

# Fine Structure and Isolation of the Hook-Basal Body Complex of Flagella from *Escherichia coli* and *Bacillus subtilis*

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The hook-basal body complex comprising the basal end of purified intact flagella from *Escherichia coli* and *Bacillus subtilis* was studied in detail with an electron microscope. The *E. coli* hook can be described as having five or six concentric helical coils. The basal body from *E. coli* is 27 nm in length and consists of four rings, 22.5 nm in diameter, arranged in two pairs and mounted on a rod. The top pair of rings is connected near their periphery, resembling a closed cylinder. In *B. subtilis* the basal body looks like that from *E. coli*, except that the top pair of rings is missing. Hook-basal body complexes from both organisms could be isolated by dissociating the filaments with either urea or acid. Based on our results, two types of basal body structures are proposed, as exemplified by *E. coli* and *B. subtilis*, which directly reflect the structure of the gram-negative and gram-positive cell envelopes.

Attempts to describe the structure of the base of the bacterial flagellum have been inadequate. Electron microscopy of sectioned cells [*Spirillum* (24), *Proteus* (33), and *Vibrio* (14, 28, 31)] or of partially degraded cells, including the flagella released from them [*Proteus* (2, 15), *Bacillus* (4), *Vibrio* (26, 30, 31), *Ectothiorhodospira* (27)], demonstrated that the basal body is a 15- to 50-nm structure closely associated with the cell envelope. In certain photographs, some details of the basal body structure are indicated (2, 4, 15, 31). However, the reported shapes and dimensions of the basal body vary widely both within and between the genera. The variation in these data is probably due to the presence of cell wall and membrane fragments which either mask the structure or form artifacts with it, as well as from real generic differences.

Compared to the work cited above, the study of Cohen-Bazire and London on three species of *Rhodospirillum* (8) shows greater detail in the basal body structure. However, their flagella preparation also contained cell wall and membrane fragments. In addition, the enzyme they used for lysing the cells is a nonspecific protease (12) and therefore could have altered the appearance of the basal body.

In our opinion, the resulting confusion about the structure of the basal body can be resolved by answering the following two questions: (i) What is the detailed structure of the basal body of flagella

that were first purified? (ii) What are the details of the relationship between the basal body and the cell envelope?

The preceding paper (9) describes a procedure for the purification of bacterial flagella in the form of a filament-hook-basal body complex ("intact flagella") free of detectable cell wall, membrane, or cytoplasmic material. Using such purified intact flagella from *Escherichia coli* and *Bacillus subtilis*, we describe in this paper the structure of the hook and basal body in detail. In the following paper (10) we identify specific interactions that exist between the cell envelope and the basal body, as well as artifacts which can occur when bacteria are lysed.

## MATERIALS AND METHODS

**Preparation of flagella.** The data presented in this paper were obtained from intact flagella purified from *E. coli* K-12 or *B. subtilis* M168 as described in the preceding paper (9).

**Electron microscopy.** Uniform negative staining was consistently obtained by using a copper grid with a Formvar membrane on one side and a carbon film on the other. The Formvar side was cleaned by dipping it onto the surface of petroleum ether for 5 sec, and the excess was drawn off with filter paper. This removes hydrocarbons which were adsorbed in the vacuum evaporator. A drop of the sample was then placed on the clean Formvar surface. After 1 to 5 min the grid was inverted onto the surface of 20% aqueous Formalin for 30 sec, and then onto deionized water for 15 sec. Excess

water was removed with filter paper and a drop of either 1% uranyl acetate, pH 4.5, or 1% phosphotungstate, pH 7.2, was placed on the grid. The excess stain was immediately removed and the grid allowed to dry in air. When an increase in stain penetration was desired, the stain was allowed to remain for 10 to 45 sec before drawing off the excess. Once the sample had been applied, the surface of the Formvar grid was not allowed to dry until the end of the procedure, otherwise poor staining and artifacts sometimes resulted.

The specimens were observed in a Siemens Elmiskop I electron microscope operated at an accelerating voltage of 80 kv with a double condenser lens using a 200- $\mu\text{m}$  condenser aperture and a 50- $\mu\text{m}$  thin metal objective aperture. A liquid nitrogen decontamination device was routinely used. Magnification was calibrated with a carbon grating replica of 2,160 lines/mm. The replica and samples were photographed under the same conditions without readjusting the microscope at magnifications of 40,000 or 80,000 $\times$ .

Quantitative data were collected from 4 to 7 $\times$  enlargements of the negative. The average of measurements on 6 to 20 different specimens was reported. Data were collected only when the component to be measured was clearly defined in the electron micrograph.

**Analysis of rotational symmetry.** The method used for analysis of rotational symmetry was that of Markham et al. (23). Images of basal rings were enlarged 11 times onto photographic paper mounted on a turntable. The exposure time ( $t$ ) was determined. The paper was then rotated  $360^\circ/n$  and exposed for  $t/n$  sec where  $n$  is an integral number. This was continued for  $n$  exposures. If  $n$  is a fundamental periodicity in the specimen, this periodicity will appear to be reinforced. Artifacts can be tested for by examining many values of  $n$ .

## RESULTS

**Flagella from *E. coli*.** Purified intact flagella from *E. coli* (9) have three major parts: the filament, the hook, and the basal body (Fig. 1). The following data deal with the structural details of this assembly with emphasis on the hook and basal body. Figure 25 is our interpretation of the structure of the base of the *E. coli* flagellum. The reader will find this model useful when examining the data.

**Filament.** The filaments from *E. coli* flagella are  $13.5 \pm 0.5$  nm in diameter. Their average length is about 5  $\mu\text{m}$ . The structural details of flagellar filaments were studied and discussed by Lowy and Hanson (22).

**Hook.** Most of the filaments had a slightly wider ( $17 \pm 0.8$  nm) hook-shaped structure at one end (Fig. 1 and various later figures). As previously observed (21), the connection between the hook and the filament often appeared to have a slightly narrowed region (Fig. 1A, 1B), and the structural design of the hook could usually be distinguished from the filament when negatively stained with uranyl acetate. The hook is  $45 \pm 2$  nm long.

Electron microscopic images of negatively

stained materials such as viruses and flagella have been shown to be ordinarily composed of the superimposed images of the top and bottom surfaces (16, 20, 22). The result is often a complex hazy pattern that appears disordered as a result of the out-of-register contributions from the two images. In addition, the contributions from each side of the particle can vary from a predominantly one-sided image to equivalent contributions from both sides.

Examination of some hooks from *E. coli* (Fig. 3, 4, and 6) and other genera (2, 21) from this point of view suggests a structure made of several helical coils. A model of a hook made of five coils (Fig. 2) shows that, if only the image of one side were seen, it would consist of parallel lines oblique to the axis of the hook. If the images of the top and bottom were seen superimposed, a cross-hatched appearance would result. In this case the model shows that the number of coils that make up the hook is equal to one more than the number of times per one-half turn a helix appears to overlap the other helices. The hooks in Fig. 3 appear to be a view of one side, and that in Fig. 4 clearly shows the cross-hatched pattern expected from a view of two sides superimposed. We estimate that the hook is composed of five or six helical coils.

**Basal body.** The hooks had a basal body attached (Fig. 1 and various later figures) which consisted of five primary components: a rod and four rings. To facilitate discussion of the basal body, we wish to introduce a nomenclature for the rings based on the basal body's relationship to the cell envelope. The details of this relationship are the subject of the following paper (10). From "top" to "bottom" (Fig. 25), the individual rings will be referred to as the L ring [for its attachment to the outer (lipopolysaccharide) membrane of the cell wall], the P ring (for its association with the peptidoglycan layer of the cell wall), the S ring (for "supramembrane," since it is located just above the cytoplasmic membrane), and the M ring (for its attachment to the cytoplasmic membrane).

The two rings proximal to the hook (the "top rings") were  $22.5 \pm 1$  nm in diameter and spaced  $9 \pm 0.5$  nm apart. These were connected via the rod to two similar rings distal to the hook (the "bottom rings"),  $22.5 \pm 1$  nm in diameter and  $3 \pm 0.3$  nm apart. The gap between the top and bottom rings was  $12 \pm 0.6$  nm. The gap between the hook and the top ring was about 3 nm, making the rod approximately 27 nm in length.

Penetration of the stain between the top rings was always significantly less than its penetration into the gap between the top and bottom rings (Fig. 1E and 1F and various later figures) or its penetration between the bottom rings (Fig. 1F),

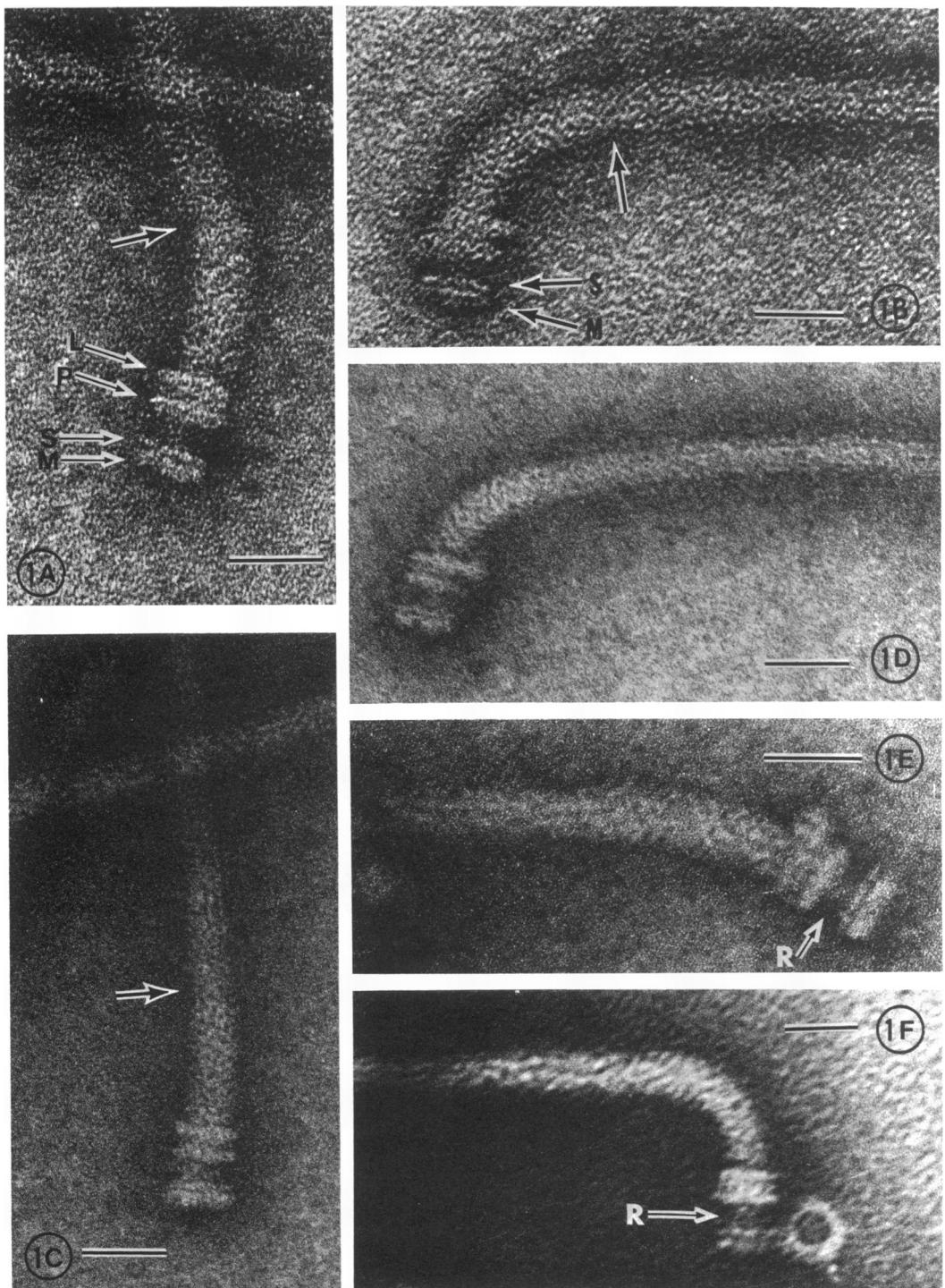


FIG. 1. Basal end of intact flagellum from *Escherichia coli*. A, Both the top and bottom edges of the L and P rings are seen, giving a circular appearance to the rings. Arrow marks the junction between the hook and the filament. Uranyl acetate;  $\times 485,000$ . The bar in this and subsequent figures represents 30 nm. B, Top edge of the S ring and the top and bottom edges of the M ring can be seen giving a circular appearance. Arrow marks the junction between the hook and filament. Uranyl acetate;  $\times 445,000$ . C, L, P, and M rings appear about twice as thick as in A, probably because the top and bottom edges of each ring are not resolved. Arrow marks the junction between the hook and the filament. Uranyl acetate;  $\times 410,000$ . D, The L ring appears extended in D and E probably because a fragment of the outer membrane remained attached (10). Uranyl acetate;  $\times 395,000$ . E, Stain has not penetrated the top rings. R marks the rod connecting the top and bottom rings. Uranyl acetate;  $\times 500,000$ . F, Stain has not penetrated the top rings. R marks the rod connecting top and bottom rings. Detached ring has associated with the bottom rings. Phosphotungstic acid;  $\times 395,000$ .

even though the bottom rings are closer together than the top rings. This fact, together with evidence presented below, suggests the existence of a structure connecting the top rings near their periphery to form a cylinder (*see* Fig. 25).

The thickness of individual rings is seen to vary from  $1.5 \pm 0.2$  nm (Fig. 1A, B) to two or three times that value (Fig. 1C, D, E). In some cases (Fig. 1C, D, E), the M ring appears thicker than the S ring; in other cases (Fig. 1A and 1B), these two rings appear equally thick. These apparent inconsistencies can be explained by viewing the electron micrographs as composed of superimposed images of the top and bottom surfaces of a basal body. Since the basal body is larger in diameter than the hook or filament, one would expect it to be somewhat tilted when lying on the surface of a grid. In addition, some rings may become "bent" as the sample is dried. Therefore, the view of the basal body is oblique. This means that the bottom edge of a ring appears adjacent to the top edge whenever the two edges are resolved (L, P, and M rings in Fig. 1A; M ring in Fig. 1B). When the two edges are not resolved, the ring appears thicker. In the case of the bottom rings, the lower edge of the S ring is hidden by the M ring, and therefore the S ring sometimes appears thinner than the M ring. Although the above interpretation of our data is difficult to prove correct in every detail, it does lead to a model (Fig. 25) which is consistent with all of our results.

**Damaged structures.** Examination of damaged parts was very useful in establishing the structure of the base of the flagellum. Damage was brought about by centrifuging intact flagella, or collecting them on a Gelman filter, and then abruptly resuspending them. Isolated basal bodies (Fig. 5), hooks (Fig. 6), and filament-hook complexes (Fig. 7) were found. Part of the rod extends above the L ring in Fig. 5; this can also be seen in Fig. 9, where the basal body is still attached. In some cases, it appeared that part of the rod remained on the hook after the other components of the basal body were gone (Fig. 8). This part of the rod evidently mounted the top rings (Fig. 10) and extended only as far as the P ring. That a part of the rod goes through the top rings is evident in Fig. 11. In some cases, the rod had its full length but the bottom rings were missing (Fig. 12). In a damaged basal body with the bottom rings missing (Fig. 17), part of the rod was seen and it appeared that the stain had entered the end, suggesting the rod is hollow.

No examples were found with only one top ring, but basal bodies were found that apparently had only one bottom ring (Fig. 13). The missing ring appeared to be the M ring. Specimens where the

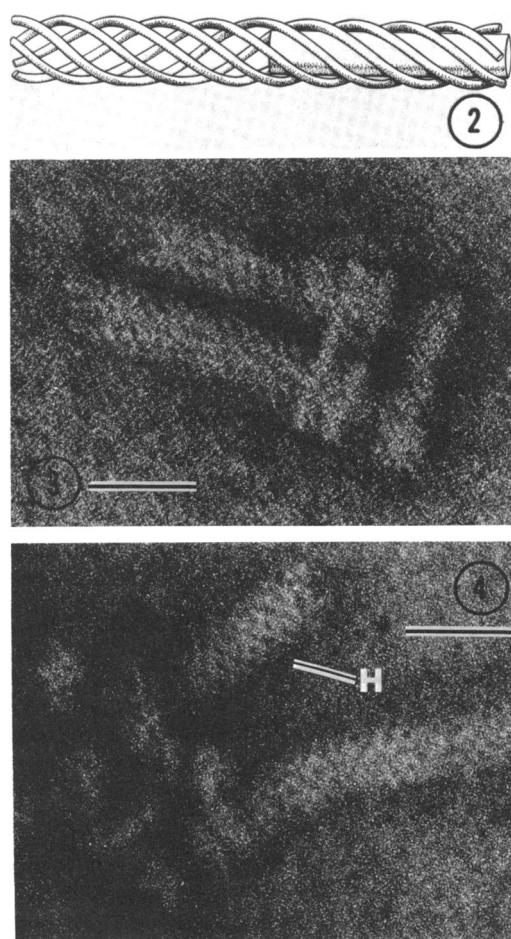


FIG. 2. Model of the surface structure of a hook based on data from *Escherichia coli* showing a view of one side, and a view of both sides superimposed. It is not known what structure, if any, occupies the center of the hook.

FIG. 3. Flagellar basal ends from *Escherichia coli* after treatment of intact flagella with 4.0 M urea. Note similarity between the appearance of the hooks and the image of one side in the model in Fig. 2. Uranyl acetate;  $\times 490,000$ .

FIG. 4. Same as material in Fig. 3. Note the cross-hatched image of the hook (H) as in the left half of Fig. 2. Uranyl acetate;  $\times 490,000$ .

M ring appeared broken and in the process of coming off supported this conclusion (Fig. 14). The fact that the M ring can come off indicates that there is no connection between the bottom rings, unlike the case of the top rings. This is supported by the fact that stain readily penetrates between the bottom rings but not between the top rings.

Isolated rings were found (Fig. 1F, 15, 17),

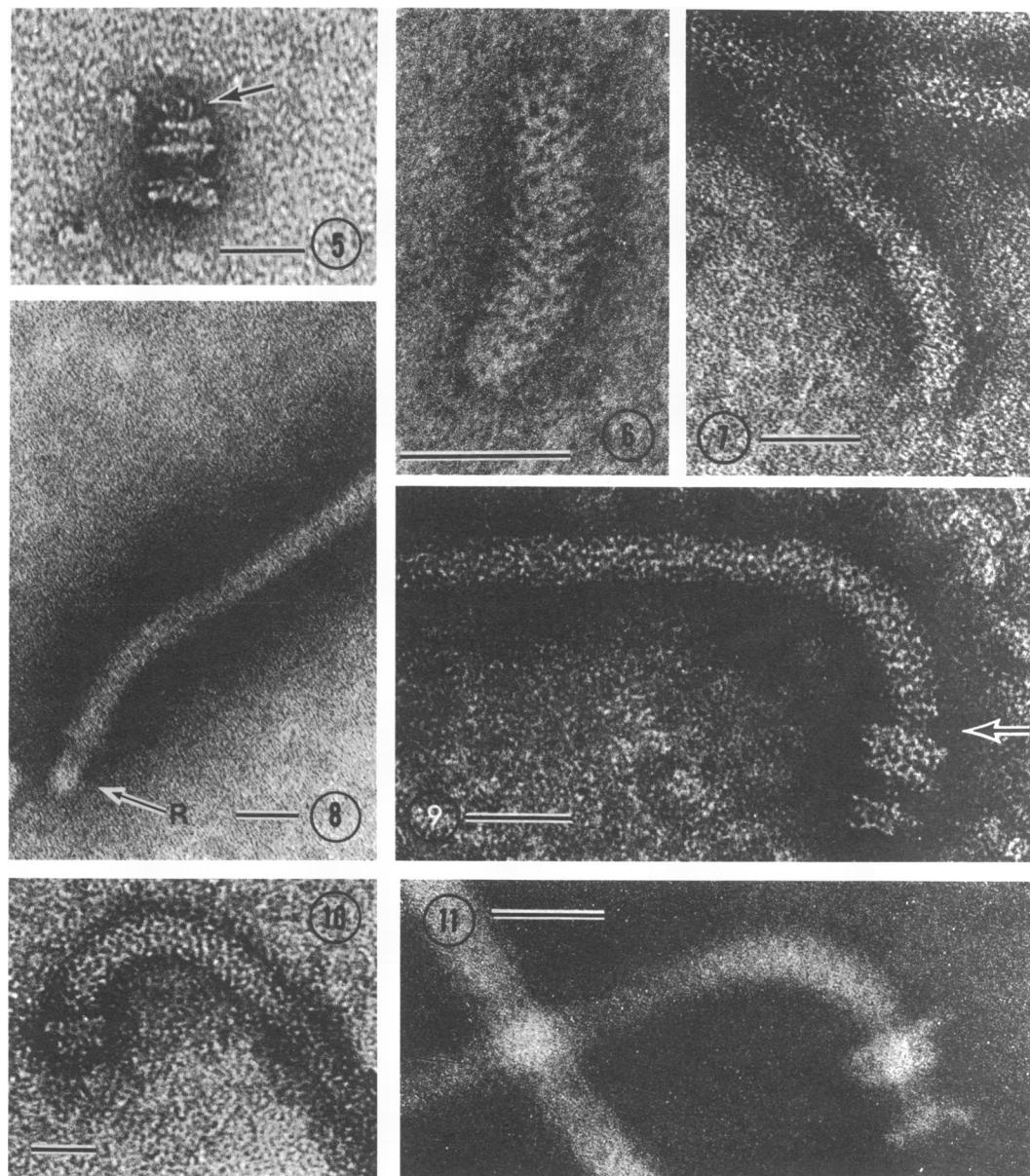


FIG. 5. *Escherichia coli* flagellar basal body detached from hook. Part of rod connecting to the hook is visible (arrow). Uranyl acetate;  $\times 395,000$ .

FIG. 6. Isolated hook found in a preparation of intact flagella from *Escherichia coli*. Note the coiled appearance. Uranyl acetate;  $\times 765,000$ .

FIG. 7. *Escherichia coli* filament-hook complex after the basal body has broken off. Uranyl acetate;  $\times 445,000$ .

FIG. 8. Flagellar end from *Escherichia coli* with part of rod (R) remaining. Uranyl acetate;  $\times 290,000$ .

FIG. 9. Basal end of *Escherichia coli* flagellum showing part of the rod extending above the L ring. Uranyl acetate;  $\times 490,000$ .

FIG. 10. Flagellar end from *Escherichia coli* with only L and P rings of the basal body still attached. Uranyl acetate;  $\times 300,000$ .

FIG. 11. Basal end of an intact flagellum from *Escherichia coli* with rod visible between L and P rings. Note the similar lack of stain penetration where two filaments cross. Phosphotungstic acid;  $\times 520,000$ . The stain was allowed to remain for 1.5 min instead of the usual 10 to 45 sec, to achieve greater penetration of the stain into the area between the top two rings.

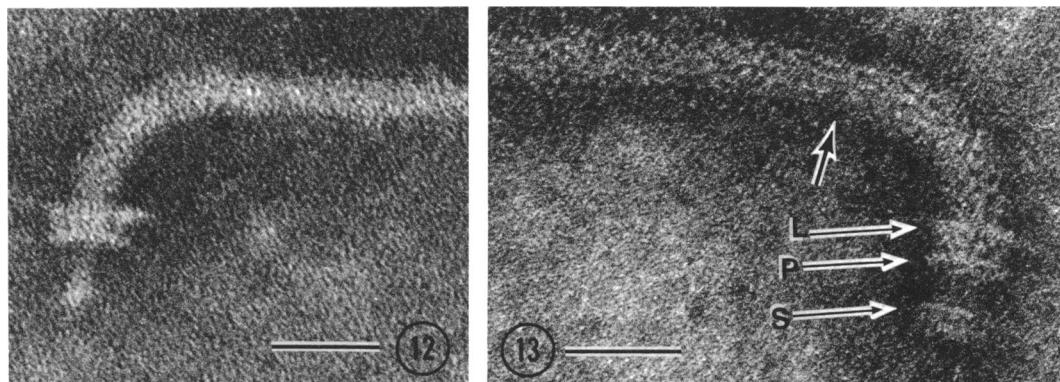


FIG. 12. Flagellar end from *Escherichia coli* with only L and P rings attached and rod visible below the rings. S and M rings are missing. Uranyl acetate;  $\times 475,000$ .

FIG. 13. Basal end of *Escherichia coli* flagellum. What might appear to be an M ring is considered to be the bottom edge of the S ring, since it is a hazy image compared to the sharp, dense images of the top edges of the L, P, and S rings. The M ring is missing. Arrow marks junction between hook and filament. Uranyl acetate;  $\times 505,000$ .

$22.5 \pm 1$  nm in diameter, which had evidently broken off from basal bodies. Some of the isolated rings appeared to have empty centers (Fig. 1F, 15A)  $10 \pm 0.4$  nm in diameter, and some contained a core (Fig. 15D, 17),  $7 \pm 0.4$  nm in diameter, which did not completely fill the opening. In Fig. 17 the core seen in the detached ring had the same diameter as the rod and was evidently part of a rod. Some of the detached rings were associated with the rings of basal bodies (Fig. 1F, 17). This aggregation phenomenon was characteristic of basal bodies.

Rotational symmetry analysis (25) was performed on several isolated rings. Patterns were produced for  $n = 4$  to  $n = 20$ , with the result that the maximum reinforcement was obtained at  $n = 16$ , suggesting that the rings are composed of 16 subunits. The best example of this analysis is shown in Fig. 16. Limitations of this method have been discussed (13).

**Isolation of the hook-basal body complex from intact flagella.** Conditions were sought for dissociating the filament without affecting the hook-basal body complex. Flagella filaments are known to dissociate under various conditions (such as low pH or heat) in which the hooks are relatively stable (3). Viscometry measurements (for method see reference 1) showed that purified sheared flagella (9) from *E. coli* were completely dissociated at 30°C after 30 min in either 4.5 M urea-0.05 M KCl or pH 3.4, or at 55°C for 10 min in 0.05 M KCl.

Samples of purified intact flagella in Tris-EDTA buffer (9) were collected on Gelman cellulose acetate filters with a diameter of 13 mm and a pore size of 100 nm. The buffer was washed through with 0.05 M KCl. The filter was immersed for 30 min at 26°C in 4 or 5 M urea-0.05

M KCl, or in either 0.04 M phthalate or 0.04 M glycine titrated to pH 3.4 with HCl. These treatments dissociate the filaments and elute the resulting hook-basal body complexes. Figures 3 and 18 show isolated hook-basal body complexes after treatment in 4.0 M urea. The basal bodies appear undamaged. The hooks average  $52 \pm 6.5$  nm in length, but some are as long as 92 nm, indicating that some filament remained. After treatment in 5.0 M urea, the filaments were completely dissolved with no apparent effect on the hook-basal body complex. The hooks were  $52 \pm 3$  nm in length. A result similar to that at 5.0 M urea was found when flagella were treated at pH 3.4 (Fig. 19): the hooks were  $47 \pm 2.2$  nm in length. Similar results were obtained by treatment at 55°C for 10 min.

The basal bodies tended to aggregate, and this resulted from association between the rings (Fig. 18 and 19). Hooks without basal bodies showed no tendency to aggregate.

To degrade the hook-basal body complex, flagella were exposed for 30 min at 26°C to 0.05 M KCl titrated to pH 2.8 with HCl. Under these conditions the hooks and rods dissolved, and only rings (Fig. 20) and hollow rectangular structures (Fig. 21) were observed. The center of the rings was either hollow or contained a core. The core may be an artifact resulting from the presence of flagellin, or may represent a specialized part of the rod. The rectangular structures had the dimensions and appearance of the top rings if connected near their periphery.

**Flagella from *E. coli* mutants.** Examination of purified intact flagella from paralyzed mutants representing each of the three known groups (5) and nonchemotactic mutants representing each of the three known genes (6) revealed no gross ab-

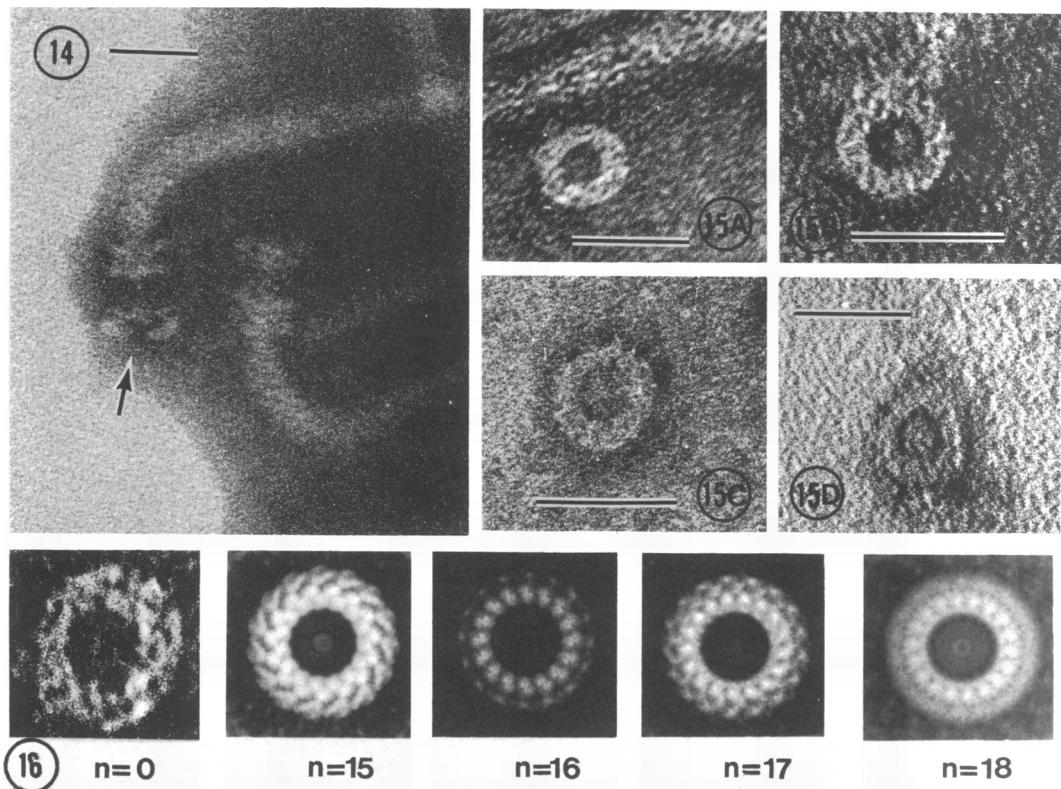


FIG. 14. Flagellar basal body from *Escherichia coli* with M ring broken and partially detached (arrow). Phosphotungstic acid;  $\times 395,000$ .

FIG. 15. Detached rings found after damaging intact flagella from *Escherichia coli*. Uranyl acetate. A,  $\times 520,000$ ; B,  $\times 670,000$ ; C,  $\times 620,000$ ; D,  $\times 520,000$ .

FIG. 16. Rotational symmetry analysis of Fig. 15A;  $\times 890,000$ . Under-focused picture was used.

normalities, i.e., there were a filament, hook, and basal body with four rings which appeared normally spaced. However, no detailed studies were made comparable to those for the wild-type parent reported in this paper.

**Flagella from *B. subtilis*.** The work of Abram et al. (4) suggested to us that the basal bodies of *Bacillus* species may have different structures from those of *E. coli*. Our studies of purified intact flagella from *B. subtilis* show a basal structure consisting of a hook and a rod with only two rings (Fig. 22). The rings are  $21 \pm 1$  nm wide and  $3.5 \pm 0.3$  nm apart and are mounted on a rod  $8 \pm 1$  nm in diameter. The distance from the hook to the bottom ring was difficult to determine accurately, but it appeared to be  $18.5 \pm 2$  nm. In Fig. 23 the rings are missing but the rod is present.

Hook-basal body complexes could be isolated by dissolving the filaments at pH 3.2 (Fig. 24). The length of the isolated complex was  $93 \pm 5$  nm; thus the hook is approximately 75 nm long.

Interactions between the basal bodies were less frequently seen than with *E. coli*, but when they did occur the associations were again between the rings.

## DISCUSSION

Figure 25 shows a scale model of the base of the flagellum consistent with the data presented for *E. coli*. The relationship of the major components, the hook, the top and bottom rings, and the rod, are clearly evident from the data. Some type of attachment between the rings and the rod is required because of the difference between the diameter of the rod and the inside diameter of the rings. The structure and number of attachments shown in the model, however, are hypothetical. The presence of a structure connecting the L and P rings near their periphery is suggested by the lack of stain penetration in this area and by the existence of structures, after the rod was dissolved at pH 2.8, having the appearance and dimensions

expected if the top rings were connected. The surface structure of the hook appears to consist of a set of helices as seen in Fig. 2.

The basal body from *B. subtilis* looks like that from *E. coli* except that it lacks a pair of rings. The two rings found on the *B. subtilis* basal body are homologous to the bottom rings described for *E. coli* on the basis of their position at the bottom of the rod and their close proximity to each other. In addition, the following paper (10) will show evidence that the bottom ring (ring M) of the basal bodies of both organisms is the only ring attached to the cytoplasmic membrane.

A critical question is whether a vital component was lost when the "intact" flagellum was isolated. Until the flagellum can function in an *in vitro* system, one cannot prove it was prepared in its complete form. Therefore the possibility remains that a fragile or unattached component of the flagellar base is missing in our preparations.

The proposed structure of the basal end of the flagellum of *E. coli* and *B. subtilis* does not appear to contain artifacts for the following reasons. First, the same structure was identified for *E. coli* in lysates from both lysozyme- and penicillin-spheroplasts, whether lysed either with Triton X-100 or by osmotic shock. The structure appeared the same when stained with uranyl acetate, phosphotungstate, or ammonium molybdate. Secondly, the hook-basal body complex did not change its appearance or dimensions when isolated by dissociation of the filament with urea, low pH, or heat, thereby demonstrating its structural stability. Finally, as mentioned next, the identification of this structure in other organisms studied with different techniques strongly supports its validity.

The structural model of the basal end of the flagellum described for *E. coli* is apparently a general structure found in flagellated bacteria that have cell envelopes comparable in structure to that of *E. coli*. Photographs of basal bodies on flagella from *Rhodospirillum* (8) and on axial filaments from *Leptospira* (25) show structures strikingly similar to *E. coli*. Data from *Proteus* (2, 15, 33), *Ectothiorhodospira* (27), and *Vibrio* (31) suggest the same structure. Thin sections through these organisms show a cell wall structure very similar to *E. coli* (7, 11, 19, 25, 27, 29, 31, 32). The basal body from *B. subtilis* lacks the top set of rings found on the *E. coli* basal body. The data of Abram et al. (4) on five other strains of *Bacillus* appear consistent with this description. These top rings specifically interact with the complex multilayered (11) cell wall of *E. coli* (10), and it appears that they are not needed for the dense, single-layered (32) wall of *B. subtilis*. Therefore, we suggest that the structure of the

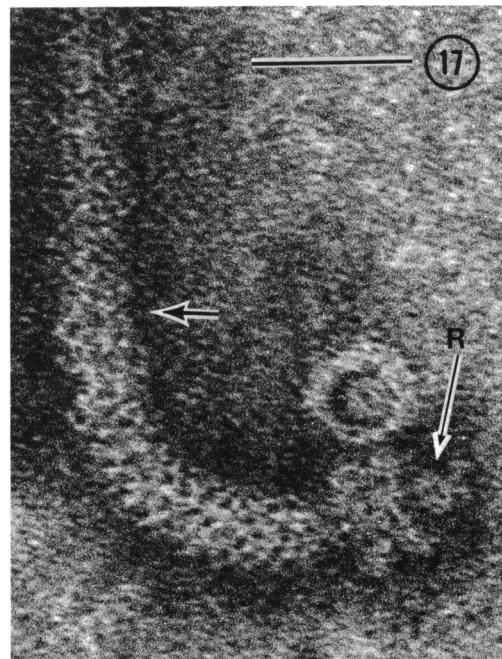


FIG. 17. Flagellar basal end from *Escherichia coli* missing the bottom rings. The rod is visible (R) and a detached ring containing a core is associated with the top rings of the basal body. Note the cross-hatched appearance of the hook. The junction between the hook and the filament is marked by arrow. Uranyl acetate;  $\times 710,000$ .

basal body directly reflects the structure of the cell envelope and expect two major classes of bacterial basal bodies as exemplified by *E. coli* for gram-negative bacteria and *B. subtilis* for gram-positive bacteria.

The complexity of the basal end of the flagellum may explain the results of genetic analyses of flagella synthesis. In *Salmonella*, mutations in 10 genes, besides the gene for flagellin synthesis, have been recognized as being responsible for the nonflagellated phenotype (17, 18). The hook-basal body complex may account for as many as eight structural genes.

The fact that paralyzed and nonchemotactic mutants showed no gross morphological differences from their wild-type parent is not surprising. Major defects in the basal body, such as loss of a ring, would more likely result in absence of flagella. However, these mutants might still be defective in the basal end.

The methods and results presented in this and the preceding paper will permit a chemical and physical characterization of the hook-basal body complex. Such a study would enable a detailed comparison to be made of the base of the flagellum of paralyzed and nonchemotactic mutants

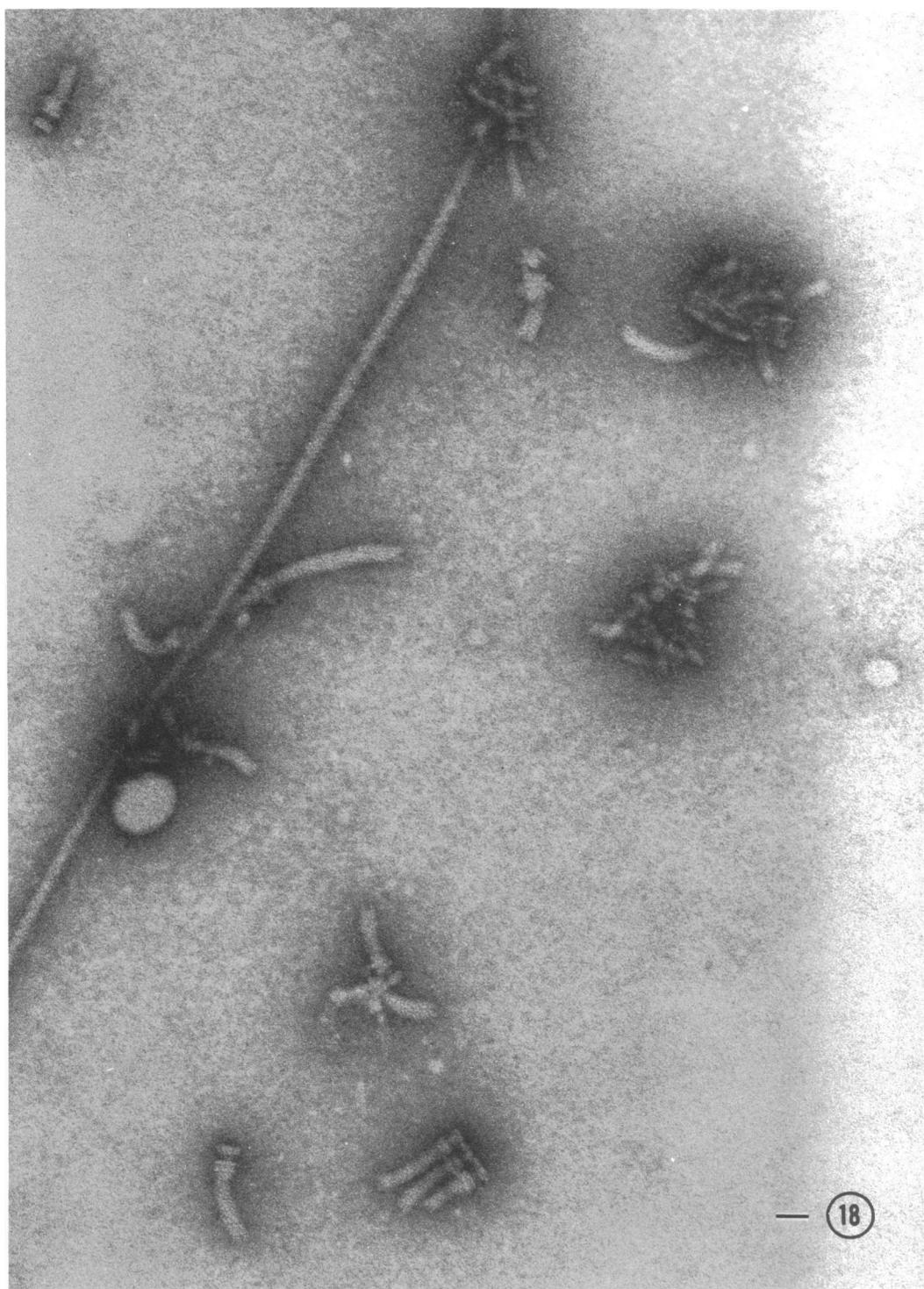


FIG. 18. Hook-basal body complexes found after treatment of intact flagella from *Escherichia coli* with 4.0 M urea. Uranyl acetate;  $\times 160,000$ .

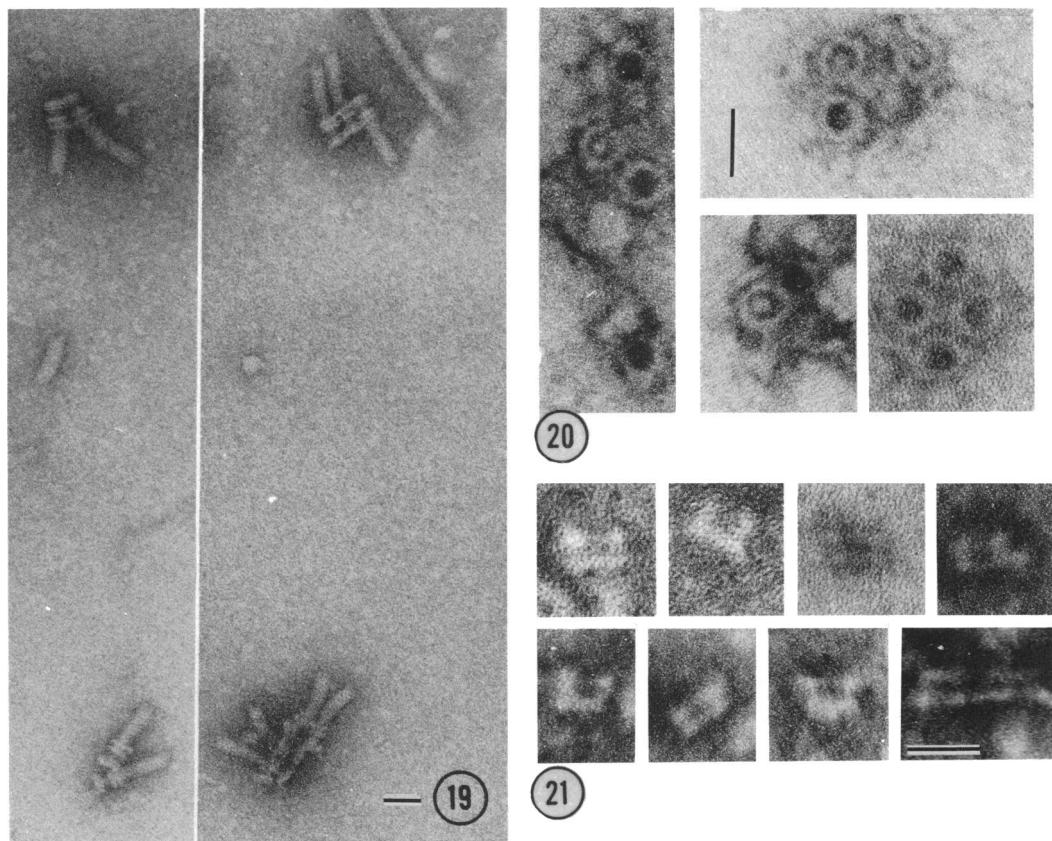


FIG. 19. Hook-basal body complexes found after treatment of intact flagella from *Escherichia coli* at pH 3.4. Uranyl acetate;  $\times 150,000$ .

FIG. 20. Material seen after treatment of intact flagella from *Escherichia coli* at pH 2.7. Note rings with and without a central core. Uranyl acetate;  $\times 310,000$ .

FIG. 21. Same preparation as Fig. 20. Note rectangular structures that appear to be side views of the top rings still attached to each other. Uranyl acetate;  $\times 310,000$ .

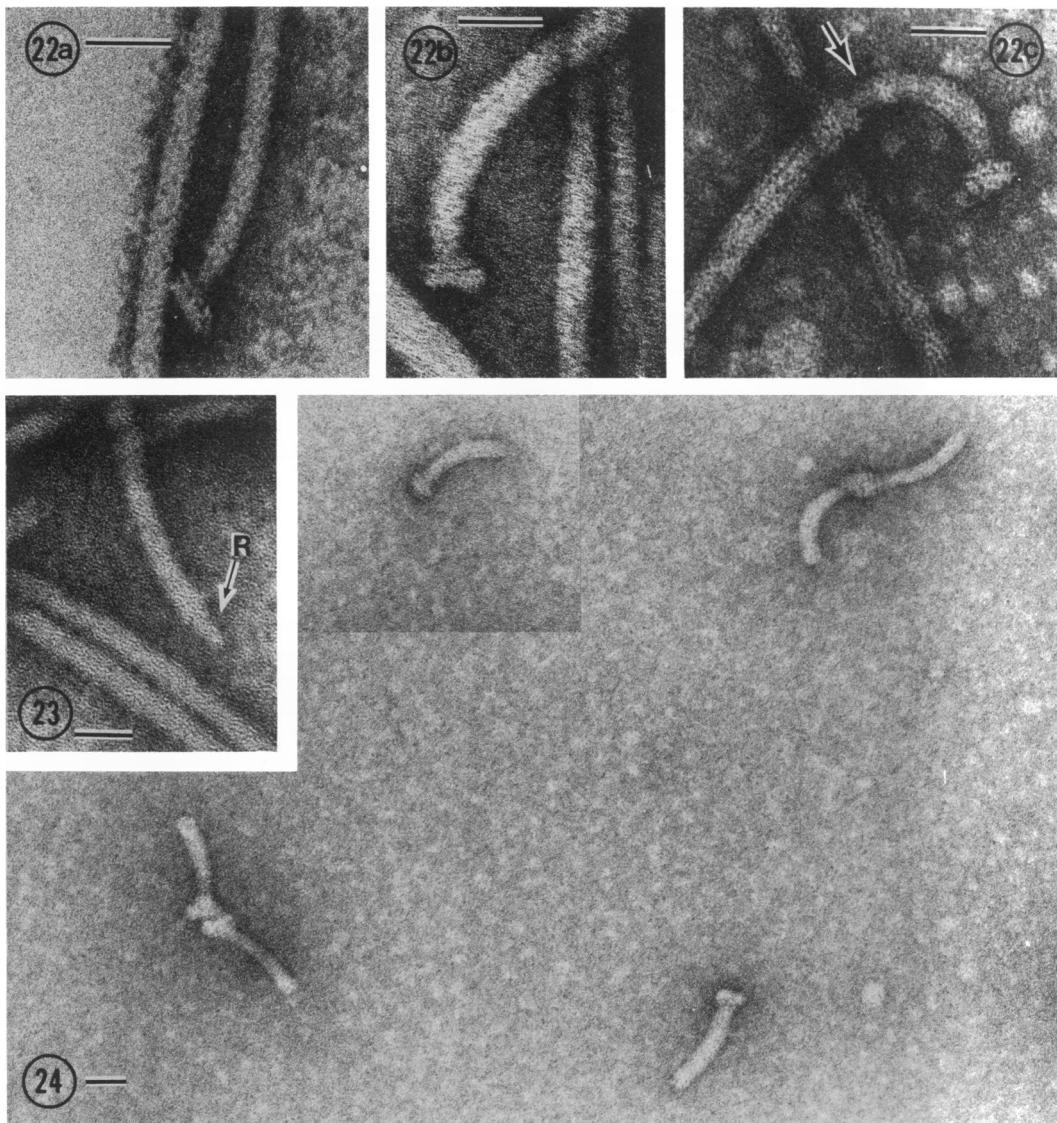


FIG. 22. Basal ends of intact flagella from *Bacillus subtilis*. A,  $\times 380,000$ ; uranyl acetate; B,  $\times 380,000$ ; uranyl acetate; C, junction between hook and filament is visible (arrow); phosphotungstic acid;  $\times 330,000$ .

FIG. 23. Basal end of intact flagellum from *Bacillus subtilis* without its rings. Rod is visible (R). Uranyl acetate;  $\times 256,000$ .

FIG. 24. Hook-basal body complexes after treatment of *Bacillus subtilis* intact flagella at pH 3.2. Uranyl acetate; 180,000.

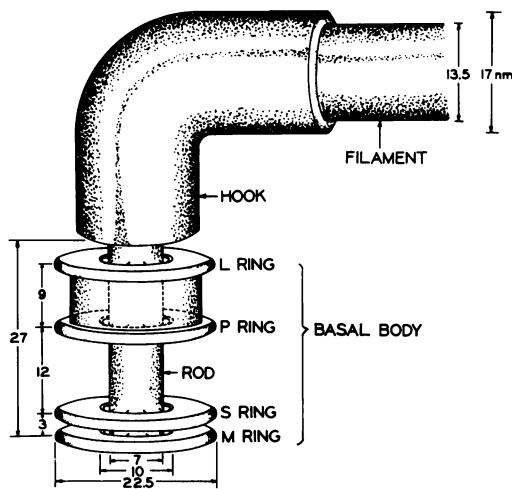


FIG. 25. Model of the basal end of a flagellum from *Escherichia coli*. Dimensions are expressed in nanometers.

with their wild-type parents in an effort to understand the mechanism of motility and chemotaxis.

#### ACKNOWLEDGEMENTS

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#### LITERATURE CITED

- Abram, D., and H. Koffler. 1964. *In vitro* formation of flagella-like filaments and other structures from flagellin. *J. Mol. Biol.* **9**:168-185.
- 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.
- Abram, D., J. R. Mitchell, H. Koffler, and A. E. Vatter. 1970. Differentiation within the bacterial flagellum and isolation of the proximal hook. *J. Bacteriol.* **101**:250-261.
- Abram, D., A. E. Vatter, and H. Koffler. 1966. Attachment and structural features of flagella of certain bacilli. *J. Bacteriol.* **91**:2045-2068.
- Armstrong, J. B., and J. Adler. 1967. Genetics of motility in *Escherichia coli*: complementation of paralyzed mutants. *Genetics* **56**:363-373.
- Armstrong, J. B., and J. Adler. 1969. Complementation of nonchemotactic mutants of *Escherichia coli*. *Genetics* **61**:61-66.
- Cohen-Bazire, G., and R. Kunisawa. 1963. The fine structure of *Rhodospirillum rubrum*. *J. Cell Biol.* **16**:401-419.
- Cohen-Bazire, G., and J. London. 1967. Basal organelles of bacterial flagella. *J. Bacteriol.* **94**:458-465.
- DePamphilis, M. L., and J. Adler. 1971. Purification of intact flagella from *Escherichia coli* and *Bacillus subtilis*. *J. Bacteriol.* **105**:376-383.
- DePamphilis, M. L., and J. Adler. 1971. Attachment of flagellar basal bodies to the cell envelope: specific attachment to the outer, lipopolysaccharide membrane and the cytoplasmic membrane. *J. Bacteriol.* **105**:396-407.
- dePetris, S. 1967. Ultrastructure of the cell wall of *Escherichia coli* and chemical nature of its constituent layers. *J. Ultrastruct. Res.* **19**:45-83.
- Ensign, J. C., and R. S. Wolfe. 1966. Characterization of a small proteolytic enzyme which lyses bacterial cell walls. *J. Bacteriol.* **91**:524-534.
- Finch, J. T., R. Leberman, C. Yu-Shang, and A. Klug. 1966. Rotational symmetry of the two turn disk aggregate of tobacco mosaic virus protein. *Nature (London)* **212**:349-350.
- 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.
- Hoeniger, J. F. M., W. van Iterson, and E. N. van Zanten. 1966. Basal bodies of bacterial flagella in *Proteus mirabilis*. II. Electron microscopy of negatively stained material. *J. Cell Biol.* **31**:603-618.
- Horne, R. W., and P. Wildy. 1963. Virus structure revealed by negative staining. *Adv. Virus Res.* **10**:101-107.
- Iino, T. 1969. Genetics and chemistry of bacterial flagella. *Bacteriol. Rev.* **33**:454-475.
- Joys, T. M., and B. A. D. Stocker. 1965. Complementation of nonflagellate *Salmonella* mutants. *J. Gen. Microbiol.* **41**:47-55.
- Keeler, R. F., A. E. Ritchie, J. H. Bryner, and J. Elmore. 1966. The preparation and characterization of cell walls and the preparation of flagella of *Vibrio fetus*. *J. Gen. Microbiol.* **43**:439-454.
- Klug, A., and D. J. DeRosier. 1966. Optical filtering of electron micrographs: Reconstruction of one sided images. *Nature (London)* **212**:29-32.
- Lowy, J. 1965. Structure of the proximal ends of bacterial flagella. *J. Mol. Biol.* **14**:297-299.
- Lowy, J., and J. Hanson. 1965. Electron microscope studies of bacterial flagella. *J. Mol. Biol.* **11**:293-313.
- Markham, R., S. Frey, and G. J. Hills. 1963. Methods for the enhancement of image detail and accentuation of structure in electron microscopy. *Virology* **20**:88-102.
- Murray, R. G. E., and A. Birch-Andersen. 1963. Specialized structure in the region of the flagella tuft in *Spirillum serpens*. *Can. J. Microbiol.* **9**:393-401.
- Nauman, R. K., S. C. Holt, and C. D. Cox. 1969. Purification, ultrastructure, and composition of axial filaments from *Leptospira*. *J. Bacteriol.* **98**:264-280.
- Pease, P. 1956. Some observations upon the development and mode of attachment of the flagella in *Vibrio* and *Spirillum* species. *Exp. Cell Res.* **10**:234-237.
- Remsen, C. C., S. W. Watson, J. B. Waterbury, and H. G. Trüper. 1968. Fine structure of *Ectothiorhodospira mobilis* Pelsh. *J. Bacteriol.* **95**:2374-2392.
- Ritchie, A. E., R. F. Keller, and J. H. Bryner. 1966. Anatomical features of *Vibrio fetus*: Electron microscopic survey. *J. Gen. Microbiol.* **43**:427-438.
- Salton, M. R. J. 1964. The bacterial cell wall. Elsevier Publishing Co., Amsterdam.
- Tawara, J. 1965. The root of flagella of *Vibrio cholerae*. *Jap. J. Microbiol.* **9**:49-54.
- Vaituzis, Z., and R. N. Doetsch. 1969. Relationship between cell wall, cytoplasmic membrane, and bacterial motility. *J. Bacteriol.* **100**: 512-521.
- van Iterson, W. 1965. Symposium on the fine structure and replication of bacteria and their parts. II. Bacterial cytoplasm. *Bacteriol. Rev.* **29**:299-325.
- van Iterson, W., J. F. M. Hoeniger, and E. N. van Zanten. 1966. Basal bodies of bacterial flagella in *Proteus mirabilis*. I. Electron microscopy of sectioned material. *J. Cell Biol.* **31**:585-601.