BASAL BODIES OF BACTERIAL FLAGELLA
IN PROTEUS MIRABILIS

II. Electron Microscopy of Negatively Stained Material

JUDITH F. M. HOENIGER, WOUTERA VAN ITERSON, 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
This paper investigates further the question of whether the flagella of Proteus mirabilis emerge from basal bodies. The bacteria were grown to the stage of swarmer differentiation, treated lightly with penicillin, and then shocked osmotically. As a result of this treatment, much of the cytoplasmic content and also part of the plasma membrane were removed from the cells. When such fragmented organisms were stained negatively with potassium phosphotungstate, the flagella were found to be anchored—often by means of a hook—in rounded structures approximately 50 m wide, thus confirming Part I of our study. In these rounded structures a more brilliant dot was occasionally observed, which we interpret as being part of the basal granule. A prerequisite for the demonstration of the basal granules within the cells was, however, the removal of both the cytoplasm and the plasma membrane from their vicinity. In some experiments, the chondrioids were "stained" positively by the incorporation into them of the reduced product of potassium tellurite. The chondrioids were here observed to be more or less circular areas from which rodlike structures extended. The chondrioids adhered so firmly to the plasma membrane that they were carried away with it during its displacement by osmotic shocking, while the basal bodies were left behind. This observation disproves our previous suggestion that the flagella might terminate in the chondrioids. The basal bodies often occur in pairs, which suggest that they could be self-reproducing particles.

INTRODUCTION

All electron microscope observations of the basal bodies of bacterial flagella have so far been made with whole mount preparations of cells, which were either shadowed or stained negatively. No records have, as yet, been published of basal bodies on bacterial flagella observed in thin sections. However, when living bacteria in preparation for ultramicrotomy are first embedded in agar and then fixed, the flagella are well preserved and may subsequently be seen in the section to pierce the cell wall and enter the cytoplasm. In a study in which Proteus mirabilis was prepared according to this adaptation of technique (20), we sometimes observed the flagella to originate from round struc-
tasures, approximately 25 to 45 mμ wide, at the periphery of the cytoplasm. Only in organisms which were very poor in their cytoplasmic content could the boundaries of the bodies be distinguished with sufficient clarity; therefore, it is desirable that further proof of the genuine existence of these bodies be provided by other techniques of investigation. To this end, we applied negative staining with potassium phosphotungstate to organisms in which much of the cellular content had been released by osmotic shock after the cell wall had been weakened by penicillin.

An earlier investigation of Proteus vulgaris (17, 18) on shadowed preparations of whole cells suggested that the flagella might emerge from the chondrioids (cf. footnote, reference 20). The chondrioids themselves can readily be made visible by incubating the bacteria with either potassium tellurite (17) or tetranitro-blue tetrazolium (23, 24). Reduced products of these compounds are deposited in rather restricted cytoplasmic areas contiguous with the plasma membrane. Such areas in Proteus do not have the membranous characteristics of chondrioids in many Gram-positive bacteria (for references, see 18).

In order to reinvestigate the relationship of the basal bodies of flagella to the chondrioids in Proteus mirabilis and to analyze further the structure of the chondrioids themselves, some cells whose walls had already been loosened by penicillin were incubated with potassium tellurite. They were then shocked osmotically and stained negatively with potassium phosphotungstate. The results of these experiments do not confirm the previous suggestion that the chondrioids function as the basal bodies of Proteus flagella.

Finally, some observations are included on the structure of free flagella which had been released from autolyzed organisms.

MATERIALS AND METHODS

In this work, two strains of Proteus mirabilis were used: one was obtained from Dr. E. Klieneberger-Nobel (cf. reference 32); the other from the Central Health Laboratories, Toronto, Canada (10-12). The bacteria were grown out overnight, usually by passing them through a motility tube of semisolid agar (heart infusion broth + 0.3% agar), then suspended in physiological saline to a concentration of about 10\(^9\) bacteria/ml. One ml of this solution was spread uniformly over the surface of heart infusion agar (Difco) in a 15-cm diameter Petri dish; the plates were incubated for 3½ to 5 hr at 35°C, i.e. to the differentiation of swarmers as checked with the light microscope (11). The bacteria were removed from the plates in heart infusion broth to which was subsequently added 2,000 IU/ml penicillin, 0.25 m sucrose, 0.01 m MgCl\(_2\), and 5% horse serum. Such suspensions were incubated for 45 to 60 min, i.e. until many of the bacteria had begun forming spheroplasts when checked under phase-contrast. The suspension was then divided into two parts: the first was centrifuged directly at 4,000 RPM and the resulting pellet shocked osmotically by diluting the organisms in distilled water; the second part was incubated under semi-anaerobic conditions for an additional hour with 0.05% potassium tellurite in stationary tubes filled to the brim and stoppered, then centrifuged, and shocked.

In a few experiments, the bacteria were removed from the plates of heart infusion agar in distilled water and allowed to autolyze by being kept overnight either in the refrigerator, for partial breakdown of the organisms, or in the 35°C incubator. The latter treatment was applied in the hope of completely freeing the flagella and their basal structures.

In all cases, the organisms—either whole or fragmented—were stained by mixing the suspension with an equal volume of 2% (w/v) potassium phosphotungstate (PTA) (41). The stained preparations were placed on electron microscope grids, coated with either carbon alone or Formvar reinforced with carbon, by breaking a thin film in a platinum loop onto the surface of the supporting membrane. The grid itself rested on a pad of filter paper so that excess fluid drained away as the film of bacteria dried down rapidly.

Electron micrographs were taken with a Philips EM 200 operating at 60 or 80 kv with the double condenser lens system, a 50-μ objective aperture, and the specimen cooling device.

OBSERVATIONS

Flagella and Basal Bodies

When cells of Proteus mirabilis are shocked osmotically in distilled water after treatment with penicillin, much of their content is released; yet many of the bacteria retain their shape and flatten on the supporting film as the potassium phosphotungstate dries down. An interesting example of such a cell is provided in Fig. 1: hardly any cytoplasm is left within the cell wall, apart from remnants of the plasma membrane (Pm) and a number of electron-transparent bodies, ca. 50 mμ wide, at the bases of the flagella. Occasionally, two such bodies are in close contact (see arrows), each bearing a flagellum (cf. also reference 2). Of interest are the several hoodlike folds (H) that
This bacterium has been almost completely emptied of its cytoplasmic content, except for a few remnants of plasma membrane (Pm). As a result, a number of basal bodies can be discerned, with one flagellum each. There are also several larger basal bodies bearing two flagella, and partially encircled by hoodlike folds (H). These latter bodies may have been in the process of growth and division, for there are several examples (indicated by arrows) of two basal bodies close together. Note the brilliant dots at d; these are interpreted as being part of the basal granule. × 150,000.
In this cell, the basal bodies of the flagella are largely hidden because the plasma membrane and much cytoplasm are still present. In contrast to the structures within the cell, comparatively thin and superficial structures like the flagella and fimbriae (f) can be distinguished readily. The arrows point to faint outlines of several basal bodies within the cell. The flagella are attached to the bacterium by means of hooks, marked h. × 104,000.
Figure 3 Basal bodies can be seen at B, i.e. where there is no plasma membrane. The basal body adjacent to the hook of the flagellum, marked h is apparently obscured by a portion of the plasma membrane. Note the fine structure of the cell wall. × 130,000.
partially encircle a large basal body with two flagella. Since the basal bodies are seen to carry one flagellum each, those with two flagella could possibly be in some stage of growth and division. The lucidity of the bodies is not quite even; in some, a more brilliant dot (d) can be discerned. This finding raises the question as to the significance of the differences in contrast in the electron micrographs of negatively stained specimens. For instance, the brilliant dots could be interpreted as being more compact in organic matter than is the rest of the basal body, the contrast of which could be lowered by small quantities of PTA either within the body or above and underneath it.

The question of the contrast in these specimens can be very involved, as is borne out by the cell in Fig. 2. Here it is doubtful whether one can discern bodies at the bases of the flagella (arrows); on the other hand, one can clearly see that some of the flagella are attached to the bacterium by means of hooks. This bacterium is not sufficiently transparent in the electron beam to reveal much internal structure, apparently because the layers of the cell wall and the plasma membrane on both sides of the organism are superimposed. Compared with such relatively large internal structures as the basal bodies (their size was determined from some 40 measurements to be 51 ± 6 m), the flagella and fimbiae (or pili) can be distinguished quite clearly. This, however, cannot serve as an argument that the basal bodies are not present, since, in spite of the considerable thickness of all the superimposed structures, the portions of the flagella lying above the bacterium in Fig. 2 have lost very little contrast when compared with those lying directly on the supporting membrane. The thickness of the flagella was found to be 133 ± 16 Å (mean ± sd), and that of the fimbiae (6, 7) or pili (4, 5) 55 to 70 Å. The organism in Fig. 2 was presumably derived from a fully fimbrate (piliated) bacterium in the inoculum. It is exceptional among our preparations because most of the other bacteria had developed beyond this stage to the fully flagellate condition (cf. reference 11).

In preparations like that of Fig. 2, the plasma membrane obscures the basal bodies of the flagella. Such bodies are only visible in those cells (e.g. Fig. 1) or at those sites (see arrows in Figs. 3 and 4) in which this membrane has disappeared. For instance, on the left in Fig. 3 a large basal body can be seen surrounded by the electron-opaque PTA, while on the right side no such structure can be discerned at the end of the flagellar hook (h) because it is hidden by a fragment of plasma membrane. In some cases, the flagella seem to extend from irregularly shaped “bodies” which are more or less elongated (Fig. 4), or have a quite fantastic configuration as in the isolated structure of Fig. 5. Quite recently, somewhat similar pictures were published by Abram et al. (2). Such structures are interpreted as fragments of plasma membrane which have been caught up by the flagellar endings and folded around them. This enfolding of the fragmented membrane probably hides the basal bodies. A similar structure was observed in thin section in Part I of this study (cf. Fig. 17 in reference 20). In Fig. 7, a basal structure—perhaps a basal body wrapped by membrane—can be discerned inside a fragment of cell wall through which the flagella have emerged.

Very rarely in our preparations did the basal body remain attached to a free flagellum, as in Fig. 8. Presumably, the bodies are fragile and break off easily (cf. reference 20). Fig. 9 shows the hooked ending of a flagellum from which the basal body has broken away, leaving a little material still attached. Fig. 10 is presented for comparison with Fig. 9. It shows in thin section two remnants of basal bodies, similar to that in Fig. 9, in a cell freed of nearly all its cytoplasm. The electron microscopy shows these remnants as structures enclosed by a double membrane.

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**Figure 4** Pm indicates elongated structures from which the flagella seem to extend. These structures are interpreted as fragments of the plasma membrane caught up by the basal bodies and folded around them. The arrow in the lower part of the figure points to a double basal body. × 73,000.

**Figure 5** This structure of unusual shape is believed to be a fragment of the plasma membrane folded around the basal bodies of the flagella. The preparation was made from bacteria which had been allowed to autolyze overnight in the cold. × 73,000.

**Figure 6** Arrows point to small protrusions on the basal bodies. × 135,000.
FIGURES 7 to 9 were made from bacteria which had been allowed to autolyze overnight at 35°C.

**FIGURE 7** Inside a fragment of cell wall, from which flagella emerge, there lies a piece of plasma membrane wrapped around one or more basal bodies. × 169,000.

**FIGURE 8** This detached flagellum shows a well preserved basal body. Note the collar, c, on the flagellum and next to it a narrowing where the flagellum is joined to the basal body. In the basal body, this point (labelled b) is a more or less disc-shaped area, apparently more solid than the rest of the structure. × 169,000.

**FIGURE 9** Nearly always the basal bodies break away from the detached flagella. Here, however, the part b of this apparatus remains. Note the collar, c, which is interpreted as being cell material. (cf. also Fig. 23, reference 2). × 169,000.

The micrograph is of material prepared according to the method described in our previous paper (20). In Fig. 10, the flagellar hooks pierce the cell wall and the plasma membrane; it is, therefore, probable that the collar in Fig. 9 (labeled c) is derived from the cell wall. Such a constriction of the flagellar hook has already been observed in negatively stained preparations by Abram et al. (2). We interpret the parts in Figs. 8 and 9, labeled b, as basal portions of the complete body, i.e. the sites at which the flagellum enters the body (cf. Figs. 15 and 17 in our previous study, reference 20). This area, b, may consist of plasma membrane material. The smooth contours of the basal body in Fig. 8 suggest that this apparatus is a true, separate entity.

An important question is whether the basal bodies of bacterial flagella are self-reproducing particles (cf. reference 20). The double bodies clearly visible in Fig. 1 give support to such a hypothesis. Moreover, the small protrusions to be seen at the arrows in Fig. 6 could perhaps be stages in the development of new basal bodies.
Figure 10  Thin section showing two flagella piercing the wall of a cell freed of nearly all its cytoplasm by being shocked osmotically (cf. reference 20). The hooklike bend of each flagellum is clearly visible. This electron micrograph is presented for comparison with Fig. 9, as it also shows the sites at which the flagella join the basal bodies; the major part of the latter is apparently removed during osmotic shocking. X 235,000.

Chondrioids and Flagellar Bases

The cells and their fragments that had been treated first with penicillin, then with potassium tellurite, and finally shocked in distilled water, clearly show the reduced product of tellurite in negatively stained preparations. The contrast at the sites where the reduced product accumulated, i.e. in the chondrioids, is very much greater than that of the potassium phosphotungstate. Penicillin treatment preceded the reduction of tellurite in the experiments because better preparations were obtained in this way. Apparently, the effect of penicillin on the cell wall was reduced if the chondrioids had first accumulated reduced tellurite. But the reduction proceeded well, even after an hour’s treatment of the bacteria with penicillin.

There was no proof for the presence of reduced tellurite at the points at which the flagella emerge from the bacterial cells (Figs. 11 to 13). On the one hand, since the several layers of cell wall and plasma membrane overlap, it is difficult to decide whether the basal bodies and the chondrioids are the same thing or two different structures (Fig. 12). On the other hand, in a bacterium like that in Fig. 11 there is obviously no reduced tellurite at the sites at which the flagella emerge from the cell (see arrows). Furthermore, the situation can be ambiguous, as indicated by the arrows in Fig. 12: within fragments of cell integument two structures are visible, one with reduced tellurite, the other without, and close to both is seen a flagellum.

Fig. 13 shows the end of a bacterium in which the plasma membrane has largely retracted: to the left are revealed the basal bodies in which the endings, often hook-shaped, of the flagella are anchored; to the right, remnants of the membrane with adherent areas of reduced tellurite, but no clearly visible flagellar bases. The structure at the base of the flagellum, indicated by an arrow,
Portion of a cell treated with potassium tellurite, a procedure that “stains” the chondrioids by the deposit of reduced product. As is the case in Fig. 2, the plasma membrane here hides the basal bodies. There is no evidence that the flagella originate from the chondrioids; the arrows indicate where the flagella emerge from the cell; there is no reduced tellurite at their bases. × 117,000.

Of particular interest are Figs. 14 and 15. Following osmotic shocking, fragments of the plasma membrane with the chondrioids still adhering can be found in these negatively stained preparations. Characteristically, these chondrioids are somewhat rounded as in Figs. 11 to 13; some of them measure about 40 mμ across. In these micrographs, one or two bundles of rods can be seen extending from the electron-opaque circular areas. This morphology of the chondrioids after tellurite reduction conforms to the previous description of them in serial sections by van Iterson and Leene (17). The rounded areas must be the part opposed to the plasma membrane; from them dense, rodlike structures protrude into the cytoplasm. In some of the rounded areas of the chondrioids (Figs. 14, 15), a mosaiclike arrangement can be membrane, a portion of which folds around a membrane has been released into the suspending liquid by shocking, the complete chondrioid still remains adherent.

DISCUSSION

The present observations of Proteus mirabilis confirm the finding, made by Abram, Vatter, and Kofler (1) with empty ghost cells of Proteus vulgaris, that the flagella “arise via hooks from spherical structures, 250 to 350 A in diameter.” Recently, however, Abram, Kofler, and Vatter (2) have amended their interpretation of the origin of Proteus flagella: “They appear to originate from spherical structures, the diameter of which is similar to or only slightly larger (110 to 140 A) than the diameter of the flagellum.” They also saw larger bodies, 20 to 70 mμ wide, at the base of many flagella. These, they suggest, “consist at least partly of fragments of the cytoplasmic membrane, a portion of which folds around a
A much fragmented plasma membrane beneath the cell wall. The flagella do not appear connected to the chondrioids. Basal bodies are visible at B because of the absence of the plasma membrane. The arrows point to two structures, one with reduced tellurite, the other without, and close to both is seen a flagellum. × 97,500.

Our interpretation is different. We believe that the basal bodies from which the flagella arise or emerge are the rounded structures, about 50 m in diameter. This belief is based on the following observations: (1) similar structures were found, in thin sections, close to the plasma membrane but separate from it (20); (2) the bodies are often paired, while others are nearly twice their normal size, as in Fig. 1, thus suggesting reproduction; (3) a basal body was found on a free flagellum (Fig. 8). The significance of the brilliant dot observed at the base of some flagella within the larger structure is not quite clear, but it must be part of the basal apparatus. It might very well be identical with the basal portion, labelled b in Figs. 8 and 9, i.e. the site at which the flagellum enters the body.

Our preparations were made from cells that were still in the logarithmic phase of growth when they were treated with penicillin. As discussed in the Introduction to our previous paper (20), many of the photographs of basal bodies which have been published previously were from preparations that had been autolyzed. Penicillin treatment is known to inhibit the synthesis of the mucopeptide component of the cell wall in Gram-negative bacteria (26-29, 34) so that spheroplasts are produced (cf. reference 12). The mucopeptide is the main component of the so called rigid layer (42) of the wall; it has recently been isolated from Proteus mirabilis (30). In our experiments, the cell wall was not greatly affected by the relatively smaller structure," i.e. the genuine basal body. The latter seems to be equivalent to the brilliant dot seen occasionally by us in the ca. 50-m bodies (cf. d in Fig. 1). Abram et al. (2) suggest, however, that the larger bodies "may not be real structural entities, but perhaps are artifacts resulting from the persistence of a part of the membrane after the rupture of the wall."

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FIGURE 13. To the right, remnants of the plasma membrane can be discerned with adherent deposits of reduced tellurite; to the left, where there is no plasma membrane, basal bodies are clearly visible. Note the hook-shaped endings on several flagella. The arrow indicates a collarlike structure resembling that on the bases of the flagella in Figs. 8 and 9. X 97,500.

short exposure to penicillin, though it usually expanded so that, when the bacteria were subsequently shocked in distilled water, much of their cytoplasmic content was released and the plasma membrane often torn to pieces.

In preparations treated with potassium tellurite, the basal bodies were left behind in the empty part of the cell when the plasma membrane had retracted, bearing with it the stained chondrioids (Figs. 12, 13). This observation makes it very unlikely that basal bodies and chondrioids, although similar in size, are identical as was postulated earlier (17). Moreover, where the plasma membrane is present (as in Figs. 11 and 12), the flagella can only rarely be traced to a chondrioid. The original supposition, that the mitochondrial equivalents in *Proteus* might function as basal granules of the flagella (17), was based on shadowed preparations of whole organisms, in which an overlapping of structures led to this presumably erroneous conclusion. It is interesting to note that the chondrioids adhere so firmly to the plasma membrane (Figs. 14, 15); they were earlier described as being contiguous with it (17, 18, 24). On the other hand, the basal granules appear to be quite separate from the membrane.

In view of the probable high energy requirement for the growth and function of flagella, it would have been gratifying to find the basal bodies and chondrioids at least close together. But the present investigation could hardly be expected to yield information on the relative positions of basal bodies and chondrioids, in view of our removal, in part, of the plasma membrane with its adherent chondrioids for the sake of revealing the basal bodies.

Asakura, Eguchi, and Iino (3) inferred, from experiments in which filaments of bacterial flagella were reconstituted from flagellin molecules in vitro, that the prerequisites for flagellar forma-
Figures 14 and 15  Fragments of plasma membrane with adherent chondrioids have been released from the cell by osmotic shocking. The chondrioids are roughly comparable in size to the basal granules, i.e. 40 mμ. Note the mosaiclike structure indicated by an arrow in some of the chondrioids. From them also one or two bundles of electron-opaque, rodlike structures are seen protruding. X 156,000.
tion in vivo are "the accumulation of flagellin and the presence of seeds or primer for polymerization," and that an energy source is unnecessary. These authors further suggest that "a basal granule might be an organelle which accumulates synthesized flagellin molecules and provides them with a seed for polymerization." Surely the function of the basal granules is broader than this. For example, they could act in the coordination of movement during locomotion.

The flagella are often anchored in the basal bodies by means of a hook, as can be seen clearly in Figs. 1, 3, 5, 10, and 13. In Fig. 2, where the bacterium is not transparent, the hook appears to terminate at the cell wall. Such hooks on the proximal ends of *Proteus* flagella were first described in 1953 (16) as follows: "Such an end may serve to hook the flagellum through the cell wall and into the granule." This interpretation is not strictly correct since—as Abram et al. (2) also observed—the hook extends as a much thinner strand from the cell wall to the plasma membrane or basal body. Hook-shaped endings were first observed by Houwink and van Iterson (13) on free flagella of *Agrobacterium radiobacter*. They have since been found for other bacteria, including *Spirillum serpens* (14, 33), *Vibrio metchnikovii* (9), *Vibrio comma* (40) and *Salmonella typhi*
vulgaris (22, 25). Rogers and Filshie (39) named them "rootlets." Recently, Abram et al. (2) and Lowy (25) have found that the globular subunits composing the entire flagellum have an ultrastructural arrangement in the hooked region which is different from that in the rest of the flagellum.

Glauert, Kerridge, and Horne (9) observed in autolyzed cells of *Vibrio metchnikovii*, negatively stained with potassium phosphotungstate, that the core of its sheathed flagellum ends in a basal disc which is situated just inside the plasma membrane. This "basal disc" or "cup," 30 to 35 μm in diameter, appeared in face view as a light central dot. These authors believe that "the larger basal granules described by previous workers in shadowed preparations are agglomerates of cytoplasmic debris surrounding the basal disc." However, in our micrographs of *Proteus mirabilis* the cells were not autolyzed, and it is perhaps for this reason that the basal bodies give the impression of being rather compact (cf. Figs. 1 to 4, 6, 12, 13). Further, it is not yet certain whether the basal apparatus of the 300-A-wide, single polar flagellum of *Vibrio* is constructed exactly like the basal bodies of the peritrichate *Proteus* with its numerous thin flagella, approximately 130 A wide. Sometimes a brilliant dot was observed in the basal body at its junction with the flagellum (Fig. 1), as if a small amount of PTA had entered the rest of the body. As pointed out under Observations, there are difficulties in interpreting negatively stained preparations. It is, therefore, highly desirable that adequate studies be made of the so-called anomalous contrast effects (31), and that these be extended to biological material.

The size of 50 μm measured in our negatively stained preparations is somewhat higher than the 25- to 45-μm value estimated on the sectioned material (20); probably, there is some shrinkage during fixation and embedding. As mentioned in Part I (20), more importance should, however, be attached to these estimates than to the "about 100 μm diameter" measurement made of the spheres in autolyzed preparations of *Proteus vulgaris* (13, 15).

The basal bodies described in this article may be "self-reproducing particles," since frequently two such bodies are seen adhering together as if in some stage of division (cf. Figs. 1, 4, 6). Further, other bodies, larger than usual, carry two flagella (Figs. 1, 4, at arrows). Similar observations were reported recently for *Proteus vulgaris* (2).

Various investigators (for references, see 38) have postulated that basal bodies and centrioles in eucaryotic cells are self-replicating organelles. Renaud and Swift (38) found that the basal body in the gamete of the fungus *Allomyces arbusculus* was not formed de novo, but from a small, pre-existing centriole. Gall and Mizukami (8) arrived at a somewhat different conclusion in the case of the fern, *Marsilea*; namely, that the basal bodies originate from compact spheres of radially oriented tubules found at the poles of the mitotic spindle, but of unknown origin. The development of basal bodies in animal cells awaits clarification. But centrioles have never been described as taking part in the nuclear division of bacteria, and it is, at any rate, extremely unlikely that the basal bodies in bacteria will prove to have the same fine structure as those in higher organisms.

Older bacteriologists referred to the basal granules in bacteria as "blepharoplasts," deriving the name by analogy from the basal apparatus of the locomotor organelles in flagellate protozoa. The soundness of this depends on the
definition of the term. Randall and his coworkers (36, 37) have established the presence of DNA in the basal bodies of protozoa. But the basal structures of our bacterial flagella are so small that it would be exceedingly difficult to discover whether they also contain DNA. The bodies line the plasma membrane, and so are located some distance from the nuclear area (cf. reference 20). At present, there is no evidence that in bacteria the nucleoplasm is confined within a membranous envelope. Moreover, the possibility that strands containing nucleic acids may extend from the nucleoplasm towards the plasma membrane is being investigated (19).

Doubt has been cast on the existence of basal bodies in Proteus (9, 21, 35) first observed in a shadowed preparation of an autolyzed swarmer (13, 15). However, Abram et al. have since found them in negatively stained preparations of empty ghosts, and in “long forms” produced by prolonged incubation at low temperature (1, 2); in some cases, the bodies occurred in pairs. The present work, based on thin sections (20) and negatively stained preparations of lysed bacteria from actively developing cultures, and on cells whose chondrioids have been “stained” with tellurite, presents evidence that the basal bodies, often seen in pairs, may indeed be genuine structures which can be distinguished from the plasma membrane proper.

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