ELECTRON MICROSCOPY OF FLAGELLATION IN SPECIES OF *SPIRILLUM*[1]

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Migula, in 1900, separated the genus *Spirillum* Ehrenberg from the other spiral bacteria on the basis of motility by means of tufts of polar flagella. Migula mentioned the fact that the tuft, in certain species, was often agglutinated into a fascicle which appeared as a single flagellum in flagellar stain preparations. Tufts of polar flagella long remained a generally accepted distinguishing morphological character of the genus. This character was not, however, always observed by all investigators. For example, Beijerinck (1925) and Dimitroff (1926) described species of *Spirillum* as having a single flagellum. Giesberger (1936) was the first investigator of the genus *Spirillum* to insist on a rigid interpretation of the character of tufts of polar flagella. He suggested that those species which had been described as having a single flagellum be transferred to the genus *Vibrio*.

Williams and Rittenberg (1957) described six species of the genus *Spirillum* as having a single flagellum in flagellar stain preparations prepared after the manner of Gray (1926). In their discussion of flagellation these authors admitted that the single flagellum might be a fascicle such as had been mentioned and illustrated by Migula (1900). They justified their description of the six species as having a single flagellum on the fact that only a single flagellum could be seen in living cells of the larger species when the cells were observed by phase contrast. In such phase contrast observations of living cells the flagellum appeared as a whiplike organelle without that fuzziness in the outline which would have been apparent if a number of flagella were being moved in unison.

This electron microscopic survey had two purposes: (i) to determine if tufts of polar flagella could be considered to be a determinative morphological character of the species of the genus and (ii) to determine which of the many flagellar staining methods revealed the closest agreement with the findings obtained by electron microscopy.

MATERIALS AND METHODS

The 13 species described by Williams and Rittenberg (1957) as well as 13 undescribed species were used. The cells were grown in broth for 48 hr, centrifuged, and washed several times in tap water. The marine species were fixed prior to centrifugation and washing by the addition to the broth tubes of an amount of 10 per cent neutral formalin equal in volume to the broth (Leifson, 1951). The mixture was allowed to stand for 30 min, after which time the cells were centrifuged and washed. This procedure permitted the removal of the salts in the sea water medium without lysis of the cells.

The specimens were mounted on collodion coated 200 mesh copper grids, dried at 37 C, and shadowed with chromium at an angle of about 4:1. The preparations were examined with an RCA EMU-2D electron microscope equipped with a 0.015-in. externally centerable (Canalco) condenser aperture and a 60 μ objective aperture in the standard pole piece.

Flagellar stains were made by the methods of Gray (1926) and Leifson (1951) and photographed with the Leitz Ortholux light microscope.

RESULTS AND DISCUSSION

Both objectives of the electron microscopic survey were achieved. All species, with one possible exception, showed multiple flagella. In one species, *Spirillum polymorphum*, which Leifson (1960) has described as having a single flagellum, it could not be determined whether the organism possessed a single, thick, compound flagellum or whether this apparent single flagellum was,

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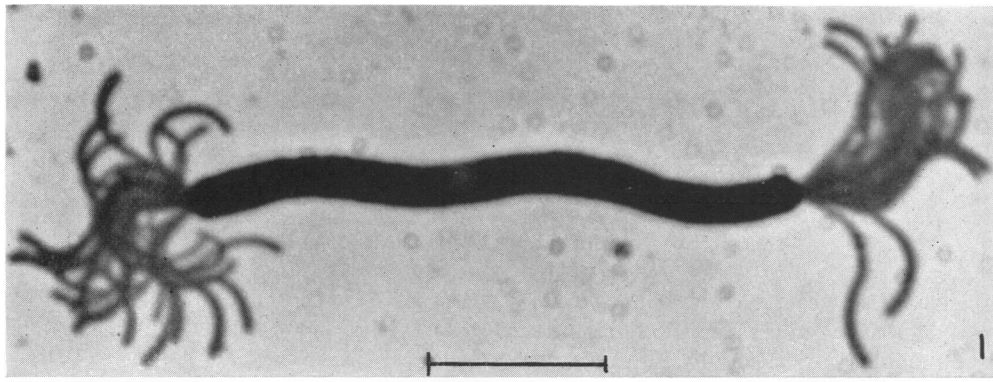


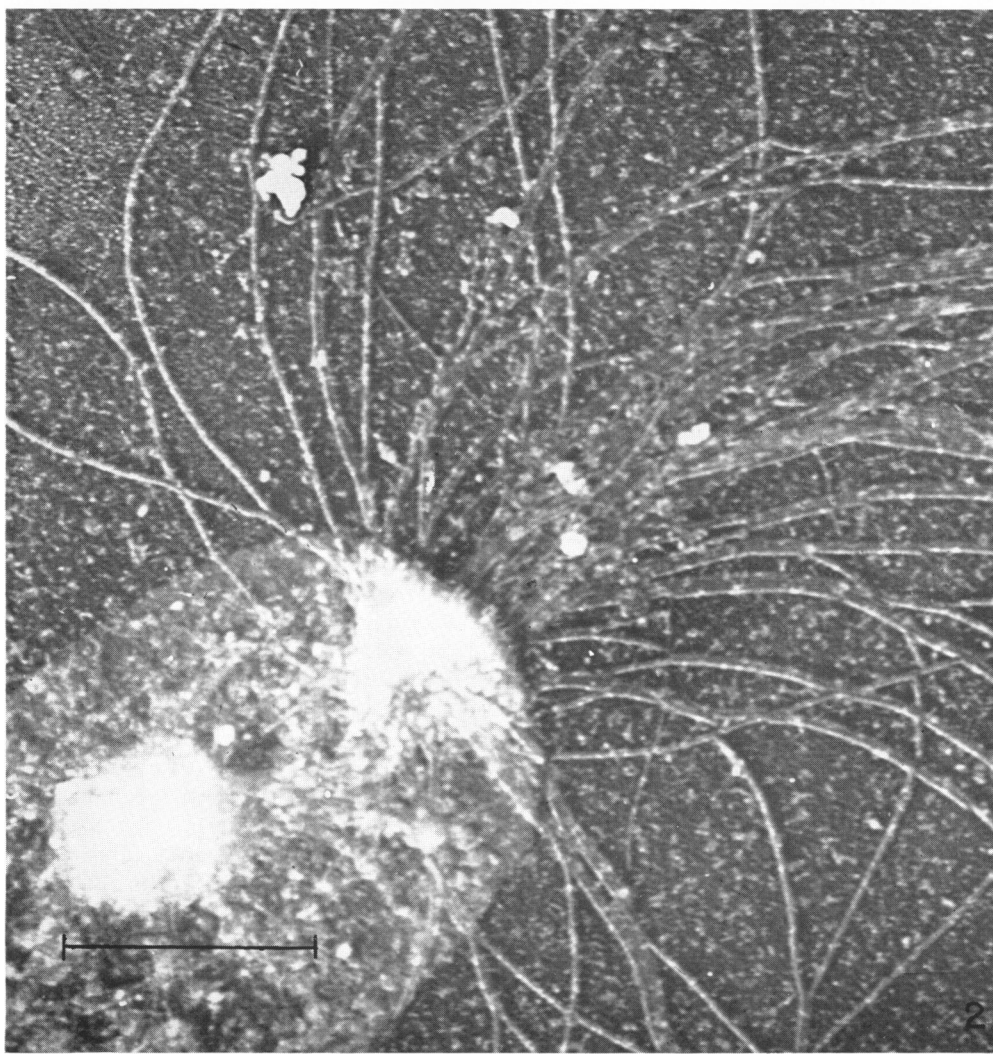


Figure 1. Leifson's flagellar stain preparation of *Spirillum serpens* var. *serpens* showing bushy tufts of flagella, typical of the majority of *Spirillum* species. The magnification mark equals 10 μ in each light micrograph.

Figure 2. Electron micrograph of the same species as in figure 1 showing the same type of flagellar arrangement. A "mass of basal granules" is also apparent as the possible site of the origin of the flagella. The magnification mark equals 1 μ in each electron micrograph.

in reality, two or more closely agglutinated flagella. Due to poor growth, it was not possible to obtain flagellated cells from agar media. The organism produces copious amounts of slime in liquid media and the possibility exists that the repeated centrifugations and washings necessary to remove the slime from the cells also removed some of the flagella. It was found that Leifson's (1951) method of flagellar staining gave the closest approximation to the results found with the electron microscope.

In flagellar stain preparations of species of *Spirillum*, three variations of flagellar arrangement are found. The majority of the species show bushy tufts, which have come to be considered typical for species of *Spirillum*. Such bushy tufts are shown in a flagellar stain preparation of *Spirillum serpens* var. *serpens* (figure 1). Electron micrographs of the same organism (figure 2) show the same type of flagellar arrangement. Structures which are apparently identical to those referred to by Bradfield (1956) as the "mass of basal granules" can be observed in figure 2. The individual flagella apparently originate from the more terminal of these masses. No definite insertion of flagella into the subterminal mass was observed and it seems, therefore, that this structure should not be equated to its terminal neighbor.

A basal structure slightly different in appearance from that in *S. serpens* var. *serpens* is seen in *Spirillum serpens* var. *azotum* (figure 3). Because these two types of basal structures are essentially similar and because individual basal granules can not be seen distinctly in either of them, the authors feel that the structures may both be appropriately termed basal bodies.

A second type of flagellar arrangement in flagellar stain preparations is that of the fascicle of flagella which might be interpreted as single flagellum were it not for the fact that tufts of flagella are also observed in the same field and

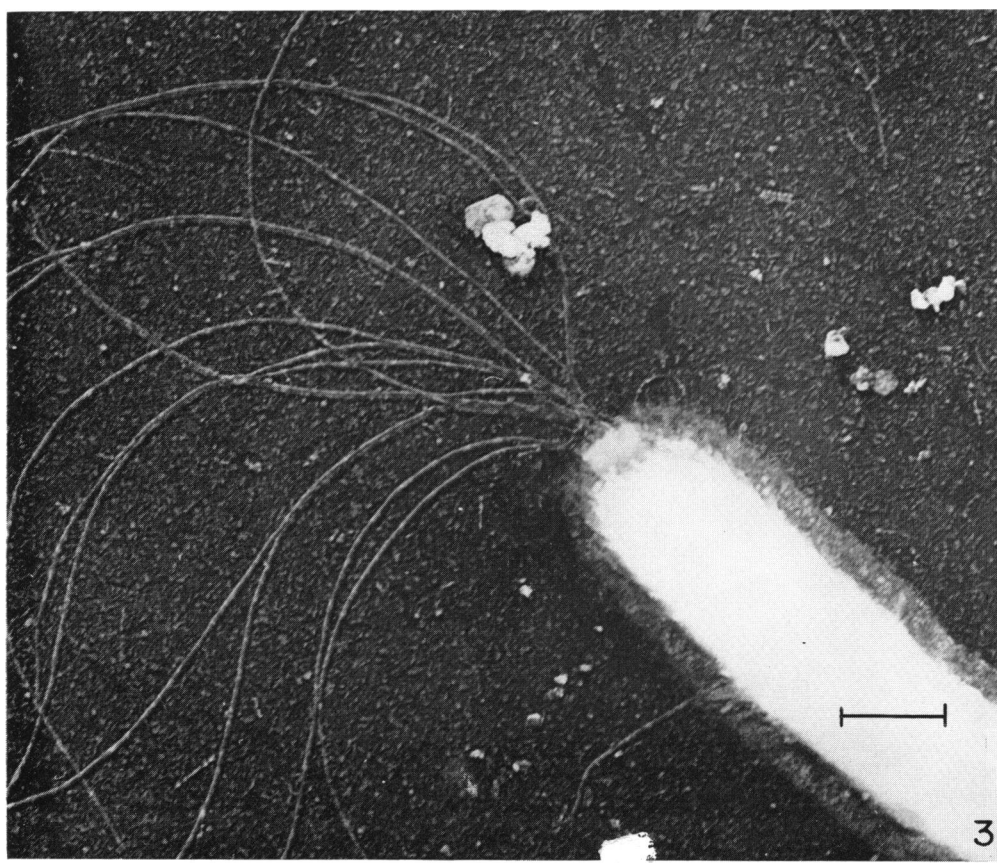
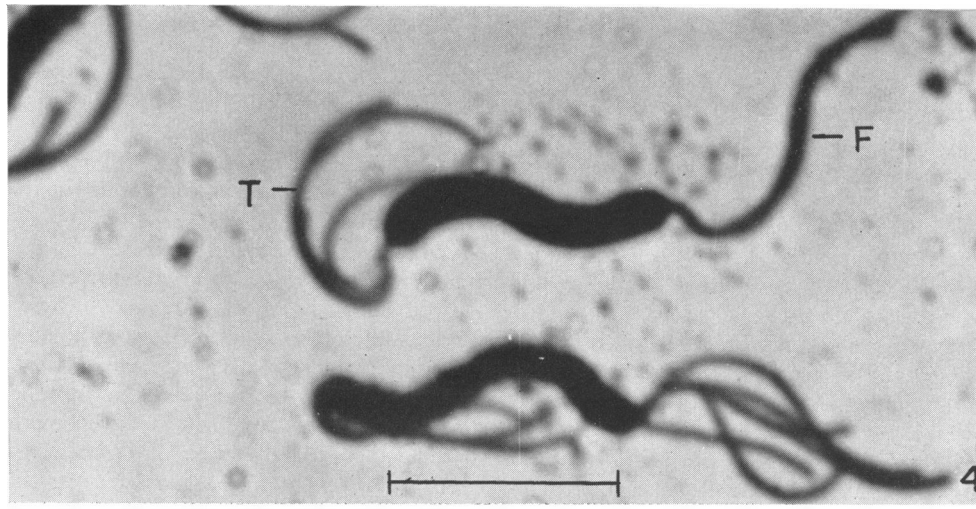


Figure 3. Electron micrograph of *Spirillum serpens* var. *azotum* showing a "basal body" from which the flagella appear to emerge.



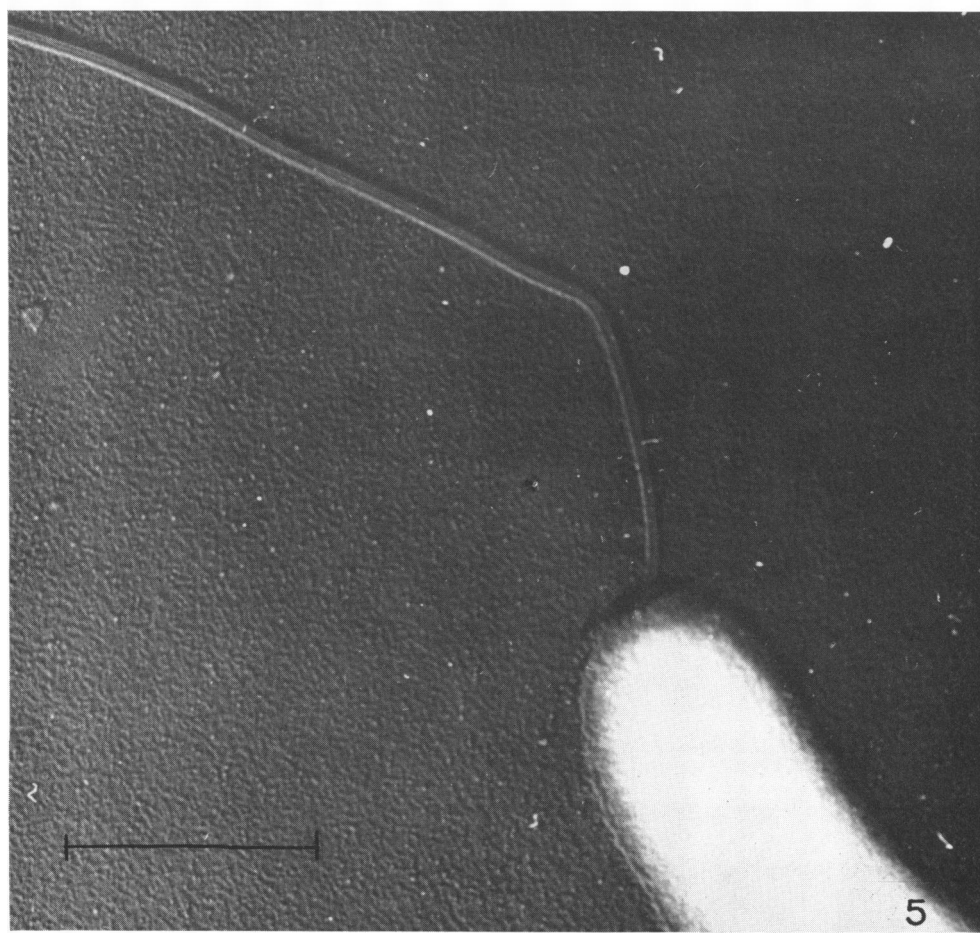
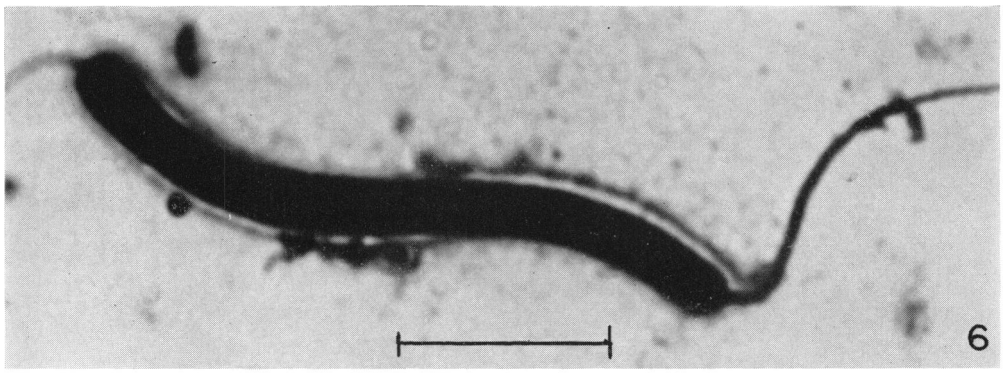


Figure 4. Leifson's flagellar stain preparation of *Spirillum sinuosum* showing fascicles (*F*) and tufts (*T*) of flagella.

Figure 5. Electron micrograph of *S. sinuosum* showing agglutination of flagella which suggests a single flagellum, but which should be considered to represent a fascicle.



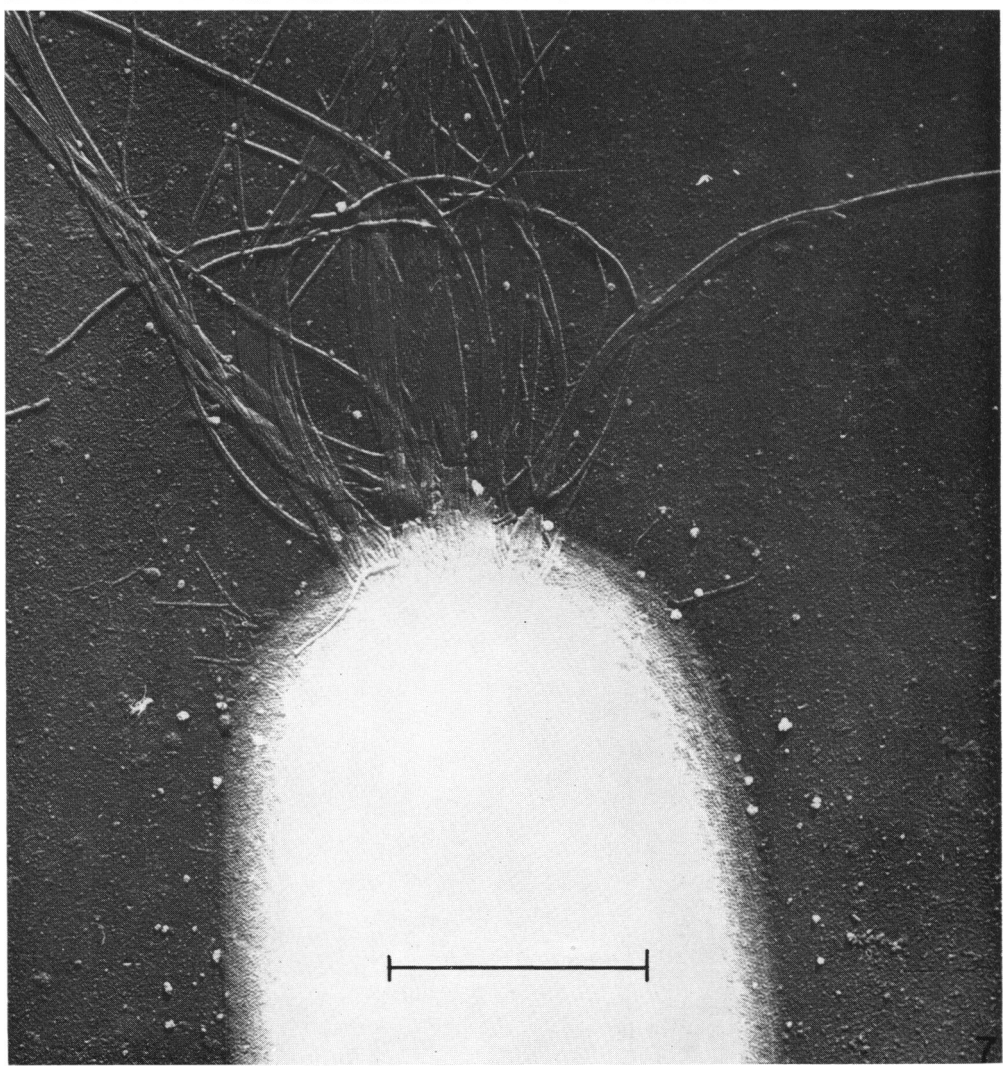


Figure 6. Gray's flagellar stain preparation of *Spirillum volutans* showing an apparent single flagellum.

Figure 7. Electron micrograph of *S. volutans* showing multiple flagella with no evidence of a fascicle arrangement.

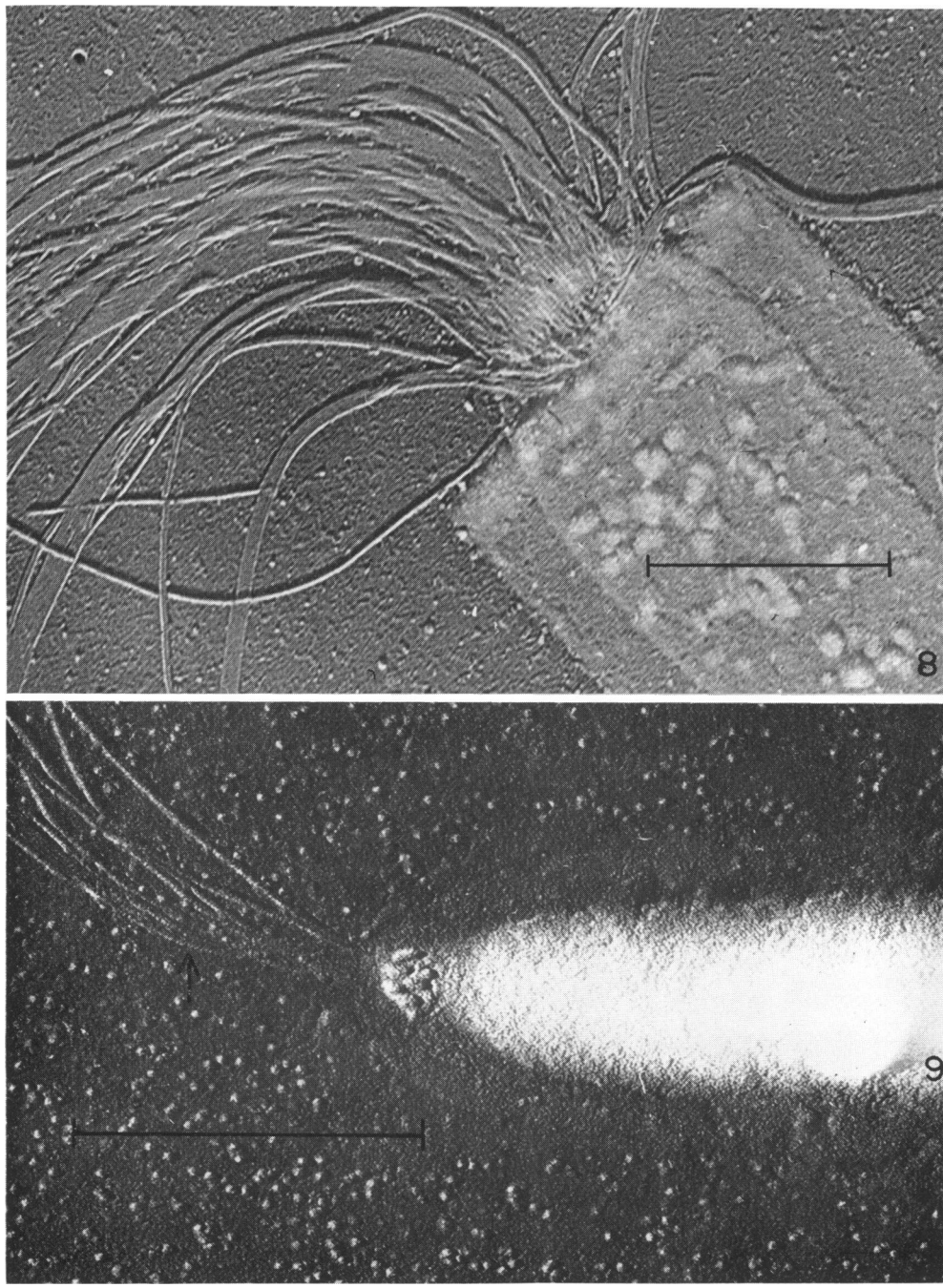


Figure 8. Electron micrograph of *Spirillum volutans*, slightly autolyzed by distilled water, in which the cell wall has retracted from the protoplast, showing the direct attachment of the flagella to the protoplast.

Figure 9. Electron micrograph of *Spirillum lunatum* showing an aggregated mass of basal granules. The location marked with arrows indicates an apparent helical structure of the flagella.

often at the pole opposite the one on which the fascicle is found. Species which possess a tuft of flagella at one pole, do not have a single flagellum at the other pole. Such an arrangement of the flagella is shown in figure 4 of *Spirillum sinuosum*. In this figure, *T* indicates a tuft of flagella and *F* indicates a fascicle. Similar arrangements of the flagella were found in electron micrographs of this organism (figure 5), and also in other species of *Spirillum*.

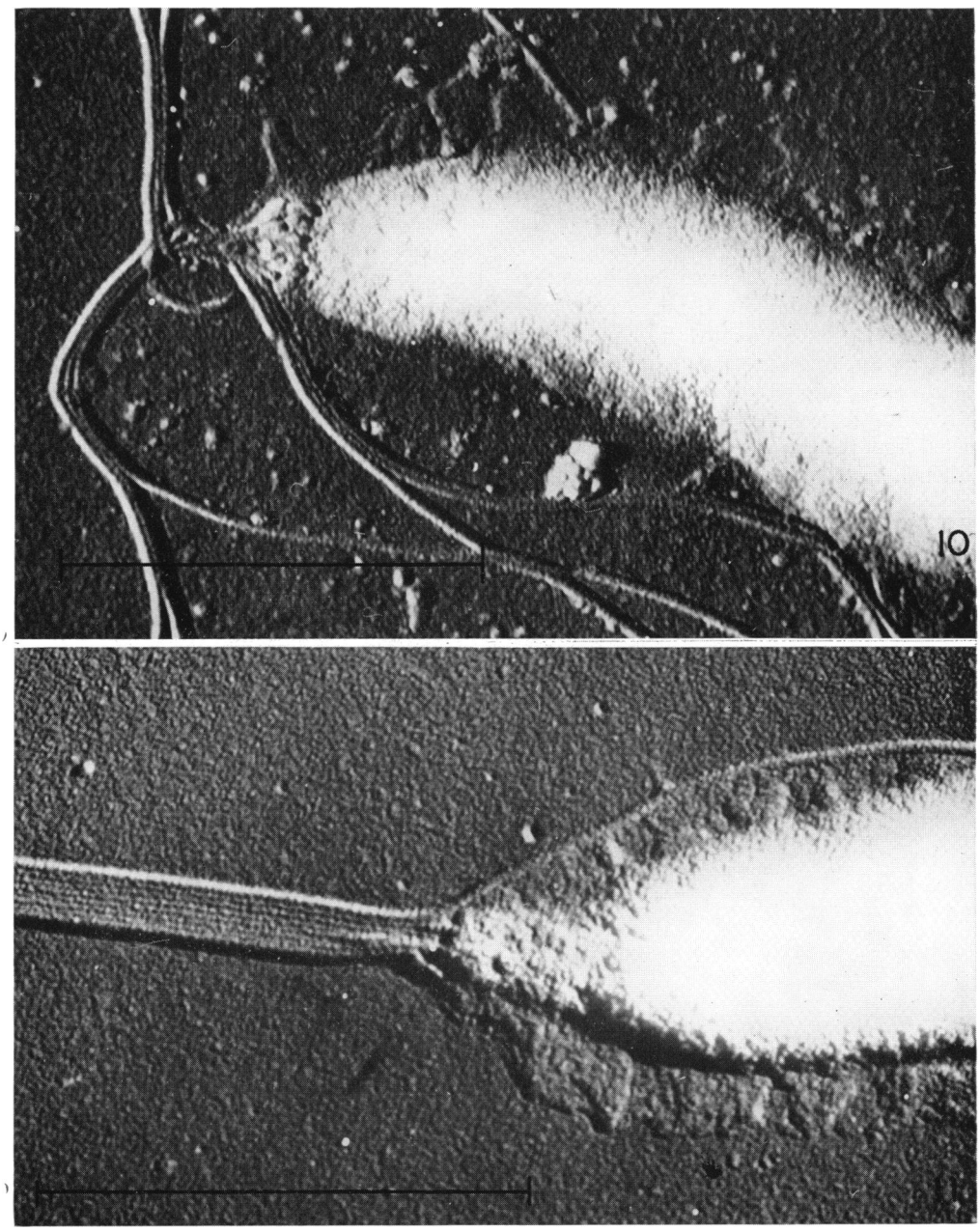


Figure 10. Electron micrograph of *Spirillum linum* showing an aggregated mass of basal granules.

Figure 11. Electron micrograph of *Spirillum atlanticum*, showing a terminal body from which or through which the flagella emerge from the cell. The arrangement of the flagella in a fascicle is evident.

A third type of flagellar arrangement found in flagellar stain preparations is one which has the appearance of a single flagellum such as is shown in figure 6 of *Spirillum volutans*. It should be noted that in this photograph there is no indication of the fuzzy outline found in the fascicle arrangement of *S. sinuosum* (figure 4). Electron micrographs of *S. volutans* (figure 7), however, showed that the apparent single flagellum found in flagellar stain preparations was in fact a number of individual flagella. Although several thick masses of flagella were often observed at one pole in the electron micrographs, there was no indication of the complete aggregation of the flagella of one pole, such as is shown in *S. sinuosum* (figure 5); nor was there any indication of a mass of basal granules such as was observed in *S. serpens* var. *serpens* (figure 2). In *S. volutans*, the flagella apparently originate directly from the surface of the protoplast as shown in a slightly autolyzed cell in which the cell wall has retracted from the cytoplasm (figure 8).

Although basal bodies, from which the flagella appeared to originate, were seen in the fresh water species used in this study, no distinct basal granules were observed in these species. A more intensive investigation will have to be made, however, before any conclusions can be drawn as to the importance of basal granules as the site of origin of the flagella in the fresh water species.

Basal granules were observed frequently in the marine species of Spirillum. In *Spirillum lunatum* (figure 9) and *Spirillum linum* (figure 10), aggregates of the individual basal granules can readily be seen. Each basal granule appears as a slightly elongated bulbous swelling of the flagellum. In *Spirillum atlanticum*, a terminal body occurs (figure 11) which appears somewhat different from both the mass of basal granules in figure 2 and from the aggregate of basal granules in figures 9 and 10. It is obvious in figure 10 that the flagella which appear as a fascicle, emerge through or from this terminal body. No individual basal granules could be observed, however.

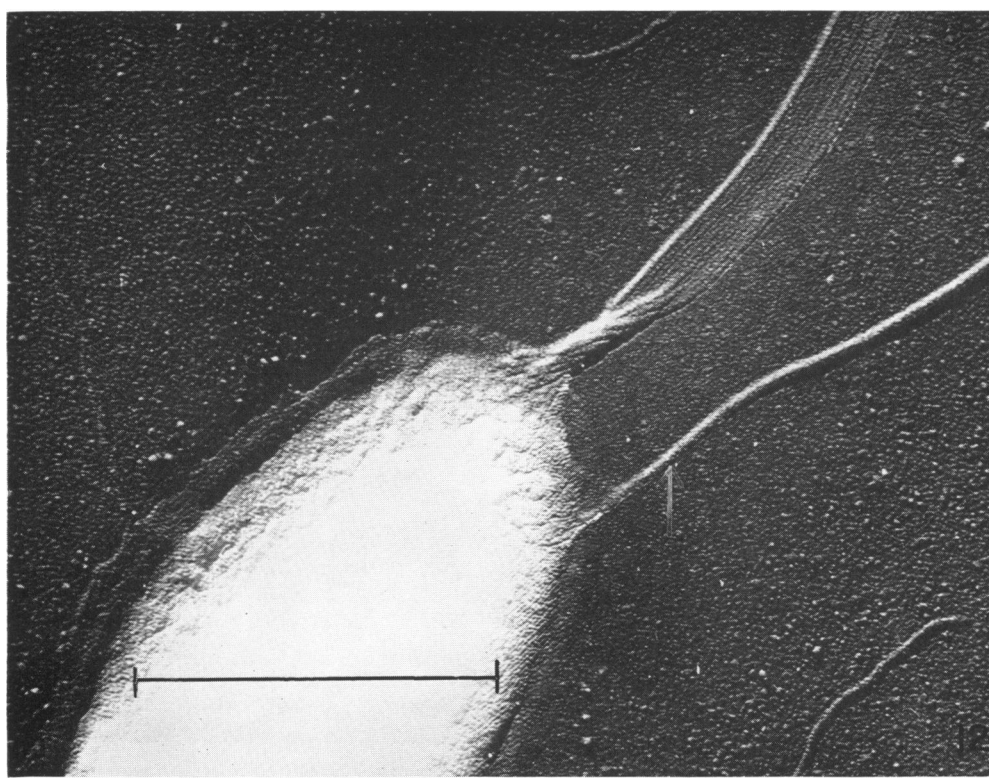


Figure 12. Electron micrograph of *Spirillum* species showing a suggestion of helical structure (*arrow*) in what is considered to be an aberrant flagellum.

Individual flagella of the marine species studied possessed an apparent helical structure, similar to that shown by Starr and Williams (1952) for the Congo diphtheroid. This suggested structure can best be observed in the area of figure 9 designated by the arrow.

In addition to the three principal variations of flagellar arrangements already described, a single lateral flagellum, somewhat removed from the main fascicle of flagella, was occasionally observed (figure 12) in an unidentified fresh water species. The rarity with which this configuration was seen suggested that it should very likely be regarded as an aberrant form. It should be noted that this single lateral flagellum is appreciably greater in diameter than the flagella of the fascicle. Also this flagellum reveals an apparent helical structure (arrow).

The data presented in this paper show that tufts or fascicles of polar flagella can be considered a morphological character of species of *Spirillum*. In those cases where, following light microscope examinations, doubt exists as to the presence of tufts or fascicles of flagella in species of the genus, it would appear to be advisable to obtain electron micrographs of the organism.

SUMMARY

An electron microscopic survey of flagellation in species of *Spirillum* has shown that tufts or fascicles of polar flagella may be considered to be a determinative morphological character of the genus. Aggregates of basal granules were demonstrated in some of the marine species, a terminal body in one marine species and basal bodies, which may be composed of aggregates of basal granules, in several fresh water species. All the marine species and certain of the fresh water species appeared to have flagella with a helical structural arrangement. The direct attachment of the flagella to the surface of the protoplast was demonstrated in *Spirillum volutans*. Leifson's method of flagellar staining was shown to give the closest approximation to the results found in electron micrographs.

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