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Basal Structure and Attachment of Flagella in Cells of *Proteus vulgaris*

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ABSTRACTS

ABRAM, DINAH (Purdue University, Lafayette, Ind.), HENRY KOFFLER, AND A. E. VATTER. Basal structure and attachment of flagella in cells of *Proteus vulgaris*. J. Bacteriol. **90**:1337–1354. 1965.—The attachment of flagella to cells of *Proteus vulgaris* was studied electron microscopically with negatively stained and shadow-cast preparations of ghosts from standard cultures and from special cultures that produced "long forms." The flagellum, the basal portion of which is hooked, arises within the cell from a nearly spherical structure, 110 to 140 A in diameter. This structure appears to be associated with the cytoplasmic membrane; it may be a part of the membrane or a separate entity that lies just beneath the membrane. Flagella associated with cell walls free from cytoplasmic membrane frequently have larger bodies, 200 to 700 A in diameter, associated with their base. These structures probably consist at least partly of fragments of the cytoplasmic membrane, a portion of which folds around a smaller structure. Flagella in various stages of development were observed in long forms of *P. vulgaris* cells grown at low temperature. The basal structure of these flagella was similar to that of the long or "mature" flagella. Strands connecting the basal structures were observed in ghosts of long forms; these strands appear to be derived from the cytoplasmic membrane. Flagella were found to be attached to fragments of cell wall and to cytoplasmic membrane in a similar manner as they are attached to ghosts. In isolates of flagella that have been separated from the cells mechanically, the organelles often terminate in hooks which almost always appear naked, but have a different fine structure than the flagellum proper.

Bacterial flagella, the organelles of locomotion, are helical filaments 120 to 190 A in diameter and several microns long. [See Stocker (1956) and Weibull (1960) for reviews.] With the exception of spirochetal flagella, these organelles lie outside the cells and are attached to them by one end. The mode of attachment has been investigated by many authors but still is not understood (Houwink and van Iterson, 1950; van Iterson, 1953; Grace, 1954; Pease, 1956; Tawara, 1957, 1964; Thornley and Horne, 1962; Glauert, Kerridge, and Horne, 1963; Houwink, 1963). In preparations in which flagella are still attached to ghosts of autolyzed cells, there are indications that they pierce the cell wall (van Iterson, 1949, 1953; Johnson, Zworykin, and Warren, 1943; Salton and Horne, 1951). Strong evidence for their protoplasmic origin is that after the cell wall of *Bacillus megaterium* is removed with lysozyme the flagella remain attached to the naked protoplast (Weibull, 1953*a*; Wiame, Storck, and Vanderwinkel, 1955). Also, a definite cytoplasmic organization was observed in the polar regions of *Spirillum serpens*, where tufts of flagella originate (Murray and Birch-Andersen, 1963). However, unlike flagella of eucaryotic organisms, bacterial flagella have not been shown to be surrounded by an extension of the cytoplasmic membrane. Hooklike bends have been observed at one end of flagella detached mechanically from cells of various bacterial species (Houwink and van Iterson, 1950; Houwink, 1953; van Iterson, 1953; Kerridge, Horne, and Glauert, 1962; Glauert et al., 1963; Rogers and Filshi, 1963). It has been suggested that the flagella arise from within the cells and the hooks serve as means of attachment (Houwink and van Iterson, 1950). These hooks were shown by Houwink (1953) at the basal end of each flagellum of a polar bundle attached to a cell of a spirillum; they appear to enter the cell. Several electron micrographs of shadow-cast specimens prepared from autolyzed cells show granules located within the cells (Houwink and van Iterson, 1950; van Iterson, 1953; Grace, 1954; Pease, 1956; Tawara, 1957, 1964; Houwink, 1963), suggesting that there are basal bodies at the cellular terminus of the flagella, perhaps analogous to blepharoplasts in higher flagellates.

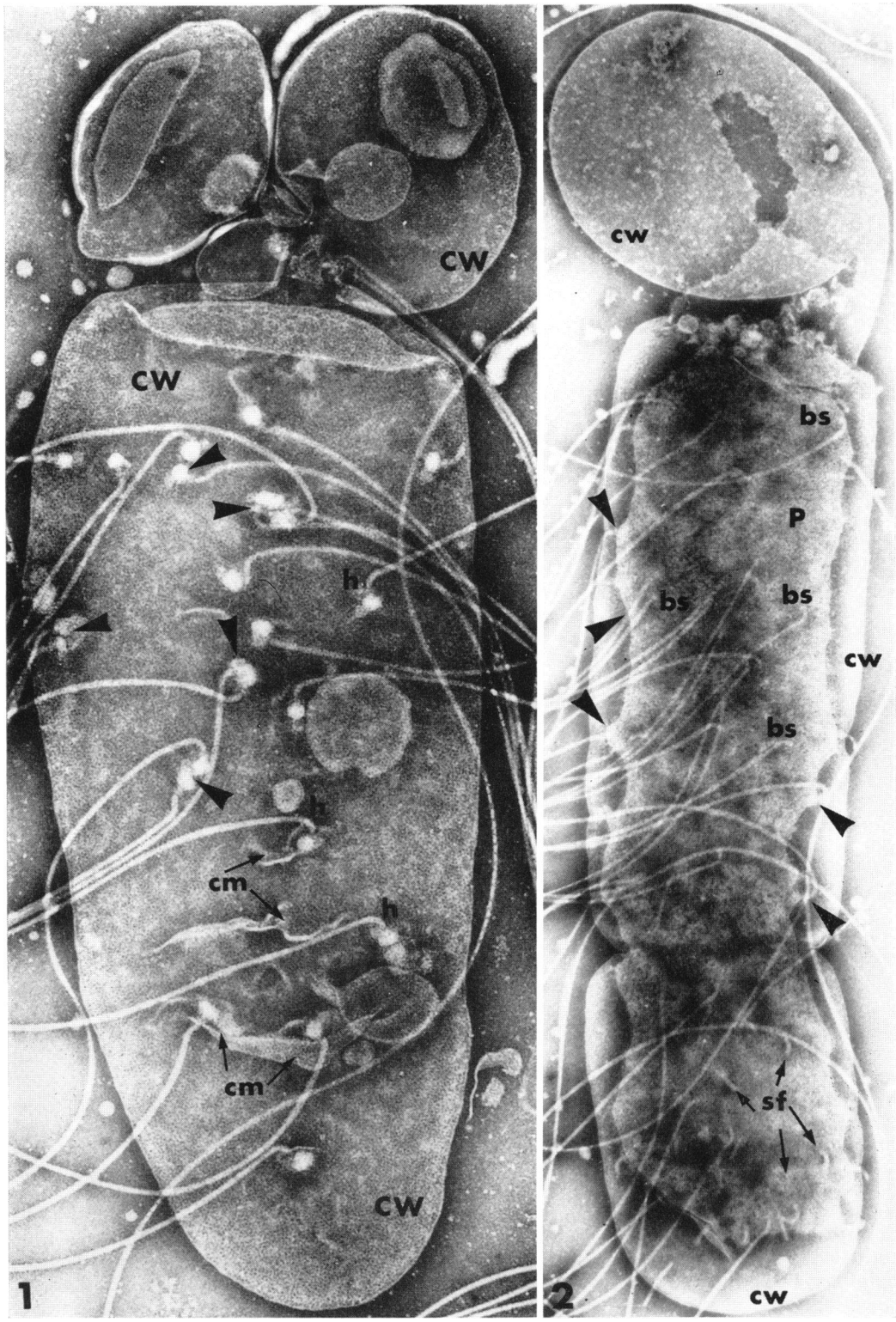

FIG. 1–2

However, only a few micrographs clearly show that the hooked ends of flagella are attached to these basal bodies. The large size of these structures, up to 0.1 to 0.2 μ in diameter, and the fact that they have been observed only in autolyzed cells, raised the question (Pijper, 1957) as to whether they are artifacts produced by autolysis. Moreover, the size of these bodies has been observed to vary within different cells of the same population [*S. undula* (Pease, 1956)]. Flagella removed from cells of *B. megaterium* by lysis with lysozyme (Weibull, 1953*b*) do not have such structures at their bases. Recently, Glauert et al. (1963) demonstrated by means of negative staining that in autolyzed cells of *Vibrio metchnikovii* the polar flagellum is attached to a basal structure by a terminal hook. Unlike the structure previously reported, this body appears to be a disc or plate, 300 to 350 A in diameter. Its location was shown to be near or in association with the cytoplasmic membrane, which had retracted from the cell wall upon autolysis. Occasionally, a structure similar to the basal disc is observed attached to fragments of flagella. Electron micrographs of thin sections suggest that the core of this flagellum is attached to the cytoplasmic membrane, or is located just within it, whereas the sheath is a continuation of the cell wall.

Because the data reported so far are not sufficient to permit either a conclusion or a generalization concerning the existence, the location, or the morphology of these specialized structures, we undertook to investigate this problem further. By preparing the specimens in various ways not previously used, we were able to observe the locus of origin of the flagellum in cells of *Proteus vulgaris*. Electron micrographs will be presented showing flagella attached to ghosts of normal and long cell forms, and to fragments of cell wall and cytoplasmic membrane of *P. vulgaris*. Of special interest is the close association and apparent connection between the flagellum and the cytoplasmic membrane. Micrographs of flagella in various stages of development from slowly growing long forms will be shown. A preliminary report of part of this study was presented previously (Abram, Vatter, and Koffler, Bacteriol. Proc., p. 26, 1964).

MATERIALS AND METHODS

P. vulgaris P (a Purdue strain) was employed in this study. Cultures were grown at 37 C on a medium containing 1% Trypticase (BBL), 0.2% yeast extract, and 1.0 to 1.5% agar. To reduce the number of piliated cells and to obtain heavily flagellated cells in the culture, subcultures were made from the swarming edges of the growth on fresh moist agar plates. Four to eight transfers were made at intervals of 12 to 18 hr until swarming growth was observed along the entire streak of the inoculation. To obtain "ghosts," i.e., cells emptied of cytoplasm, or cytoplasm and membrane, swarming cells from cultures in the exponential phase of growth were incubated for 6 to 8 hr at 37 C and were then held at 6 to 10 C for 2 to 6 weeks. Cells were washed off the medium with deionized water at room temperature, yielding suspensions containing 10^8 to 10^9 cells per milliliter. Cells produced under these conditions were 1.5 to 2.5 μ in length. No more than 1% of the cells were "ghosts."

Another type of cell, the "long form," was cultured as follows. Cultures in the early exponential phase of growth (4 to 6 hr at 37 C) were incubated at 2 to 4 C for 2 days to 6 weeks. The swarming edges of the growth continue to extend slowly in the cold, and cells at these edges continue to increase in length until they become 5 to 50 μ and occasionally up to 125 μ long, as compared with 1.5 to 2.5 μ for cells grown at 37 C. Suspensions of the long forms containing 10^7 to 10^8 cells per milliliter were prepared in deionized water. These preparations were examined electron microscopically for structures to which flagella might still be attached (empty cells, fragments of cell wall, and cell walls in association with cytoplasmic membranes).

Isolates of flagella were prepared according to the procedure described previously (Abram and Koffler, 1964). Both the final purified preparations and the debris obtained during purification of flagella were examined for flagella detached from cells but still attached to cell fragments; the debris constituted the fraction isolated by centrifugation (16,000 $\times$ *g* for 10 to 15 min) of suspensions of flagella from which most of the deflagellated cells had been removed.

Specimens were negatively stained with 1% potassium phosphotungstate (PTA) at *p*H 6.8 to

FIG. 1. *Ghost cell, from a standard culture kept 2 weeks at 6 to 10 C, almost completely devoid of cytoplasmic membrane. The attached flagella arise inside the cell from hooks (h) associated with spherical electron-lucid bodies, 200 to 500 A in diameter. Some of these structures are arranged in pairs (arrows). The electron-lucid material that is attached to the spherical bodies probably represents fragments of the torn cell membrane (cm). The meshlike fine structure of the cell wall (cw) is apparent. Negatively stained with PTA.* $\times$ *98,000.*

FIG. 2. *Ghost of a long-form cell from a water-shocked culture that previously had been incubated for 2 days at 2 to 4 C. Through the relatively transparent cell wall (cw), intact flagella can be seen to arise from hooks. Note the plasmolyzed protoplast (P). The flagella inserted along the stretched edges of the protoplast (arrows) appear to originate in or near the cytoplasmic membrane. In some instances, the point of origin of the flagella is associated with spherical structures having a diameter of 110 to 140 A (bs). Newly formed, short flagella (sf) can be seen at one end of the cell which seems to be dividing. Negatively stained with PTA.* $\times$ *68,000.*

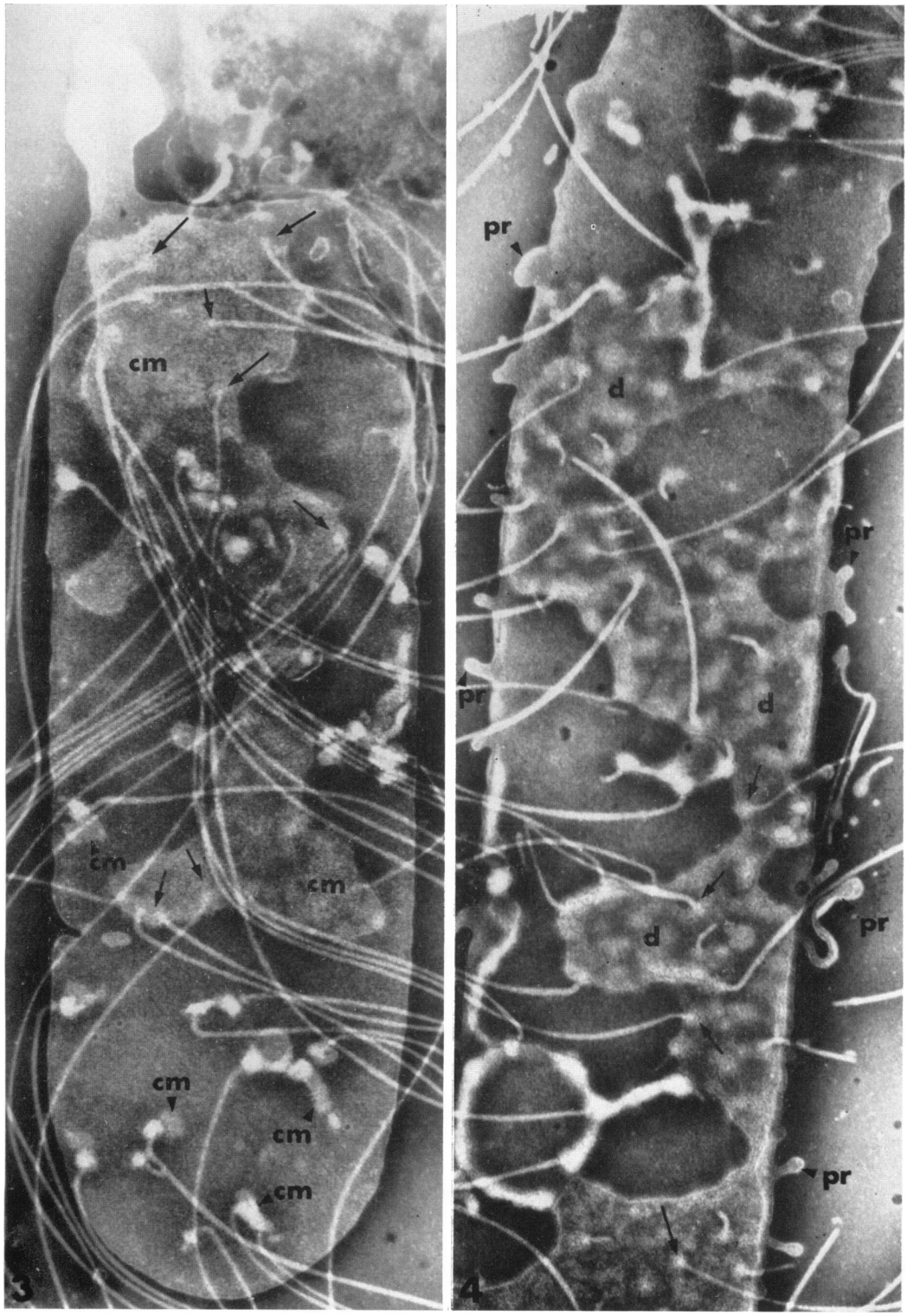

FIG. 3–4

7.2 or with 0.25% uranyl acetate, and were shadow-cast with palladium. The preparation of the specimens and their examination in the electron microscope were the same as described previously (Abram, 1965).

RESULTS

Ghost cells. It is not possible to observe flagella within intact negatively stained or shadow-cast cells, because such cells are electron-opaque. In negatively stained preparations of ghosts (Fig. 1 to 6 and 9 to 11), on the other hand, the intracellular origin or base of the flagellum can be seen through the cell wall, or the complex of cell wall and cytoplasmic membrane. In shadow-cast preparations of ghosts (Fig. 7 and 8), the attached flagella show up in relief, and unlike the situation in intact cells their origin is observable. The ghost cells illustrated in Fig. 1 to 11 were found in the two types of cultures described above. In some ghosts (Fig. 1 and 3), the cell wall is damaged locally, but the rod shape of the intact cell still is retained. The meshlike fine structure of the wall can be seen in cells that are nearly devoid of cytoplasmic membrane (Fig. 1). In suspensions of such cells, various fragments, for example, of walls and membranes, are also present (as in Fig. 12 to 17).

Cells in the early exponential phase of growth, cultured at 37 C, continue to elongate when incubated at lower temperatures, i.e., from 2 to 4 C. In the cold, the capacity to develop cross walls appears to be impaired; however, other components of the cells, including cell wall, cytoplasmic membrane, and flagella, continue to form, though slowly. During the first week of incubation in the cold, while the solid medium surface is still moist, at the edges of the swarming cultures long forms can be seen to move actively on the agar surface when observed at room temperature by light microscopy. When these cells are suspended in water, many rupture and appear as ghosts when examined by electron microscopy. The proportion of ghosts obtained in this manner increases as the time of incubation in the cold and the length of the cells increase. In some of the ghosts of long forms, the exposed cytoplasmic membrane appears to be nearly intact throughout the entire cell (Fig. 5), or consists of flattened fragments (Fig. 4). However, as in the case of the ghost cells described previously (Fig. 1 and 3), portions of some of the ghosts of the long forms are almost completely devoid of cytoplasmic membrane (Fig. 9 and 11). After 2 days of incubation in the cold, some cells, which are only slightly elongated, rupture when suspended in water. In some of these cells, the cell wall appears to be damaged locally, and a nearly intact plasmolyzed protoplast is observable through the relatively transparent cell wall (Fig. 2). Many of the rod-shaped long forms exhibit protrusions (Fig. 4, 5, and 9 to 11) or blebs; the latter probably form when the wall ruptures (Fig. 6). In addition, many pieces of cell wall and cell wall-membrane fragments persist in these preparations (Fig. 12 to 17), apparently derived from cells that are more completely disrupted.

Flagella attached to ghosts. In all instances, intact flagella attached to ghost cells were observed to arise from basal hooks. These terminal structures are integral but differentiated parts of these organelles (Fig. 18 to 20). The cell shown in Fig. 2 is the least damaged of the ghosts illustrated in this paper. Through the relatively transparent cell wall, one can see a practically intact plasmolyzed protoplast to which many flagella are attached. The appearance of the stretched edges of the protoplast suggests that the flagella arise from the surface or just beneath the surface of the cytoplasmic membrane. At the base of some of these attached flagella, one can recognize a lucid spherical structure, the diameter of which is

FIG. 3. *Ghost cell from a standard culture kept for 3 weeks at 6 to 10 C, with fragments of the cytoplasmic membrane (cm). A flattened membrane fragment inside the ghost shows scattered areas that are slightly more lucid than the background. Some flagella arise in these areas from hooks associated with spherical structures, 110 to 140 A in diameter (arrows); these flagella appear to be associated intimately with the membrane. The flagella that originate in the areas of the ghost devoid of flattened cytoplasmic membrane arise from electron-lucid, approximately spherical bodies, 200 to 700 A in diameter. Some of these structures are arranged in pairs. The electron-lucid material that is attached to these bodies probably constitutes fragments of torn cytoplasmic membrane (cm). Negatively stained with PTA.* × *72,000.*

FIG. 4. *Portion of a long-form ghost derived from water-shocked cells that previously had been incubated at 2 C for 6 days. Almost all the flagella, including short ones, appear to be connected to the flattened cytoplasmic membrane. Some of the flagella arise in lucid structures, 200 to 700 A in diameter, or in lucid areas of folded edges of the membrane. Other flagella arise in slightly differentiated dispersed areas of the flattened membrane, which are only slightly more lucid than the background. A few originate in spherical structures, 110 to 140 A in diameter, within these areas (arrows). Many dispersed areas can be observed on the flattened membrane with which no flagella are associated (d). Notice the protrusions (pr) of the wall along the edges of the cell. Negatively stained with PTA.* × *68,000.*

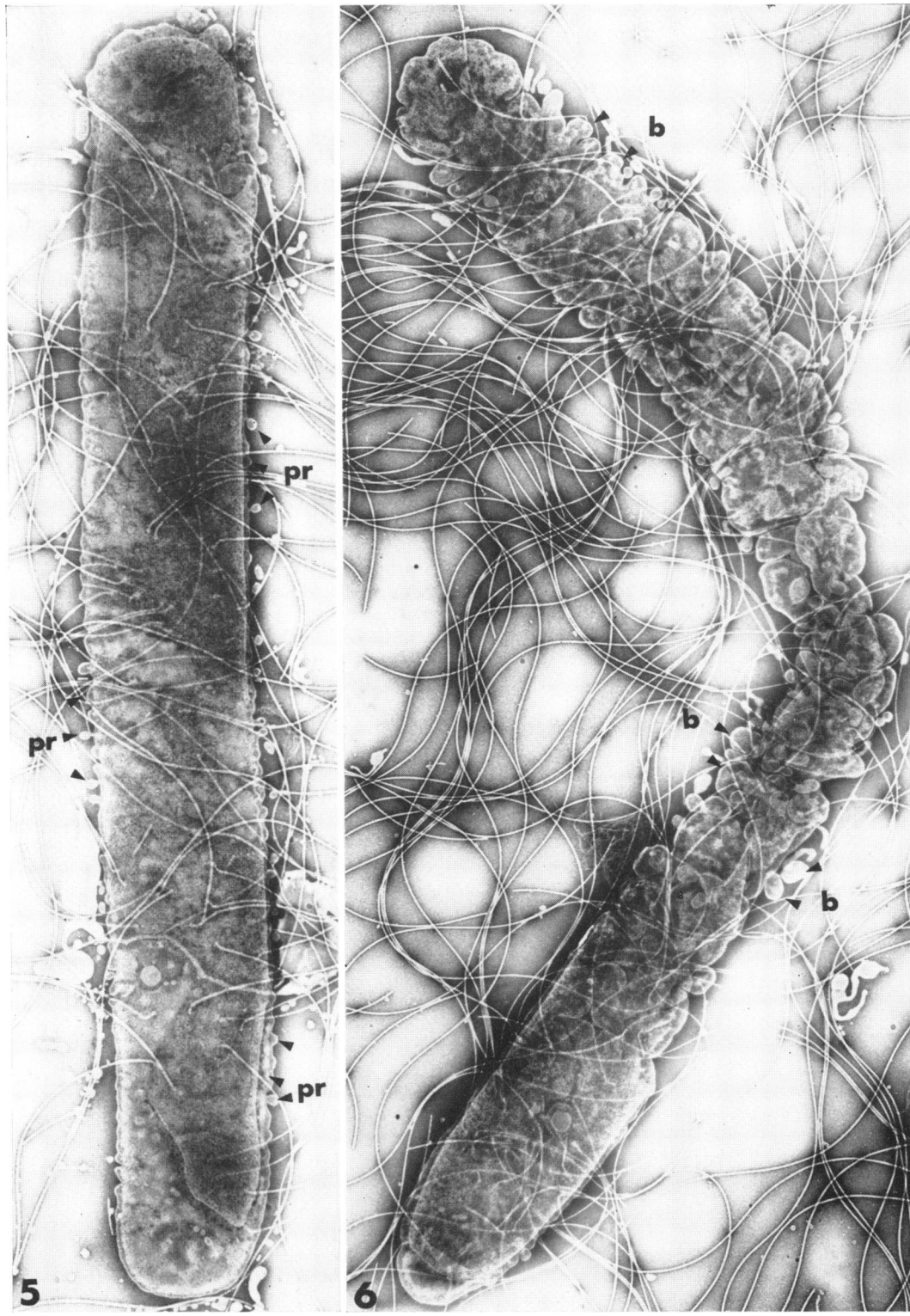

FIG. 5 and 6. *Low-magnification view of two long-form ghosts to which many flagella are still attached. In Fig. 5 the rod shape of the cell is retained. The cytoplasmic membrane occupies most of the space inside the cell. Almost all the attached flagella are seen to arise from hooks associated with electron-lucid structures 110 to 140 A in diameter. Notice the protrusions (pr) of the cell wall along the edges of the cell. In Fig. 6 a portion of the cell is ruptured and forms blebs (b). However, the rod shape of the cell is still recognizable. Negatively stained with PTA. Figure 5, × 40,000; Fig. 6, × 33,000.*

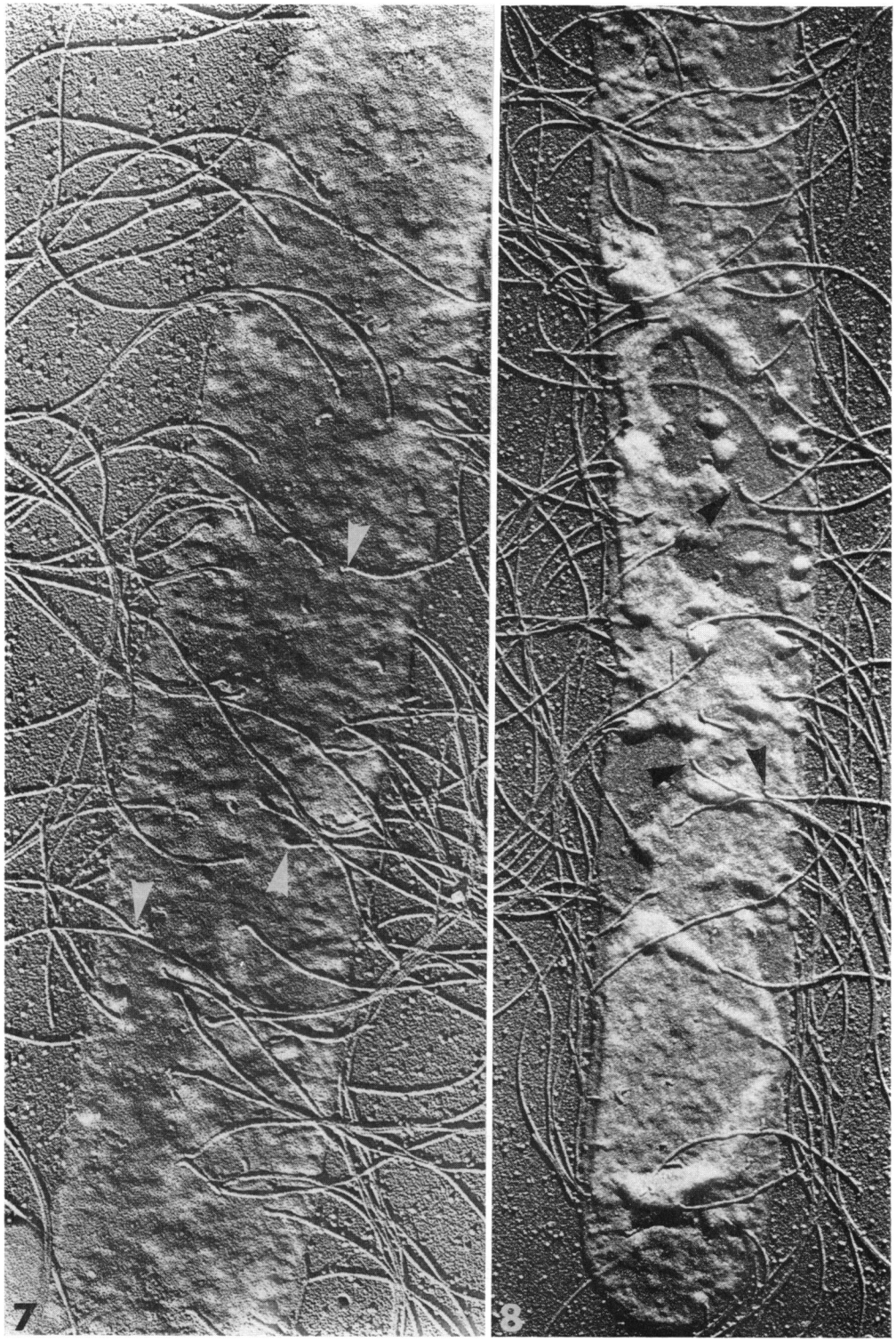

FIG. 7 and 8. *Shadow-cast preparation showing flagella attached to portions of long-form ghosts. In Fig. 7 one can see terminal hooks at the basal region of the flagella. In this ghost the cytoplasmic membrane is probably intact. In Fig. 8 the areas in which the cytoplasmic membrane is present appear elevated. The terminal hooks and, in a few cases, also small spherical structures from which the flagella originate can be seen (arrows). In the areas of the cell devoid of cytoplasmic membrane, the flagella arise from hooks associated with the larger spherical structures. Figure 7, × 60,000; Fig. 8, × 36,000.*

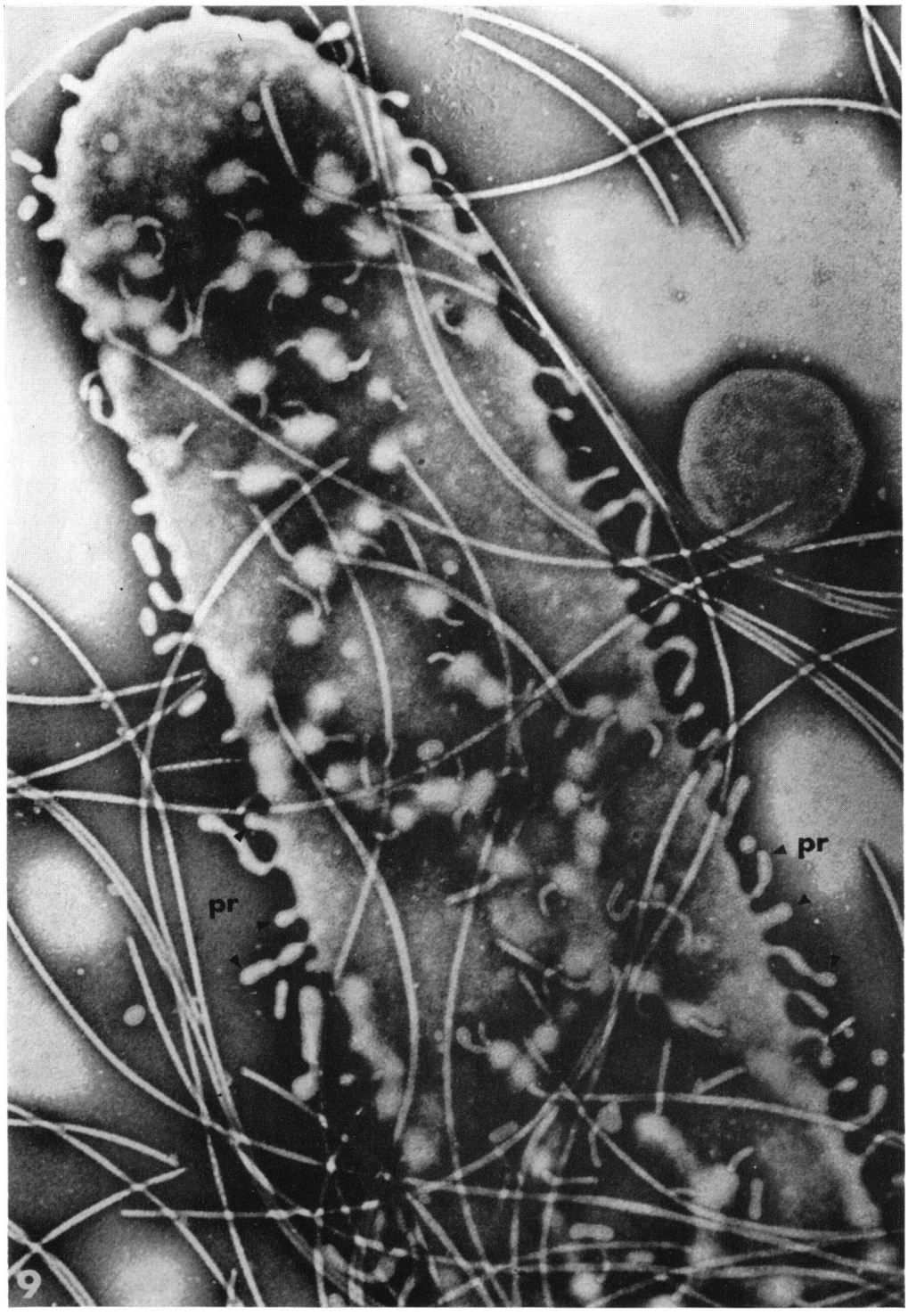

FIG. 9. *Portion of a long-form ghost after incubation at 4 C for 4 days, showing many short flagella, interpreted to be inside the cell. All the flagella, the short as well as the long ones, possess hooks and arise from large spherical bodies, 300 to 600 A in diameter. Many of these bodies are in pairs, some of which appear merged. Flagella arising from the paired bodies often differ in length. Notice the protrusions (pr) of the cell wall along the edges of the cell. Negatively stained wlh PTA.* × *90,000.*

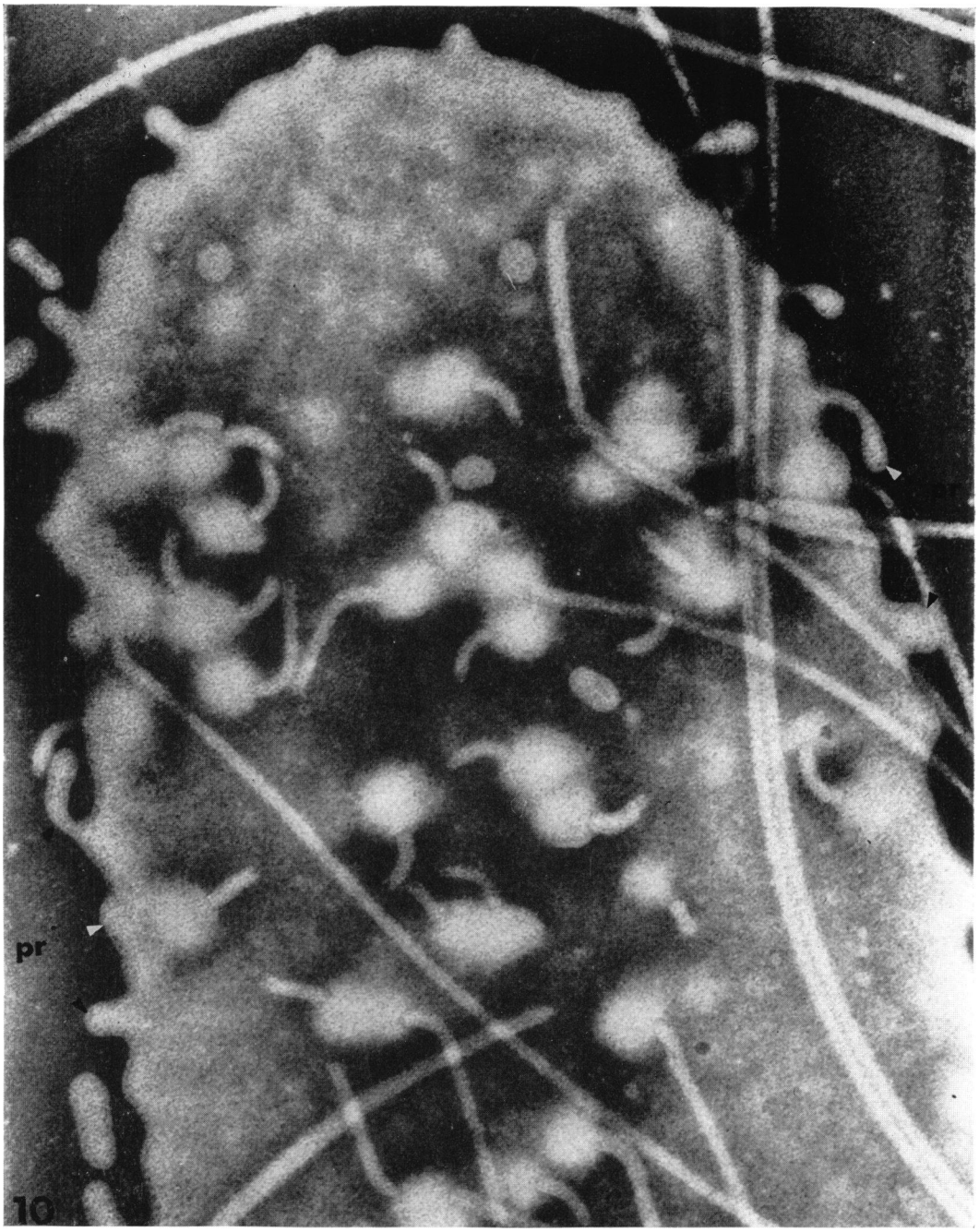

FIG. 10. *Same preparation as the one shown in Fig. 9 at a higher magnification (pr = cell-wall protrusions).* × *200,000.*

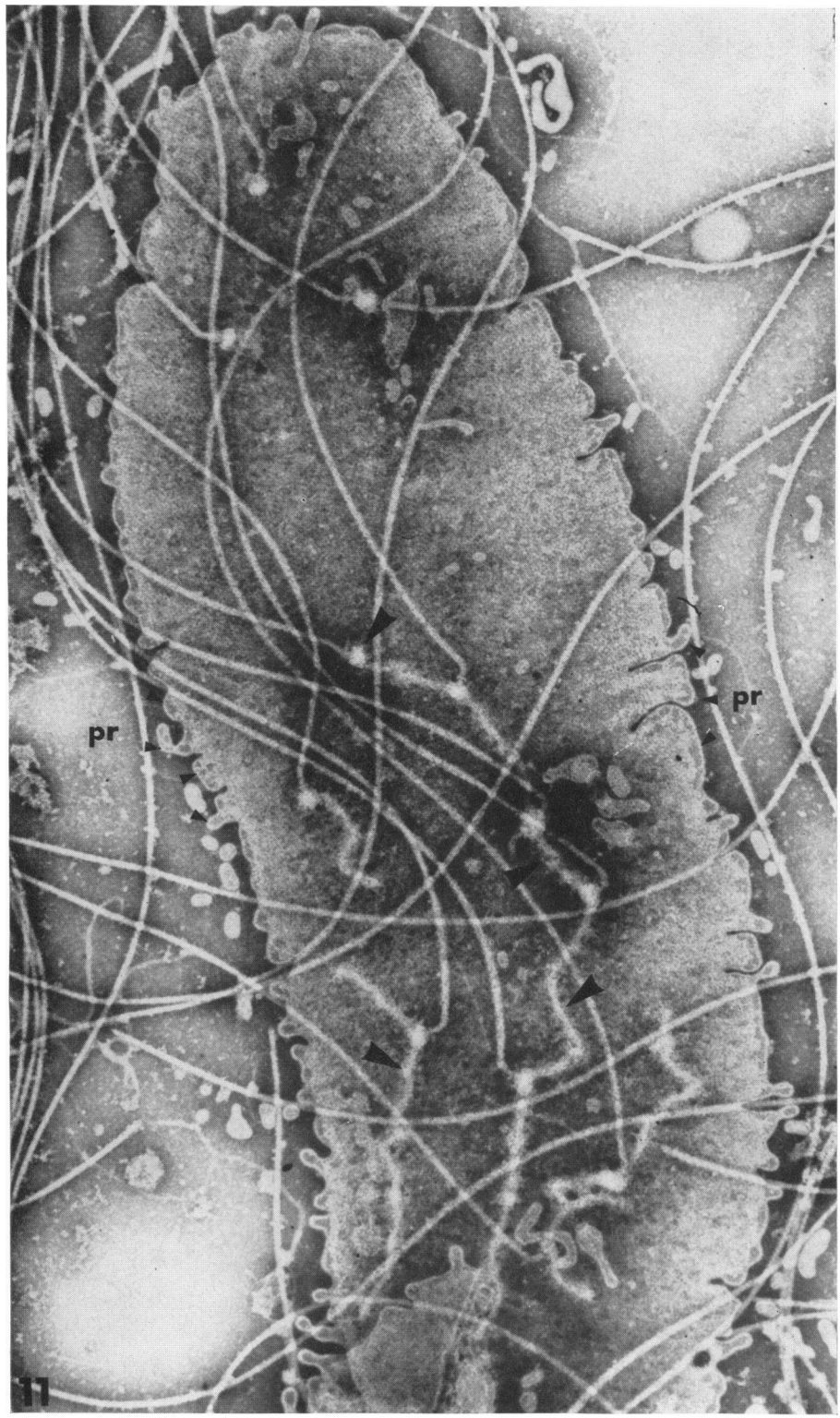

FIG. 11. *Portion of a long-term ghost (after incubation at 4 C for 4 weeks). The bases of intact flagella are hooked and attached to large electron-lucid bodies. Some of the latter are interconnected by strands of material (arrows) that probably represent fragments of the cytoplasmic membrane. The edges of the cell show bleb protrusions (pr). The meshlike structure of the cell wall can be recognized. Negatively stained with PTA.* × *90,000.*

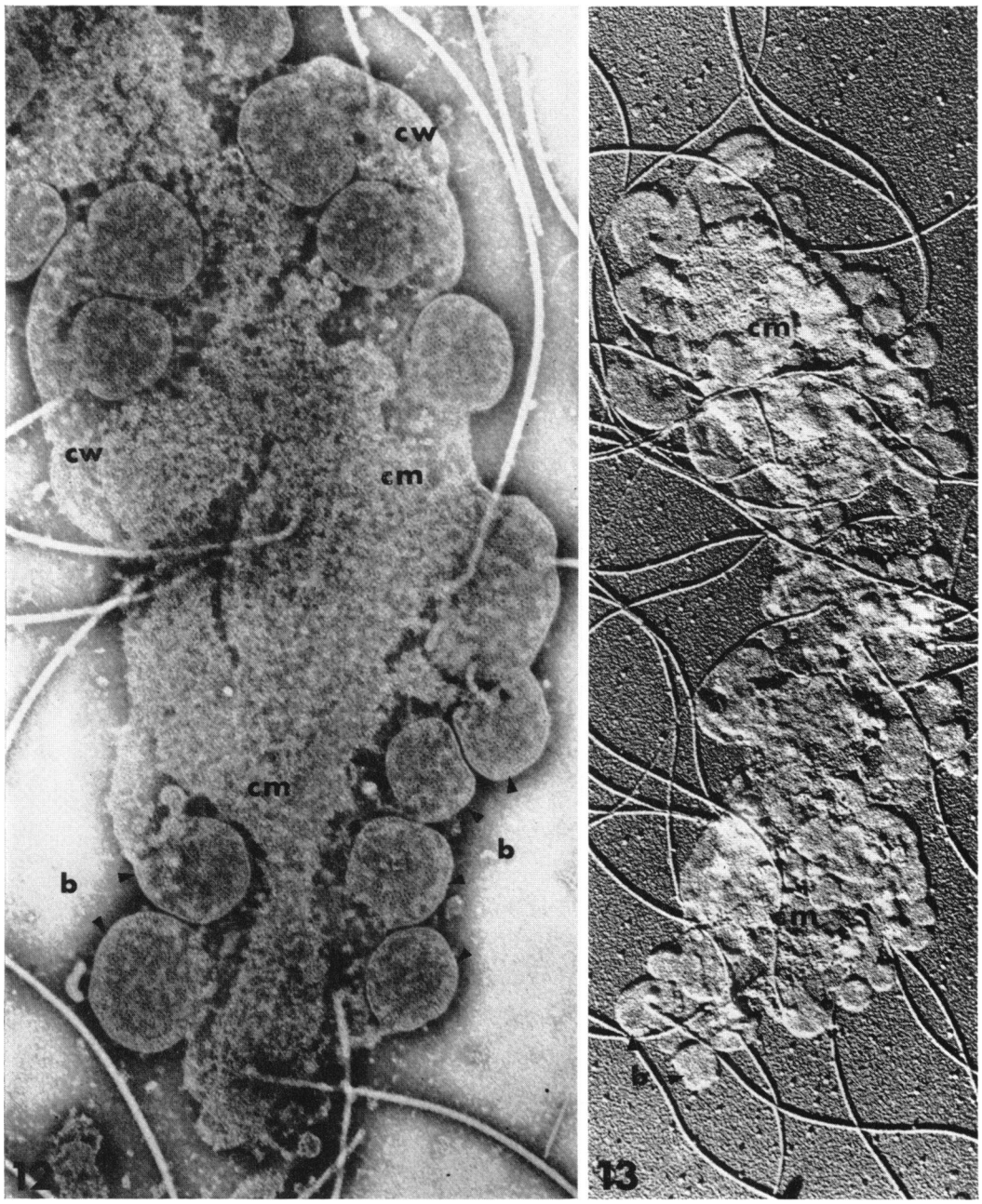

FIG. 12 and 13. *Large fragments of cell envelope observed in suspensions of long forms. The cytoplasmic membrane (cm) has not separated from the cell wall (cw), while the latter forms bleblike fragments (b) that show the characteristic fine structure of the cell wall. In Fig. 12 the intact flagella appear to be attached to the cytoplasmic membrane. The terminal hooks are visible. The observations in the negatively stained (Fig. 12) and the shadow-cast preparations (Fig. 13) are in good agreement. Figure 12, × 100,000; Fig. 13, × 60,000.*

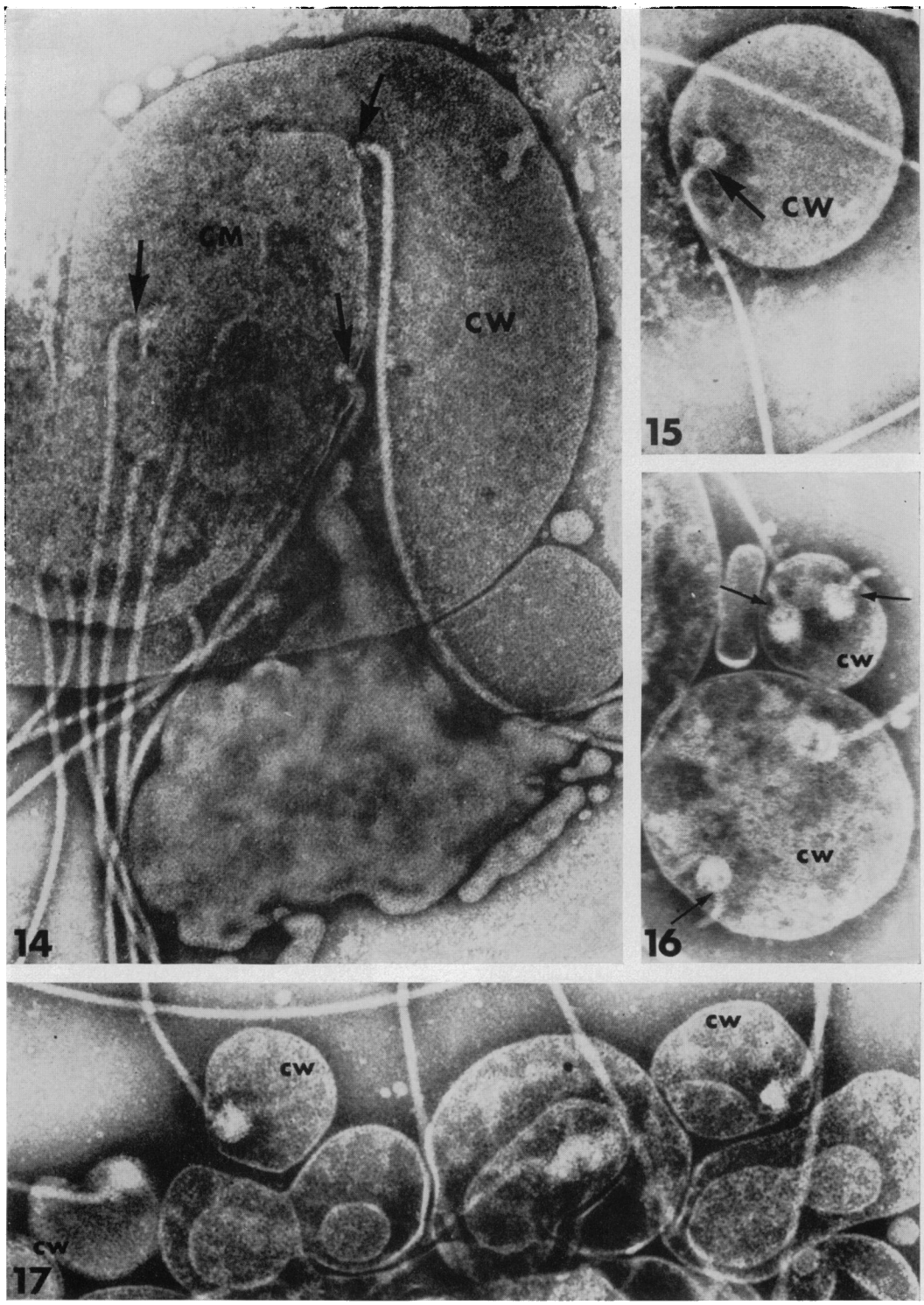

FIG. 14. *Similar preparation to that shown in Fig. 12. The flagella attached to pieces of the cytoplasmic membrane (cm) and adherent cell wall (cw). Some of the flagella originate in small spherical lucid structures, 110 to 140 A in diameter (arrows). Notice the meshlike structure of the rounded cell wall fragment.*

FIG. 15 to 17. *The bases of the flagella attached to rounded cell wall fragments originate in spherical structures, 500 to 700 A in diameter. Within some of these large bodies, smaller spherical structures can be observed (arrows). The fine structure of the cell wall can be seen clearly in Fig. 17. Negatively stained with PTA. Figure 14, × 120,000; Fig. 15, × 95,000; Fig. 16 and 17, × 100,000.*

the same or only slightly larger than that of the organelle (110 to 140 A). In other ghosts, as in Fig. 3 and 4, flagella that are attached to fragments of cytoplasmic membrane appear to originate from differentiated areas of the membrane. These areas, 200 to 700 A in diameter, are dispersed and less opaque than the remainder of the membrane. Occasionally, a distinct spherical structure, 110 to 140 A in diameter, can be recognized at the origins of the flagella. On the other hand, some of the flagella attached to ghost cells shown in Fig. 1, 3, 4, and 8 to 11 arise via hooks from larger bodies than the ones described above. In the latter case, the cells have lost either part (Fig. 3 and 4) or most (Fig. 1 and 8 to 11) of their cytoplasmic membrane, or the flagella originate at the edges of the flattened cytoplasmic membrane (Fig. 4 and 8). Figure 1 shows an example of a ghost practically devoid of cytoplasmic membrane. The flagella attached to this ghost originate from spherical structures 200 to 500 A in diameter, which often are paired. Material attached to these structures probably represent fragments of torn cytoplasmic membrane. In Fig. 3, flagella are associated with similar structures. These can be observed in regions of the cell which are devoid of their cytoplasmic membrane. In Fig. 4, part of a ghost of a long form is shown in which the cytoplasmic membrane is more intact. Almost all the flagella are inserted in the membrane. Numerous short flagella (Fig. 2, 4, 9, and 10) are present in long forms during the first week of incubation at 2 to 4 C. These probably result from the slow growth rather than from the breaking of flagella, since after longer periods of incubation in the cold almost all the flagella attached to cells are intact and long. Furthermore, purified flagella are essentially stable at 2 to 4 C. Some flagella attached to cells (as in Fig. 4) can be seen to have their origin at specific sites. Similar sites without flagella are scattered over the membrane, and it is possible that these are sites from which new flagella arise. Some flagella are attached to larger structures. Another aspect can be observed in the cell shown in Fig. 9 and 10. This cell contains little cytoplasmic membrane, and has many short flagella. The flagella, irrespective of their length, arise from structures 300 to 600 A in diameter, many of which are paired. Flagella arising from adjacent basal structures often differ in length. In this cell, only a few of the spherical structures are without the accompanying flagella.

Shadow-cast preparations (Fig. 7 and 8) of long-form ghosts show the hooked base of the flagellum as being inserted into the cell. The surface of the cell shown in Fig. 8 appears uneven; the elevated interconnected areas of the cell are probably occupied by cytoplasmic membrane. Most of the flagella arising in these areas have no basal structure other than a hook. Occasionally, a small structure, the diameter of which is slightly larger than that of the flagella, is seen at the base of the hook. Larger and more distinct structures, 300 to 700 A in diameter, are seen separately or associated with the cytoplasmic membrane (Fig. 8). Flagella, some of which are short, arise from these complexes. The observations made on negatively stained preparations reveal similar structures. The cell illustrated in Fig. 7 is comparable to those shown in Fig. 2 and 5; the cell in Fig. 8, to the cells in Fig. 3 and 4.

Attached flagella are present in ghosts after incubation for 4 to 6 weeks at 2 to 4 C. Some of these ghosts appear devoid of almost all cytoplasmic membrane (Fig. 11). The flagella arise from larger spherical structures. Occasionally, these structures are connected by strands of material. These may be remnants of the cytoplasmic membrane.

Flagella detached from cells. Cell suspensions examined for ghosts (see above) and preparations of partially purified isolates of flagella contained fragments of cell wall and cytoplasmic membrane to which flagella still were attached (Fig. 12 to 17 and 24 to 31). The meshlike fine structure of the cell wall is evident on rounded fragments (Fig. 12); similar blebs can be recognized in shadow-cast preparations (Fig. 13). The flagella (Fig. 12 and 14) arise from sites in which the fine structure of the cell wall cannot be observed. The hooks are clearly distinguishable, and some flagella originate in structures 110 to 140 A in diameter (Fig. 14). In the shadow-cast specimen (Fig. 13), most of the flagella possess terminal hooks, but only few flagella arise from a prominent structure. In Fig. 15 to 17, flagella are attached to rounded fragments which show the characteristic meshlike fine structure of the cell wall. Here the bases of the flagella arise from structures that are 300 to 700 A in diameter. The manner in which flagella are attached is similar when they are connected to cell wall, to membrane fragments, or to ghosts. The flagella seen in Fig. 12 to 14 are attached in the same manner as the flagella attached to ghosts in the areas that are occupied by flattened cytoplasmic membrane (Fig. 2, 3, 4, and 5). On the other hand, in Fig. 15 to 17 the flagella are attached by structures similar to those observed in ghosts (Fig. 1, 3, and 9 to 11) that are devoid of cytoplasmic membrane.

Hooks can be observed on many flagella that have been mechanically removed from cells and partially purified by differential centrifugation

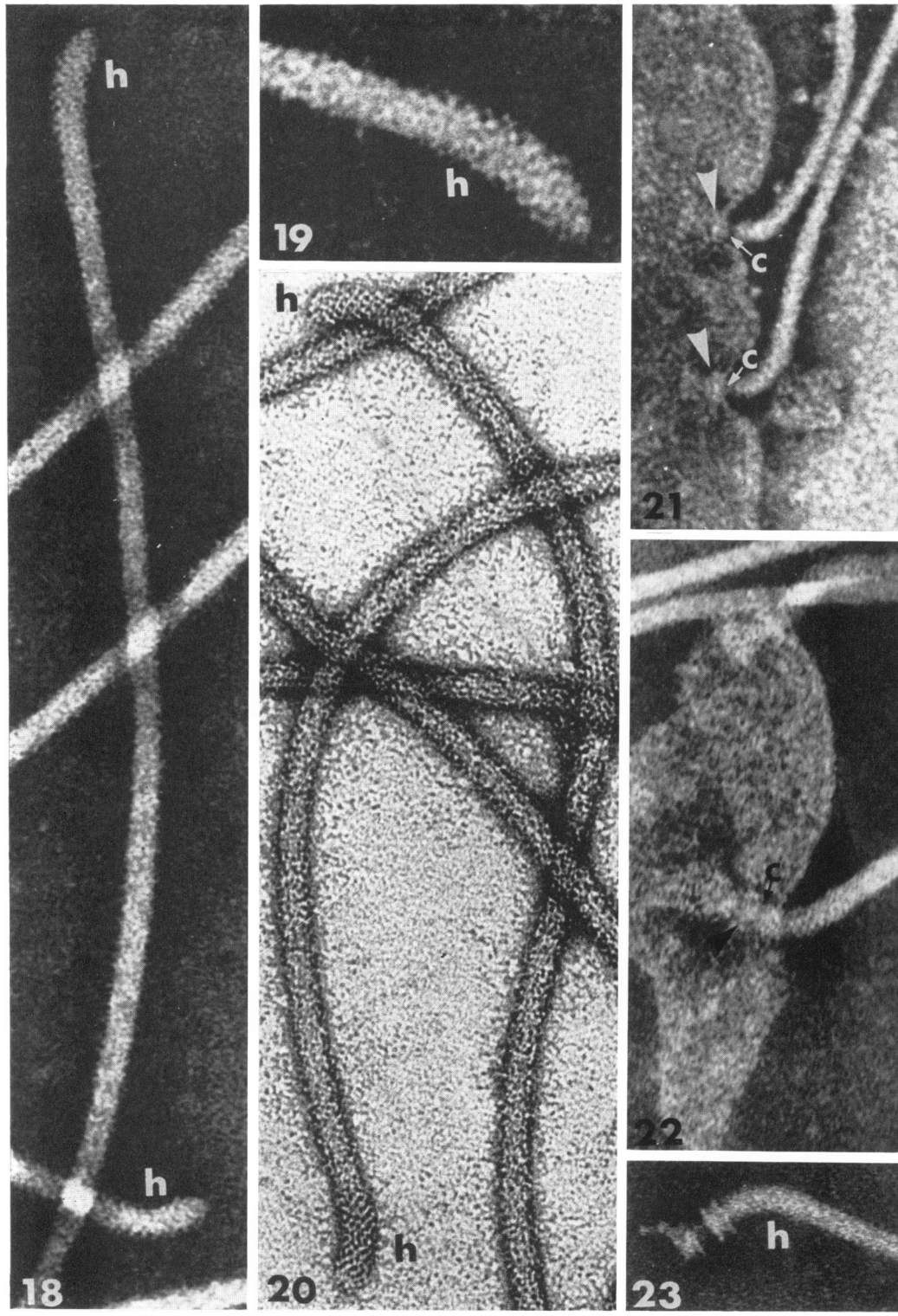

FIG. 18–23

(Fig. 18 to 20). The hooked region is greater in diameter than the flagellum proper, and the end of the hook is tapered and rounded. The hooked region of the flagella almost always is naked, and at high magnification it is possible to see that the subunits in that region are arranged differently than they are in the flagellum proper. Occasionally, material attached to the hooks can be observed (Fig. 23). Spherical structures, 110 to 700 A in diameter, associated with the base of the flagellum within ghosts of either normal or long-form cells, never were found to be connected to hooks of isolated flagella. Occasionally, flagella attached to fragments of cell wall were observed in purified isolates (Fig. 21 and 22). More frequently, flagella still attached to large (Fig. 29) and smaller (Fig. 25 to 31) fragments of cell wall, or membrane-wall complexes, can be seen in debris isolated during the purification of flagella. The hooks are associated with a basal structure, part of which is spherical, (110 to 140 A in diameter). The basal structures appear to be attached to the base of the hook, which creates the impression that the hook is constricted (Fig. 21, 22, and 25 to 31). It is possible that the constriction is the point at which flagella are detached when cells are manipulated.

DISCUSSION

The observations described in this paper provide information concerning the attachment of flagella to cells of *P. vulgaris*. Both the ghosts of normal cells, which are present in suspensions of cells stored at low temperature, and ghosts of long forms, which result by spontaneous rupture when cells of long forms are placed in water, appear to be altered less than the autolyzed cells described in previous reports by others. Autolysis after prolonged incubation at 26 to 37 C results in disorganization of the cellular components of the cells. On the other hand, in the forms described in this paper one still can distinguish the cell wall and the cytoplasmic membrane, and occasionally an intact plasmolyzed protoplast is observed through the relatively transparent cell wall. One can observe the site of origin of flagella within the cells even after many weeks of incubation at low temperature. In some ghosts, the flagella are attached to the intact plasmolyzed protoplasts. They appear to originate from spherical structures, the diameter of which is similar to or only slightly larger (110 to 140 A) than the diameter of the flagellum. These structures appear to be associated with the cytoplasmic membrane and probably constitute a part of the membrane or a separate entity in close proximity to the membrane.

In cells partially or completely devoid of cytoplasmic membrane, the flagella appear to originate in larger structures, the diameter of which ranges from 200 to 700 A. In negatively stained preparations, at times the smaller basal portion of the flagellum can be distinguished within these larger structures. This structure also can be seen in shadow-cast preparations. The large bodies at the base of the flagella may not be real structural entities, but perhaps are artifacts resulting from the persistence of a part of the membrane after the rupture of the wall. In this interpretation, the attached membrane is thought to fold around the smaller basal structure of the flagellum, thus making it appear larger. These structures also are seen on flagella still attached to cell-wall fragments. If flagella indeed originate in or near the cytoplasmic membrane, the formation of these large bodies in this manner seems plausible. Cells of *P. vulgaris* develop into long forms at 2 to 4 C, a temperature at which the growth processes, including the formation of flagella, are retarded. The short flagella attached to the elongated cells probably represent these organelles at an early stage of their development. The bases of both short and long flagella are associated with similar-appearing sites within the cell, namely, small structures 110 to 140 A in diameter. The basal structure of the flagellum thus appears to exist before this organelle is fully formed.

The function of the basal structure is unknown; however, several can be postulated. First, the basal structure may be the site at which flagellin, the protein constituent of flagella, is either synthesized or assembled to form the flagellum; it thus could function as a compartment in which a sufficiently high concentration of flagellin can be achieved locally to result in polymerization (Ada et al. 1963; Abram and Koffler, 1964; Asakura, Eguchi, and Iino, 1964; Lowy and McDonough, 1964). Second, the basal structure may function

FIG. 18 to 20 and 23. *Flagella from purified isolates prepared by mechanical removal of the organelles from cells and differential centrifugation. The hooks (h) are almost always naked (Fig. 18 to 20) and reveal a fine structure different from that of the flagellum proper. Only seldom is material attached to the hooks as in Fig. 23.*

FIG. 21 and 22. *Fragments of cell wall and membrane in purified isolates of flagella to which the organelles are still attached. In Fig. 22 characteristic fine structure of the hook can be observed. There is a constricted region (c) where the hook is connected to the basal unit, which is a spherical structure, 100 to 140 A in diameter (arrows). Negatively stained with PTA: Fig. 18, × 400,000; Fig. 19, × 710,000; Fig. 21, × 300,000; Fig. 22, × 400,000; Fig. 23, × 330,000. Negatively stained with uranyl acetate: Fig. 20, × 355,000.*

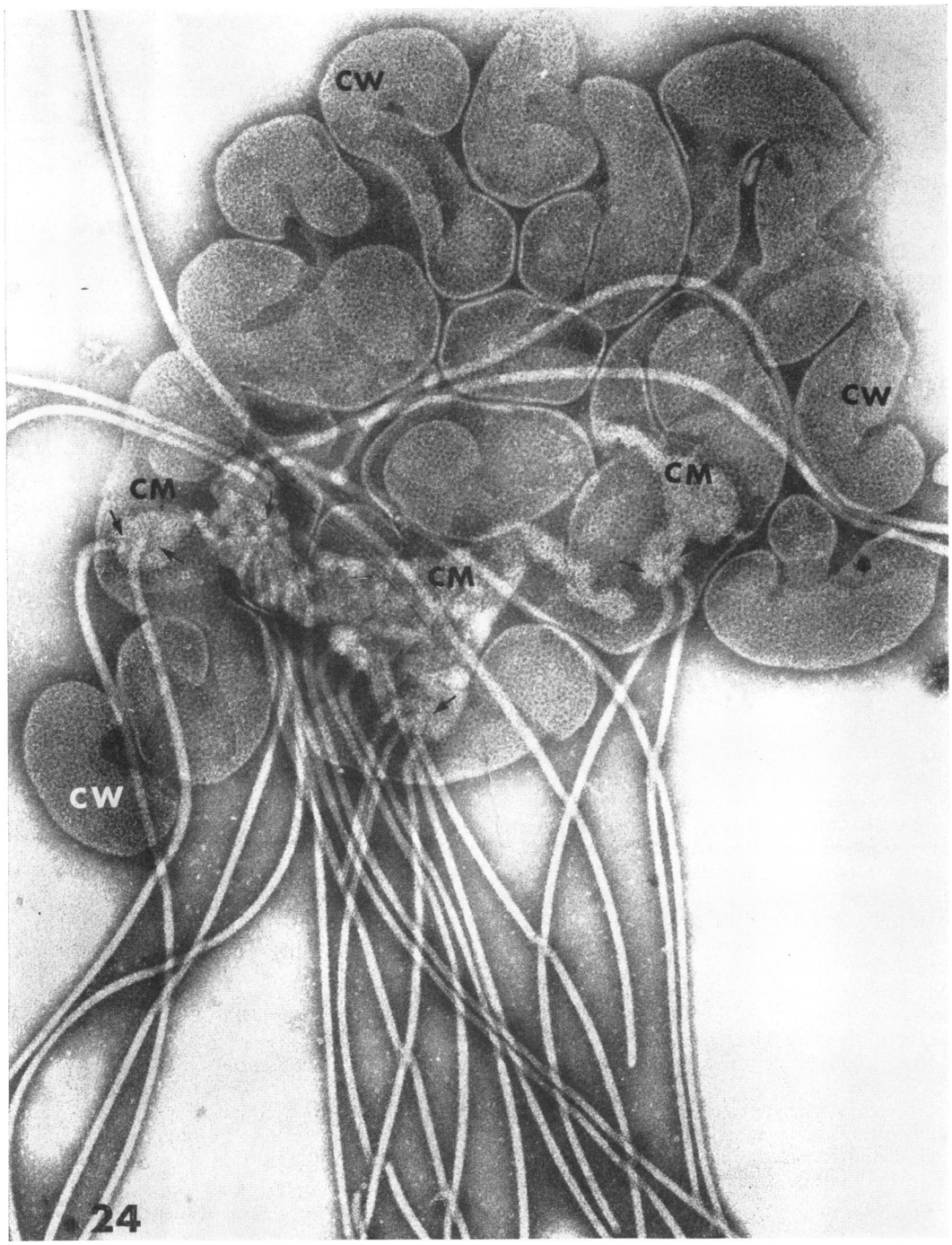

FIG. 24 to 31. *Preparations from a fraction containing cells and cell debris obtained in the process of purifying mechanically detached flagella by differential centrifugation.*

FIG. 24. *Large fragment of interconnected cell-wall blebs (cw). Flagella are attached in areas at which cytoplasmic membrane (cm) is still adherent to the cell wall. In these areas, the meshlike fine structure of the cell wall cannot be seen. Some flagella originate in basal structures (arrows) 110 to 140 A in diameter.*

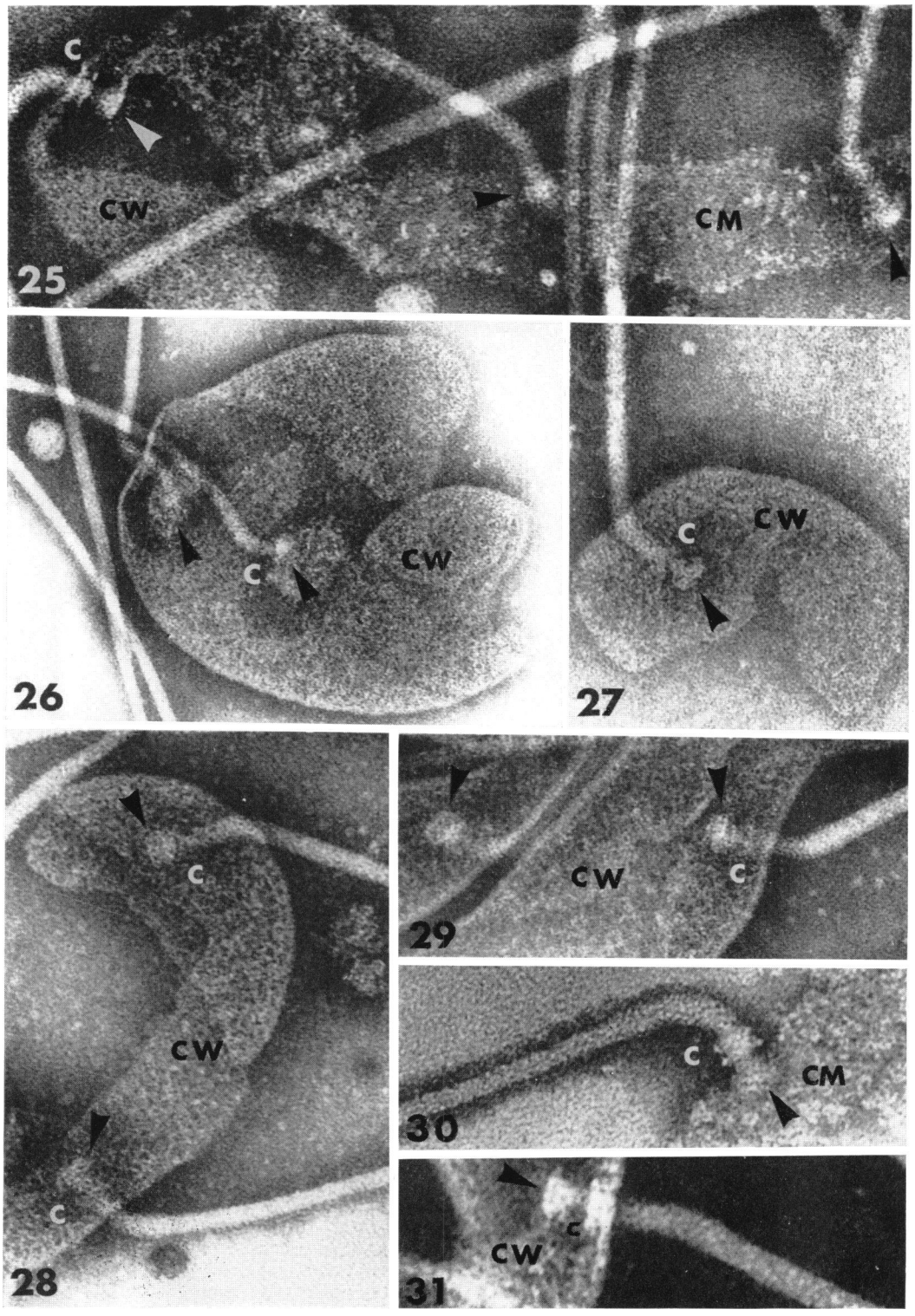

FIG. 25 to 31. *Examples of flagella attached to smaller fragments of cell wall (cw) and cytoplasmic membrane (cm). The flagella originate in a basal structure 110 to 160 A in diameter (arrows). In some cases, a constricted region (c) where the hook is connected to the basal unit can be recognized. Negatively stained with PTA. Figure 24, × 114,000; Fig. 25, × 298,000; Fig. 26, × 75,000; Fig. 27 to 29, × 240,000; Fig. 30, × × 280,000; Fig. 31, × 470,000.*

in anchorage. Third, it may be involved in motility. The apparently close association of this structure with the cytoplasmic membrane, at which enzyme and cofactors involved in energy-yielding reactions reside, makes this role plausible. The strands that appear to be cytoplasmic membrane connecting the large basal bodies in ghosts of the long forms may be a device for the coordination of flagella when they function as organelles of locomotion or artifacts produced by the disorganization resulting from the rupture of the wall. In any case, this observation indicates the close association of this basal structure with the cytoplasmic membrane.

The hooks appear to have their own identity, and differ from the flagellum proper not only morphologically but also in fine structure. Their function is not yet understood.

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