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ELECTRON MICROSCOPY OF AXIAL FIBRILS, OUTER ENVELOPE, AND CELL DIVISION OF CERTAIN ORAL SPIROCHETES

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ABSTRACT

LISTGARTEN, M. A. (Harvard School of Dental Medicine and Forsyth Dental Center, Boston, Mass.), AND S. S. SOCRANSKY. Electron microscopy of axial fibrils, outer envelope, and cell division of certain oral spirochetes. J. Bacteriol. **88**:1087-1103. 1964.—The ultrastructure of axial fibrils and outer envelopes of a number of oral spirochetes was studied in thin sections and by negative contrast. The axial fibrils measured 150 to 200 A in diameter. Only one end of each fibril was inserted subterminally into the protoplasmic cylinder by means of a 400 A wide disc. The free ends of fibrils inserted near one end of the cylinder extended toward, and overlapped in close apposition, the free ends of fibrils inserted at the other end. In thin sections, some axial fibrils showed a substructure, suggestive of a dense central core. The outer envelopes of most spirochetes appeared to consist of 80 A wide polygonal structural subunits. However, in one large spirochete, the outer envelope demonstrated a "pin-striped" pattern. Cell division in a pure culture of *Treponema microdentium* was studied by negative contrast. Results suggested that this organism divides by transverse fission, the outer envelope being last to divide. During the course of division, new axial fibrils appeared to originate on either side of the point of constriction of the protoplasmic cylinder. Flagellalike extensions which were found in rapidly dividing organisms were due to protruding axial fibrils, and appeared to be the result of cell division. Some evidence is presented to support the concept of a homologous origin for axial fibrils and flagella.

Relatively little information was available on the detailed morphology of bacteria until the electron microscope was employed to investigate their structure. The identification of spirochetal morphology was hampered still further by the obstacles encountered in cultivating spirochetes on artificial media. However, soon after the introduction of the electron microscope, a number of investigators began to shed some light on the ultrastructure of spirochetes obtained both from artificial culture media and directly from pathological lesions (Morton and Anderson, 1942; Wile, Picard, and Kearney, 1942; Wile and Kearney, 1943; Mudd, Polevitzky, and Anderson, 1943; Hampp, Scott, and Wyckoff, 1948; Watson et al., 1951; Morton, Rake, and Rose, 1951). The majority of these investigators employed shadowed preparations which revealed flagellalike structures inserted subterminally on the spirochetal cell. According to Swain (1955), excessive washing of the organisms with distilled water resulted in the disruption of the spirochetes, which led many investigators to the mistaken conclusion that spirochetes were flagellated organisms. Later, workers using careful methods of preparation showed that spirochetes consisted of three main structures, namely, a protoplasmic cylinder, varying numbers of axial fibrils with terminal or subterminal insertions, and an outer envelope (Bradfield and Cater, 1952; Czekalowski and Eaves, 1955; Swain, 1955, 1957; Berger, 1958; Varpholomeeva and Stanislavsky, 1958; Babudieri, 1960). The controlled enzyme digestion of the outer envelope (Bradfield and Cater, 1952; Swain, 1955) or its dissolution with deoxycholate (Swain, 1957) allowed these investigators to demonstrate that in most species the fibrils were probably contained in the space between the protoplasmic cylinder and the outer envelope. More recent studies of ultrathin sections supplied additional information on the fine structure of the protoplasmic cylinder (Kawata, 1957, 1961; Simpson and White, 1961; Miller and Wilson, 1962; Ryter and Pillot, 1963; Listgarten, Loesche, and Socransky, 1963), and provided conclusive proof that the axial fibrils are located between the protoplasmic cylinder and the outer envelope. Most of these recent studies described the existence of a

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more or less granular cytoplasm containing clear central areas of a filamentous or reticular nature. This reticular area is not separated from the remaining cytoplasm by any detectable membrane, and is thought to represent a nucleoid body similar to that found in other bacteria (Kawata, 1957, 1961; Ryter and Pillot, 1963). Listgarten et al. (1963) also noted in *Treponema microdentium* the existence of concentric laminations in a few cross-sections of the protoplasmic cylinder, the significance of which is not understood. In the Reiter treponeme, concentric lamellations have been described in localized areas within the cytoplasm (Ryter and Pillot, 1963). Similar observations were made by Ritchie (1963) in *Leptospira pomona*. Ryter and Pillot (1963) and Ritchie (1963) stated that these lamellated areas are suggestive of mesosomes, which are cytoplasmic organelles of uncertain function previously described in a variety of bacteria and thought to be related to the formation of transverse septa. Ryter and Pillot (1963) considered the outer envelope of the Reiter treponeme to represent the cell wall of the organism. They described it as a structure of 70 to 90 A in width, consisting of two dense outer leaflets and an inner less-dense leaflet. The existence of a cytoplasmic membrane consisting of two dense leaflets closely adapted to the protoplasmic cylinder was also described in spirochetes (Ryter and Pillot, 1963; Listgarten et al., 1963).

The use of negatively stained preparations of treponemes has supplied new information regarding the insertion of the axial fibrils into the protoplasmic cylinder. Ryter and Pillot (1963) stated that these fibrils are inserted at both ends of the organism, in a subterminal position, by means of "annular swellings." These authors believe that these fibrils form a bundle which is normally stretched from one tip to the other in a manner reminiscent of the axostyle of leptospira. They state that because of their fragility these fibrils break during the spreading of the organisms on a flat surface. Until recently, no evidence had been presented to suggest the existence of a substructure in the axial fibrils of spirochetes, although a number of investigators described some substructure in a variety of bacterial flagella (Burge, 1961; Kerridge, Horne, and Glauert, 1962). However, Bladen and Hampp (1964) recently observed cross-striations on the axial fibrils of some oral spirochetes.

The purpose of the present investigation was to study in greater detail the substructure of ths axial fibrils and the outer cell envelope of varioue oral spirochetes, as well as the process of cell division in a pure culture of *T. microdentium*.

MATERIALS AND METHODS

Isolation and cultivation of T. microdentium. *T. microdentium* was separated from other oral organisms by inoculating gingival debris onto the surface of 100-mμ Millipore filters (Millipore Filter Corp., Bedford, Mass.) placed on the surface of a medium consisting of PPLO broth without crystal violet or serum (BBL) containing 0.7% Ionagar #2 (Consolidated Laboratories Inc., Chicago Heights, Ill.). After 7 days of incubation at 35 C in 95% H_2 and 5% CO_2, the spirochetes were transferred to a medium consisting of PPLO broth (BBL); 0.1%, glucose; L-cysteine, 800 μg/ml; nicotinamide, 400 μg/ml; cocarboxylase, 5 μg/ml; sodium isobutyrate, 20 μg/ml; and spermine tetrahydrochloride, 150 μg/ml. The cysteine and cocarboxylase were filter-sterilized and added aseptically to the rest of the medium which had been previously autoclaved at 121 C for 10 min. Pure cultures were obtained from the mixed spirochetes by use of a combination of pour plates and shake tubes, as well as by streaking in an anaerobic chamber as previously described (Socransky, Macdonald, and Sawyer, 1959).

Preparation of T. microdentium for thin sectioning. Pure cultures of *T. microdentium* were collected on the surfaces of 0.45-μ Millipore filters and fixed on the filter surface by use of a modification of the method described by Kellenberger, Ryter, and Séchaud (1958). The details of the procedure were described in a previous publication (Listgarten et al., 1963). The filters supporting the spirochetes were dehydrated in graded ethanol solutions saturated with sucrose (50, 75, 95, and two 100% solutions). Before immersing the filter and spirochetes into the 100% alcohol, the layer of organisms was separated from the filter and subdivided into small blocks suitable for embedding, by use of a sharp razor blade. The dehydrated blocks were then embedded in Epon No. 812 (Shell Oil Co., New York, N.Y.) according to the method of Luft (1961). Sections (0.1 μ) were cut on a Porter-Blum microtome and collected on carbon-reinforced Formvar-coated grids. The specimens were stained by immersing the

grids in a freshly filtered concentrated solution of uranyl acetate in 50% ethanol for 20 to 60 min. The grids were then washed with 50% ethanol, blotted with filter paper, and left to dry.

Preparation of ultrathin sections of gingival lesions. Typically ulcerated gingival lesions were obtained from eight patients of both sexes ranging from 17 to 35 years of age, which included one Negro, one Mongolian, and six Caucasians. All patients had obvious clinical evidence of acute necrotizing ulcerative gingivitis (ANUG), as evidenced by the presence of severe interdental papillary necrosis on four or more gingival papillae. The gingival ulcers were excised by sharp dissection, with minimal displacement of the tissues by means of a No. 12 Bard-Barker blade under regional or nerve block anesthesia, by use of 2% Xylocaine with epinephrine in a concentration of 1:100,000. The excised sample was immediately immersed in ice-cold fixative for 30 to 45 min. The tissue was then subdivided into thin slices and replaced in the fixative for a varying length of time, depending on the fixative used. The fixatives included (i) 2% osmic acid, buffered to pH 7.4 with Veronal-acetate and osmotically balanced by the addition of 8% (w/v) sucrose (Caulfield, 1957), and (ii) "Kellenberger fixative" as described by Kellenberger et al. (1958). The total fixation time was 2 hr in the 2% osmic acid fixative and 3 to 4 hr in the Kellenberger fixative. The tissues were then dehydrated in graded solutions of ethanol, embedded in Epon, and sectioned as described above. The tissue sections were stained by immersing the grids in a freshly filtered, saturated solution of uranyl acetate for 5 min and rinsing in 50% ethanol. The sections were stained further with lead salts according to the method of Millonig (1961) or Reynolds (1963). The grids were then washed with 0.02 N NaOH and 50% ethanol, blotted, and left to dry.

Negatively stained preparations. Gingival scrapings were obtained from the patients in the study by gentle curettage with a sterile instrument of the necrotic material overlying the ulcerated lesions. This material was kept on ice in a stoppered glass vial until it could be mixed with distilled water to form a cloudy suspension. In all instances, the material was suspended within 1 hr of its collection. The suspension was mixed with an equal volume of 3% phosphotungstic acid buffered to pH 6.8 with 5 N NaOH. After gentle agitation to allow thorough blending, a drop of the mixture was placed on a coated grid which was blotted with filter paper after a few seconds.

Spirochetes grown in broth were examined by negative contrast after mixing equal volumes of broth and 3% phosphotungstic acid, and following the procedure described above.

All specimens were examined and photographed in an electron microscope (model EMU-3G, RCA Broadcast & Communications Products Division, Camden, N.J.).

RESULTS

Axial fibrils. The axial fibrils of the various spirochetes examined in this study measured between 150 and 200 A in diameter, and were inserted subterminally by means of disclike attachments approximately 400 A wide (Fig. 1 and 2), similar to the "annular swellings" described by Ryter and Pillot (1963) in the Reiter treponeme. The fibrils made a sharp bend before becoming inserted into the protoplasmic cylinder. The axial fibrils were not continuous from one end of the spirochete to the other. Each fibril seemed to be inserted at one end of the organism only, the free end of each axial fibril extending toward the opposite end for a distance which varied from cell to cell. Usually, the fibrils originating from opposite ends overlapped for a considerable portion of their length, giving the impression that the organism had twice as many fibrils near the middle than were actually inserted at either end (Fig. 3, 4, 5, and 6). This was most readily observed in small spirochetes with few axial fibrils. In rapidly dividing cultures of *T. microdentium*, axial fibrils frequently extended past the opposite end of the organism in a flagellumlike appendage (Fig. 7). The formation of this fibrillar extension appeared to follow cell division (see below).

No evidence of periodicity was observed on the surface of the axial fibrils in any of the negatively stained preparations. This is contrary to the recent findings by Bladen and Hampp (1964). However, in ultrathin sections of some large spirochetes seen in the lesions of ANUG, a suggestion of a dense central core was visible in some of the fibrils (Fig. 8).

Outer cell envelope. The outer envelopes of all the spirochetes examined in this study were morphologically identical, with the exception of a large spirochete found in the lesion of ANUG.

In negatively stained preparations, the outer envelopes of most spirochetes appeared to be

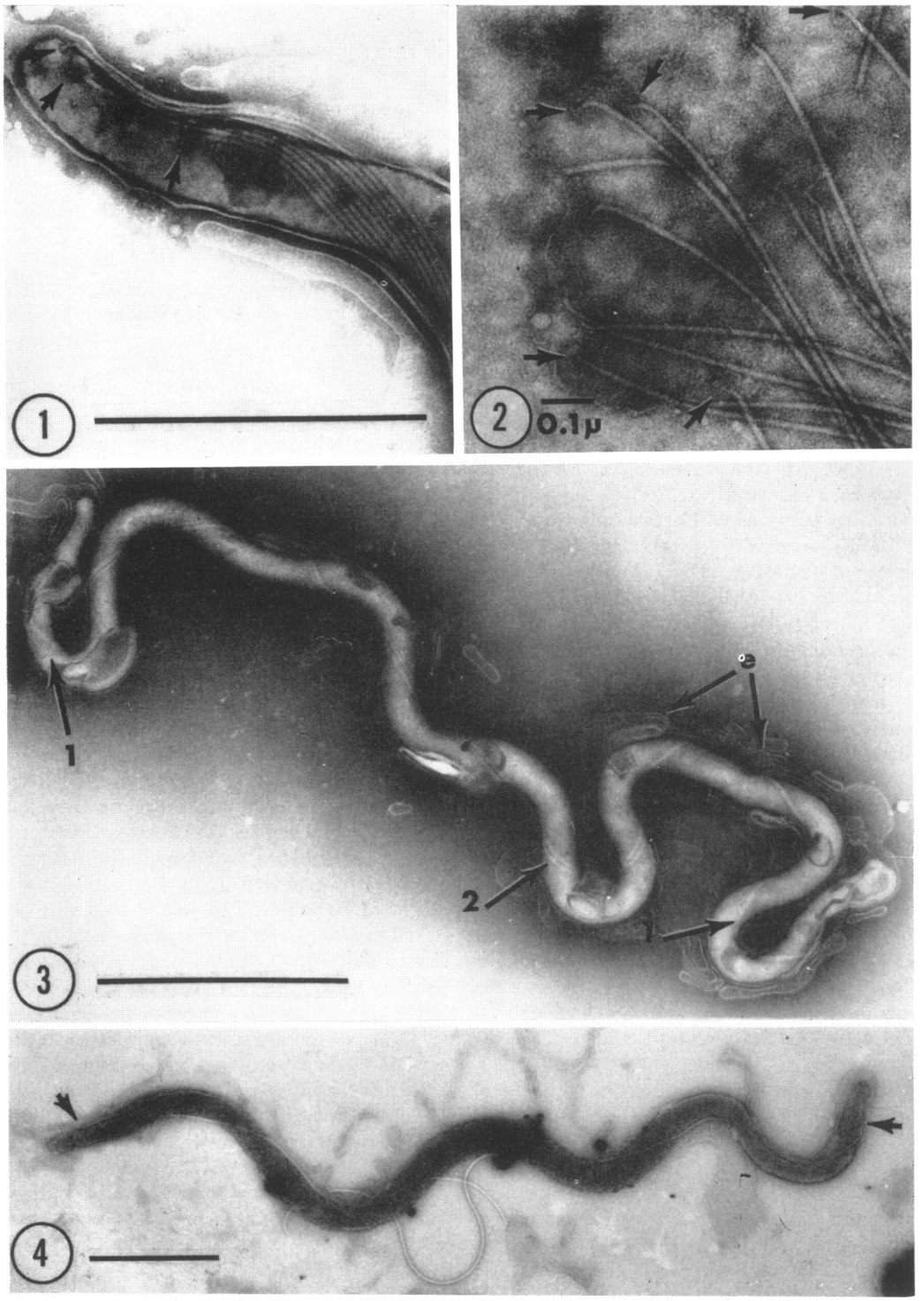


FIG. 1. *Extremity of spirochete in gingival scrapings. Note terminal bend in axial fibrils leading to attachment discs in protoplasmic cylinder (arrows). Negative contrast.* × *50,000.*

FIG. 2. *Axial fibrils inserted into a crushed protoplasmic cylinder by means of attachment discs (arrows). Negative contrast.* × *69,500.*

FIG. 3. *Spirochete from Treponema microdentium culture showing "1-2-1" arrangement of axial fibrils. Axial fibrils are in good relation to protoplasmic cylinder; (e) disrupted outer envelope. Negative contrast.* × *38,400.*

FIG. 4. *Partially disrupted spirochete from Treponema microdentium culture. Note point of insertion of axial fibrils (arrows) and overlapping free ends. Negative contrast.* × *18,400.*

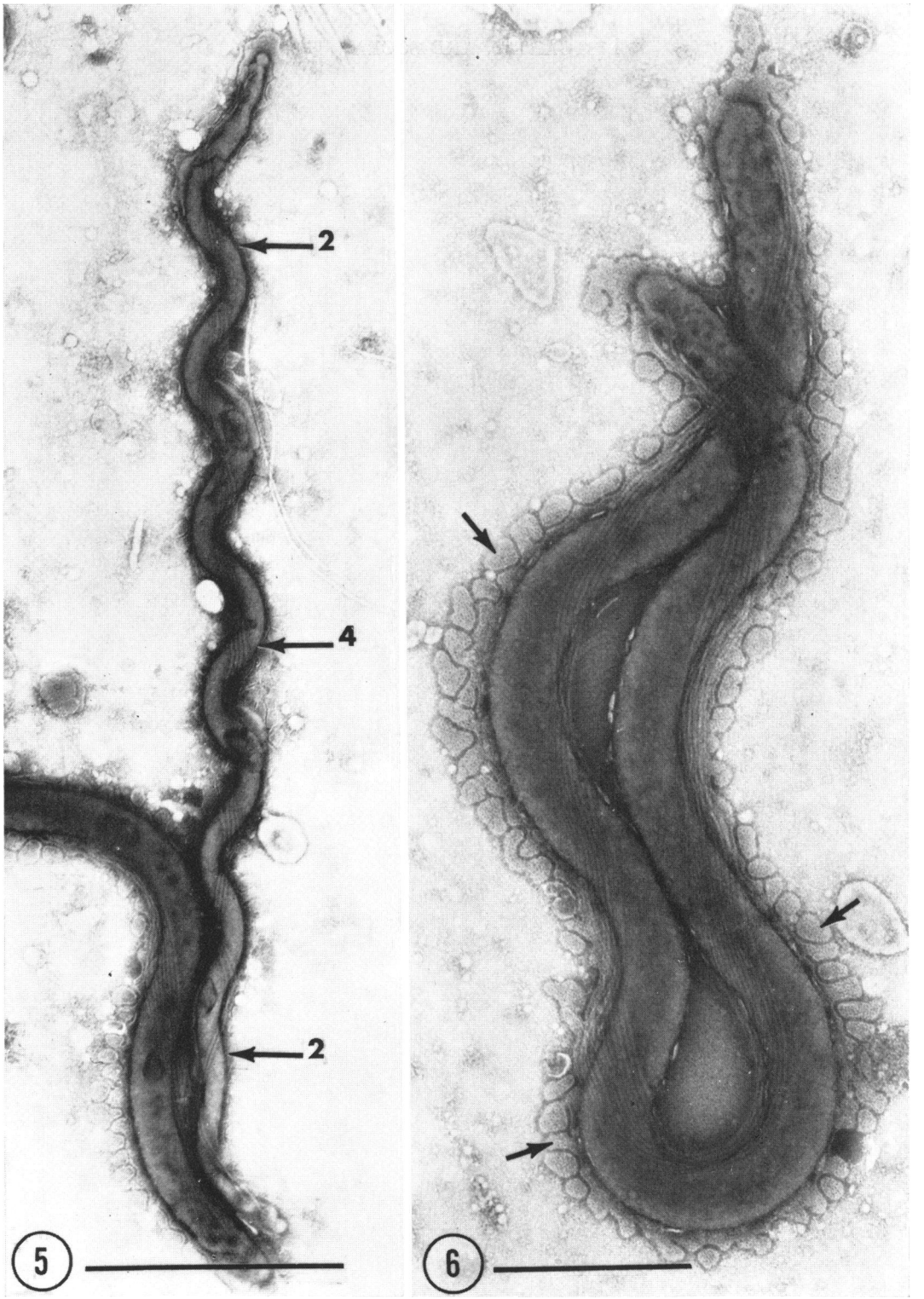
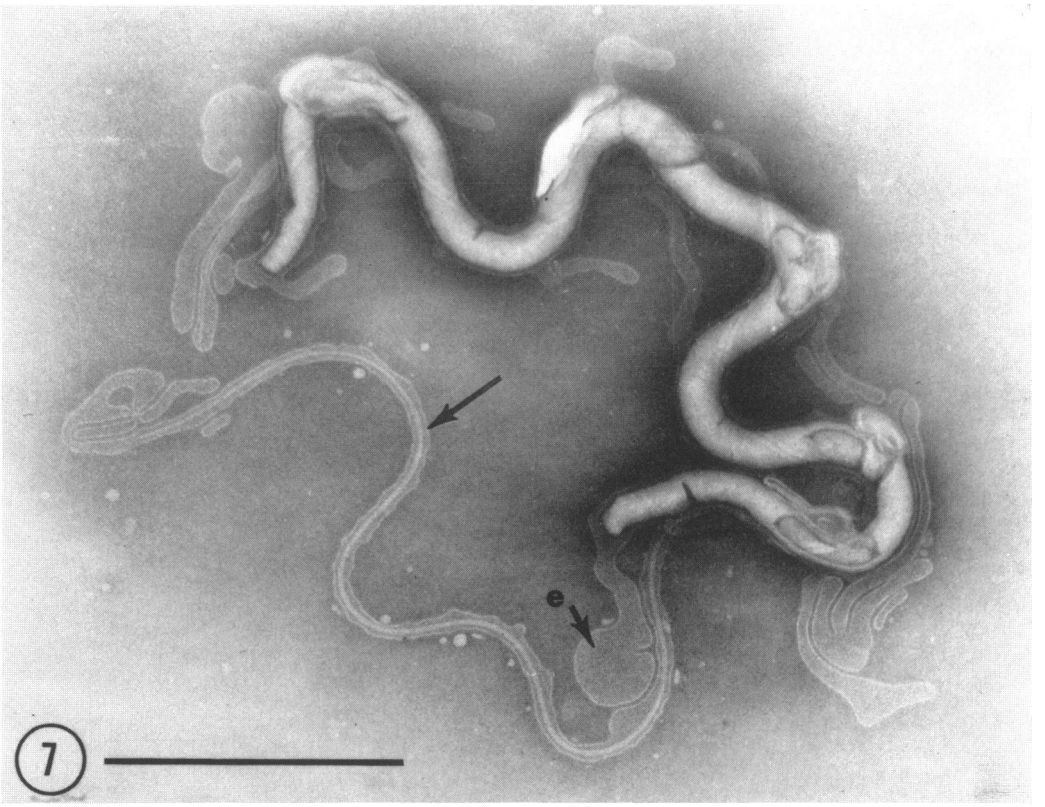
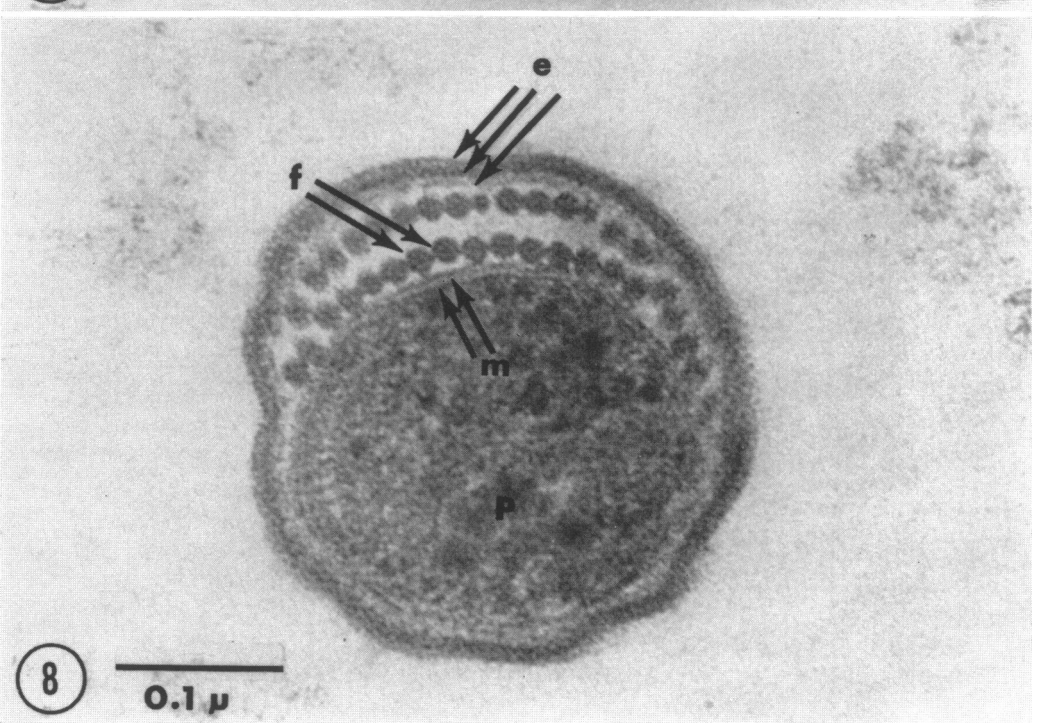


FIG. 5. *Small spirochete in gingival debris showing "2-4-2" arrangement of axial fibrils. Note well-preserved relationship of axial fibrils to one another and to protoplasmic cylinder. Overlapping of axial fibrils from each end is well demonstrated. Negative contrast.* × *39,500.*

FIG. 6. *Spirochete of intermediate size in gingival debris demonstrating overlap of axial fibrils from opposite ends. Note disrupted outer envelope (arrows). Negative contrast.* × *29,900.*





FIGS. 7–8

composed of polygonal structural subunits with a diameter of 80 A separated from each other by 20-A wide partitions (Fig. 9, 10, and 11). In the center of each unit, a central core approximately 25 A in diameter was frequently found. The polygonal subunits could also be seen in favorable tangential sections (Fig. 12). In true cross-sections, the envelope of *T. microdentium* and of some unidentified spirochetes in the ANUG lesion appeared to be composed of two electron-dense lamellae separated by a less-dense space, the total width of the envelope being approximately 140 A (Listgarten et al., 1963).

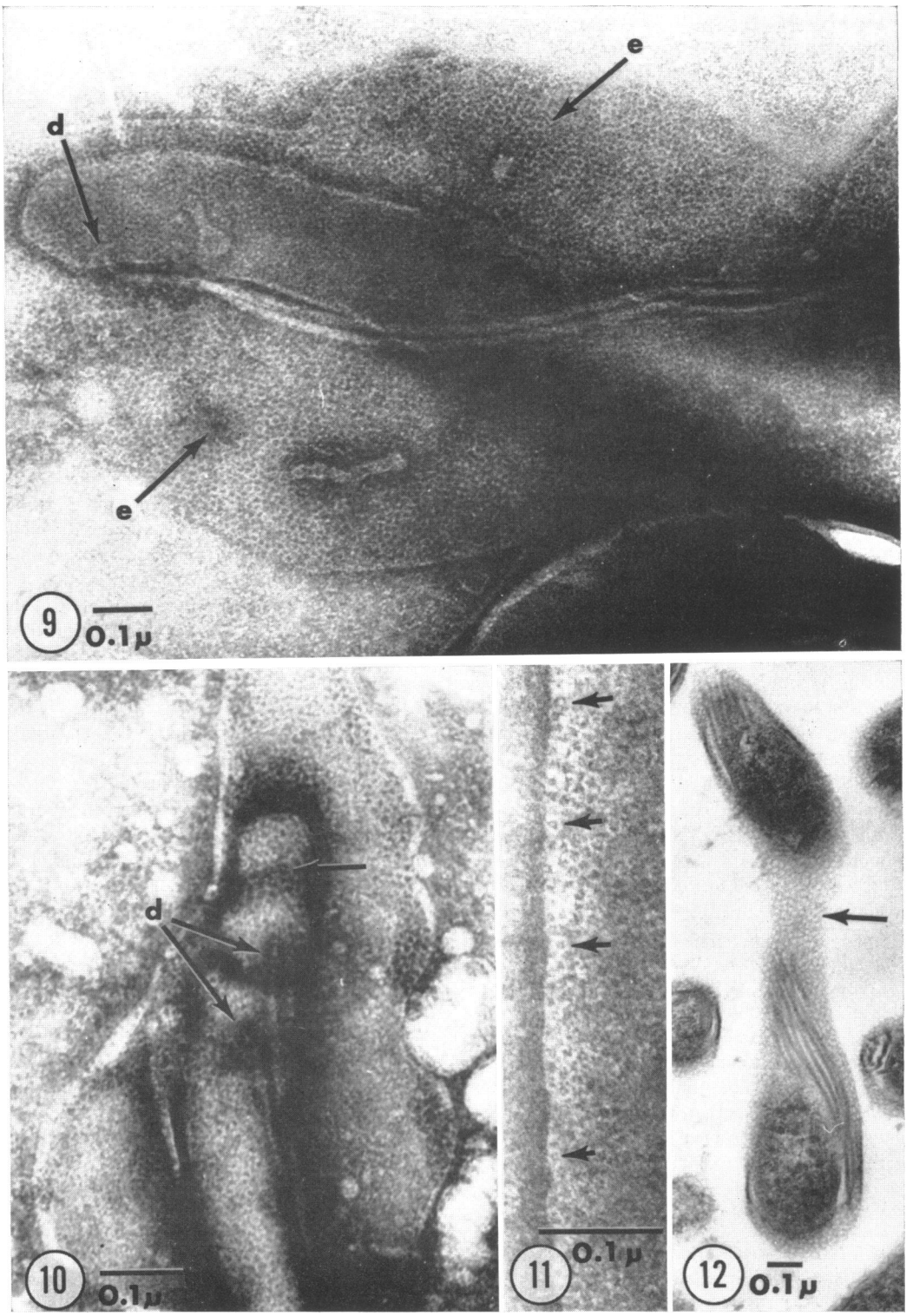
A large spirochete was occasionally found in the lesion of ANUG which was characterized by a different outer cell envelope. This envelope was more resistant to disruption (Fig. 13), and did not exhibit the flowing qualities and the tendency to break up commonly observed with the other type of envelope (Fig. 3, 5, and 6). In negatively stained preparations, this envelope appeared to have a "pin-striped" appearance (Fig. 12). In areas where two layers of the envelope were superimposed, as in the intact envelope, a "cross-hatched" appearance was observed (Fig. 12 and 15). The parallel stripes of the envelope were 150 to 160 A wide, and were separated by a space 50 to 60 A wide. This envelope was recognizable in encysted forms (Fig. 16) as well as in intact spirochetes. The pin-striped pattern was also noticeable in tangential sections of the outer envelope in the large spirochetes occurring in the lesion of ANUG (Fig. 17). However, it was occasionally difficult to distinguish in such sections between the stripes of the outer envelope and the axial fibrils.

Cell division. Because of the unusual relationship of axial fibrils, outer envelope, and protoplasmic cylinder, it seemed worthwhile to investigate the method of cell division of these organisms. To obtain a large number of cells in division, the spirochetes were examined during their log phase. The pure strain of *T. microdentium* seemed best suited for this purpose, since it demonstrated the typical structure of spirochetes with a minimum of axial fibrils, i.e., only one fibril originating at either end of the protoplasmic cylinder (Fig. 3 and 4).

Log-phase cultures were characterized by organisms attached end to end to each other, apparently as a result of transverse fission (Fig. 19). Numerous organisms could be found which, although well preserved, demonstrated what resembled flagellalike extensions, consisting of a central fibril morphologically identical to an axial fibril, surrounded by a sleeve of material continuous with the outer envelope of the organism. Furthermore, organisms could be found on occasion which had more than the two fibrils characteristic of resting cells of this strain of spirochetes. A careful study of a large number of organisms in various stages of division suggested the pattern of division diagrammatically illustrated in Fig. 18. Figure 18A represents a normal resting cell of *T. microdentium* (also see Fig. 3). The first step in cell division consists of a pinching of the protoplasmic cylinder, the outer envelope remaining intact. New axial fibrils appear to arise on either side of the point of constriction (Fig. 18B). Figures 20 and 21 are electron photomicrographs of this stage of division. Although the axial fibrils are shown widely separated in the diagrams, they normally run in close apposition to one another. Thus, diagram 18A represents the normal "1–2–1" axial fibril arrangement illustrated in Fig. 3, whereas 18B represents a "1–2–3–3–2–1" arrangement seen in dividing cells (Fig. 20 and 21). In a later stage of division (Fig. 18C), the protoplasmic cylinders separate further so that the organisms are united only by means of the outer cell envelope (Fig. 22 and 23). Note that in Fig. 18C and 22 one axial fibril from each daughter cell extends beyond the protoplasmic cylinder. Depending on the length of the original axial fibrils in the parent cell, one, two, or no fibrillar protrusions may be observed as the daughter cells separate. After division is complete, the protruding axial fibril surrounded by its sheath of outer envelope may produce a flagellumlike appendage (Fig. 7 and 24). Figure 18D represents four incompletely separated organisms. Note that in this situation more than

FIG. 7. *Flagellum-like projection of one axial fibril (arrow) in Treponema microdentium, probably following recent division. Note that sheath surrounding fibrillar protrusion is of a structure similar to that of disrupted envelope (e). Negative contrast.* × *39,000.*

FIG. 8. *Cross-section through large spirochete in gingival lesion of acute necrotizing ulcerative gingivitis (ANUG). Note substructure of axial fibrils (f) suggestive of the presence of a dense central core. Fibrils are located between three dense layers of outer envelope (e) and two dense layers of membrane (m) covering protoplasmic cylinder (p). Kellenberger fixation.* × *216,000.*



FIGS. 9-12

three fibrils can be seen in apposition on the protoplasmic cylinder. Figure 18E represents the separated organisms of Fig. 18D, with various patterns of axial fibrillar protrusion.

Spirochetes either in the resting phase or in various stages of division frequently demonstrated localized disruptions in the integrity of the protoplasmic cylinder. These protrusions frequently occurred adjacent to a site of division (Fig. 20 and 22). It is not known whether these disruptions are artifacts of preparation or the result of a local alteration of the protoplasmic cylinder.

DISCUSSION

The examination of tissue sections and scrapings from gingival lesions of patients with ANUG has allowed us to study oral spirochetes as they occur within diseased tissues and in the debris overlying the lesions, with minimal handling of the organisms themselves.

Spirochetes in the tissues were easily recognizable because of their characteristic structure consisting of a protoplasmic cylinder, surrounded by axial fibrils, with an outer envelope covering both cylinder and fibrils. In negatively stained preparations, these structures could also be observed but, in addition, the shape of the whole organism was helpful in identifying it as a spirochete.

Our observation that axial fibrils originate at each end of the protoplasmic cylinder and have overlapping free ends closely adapted to the protoplasmic cylinder is not in accord with the findings of Ryter and Pillot (1963), in the Reiter treponeme. These authors stated that axial fibrils form a continuous bundle stretched from one extremity of the organism to the other, in a manner similar to the axostyle of leptospira. It is of interest to note in this regard that, in a recent report, Ritchie (1963) stated that in *L. pomona* the axostyle was not continuous between its terminal insertions, as has been formerly believed, and that in some regions of the organism this structure appeared to be doubled. No one, to date, has given conclusive evidence that axial fibrils are continuous from their insertion at one end of the protoplasmic cylinder to their postulated insertion at the opposite end. Van Iterson (1953), while noting the resemblance of axial fibrils to bacterial flagella, stated that their main difference depended on the fact that the former were inserted at two points, probably both poles of the spirochete, whereas the latter only had one point of insertion. The results presented here will necessitate a reconsideration of the validity of this difference as a criterion to distinguish between bacterial flagella and axial fibrils. The observations made by earlier workers (Morton and Anderson, 1942; Mudd et al., 1943; Wile and Kearney, 1943; Hampp et al., 1948; Moureau and Giuntini, 1956; Morton et al., 1951) of terminal or subterminal flagellation in spirochetes were most likely due to the disruption of the outer envelope and the consequent release of the axial fibrils, which were only retained at the single end where they were inserted, rather than to the breakage of these fibrils, as has been suggested (Bradfield and Cater, 1952; Swain, 1955; Ryter and Pillot, 1963).

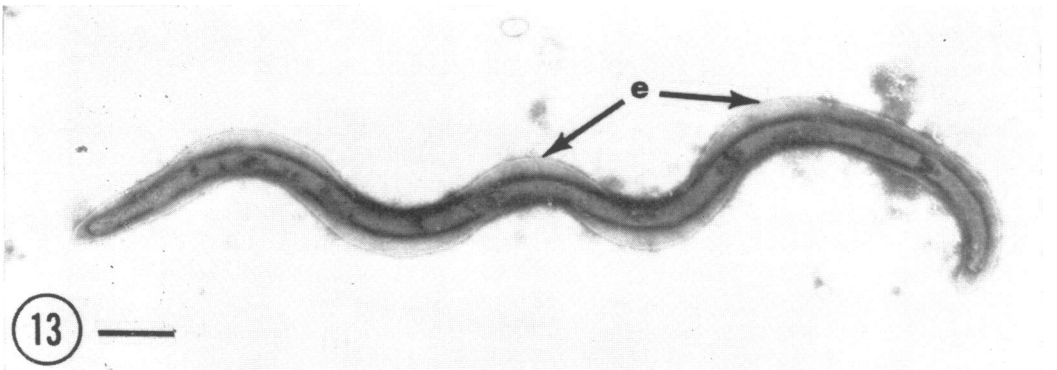
The resemblance of axial fibrils to bacterial flagella is worth noting. In addition to their similarity in size, both types of fibrils demonstrate a terminal "hook-like bend" (Van Iterson, 1953) near their site of insertion, and both are attached by means of specialized structures variously described as "annular swellings" (Ryter and Pillot, 1963), "basal granules," or "blepharoplasts" (Houwink and Van Iterson, 1950; Grace, 1954; Pease, 1956; Tawara, 1957), "terminal knobs" (Czekalowski and Eaves, 1955; Miller and Wilson, 1962; Ritchie, 1963), or "basal discs" (Glauert, Kerridge, and Horne, 1963). There is also some evidence, based on the enzymatic digestion of cell walls (Wiame, Storck, and Vanderwinkel, 1955; Weibull, 1960), that bacterial flagella are attached to the cytoplasmic body, in a manner similar to the attachment of axial fibrils demonstrated above. It is tempting to propose

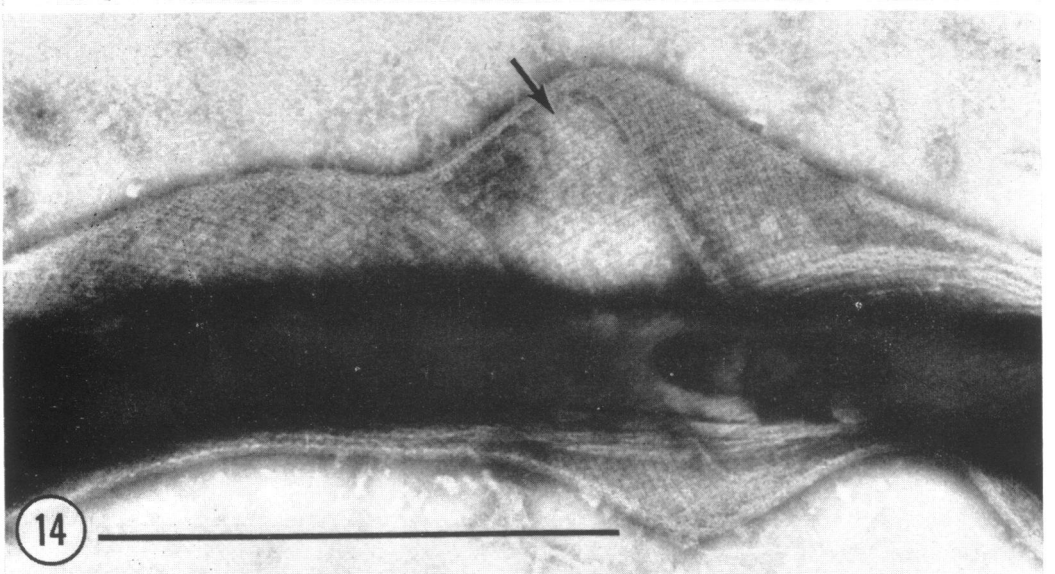
FIG. 9. *Polygonal structural subunits in outer envelope (e) of a disrupted spirochete in gingival debris; (d) attachment disc. Negative contrast.* × *90,000.*

FIG. 10. *Polygonal subunits in outer envelope of a small spirochete in gingival debris. Arrow points at a subunit with central core. Note insertion of axial fibrils into attachment discs (d) in protoplasmic cylinder. Negative contrast.* × *121,500.*

FIG. 11. *Polygonal subunits in outer envelope of a spirochete of intermediate size, in gingival debris. Arrows point at typical subunits with central core. Negative contrast.* × *187,300.*

FIG. 12. *Ultrathin section of a spirochete of intermediate size in a gingival lesion of ANUG. Note polygonal subunits in tangential section of outer envelope (arrow). Kellenberger fixation.* × *46,000.*





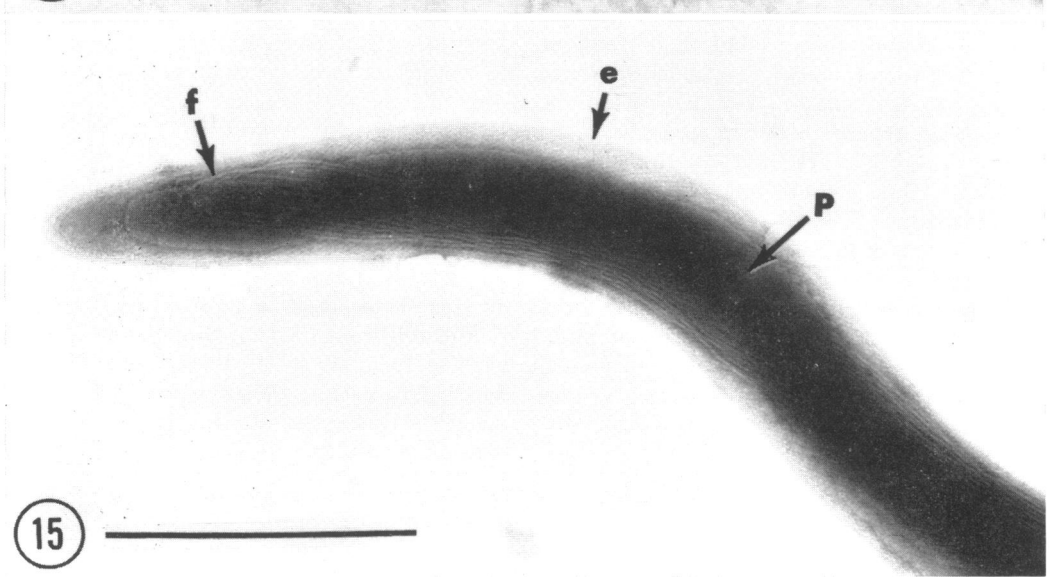
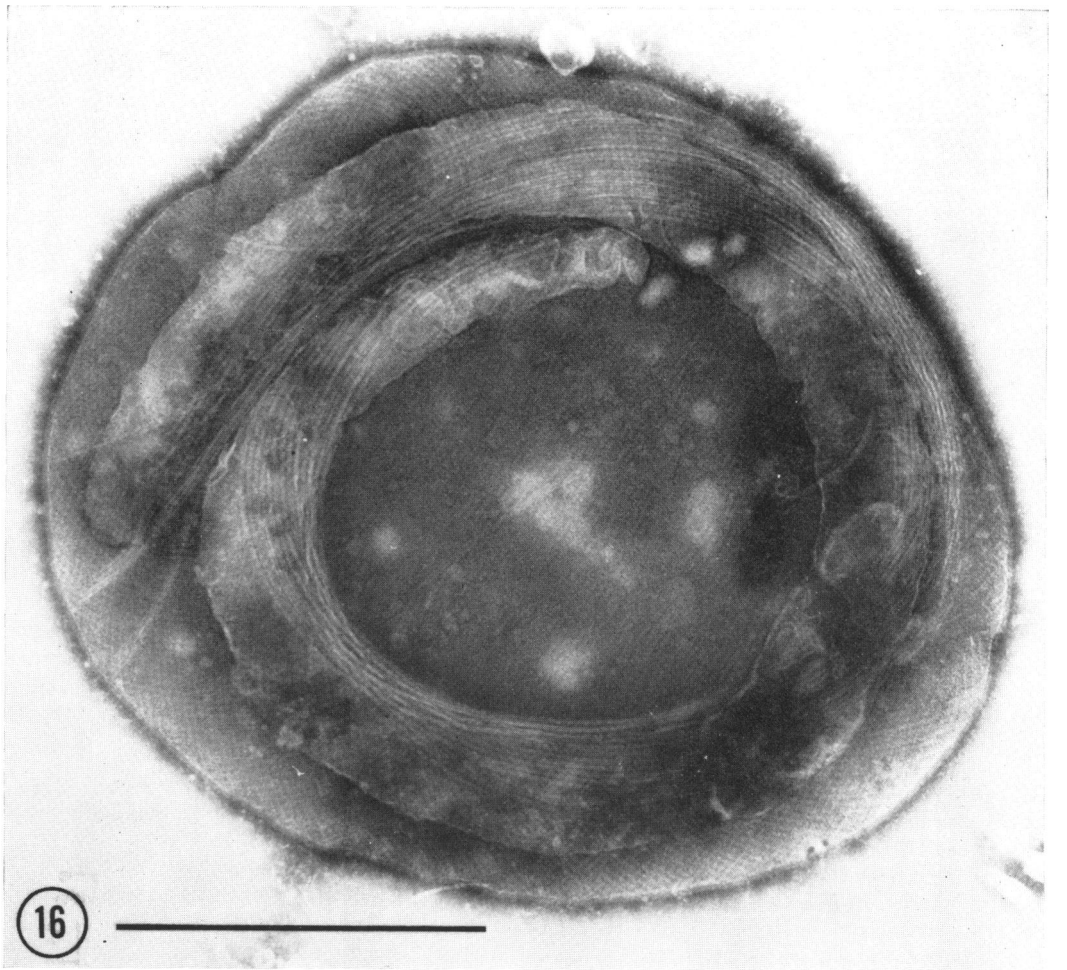


FIG. 13. *Large spirochete in gingival debris demonstrating well-preserved outer envelope (e). Negative contrast.* × *9,300.*

FIG. 14. *Portion of a large spirochete in gingival debris similar to organism in Fig. 13. Note "pin-striped" pattern in torn portion of outer envelope (arrow). Where envelope is intact, a "cross-hatched" pattern is formed by superimposition at right-angle of "pin-striped" pattern. Negative contrast.* × *69,000.*

FIG. 15. *Extremity of a large spirochete in gingival debris demonstrating characteristic well-preserved outer envelope (e), containing protoplasmic cylinder (p) and a number of axial fibrils (f). Relationship of these structures is typical of spirochetes. Negative contrast.* × *41,000.*



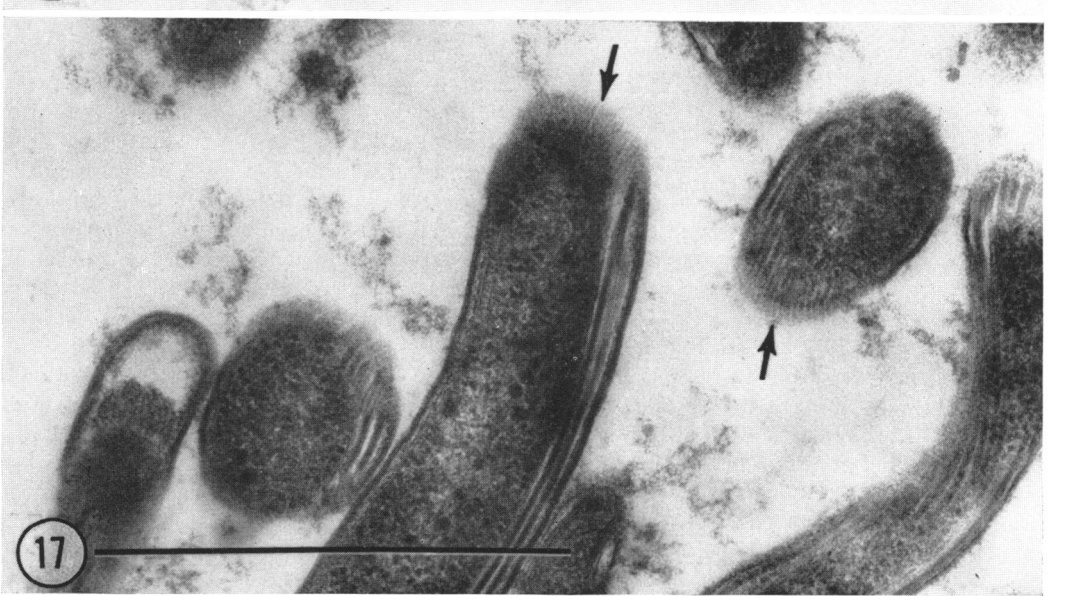


FIG. 16. *Encysted form of a large spirochete in gingival debris. Note "pin-striped" pattern in center, and "cross-hatched" pattern on periphery where stripes overlap at right-angle. Negative contrast.* × *47,000.*

FIG. 17. *Ultrathin section of large spirochetes in gingival lesion of ANUG. Note striped pattern of outer envelope in tangential sections indicated by arrows. A spirochete sectioned near its tip is visible in lower left corner. Kellenberger fixation.* × *63,000.*

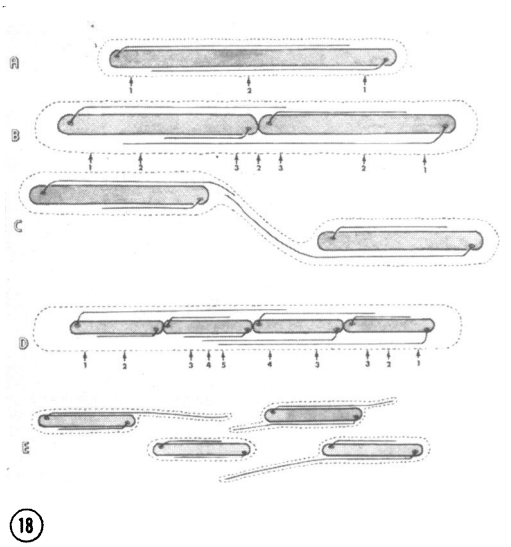


FIG. 18. *Diagrammatic representation of cell division in Treponema microdentium. (A) Spirochetal cell in resting stage. Note attachment of one axial fibril (solid line) near each end of protoplasmic cylinder (shaded portion) giving typical "1-2-1" pattern. Fibrils are separated for clarity. Outer envelope is represented by dotted line. (B) Dividing cell. Protoplasmic cylinder has pinched off in middle and new axial fibrils are originating on either side of division point producing a "1-2-3-3-2-1" pattern of fibrils. Outer envelope is intact. (C) Daughter cells in process of separation. Note trailing of original axial fibrils. Outer envelope will be last to divide. (D) Chain of four daughter cells within common outer envelope. Numbered arrows correspond to number of fibrils that would be seen at each respective site on protoplasmic cylinder. (E) Patterns of fibrillar protrusion which would be produced by separation of daughter cells shown in D. Note possibility of one, two, or no flagella-like appendages.*

that axial fibrils and flagella are homologous structures, the flagella having become oriented away from the cytoplasmic body. Flagella either may pass directly through the cell wall, or may form an outpouching in the wall, taking it along as a surrounding sheath. This relationship has been most convincingly demonstrated in *Vibrio metchnikovii* by Glauert et al. (1963), and is also found in *Bacillus brevis* (De Robertis and Franchi, 1952). A comparison of the chemical composition of flagella and axial fibrils would be of utmost interest in confirming their homologous origin.

The function of the axial fibrils of spirochetes is still not clear. The observation that axial fibrils in *T. microdentium* and other oral spirochetes are not continuous between the ends of the organisms makes it more difficult to visualize how these fibrils play a role in maintaining the spiral shape of the organism or in assisting in its locomotion. These fibrils are usually in close apposition in well-preserved specimens. This suggests the possibility that lateral bonds may be present between adjacent fibrils. These bonds might allow fibrils from opposite ends to slide over each other in a fashion similar to that of muscle fibers (Hanson and Lowy, 1962), thereby shortening the end-to-end distance of the organism. However, we have no evidence of any resemblance between axial fibrils and muscle fibers. Weibull (1950) investigated the composition of muscle fibers and bacterial flagella, and found important differences between the two in their qualitative amino acid composition. In addition, flagella were devoid of sulfhydryl groups. A tendency for bacterial flagella to aggregate has been known for many years (Weibull, 1960), and the close apposition of axial fibrils may simply be due to the manifestation of a similar phenomenon.

The substructure of some axial fibrils shown in Fig. 8 resembles the substructure of α-keratin fibrils described by Rogers and Filshie (1963). Astbury, Beighton, and Weibull (1955) showed that bacterial flagella are protein in nature, and give an X-ray diffraction pattern of a type closely related to that of α-keratins. However, the "9 + 2" arrangement of subfibrils described by Rogers and Filshie (1963) in wool fibers has never been definitely shown to exist, as had originally been suggested by Astbury et al. (1955), in the bacterial flagella examined to date by electron microscopy. Although protofibrils in various helical arrangements have been described in individual bacterial flagella, none has shown a "9 + 2" pattern of organization.

The polygonal pattern observed in the outer envelope of most oral spirochetes is similar to the structural organization recently described in a variety of membranous structures. Robertson (1963) observed a morphologically identical structural subunit in the cell membrane of club endings in Mauthner cell synapses of the goldfish brain. Furthermore, the capsids of the *Herpes simplex* virus, as illustrated by Smith (1963), also show the typical substructure described above, each capsomere being the equivalent of a structural

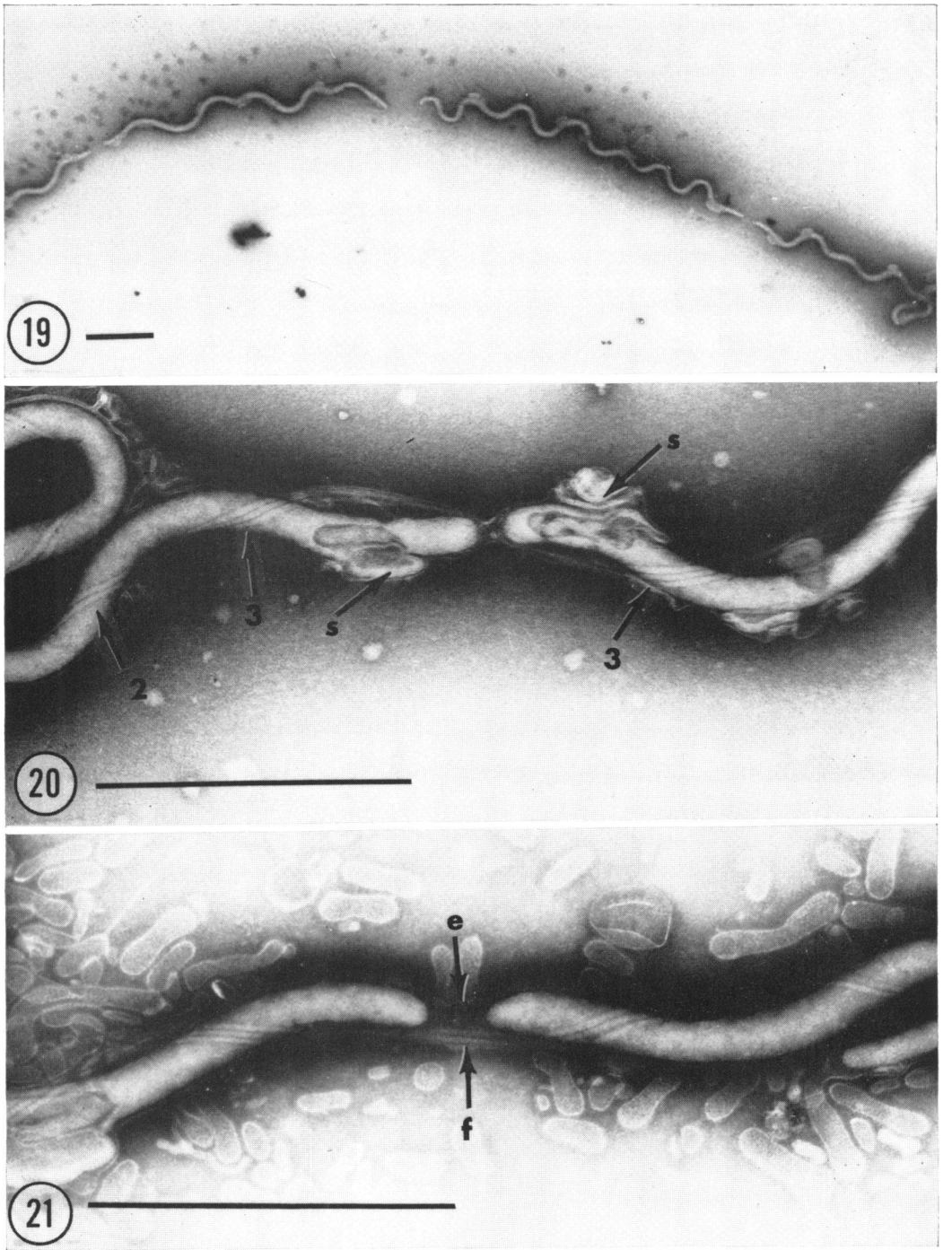
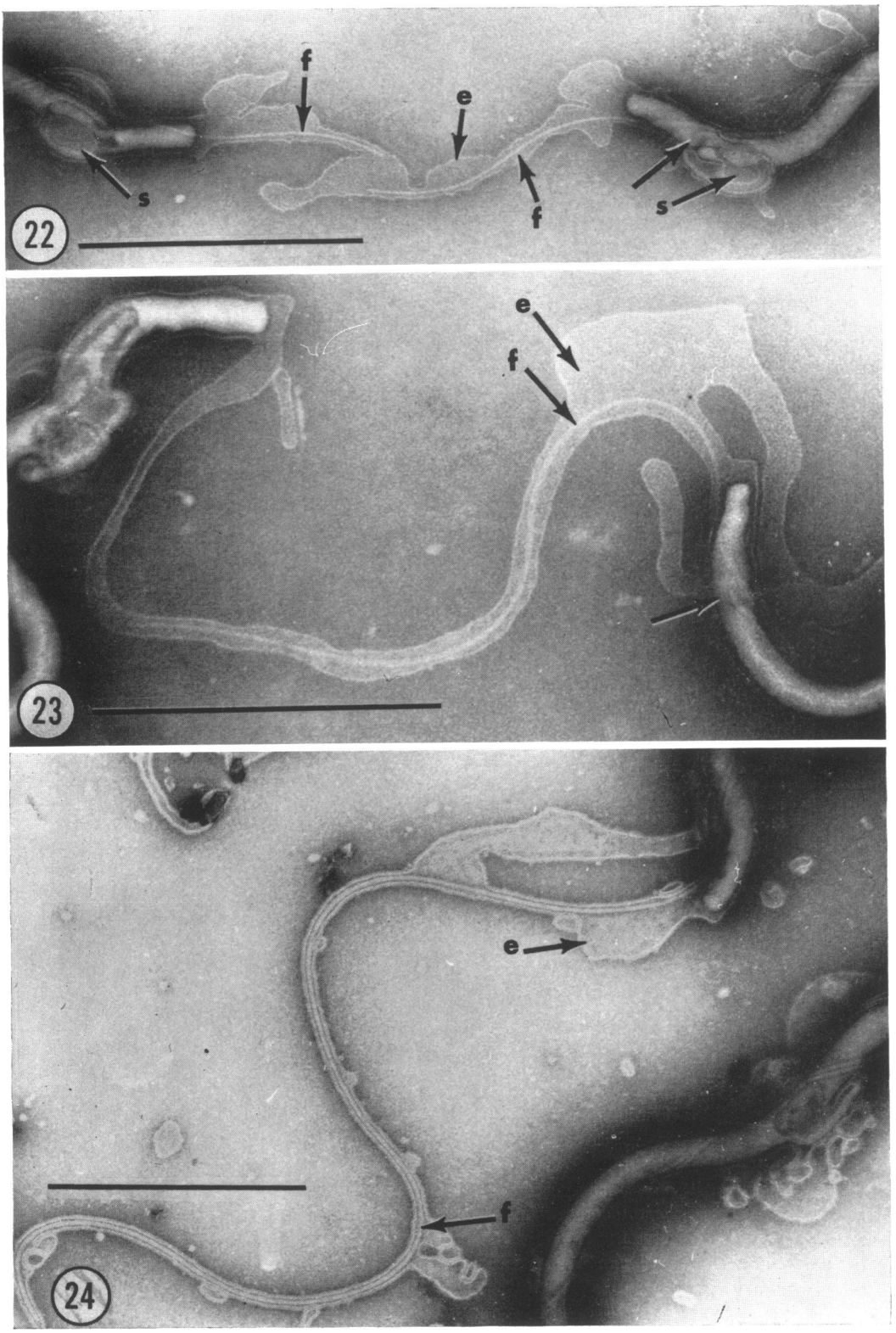


FIG. 19. *Chain of spirochetes in a pure culture of Treponema microdentium in log phase. Negative contrast.* × *9,500.*

FIG. 20. *Dividing Treponema microdentium. Note three fibrils on either side of division site and two fibrils farther away. Compare with Fig. 18B. Also note localized disruptions of protoplasmic cylinder* (s) *on either side of point of division. Negative contrast.* × *46,000.*

FIG. 21. *Dividing Treponema microdentium. Note that daughter cells are beginning to separate, but are still connected by outer envelope* (e) *and two axial fibrils* (f). *Compare arrangement of fibrils with Fig. 18B and C. Negative contrast.* × *54,000.*



FIGS. 22–24

subunit. Murray (1963) reported a complex hexagonal prismatic subunit in the cell wall of *Spirillum serpens* which he described as giving the overall impression of a central 100 A polygon with a hole in the middle. It may be that the polygonal structural subunit is a universal building block possibly found in a number of membranous structures.

The appearance of a pin-striped pattern in the cell envelope of the large spirochete seen in the lesion of ANUG patients does not necessarily imply the existence of an entirely different structural subunit. The difference may simply be due to masking of the basic subunit by other components of the envelope. It is interesting to note in this connection that Pease (1956) observed a striated cell wall in *S. undula* and that Houwink (1953), in an unidentified *Spirillum* and in *S. serpens*, described globular subunits arranged in a hexagonal pattern which produced a striated appearance on the outer face of the wall.

The three dense layers of the outer envelope of the large spirochete found in ANUG lesions (Fig. 8) closely resemble the "outer membrane" and "intermediate layer" described by Claus and Roth (1964) in the cell wall of *Acetobacter suboxydans*. These authors believe the intermediate layer to be characteristic of the cell wall of gram-negative bacteria. This morphological evidence suggests the possibility that the outer envelope of at least this spirochete is a modified cell wall.

Although we are aware of the difficulties in studying a dynamic process by means of electron microscopy, it was deemed worthwhile to synthesize a working hypothesis of spirochetal cell division which would satisfy the various observations recorded in log-phase spirochetal cultures. Our observations confirmed the evidence presented by Ryter and Pillot (1963) that spirochetes divide by transverse fission. In addition, our hypothesis provides an explanation for the observation that three or more axial fibrils can occasionally be encountered in dividing cells of *T. microdentium*.

It has been shown that axial fibrils, immediately after cell division, may extend beyond the end of the protoplasmic cylinder into a flagellum-like appendage. Although this fibrillar extension could conceivably play some role in cell locomotion, its role would be limited, since its presence is probably only temporary. It seems to appear after cell division and, because of its transitory nature, is not likely responsible for the motility of resting cells.

The concept presented here regarding the development of new axial fibrils from the protoplasmic cylinder adjacent to the site of division is not unique. Pease (1956) described the appearance in a lateral position of extracellular flagella in *S. undula*. She stated that the appearance of flagella in this position frequently preceded the fission of exceptionally long spirilla, the newly divided daughter spirilla being thus provided with a full tuft of flagella. These observations by Pease (1956) regarding the method of cell division in a spirillum with "extracellular" flagella further supports the concept that flagella and axial fibrils are homologous structures.

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FIG. 22. *Dividing Treponema microdentium. Note that cells are still connected by outer envelope (e) which surrounds flagella-like extensions of axial fibrils (f). Arrow indicates origin of a new axial fibril. Also note localized protoplasmic disruption (s). Compare to Fig. 18C. Negative contrast.* × *44,000.*

FIG. 23. *Dividing Treponema microdentium. Similar stage to Fig. 22. Note that only one axial fibril (f) is enclosed by outer envelope (e) connecting both daughter cells. Arrow indicates origin of new axial fibril. Compare with Fig. 18C. Negative contrast.* × *54,800.*

FIG. 24. *Recently divided cell in culture of Treponema microdentium. Note flagellum-like appendage (f) surrounded by sheath of outer envelope (e). Compare with Fig. 18E. Negative contrast.* × *39,000.*

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