BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE

V. TREPONEMA PALLIDUM, T. MACRODENTIUM AND T. MICRODENTIUM

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Received for publication January 25, 1943

The treponemata present morphological features of singular interest which have been the subjects of a multiplicity of interpretations over a period of more than thirty-five years. Electron micrographs permit the examination of these structures with greater precision, and hence can contribute, within limits, to their interpretation.

An electron microscopic study of *Treponema pallidum* directly from syphilitic lesions has recently been published by Wile, Picard and Kearny (1942). Morton and Anderson (1942) have examined the cultured Nichols-Hough strain of *T. pallidum*. A previous paper by the present authors (Mudd, Polevitzky and Anderson, 1942), and the present article include electron micrographs of the cultured Noguchi, Nichols-Hough, Kroó and Reiter strains of *T. pallidum* and of *Treponema microdentium* and *Treponema macrodentium*. A picture of treponemata of the virulent Nichols-Hough strain from a testicular lesion in a rabbit is also included.

From these studies it is clear that the inner protoplasm of treponemata, like that of all bacteria studied, is enclosed in a definite cell-wall. In the case of the treponemata this cell-wall is a sheath or periplast of extreme delicacy, which may be continuous between incompletely divided spirochetal cells and may extend beyond the cell protoplasm as a terminal filament. Flagella are frequently or regularly present; these appear in groups either along the sides or near the ends of the spirochetal cells. Dense granules in certain preparations are present within the cell protoplasm. Finally, and most challenging to further investigation, dense spheroidal bodies are often found attached to the treponemata by short stalks or free in the medium.

The strains studied were all received from Miss Clara C. Kast, Research Institute of Cutaneous Medicine, Philadelphia; these strains and the media used for their cultivation have been described by Kast and Kolmer (1940). The cultures of *T. pallidum* were in the cysteine medium described, and those of *T. macrodentium* and of *T. microdentium* in 0.5 per cent glucose hormone broth with 10 per cent ascitic fluid to which a small piece of sterile neutralized kidney tissue had been added. The cultures were vaseline sealed and incubated 2 or 3 days or longer.

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For electron microscopic study a sample of the culture was removed by a capillary pipette and mixed with sterile distilled water; the suspension was centrifugalized at about 1500 r.p.m. for 20 minutes. The sediment was resuspended in distilled water and recentrifugalized. The process was repeated a third time. The final sediment was resuspended in distilled water and a droplet of the suspension immediately dried on the collodion mount as usual for introduction in the electron microscope. It is probable that the size of the cells and their spirals may have been altered by the manipulations described; these features are therefore not discussed in the text below.

The cell-wall. The cell-wall ("Periplast," Schaudinn, 1907; "périplasme," Manouélian, 1940) in some preparations encloses the protoplasm so closely that it cannot be distinguished separately. In other preparations, however, the delicate cell-wall unites daughter spirochetes which have not completed their transverse division, or extends beyond the protoplasm at the end of a spirochetal cell (figs. 1 and 2); other pictures show the protoplasm shrunken away from the surrounding cell-wall (fig. 3); or the cell-wall may remain as a "ghost" of a cytolyzed cell from which the protoplasm has escaped (fig. 7). The periplast is obviously not an "undulating membrane" as believed by Schaudinn and other early observers who regarded *Spirochaeta pallida* as a protozoön.

According to Noguchi (1928), "The cell-body consists of a spiral, elastic axial filament, a layer of protoplasm of varying thickness around the filament and a delicate flexible membrane covering the whole body." In none of the electron pictures in this or earlier studies have we found evidence of the existence of such an elastic axial filament as a differentiated structure. The "axial filament" connecting adjacent cells in Noguchi's figures we believe to have been the delicate cell-wall joining incompletely divided cells. Noguchi believed the elastic axial filament to be requisite to explain motility; he was not, however, aware of the existence of flagella, which are demonstrated in this and preceding papers (Morton and Anderson, 1942; Mudd, Polevitzky and Anderson, 1942).

Flagella. Flagella are seen in electron micrographs of all of the strains of *T. pallidum* (figs. 1, 2, 3, 4 and 6) and the strain of *T. macrodentium* studied (fig. 7). We have not been able to demonstrate flagella on our pictures of *T. microdentium.* The flagella are lophotrichate, in many of the pictures occurring in groups of four, situated either near the end or along the sides of the spirochetal cell. The flagella are from 14 to 17 mμ in diameter. The uniformity of the flagellar diameters of the several strains of treponemata (when calculated to the same magnification) is noteworthy.

Under *Treponema pallidum* in Bergey's Manual (1939) the plain statement "flagella absent" is made, but the genus *Treponema* is described as "with or without flagelliform tapering ends." Many of the early descriptions of stained preparations of spirochetes (Herxheimer and Löser, 1905; Schaudinn, 1907; Uhlenhuth and Haendel, 1907; Noguchi, 1912a) describe a terminal flagellum at one or both ends of the spirochetal cell. The flagella of typhoid bacilli in locomotion are plaited together to form single spiral filaments (Pijper, 1941); these are visible in the dark-field microscope with the special equipment of

Pijper, but not with ordinary equipment. The flagella of a lophotrichate water bacterium recently isolated by Hutchinson and McCracken (1942) form in loco-

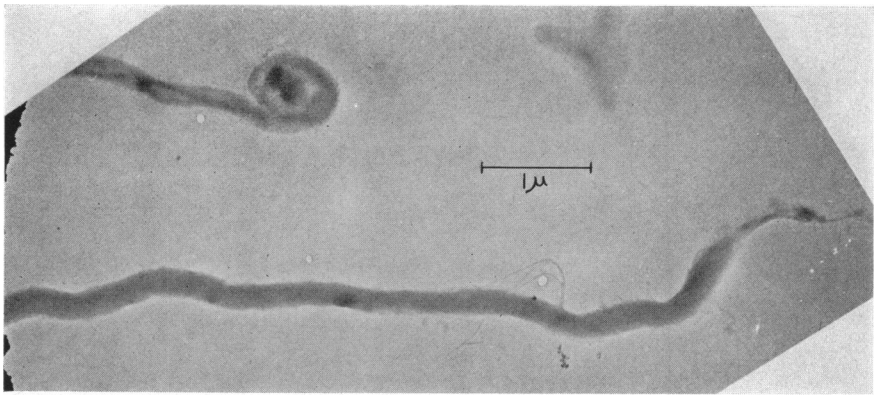

FIG. 1. T. PALLIDUM (REITER STRAIN). × 14,000

The segment of a spirochetal cell below shows a terminal extension of the cell-wall or periplast beyond the protoplasm; a tuft of flagella arises from the side of the cell. The upper segment shows a terminal "end-body"; a somewhat vague granule within the protoplasm is shown proximal to the "end-body."

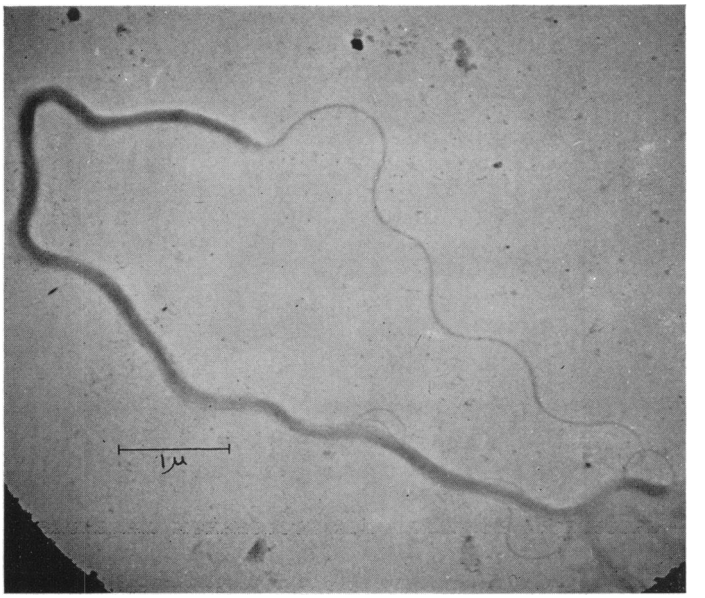

FIG. 2 . T. PALLIDUM (NICHOLS-HOUGH STRAIN). × 14,500

The cell-wall or periplast is continued beyond the protoplasm of the spirochetal cell as an end-filament. Flagella are seen arising from the side of the cell.

motion spirally-wound filaments attached to each end of the bacterium, which may be seen with ordinary dark-field equipment; each filament appears in the dark-field as single, but each may be resolved with the electron microscope into

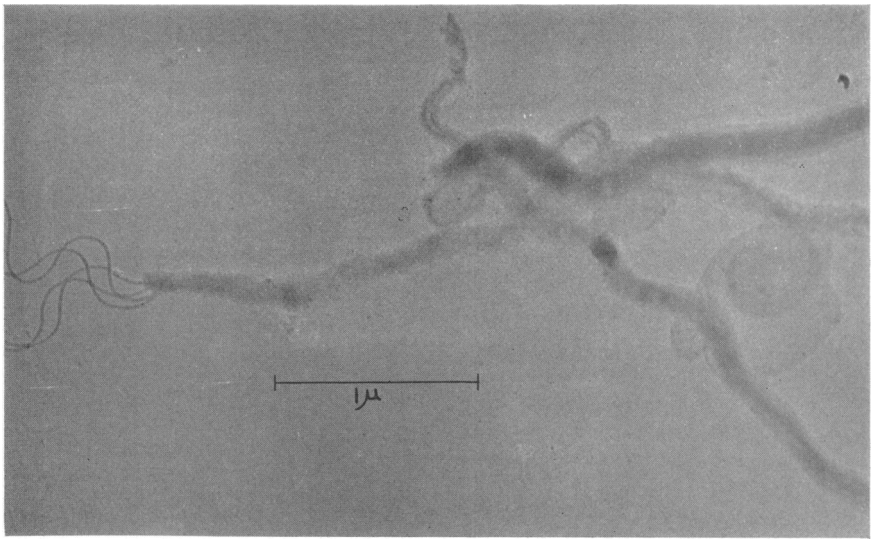

FIG. 3. T. PALLIDUM (KROÓ STRAIN). × 27,000

The spirochetal cells in this preparation appear to be more or less cytolyzed. To the left a tuft of four distinct flagella are seen. Above to the right is a segment of a spirochetal cell in which the dark inner protoplasm is shrunken away from the cell-wall surrounding it. Above is what appears to be a tuft of flagella plaited together.

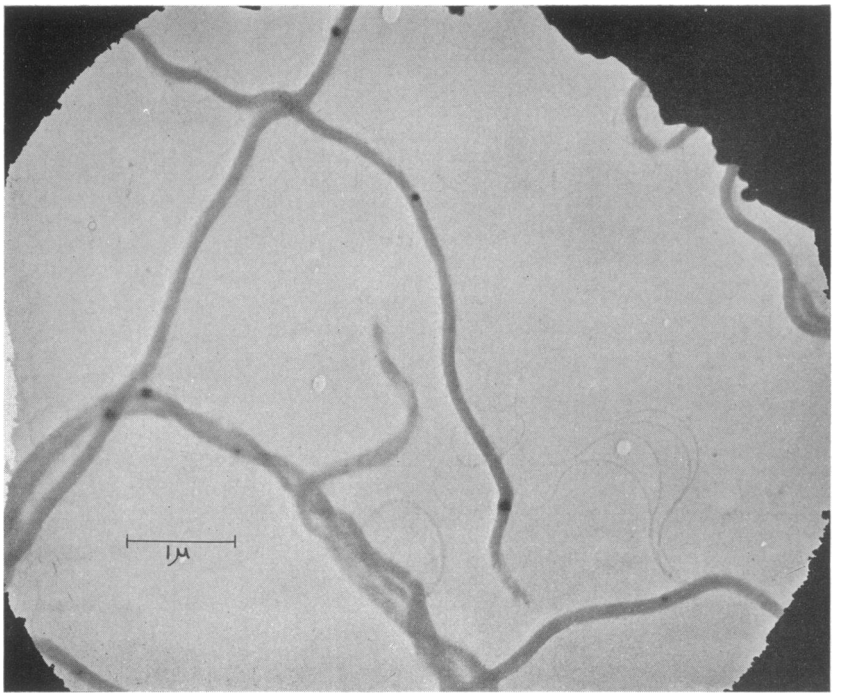

FIG. 4. T. PALLIDUM (NICHOLS-HOUGH STRAIN). × 14,000

Intertwined spirochetal cells. Granules, 40 to 90 mμ in diameter, are clearly shown within the protoplasm. One tuft of four flagella is clearly and other tufts are somewhat vaguely seen.

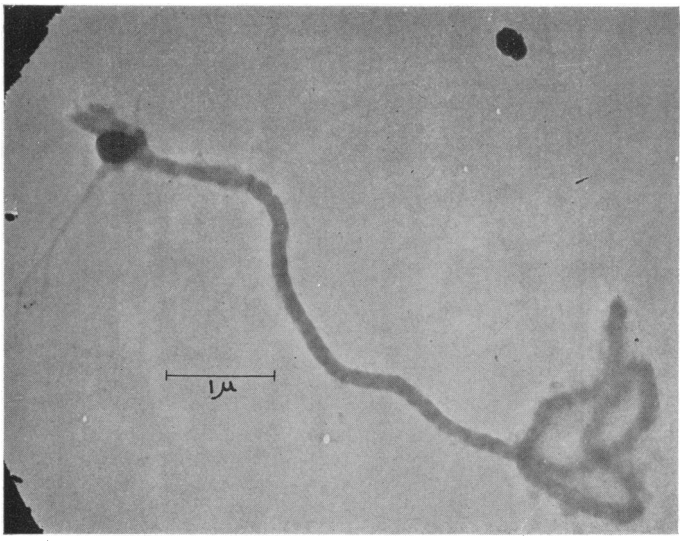

FIG. 5. T. PALLIDUM (REITER STRAIN). × 14.000

The protoplasm is uneven in density and may well have been in process of degeneration. A dense spheroidal body, 290 to 400 mμ in diameter, is attached to the spirochetal cell near its left end (cf. Wile, Picard and Kearny, 1942, figs. 1, 2, 4; and Morton and Anderson, 1942, figs. 3, 6, 7 and 8).

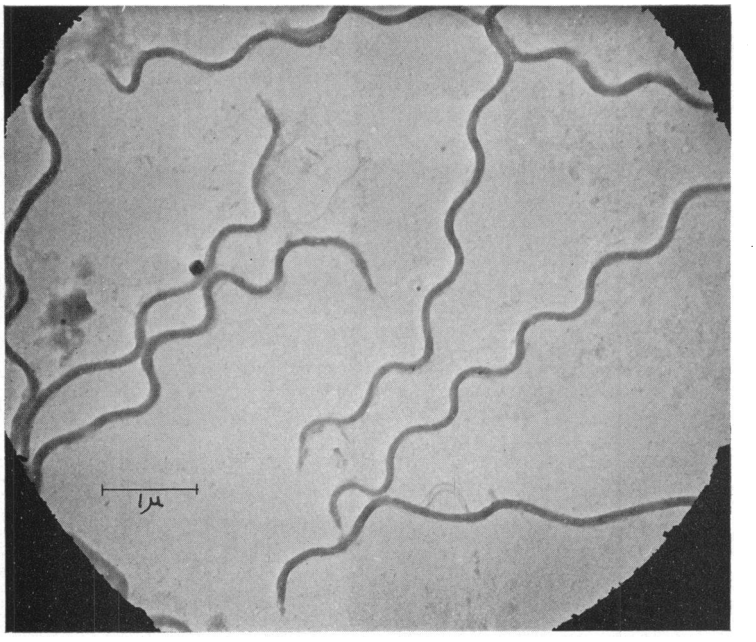

FIG. 6. T. PALLIDUM (VIRULENT NICHOLS-HOUGH STRAIN FROM RABBIT SYPHILOMA). × 12,500

A dense, spheroidal body about 155 mμ in diameter is attached to one spirochete; a tuft of flagella appears on another cell.

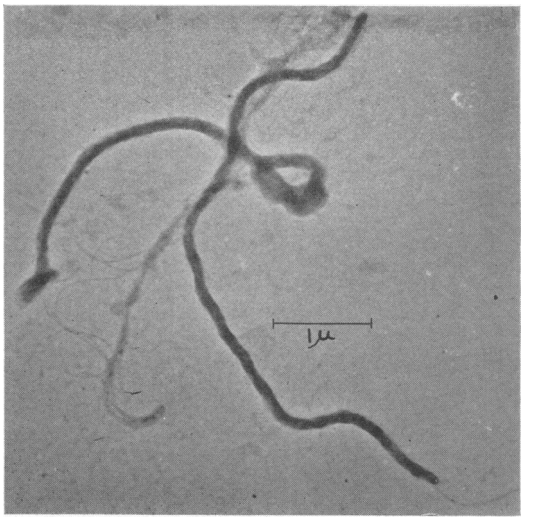

FIG. 7. T. MACRODENTIUM. × 13,000
Two intact cells and one "ghost," or cell-wall empty of protoplasm, are seen. Two tufts of flagella remain attached to the empty periplast of the "ghost."

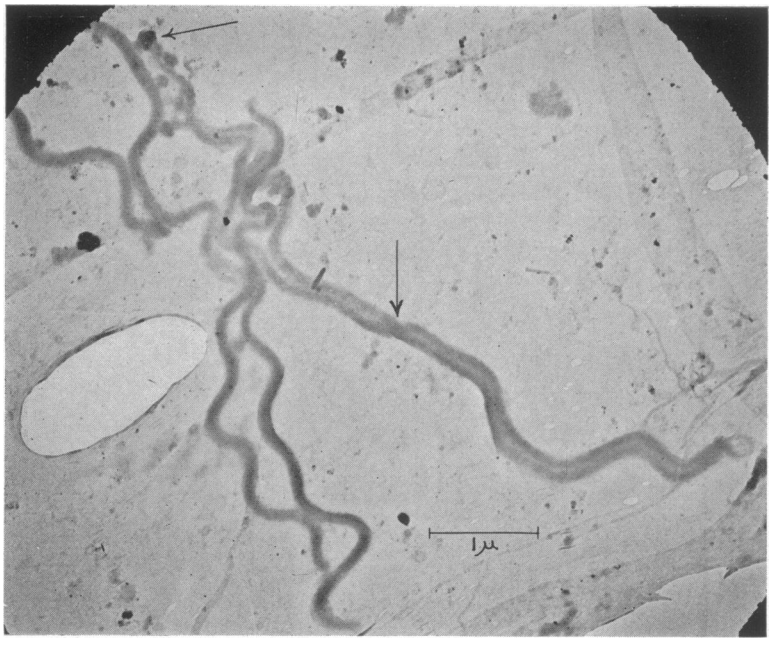

FIG. 8. T. MICRODENTIUM. × 14,500
Intertwined spirochetal cells simulate the appearance of longitudinal division. The point where one cell crosses over the other is indicated by an arrow. A dense spheroidal body, about 170 mμ in diameter is similarly indicated.

multiple flagella. It is possible that the flagella of spirochetes, similarly plaited together, may have been seen in stained preparations by the early observers; it is also likely, however, that terminal extensions of the periplast may have been mistaken for flagella in some instances. Restudy of the locomotion of spirochetes with equipment of the excellence of Pijper's would be of interest.

Granules within the protoplasm. Granules within the spirochetal protoplasm are seen in figs. 1 and 4 (and in fig. 4A, Mudd, Polevitzky and Anderson, 1942). These appear to be dense spheres 40 to 90 mμ in diameter; they resemble granules seen within the protoplasm in micrographs of a variety of bacteria (Mudd, Polevitzky and Anderson, 1942; Knaysi and Mudd, 1943). In the absence of adequate cytological data on these granules in spirochetes we would rather not attempt to interpret them. Granules within the protoplasm were shown in a drawing of a stained spirochete by Herxheimer (1905) and distinguished by him from the spheroidal bodies next to be described.

"*Granules spirochétogènes.*" Dense, irregularly spheroidal bodies may be attached to the spirochetal cells, frequently near the ends of the cells. Some of these dense bodies are closely applied to the sides of the spirochetes; others are attached to the side of the spirochetal cell by short stalks, and others are found free near the spirochetes. Such dense bodies are shown in figs. 5 and 6, in Morton and Anderson (1942) figs. 3, 6, 7 and 8, and in Wile, Picard and Kearny (1942) figs. 1, 2 and 4. These bodies have been described by a long series of investigators; the terms frequently applied to them, "Knospen" or "buds," (Meirowsky, 1913, 1925); "spore-like spherical bodies," (Noguchi, 1912c); "granules spirochétogènes," (Manouélian, 1935, 1940), express implicitly the interpretation often explicitly made that these are asexual reproductive bodies. The very impressive accumulation of evidence supporting this interpretation has been reviewed by Meirowsky (1930) and Ingraham (1932), and more recently by Manouélian (1940).

The extreme monomorphic view has, however, not been without its proponents. Thus Bessemans (1938) writes: "The so-called atypical forms of *T. pallidum* are only fragmented and altered organisms. . . . The organism causing syphilis is and remains a treponeme. In other words its morphologic appearance is always the same, barring the slight variations in its dimensions and motility which have been described."

The spheroidal bodies shown in the electron micrographs cited we certainly do not believe can reasonably be interpreted as degeneration products. They are definite and characteristic bodies originating from the spirochetal cell.[2] Morphologically they resemble endospores (Mudd, Polevitzky, Anderson and Chambers, 1941) in their high density relative to the protoplasm of the vegetative cells; the spheroidal bodies of spirochetes are of course smaller than endospores and differ from them also in their positions at the sides of the vegetative cells. The spheroidal bodies are similar in size to the reproductive granules of the micro-organisms of the pleuropneumonia group (Ledingham, 1933; Sabin,

[2] These bodies were also observed by the senior author many years ago in studying culture spirochetes in oil-water interfaces.

1941). Pleuropneumonia-like micro-organisms have recently been described by Dienes (1942) as associated, he believes as variants, with a number of kinds of bacteria. In their position at the sides of vegetative cells, at times on little stalks, the spirochetal spheroidal bodies recall the conidia and chlamydospores of higher fungi (Weidman, 1933, 1939). Whatever the taxonomic relationships of these spirochetal spheroidal bodies may be, the important fact is that they are not artefacts, impurities from the medium, or products of degeneration; hence they must have a positive significance, and it is difficult to understand, considering all the evidence, what this significance might be if not that of asexual reproductive bodies.

"*End-bodies.*" A peculiar structure which appears to be formed by a rolled-up end of a spirochetal cell is shown in figure 1. Such structures have been observed by many students of the spirochetes. Meirowsky (1925) identified them with the spore-like bodies we have described above; this appears, however, to have been a misinterpretation. The most exact descriptions of such "corpuscules arrondis" we have found are given by Manouélian (1935, 1940) who regarded them as probably involutionary.[3]

Division. Schaudinn (1907) and many other early investigators believed that cell-division among the treponemata was longitudinal. Noguchi (1912b) in his earlier work definitely supported this view; later, however (1917), he wrote: "It is highly probable that the usual mode of division in culture is transverse, although the possibility of longitudinal division cannot be excluded." Finally Noguchi (1928) simply described division of the treponemata as transverse.

Figure 8 shows the type of morphologic appearance upon which the interpretation of longitudinal division was based (the light microscopes used did not resolve the separate but intertwined treponemal cells). The point at which one treponemal cell of an intertwined pair crosses over the other is indicated by an arrow.[4] Division is now generally agreed to be transverse (Manouélian, 1940).

A second study on *T. pallidum* from human lesions appeared after galley proof had been read on the present communication (Wile, U. J. and Kearney, E. B., The Morphology of Treponema Pallidum in the Electron Microscope. Demonstration of Flagella. J. Amer. Med. Asso., 1943, **122,** 167).

SUMMARY

Electron micrographs of the Nichols-Hough, Kroó and Reiter cultured strains of *Treponema pallidum*, of treponemes of the virulent Nichols-Hough strain from a rabbit syphiloma, and of cultured strains of *Treponema macrodentium* and *Treponema microdentium* are presented and the morphology of the treponemal cells described.

A delicate cell-wall or periplast encloses the inner protoplasm of treponemata;

[3] A consultant mycologist has suggested, in view of the spiral arrangement of this structure and the fact that granules appear within it, a possible relationship to an abortive perithecium such as appears in the ascomycetes.

[4] This is an interesting illustration of the fact that the depth of focus with the electron microscope is considerably in excess of the diameter of such objects as bacteria (Anderson, 1942).

this periplast may connect adjoining cells until transverse cell division is completed; thereafter it may extend beyond the cell protoplasm as a terminal filament. No evidence of a differentiated axial filament within the protoplasm is found.

Flagella, often in groups of four, are found along the sides or near the ends of the cells of *T. pallidum* and *T. macrodentium.*

Dense granules, 40 to 90 mμ in diameter are often found within the spirochetal protoplasm.

Irregularly spheroidal, dense bodies, 150 to 500 mμ in diameter, are often found attached to the spirochetal cell, frequently near the end; such a dense body may be in close apposition to the outside of the spirochetal cell-wall or may be connected to it by a short stalk. The evidence concerning these bodies seems to support the interpretation that they are asexual reproductive bodies.

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