A "MICROTUBULE" IN A BACTERIUM

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

A study of the anchorage of the flagella in swarmers of *Proteus mirabilis* led to the incidental observation of microtubules. These microtubules were found in thin sections and in whole mount preparations of cells from which most of the content had been released by osmotic shock before staining negatively with potassium phosphotungstate (PTA). The microtubules are in negatively stained preparations about 200 A wide, i.e. somewhat thicker than the flagella (approximately 130 A). They are thus somewhat thinner than most microtubules recorded for other cells. They are referred to as microtubules because of their smooth cylindrical wall, or cortex, surrounding a hollow core which is readily filled with PTA when stained negatively. Since this is probably the first time that such a structure is described inside a bacterium, we do not know for certain whether it represents a normal cell constituent or an abnormality, for instance of the type of "polysheaths" (16).

INTRODUCTION

Microtubules are filaments 120 to 270 A wide with a core of low electron opacity. They have long escaped the attention of electron microscopists, presumably because of inadequate techniques of cellular fixation. But since fixation with aldehydes, in particular with glutaraldehyde followed by osmium tetroxide, has become standard procedure, tubular structures of varying dimensions have been found in practically all types of plant and animal cells which have been examined, and in the protozoa. These findings are recorded in an ever increasing number of publications which cannot be reviewed here (*e.g.* 1–3, 5, 7–11, 17, 18, 25–29).

These articles convey the general impression that the microtubules are to be found in nondividing cells in the cytoplasm, while in dividing cells they are constituents of the mitotic spindle. Comparatively early, attention was directed to the fibrous or tubular nature of the achromatic apparatus. Using divalent cations in combination with fixation in osmium tetroxide, Roth and Daniels (22) found 14-mμ-wide fibrils in the spindle of dividing nuclei from the giant ameba, *Pelomyxa carolinensis*, which were characterized by a dense cortex and a less dense center. Later, Roth and Shigenaka (23) and Carasso and Favard (5) described similar tubules in dividing macro- and micronuclei of ciliates. The former authors considered these tubules to be similar in diameter and appearance to the filaments composing the cilia. Ledbetter and Porter (17) also state: "In their form, if not dimensions, these structures of the mitotic spindle are not unlike the filaments which make up the 9 + 2 filamenture of the cilium and flagellum." The dimensions of the filaments in cilia and flagella match those of the microtubules found by Porter and Ledbetter in

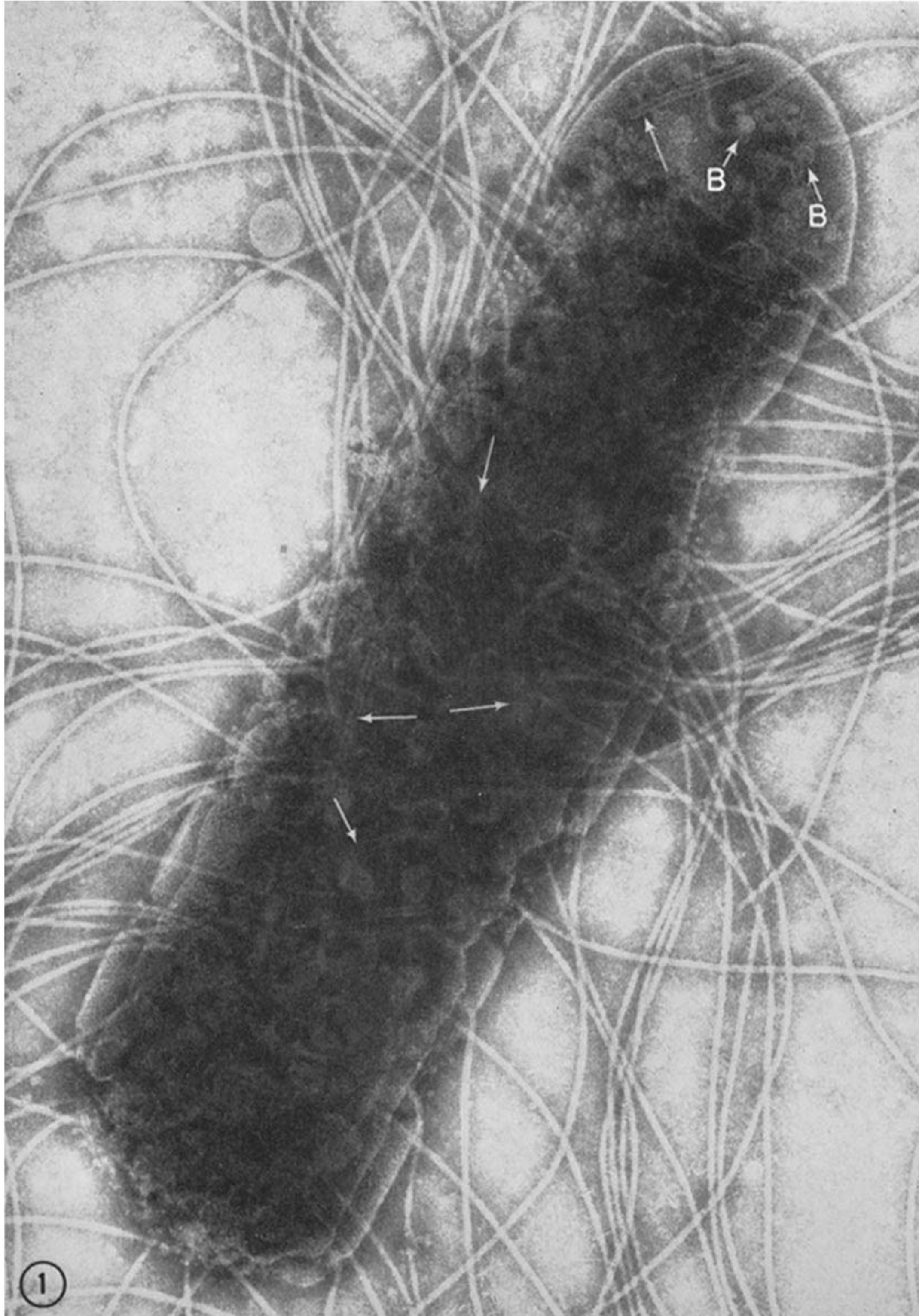


FIGURE 1 A cell of *Proteus mirabilis* from which most of the contents were released by osmotic shocking in distilled water after a light treatment with penicillin. It will be seen that in this negatively stained preparation the microtubules (arrows) have a random configuration. Basal bodies of the flagella can be observed at *B*, *i.e.* where the plasma membrane has disappeared. (cf. also Fig. 5.) × 158,000.

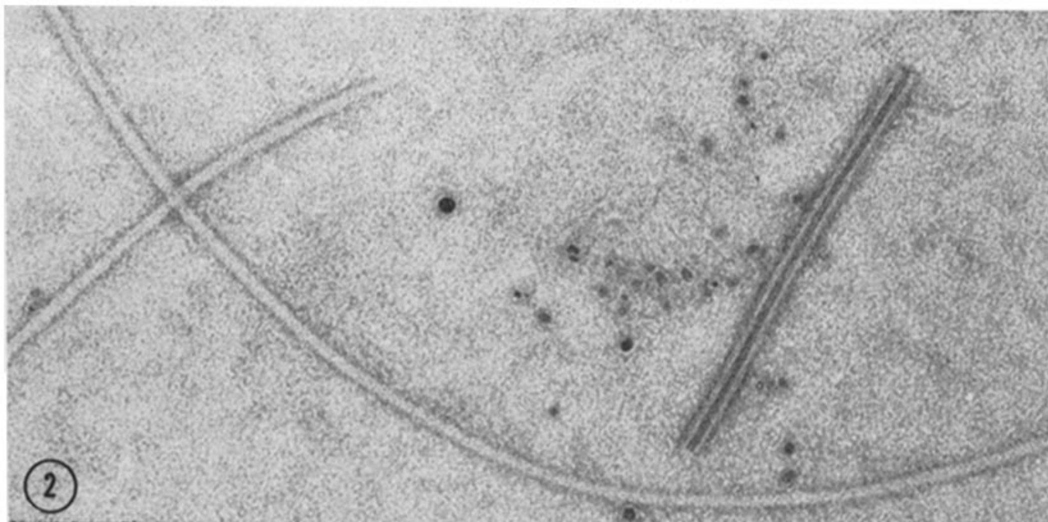


FIGURE 2 This negatively stained preparation shows a microtubule which has dried down directly on the supporting membrane following its release from an osmotically shocked cell. The center of the microtubule is filled with PTA. The tubule is somewhat wider than the two flagella. × 130,000.

plant cells (17; and for a spermatozoid, cf. reference 27), but are greater than those reported for the mitotic spindle of animal cells.

To the best of our knowledge, bacteria and blue-green algae are not yet listed among the cell types for which microtubules have been recorded. The question of whether microtubules similar to those in higher cells and protozoa occur in the lower protists is a fundamental one because the latter are believed to divide nonmitotically. Moreover, if the comparison between microtubules and the filaments comprising cilia and flagella is a sensible one, it should be borne in mind that bacterial flagella are not built according to the 9 + 2 pattern. A single flagellum of *Proteus mirabilis* has a diameter of about 130 A (in negatively stained preparations) and is thus slightly thinner than the filaments comprising the eucaryotic flagellum or cilium. It could be that filaments in the bacterial cell, comparable in function to microtubules of the larger cell types, would be too thin to be detected readily in thin sections.

It was, therefore, somewhat astonishing that, in the course of a study of the basal structures of the flagella of *Proteus mirabilis*, we occasionally observed in this organism tubules which were about 200 A wide and presumably hollow. These microtubules were seen in negatively stained preparations of cellular debris and in thin sections of bacteria fixed directly through the agar in the osmium tetroxide solution of Ryter and Kellenberger (24). These microtubules were found to be somewhat thicker than the flagella of *Proteus*.

MATERIALS AND METHODS

The strains of *Proteus mirabilis*, the procedure for culturing them, and the techniques of electron microscopy were all as described previously (12, 15). After swarmers had developed on a moist agar surface, the bacteria were treated with penicillin G (2,000 IU/ml for 60 to 75 min) to loosen their cell walls, and some of these were subsequently shocked osmotically in distilled water. Many preparations were exposed to potassium tellurite, the reduction of which enhances contrast in the chondrioids (14).

OBSERVATIONS

In preparations stained negatively with potassium phosphotungstate, microtubules were occasionally found inside the remnants of cells (Figs. 1, 3, 4) or free on the supporting membrane (Fig. 2). Inside the cells, they became visible only after sufficient cytoplasmic material had been released by osmotic shock following penicillin treatment (12). The structures were comparatively short rods, approximately 0.1 to 0.45 μ long and 200 A wide, with a core of about 64 A. The microtubules

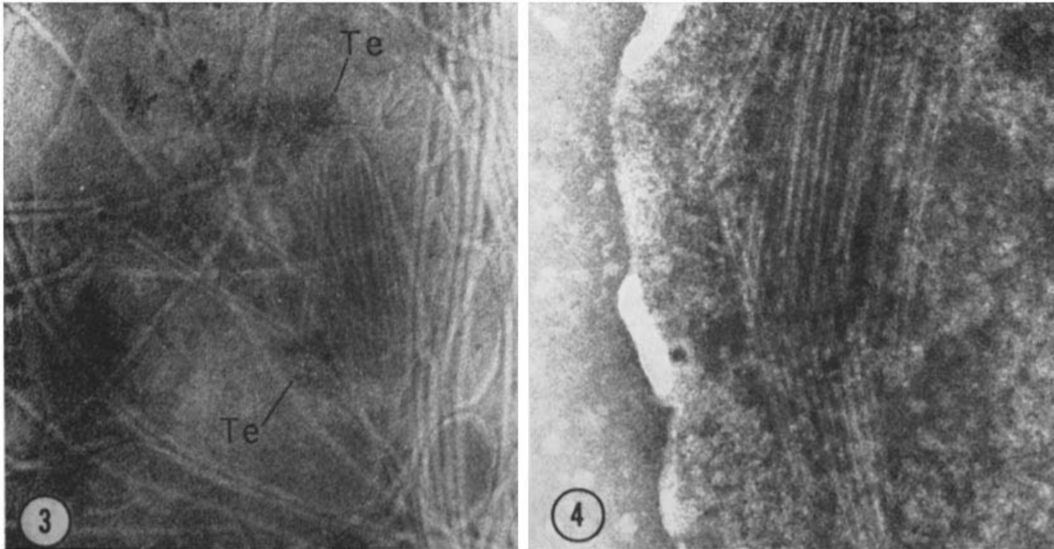


FIGURE 3 A package of microtubules within a partly emptied cell negatively stained with PTA. Some rods are orientated obliquely to others that are parallel. The specimen had been treated with tellurite to enhance the contrast of the chondrioids. The deposits of reduced product are labeled *Te*. The chondrioids are attached to the plasma membrane and here appear in the vicinity of the microtubules. × 143,000.

FIGURE 4 Array of microtubules within a fragment of the plasma membrane that was found to be free from cell wall material. × 112,500.

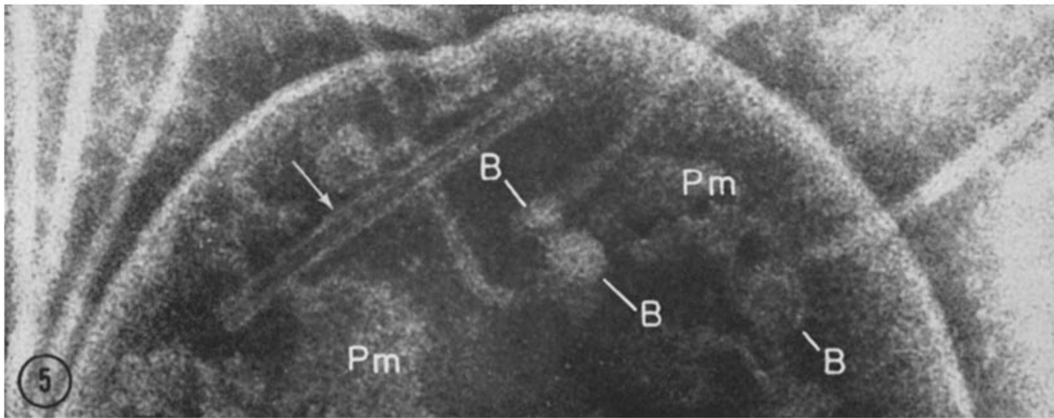
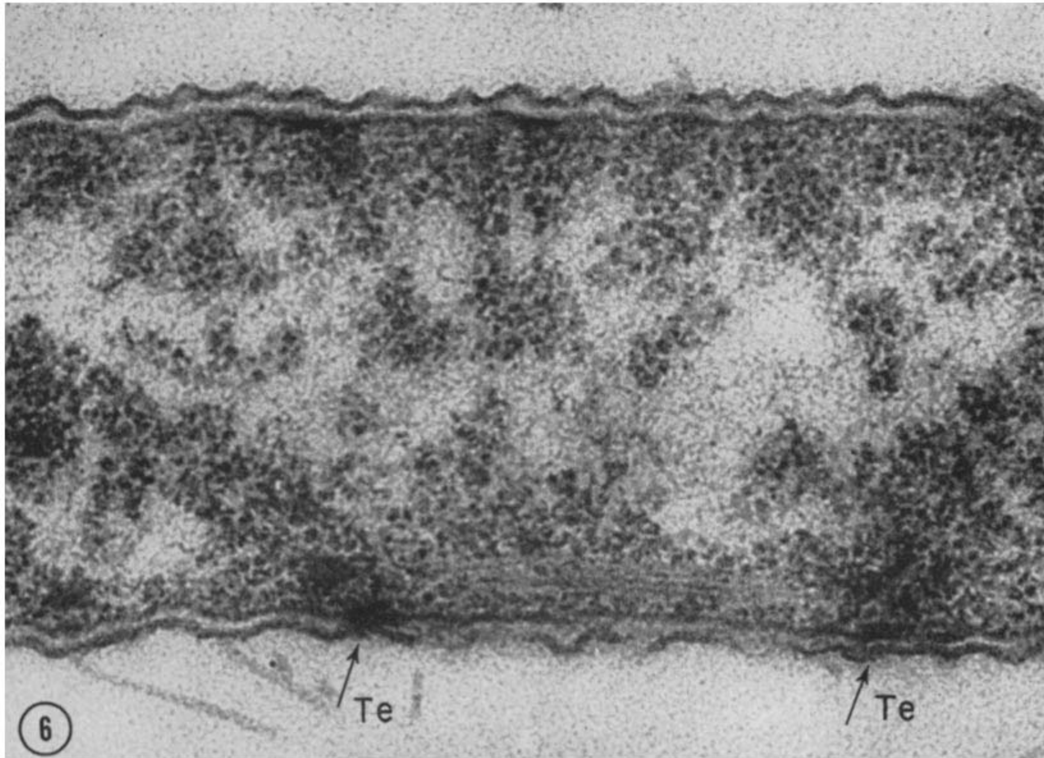
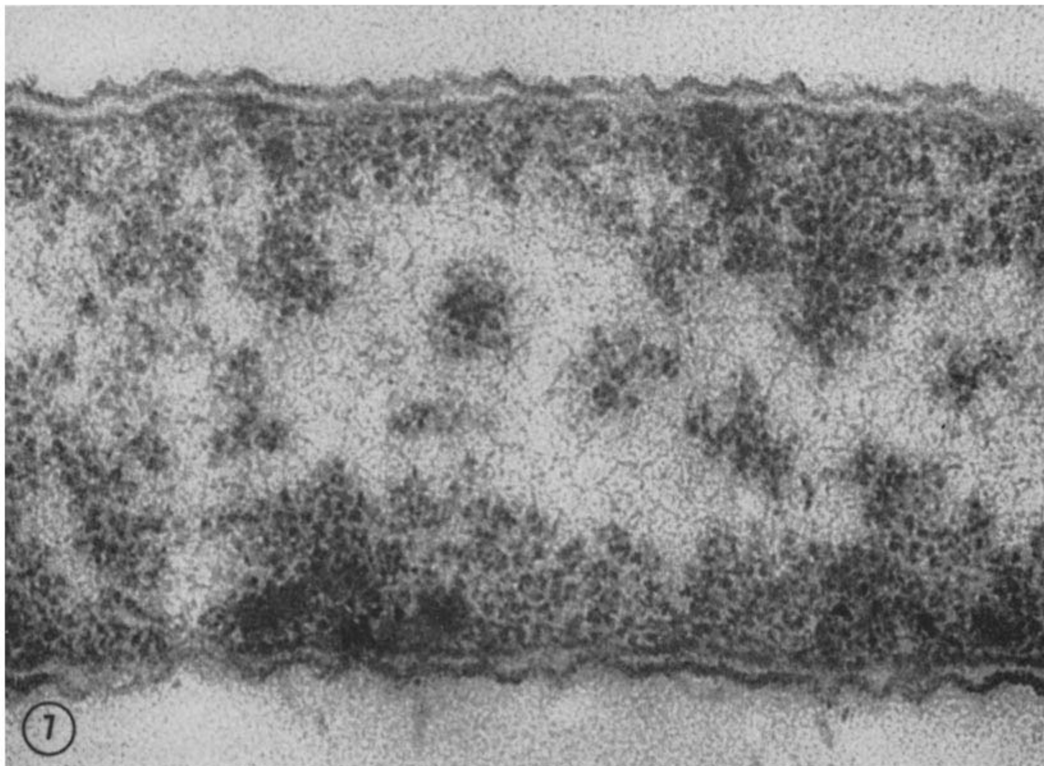


FIGURE 5 The tip of the bacterium in Fig. 1 shown at higher magnification. Except for a few fragments of the plasma membrane (*Pm*), the cell wall is comparatively clear and the basal bodies of the flagella (*B*) can readily be seen. The microtubule (arrow) looks as if it could well be open at both ends, and is somewhat stouter than the flagella. × 273,000.

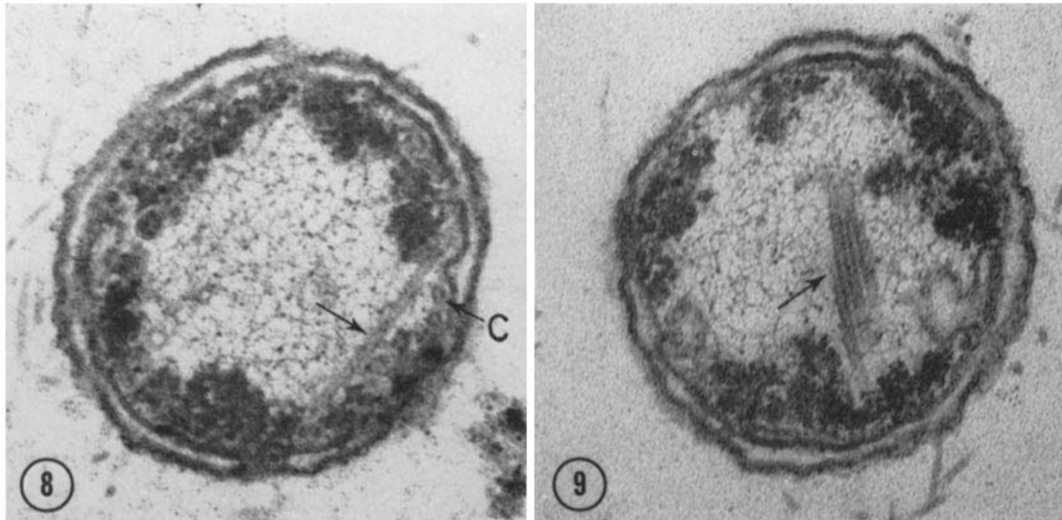
are definitely wider than the flagella. The hollowness of the core is indicated by its being stained strongly with potassium phosphotungstate (Fig. 2) and by its relatively low contrast in both cross-sections (Figs. 8, 10 to 12) and longitudinal sections (Figs. 6, 8–9).

The distribution of microtubules within a cell can be studied in Fig. 1 where they are seen singly





FIGURES 6 and 7 Two longitudinal sections in series. A bundle of microtubules parallel to the cell surface has been cut almost longitudinally. The fibers could perhaps be connected with two chondrioids (*Te*) at the arrows. The section in Fig. 7 is already free from microtubules. In the vicinity of the two chondrioids flagella are seen to emerge. × 143,000.



FIGURES 8 and 9 Two single sections from a long series of cross-sections through the same organism. The microtubules indicated by arrows are sectioned more or less lengthwise. They run obliquely with respect to the cell's axis, but others, close to the periphery in Fig. 8, appear in cross-section (*C*). × 104,000.

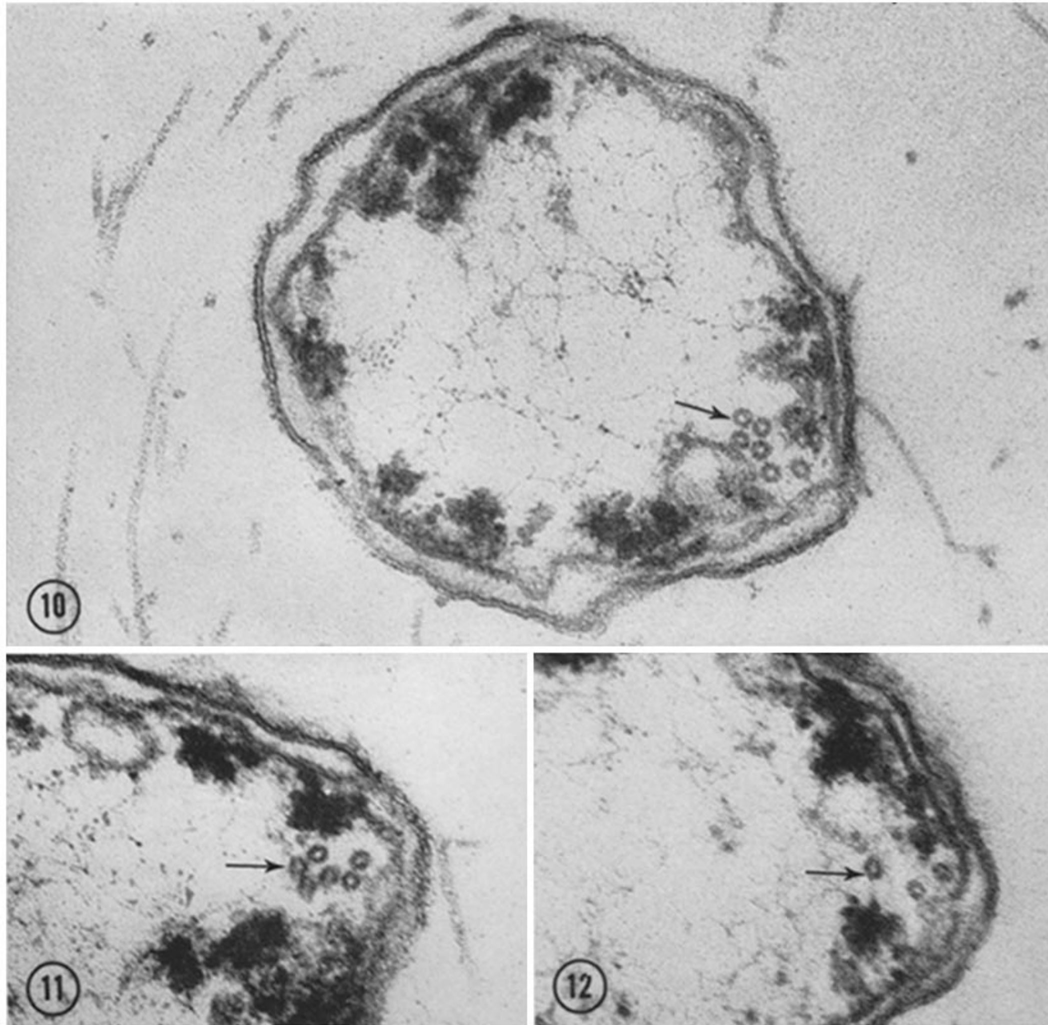
(arrows). But this is not always the case: sometimes, more or less parallel packages are observed, as in Fig. 3; here, other rods are orientated obliquely to the package. The orientation of the packages or single rods within cells seems rather random in the flattened material (Fig. 1); such a distribution is probably influenced by preparation of the specimen. In sectioned cells the orientation of the microtubules can be lengthwise, as in Fig. 6 and Figs. 10 to 12 (cf. serial cross-sections), or it may be oblique to the cell's axis (Figs. 8 and 9). In Fig. 10, a substructure consisting of a number of subunits is just discernible in the periphery of the microtubules; this recalls the observation of Ledbetter and Porter (18).

The organisms in Figs. 3 and 6 to 12 had all been treated with potassium tellurite. It is interesting to note that the fibers in Fig. 6 appear to connect two deposits of reduced tellurite (see arrow, *Te*). In the following serial section (Fig. 7), the longitudinal striations at the lower side of the cell have disappeared, and flagella are now seen to be inserted close to the sites of accumulation of reduced product. It would, of course, be too rash to conclude from this single observation that the location of microtubules is normally between two chondrioids.

Fig. 5 shows an exposure of the same cell as in Fig. 1, but taken at higher magnification. The tip of the bacterium is comparatively free from material of the plasma membrane, though some remnants may be attached to the tubule. Without the adherent plasma membrane, the microtubule looks as if it could well be open at both ends. It is obviously thicker than the flagella, some of which are seen to arise from basal bodies, marked *B*.

DISCUSSION

The fact that the microtubules in *Proteus* are found in sections of entire cells as well as in negatively stained preparations of cellular debris makes it likely that they are genuine structures, and not an artifact of specimen preparation. They must be comparatively robust structures since they are well preserved outside the cell following osmotic shock (Fig. 2). In the literature the most successful preparations of microtubules, apart from those fixed in glutaraldehyde, have been obtained in the presence of divalent cations (22, 28), and our specimens were routinely prepared in the presence of magnesium ions. The reason for referring to these rods as microtubules is that they are constructed as a smooth circular wall, or cortex, surrounding a hollow center which is readily filled with PTA when stained negatively. In cross-section, a substructure is just visible resembling the one ob-



FIGURES 10 to 12 Three cross-sections from a cell with very little cytoplasm. Tubules parallel to the cell's axis appear in cross-section. Their original number of 6 in Fig. 10 is reduced to 3 in Fig. 12. In Fig. 10, a substructure in the periphery of the tubules is just discernible in the original photographic print. × 130,000.

served by Ledbetter and Porter (18) in the microtubules of plant cells; but the present tubules are thinner. Remarkably enough, the microtubules in *Proteus* with their diameter of about 200 A are in the same order of size as those recorded in the literature for the more highly differentiated cells, i.e. 120 to 270 A. Slautterback (28), however, has pointed out that the reported dimensions may suffer from inaccuracy. Comparison with published micrographs suggests that the microtubules of *Proteus* are somewhat thinner than those in most other cells.

It is remarkable that tubules of these dimensions have not previously been described inside the bacterial cell. In our study of flagellation in *Proteus mirabilis* they were overlooked at first, but later they were observed quite frequently. So far, they have been recorded in over 60 different electron micrographs, and it would be quite easy to extend this number significantly.

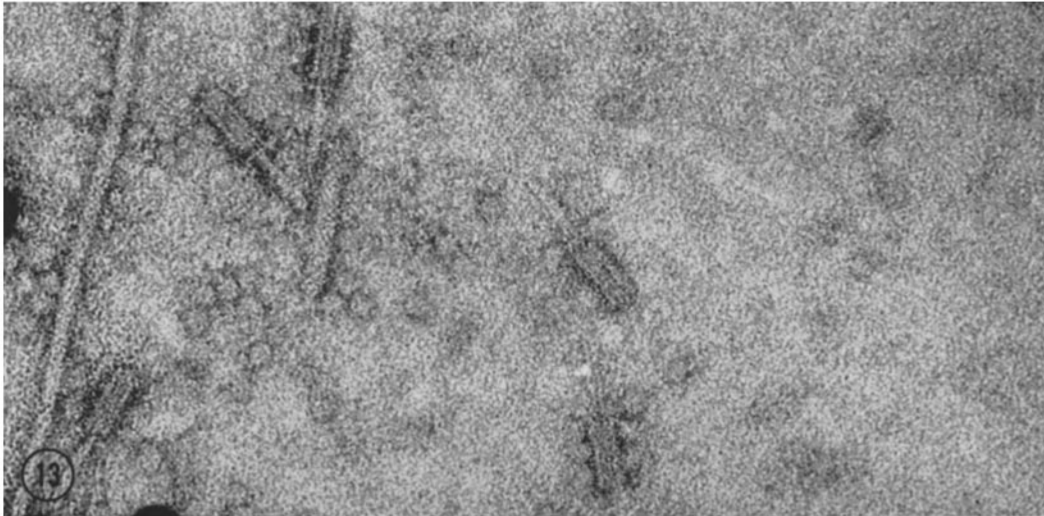


FIGURE 13 On treatment with mitomycin C in the cultures of two of the investigated strains of *Proteus*, particles appear looking like headless bacteriophages. × 294,000.

The arrangement and position of microtubules in *Proteus* cells do not provide us with an obvious clue to their function or significance. They could be involved in some way with the curious swarming motion of this organism over the surface of moist agar. The main question as to whether such microtubules represent a normal and constant constituent of particular bacterial cells, or are an abnormality, remains unsolved at present; indeed it may prove to be a much involved problem. Swarmers of three different strains of *Proteus mirabilis* were checked for the presence of microtubules; they were found in all three. But our methods are inadequate for deciding whether they occur in all cells of a culture.

The microtubules might also be considered to represent some kind of abnormality, such as a virus infection; one could think, for instance, of abortive formation of phage tails. In an earlier version of this paper, this supposition was considered unlikely in view of the smooth contours of the tubules in contrast with the morphological subunits which make up the tail of bacteriophages and may be regarded as true capsomeres (4). Even at good resolution cross-striations are not a distinct feature of the present tubules. But we have come to realize that elongated hollow structures bearing resemblance to our microtubules have already been recorded. In Fig. 11 of Kellenberger and Boy de la Tour's (16) paper "On the fine structure of normal and 'polymerized' tail sheath of phage T4," the chemically isolated tail sheaths resemble in contours and central hole the much longer tubules of *Proteus*. Therefore, the microtubules may be interpreted as the smooth form of polysheaths (16) which are aberrant assemblies of the tail material of phages. But there were no indications that our *Proteus* strains carry bacteriophages. To check this latter possibility, cells in the logarithmic phase of growth were treated with mitomycin C, a procedure which, in many cases, is known to induce bacteriophages or "phage-like objects" (*cf.* reference 4). In two of the strains "phage-like objects" did, indeed, appear (Fig. 13), looking like headless phages and strikingly resembling those described by Ishii *et al.* (13) for *Pseudomonas* as pyocin, a bacteriocin. The width of the sheath of these particles approaches that of the microtubules. Further investigation is required to establish whether a relationship exists between these phagelike particles and the tubules. The particles also resemble the "rhapidosomes" observed by Lewin and others in the gliding bacteria, *Saprospira* (6, 19) and *Archangium* (21) which contain protein and ribonucleic acid. In some illustrations, these rhapidosomes are accompanied by short, hollow tubules resembling fragments of the long microtubules of *Proteus*. In thin section, rhapidosomes were ob-

served in the nuclear area of *Saprospira* cells (19) which had passed the logarithmic phase. In view of these various data, the significance of the long, smooth microtubules in the cytoplasm (Fig. 6) and nucleoplasm (Figs. 8 to 12) of *Proteus* is somewhat problematic.

The observation of microtubules in *Proteus* was made quite incidentally. Further investigation is necessary to ascertain whether such structures are normal constituents of bacteria in general, or the consequence of induction by a stimulus of foreign origin. One explanation for their strong resemblance to the microtubules of ubiquitous distribution might be that they all fulfill a general structural principle as do, for instance, the lipoprotein membranes which, while showing some differences, still have important features in common. In this sense, it would be possible to regard microtubules as "elementary structures of the cell" (20). In spite of the problematic aspects, the existence in a bacterial cell of a structure similar in dimensions to the microtubules of larger and more highly differentiated cell types is in itself a finding of considerable interest.

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 18 April 1966.

REFERENCES

1. Bassot, J.-M., and Martoja, R., Présence de faisceaux de microtubules dans les cellules du canal éjaculateur du Criquet migrateur, *J. Micr.*, 1965, **4**, 87.
2. Behnke, O., A preliminary report on "microtubules" in undifferentiated and differentiated vertebrate cells, *J. Ultrastruct. Research*, 1964, **11**, 139.
3. Behnke, O., Further studies on microtubules. A marginal bundle in human and rat thrombocytes, *J. Ultrastruct. Research*, 1965, **13**, 469.
4. Bradley, D. E., The morphology and physiology of bacteriophages as revealed by the electron microscope, *J. Roy. Micr. Soc.*, 1965, **84**, 257.
5. Carasso, N., and Favard, P., Microtubules fusoriaux dans les micro et macronucleus de Ciliés Péritriches en division, *J. Micr.*, 1965, **4**, 395.
6. Correll, D. L., and Lewin, R. A., Rod-shaped ribonucleoprotein particles from *Saprospira*, *Canad. J. Micr.*, 1964, **10**, 63.
7. Crownshaw, J., and Bouck, G. B., The fine structure of differentiating xylem elements, *J. Cell Biol.*, 1965, **24:** 415.
8. De-Thé, G., Cytoplasmic microtubules in different animal cells, *J. Cell Biol.*, 1964, **23**, 265.
9. Fawcett, D. W., and Witebsky, F., Observations on the ultrastructure of nucleated erythrocytes and thrombocytes, with particular reference to the ultrastructural basis of their discoidal shape, *Z. Zellforsch. u. Mikr. Anat.*, 1964, **62:** 785.
10. Gall, J. G., Fine structure of microtubules, *J. Cell Biol.*, 1965, **27**, 32A.
11. Haydon, G. B., and Taylor, D. A., Microtubules in hamster platelets, *J. Cell Biol.*, 1965, **26**, 673.
12. Hoeniger, J. F. M., Iterson, W. van, and Nijman van Zanten, E., Basal bodies of bacterial flagella in *Proteus mirabilis*. II. Electron microscopy of negatively stained material, *J. Cell Biol.*, 1966, **31**, 602.
13. Ishii, S., Nishi, Y., and Egami, F., The fine structure of a pyocin, *J. Mol. Biol.*, 1965, **13**, 428.
14. Iterson, W. van, and Leene, W., A cytochemical localization of reductive sites in a Gram-negative bacterium. Tellurite reduction in *Proteus vulgaris*, *J. Cell Biol.*, 1964, **20**, 377.
15. Iterson, W. van, Hoeniger, J. F. M., and Nijman van Zanten, E., Basal bodies of bacterial flagella in *Proteus mirabilis*. I. Electron microscopy of sectioned material, *J. Cell Biol.*, 1966, **31**, 585.
16. Kellenberger, E., and Boy de la Tour, E., On the fine structure of normal and "polymerized" tail sheath of phage T4, *J. Ultrastruct. Research*, 1964, **11**, 545.
17. Ledbetter, M. C., and Porter, K. R., A "microtubule" in plant cell fine structure, *J. Cell Biol.*, 1963, **19**, 239.
18. Ledbetter, M. C., and Porter, K. R., Morphology of microtubules of plant cells, *Science*, 1964, **144**, 872.
19. Lewin, R. A., and Kiethe, J., Formation of rhapidosomes in *Saprospira*, *Canad. J. Microbiol.*, 1965, **11**, 935.
20. Palade, G. E., The organization of living matter, *in* The Scientific Endeavor, Centennial Celebration of the National Academy of Sciences, New York, The Rockefeller University Press, 1963, 179.
21. Reichenbach, H., Rhapidosomen bei Myxobakterien, *Arch. Mikrobiol.*, 1965, **50**, 246.

22. ROTH, L. E., and DANIELS, E. W., Electron microscopic studies of mitosis in amebae. II. The giant ameba *Pelomyxa carolinensis*, *J. Cell Biol.*, 1962, **12**, 57.
23. ROTH, L. E., and SHIGENAKA, Y., The structure and formation of cilia and filaments in rumen protozoa, *J. Cell Biol.*, 1964, **20**, 249.
24. RYTER, A., and KELLENBERGER, E., Etude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active, *Z. Naturforsch.*, 1958, **13b**, 597.
25. SANDBORN, E., KOEN, P. F., McNABB, J. D., and MOORE, G., Cytoplasmic microtubules in mammalian cells, *J. Ultrastruct. Research*, 1964, **11**, 123.
26. SATIR, P., and STUART, A. M., A new apical microtubule-associated organelle in the sternal gland of *Zootermopsis nevadensis* (Hagen), Isoptera, *J. Cell Biol.*, 1965, **24**, 277.
27. SILVEIRA, M., and PORTER, K. R., The spermatozoids of flatworms and their microtubular system, *Protoplasma*, 1964, **59**, 240.
28. SLAUTTERBACK, D. B., Cytoplasmic microtubules. I. Hydra, *J. Cell Biol.*, 1963, **18**, 367.
29. WOLFE, S. L., Isolated microtubules, *J. Cell Biol.*, 1965, **25**, 408.