JOURNAL OF BACTERIOLOGY, May, 1966
Vol. 91, No. 5

Printed in U.S.A.

Attachment and Structural Features of Flagella of Certain Bacilli

DINAH ABRAM,[1] A. E. VATTER, AND HENRY KOFFLER

Department of Biological Sciences, Purdue University, Lafayette, Indiana, and Department of Pathology, The University of Colorado Medical Center, Denver, Colorado

Received for publication 4 February 1966

ABSTRACT

ABRAM, DINAH (Purdue University, Lafayette, Ind.), A. E. VATTER, AND HENRY KOFFLER. Attachment and structural features of flagella of certain bacilli. J. Bacteriol. **91**:2045–2068. 1966.—The attachment of flagella to cells of various mesophilic and thermophilic strains of *Bacillus* was studied electron microscopically. Studies of ghost cells and membrane fragments indicate that flagella are connected to the cytoplasmic membrane. Flagella removed from cells mechanically, during autolysis, or by phage lysis, have attached to the base of their proximal hooks material that is heterogeneous in character. In part, this material consists of cytoplasmic membrane; its varied shape appears to be caused by the folding of the membrane around the proximal end of the flagellum at the site of attachment. It is uncertain whether this material represents a real structure or an artifact. High-resolution microscopy reveals differences in the fine structure of intact flagella of the various strains studied. The proximal hook and the flagellar filament are distinct in morphology and fine structure. A specialized structure is associated with the hook of flagella of *B. brevis* and *B. circulans*. The filament of flagella of *B. stearothermophilus* 2184 has two regions that show marked differences in the manner in which the subunits appear to be organized. No correlation was found between the site of origin of flagella and the location of reduced tellurite when the reduction of potassium tellurite was used to indicate the loci of enzymatic respiratory activities.

In a previous paper, we reported on the basal structure and attachment of flagella of *Proteus vulgaris* (3). Most of these observations were made on flagella attached to ghost cells and to fragments of cell wall-membrane complexes. Bearing in mind the different properties of the cell envelope of various bacteria (12) and the relative ease with which the cell wall and the cytoplasmic membrane in some bacilli can be separated and identified (1), we extended our study to several mesophilic and thermophilic strains of *Bacillus*. Observations of these organisms yielded information concerning the intracellular origin and the attachment of the flagella to the cytoplasmic membrane, differences in the fine structure of intact flagella, and differentiation within these organelles. Electron micrographs of detached flagella and of flagella attached to intact and ghost cells or to fragments of cytoplasmic membrane will be presented. A preliminary report of part of this study was given previously (Abram, Vatter, and Koffler, Bacteriol. Proc., p. 26, 1964).

[1] Present address: Section of Biological Ultrastructure, The Weizmann Institute of Science, Rehovoth, Israel.

MATERIALS AND METHODS

Five strains of the genus *Bacillus* were studied: *B. pumilus* NRS 236, *B. brevis* 8185 NA, *B. circulans* Q19, and *B. stearothermophilus* 2184 and 194 (the latter was obtained from H. Sobotka, Mount Sinai Hospital, New York, N.Y.). A medium containing 1% Trypticase (BBL), 0.2% yeast extract (Difco), and 1.5 to 2% agar was used. The mesophilic organisms were grown at 37 C, and the cells were harvested at the exponential or stationary phase of growth, after 6 to 8 and 14 to 18 hr of incubation, respectively. The thermophiles were grown at 55 to 60 C. Cells of *B. stearothermophilus* 2184 were harvested at the exponential growth phase of the culture after 4 to 6 hr of incubation, and at the stationary phase of growth after 10 to 14 hr. *B. stearothermophilus* 194 was harvested after 4 to 5 hr of growth. This strain is phage-infected, and at this stage of growth many cells were lysed.

Cells were washed from the medium with deionized

water at room temperature to give suspensions containing 10^8 to 10^9 cells per milliliter. The cultures of the thermophiles were cooled to room temperature prior to preparation of the suspensions.

Cultures of *B. pumilus* were grown for 4 to 6 hr at 37 C (the early exponential phase of growth), and then placed at 5 to 10 C. After 3 days to 2 weeks, cell suspensions were prepared as described above.

Cells in the exponential phase of growth were used to study the reduction of potassium tellurite. A 1-ml amount of a 0.05 to 0.1% aqueous solution of potassium tellurite was added to each culture on agar slants containing 6 to 8 ml of medium. The cultures were incubated in the presence of tellurite for 1 to 4 hr at various temperatures. The tubes were placed in a slanted position so that the entire surface of the agar slant was covered with the tellurite solution. The cells from each slant were washed from the agar with 10 ml of deionized water and were sedimented. The pellets were washed twice in 15 ml of deionized water (4,000 to 5,000 $\times$ *g* for 10 min). Finally, the cells were suspended in water to give 10^8 to 10^9 cells per milliliter. The pellets of the cells that had reduced tellurite appeared black; an examination in the electron microscope revealed elongated electron-opaque crystals of reduced tellurite inside the cells or attached to extruded cytoplasmic membrane.

Isolated flagella from cells in the exponential and stationary phases of growth were prepared according to the procedure described previously (2). Both the crude and the final purified preparations were examined for detached flagella.

Specimens for electron microscopy were prepared from cell suspensions kept at room temperature for no longer than 30 min, or from freshly isolated flagella that were kept for no longer than 5 days at 5 C. In the experiment represented by Fig. 28g, a flagellar isolate frozen at -5 C was used. The specimens were negatively stained with 1% potassium phosphotungstate (PTA) at *p*H 6.8 to 7.2 or with 0.25 to 0.5% uranyl acetate at *p*H 4.4 to 4.6, or were shadowcast with palladium. The preparation of the specimens and their examination in the electron microscope were the same as described previously (1).

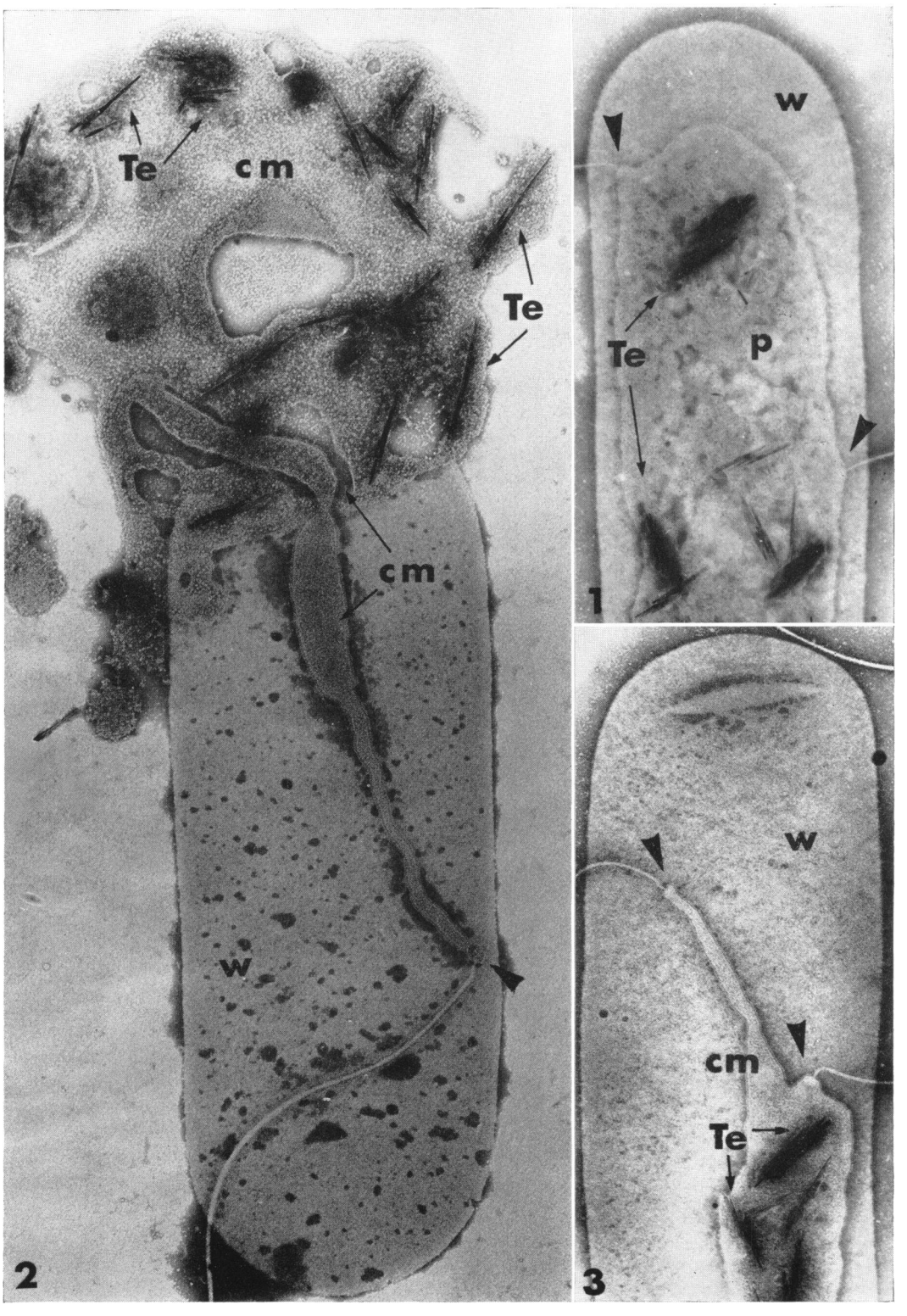
RESULTS

Attachment of flagella to the cytoplasmic membrane. Selected examples from many preparations show that there is a close association and apparent connection between flagella and the cytoplasmic membrane (Fig. 1 to 5, 7 to 11, 13 to 15, 18, 28 to 31). Individual differences exist among the flagella of various organisms as well as in different preparations. However, the attachment of flagella to the cytoplasmic membrane is common to all the organisms studied.

The protoplastic origin of the flagella can be observed through the relatively electron-lucid cell wall of ghosts of *B. stearothermophilus* 2184 (Fig. 1 to 3). These ghosts are from cells that had been exposed for 4 hr to tellurite (see Materials and Methods). In Fig. 1, the attachment of flagella to the plasmolyzed protoplast is shown by the concomitant stretching of the membrane at the points of flagellar insertion. In other ghosts from which most of the protoplast is extruded, the flagella are attached to the stretched membrane that remains within the cell wall (Fig. 2 and 3). This observation indicates that the flagellum and the membrane are firmly connected, and in these cases the flagellum prevents the membrane from being extruded. In some instances, flagella associated with fragments of ghost cells are attached to smaller membrane fragments (Fig. 4 and 29). However, in other specimens only spherical, mushroom- or disc-shaped structures are observed at the base of the flagella inserted in ghost cells (Fig. 5 and 29). Figure 5 shows a portion of a ghost cell of *B. stearothermophilus* 2184; the structures attached to the bases of flagella vary in morphology and size (from 250 to 600 A). The bases of the attached flagella of a ghost of *B. circulans* (Fig. 29) exhibit mushroom- or disc-shaped structures, 110 to 140 A in diameter. The shape and size of such structures in ghost cells of *B. brevis* may depend on the physiological state of the cells and appear to be related to the integrity of the cell envelope (Fig. 6 and 7). The flagella attached to the ghost shown in Fig. 6 originate in small disc-shaped structures; the cell wall appears to be almost completely disintegrated. The cell wall of the ghost shown in Fig. 7, on the other hand, appears to be intact; fragments of membranous material are associated with the bases of the flagella.

Specimens of cell suspensions of phage-lysed cultures of *B. stearothermophilus* 194 contain many large fragments of cytoplasmic membrane with attached flagella. The membranes are easily identified by their characteristic fine structure (Fig. 8 to 11). Spherical, mushroom-, or disc-

FIG. 1–3.—*Ghost cells of Bacillus stearothermophilus 2184 from cultures that were incubated for 4 hr at 55 C in the presence of 0.05% potassium tellurite. In Fig. 1 flagella are attached to a nearly intact plasmolyzed protoplast (p). Note the stretching of the membrane at the points of attachment (arrows). Figures 2 and 3 show flagella attached to the cytoplasmic membrane (cm) that remained within the cell wall (w) in ruptured ghost cells; again note that the membrane is stretched at these points (arrows). In Fig. 1 and 3, electron-opaque crystals of reduced tellurite (Te) can be observed in the protoplast. In Fig. 2 these crystals appear to adhere to the flattened cytoplasmic membrane (cm) that was extruded from the cell wall. Negatively stained with PTA. Figure 1, $\times$ 36,000; Fig. 2, $\times$ 55,000; Fig. 3, $\times$ 48,000.*


Te
cm
Te
cm
w
2
w
Te
p
1
w
cm
Te
3

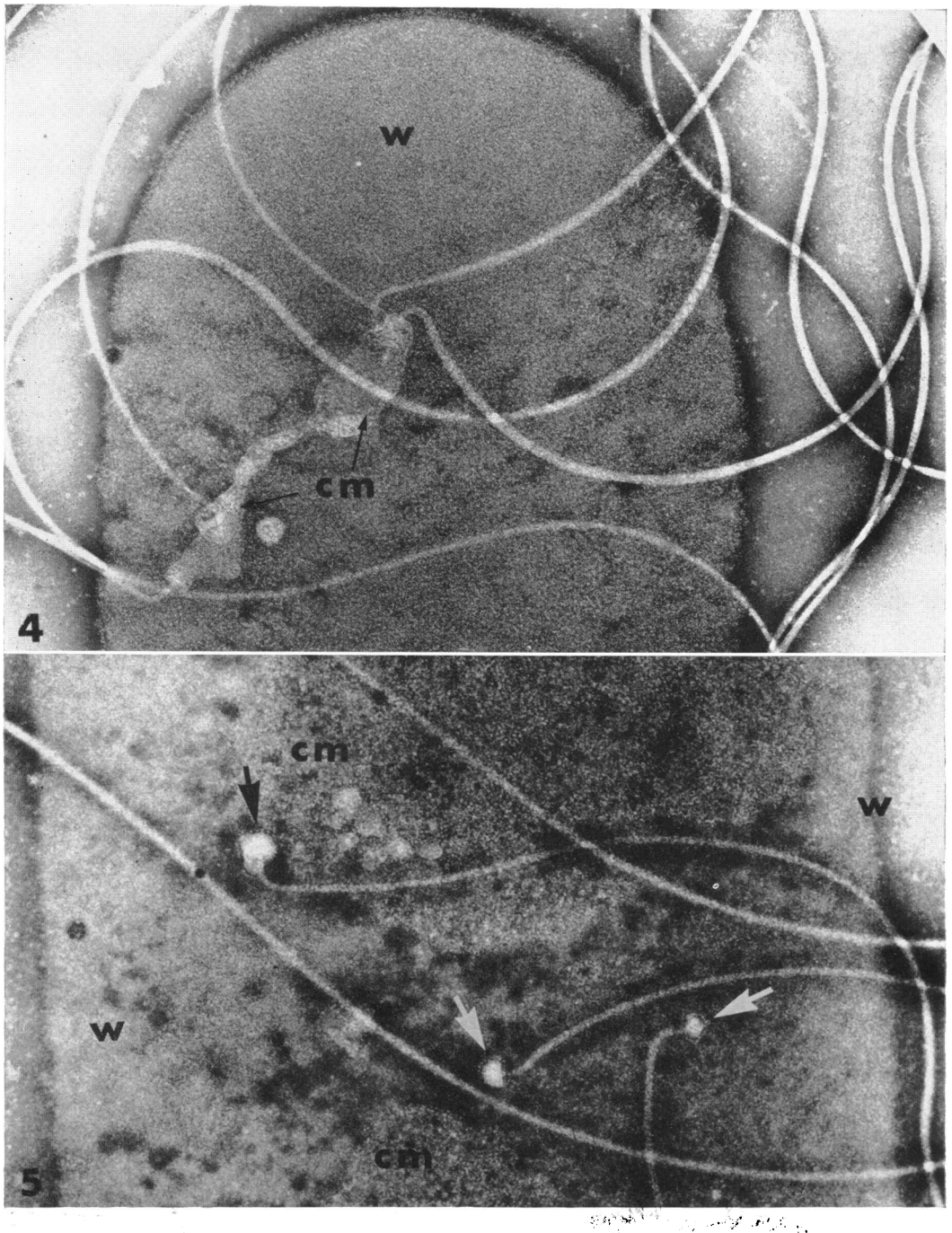


FIG. 4. *A portion of a ruptured ghost cell of Bacillus stearothermophilus 194. Through the relatively transparent cell wall (w), one can see several intact flagella attached to a fragment of cytoplasmic membrane (cm). Negatively stained with PTA.* × *110,000.*

FIG. 5. *Portion of autolyzed but nearly intact ghost of Bacillus stearothermophilus 2184. The flagella attached to this ghost originate in spherical or mushroom-shaped bodies (arrows), 250 to 600 A in diameter. Fragments of the disrupted cytoplasmic membrane (cm) can be seen through the lysed cell wall (w). Negatively stained with PTA.* × *108,000.*

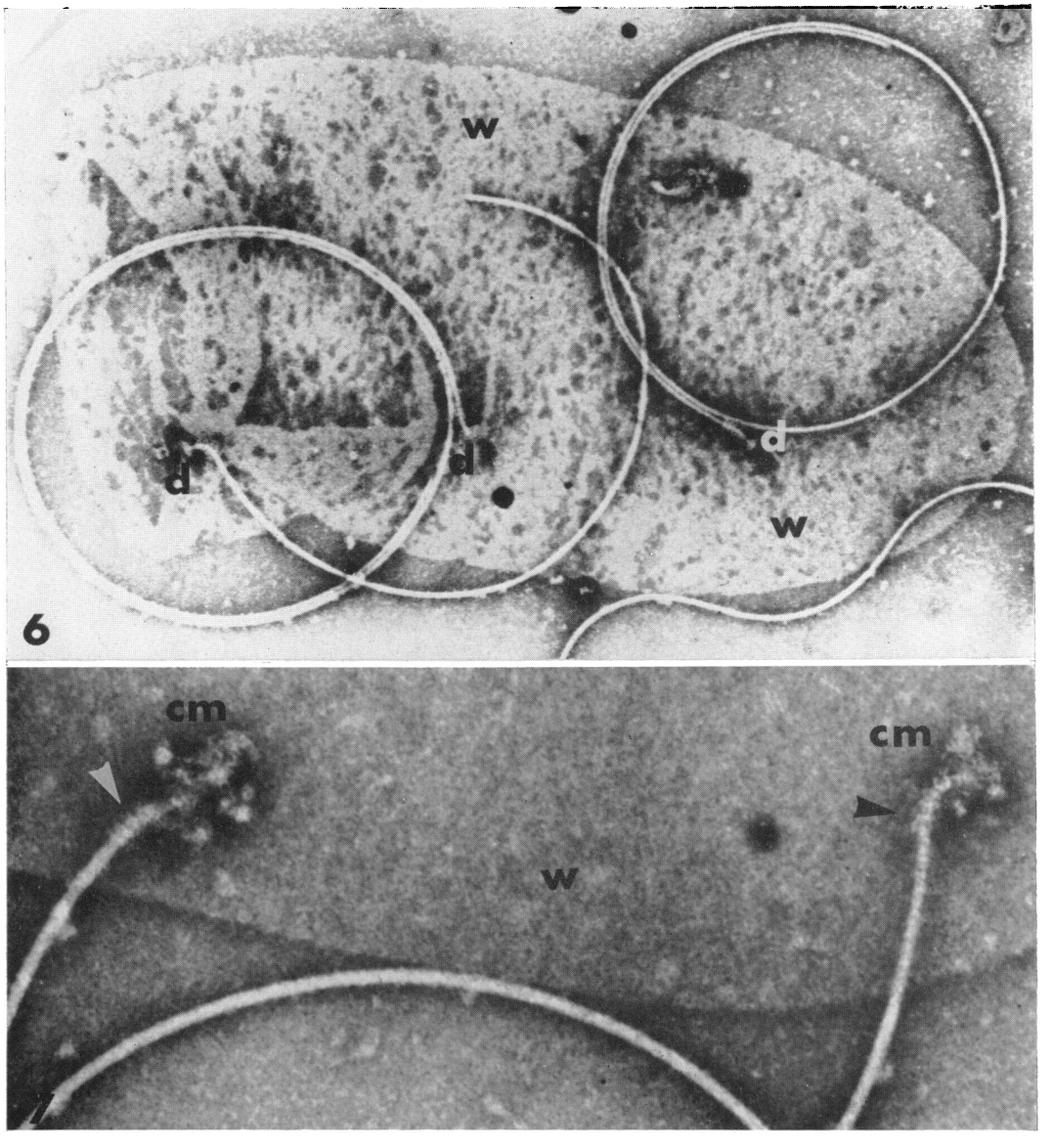


FIG. 6 and 7. *Two cell wall ghosts of autolyzed cells of Bacillus brevis from a culture in the stationary phase of growth. In Fig. 6 the cell wall (w) is almost completely disintegrated, and the flagella originate in small disc-shaped bodies (d). In Fig. 7 the cell wall appears intact, and the flagella attached to it are associated with what are believed to be fragments of cytoplasmic membrane (cm). The hooks have a cross-banded surface structure (arrows). Negatively stained with PTA. Figure 6, × 74,000; Fig. 7, × 160,000.*

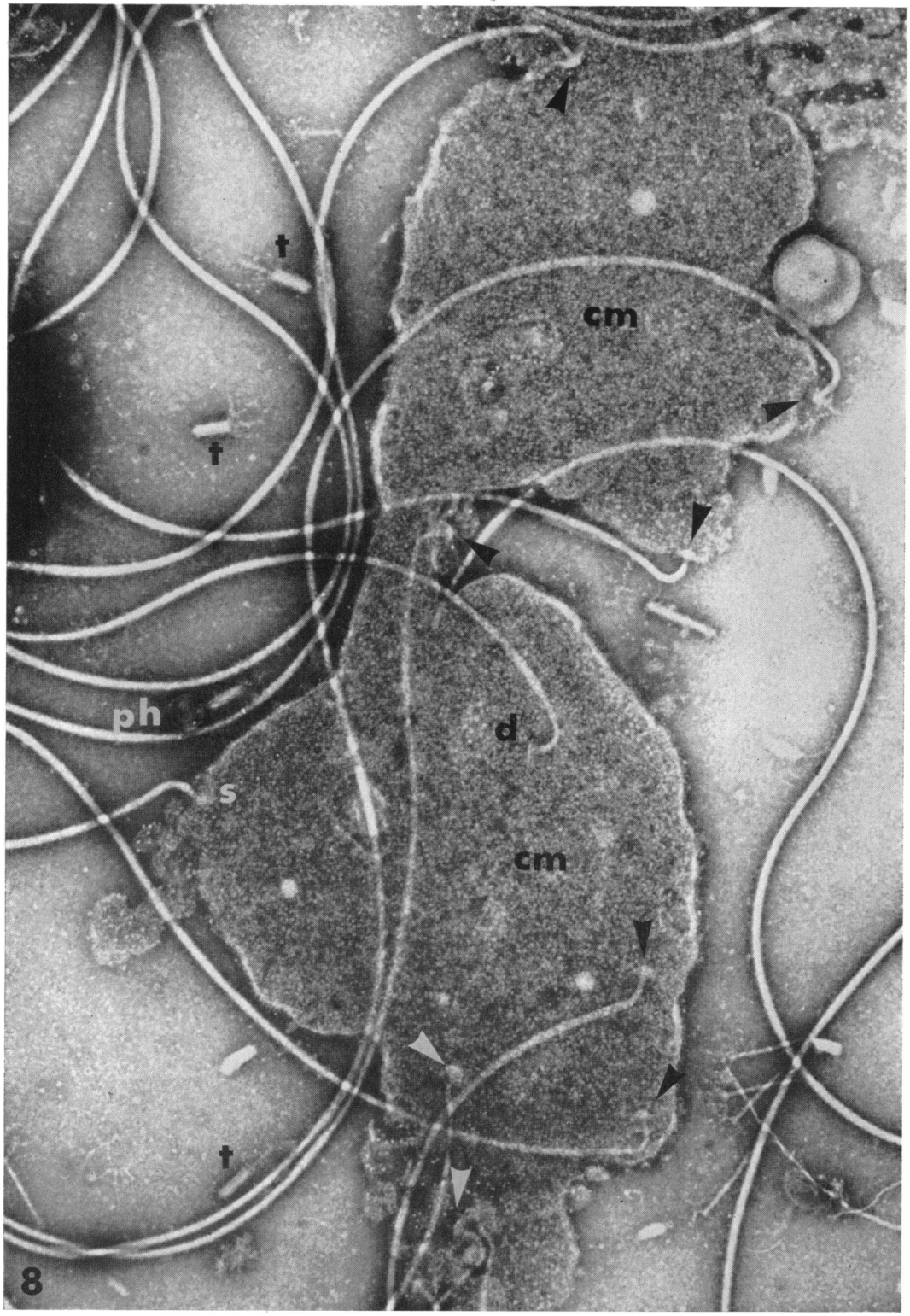


FIG. 8. *Large fragment of cytoplasmic membrane (cm) in a preparation of phage-lysed cells of Bacillus stearothermophilus 194. Most of the flagella attached to the membrane originate in spherical (s), disc-shaped (d), or mushroom-shaped structures (arrows). A phage (ph) and phage tails (t) can be seen in the background. Negatively stained with PTA.* × *85,000.*

shaped material is present at the sites where the flagella are inserted (Fig. 8 and 10). However, in many cases such structures are absent (Fig. 9 and 11). The frequency with which the different types of basal arrangements are observed varies from preparation to preparation and in different fields examined in the same specimen. The structures at the base of the flagella can be seen more clearly when the latter are connected to edges of membrane fragments, especially when folding of the membrane occurs. Flagella attached to membranes exhibiting the above-mentioned characteristics were also observed in cell suspensions of *B. stearothermophilus* 2184 prepared from cultures in the stationary phase of growth that contained many autolyzed cells (Fig. 13), and of *B. pumilus* prepared from cultures chilled during their early exponential phase of growth (Fig. 14 and 15). In the latter case many ruptured cells and large cell wall-free fragments of cytoplasmic membrane are present. In these membrane fragments, holes and tears are common. Flagella with either disc- or mushroom-shaped basal complexes that seem to be constituted of folded membrane are often associated with these tears. This attachment of flagella to the edges of the holes again suggests a firm connection of these organelles to the membrane.

Flagella of all the organisms employed in this study, detached from cells by mechanical means, or as a result of autolysis, or of lysis by bacteriophage exhibit material attached to the base of the proximal hook (Fig. 12, 18 to 21, 24, 25, 27 to 29, 31 and 32). This material was seen in some but not all of the preparations, and its shape and size vary among flagella present in the same preparation as well as among different preparations of flagella from the same or different organisms; it is either spherical, mushroom-shaped, or disc-shaped. Frequently, membranous material, which can be identified by its characteristic fine structure to be in part cytoplasmic membrane, is attached to the proximal end of the flagellum (Fig. 18a, d, f, g, i, k and l). This element, located at the attachment site of the flagellum, sometimes appears more electron-lucid than the surrounding material (Fig. 18a, b, d, f, i, and l); on rare occasions, such elements appear to have structural identity (Fig. 28g). However, in other cases this membranous material does not show any differentiation (Fig. 18c and h). The heterogeneity of the material associated with the bases of flagella of *B. stearothermophilus* 194 is illustrated in Fig. 18 and 19. The disc-shaped basal complex of the flagellum is seen more frequently in preparations of *B. pumilus* and *B. circulans* (Fig. 28 and 32) than in preparations of flagella of other organisms.

It was observed that the number of detached flagella that have material attached to their proximal ends can be increased if these organelles are mechanically detached from cells in the stationary rather than exponential phase of growth or are obtained from phage-lysed cells. Apparently, the cell wall is partially digested during autolysis or lysis by bacteriophage, and membrane fragments remain connected to the detached flagella. On the other hand, in cells from cultures that are in the exponential phase of growth, the flagella tend to separate more cleanly from the cells.

From the observations noted thus far, it is not clear whether the material at the base of the flagellum is a separate structural entity. It might be a portion of the cytoplasmic membrane that assumes different shapes when it folds around the proximal end of the flagellum. This structure can be identified only when it is associated with the base of the flagellum, since it possesses no distinct morphological features of its own.

Attempts were made to correlate the origin of flagella in cells with sites at which the reduction of potassium tellurite occurs. The deposition of reduced tellurite had been used previously to indicate sites of respiratory enzyme systems (4, 10, 13–15). van Iterson and Leene (13) suggested that the thin rodlike crystals of reduced tellurite at the cell periphery may be the sites from which the flagella emerge. The enzymatic nature of the reduction of potassium tellurite was confirmed by our observation that the optimal temperature for this reaction in mesophilic organisms was 35 to 40 C, whereas it was 55 to 65 C in the case of the thermophilic organisms. No reduction occurred at 55 and 26 C in the case of mesophiles and thermophiles, respectively. The cells that were incubated in the presence of tellurite were negatively stained. Figures 16 and 17 show cells of *B. stearothermophilus* 2184 that were incubated at 58 C in the presence of potassium tellurite for various periods of time. They are representative of results obtained when other organisms were used. However, the number, shape, and size of the crystals of reduced tellurite are not constant from one organism to another, and in different preparations of one strain (Fig. 16 and 17). Incubation for 4 hr in the presence of tellurite results in an accumulation of many elongated reduced tellurite crystals within the protoplast (Fig. 16). Few details within the protoplasts can be seen because of the size and number of the crystals. Many cells in these suspensions are ruptured and appear as ghosts (Fig. 1 to 3). In the case of shorter incuba-

tion periods (1 to 2 hr), fewer or smaller crystals are present, and the intracellular infoldings of the cytoplasmic membrane can easily be distinguished (Fig. 17a, b, c). Because of the size of the reduced tellurite crystals, the accurate localization of the reducing activity within the cells is not possible. The few flagella that can be seen attached to treated cells (Fig. 17a, b, c) originate in areas of the protoplast that are devoid of such crystals. Although the reduced tellurite crystals adhere to the cytoplasmic membrane that is extruded from ruptured cells (Fig. 2), in intact cells they are scattered irregularly all over the protoplast; also, in intact cells they rarely are observed to be associated with the mesosomes (Fig. 17a, b, c). However, it is possible that a small amount of reduced tellurite may be associated with the mesosome and can be visualized in sectioned preparations as was demonstrated by van Iterson and Leene (13).

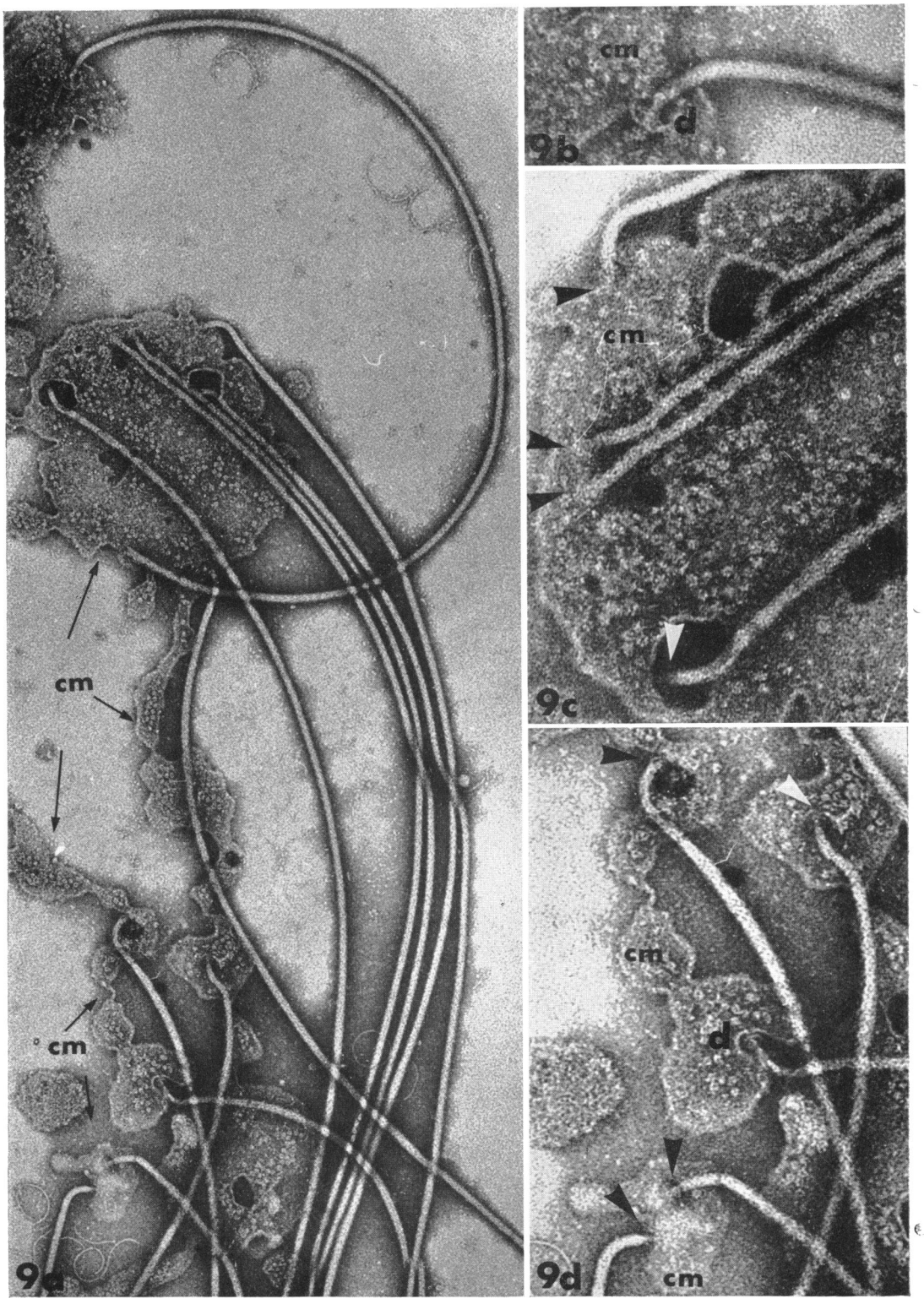
Structure of the flagella. Terminal hooks were previously described in shadow-cast (6, 7) and in negatively stained (5, 9, 11) preparations. The differentiation in fine structure of the flagellum into a proximal hook region and its continuation, the filament, was shown in flagella from cells of *P. vulgaris* (3). Such differentiation, both in morphology and in fine structure, was observed in the flagella of all strains of bacilli employed in this study (Fig. 18, 19, and 21 to 32). The following differences in the fine structure of intact flagella from the various organisms were also observed (Fig. 18, 23 and 27).

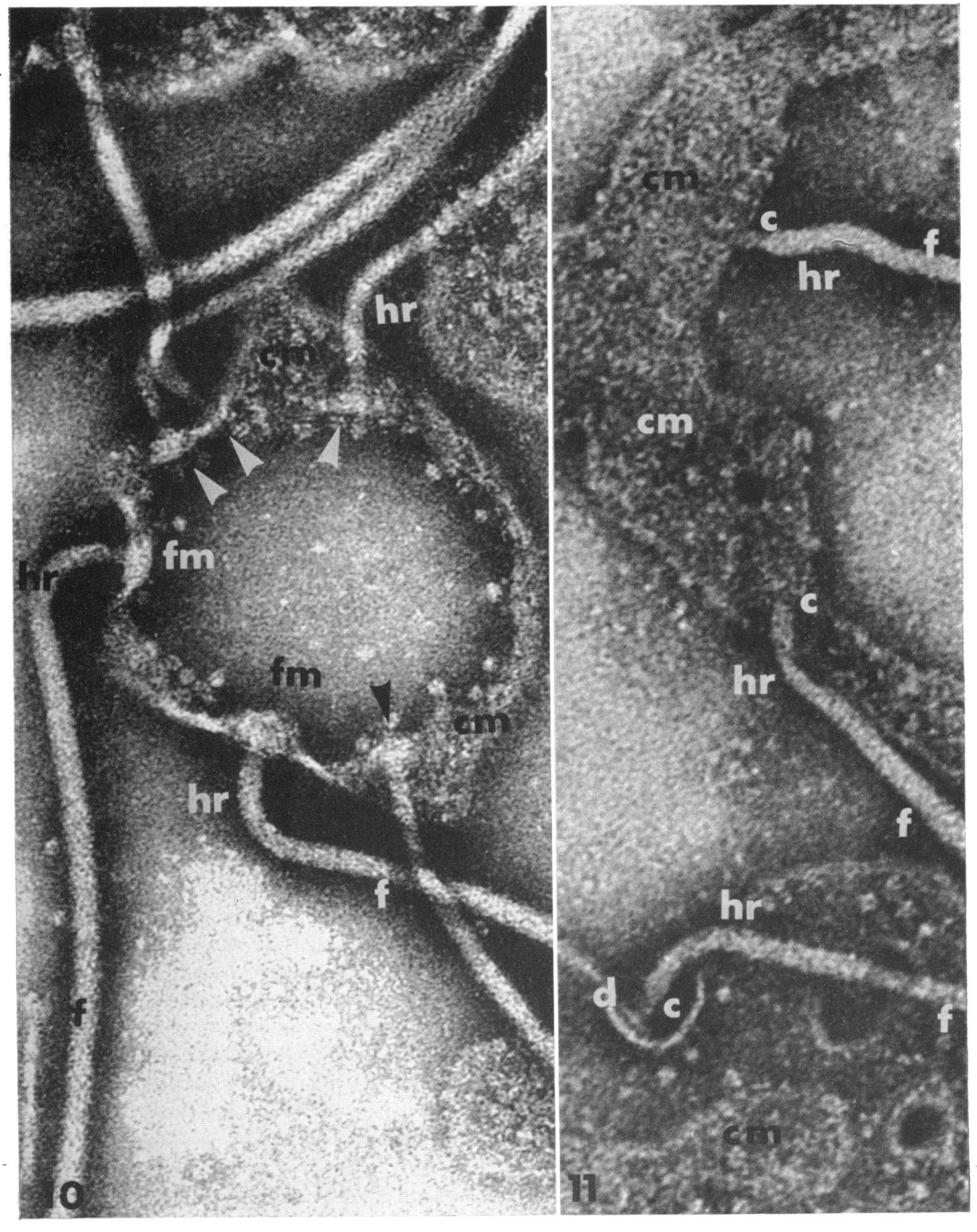
(i) In flagella of *B. stearothermophilus* 194 (Fig. 19 and 21), the diameter of the filament is 125 to 145 A, and its edges are somewhat diffuse. An ordered pattern of subunits, 30 to 40 A in diameter, results in an oblique array that suggests a spirally arranged filament. The diameter of the hook, 110 to 120 A, is smaller than that of the filament; its edges are smooth and the subunits are not arranged in a distinct pattern. The fine structure characteristic to the proximal hook region extends into the spiral portion of the filament, and the length of this region is from 50 to 150 mμ; it varies in different flagella, even when they originate from the same cell (Fig. 10).

(ii) Flagella from cells of *B. stearothermophilus* 2184 show two flagellar structures (Fig. 22 to 26). The filament of a typical flagellum is 150 to 160 A in diameter with a finely granular surface free from appendages. In contrast, portions of the filament are 300 to 350 A in diameter and seem to consist of a central filament, 100 to 120 A in diameter, surrounded by a mat of fine fibers, 10 to 20 A in diameter, which are helically arranged around the central filament. The helix is right-handed (Fig. 22) and usually cannot be followed for more than several turns. The most common arrangement of the fibers around the central filament, as judged by stereoelectron microscopy, appears to be that of many short frayed ends. The change in the diameter of the filament that occurs at the point where the surrounding mat begins or ends can be readily observed (Fig. 22, 24, and 25a and c). The matted region of the filament is usually basal, starting just beyond the bend of the hook, which is smooth and has a diameter of 120 to 130 A. This region varies in length, extending along the filament for distances of less than 0.1 μ to more than 1 μ. Rarely, a short matted region occurs at nonbasal locations (Fig. 26). The two flagellar structures have been observed in purified flagellar isolates as well as on flagella attached to cells. Whether the enlarged matted region of the filament represents disintegration or assembly, or whether it might be related to the movement of the flagella, is not known. The differentiated regions of the flagella of *B. stearothermophilus* 194 and 2184 can be seen in preparations stained negatively with either PTA or uranyl acetate (Fig. 18 to 25).

(iii) The differentiation of the flagellum into two regions is supported by the presence of a unique basal structure that is limited to the hook in flagella of *B. brevis* and *B. circulans* (Fig. 6, 7, and 29 to 32). In the flagella of *B. brevis*, this structure was seen only when the organelles were attached to ghost cells (Fig. 6 and 7), but was never present on detached flagella. In the case of the flagella of *B. circulans*, on the other hand, this structure is present regardless of whether the flagella are detached (Fig. 32) or still attached to ghost cells (Fig. 29) or membrane fragments (Fig. 30 and 31). This element is cross-banded and has been observed to exist in various states of

FIG. 9–11. *Micrographs from a preparation similar to the one shown in Fig. 8. In this specimen many of the flagella (arrows) that are attached to the cytoplasmic membrane (cm) do not originate at sites that are morphologically distinct (Fig. 9 and 11). Other flagella attached to a circular fragment of membrane (cm) in Fig. 10 originate from spherical or mushroom-shaped structures; these might result from a folding of the membrane (fm). The proximal ends of some flagella are attenuated, as if the point of attachment has been stretched (c). The proximal end of several flagella is separated from the cytoplasmic membrane by a thin disc plate (d) (Fig. 9b and d, and 11). Notice the differentiated regions of the flagella (hook, hr; filament, f) and the fine granular texture of the membrane. Negatively stained with PTA. Figure 9a, × 92,000; Fig. 9b and c, × 220,000; Fig. 9d, × 160,000; Fig. 10, × 230,000; Fig. 11, × 340,000.*


cm
cm
9a
cm
d
9b
cm
9c
cm
d
9d
cm


hr
cm
fm
hr
fm
cm
hr
f
f
10
cm
c
f
hr
cm
c
hr
f
hr
d
c
f
cm
11

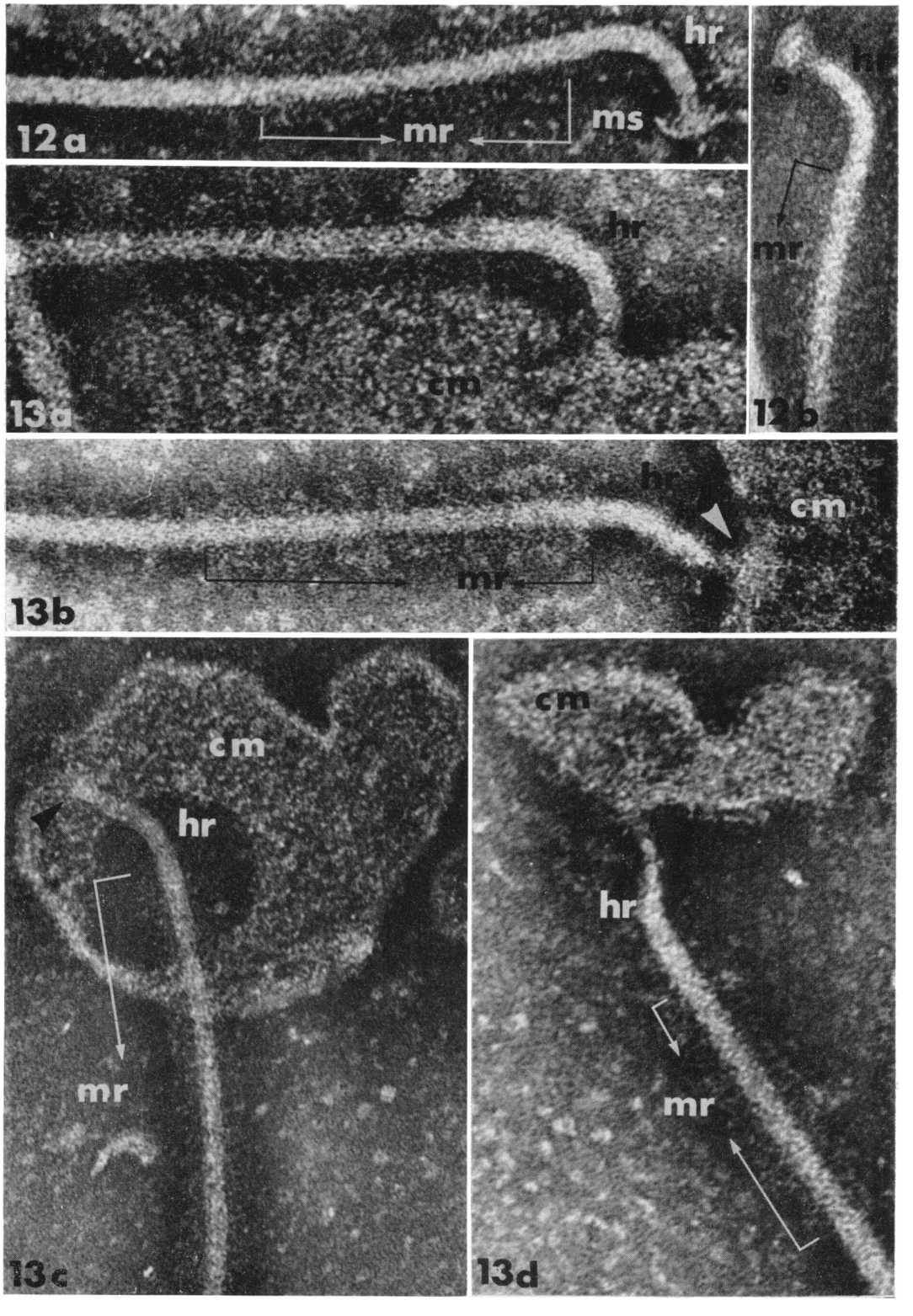


FIG. 12 and 13. *Flagella of Bacillus stearothermophilus 2184 from cultures in the stationary phase of growth. Figures 12a and b show detached flagella having spherical (s) or mushroom-shaped (ms) structures at their proximal ends. Figures 13a to d show the attachment of flagella to fragments of cytoplasmic membrane (cm). In Fig. 13b and c, electron-lucid material of varying appearance is present at the connecting sites between flagella and the membrane (arrows); in Fig. 13a and d, such material is less clearly defined or absent. In Fig. 13d the membrane and the hook appear stretched. Notice the region of the filament showing a diffused matlike surface structure (mr) that starts beyond the proximal hook (hr). Negatively stained with PTA. Figures 12a, b, and Fig. 13a, b, and d, × 350,000; Fig. 13c, × 284,000.*

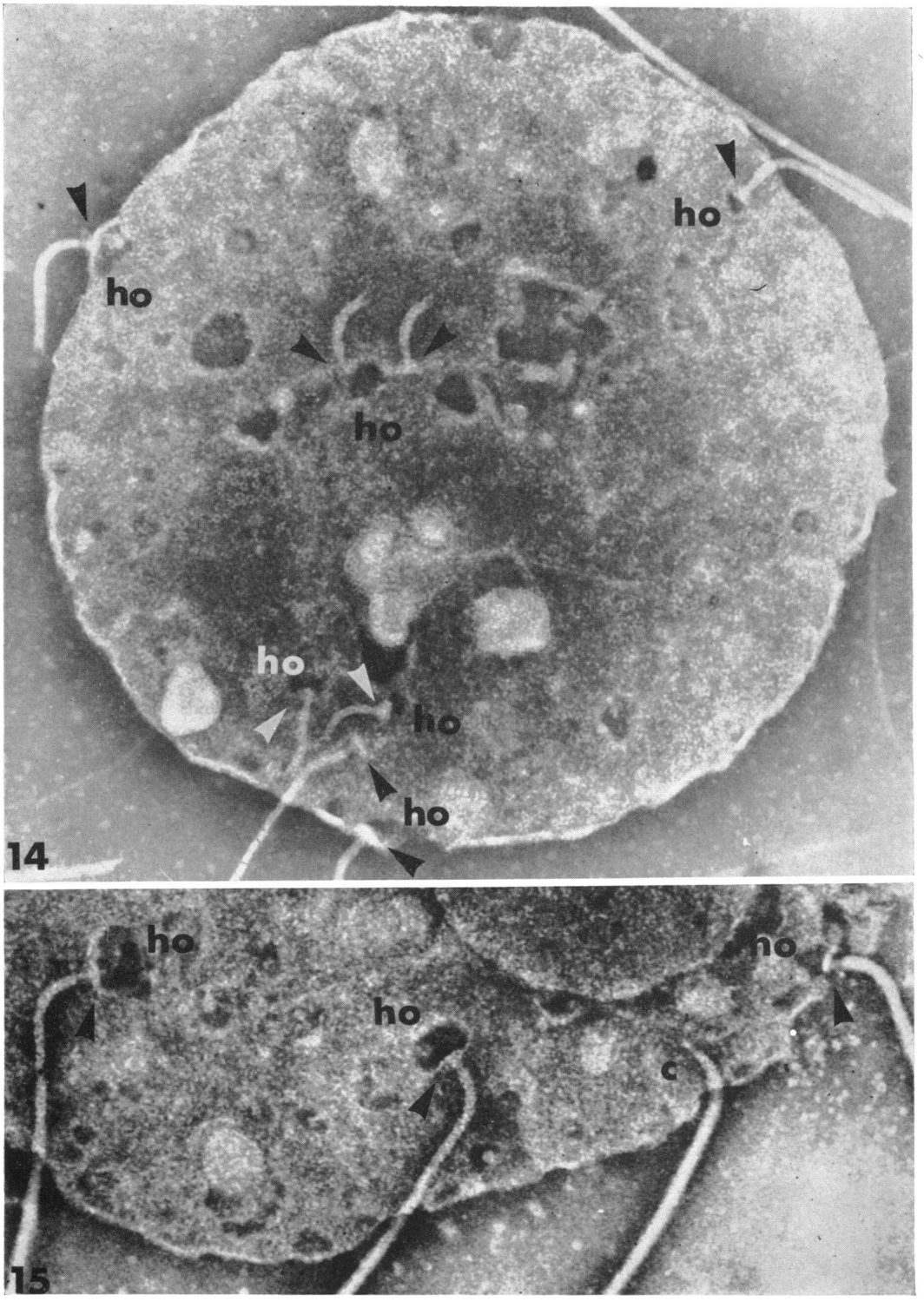
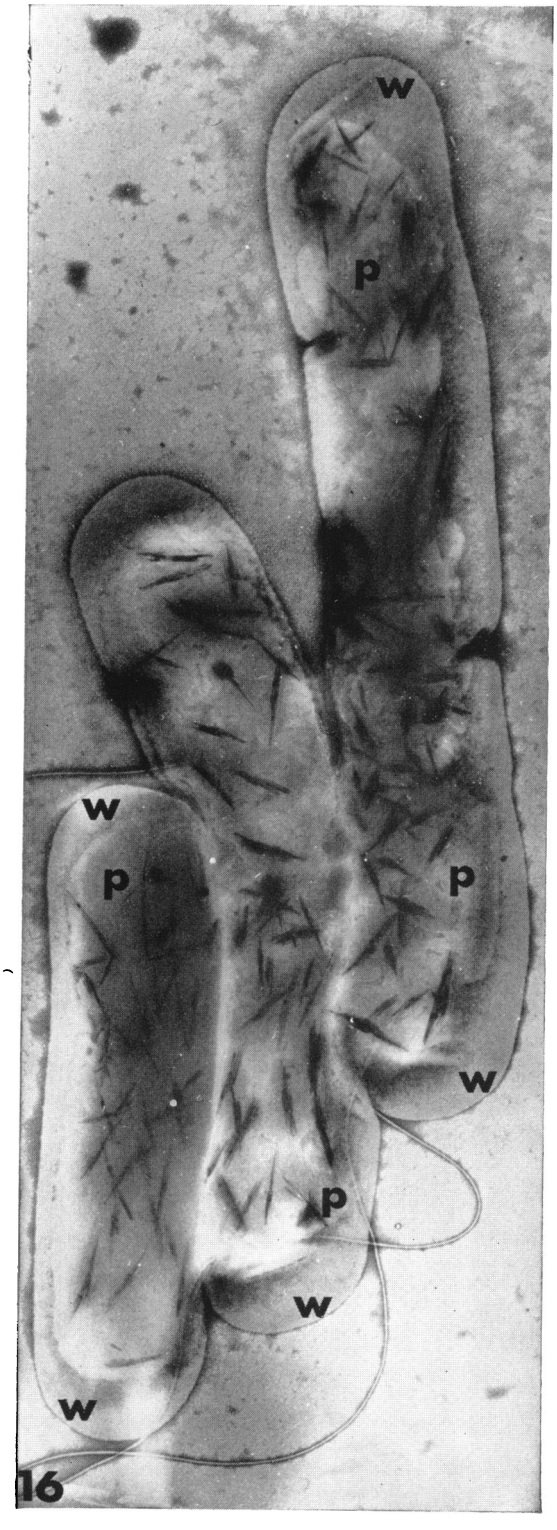


FIG. 14 and 15. *Flagella attached to fragments of cytoplasmic membrane in suspensions of cells prepared from cultures of Bacillus pumilus that were chilled for 6 days at their early exponential phase of growth. Holes or tears of the cytoplasmic membrane (ho) are present at the base of many of the flagella, which originate in disc- or mushroom-shaped structures (arrows). These structures may result from the folding of the disrupted membrane. Notice the flagellum in Fig. 15 that is attached to intact membrane at the constricted end (c) of its proximal hook and does not appear to originate in morphologically distinct material. Negatively stained with PTA. Figure 14, × 140,000; Fig. 15, × 180,000.*



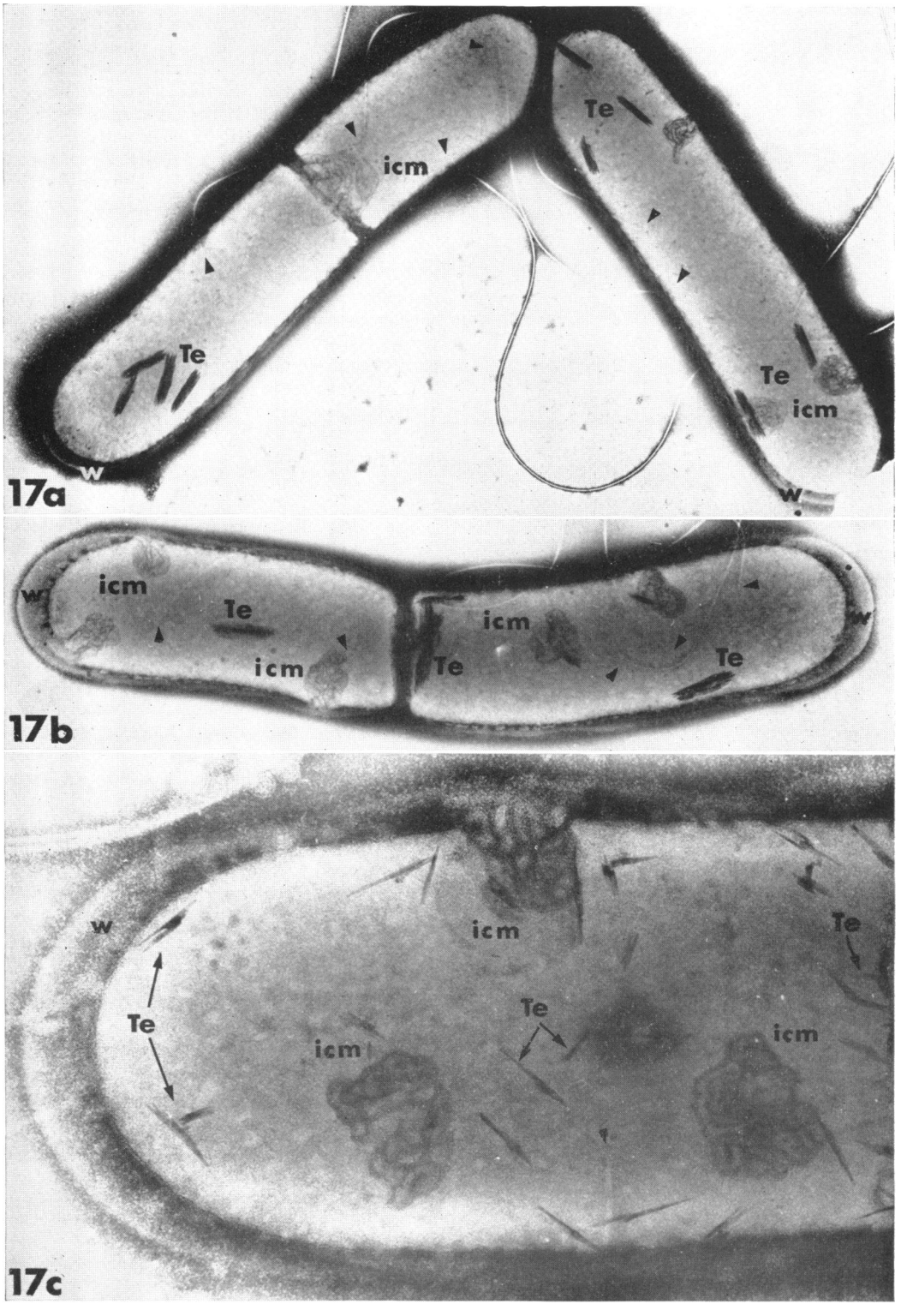
integrity (Fig. 32a to o). When intact, it shows six bands or levels (Fig. 32a to f). Various states of disorganization of this element can be seen in Fig. 32g, h, i, and m. Sometimes this structure appears so dispersed that the cross bands can no longer be distinguished (Fig. 32k and l). Only a few hooks of detached flagella of *B. circulans* are free from this structure (Fig. 32n and o); that this may be a single element which separates from the filament is suggested in Fig. 32i.

(iv) The differentiation into the hook region and the filament in flagella of *B. pumilus* is less distinct; however, the hook has a diameter slightly smaller than that of the filament (100 to 110 A versus 110 to 120 A), and a slight constriction marks the beginning of the filament (Fig. 27 and 28). Although no function can as yet be ascribed to the hook or basal complex, it is an element that can be characterized on the basis of morphology and fine structure.

DISCUSSION

The observations noted in this paper add to those previously discussed (3) concerning the intracellular origin of flagella in cells of various bacilli, and contribute evidence for their attachment to the cytoplasmic membrane. In contrast to cells of *P. vulgaris* and other gram-negative organisms, the cytoplasmic membrane of the bacilli can be distinguished from the cell wall not only when it is present inside ghost cells but also when separated from the cell wall. Flagella can be observed to be connected to this membrane when attached either to ghost cells or to membrane fragments. Furthermore, material associated with the proximal ends of detached flagella can be identified to be constituted in part of cytoplasmic membrane.

FIG. 16 and 17. *Cells of Bacillus stearothermophilus 2184 from a culture that was incubated with 0.05% potassium tellurite for 4 hr at 55 C are shown in Fig. 16. The elongated crystals of reduced tellurite are present inside the plasmolyzed protoplast (p). The sites at which the flagella originate from the cells cannot be detected. After incubation for 2 hr at 55 C with 0.05% tellurite (Fig. 17a to c), the cells appear to be less damaged, and infolded cytoplasmic membranes (icm) or mesosomes are easily identified; they are seen through the relatively electron-transparent cell wall (w). A few large (Fig. 17a, b) or many small (Fig. 17c) elongated crystals of reduced tellurite (Te) are seen inside the protoplasts. They show no specific association with the mesosomes. Most of the flagella that are attached to the cells originate in areas of the protoplast devoid of reduced tellurite deposits (arrows). Negatively stained with PTA. Figure 16, × 50,000; Fig. 17a, × 34,000; Fig. 17b, × 40,000; Fig. 17c, × 120,000.*


Te
icm
Te
Te
icm
W
W
17a
W
icm
Te
icm
icm
Te
Te
W
17b
W
Te
icm
Te
Te
icm
icm
Te
icm
17c

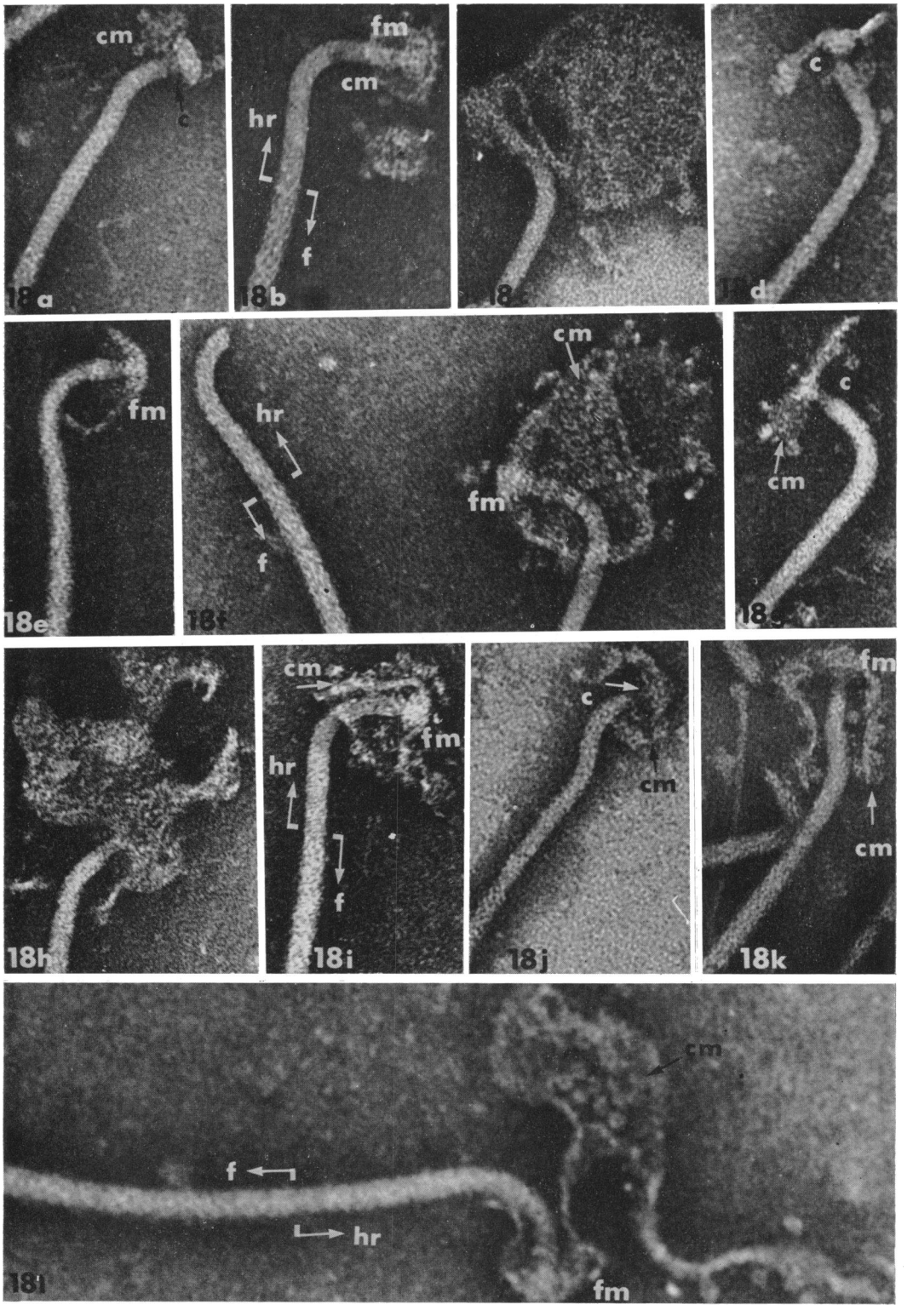


FIG. 18. *A few flagella of Bacillus stearothermophilus 194 detached from cells mechanically or as a result of lysis by phage infection. The membranous material attached to the proximal ends of the hooks is heterogeneous both in shape and size. Often it can be identified by its characteristic fine structure to consist, at least in part, of cytoplasmic membrane (cm). In some cases, it appears that the electron-lucid material at the end of the hook results from folding of the membrane (fm) around the proximal end of the flagella. Notice the constriction (c) at the end of the hooks, and differences in the fine structure of the hook region (hr) and the filament (f). Negatively stained with PTA. Figure 18a to k, × 250,000; Fig. 18l, × 360,000.*

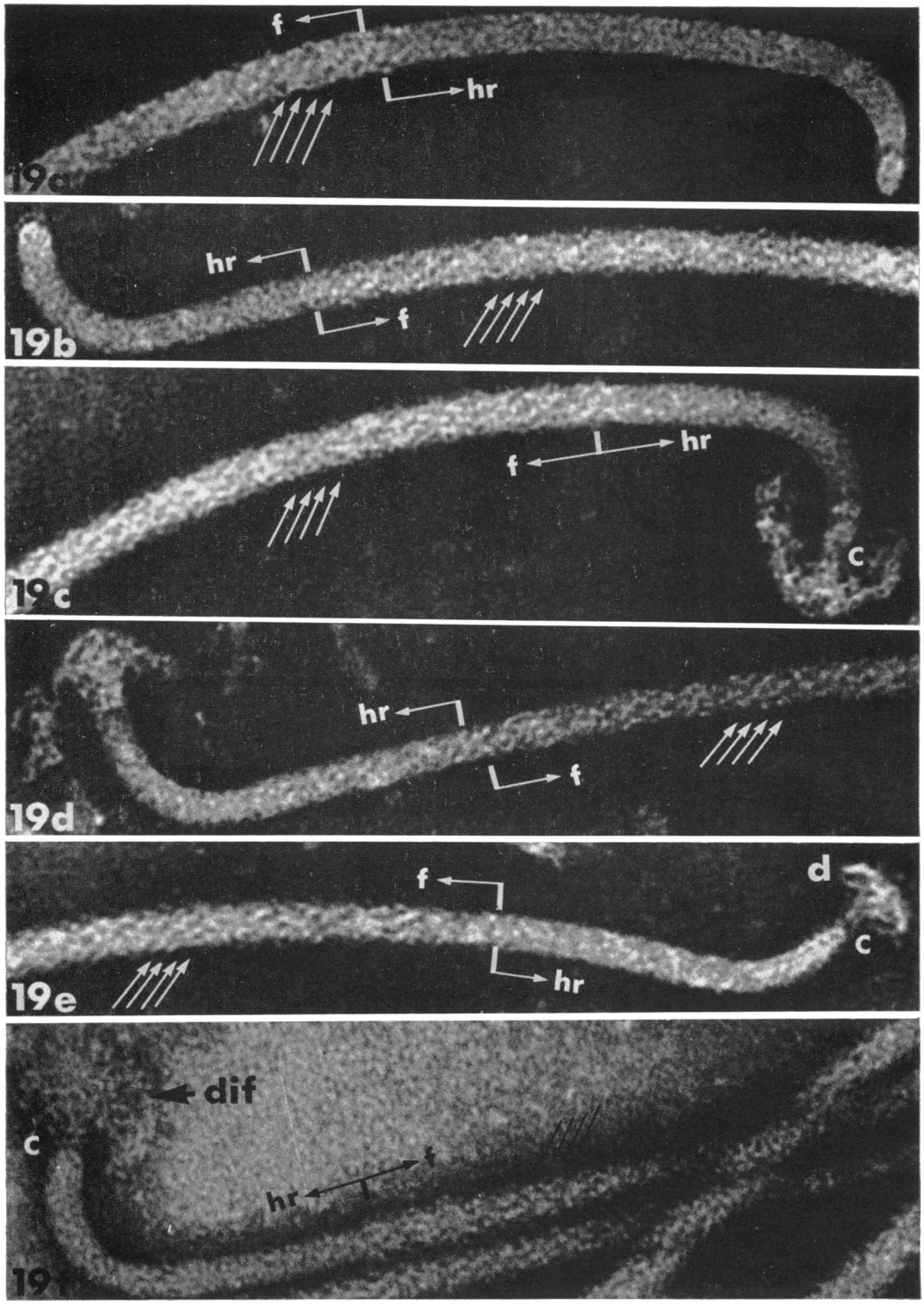
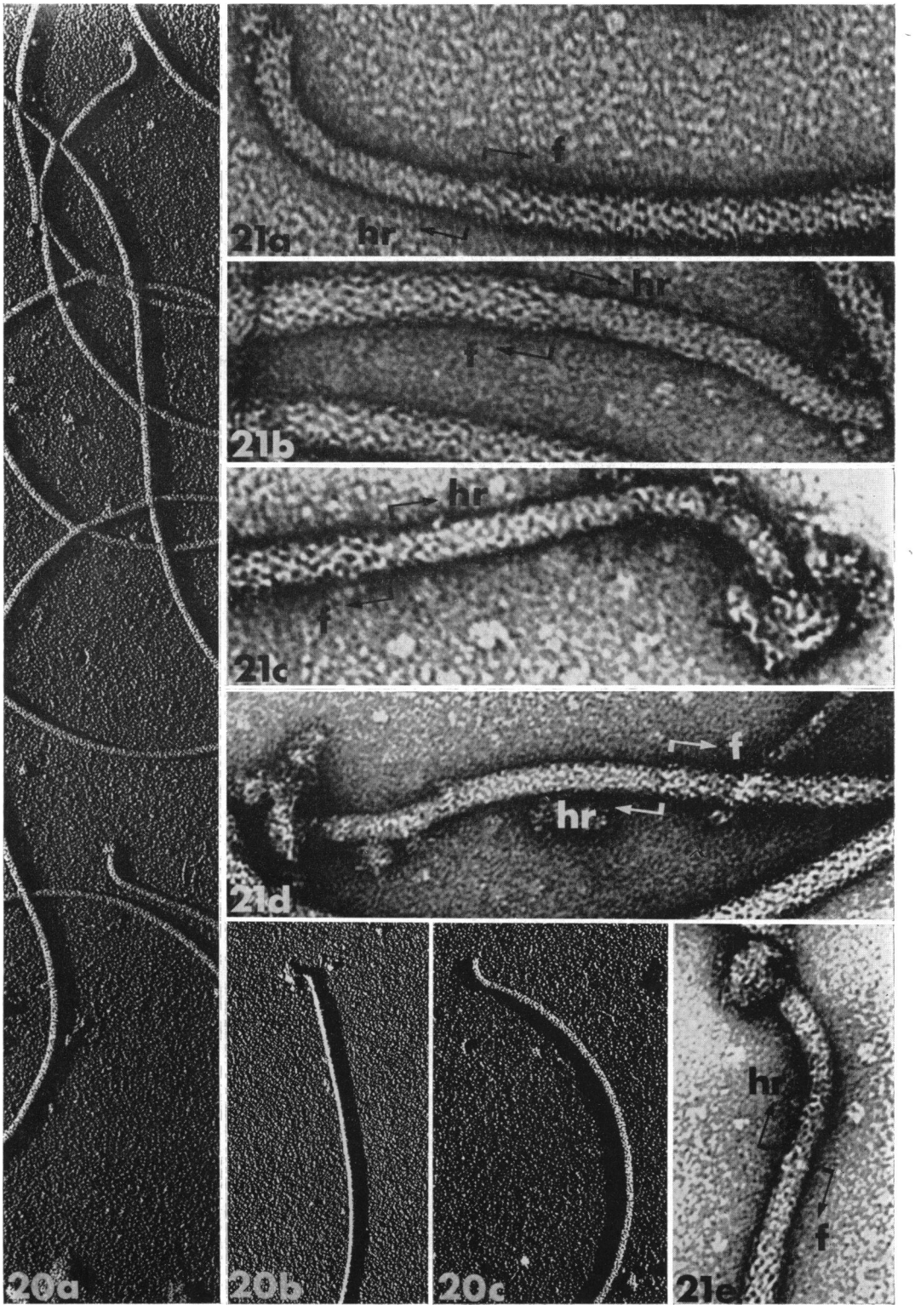
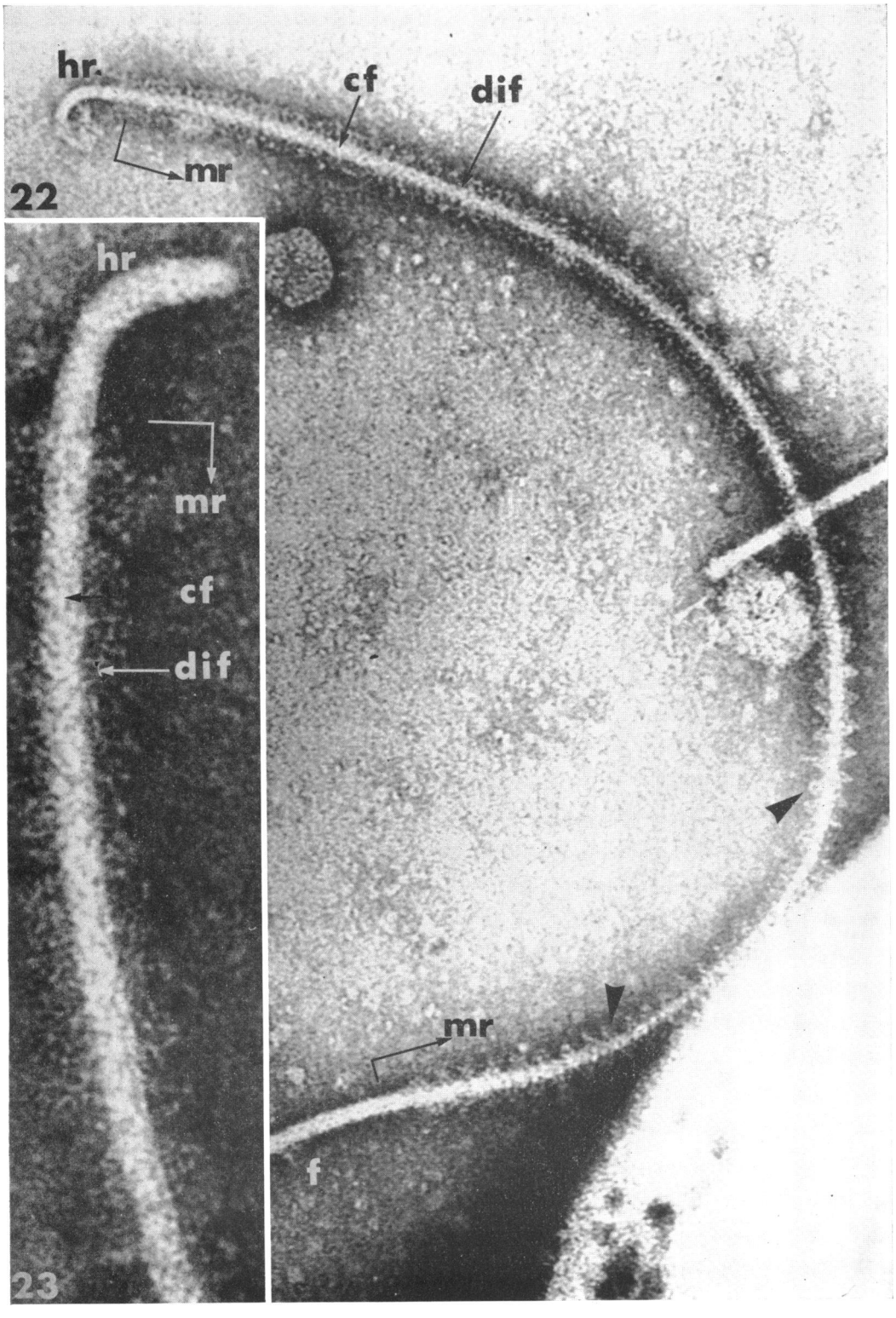


FIG. 19–21. *Detached flagella of Bacillus stearothermophilus 194 from preparations similar to the ones shown in Fig. 18. The micrographs shown in Fig. 19 and 21 are at a higher magnification, and reveal the fine structure of the differentiated regions of the flagella: the proximal hook region (hr) and the filament (f). In Fig. 19 the oblique direction of the subunits on the filament is marked with arrows. Note that the length of the hook region (hr) may vary; also note the constriction (c) at the end of the hook and the material attached to the base of the flagella, especially the disc-shaped (d) structure in Fig. 19c, and the diffuse (dif) appearance of the attached material in Fig. 19f. The shadow-cast preparations show differentiation in morphology of the flagella but not in their fine structure. The observations made with specimens stained with PTA and uranyl acetate are in good agreement. Figures 19 and 21 are negatively stained with PTA and uranyl acetate, respectively. Figure 20 is shadow-cast with palladium. Figure 19, × 500,000; Fig. 20a and c, × 92,000; Fig. 20b, × 56,000; Fig. 21a to c, × 500,000; Fig. 21d and e, × 350,000.*


f
hr
21a
hr
f
21b
hr
f
21c
f
hr
21d
20a
20b
20c
hr
f
21e


hr.
cf
dif
mr
22
hr
mr
cf
dif
23
mr
f

The existence of specialized structures at the proximal ends of flagella, analogous to the basal structures of flagella of eucaryotic organisms, cannot be established unequivocally. The material associated with the proximal ends of the flagella either attached to ghosts or membrane fragments, as well as of detached flagella, appears to be heterogeneous. Doubtlessly, a portion of this material is cytoplasmic membrane, and its shape is caused by the folding of the membrane around the site of attachment of the flagellum. Whether part of this electron-lucid, mushroom- or disc-shaped material is a true structural entity cannot be established from the data available. The possibility exists that this structure, if it is a real entity, is unstable under the experimental conditions employed, and therefore not always observable. Yet, the fact that this structure is more easily demonstrable in cases of greater cytolysis speaks against such a possibility. Since it has not been possible to ascribe specific morphological characteristics to it, this material cannot be identified when separated from the flagellum. Spherical structures, 110 to 140 A in diameter, which were observed at the base of flagella of *P. vulgaris*, were not seen at the bases of the flagella of the bacilli. However, in cells of *P. vulgaris*, the structures always were observed in instances in which flagella were attached to ghosts or to cell wall-membrane complexes, and may therefore be the result of the close association of the wall and membrane.

The functional relationship, if any exists, between cytoplasmic membrane and the flagellum is still unknown. Attempts to correlate the sites of origin of flagella on the membrane with sites of reducing activity by observing the deposition of crystals of reduced tellurite failed. These crystals accumulate inside the protoplast and were not observed to be associated with mesosomes, which have been suggested to be the sites of respiratory enzymes (8, 13). The deposits were too large to serve as sensitive indicators of reducing sites on the cytoplasmic membrane, but they did adhere to the membrane when it was extruded from ruptured cells. Even then the sites at which flagella were attached were devoid of crystals of reduced tellurite; this is contrary to the suggestion by van Iterson and Leene (13) that reduced tellurite crystals accumulate near sites from which flagella emerge.

Differences in the fine structure of intact flagella from the various organisms have been investigated by high-resolution electron microscopy. The flagella of all the bacilli studied exhibit differentiated regions, which can be identified on the basis of their fine structure, namely, a proximal hook and the filament. This is in agreement with previous results (3) showing different patterns of the fine structure in comparable regions in flagella of *P. vulgaris*. As will be presented more fully in a separate communication, the flagellar hooks of certain bacilli are more stable to acid, alcohol, or heat than are the spiral filaments; this difference in stability increases confidence in the reality of the hooks and serves as a basis for separating them from the spiral filaments. Two regions having different fine structure in the filament of *B. stearothermophilus* 2184, and a specialized surface structure or basal complex associated with the hooks of the flagella of *B. brevis* and *B. circulans*, which appear as six cross bands, were shown. The function of the hooks and these specialized structures is not understood. However, these observations may open new approaches toward investigating the corrrelation between molecular architecture and the function of bacterial flagella.

ACKNOWLEDGMENTS

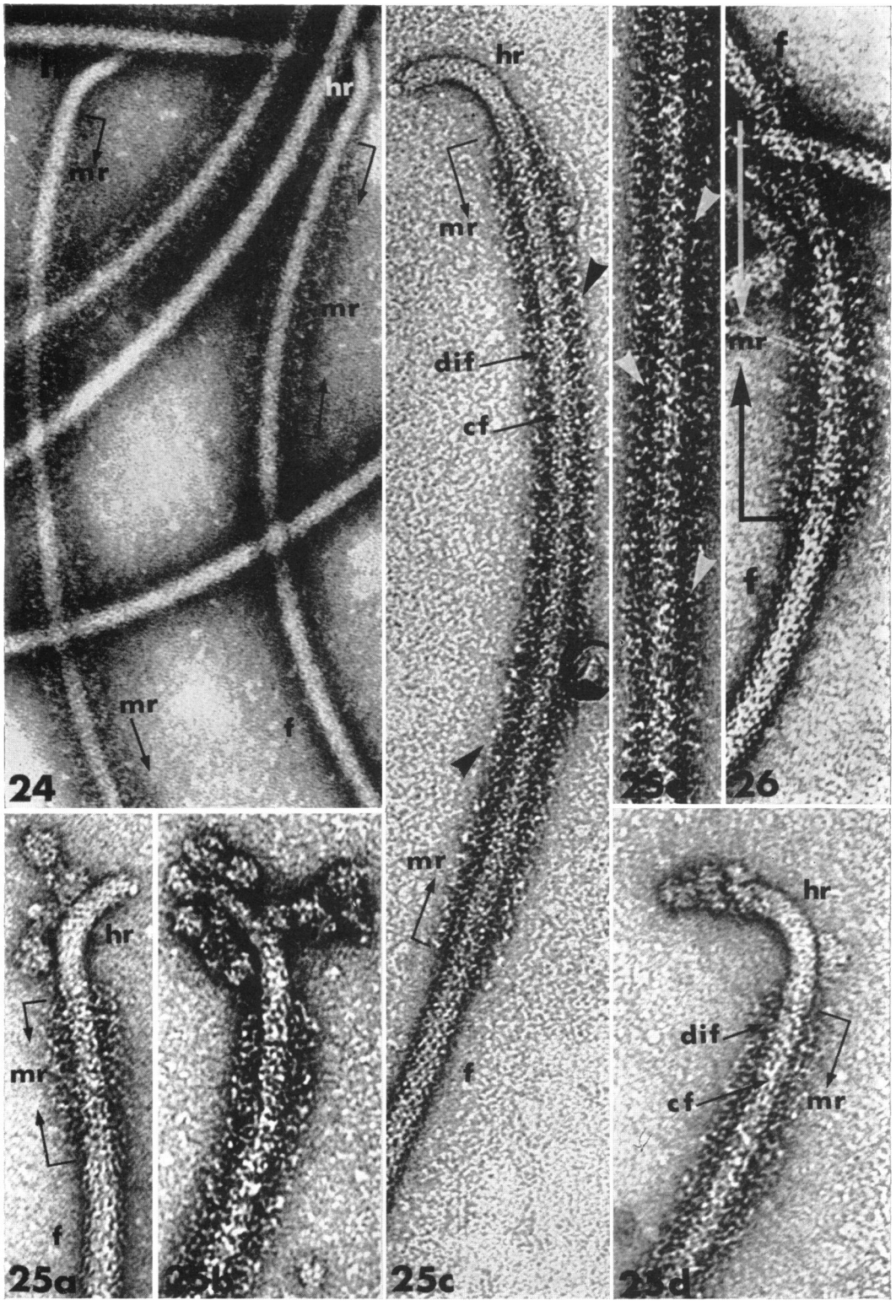
We acknowledge the capable technical assistance of Florence Shen and the help of Estelle Beihl in preparing the photographic plates.

This investigation was supported by Public Health Service grant AI-00685 from the National Institute of Allergy and Infectious Diseases and by National Science Foundation grant G-24237.

LITERATURE CITED

1. ABRAM, D. 1965. Electron microscope observations on intact cells, protoplasts, and the cytoplasmic membrane of *Bacillus stearothermophilus*. J. Bacteriol. **89**:855–873.
2. ABRAM, D., AND H. KOFFLER. 1964. *In vitro* forma-

FIG. 22–26. *Isolated flagella of Bacillus stearothermophilus 2184 showing the fine structure of the organelle. The proximal hook (hr), 120 to 130 A in diameter, is short and has smooth edges. The portion (mr) of the filament that follows the bend of the hook has a diameter nearly three times that of the hook (300 to 350 A); it consists of a central filament (cf), 100 to 120 A in diameter, surrounded by a diffuse (dif) mat of fine fibers. In certain instances, a single fiber may be observed to form a helical structure around the filament (arrows, Fig. 22 and 25c and e). The length of the matted region varies. The diameter of the filament (f) beyond the matted region is 150 to 160A, and its edges are not dispersed. Notice the material attached to the proximal hook of the flagella in Fig. 25b and d. Figure 26 shows a rare case of a short matted region (mr) that is not basal. There is good agreement between observations on specimens stained negatively with PTA (Fig. 22 to 24) and uranyl acetate (Fig. 25a to e and 26). Figure 22, × 200,000; Fig. 23, × 500,000; Fig. 24, × 194,000; Fig. 25a to e and 26, × 350,000.*


hr
mr
mr
mr
f
24
hr
mr
dif
cf
mr
f
25c
f
mr
26
f
hr
mr
f
25a
hr
dif
cf
mr
25d

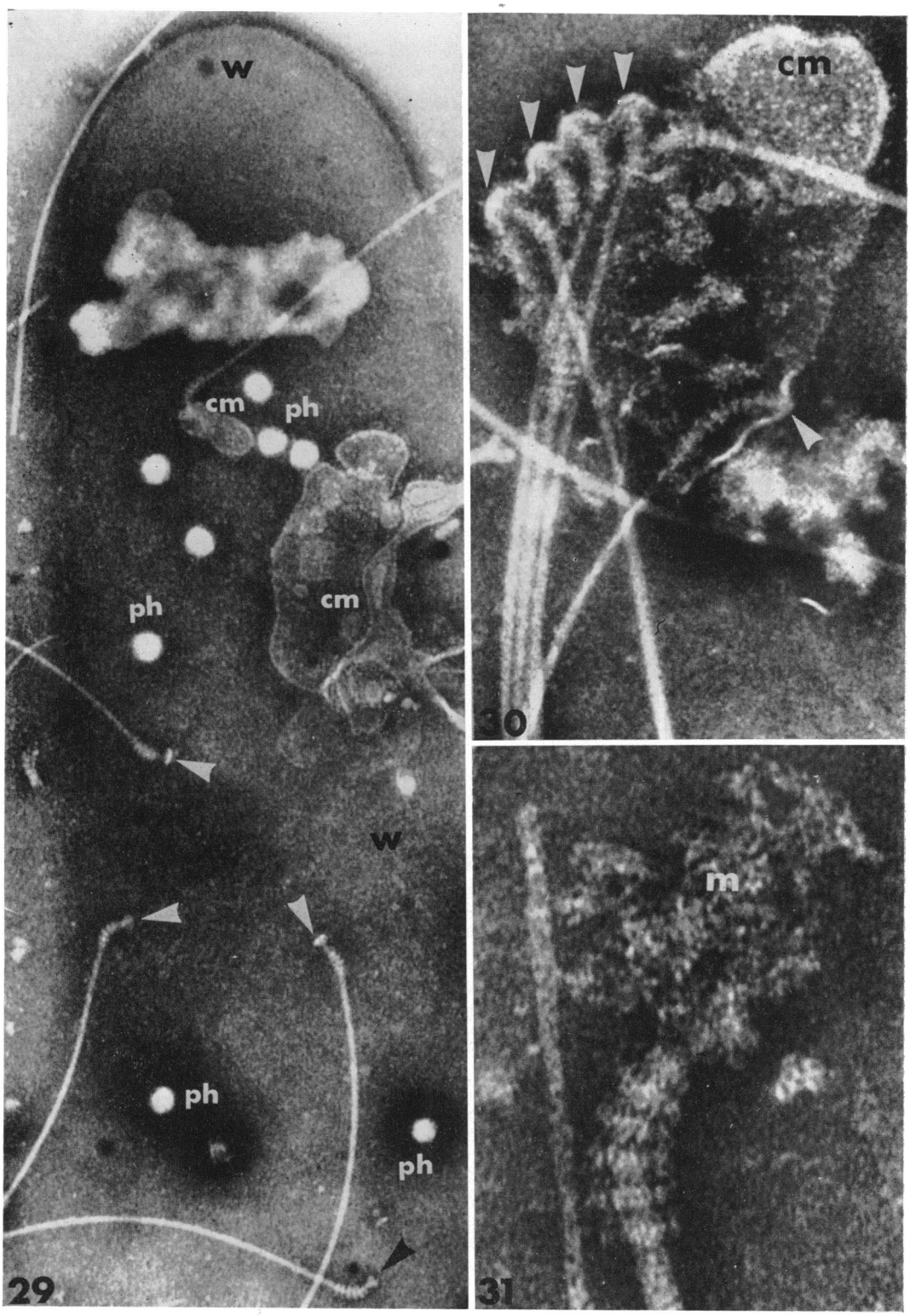


FIG. 29. *Phase-lysed ghost cell of Bacillus circulans. The flagella attached to the cell wall originate in mushroom- or disc-shaped structures (arrows); the structure associated with the hook appears to be a series of cross-banded elements. One flagellum is attached to a small fragment of cytoplasmic membrane (cm) still inside the cell wall (w). Notice the round phage particles (ph) inside the ghost. Negatively stained with PTA.* × *70,000.*

FIG. 30 and 31. *A few flagella attached to a fragment of cytoplasmic membrane (cm) in a suspension of cells from a phage-lysed culture of Bacillus circulans are seen in Fig. 30. The flagella that are attached to the edge of the membrane fragment originate in disc- or mushroom-shaped structure (arrows). A flagellum attached to dispersed membranous (m) material is seen in Fig. 31. Notice the cross-banded structure associated with the hooks. Negatively stained with PTA. Fig. 30,* × *125,000; Fig. 31,* × *500,000.*

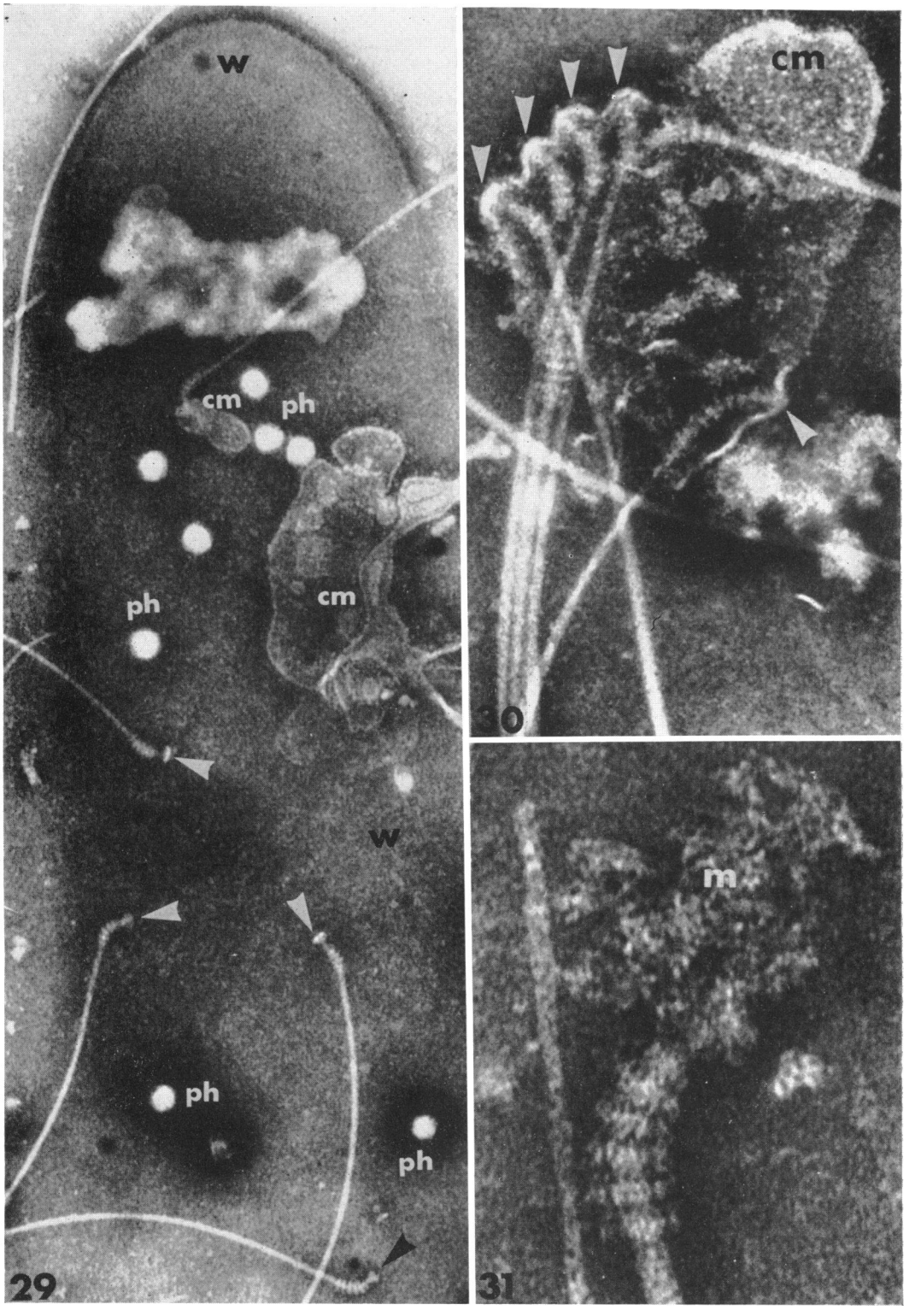


FIG. 29. *Phase-lysed ghost cell of Bacillus circulans. The flagella attached to the cell wall originate in mushroom- or disc-shaped structures (arrows); the structure associated with the hook appears to be a series of cross-banded elements. One flagellum is attached to a small fragment of cytoplasmic membrane (cm) still inside the cell wall (w). Notice the round phage particles (ph) inside the ghost. Negatively stained with PTA.* × *70,000.*

FIG. 30 and 31. *A few flagella attached to a fragment of cytoplasmic membrane (cm) in a suspension of cells from a phage-lysed culture of Bacillus circulans are seen in Fig. 30. The flagella that are attached to the edge of the membrane fragment originate in disc- or mushroom-shaped structure (arrows). A flagellum attached to dispersed membranous (m) material is seen in Fig. 31. Notice the cross-banded structure associated with the hooks. Negatively stained with PTA. Fig. 30,* × *125,000; Fig. 31,* × *500,000.*

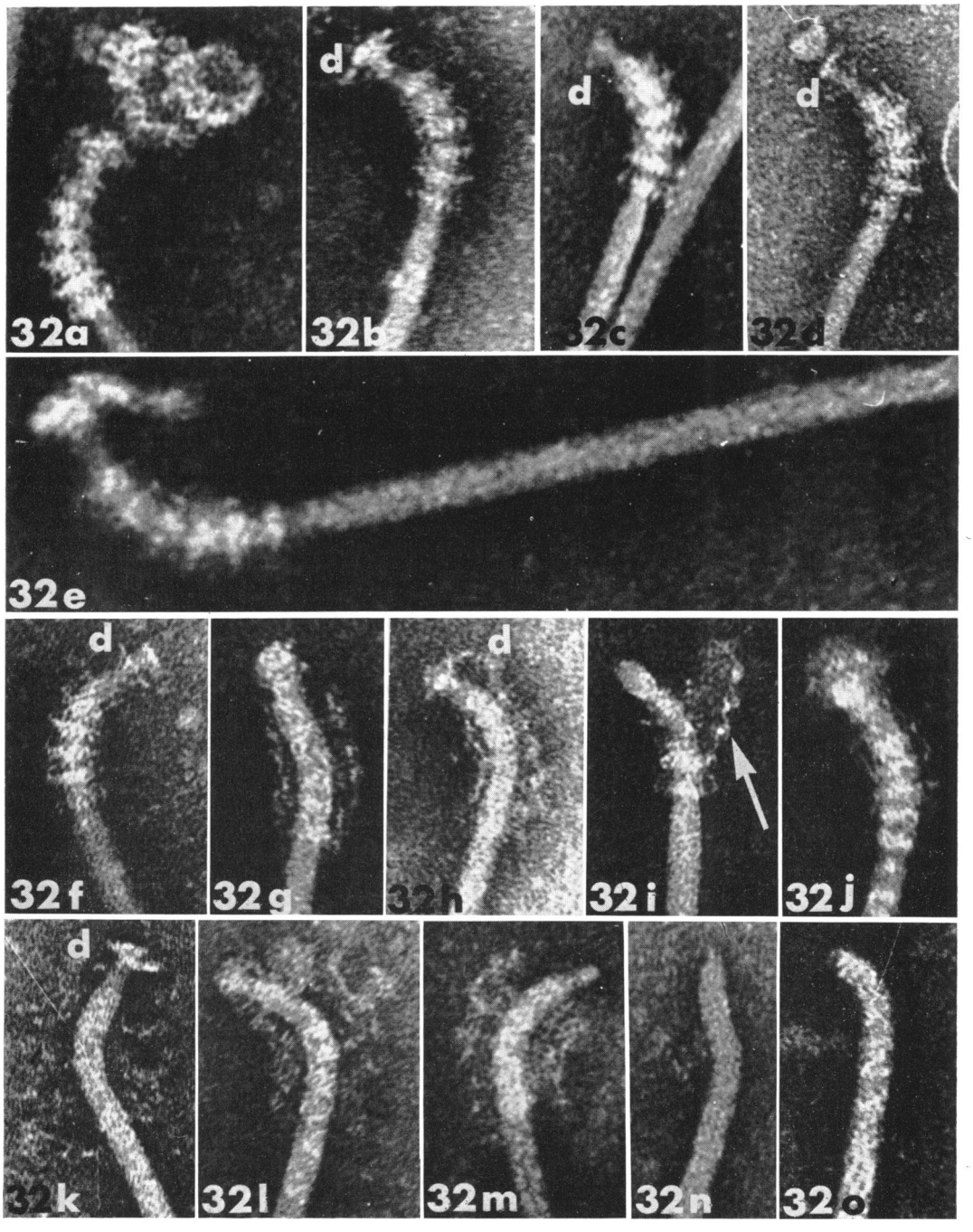


FIG. 32. *A few detached flagella of Bacillus circulans from either flagellar isolates or cells in the stationary phase of growth. The surface structure on the hooks is seen in various states of integrity. In Fig. 32a to f, the striated element is intact and appears to be composed of six bands. The cross bands appear partially disrupted in Fig. 32g, h, i, and m; they can no longer be recognized in Fig. 32k and l. In Fig. 32i, the surface structure appears to peel off the hook as a single unit (arrow), and flagella showing naked hooks are seen in Fig. 32n and o. The material attached to the proximal bases of the flagella shows heterogeneity both in shape and size; often it is disc-shaped (d); in other cases, it is rounded or mushroom-shaped, or it may appear as a larger membranous fragment (Fig. 32a). Negatively stained with PTA. Figures 32a to d and f to o, × 300,000; Fig. 32e, × 480,000.*

tion of flagella-like filaments and other structures from flagellin. J. Mol. Biol. **9**:168–185.

3. ABRAM, D., H. KOFFLER, AND A. E. VATTER. 1965. Basal structure and attachment of flagella in cells of *Proteus vulgaris*. J. Bacteriol. **90**:1337–1354.
4. BARRNETT, R. J., AND G. E. PALADE. 1957. Histochemical demonstrations of the sites of activity of dehydrogenase systems with the electron microscope. J. Biophys. Biochem. Cytol. **3**:577–588.
5. GLAUERT, A. M., D. KERRIDGE, AND R. W. HORNE. 1963. The fine structure and mode of attachment of the sheathed flagellum of *Vibrio metchnikovii*. J. Cell Biol. **18**:327–336.
6. HOUWINK, A. L. 1953. A macromolecular monolayer in the cell wall of *Spirillum* spec. Biochim. Biophys. Acta. **10**:360–366.
7. HOUWINK, A. L., AND W. VAN ITERSON. 1950. Electron microscopic observations on bacterial cytology II. A study on flagellation. Biochim. Biophys. Acta **5**:10–44.
8. HUGHES, D. E. 1962. The bacterial cytoplasmic membrane. J. Gen. Microbiol. **29**:39–46.
9. KERRIDGE, D., R. W. HORNE, AND A. M. GLAUERT. 1962. Structural components of flagella from *Salmonella typhimurium*. J. Mol. Biol. **4**: 227–238.
10. MUDD, S., K. TAKEYA, AND H. I. HENDERSON. 1956. Electron-scattering granules and reducing sites in mycobacteria. J. Bacteriol. **72**:767–783.
11. ROGERS, G. E., AND B. K. FILSHIE. 1963. Some aspects of the ultrastructure of α-keratin, bacterial flagella, and feather keratin, p. 123–138. *In* R. Borasky [ed.], Ultrastructure of protein fibers. Academic Press, Inc., New York.
12. SALTON, M. R. J. 1964. The bacterial cell wall. Elsevier Publishing Co., Amsterdam.
13. VAN ITERSON, W., AND W. LEENE. 1964. A cytological localization of reductive sites in a Gram-positive bacterium. Tellurite reduction in *Bacillus subtilis*. J. Cell Biol. **20**:361–375.
14. VAN ITERSON, W., AND W. LEENE. 1964. A cytological localization of reductive sites in a Gram-negative bacterium. Tellurite reduction in *Proteus vulgaris*. J. Cell Biol. **20**:377–387.
15. WACHSTEIN, M. 1949. Reduction of potassium tellurite by living tissues. Proc. Soc. Exptl. Biol. Med. **72**:175–178.