ELECTRON MICROSCOPIC OBSERVATIONS OF FLAGELLATION IN SOME SPECIES OF THE GENUS TREPONEMA SCHAUDINN

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Bacterial flagella are generally considered as structures of taxonomic value, but the mode of penetration of flagella and their significance and origin are the subjects of controversies that have lasted since the early times of bacteriology. Although most authors contend that flagella are organs of locomotion, or at least definite structures, several workers consider flagella either functionless appendages or plain artifacts. A similar controversy exists concerning whether flagella originate from the cytoplasm or from the cell wall. Most of the reviews of these subjects deal with arguments drawn from light-microscopic evidence, e.g., Pijper (1930, 1938, 1946). On the other hand, van Iterson (1947), Conn and Elrod (1947), and Houwink and van Iterson (1950) have reviewed the subject of flagellation from the standpoint of electron microscopy.

In the present paper, the observations made with the electron microscope on flagellation in *Treponema carateum* Brumpt, in *Treponema* sp. from the so-called Cuban form of pinta, and in *Treponema pertenue* Castellani are presented.

MATERIAL AND METHODS

Flagellation was studied in 89 specimens of *Treponema* sp. obtained from a Cuban case of the so-called Cuban form of pinta, in 9 specimens of *T. carateum* from a Mexican case of pinta (known in Mexico as *mal del pinto*), and in 30 specimens of *T. pertenue* from a Jamaican case of yaws. The characteristics of these cases as well as the techniques used in preparing the tissue fluid samples for electron microscopy are described in previous reports (Angulo, Watson, and Olarte, 1950; Angulo, Watson, Wedderburn, León-Blanco, and Varela, 1951).

OBSERVATIONS

Flagella occurred in bundles along the treponeme body (figures 1 and 3) and were observed here in *T. carateum* and in *Treponema* sp. specimens. Out of 9 *T. carateum* specimens wholly or partially visible on the screens, only one was definitely observed to possess flagella. It was an unshadowed field of such thickness that resolution was inferior, but the flagella were easily visible and had a thickness of about 0.09 to 0.06 microns. Of 89 specimens of *Treponema* sp. from the Cuban form of pinta, 57 had visible flagella, but among 30 *T. pertenue* specimens only one gave any evidence of having flagella, and this evidence was

Figure 1. Portion of a *Treponema* sp. specimen from a case of the so-called Cuban form of pinta. Note winding of flagella tufts around the organism spirals, thus mimicking the "axial filament" of *Spirochaeta* species and the undulating membrane of protozoa. Shadow-cast with chromium at about 12° . $\times 36,000$.

doubtful. Because *Treponema* sp. yielded the heaviest population of flagella-bearing treponemes, the following observations concerning flagella were taken from preparations of this material.

In 26 of the 57 flagella-bearing specimens, the flagella ran mutually parallel

in bundles of 2, 3, or 4 (figures 1, 2, and 3). Although sometimes separated from the treponeme body by as much as a half-micron or more, they were usually

Figure 2. Another portion of the same specimen shown in figure 1. Findings in this figure are the same as in the previous one. Same treatment with chromium. $\times 36,000$.

wrapped around it and in close contact with it as far as its tip. Only one example was seen of a flagellum broken completely away from an organism.

The spiraling character of the flagella made any reliable measurement of their

lengths impossible. Many were estimated to have lengths comparable to those of the organisms themselves, about 10 microns, but others were a minimum of

Figure 3. A portion of a *Treponema* sp. specimen from a case of the Cuban form of pinta. Shadow-cast with chromium at angle of about 12° . $\times 36,000$.

2, 5, or 6 microns. The thickness of the flagella was very uniform at about 0.05 microns. The remarkable uniformity in thickness agrees with a similar observation made by Morton and Anderson (1942) in *Treponema pallidum*. Among

the 57 flagella-bearing organisms, the number of flagella varied as shown in table 1. As many as 15 were counted on a single specimen, and the average number per cell was 4.75. Tufts of flagella were shown by 47 of the 57 specimens, and of these, 29 showed one tuft, 10 showed two, 7 showed three, and one had six tufts. There was an average of 1.6 tufts per flagella-tuft-bearing specimen.

In most cases, flagella were in close contact with the cell, winding about it singly or, more frequently, in bundles (figures 1, 2, and 3). The flagella were observed to wind around the treponeme body so often (in about two-thirds of the flagella-bearing specimens) that this arrangement seems to be more than coincidence. Although mention has frequently been made in the literature of the probability that other bacterial flagella constitute bundles winding and unwinding parallel to each other, there is no mention in light or electron microscopic studies of the wrapping of flagella around the spirals of the treponeme

TABLE 1
Number of flagella per flagella-bearing specimen
(*Treponema* sp. from the so-called Cuban form of pinta)

Number of flagella per organism..	1	2	3	4	5	6	7	8	9	10	11	15
Number of organisms.....	3	10	7	13	8	3	3	3	4	1	1	1

body in members of the family *Treponemataceae*. This phenomenon is, however, apparent in some published micrographs (Hampp, Scott, and Wyckoff, 1948).

DISCUSSION

There is the possibility that flagella result from disintegration of the cell wall or, in general, of the outer layers of the treponeme cell. Electron micrographs of *Borrelia vincentii* (Lofgren and Soule, 1945) suggest this interpretation, but it may be questioned since the preparations were concentrated by electrodialysis, a procedure that provokes disintegration of *Proteus vulgaris* flagella (Weibull, 1948). In addition to the numerous arguments either raised or reviewed by van Iterson (1947), Conn and Elrod (1947), and Houwink and van Iterson (1950), the following observations, which are drawn mainly from the present study, are also against the hypothesis that the flagella are artifacts: (a) the striking uniformity in flagellum diameter shown not only by a given treponeme specimen but among all flagella-bearing specimens in the present work; (b) the regularity and definition of the contours of the flagella, where no jaggling or fuzziness is observed (this has been noticed even in flagella attached to disintegrating cell ghosts [Mudd, Polevitzky, and Anderson, 1943]; in this case, flagella kept their regularity of shape, contour, and dimensions, whereas the cell wall showed ill-defined contours and different density); (c) the frequency of occurrence of the flagella; (d) the occurrence of flagella in tufts; (e) the number and number uniformity of flagella in a single tuft (a flagellum isolated entirely from the organism was observed only once and is probably a result of specimen preparation); (f) the similarity of treponemal flagella to typical flagella of other bacteria together with consistency in diameter, diameter uniformity, length, density,

degree of contortion, and appearance in all these cases; (g) the fact that flagella may be seen either in apparently normal treponemes or in degenerating ones (figures 1, 2, and 3) and the lack of a relation between the number of flagella and the degree of disintegration of the specimen. The disintegration is indicated by a certain granularity of the cytoplasm or perhaps by the straightening out of the spirals.

The lack of flagella shown by the *T. pertenue* specimens is thought to be an artifact because it is very difficult to accept the existence of such a sharp difference between species so closely related as are *T. pallidum* and *T. pertenue* and because little is known about the factors influencing the presence or absence of flagella in preparations for electron microscopy. Pijper (1949) could amputate flagella from *Salmonella typhosa* by merely shaking cultures of this organism vigorously. No flagella were found by Angulo, León-Blanco, and Rake (1948) in a 12-treponeme batch obtained from the same patient with the Cuban form of pinta.

Since the cells examined here did not show retraction of the cytoplasm or similar changes, the origin of flagella could not be studied.

Mudd, Polevitzky, and Anderson (1943) showed evidence that the so-called terminal filament, considered to be of taxonomic value (Bergey *et al.*, 1939; Breed, Murray, and Hitchens, 1948), is a prolongation of the cell wall in *T. pallidum* and that it is probably an artifact. These observations were supported by those reported by Lofgren and Soule (1945) for *Borrelia novyi*. Lateral flagellation has been observed in *T. pallidum* by Morton and Anderson (1942), Wile and Kearney (1943), Mudd, Polevitzky, and Anderson (1943), and Hampp, Scott, and Wyckoff (1948), as well as by Mudd, Polevitzky, and Anderson (1943) in *T. macrodentium* and by Hampp, Scott, and Wyckoff (1948) in *Borrelia vincentii*. In the present studies, both *T. carateum* and *Treponema* sp. showed identical flagellar bundles also situated along the cell bodies. This evidence indicates that these lophotrichate flagella deserve more taxonomic value than that given to the so-called terminal filament, and it is suggested that taxonomic value be given them on a tentative basis until the significance of flagella in species of the genus *Treponema* Schaudinn is definitively established.

SUMMARY

Electron micrography of flagellation in 9 specimens of *Treponema carateum* Brumpt, 89 specimens of *Treponema* sp. from the so-called Cuban form of pinta, and 30 specimens of *Treponema pertenue* Castellani is reported. Tufts of flagella were observed along the treponeme cell bodies except in *T. pertenue*, where their absence seems to be an artifact. No sign of the cytoplasmic origin of the flagella was found, but the sample characteristics were not favorable for observing this origin. Some data on the number and dimensions of flagella were obtained. Evidence is presented that suggests that these flagella are not artifacts. An appearance similar to the "axial filament" of *Spirochaeta* species and to the undulating membrane of some protozoa was found to be caused by a winding of flagella

around the treponeme body. Tentative consideration of the laterally placed lophotrichate flagella as a taxonomic characteristic is proposed.

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