BASAL BODIES OF BACTERIAL FLAGELLA
IN PROTEUS MIRABILIS

I. Electron Microscopy of Sectioned Material

WOUTERA VAN ITERSON, JUDITH F. M. HOENIGER, and
EVA NIJMAN VAN ZANTEN

From the Laboratory of Electron Microscopy, University of Amsterdam, Amsterdam, The
Netherlands. Dr. Hoeniger's present address is the Department of Microbiology, School of Hygiene,
University of Toronto, Toronto, Canada

ABSTRACT

Years ago (16, 18, 19), in a study of shadowed preparations of Proteus vulgaris that had been
autolyzed in the cold, the observation was made that the flagella arose from basal bodies.
However, recently (3, 7, 24, 33) doubt has been cast on the conclusion that the flagella of
bacteria emerge from sizable basal bodies. This problem has, therefore, been reinvestigated
with actively developing cultures of Proteus mirabilis, the cell walls of which had been ex-
panded slightly by exposure to penicillin. Two techniques were applied: ultramicrotomy,
and negative staining of whole mount preparations. This paper deals with the thin sections
of bacteria after the usual fixation technique had been altered slightly: the cells were em-
bedded in agar prior to their fixation and further processing. The flagella then remained
attached to the cells and were seen to extend between the cell wall and the plasma mem-
brane. Occasionally, the flagella appeared to be anchored in the cell by means of a hook-
shaped ending. In sections of cells rich in cytoplasm, the basal bodies are particularly diffi-
cult to visualize due to their small size (25 to 45 mµ) and the lack of properties that would
enable one to distinguish them from the ribonucleoprotein structures; in addition, their
boundary appears to be delicate. However, when the cytoplasm is sparse in the cells, either
naturally or as a result of osmotic shocking in distilled water, the flagella can be observed
to emerge from rounded structures approximately 25 to 45 mµ wide. Contrary to a previous
suggestion (21), the flagella do not terminate in the peripheral sites of reduced tellurite,
i.e. the chondrioids. The observations in this part of the study agree with those described
in the following paper (15) dealing with negatively stained preparations.

INTRODUCTION

Cilia and flagella of all cells examined so far, with the exception of the bacteria, show a re-
markable uniformity in their structure and in the organization of their basal apparatus. This basal
apparatus, often called for the flagellate protozoa "blepharoplast" and for the ciliates "kinetosome,
" can be referred to by the general term "basal
body" (cf. reference 9). Since the last century, it
has been suggested that basal bodies and centrioles
are homologous structures (11, 26). Lwoff (31)
in particular focused attention on these structures
by emphasizing that they may be endowed with
genetic continuity and would provide a beautiful
model of a self-reproducing particle. With the aid
of the electron microscope, it has indeed been found that basal bodies are strikingly similar to centrioles in their fine structure (6, 10, 44). The expectation, that basal bodies and centrioles carry genetic information which may function both in instigating their reproduction and in forming the protein fibers that constitute the cilium or flagellum and the mitotic spindle, has been confirmed, at least for the basal bodies, by the demonstration in them of DNA (38, 40).

The existence of such bodies at the base of flagella and cilia in protozoa and other cells prompted some early investigators to look for similar structures in bacteria with the light microscope, and, indeed, so-called basal granules (biepharoplasts) were recorded by several authors (for relevant literature, see reference 16). The first demonstration with the electron microscope was made in *Proteus vulgaris* by one of the authors in cooperation with Professor C. F. Robinow in 1948 (16, 18, 19). Astbury (5) confirmed this finding, and subsequently basal granules were recorded with the electron microscope for *Chromobacterium violaceum* and *Vibrio metchnikovii* (20), *Rhodospirillum rubrum* (8), and *Vibrio comma* (8, 45), for *Vibrio* spp. (35) and *Spirillum* spp. (35, 48).

However, all these observations were made on shadowed preparations of cells that either were old or had been allowed to autolyze in order to make them more transparent to the electron beam. Pijper (36) and Kerridge (24) therefore suggested that the basal bodies might be artifacts caused by cytoplasmic coagulation.

Since Weibull’s observation (47) that, in *Bacillus megaterium*, flagella remain attached to the naked protoplasts after the cell wall had been removed with lysozyme, it is generally agreed that flagella terminate within the plasma membrane. But the question whether basal bodies really exist still remained unanswered. Recently several groups of workers have looked for the exact site of implantation of bacterial flagella in sectioned material and in cells negatively stained with potassium phosphotungstate. Murray and Birch-Andersen (32) could see in thin sections of *Spirillum serpens* that the polar flagella penetrate the cell wall and terminate within the plasma membrane, but the detailed mode of anchorage was not apparent. Glauert, Kerridge, and Horne (7), examining the sheathed flagellum of *Vibrio metchnikovii*, found that although the sheath was continuous with the cell wall, the core of the flagellum terminated in a basal disc, 30 to 35 nm wide, just inside the plasma membrane. Abram, Vatter, and Koflter (2) reported that in negatively stained preparations of *Proteus vulgaris* and in various strains of *Bacillus* the flagella can be seen to “arise via hooks from spherical structures, 250 to 350 Å in diameter.” But Newton and Kerridge (33) suggested again in 1965 that the larger “granules” seen in the electron microscope could be artifacts.

Because of this uncertainty regarding the basal apparatus of bacterial flagella, it remains an open question how far these locomotor organelles may, in their fundamental features, be compared to the cilia and flagella of eucaryotic organisms. In their fine structure, bacterial flagella, of course, differ strikingly from the beautifully symmetrical “9 + 2” pattern established for cilia and flagella of diverse origin. Since the over-all width of bacterial flagella falls between 100 and 190 Å, these filaments can then be compared with the 100- to 300-Å-wide fibrils that constitute the higher cilia and flagella (6, 17, 33, 39). At the molecular level, bacterial flagella were seen to consist of helically or longitudinally arranged rows of globular subunits of the protein flagellin, there being between 3 and 10 such rows, depending on the organism (1, 25, 27, 28). The fibrils of higher cilia and flagella may possess comparable features (4, 34).

The aim of the present study was to reinvestigate by means of current techniques of electron microscopy whether the bacterial flagella arise from structures comparable to the basal bodies (kinetosomes or biepharoplasts) in other cells. The thin-sectioning technique has been applied, together with negative staining of whole mounts with potassium phosphotungstate (46). In order to preserve in the thin sections the flagella attached to the cells, the usual procedure had to be altered by embedding the organisms in agar prior to fixation and further processing.

After this work had been completed, an article appeared by Abram, Koflter, and Vatter (3) containing beautiful illustrations of the basal structures of the flagella of *Proteus vulgaris*. They examined negatively stained and shadow-cast preparations of ghost cells and “long forms” obtained by treating cultures in the cold for 2 days to 6 wk, and concluded that the flagellum arises from a small body, i.e. a spherical structure 11
to 14 µm in diameter, associated with the plasma membrane.

This first communication deals with the sectioned cells. The organisms studied were actively motile swarmers of *Proteus mirabilis*, since they possess multitudes of flagella (13). The cells were treated with penicillin, with a view to loosening the cell wall and the texture of the cytoplasm so as to facilitate tracing the flagella to their cellular origin. In order to ascertain whether a relation exists between the chondrioids and the sites of flagellar implantation, some cells were incubated with potassium tellurite, a treatment which enhances the contrast of the peripherally located chondrioids by the incorporation of reduced product (21).

In this study of flagellation in *Proteus*, and in the next (15), the term “chondrioid” is used to denote the rounded areas contiguous with the plasma membrane in which the reduced product of potassium tellurite first appears following incubation under suitable conditions. Obviously, one difficulty in staining the chondrioids by means of tellurite reduction is how to determine the moment at which to stop the experiment. Therefore, the length of the incubation period must be arbitrary; so also is the delineation of the true sites of reductive activity. During this unnatural treatment, the optical density of the culture gradually stops rising (21); the cells autolyze and gross morphological changes take place, including the formation of large electron-scattering crystals. As described previously (21), the structure of the chondrioids in *Proteus* differs fundamentally from that in *Bacillus subtilis*.

---

The second article (15) will deal with negatively stained material from *Proteus mirabilis* cells.

---

![Figure 1](image)

**Figure 1.** An oblique section through a cell in which the plasma membrane has partly receded from the cell wall, leaving an empty space within which four rounded structures are to be seen. A fifth body lies outside the cell. The arrows point to flagella emerging from the bodies; Te indicates deposit of reduced tellurite. Note the tubules in the lower part of the cell; such organelles are the subject of a separate paper (28). X 130,000.
MATERIALS AND METHODS

The strain of Proteus mirabilis used in this work was isolated from a stool specimen and maintained on egg slopes as described previously (12-14). Organisms which had been passed through a motility tube of semisolid agar (heart infusion broth + 0.3% agar) were suspended in physiological saline to a concentration of about $10^9$ bacteria/ml. One ml of this suspension was spread uniformly over the surface of heart infusion agar (Difco) in a 15-cm diameter Petri dish; such plates were incubated for 3½ to 5 hr at 35°C, i.e. to the peak of swarmer differentiation when checked with the light microscope (13). The organisms were removed from the plates in heart infusion broth, containing 2000 IU/ml penicillin G, 5% horse serum, 0.25 M sucrose, and 0.003 M MgCl$_2$; they were left in this medium for 75 min at room temperature. In many cases, the bacteria were incubated with 0.05% potassium tellurite (K$_2$TeO$_3$) under semi-anaerobic conditions for 60 to 75 min in order to enhance contrast of the chondrioids (21). The cultures were then centrifuged, and embedded before fixing in 1% agar made up in modified Michaelis buffer containing 0.25 M sucrose (43). The fixation procedure and posttreatment with uranyl acetate of Ryter and Kellenberger (43) was employed on the agar-embedded bacteria; they were dehydrated through graded series of alcohol, transferred to propylene oxide, and embedded in Epon 812 (30).

In later experiments parallel to the work of Part II (15), the cell walls were loosened during 60 min with 2000 IU/ml penicillin, whereupon the cells were shocked in distilled water with 0.01 M MgCl$_2$, and then embedded in agar and prepared for ultramicrotomy as usual. These preparations (Figs. 15, 17, and 18) were embedded in Vestopal W.

Thin sections were cut on an LKB Ultratome with a glass knife, and stained with lead citrate (41). Electron micrographs were taken with a Philips EM 200 operating at 80 kv, with a double condenser lens system and an objective aperture of 50 μ. Most micrographs were made with the specimen-cooling device in operation.

Figures 2 to 6 Five serial sections through the tip of an elongated organism rich in cytoplasm. The arrows in Figs. 2 and 3 indicate faintly distinguishable rounded structures; those in Figs. 5 and 6 point to flagellar extensions which can be traced in the cytoplasm. In Fig. 3, a deposit of reduced tellurite is marked Te. The dense track in Fig. 5 may represent an arrangement of the ribonucleoprotein structures. × 180,000.

588 THE JOURNAL OF CELL BIOLOGY • VOLUME 31, 1966
OBSERVATIONS

Embedding of bacteria prior to their fixation proves to be a good method for keeping flagella attached to the cells so that in thin sections one can study their mode of insertion. In the sections, they are found to be distributed either singly (Figs. 1 to 12) or occasionally in small bundles, as in Fig. 7 (cf. reference 20). In many instances (Figs. 6 to 8, and 10 to 18), the flagella do not stop at the cell wall, but extend between the loosened cell wall and the plasma membrane, sometimes even farther, as will be described below. The width of the flagella in the sectioned material was calculated from some 200 measurements: after penicillin treatment only, it was found to be 88 ± 21 A (mean ± se); after penicillin treatment and water shocking, it was found to be 97 ± 17 A. The considerable variation in the two sets of data may be due either to individual differences between the flagella or to inaccuracies in measurement. Moreover, there was apparently some shrinkage of the flagella during fixation and embedding (cf. diameter of 133 ± 16 A for flagella in negatively stained preparations—reference 15).

Since our work concerned mainly Proteus swarmers, observations of the type of short rod-shaped cell illustrated in Fig. 1 were rarely made. In this oblique section the plasma membrane has partly receded from the cell wall; in the empty space between the two integuments four rounded structures can be seen which strongly resemble those observed years ago in a shadowed, autolysed swarmer (16, 18). The arrows in Fig. 1 point to sections of flagella emerging from the rounded structures. A similar rounded element is also seen outside the cell in Fig. 1; presumably it had been released from a broken part of the cell.

The bacteria examined had been exposed to penicillin. It was to be expected that this treatment would decrease the rigidity of the cell wall, thus enabling the contents of the cell to swell. Yet the cytoplasm, in many cases, remained so compact that few structures can be discerned between the ribonucleoprotein. Figs. 2 to 6 are examples of serial sections through the end of such a compact cell. There are indications of rounded structures near the cell periphery (see arrows in Figs. 2 and 3) but these are faint, and indeed one could have reason to question their existence on the basis of such evidence alone. In Figs. 5 and 6 the direction of the sectioning is such that several flagellar extensions can be traced in the cytoplasm at the tip of the cell. In Fig. 5, the electron-opaque track (see arrows) through the cytoplasm and probably extending from a flagellum on the lower surface may prove of particular interest (cf. Discussion) at a more advanced stage in our research. In this series of five sections, deposits of reduced tellurite (labeled Te) are so few that one cannot decide whether any correspondence exists between such sites and the implantation of the flagella, as originally suggested by van Iterson and Leene (21).

Some swarmers were found to be unusually poor in cytoplasmic material, and to have a much swollen nucleoplasm. Figs. 7 and 8 together depict a single longitudinal section from such an organism. In the periphery of this very transparent swarmer there are rounded structures, labeled B. The borders of these rounded bodies are distinct from the plasma membrane proper. In most electron micrographs, the bodies lack a distinctly visible limiting membrane (Figs. 12 to 18), and this makes them difficult to recognize. The width of the rounded structures in the sections is about 25 to 45 μ. The reduced tellurite can be recognized by its contrast (arrows, Te), and at the arrow in Fig. 8 a flagellum seems to come out of the cell in the neighborhood of such an area. However, these and similar pictures do not seem to support the contention (21) that the deposits of reduced tellurite coincide with the sites of the rounded bodies.

Figs. 9 to 11, representing three serial sections through a swarmer, further illustrate the difficulty of analyzing the structures at the bases of the flagella in cells with a more or less normal content of cytoplasm. (It will be noted that the sister cell on the left of the swarmer became swollen during the penicillin treatment.) Flagella that can be seen emerging from the long bacterium in one of these three sections are numbered A to G. Several features should be noted. Of interest is the cytoplasmic structure at the site of penetration of flagellum A in Fig. 10 (see arrows). In the previous section (Fig. 9), this location may correspond with a deposit of reduced tellurite. To the right of this flagellum in Fig. 9 is a delicately striated area surrounded by reduced tellurite; it is unlikely that this area would correspond to the basal granule of the flagellum. Flagellum G, in Fig. 11, shows, between the cell wall and the plasma membrane, a bend above which the outlines of a basal granule may perhaps be recognizable in the cytoplasm.
Figures 7 and 8  Longitudinal sections from a Proteus swarmer which is unusually poor in cytoplasm. It should be noted that both extremities of this cell show on their left side signs of compression during sectioning due to defective polymerization of the Epon. In the periphery of this organism deposits of reduced tellurite (Te) can be seen, as well as rounded structures (B) which are interpreted as possibly representing the basal bodies of the flagella. However, here, contrary to their condition in cells from
which the cytoplasm had been released artificially (see Figs. 15 to 18), the basal bodies appear to be more or less empty. In Fig. 8 a, the flagellum at h is seen to have a hook-shaped bend. At the arrow in Fig. 8 b, a flagellum appears to extend in the direction of a deposit of reduced tellurite, but this was not usually found to be the case. Figs. 7 and 8, × 75,000; Figs. 8 a and b, × 150,000.
(see arrows). On the whole, the details to be discerned in the serial sections are disappointing, and this is not surprising, since the basal structures are likely to be smaller (25 to 45 m/z) than the section thickness, which has not been estimated here. Moreover, contrast in the sections and the properties of the boundary of the bodies seem to militate against their clear demonstration in thin sections.

The penetration of a flagellum through the cell wall and into the cytoplasm is shown particularly well in Fig. 12. Again, the basal structure is only faintly distinguishable because of its delicacy and its lack of conspicuous properties and because the section is comparatively thick. Of special interest is the electron-opaque, very thin track in the cytoplasm (indicated by arrows) which may originate from the basal body.

Finally, in Figs. 13 to 18, a selection of cell fragments is shown in order to obtain further proof for the true existence of specific cytoplasmic structures at the bases of the flagella. With the exception of Figs. 13, 14, and 16, these electron micrographs were made parallel with the work described in Part II (15), i.e. from cells that had released most of their cytoplasm after being osmotically shocked in distilled water. In all of these illustrations, flagella can be seen entering the cell and terminating in basal bodies. In Fig. 13, there appear to be two such bodies about 30 m/z wide. The presumably double body in Fig. 14 again is difficult to distinguish from the surrounding cytoplasm. In cells from which the cytoplasm had been released by osmotic shock the bodies sometimes—but not always (Fig. 18)—appear incomplete, like those indicated by arrows in Fig. 15. The one indicated by two arrows (Fig. 15) may represent a more complete basal body. To its left can be seen a circular, clearly membranous structure. Fig. 17 is of interest because it is a section through a cell comparable to the one in Fig. 4 of Part II (15) in which an elongated structure inside a negatively stained cell is interpreted as a fragment of the plasma membrane “caught up by the flagellar endings and folded around them.” That this interpretation holds true, and that “this enfolding of the fragmented membrane probably hides the basal bodies” can be deduced from Fig. 17, in which the arrow indicates a basal structure attached to a flagellum which passes both the cell wall and the fragmented plasma membrane.

In Fig. 18, several bodies are shown at high magnification. It is interesting to note that in this young cell, quickly released of its contents by osmotic shocking, there remains around the basal bodies no clearly visible limiting membrane of a structure comparable to that of the plasma membrane. Thin filaments appear to interconnect remnants of the cellular content, including the basal bodies (cf. reference 22).

**DISCUSSION**

Fixation through agar is an easy way to obtain satisfactory embeddings for the sectioning of material in which the flagella are well preserved. The usual failure to demonstrate flagella in thin sections is probably due to their being broken off during centrifuging after fixation. In thin sections of swarovers of *Proteus mirabilis* prepared in this manner, numerous flagella are seen to penetrate the cells and to extend between the cell wall and the plasma membrane. When serial sections were superimposed, the flagella could be traced over considerable distances.

The present state of the electron microscope technique does not yield preparations in which the basal granules on *Proteus* flagella are easily demonstrated. But the evidence is sufficiently convincing when considered together with the results of negative staining (15). One of the difficulties in demon-

---

**FIGURES 9 to 11** Three serial sections through a swarmer. The sister cell just visible on the left in this series was observed to be quite enlarged, presumably as a result of penicillin treatment. Flagella emerging from the cell in at least one of these three sections have been numbered 1 to 8; their position is indicated in the other two sections so that the corresponding cytoplasmic areas can be examined. For example, note in Fig. 10 the cytoplasmic fine structure (arrows) at the site of penetration of flagellum 1; in Fig. 9 this location may correspond to a deposit of reduced tellurite, while in Fig. 11 it looks like a basal granule. The thickness of the section, when compared with the diameter of the basal granules (25 to 45 m/z), is too great to permit really profitable study. × 78,000.
Figure 12  Note the penetration of a flagellum through the cell wall and into the cytoplasm at the left side of this organism. The basal structure can be distinguished only faintly (at the lower arrow) owing to the relative thickness of the section, and to the lack of properties which would make it stand out from the surrounding cytoplasm. The upper arrows indicate a very thin track in the cytoplasm; deposits of reduced tellurite are marked Te. × 81,000.
strating such bodies in sections of young cells is that in the electron micrographs limiting membranes of a structure comparable to that of the plasma membrane cannot readily be recognized around them. There are possibly two reasons for this: first, the bodies may not be bounded by a membrane similar in thickness and construction to the plasma membrane; second, such a membrane, when present, may not show up well in the sections, due to curvature of the small bodies. These "membranes" are best shown in Figs. 7, 8, and 18. They should perhaps be studied in cells that have passed beyond the logarithmic phase. The "membrane" may be derived from the plasma membrane, but is separated from it (cf. insets, Fig. 8). The impression obtained from cells with a well developed cytoplasm suggests that the boundary is more delicate than the plasma membrane, and that this makes it nearly impossible technically to observe it in such comparatively thick sections. The results obtained by negative staining, as shown in particular by Fig. 8 in Part II (15), appear to confirm the present suggestion that the basal bodies have very delicate boundaries.

The study of serial sections is of interest in view of the relative positions of the sites of flagellar implantation; but it is of no help in shedding light on the structure of the bodies themselves, their size being presumably in the same range as the section thickness. The results obtained with other autolyzed organisms (8, 35, 45, 48) now requires reinvestigation with more modern techniques (cf. reference 7).

The flagella are sometimes seen to be anchored to the cell by means of a hook (Figs. 1, 8 a, 11, 17), which agrees with earlier observations (3, 13, 16, 20, 29, 42).

In some electron micrographs (Figs. 8 a and b, 12, 16, and 18), thin filamentous structures, which may contain nucleic acids (22), appear to be in contact with the basal bodies. But it is considered premature to speculate on the possible contact of these bodies with nucleic acids, particularly DNA, although this would make their comparison with self-reproducing particles of great interest, as mentioned in the Introduction.

We wish to thank Mr. P. J. Barends, Mrs. J. Raphaël-Snijer, Miss N. M. Slikker, and Miss E. Bon for their capable assistance. One of us (J.F.M.H.) is indebted to the Medical Research Council of Canada for a travel grant.

Received for publication 31 March 1966.

VAN ITERSON, HOENIGER, AND NIJMAN VAN ZANTEN  Basal Bodies of Flagella. I  597
FIGURE 13 At the arrows two basal bodies may be seen, each about 30 m\(\mu\) in diameter. \(\times 160,000\).

FIGURE 14 The arrows indicate what is presumed to be a double basal body. \(\times 156,000\).

FIGURE 15 Fragment of a cell from which most of the cytoplasm has been released by osmotic shocking in distilled water. The large, single arrows point to the origins of flagella, and these are interpreted as being fragments of basal bodies. The smaller arrows indicate a complete basal body, to the left of which a rounded fragment of membrane can be seen. \(\times 156,000\).

FIGURE 16 A flagellum appears to be emerging from a basal body which has dimensions 27 \(\times\) 40 m\(\mu\). Note the fine, central fibril which lies within or over the basal body. \(\times 147,000\).

FIGURE 17 This micrograph is interesting because it represents in section the same sort of situation as is seen in the negatively stained preparation of Fig. 4 in Part II (15). A fragment of the plasma membrane has been caught up by the flagellar endings and appears to be folded around them. The arrow points to a basal structure of a flagellum which penetrates both the cell wall and the plasma membrane; note the hooklike bend in this flagellum. The fine dots which speckle the micrograph are due to dirt picked up during preparation when the section was floated. \(\times 110,000\).

FIGURE 18 As in Figs. 16 and 17, most of the cytoplasm has been released by osmotic shocking; this figure can thus be compared with those of negatively stained preparations in Part II (15). Several basal granules appear to be present which resemble in size and situation those illustrated in Part II (15). No distinct limiting membrane can be distinguished, even at this high magnification. The bodies appear to be in contact with fibrillar material remaining from the cytoplasm. \(\times 190,000\).
REFERENCES


33. Newton, B. A., and Kerridge, D., Flagellar and ciliary movement in microorganisms, in
Function and Structure in Micro-organisms, 


45. Tawara, J., Electron-microscopic study on the flagella of *Vibrio comma*, *J. Bact.*, 1957, 73, 89.

