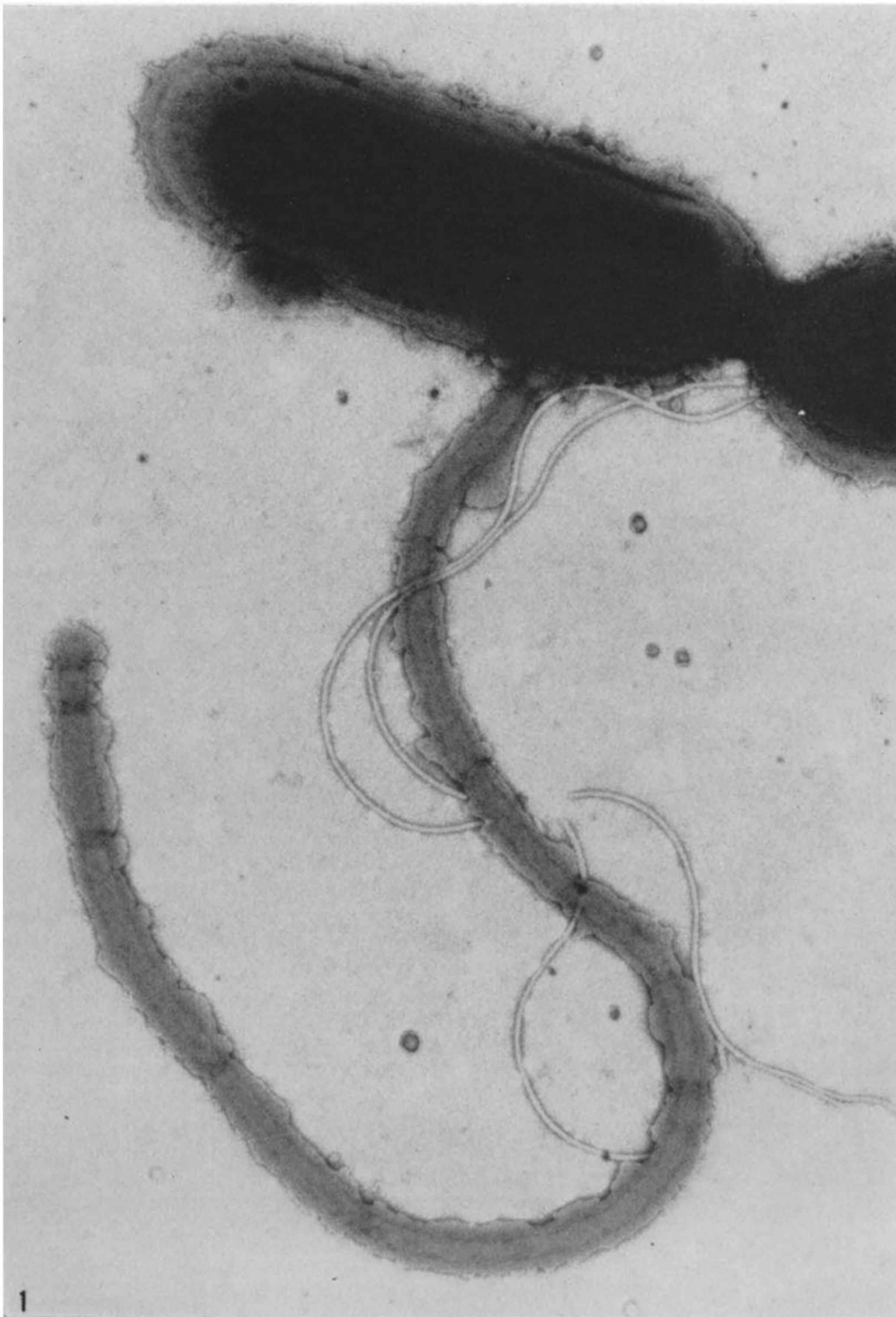
Electron Microscopy

PHOSPHOTUNGSTIC ACID (PTA) PREPARATIONS: A 2 per cent solution of phosphotungstic acid in distilled water was adjusted to pH 7 with NaOH. Slightly turbid bacterial suspensions were mixed with the PTA solution in a 1:1 ratio. The material was placed on carbon-coated grids by dipping a wire loop, slightly larger in diameter than the grids, into the suspension, withdrawing the loop, and placing it over a grid on filter paper.

SHADOW CAST PREPARATIONS: Bacteria harvested from liquid cultures or from the surface of an agar plate were placed in a small amount of distilled water to give a slightly turbid suspension. Electron microscope grids with parlodion support films were dipped into these suspensions, and the excess fluid was removed with filter paper. The grids were then placed in a Mikros vacuum evaporator model VE-10, and palladium wire or carbon and platinum pellets were evaporated over their surfaces.

SECTIONED MATERIAL: Cells were harvested from liquid or solid media, then fixed and dehydrated according to the method of Ryter and Kellenberger (27). The embedding material used was Epon 812 (14). The sections were cut on an LKB ultratome using glass or diamond knives, picked up on grids with carbon support films, and stained for 2 to 3 hours with 2 per cent uranyl acetate in distilled water or for 15 minutes with 2 per cent uranyl acetate followed by a 10 minute treatment with lead citrate (25). All of the electron micrographs were made using RCA EMU 3G microscopes.

FIGURE 1 PTA preparation of *A. excentricus* cells. A flagellated cell is attached to a cell with a pseudostalk. The attachment is at the poles of the cells and not at the tips of the pseudostalks, as in *Caulobacter*. The flagella emerge from the cell at an excentral position rather than from one pole as in *Caulobacter*. × 70,000.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RESULTS

Shadow Cast Preparations

The surface of the pseudostalks of both organisms appears identical with the surface of the bacterial cells. The three-dimensional appearance of the pseudostalks (Fig. 2) is that of a long slender casing covering a cylinder of slightly smaller diameter. The casing appears to have collapsed against the cylinder so that the cylinder is outlined under the casing. At irregular intervals along the length of the pseudostalks, the smooth cylinders are interrupted by areas about 20 mμ wide which are raised slightly above the surface and which traverse the diameter of the pseudostalks. These slightly raised areas correspond to the structures described as "querbalken" or "bulkheads" in the *Caulobacter* stalk (9, 23). The pseudostalk, then, gives the appearance of a smooth cylinder over which several rings had been slipped and left at irregular intervals, and which was then covered with a thin casing.

Since a cell of the *A. excentricus* strain having both a flagellum and a pseudostalk has not been observed, it has not been determined whether the pseudostalk is formed at the exact site of flagellar insertion, which is the case with the stalks of *Caulobacter* (23). This may occur since both structures project from excentral positions on the surface of the cell (Figs. 1 and 3). But there is clearly no connection between the site of flagellar insertion and the sites of pseudostalk formation in the case of strain C-19, where the two pseudostalks are located as much as half a cell length away from the single polar flagellum. A flagellated cell is shown in Fig. 4.

Both *A. excentricus* and strain C-19 produce the holdfast substance at the flagellated poles as does *Caulobacter*. The cells of both strains form rosettes by attaching to one another at the poles of the cells. Electron micrographs of rosettes (Fig. 5) show that the cell walls of the attached bacteria are not in close contact at the point of attachment; the rosettes are apparently formed by the attachment of the adhesive substance of one bacterium to the adhesive substance of other bacteria and not to their cell walls. The adhesive substance is sometimes seen at one pole of the cell (Fig. 2). This substance appears to be structureless and is probably a secretion of the cell. A capsular material surrounds the cell (Fig. 2). Measurements obtained from electron micrographs of whole shadowed cells of both organisms gave an average length of 1.0 μ for the main body of the cell. The average diameter of the cells is 0.5 μ. The diameter of the pseudostalks of both strains is approximately 0.09 μ.

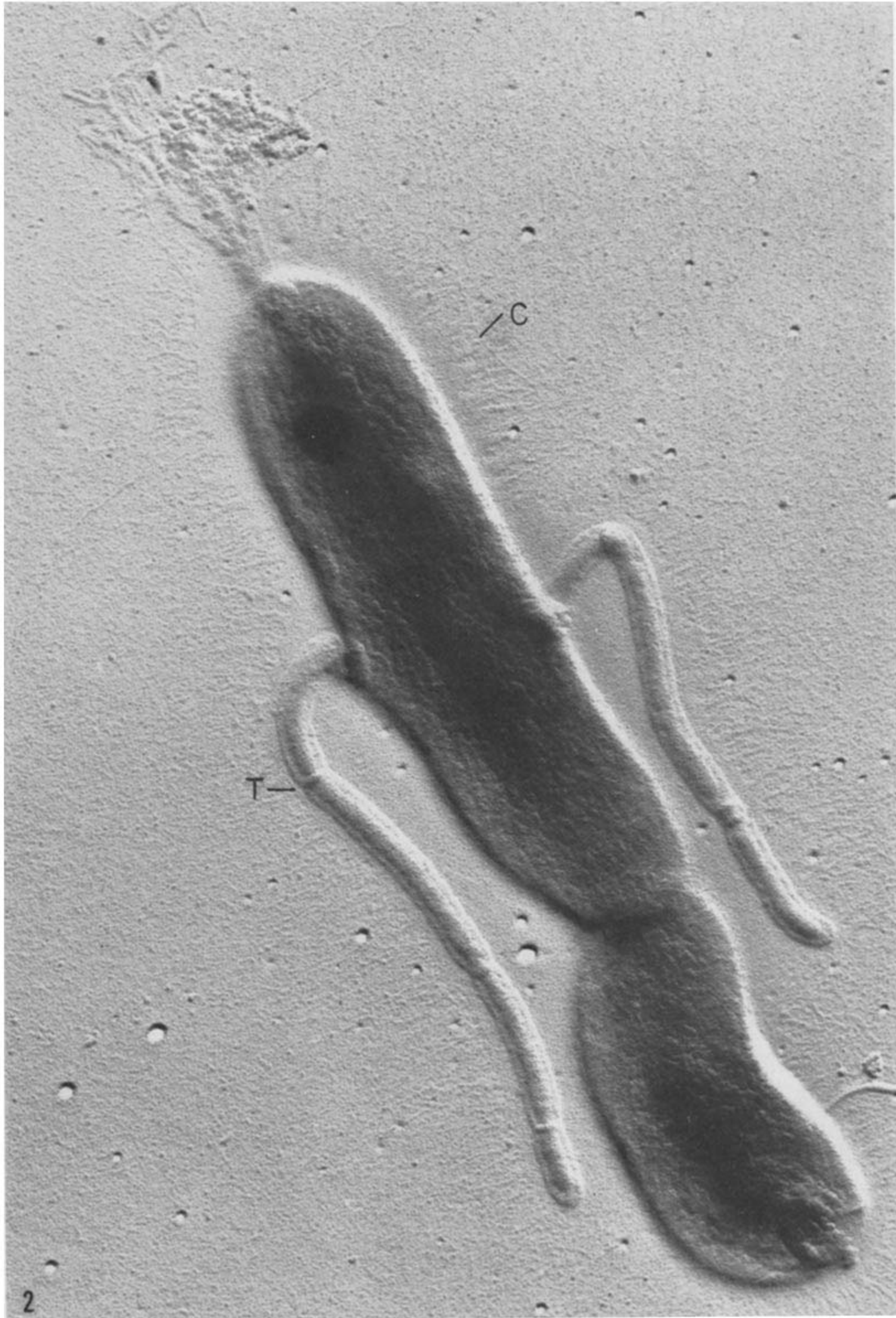
Negative Staining

The average diameter of pseudostalks which have been treated with PTA is 0.085 μ. The central core of negatively stained pseudostalks appears as a light region which extends the full length of the pseudostalk, interrupted at irregular intervals by the transverse thickenings (Fig. 6). The core narrows and then widens at the regions where it comes in contact with the thickenings, giving the appearance of attachment of the core to the bulkheads.

Sectioned Material

CAPSULAR MATERIAL AND PERIPHERAL MEMBRANES: In most cases, the peripheral membranes of cells of both strains appear to consist of two structures. The outermost structure is a unit membrane made up of two dense layers separated by a layer of less density. The two dense layers are about 30 A thick and the less dense layer is about 25 A thick. Fig. 7 shows an example of this structure in strain C-19. The outermost structure has an irregular wavy outline and is not closely associated with the inner structure of the cell wall–plasma membrane complex. The profile of the inner structure is smooth and has a thickness of approximately 140 A. In a few cases, electron micrographs were obtained showing a separation of the inner structure into one dense layer approximately 40 A thick plus an inner

FIGURE 2 C-19 cells shadowed with carbon and platinum. The holdfast material can be seen at one pole, and there is a suggestion of capsular material (*C*) surrounding the cell. A cell possessing pseudostalks is shown dividing to give rise to a cell with no pseudostalks. Notice the transverse thickenings of the pseudostalks (*T*). × 70,000.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membrane identical in appearance to the outer unit membrane. This is shown for a cell of the C-19 strain in Fig. 8. Thus, the cell envelope of these bacteria appears to be composed of two unit membranes separated by a single dense layer which is contiguous with the inner unit membrane. None of the sections showed any invaginations of the inner membrane into the cytoplasm, nor was there any evidence of an intracytoplasmic membrane system.

POLAR STRUCTURE: Fig. 9 shows a structure (*PS*) at one pole of a cell of strain C-19 which appears to be two unit membranes separated by a distance of approximately 130 A and linked together by many tiny cross-bars. The whole structure extends across the width of the cell at the pole and presents a convex surface to the interior of the cell. This structure may be analogous to the "polar membrane" of *Spirillum serpens* (22) and the "polar cap" of *Rhodospirillum rubrum* (6).

ADHESIVE SUBSTANCE: Sections taken through the adhesive substance showed, as did the shadow cast preparations, that this material is structureless. Fig. 10 shows a relatively thick section through two attached cells of strain C-19. It can be seen that the adhesive substance of one cell is attached to the adhesive substance of the other and not to the cell wall.

CYTOPLASM AND NUCLEOPLASM: The internal organization of these cells is typical of bacterial cytoplasm as shown by previous reports on bacterial fine structure (20, 26). The cytoplasm is filled with granules of ribosomal dimensions intermixed with a less electron-opaque region, which may assume various configurations and which is considered to be the nucleus (15). No

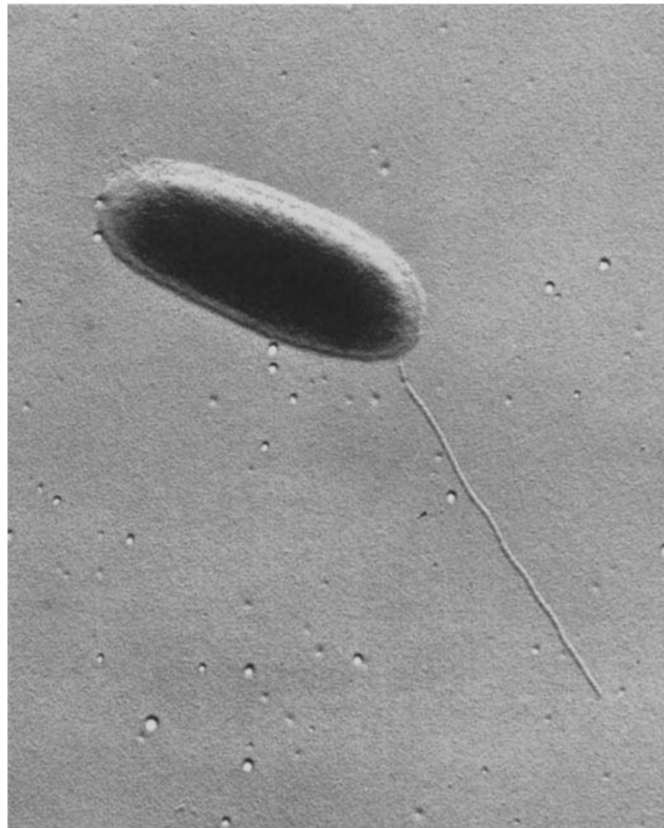


FIGURE 3 *A. excentricus* swarmer cell shadowed with carbon and platinum. Notice excentral location of flagellum. × 33,000.

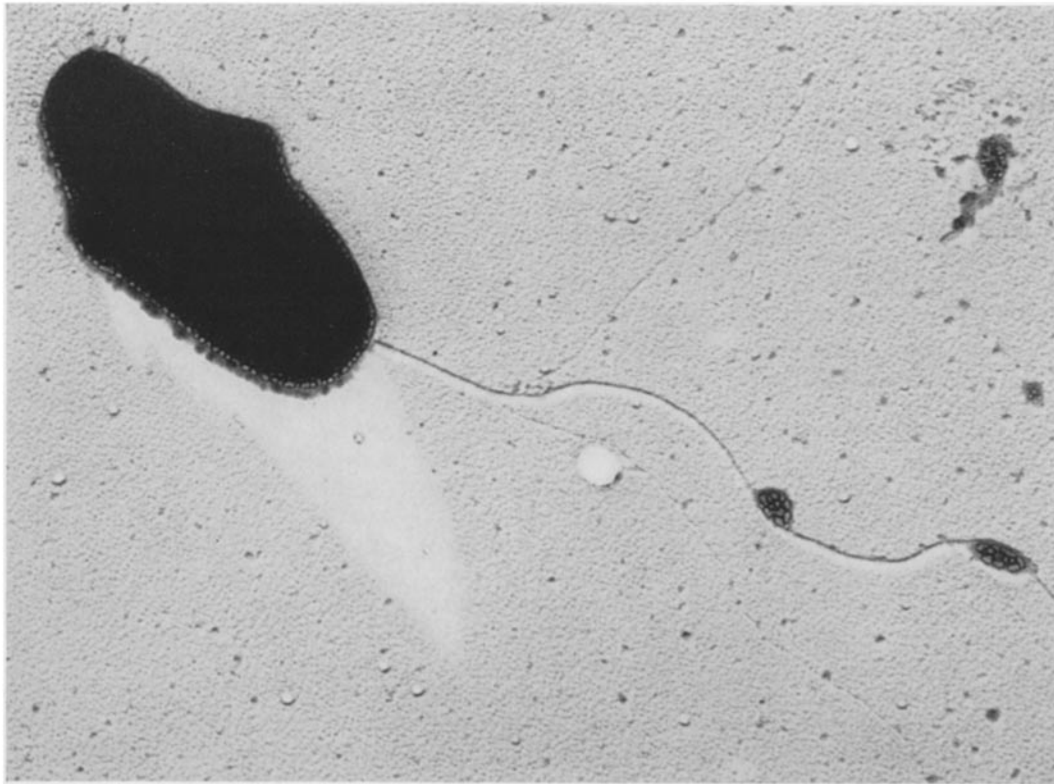


FIGURE 4 C-19 cell shadowed with palladium. A swarmer cell has just started to produce the pseudostalks. The flagellum is polar while the pseudostalks are produced from the lateral surfaces of the cell. × 33,000.

internal organization of the cell has been seen in the cytoplasm directly adjoining the pseudostalks.

THE PSEUDOSTALKS: All of the components of the cell wall–plasma membrane complex continue out into the pseudostalks, and the disposition of these membranes in the pseudostalks is the same as in the main body of the cell: an outer unit membrane separated by a 150 to 200 A gap from the inner structure, which is made up of a single dense layer plus a unit type membrane (Fig. 9). The sectioned pseudostalks measure 0.06 to 0.08 μ in diameter. This is slightly smaller than they appear in PTA-treated and shadowed preparations. The cytoplasm of the main body of the cell is also seen to continue out into the pseudostalks. None of the ribosomal granules which are so prominent in the cytoplasm of the cell are ever seen in the cytoplasm of the pseudostalks, although there appears to be no physical barrier to prevent their entering this structure. Poindexter (23) has found a membranous organelle separating the ribosomal region of the cell from the core of the stalk in the organisms she studied. No such organelle was found in our two strains.

In most cases, the bulkheads appear to extend completely across the pseudostalks and to be attached to the inner layer of the outer unit membrane (Fig. 11). The thickness of the bulkheads varies from 40 to 200 A. The different appearances of the bulkheads might be the result of sectioning during different stages in the formation of these structures. None of the sections through the pseudostalks have demonstrated a unit membrane structure for the bulkheads, although sections through the median plane of pseudostalks have been obtained which show the structure of the outer unit membrane clearly (Fig. 11). The bulkheads are made up of a more densely staining substance than the peripheral membranes.

DISCUSSION

Peripheral Membranes

Since the intermediate layer of the Gram-negative cell wall has not been shown to be contiguous with the inner unit membrane in any other species examined by other workers, it was questioned whether the methods used in preparing the cells of our two strains of *Asticcacaulis* might have been responsible for this appearance. To test this, cells of the C-19 strain were harvested and mixed with *Escherichia coli* cells which had been grown under identical conditions. These two strains were fixed, dehydrated, and embedded in the same block. When sections were cut from this block and examined in the electron microscope, the cell envelope of strain C-19 appeared the same as before; the intermediate layer was contiguous with the inner unit membrane. The envelope of *E. coli* was also shown to be made up of two structures. The inner structure immediately surrounding the cytoplasm is a typical unit membrane. The outer structure has five layers; *i.e.*, three dense layers separated by two less dense layers (Fig. 12). Another Gram-negative strain, *Chondrococcus columnaris*, was grown and prepared for sectioning using the same conditions, and the results were different from those obtained with either *Asticcacaulis* or *E. coli*. In this case, the envelope is composed of two unit membranes and an intermediate dense layer. The dense layer is more closely associated with the inner membrane than with the outer, but is not contiguous with it (Fig. 13). Both unit membranes are wavy and irregular, and the dense layer is smooth. The inner membrane makes contact with the dense layer at various points, but the outer membrane appears to be completely separated from it.

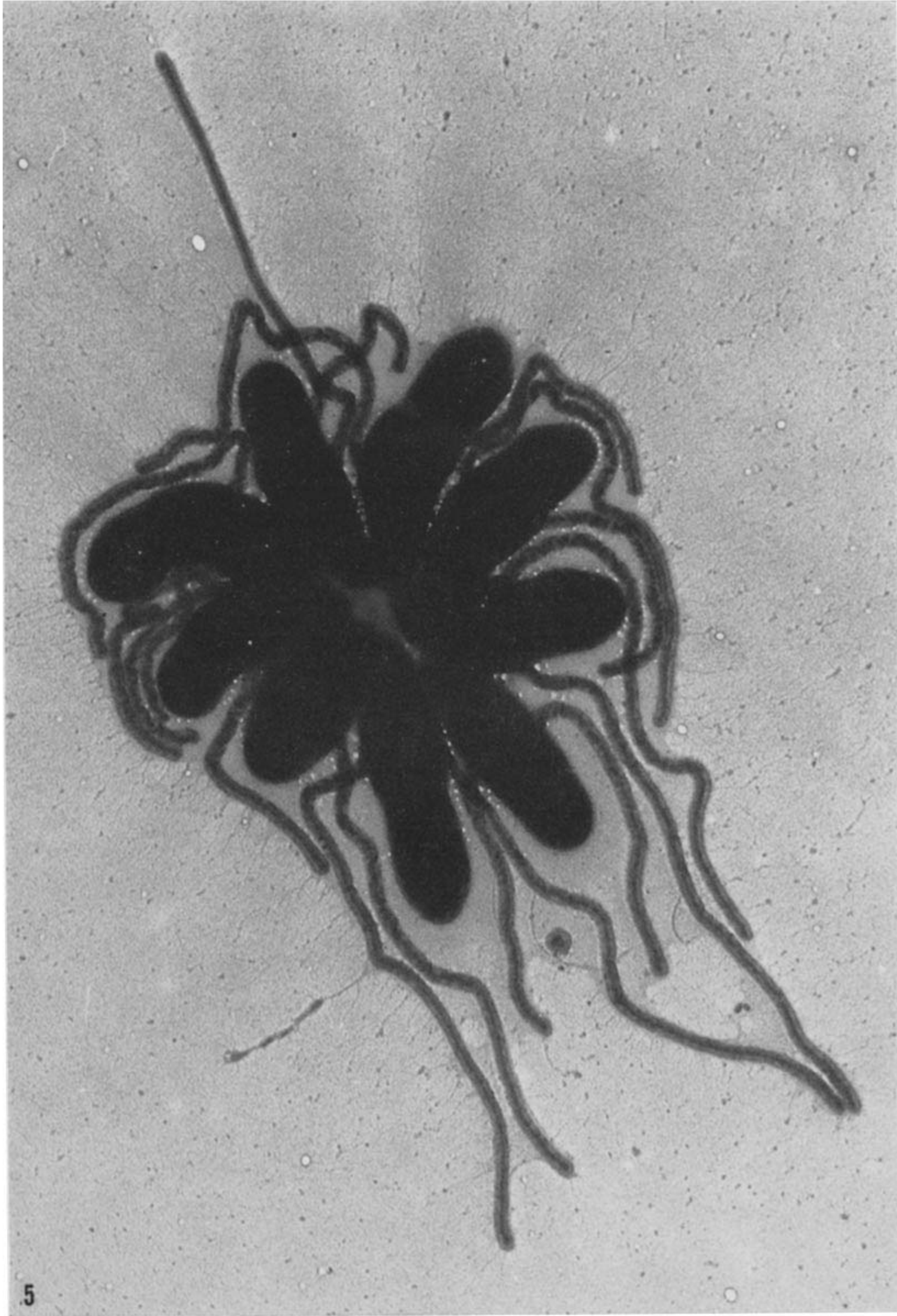
Other investigators working with Gram-negative bacteria have reported the existence of the same three components of the peripheral membranes: two unit membranes and a dense intermediate layer (21, 6, 23). The cell envelopes of some other Gram-negative organisms have been shown to be made up of two unit membranes separated by a space of varying dimensions which is filled with a homogeneous material of moderate density (1, 4, 5). Lautrop *et al.* (13) studied the cell envelopes of 27 species of Gram-negative bacteria. They found that in most cases the space between the two unit membranes contained a moderately electron-opaque zone contiguous to the outer membrane or a well defined line of varying width which was contiguous to neither membrane. Thus, the structure of the cell envelopes of the two species of *Asticcacaulis* appears to conform to the general pattern which is emerging for Gram-negative bacteria. The differences between strains seem to be chiefly in the amount and disposition of the intermediate layer present.

The rigid mucopeptide layer of Gram-negative cell walls can be removed by treatment with lysozyme and Versene. When cells which have had the rigid layer removed are sectioned and examined in the electron microscope, no dense intermediate layer is seen; both unit membranes are still present, but the outer unit membrane is often in various stages of disruption (2, 8). Thus, the intermediate layer seen in Gram-negative bacteria appears to be identical with the mucopeptide structural layer identified by chemical analysis (29).

The close association of the rigid mucopeptide layer with the inner unit membrane, as seen in the cell envelopes of *A. excentricus* and C-19, is probably not due to the effects of the fixation procedure. The appearance of pseudostalks in preparations of whole, unfixed cells in shadow cast preparations is one of a flexible outer membrane collapsed against a rigid inner structure. This appearance is in agreement with the arrangement seen in sectioned material with the rigid intermediate layer adherent to the inner unit membrane (Fig. 9).

STRUCTURE OF THE PSEUDOSTALKS: Since the central core of the pseudostalks appears light in electron micrographs of PTA preparations

FIGURE 5 Rosette formed by cells of C-19 attaching at the poles of the cells. None of the pseudostalks are involved in the attachment. The secreted adhesive material of one cell is attached to the secreted adhesive material of other cells and not to the walls of other cells. Attachment of one *Asticcacaulis* cell to the wall of another *Asticcacaulis* cell has not been seen. Shadowed with palladium. × 26,000.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(Fig. 6), it can be concluded that there is no cavity in this structure; the core is occupied by a material that is not permeable to the PTA and is, therefore, less dense to the electron beam than is the surrounding area. The PTA appears to have penetrated to the area between the central core and the wall of the pseudostalks in these preparations since the walls appear darker than the core (Fig. 14). The wall of the pseudostalks appears constricted at the sites of the transverse thickenings, but comparison of these areas with shadow cast preparations and sectioned pseudostalks indicates that this constricted appearance is due to the method of preparation. Upon drying, the outer membrane of the pseudostalk collapses against the cytoplasmic cylinder, but the bulkheads support the outer membrane and prevent its collapse. The regions where the membrane has collapsed are wider than where it is supported, and this effect gives the appearance of constrictions when seen in PTA preparations. When seen from the surface (shadowed preparations), the same regions appear raised slightly above the surface of the rest of the pseudostalk. This explanation also accounts for the wider appearance of the pseudostalks in shadowed and PTA preparations than in sections.

It has not been possible to determine the structure of the bulkheads from PTA preparations of the pseudostalks. The cytoplasmic core is seen to widen on both sides of the bulkheads just before coming into contact with this structure, but whether the core actually passes through the bulkhead or comes to an end at this point is not revealed.

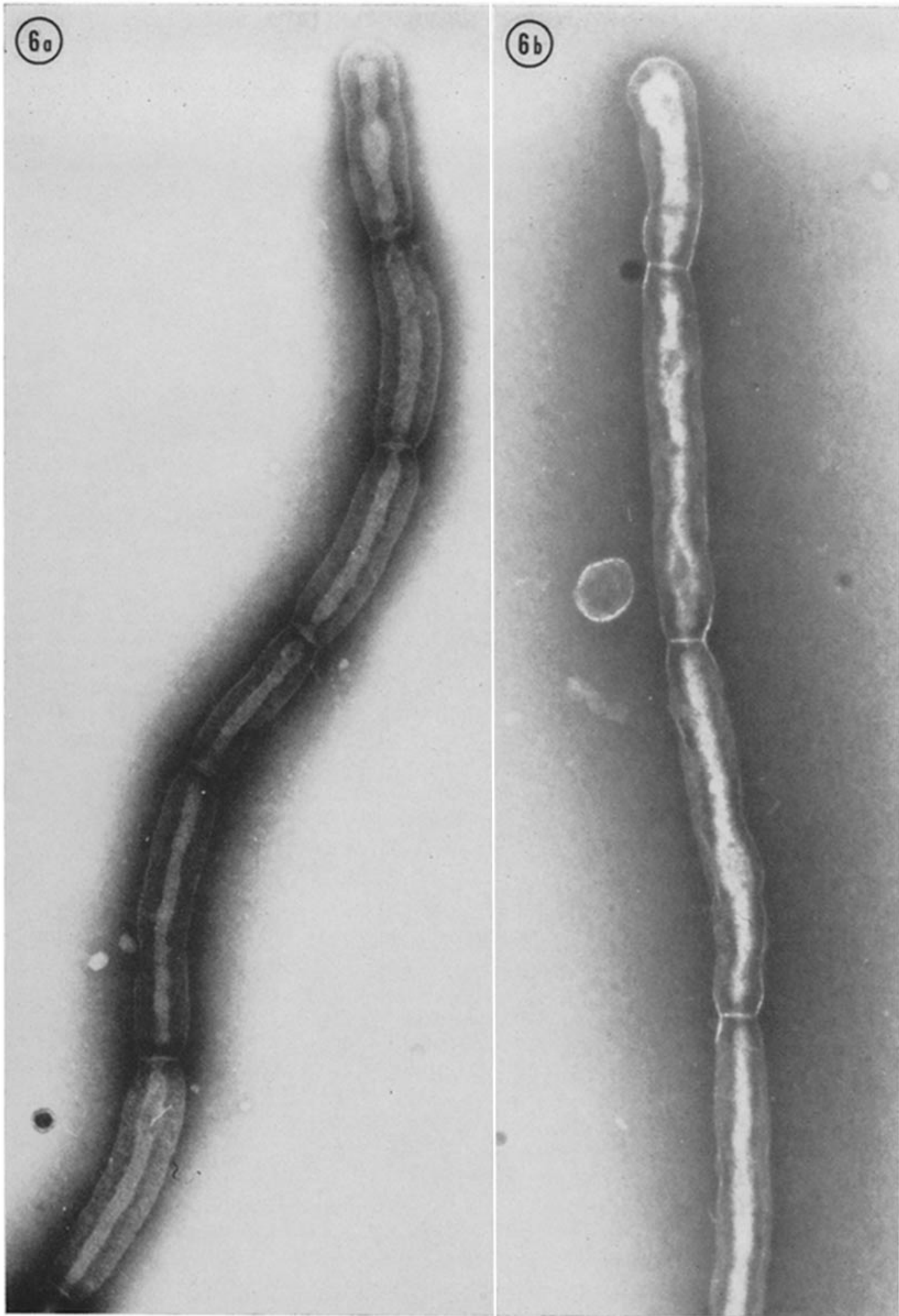
Several electron micrographs of sections through the median plane of the pseudostalks were obtained (Fig. 11), and in none of these is there clear evidence for a central pore in the bulkhead. The bulkheads appear to extend from the outer unit membrane on one side of the pseudostalk to the outer unit membrane on the other side without any openings. Although an opening large enough to permit the core to pass through has not been demonstrated, electron micrographs of sections through the pseudostalks give the appearance of continuity of the core from one side of a bulkhead to the other.

A MODEL OF THE PSEUDOSTALKS: A study of the electron micrographs at first suggests that the areas of transverse thickenings are true bulkheads dividing the pseudostalks into discrete segments. No pore can be seen in the bulkheads in sectioned pseudostalks, and in PTA preparations the core appears to attach to the bulkheads rather than to pass through them. In spite of this appearance, for the reasons set forth in the following discussion it seems likely that these are not true bulkheads but annular structures.

The diameter of the pseudostalks in section is approximately 700 A. The small radius of curvature makes it necessary to obtain median sections of the pseudostalks in order to resolve the unit membrane structure. Since most of the sections are on the order of 400 to 500 A thick, any section through the median plane of the pseudostalk would include the entire half of the pseudostalk. When viewing this kind of section in the electron microscope, it would not be possible to determine whether the density to the electron beam was due to the bulkhead material located in the median plane of the pseudostalk or farther back in the section, and the result would be that any opening in the bulkhead could not be detected.

The structure of the pseudostalk is presented in diagrammatic form in Fig. 15. This model has been constructed from electron micrographs of shadowed, negatively stained, and sectioned pseudostalks. According to this interpretation, the outgrowth of the peripheral membranes of the cell results in the formation of a structure which is a tube within a tube, the innermost tube being an extension of the inner unit membrane and the outermost tube an extension of the outer unit membrane. Between the two membranes and contiguous with the inner membrane is the rigid intermediate layer of the cell wall–plasma membrane complex. The bulkheads are represented as annular structures formed by localized attachments of the rigid intermediate layer to both unit membranes.

FIGURE 6 *a* PTA preparation of a pseudostalk of *A. excentricus*. The core of the pseudostalk appears to be attached to the transverse thickenings. Diameter of the pseudostalk is about 85 mμ. *b* PTA preparation of a pseudostalk of C-19. Fig. 6 *a* $\times$ 100,000; Fig. 6 *b* $\times$ 100,000.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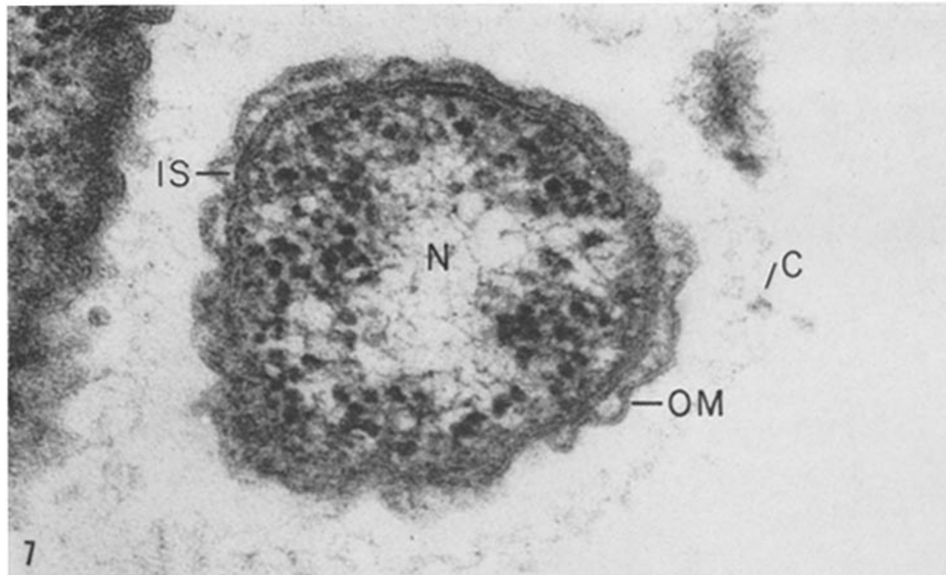


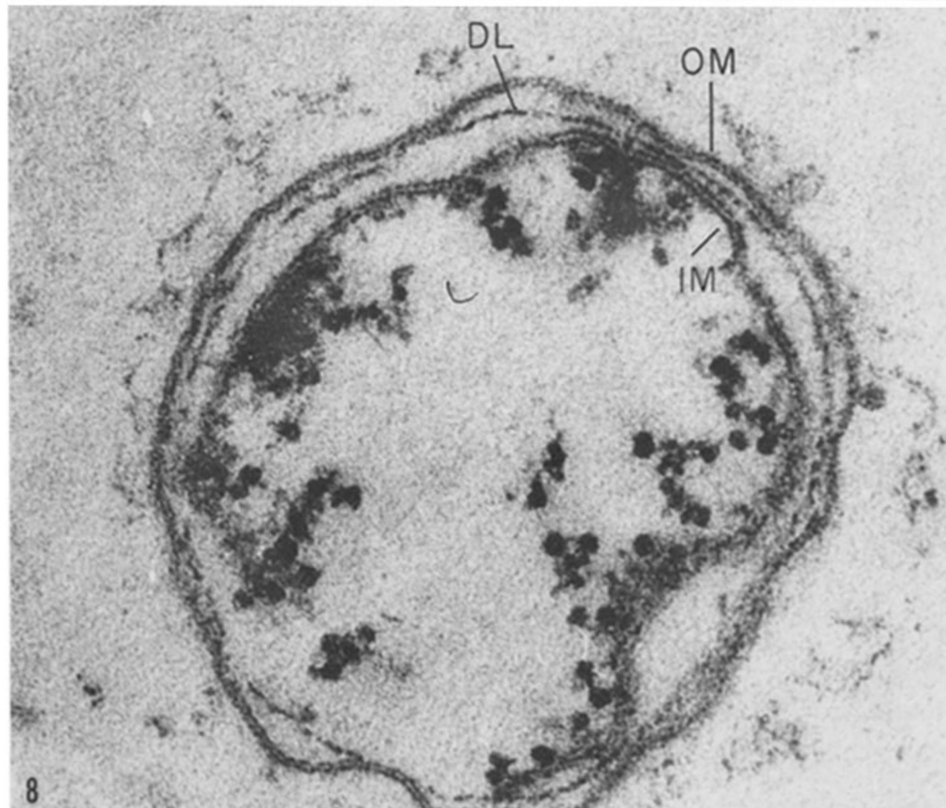
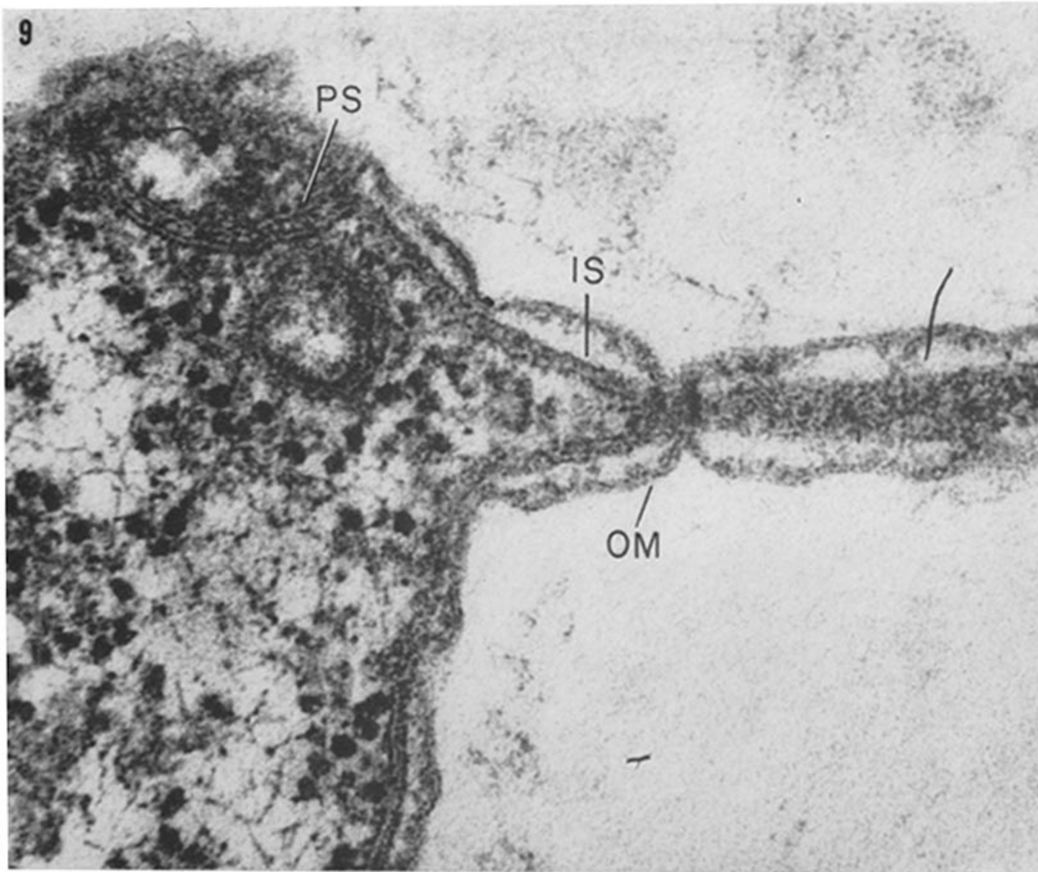


FIGURE 7 Section through a cell of C-19. The cell envelope appears to consist of two structures: an outer unit membrane and an inner structure about 140 A thick. *OM*, outer membrane, approximately 70 A; *IS*, inner structure, approximately 140 A; *C*, capsular material; *N*, nuclear material. × 150,000.

FIGURE 8 Section through a cell of C-19. The 140 A inner structure is separated showing an inner membrane (*IM*) identical in appearance to the outer membrane (*OM*). A dense layer (*DL*), about 40 A thick, is located between the two unit membranes. × 182,000.



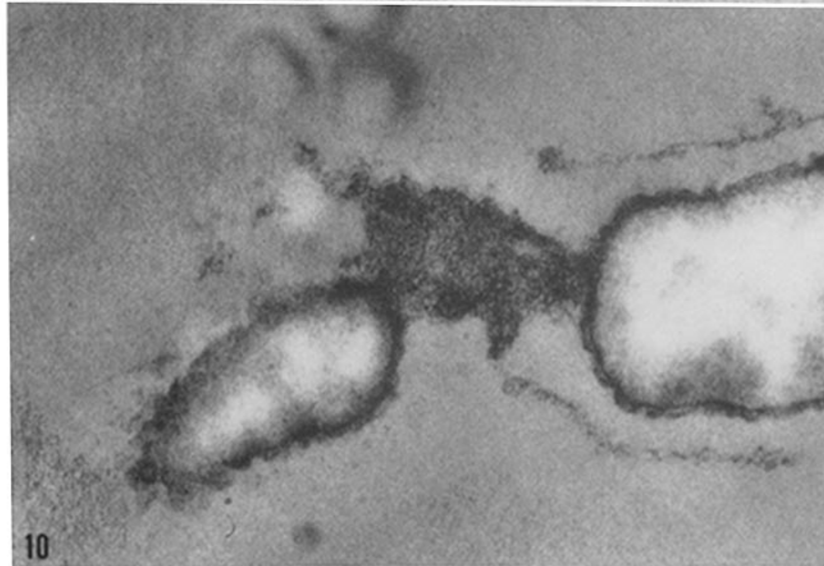


FIGURE 9 Section through cell and pseudostalk of C-19. The components of the cell wall–plasma membrane complex continue out into the pseudostalks. A structure at the pole of the cell (*PS*) has the appearance of two unit membranes linked together by cross-bars. × 182,000.

FIGURE 10 Section through two C-19 cells attached by the holdfast material demonstrating the structureless nature of the holdfasts. × 65,000.

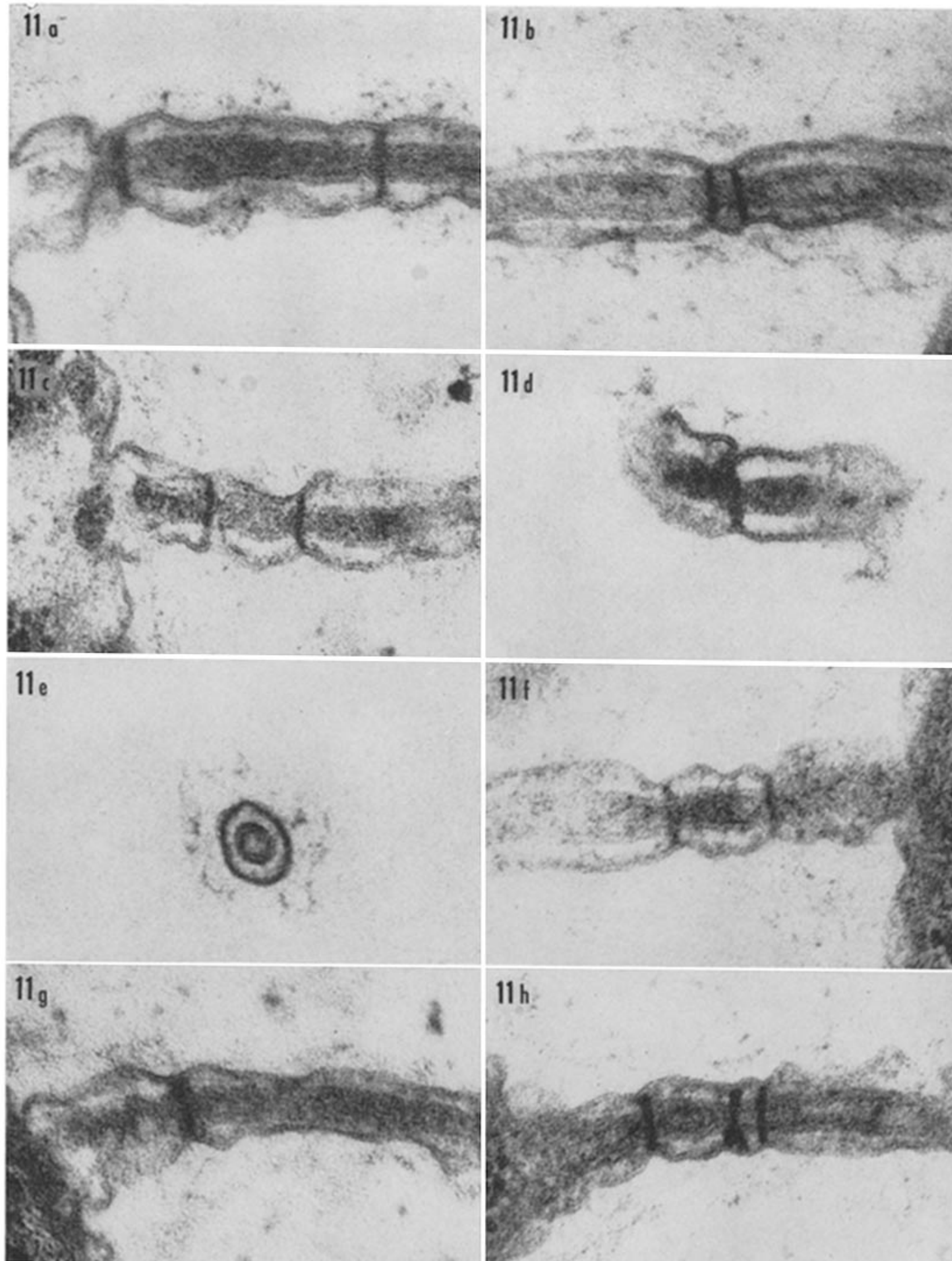
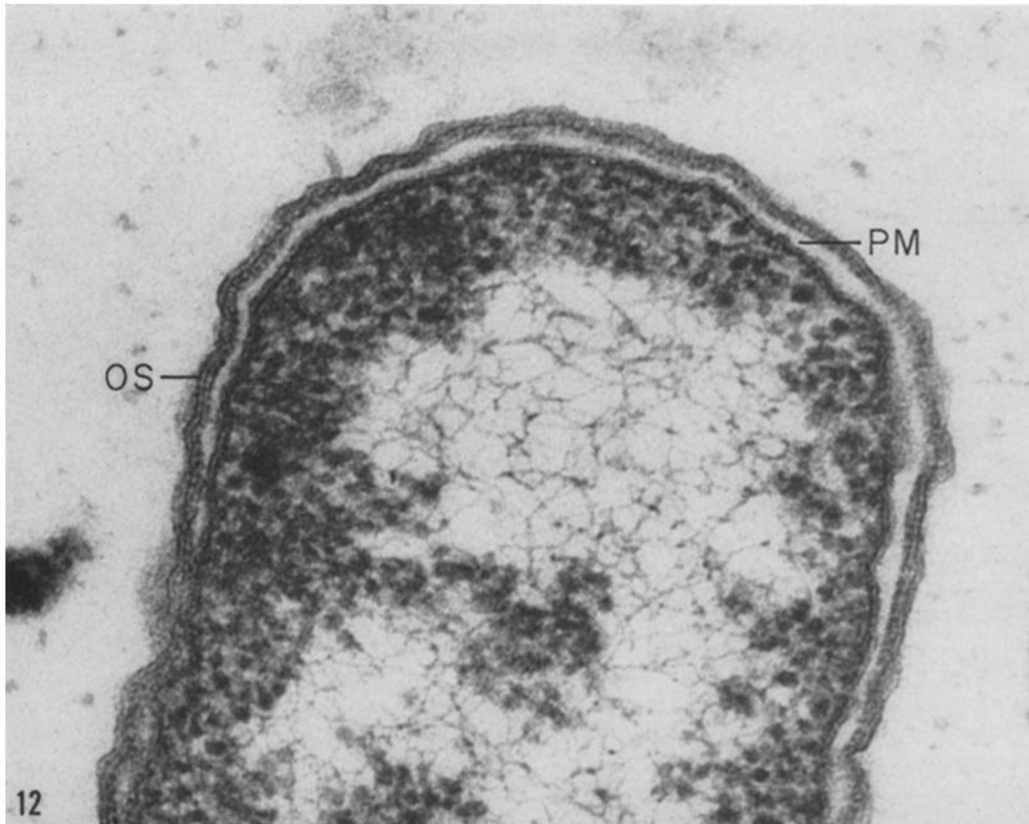


FIGURE 11 Sections through pseudostalks of *A. excentricus* and C-19. The bulkheads appear to extend from the outer unit membrane on one side of the pseudostalks to the outer unit membrane on the other side. No definite pore is shown in the bulkheads, but there is an apparent continuity of the core from one side of the bulkhead to the other. In *g* and *h*, the bulkheads appear thicker than in the other sections. The bulkheads do not give the appearance of membranous structures. *b* and *d* are *A. excentricus*; the others are strain C-19. *e* is a cross-section of a pseudostalk. × 160,000.



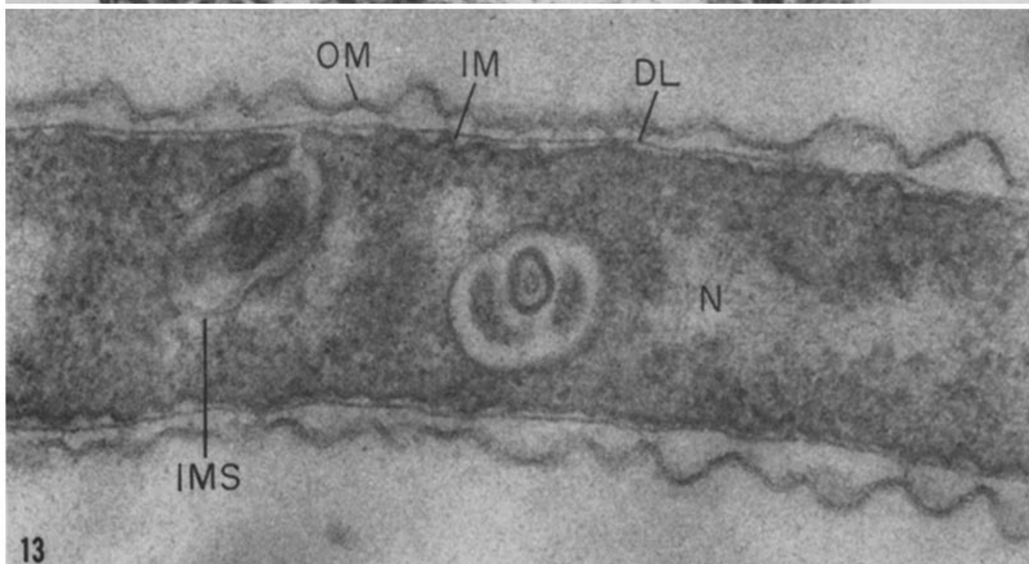


FIGURE 12 Section through *E. coli* cell. *OS*, outer 5-layered structure of the cell envelope; *PM*, plasma membrane. × 100,000.

FIGURE 13 Section through *Chondrococcus columnaris* showing the disposition of the membranes of the cell envelope. *OM*, outer membrane; *IM*, inner membrane; *DL*, dense layer; *IMS*, intracytoplasmic membrane structure; *N*, nuclear material. × 150,000.

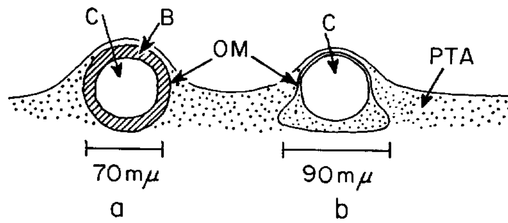


FIGURE 14 Diagram of an end-on view of pseudostalks embedded in PTA. In *b* the wall of the pseudostalk has collapsed against the core, but in *a* it is supported by the bulkhead. When a preparation such as this one is examined in the electron microscope, the areas between the bulkheads appear wider than the areas containing bulkheads. *B*, bulkhead; *C*, core; *OM*, outer membrane.

The identification of the bulkhead material as the mucopeptide layer is not positive, but it seems probable for the following reasons: (*a*) in section, the bulkhead appears not to be a membrane but a homogeneous substance, the thickness of which can vary from 40 to 200 A. This variation in thickness might be due to later accretions of the mucopeptide-containing layer after the bulkhead is initially laid down; (*b*) the mucopeptide layer is responsible for conferring rigidity on the cell envelope. Therefore, bulkheads composed of this material should have the rigid appearance seen in shadowed preparations; and (*c*) an adhesion of the central core to the bulkheads is necessary to explain the appearance of negatively stained preparations of pseudostalks. That the inner unit membrane and the dense intermediate layer usually give the appearance of one structure when seen in section indicates an adhesion of these two structures.

The function of the rigid annular structure is probably to anchor the inner unit membrane to the outer membrane, thus preventing a wide separation of the two membranes of the pseudostalk.

POSSIBLE FUNCTIONS OF THE PSEUDOSTALKS: A consideration of the structural similarities of the stalks of *Caulobacter* and the pseudostalks of *Asticcacaulis* has led us to conclude that these are homologous structures. Just as attachment is not the function of the pseudostalk of *Asticcacaulis*, neither is it the function of the stalk of *Caulobacter*. Attachment is a property of the secreted holdfast and can occur in the absence of the stalk (23). The involvement of the stalk of *Caulobacter* in attachment appears to be due to the production of the stalk at the site of attachment. The real function of the *Caulobacter* stalk has not yet been discovered.

Poindexter (23) has suggested that the advantage gained by the formation of stalks by the caulobacters is one of increased buoyancy. She argues that, since these organisms are strictly aerobic, it would be to their advantage to produce organelles which would help to keep them at the liquid-air interface where the oxygen concentration is maximum. However, it is questionable

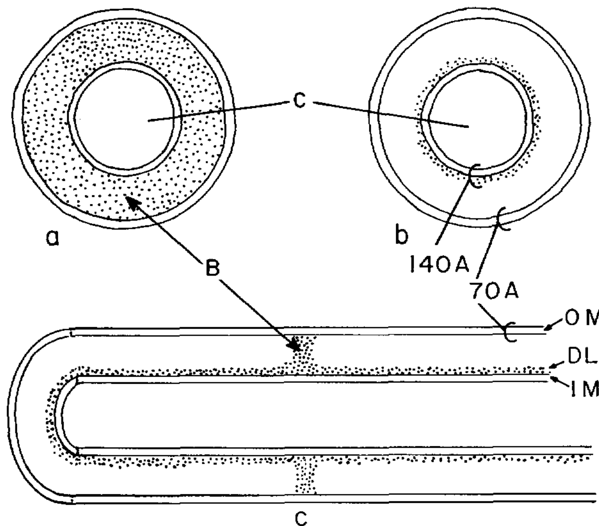


FIGURE 15 Model of pseudostalks. *a* Cross-section through bulkhead. *b* Cross-section through region between bulkheads. *c* Longitudinal section containing bulkhead; *OM*, outer membrane; *IM*, inner membrane; *DL*, dense layer; *D*, core; *B*, bulkhead.

whether increased buoyancy would benefit an organism which spends the stalked portion of its life cycle attached to other microorganisms or to the surface of some submerged structure.

The structural arrangement of the pseudostalks, as revealed by electron microscopy, results in an increase in the surface area of the two unit membranes with a small corresponding increase in cytoplasmic volume. Thus, the function of the pseudostalks is likely to be reflected by the properties of bacterial membrane systems.

The plasma membrane of bacteria is known to be a permeability barrier (12, 19). Although it has not been demonstrated that the outer unit membrane is also a permeability barrier, it very likely is, since similar membranes found in all cellular organisms so far examined appear to control permeability (4, 28). The cytochrome system and enzymes involved in oxidative phosphorylation are localized on particles derived from bacterial plasma membranes (12, 17). The pseudostalks of C-19 have been shown to contain a cytochrome system (unpublished data), and it is probable that any respiratory enzymes localized on the plasma membrane of the cell would also be found on the plasma membrane of the pseudostalks. Catalysts of membrane transport are also situated in the membrane (16, 18). Though several models exist which attempt to explain the mechanism of these transport enzymes, the end result is the same for all of them: a higher concentration of the transported molecules inside the cells than in the environment. The present understanding of these transporting enzymes is that they are located in the surface membranes of bacteria, are substrate specific, and that they control the rate-limiting step of the entry of nutrient molecules from the medium into the metabolic processes of the cell. Whatever the mechanism of the transport enzymes, it is first necessary for the substrate molecules to come into contact with these enzymes. This would be brought about by a diffusion of the molecules in the environment to the sites of enzyme localization on the bacterial membranes. Thus, the first step in active transport is dependent upon the concentration of the substrate. This step would also be dependent upon the amount of enzyme-containing surface area available.

Since the pseudostalks have been shown to have no reproductive function and to be unnecessary for attachment, and from the observations on fine structure, it appears that the formation of these structures accomplishes little more than an extension of the peripheral membranes into the growth medium. From the discussion on transport enzymes, it can be seen that the increased surface area resulting from pseudostalk formation might be an advantage to organisms in an environment of low nutrient concentration. The caulobacters are commonly found in environments where the nutrient concentration is extremely low (23).

The dimorphic life cycle of the caulobacters could be the result of evolutionary processes selecting on the basis of two traits: an increased surface area and motility. In this case, the two traits are expressed at different periods in the development of the organism rather than simultaneously. Motility is manifested immediately upon division and makes dispersal possible, while an increased surface area is not realized until later, after dispersal has been achieved. While this explanation of the *Caulobacter* life cycle is attractive, it has not been proven. It is hoped that further research to determine the function of the stalks and pseudostalks of the caulobacters and the mechanisms controlling the dimorphic life cycle will provide us with the information necessary to determine the selective advantage gained by an organism that produces these cellular extensions.

This investigation was supported in part by Public Health Research Grant, 5-F1-GM-19, 197, from the National Institute of General Medical Sciences, and by Grant No. Nonr-477(43) from the Office of Naval Research (United States).

Received for publication, April 6, 1965.

BIBLIOGRAPHY

1. Beer, M., Disposition of membranes in *Alcaligenes faecalis*, *J. Bacteriol.*, 1960, **80**, 659.
2. Boatman, E. S., Observations on the fine structure of spheroplasts of *Rhodospirillum rubrum*, *J. Cell Biol.*, 1964, **20**, 297.
3. Bowers, L. E., Weaver, R. H., Grula, E. A., and Edwards, O. F., Studies on a strain of *Caulobacter* from water. I. Isolation and identification as *Caulobacter vibrioides* Henrici and Johnson with emended description, *J. Bacteriol.*, 1954, **68**, 194.
4. Brown, A. D., Drummond, D. G., and North,

R. J., The peripheral structures of Gram-negative bacteria. *Biochim. et Biophysica Acta* 1962, **58**, 514.

5. Claus, G. W., and Roth, L. E., Fine structure of the Gram-negative bacterium *Acetobacter suboxydans*, *J. Cell Biol.*, 1964, **20**, 217.
6. Cohen-Bazire, G., and Kunisawa, R., The fine structure of *Rhodospirillum rubrum*, *J. Cell Biol.*, 1963, **16**, 401.
7. Henrici, A. T., and Johnson, D. E., Studies of freshwater bacteria, *J. Bacteriol.*, 1935, **30**, 61.
8. Hofschneider, P. H., Zur Wandstruktur von *Escherichia coli* Sphaeroplasten, European Regional Conference on Electron Microscopy, Delft, 1960, 1028.
9. Houwink, A. L., *Caulobacter* versus *Bacillus* species, *Nature*, 1951, **168**, 654.
10. Houwink, A. L., *Caulobacter*, its morphogenesis, taxonomy and parasitism, *Antonie van Leeuwenhoek, J. Microbiol. and Serol.*, 1955, **21**, 49.
11. Houwink, A. L., and van Iterson, W., Electron microscopical observations on bacterial cytology. II. A study on flagellation, *Biochim. et Biophysica Acta*, 1950, **5**, 10.
12. Hughes, D. E., The bacterial cytoplasmic membrane, *J. Gen. Microbiol.*, 1962, **29**, 39.
13. Lautrop, H., Reyn, A., and Birch-Andersen, A., A comparative electron microscope study of the cell walls of Gram-negative bacteria, *in* Proceedings of the 14th Scandinavian Congress of Pathology and Microbiology, Parts I and II, 1964, 157.
14. Luft, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
15. Kellenberger, E., The physical state of the bacterial nucleus, *Symp. Soc. Gen. Microbiol.*, 1960, **10**, 39.
16. Kepes, A., and Cohen, G. N., Permeation, *in* The Bacteria, (I. C. Gunsalus and R. Y. Stanier, editors), New York, Academic Press, Inc., 1962, 4, 179.
17. Marr, A. G., Localization of enzymes in bacteria, *in* The Bacteria, (I. C. Gunsalus and R. Y. Stanier, editors), New York, Academic Press, Inc., 1960, **1**, 443.
18. Mitchell, P., Structure and function in microorganisms, *Biochem. Soc. Symp.*, Cambridge, England, 1958, **16**, 73.
19. Mitchell, P., and Moyle, J., Osmotic function and structure in bacteria, *Symp. Soc. Gen. Microbiol.*, 1956, **6**, 150.
20. Murray, R. G. E., Fine structure and taxonomy of bacteria, *Symp. Soc. Gen. Microbiol.*, 1962, **12**, 119.
21. Murray, R. G. E., On the cell wall structure of *Spirillum serpens*, *Canad. J. Microbiol.*, 1963, **9**, 381.
22. Murray, R. G. E., and Birch-Andersen, A., Specialized structure in the region of the flagella tuft in *Spirillum serpens*, *Canad. J. Microbiol.*, 1963, **9**, 393.
23. Poindexter, J. S., Biological properties and classification of the *Caulobacter* group, *Bact. Rev.*, 1964, **28**, 231.
24. Poindexter, J. S., and Cohen-Bazire, G., The fine structure of stalked bacteria belonging to the family Caulobacteraceae, *J. Cell Biol.*, 1964, **23**, 587.
25. Reynolds, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.
26. Robinow, C. F., Outline of the visible organization of bacteria, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York and London, 1960, **4**, 45.
27. Ryter, A., and Kellenberger, E., L'inclusion au polyester pour l'ultramicrotomie, *J. Ultrastruct. Research*, 1958, **2**, 200.
28. Stoeckenius, W., The molecular structure of lipid-water systems and cell membrane models studied with the electron microscope, *Symp. Internat. Soc. Cell Biol.*, 1962, **1**, 349.
29. Work, E., Biochemistry of the bacterial cell wall, *Nature*, 1960, **170**, 841.
30. Zavarzin, G. A., Budding bacteria, *Microbiologia*, 1961, **30**, 952.