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# Ultrastructure of Gliding Bacteria: Scanning Electron Microscopy of *Capnocytophaga sputigena, Capnocytophaga gingivalis,* and *Capnocytophaga ochracea*

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When examined by both light and scanning electron microscopy, *Capnocytophaga gingivalis, C. sputigena,* and *C. ochracea* displayed three distinct growth zones: the original streak, an intermediate zone, and the advancing edge, or halo zone. On Trypticase (BBL Microbiology Systems)-soy-blood agar, the cells translocated by gliding. *C. gingivalis* and *C. sputigena* formed large, irregular isolated colonies, while *C. ochracea* formed a more confluent cell mass. The cells within the streak zone and in most of the intermediate zone were heaped into mounds, with the individual cells displaying a definite flow pattern, the latter characteristic of *C. sputigena* and *C. gingivalis.* The halo zone consisted of tracks of cells which appeared to have translocated back upon themselves, or were restricted in their outward movement by adjacent cells. Also present within the halo zone were small aggregates of cells, referred to as pioneer colonies. The cell surfaces of *C. gingivalis* and *C. ochracea* were smooth and free of any apparent extracellular material, whereas *C. sputigena* was covered with a thick amorphous material, as well as long, thick, cell surface-associated fibrils.

Small gram-negative fusiform chemoorganotrophic bacteria have been isolated from periodontal lesions and from individuals with rapidly progressing periodontitis (29, 30) as well as from individuals displaying no clinical manifestations of periodontal disease (24). These microorganisms have been classified in the (new) genus *Capnocytophaga* (24). *Capnocytophaga sputigena* (strain no. 4) possessed bone-resorptive capabilities in the periodontium of gnotobiotic rats (21). Of interest in this observation, as well as in others (9), was that the microbial population of the developed pocket did not infiltrate the epithelial tissues of the periodontium, suggesting either destruction of the periodontium by extracellular enzymes, other bacterial products, or by host reactions to the presence of the microorganisms or their products within the pocket (32).

Recent transmission electron microscopic examination of *Capnocytophaga* (19) revealed a morphology typical of other gram-negative bacteria. Several of the representative capnobacters possessed, external to the outer cell membrane, a ruthenium red-positive layer which appeared to join adjacent cells into a matrix or network. Scanning electron microscopy (SEM), because of its ability to represent cell structure in three-dimensional arrangement, provided an opportunity to study characteristics of cell migration and colony enlargement superior to that possible with light and transmission electron microscopy.

## MATERIALS AND METHODS

**Bacterial strains.** In addition to the type strains of *Capnocytophaga* (41), fresh isolates were recovered from laboratory personnel by probing the gingival region of the distal molars with a sterile toothpick.

**Media and growth conditions.** The *Capnocytophaga* examined in this study were maintained on commercially prepared 5% sheep blood agar plates (BBL Microbiology Systems) in an atmosphere of 10% $CO_2$ + 90% $H_2$ in a Brewer anaerobic jar at 4°C.

**SEM of gliding motility.** For SEM, the *Capnocytophaga* were streaked initially to a Trypticase (BBL Microbiology Systems)-soy (TS)-blood agar plate and incubated approximately 3 days as described above. A fresh thin streak of the growth from this plate was made to another TS-blood plate and again incubated under Brewer Anaerobic Jar conditions. At 24-h intervals the plates were removed and cells were prepared for SEM. Suitable gliding could also be obtained on laboratory-prepared medium consisting of Todd-Hewitt broth (BBL Microbiology Systems), 5% (vol/vol) defibrinated sheep blood (GIBCO Diagnostics), 5 μg of hemin per ml (Eastman Organic Chemicals), 0.1 mg of sodium succinate per ml, final concentration (Fisher Scientific Co.), and 1.5% purified agar (BBL Microbiology Systems). The capability of the capnobacteria to grow below an agar surface or to move through small holes or crevices in agar was determined by utilizing "holey" agar, prepared by allowing the autoclaved agar to cool to 50°C, adding sheep blood, aerating by agitation, and pouring into sterile petri plates which were then placed at 4°C for solidification.

**SEM.** The entire colony was fixed in situ by flooding the cell-agar surface with freshly prepared 2% (vol/

vol) glutaraldehyde (Polysciences, Inc.) in 0.2 M potassium phosphate buffer (27), pH 7.2, for 24 h at 4 to 6°C. The glutaraldehyde was removed by aspiration, and the cell-agar surface was washed gently with cold Millonig buffer for approximately 15 min. The buffer was withdrawn by aspiration, and the agar was cut into 1-cm cubes; special note of the streak orientation was made. The individual cubes were postfixed with 1% (wt/vol) osmium tetroxide in Millonig buffer for approximately 12 h at 4 to 6°C. Osmium was removed from the fixed samples by aspiration, and the cubes were washed twice and dehydrated through a graded aqueous acetone series. All samples were dried by the critical-point drying procedure of Anderson (1) in a Polaron E3000 Critical Point Drying Apparatus (Polaron Instruments, Warrington, Pa.) employing liquid $CO_2$ replacement. The dried blocks were mounted on aluminum Cambridge pin-type stubs with double coated tape, covered with a thin carbon coating (approximately 10 nm) and coated with gold to a thickness of 10 to 25 nm in a Polaron E5000 Coating Unit. All samples for SEM were examined in a JEOL JSM 35 Scanning Electron Microscope operating at 15 or 25 kV.

**Light microscopy.** Light photomicrographs of surface translocating capnobacteria were obtained by growing cells directly on the surface of sterile glass microscope slides overlaid with the Todd-Hewitt broth, hemin, succinate medium plus 1.5% (wt/vol) purified agar (BBL Microbiology Systems). For measurement of translocation (i.e., gliding), freshly prepared liquid cultures were streaked to the surface of prereduced growth medium, then incubated for a specified time (36 h), and measurements of distance from the streak were determined. The developing colonies and associated translocating cells were examined by using differential-interference contrast microscopy.

**Negative stain.** Cells from 4-day growth on TS-blood plates were suspended in several milliliters of 3% (wt/vol) phosphotungstic acid (pH 6.95), and one drop of this suspension was placed on a Formvar-coated, carbon-reinforced grid. Excess material was removed, and the grid was air-dried and examined in a Philips 200 transmission electron microscope operating at 60 kV.

## RESULTS

Gliding colonies of *Capnocytophaga* were separated into three distinct zones of growth (Fig. 1a, b, c): the original streak line which appeared as a heaped region, an intermediate zone characterized by a speckled, evenly dispersed cellular morphology, and an advancing sharp edge, or halo zone. When inoculated to the surface of

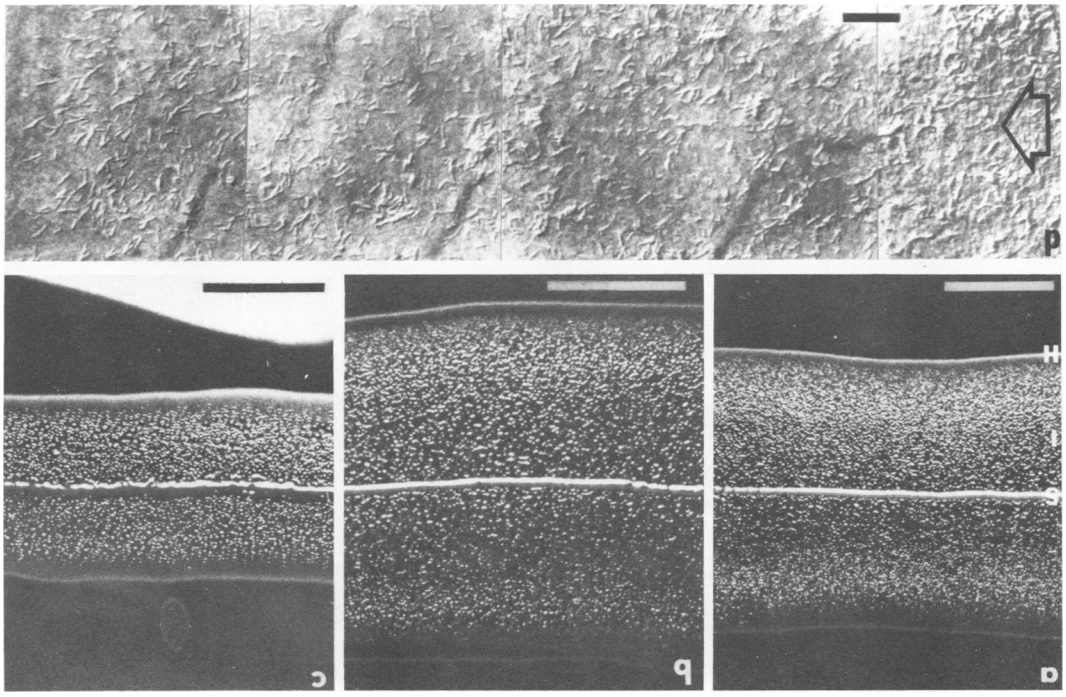


FIG. 1. *Photographs of C. sputigena (a), C. gingivalis (b), and C. ochracea (c) grown on the surface of TS-blood agar for 36 h. The streak zone (S) exhibits a tight packing of cells, whereas the intermediate zone (I) consists of a speckled or more diffuse arrangement of microorganisms. The halo zone (H) is seen as a thin advancing front of cells. Note the sparseness of cells within the leading edge of the halo zone of C. gingivalis (d). Groups of single cells tend to form small pioneer colonies several millimeters from the original streak; preparation was grown on Todd-Hewitt for 36 h. Arrow = direction of colony expansion. (a to c) Bar = 1 cm; (d) bar = 10 μm.*

TS-blood, the cells moved out from the streak in an almost perpendicular direction with an average colony expansion rate of 6.5 to 15.1 mm/36 h. *Capnocytophaga gingivalis* and *C. sputigena* colonies expanded at approximately the same rate, 10.8 to 15.1 mm/36 h, whereas *Capnocytophaga ochracea* moved more slowly, at 6.5 to 10.8 mm/36 h.

**Colonial characteristics.** *C. gingivalis* and *C. sputigena* (Fig. 2a, b) formed large, irregular isolated colonies, whereas *C. ochracea* formed a more confluent cell mass, with no clear separation of individual colonies (Fig. 2c). The individual colonies of *C. gingivalis* and *C. sputigena* (Fig. 2a, b) were easily lifted from the agar surface during sample preparation for SEM, whereas *C. ochracea* more firmly adhered to the agar surface.

**Streak zone.** The streak and the first two-thirds of the intermediate zone consisted of cells clearly heaped to form mounds (microcolonies; Fig. 2) and were arranged in a whirl pattern, with the cells of the whirl being in a characteristic end-to-end arrangement (Fig. 3a, b).

The cells in the streak zone were approximately 0.18 to 0.23 μm or 0.25 to 0.30 μm wide by 1.7 to 6.8 μm long. In some instances, seemingly where several of the capnobacteria had been freed of their cell-to-cell association, they reached lengths of 30 μm or greater.

**Intermediate zone.** The intermediate growth zones of *C. sputigena* and *C. gingivalis* differed from that of *C. ochracea* (Fig. 2a, b, c). With *C. sputigena* and *C. gingivalis* this zone consisted of individual irregular to circular, raised and convex pioneer microcolonies which frequently abutted one another. Their diameters ranged from 500 to 50 μm, the smaller colonies being representative of the advancing edge area. The cell masses exhibited eddy patterns, and cells were in close association with each other (Fig. 3a). At greater distances from the streak, colonies were progressively smaller, rounder, dome-like, and separated from each other (Fig. 3b). At colony diameters of approximately 100 μm or less, the swirl patterns were not seen; instead cells were arranged collaterally about the center of the pioneer colony with (presumably) migrating bacteria between colonies. At the extreme edge of the intermediate zone the centers of these colonies were devoid of cells (Fig. 3c). The capnobacteria left the heaped microcolonies in a flow pattern at several points (Fig. 3b) and moved out from the colony to occupy and colonize regions far removed from the original streak line (pioneer colonies).

**Halo zone.** The morphology of the halo zone was similar for all the capnobacteria examined and is represented here by Fig. 3d. The cells in this region appeared to be moving back upon themselves, or were limited in their outward movement by those cells involved in colony development.

In addition to the individual cells and colonies which occupied the far reaches of the intermediate zone and entire halo zone, small aggregates of cells (pioneer colonies) were observed (Fig. 4a to c). These pioneer colonies consisted of between 50 and 200 cells, again arranged in an end-to-end pattern (Fig. 4c). In Fig. 4d an early pioneer colony (white arrow) as well as smaller aggregates of cells (double arrow) in the halo zone were seen (cf. Fig. 4d and Fig. 4a, c). The leading edge of the halo zone was composed

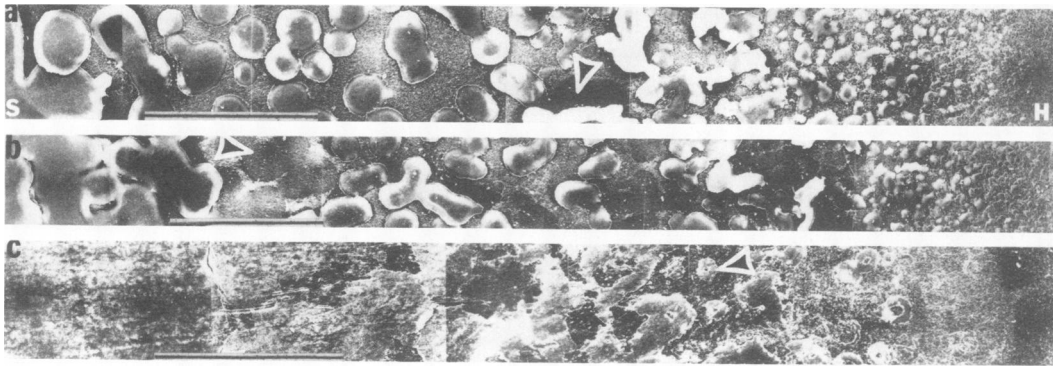


FIG. 2. *Low-magnification scanning electron photomicrographs of the streak-to-halo zone in C. sputigena (a), C. gingivalis (b), and C. ochracea (c) grown on the surface of TS-blood agar for 36 h. The individual cells of the streak zone are tightly packed and become more diffuse and separated as the cells move toward the leading edge of the halo. Microcolonies have been removed (arrow) by the preparative procedures for SEM (a, b). C. ochracea (c) exhibits numerous isolated microcolonies (arrow) in the latter one-third of the intermediate zone. Note the absence of cells in the central region of these colonies. Bar = 1 mm.*

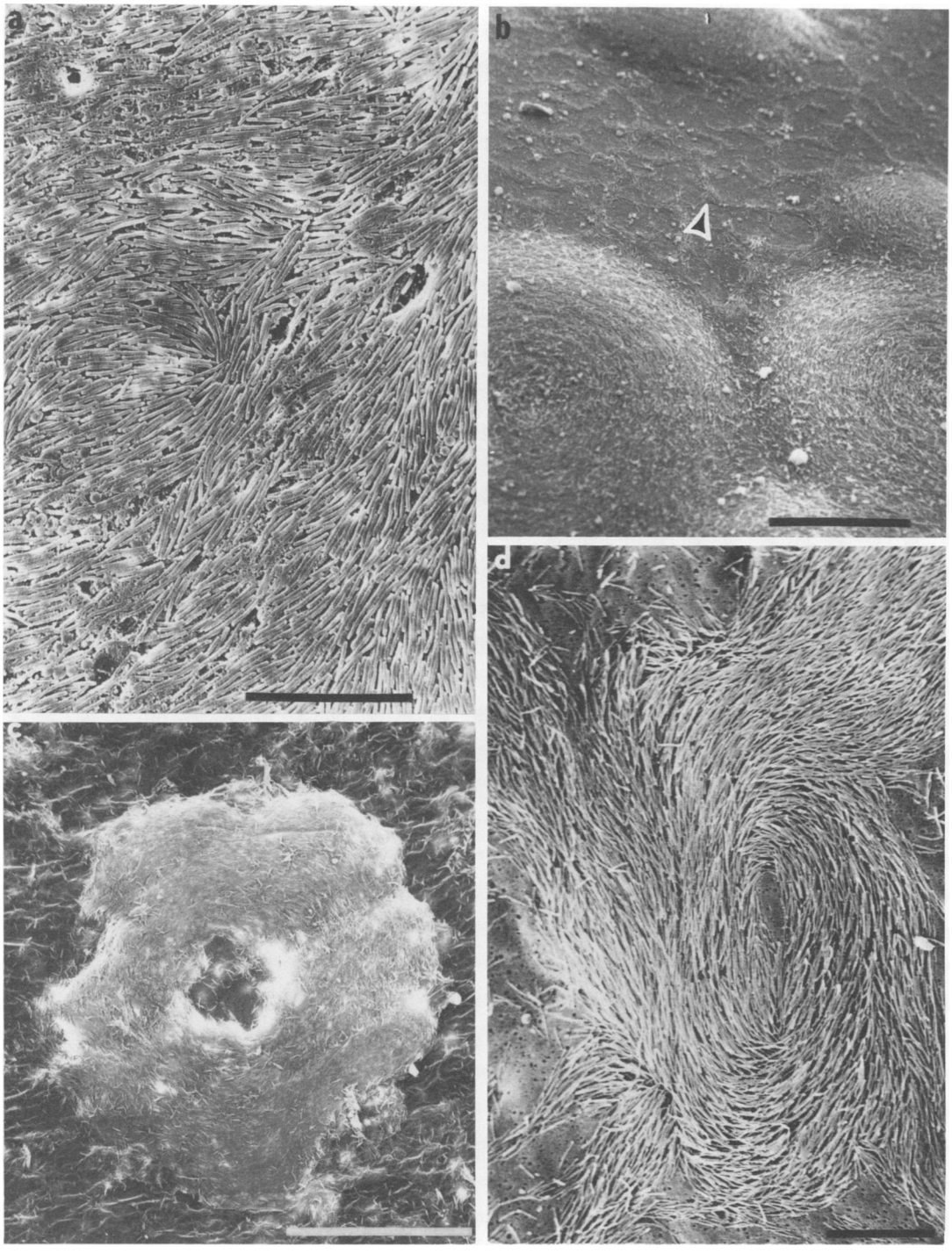


FIG. 3. *SEM of the streak and intermediate zones of Capnocytophaga grown on the surface of TS-blood agar. (a) C. sputigena shows the flow pattern typical of these oral bacteria in the region of maximum confluent growth. Note the end-to-end arrangement of the cells as well as the lateral attachment of adjacent cells. (b) C. sputigena and C. gingivalis in the intermediate-growth zone. The individual cells of the colony are aligned colaterally about the colony center. Numerous cells can be seen to have migrated from the individual colonies in a directed pattern (arrow). (c and d) C. ochracea microcolony and swarm cell, respectively. The cells are held together in a tight, oriented pattern, with the central region of the colony devoid of cells (cf. Fig. 2c). (d) The swarm of cells appears to have looped back upon themselves. (a and d) Bar = 10 μm; (b and c) Bar = 50 μm.*

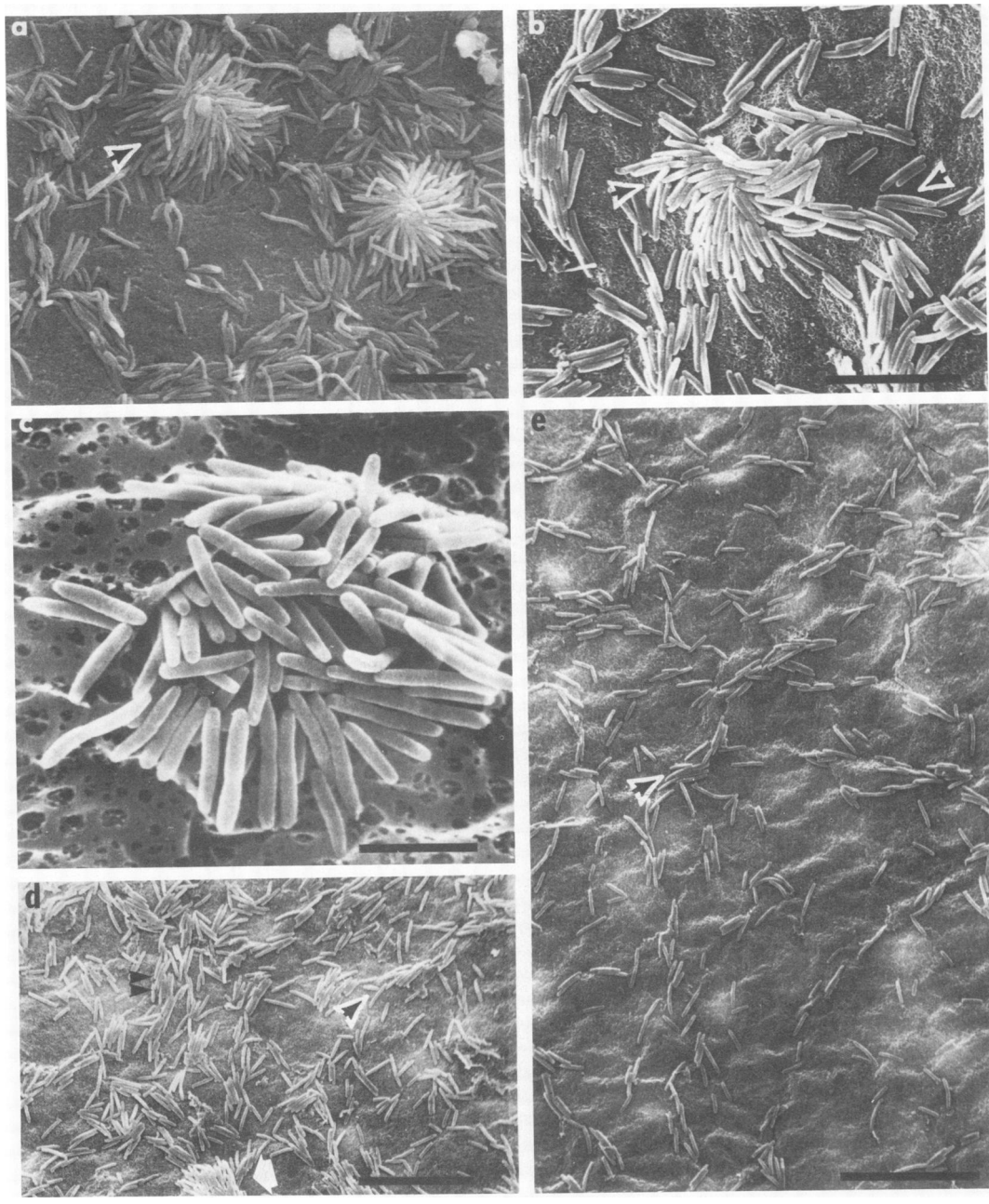


FIG. 4. *SEM of the halo zone of C. gingivalis (a), C. ochracea (b), and C. sputigena (c). The pioneer colonies in this region consist of individual cells in an oriented, heaped, end-to-end arrangement. The individual cells (arrow) appear to have migrated in a directed fashion to these pioneer colonies. (d) The oriented cells of C. ochracea were approaching the outermost edge of the translocating colony at fixation. (e) The leading edge of the colony of C. ochracea is seen. The cells are sparsely arranged, but again reveal an oriented pattern (arrow). (a and b) Bar = 5 μm; (c) bar = 2 μm; (d and e) bar = 10 μm.*

primarily of individual and randomly arranged cells (Fig. 1d, 4e).

**Subsurface growth.** The *Capnocytophaga* species examined in this study were capable of penetration and movement below the agar surface (Fig. 5). Single capnobacteria can penetrate

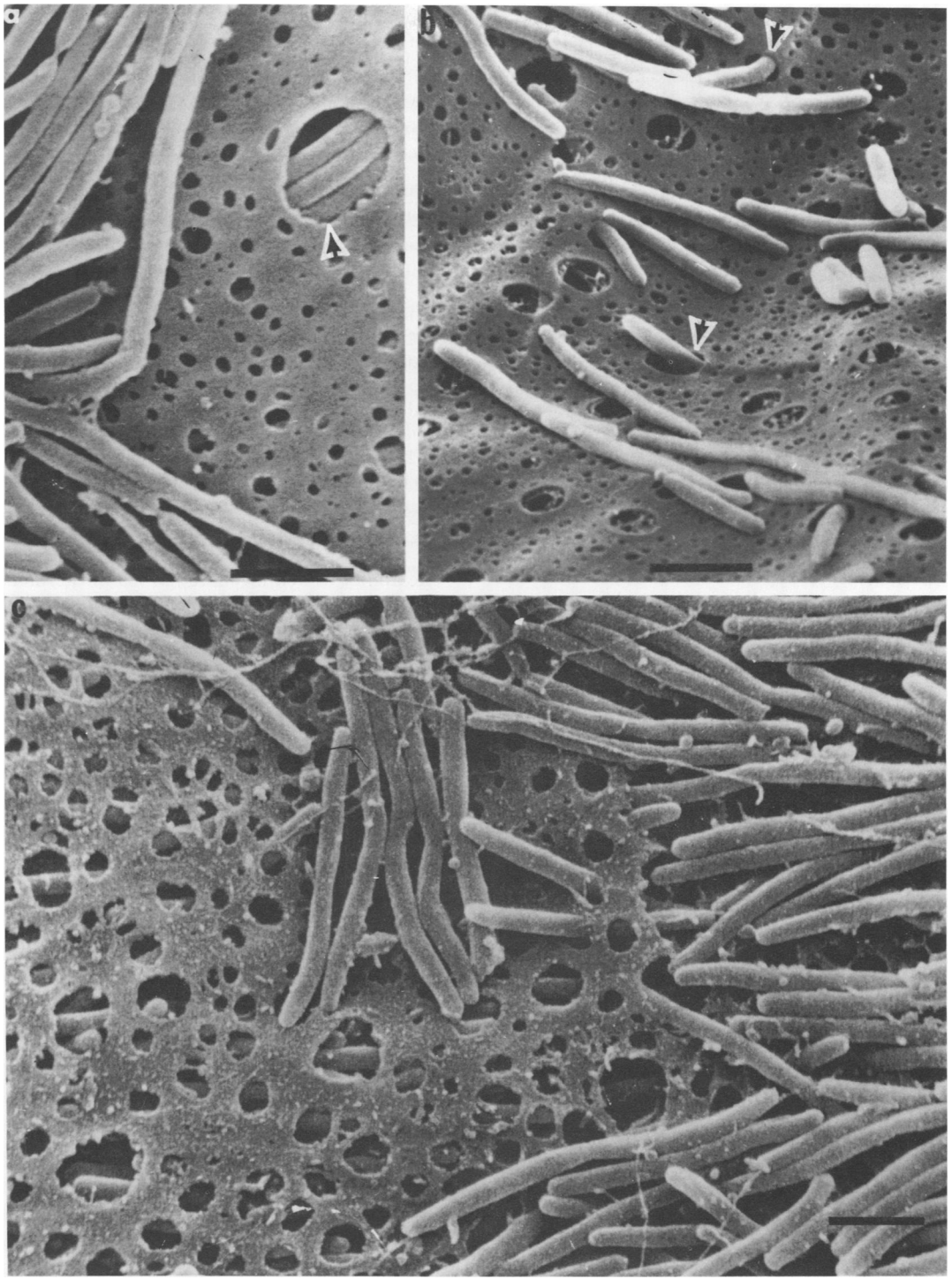


FIG. 5. *SEM of C. ochracea (a), C. gingivalis (b), and C. sputigena (c) growing both above and below the surface of holey agar. The numerous small holes within the agar permit the thin capnobacteria to migrate below the agar surface. At least three juxtaposed cells (arrow) are apparent in (a) below the agar surface, whereas portions of cells (arrows) occur above and below the agar surface (b). Where the agar has been torn (c) or the holes are large, numerous capnobacteria are apparent below the agar surface. Cell surface-associated filaments are also apparent (c). Bar = 1 μm.*

through holes in the agar, and where the holes were large enough (Fig. 5a), several juxtaposed cells were seen. In Fig. 5b, portions of cells both above and below the agar surface were apparent.

**Cell characteristics.** The outermost layers of *C. gingivalis* and *C. ochracea* were smooth surfaced and free of extracellular material, whereas *C. sputigena* was covered with a thick amorphous material (Fig. 7a, 7b, 6, respectively). *C. sputigena* also contained long, thick fibrils (Fig. 6a). The fibrils traversed long distances and covered numerous juxtaposed cells. Figure 6c and negatively stained cells (see Fig. 9a) clearly showed that the fibrils stemmed from and were continuous with the outermost, amorphous cell surface. They were, in morphological respects, similar to the lipopolysaccharide of other gram-negative bacteria. However, as judged by SEM, this amorphous layer described for *C. sputigena* was external to the outer membrane.

Depending upon the growth stage of *C. sputigena*, several types of cell surface material were evident. At 24 h, numerous blebs between 50 and 180 nm in diameter covered the outer membrane (Fig. 6a). After 72 h of incubation, the cells within the streak and intermediate zone showed fewer surface-associated blebs, and increased in the amount of extracellular matrix material (Fig. 6b). The cells during this stage of growth appeared to be intimately associated with this developing extracellular matrix material (Fig. 6b). After approximately 120 h there was an aggregation or coalescing of the extracellular matrix into an amorphous covering which outlined and delineated individual cells (Fig. 6c). The extracellular strands were relatively thick (100 to 160 nm) and traversed numerous juxtaposed cells.

A fresh oral isolate was morphologically similar to the laboratory-maintained strain of *C. sputigena*. This isolate (Fig. 8) was also covered with an amorphous outer material; however, numerous radiating strands or fibrils interconnected the cell mass. There appeared to be a marked reduction in these radiating strands in the laboratory-maintained capnobacteria.

Negative staining of the *Capnocytophaga* species revealed a variable morphology in the outer cell membrane (Fig. 9). Ninety-six-hour cultures of *C. sputigena* (Fig. 9a) were covered with a large amount of extracellular material of varying length, but with an average diameter of 8 to 16 nm. Blebs, 20 to 80 nm in diameter, also originated at the surface of the outer membrane. In contrast, *C. gingivalis*, which was free of any apparent cell surface material by SEM, possessed loose-fitting, thin strands (lipopolysaccharide) when examined by phosphotungstic acid-negative staining (Fig. 9b). The fact that in *C. gingivalis* this outermost material is loose fitting and sparse may render it nondetectable in the SEM. It may also possess a chemical or physical composition different from that of *C. sputigena*, which might also render it morphologically different when examined in the SEM. *C. ochracea* (Fig. 9c) possessed outer-membrane-associated material which originates from the surface of the membrane. This may be analogous to the material which gave *C. ochracea* the knobby texture seen with SEM (Fig. 7b). As with *C. gingivalis*, the lack of visualization of this material on *C. ochracea* by SEM may be due to a difference in its chemistry or degree of hydration, or to physical parameters reflecting the material collapsing onto the surface of the outer membrane during preparative procedures, or its being lost within the architecture of the membrane.

## DISCUSSION

The ability of nonflagellated procaryotic rods to migrate on solid surfaces has been long recognized, although the mechanism(s) responsible for this motility remain obscure. Henrichsen (16) has contrasted the characteristics of the sliding, twitching, darting, and gliding sorts of surface mobilities.

Certain aspects of gliding motility have been well described for gram-negative genera, among the *Beggiatoa, Chondromyces, Cytophaga, Stigmatella, Myxococcus, Simonsiella*, and now *Capnocytophaga* (2, 3, 7, 13, 26, 33, 34, 46). This motility is characterized by cellular migration which, in comparison with that of flagellated bacteria, is rather slow; cell movement may be either forward or backward relative to the longitudinal axis of the cell. Cells often flex or bend, and then the direction of movement may change. Although such movement clearly is a property of healthy cells, the latter do not always move, but may show spurts of motility. Most attention has been paid to aggregates of cells at the leading, or migrating, edge of colonies, perhaps because colonies formed by gliding bacteria do not enlarge passively (i.e., as a result of cell growth alone) but rather because of cellular motility.

The results of this study revealed both similarities and differences in the pattern of motility of these capnobacters and other chemoorganotrophic gliding bacteria (e.g., *Cytophaga, Flexibacter*). The capnobacters differ more markedly from fruiting myxobacters such as *Myxococcus*, where colony spreading per se is complicated by often concomitant fruitification events (4, 38).

As has been both inferred (43) and demonstrated (3, 16) for other gliding bacteria, capnobacterial cells may be motile either when single

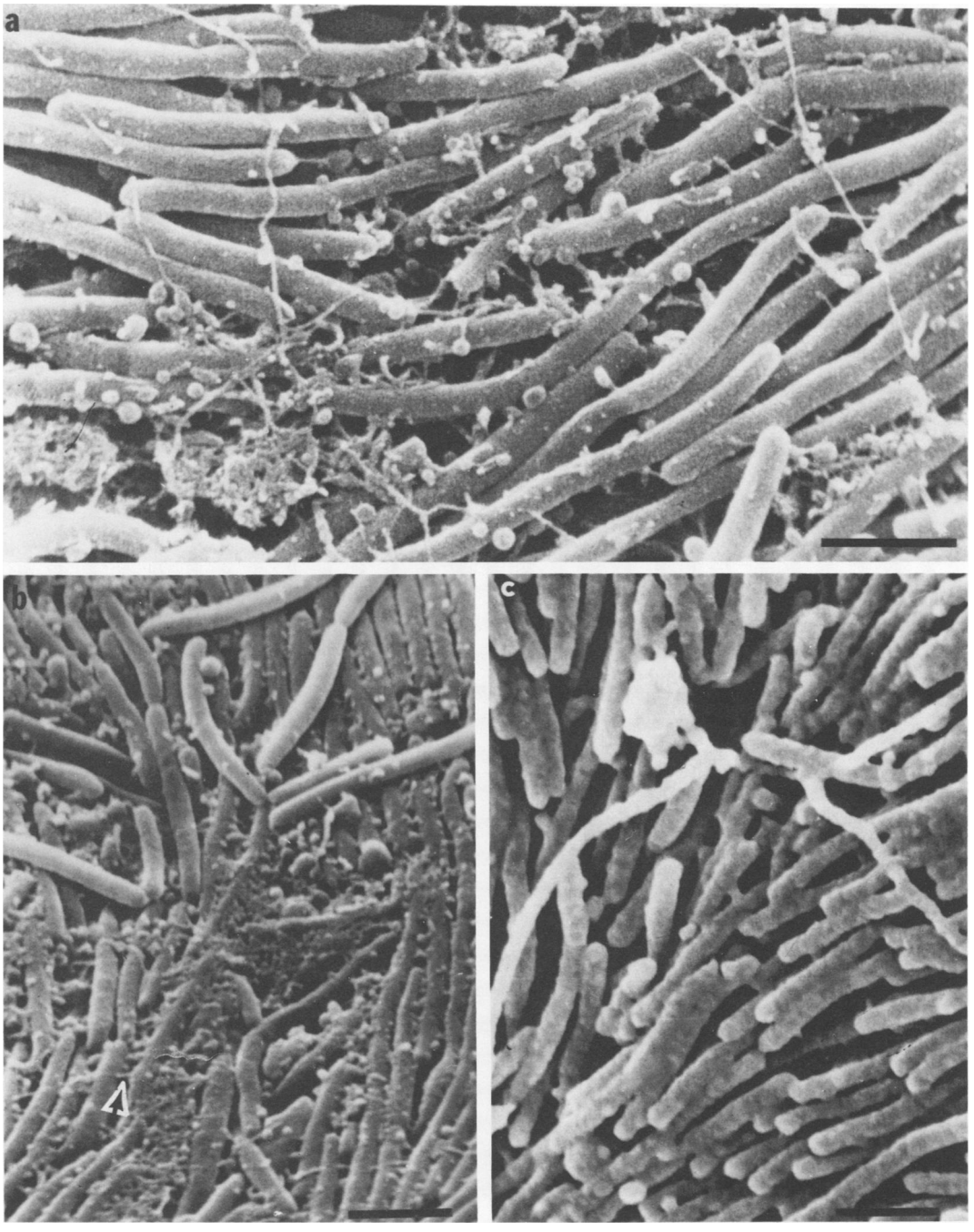


FIG. 6. *SEM of C. sputigena after 24-h (a), 72-h (b) and 120-h (c) growth on the surface of TS-blood agar. At 24 h, numerous surface-associated blebs and strands covered the smooth-surfaced cells. After 72 h, a thick filamentous network attached adjacent cells (arrow), with many juxtaposed cells attached by short filaments. The cell surface was then rough textured. After 120 h, C. sputigena was covered with thick filaments which emerged from the extremely rough outer surface material. Bar = 1 μm.*

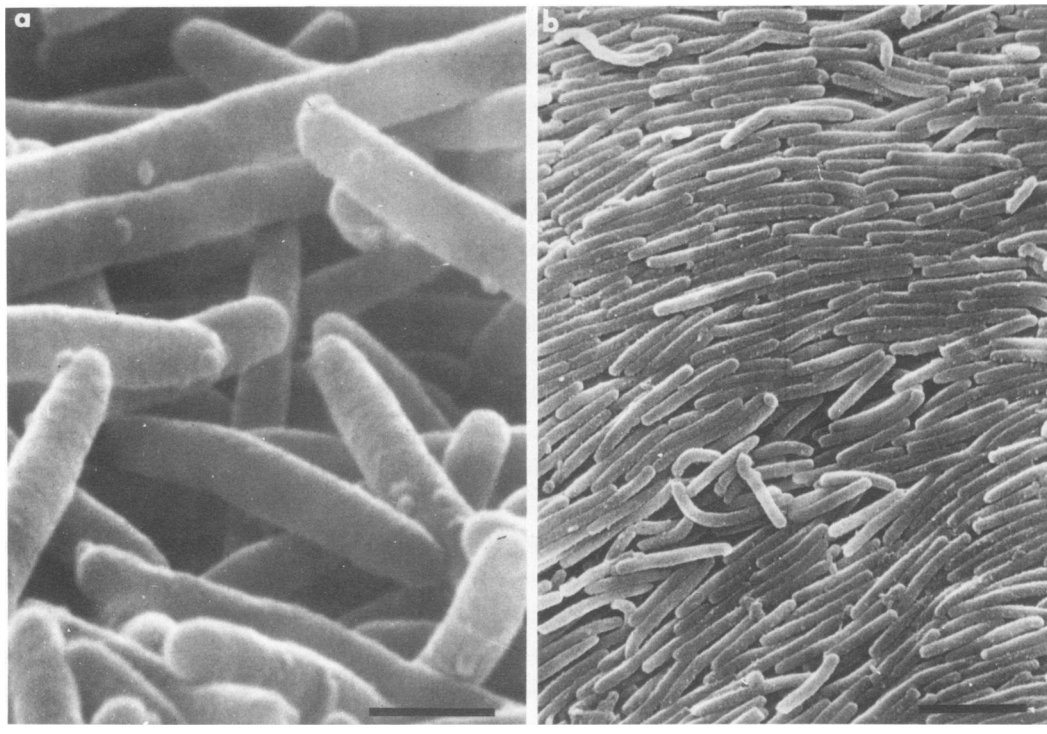


FIG. 7. *SEM of C. gingivalis (a) and C. ochracea (b) grown on TS-blood agar for 120 h. The cells are generally smooth and lack the extracellular material found on C. sputigena (Fig. 6). Occasional surface blebs can be seen in both species. (a) Bar = 0.5 μm; (b) bar = 2 μm.*

or as cell aggregates, and the individual colonies may be divided into three regions: the streak or deposition zone, the spreading or intermediate zone, and the halo or leading edge of the colony. It is in the halo zone that the capnobacters differ most from other gliding procaryotes, for a large proportion of the capnobacterial cells exist singly or as small aggregates removed from the majority of cells making up the colony. In other gliders, cells which migrate away from the colony core usually do not remain separated and initiate microcolony formation, but rather return to the main swarm and grow and migrate possibly in cooperation with other cells.

Microcolonies of *C. sputigena*, in particular, develop in a manner similar to the fruiting myxobacters *Chondromyces crocatus* and *Stigmatella aurantiaca*: young aggregation centers before mound or "yolk" region formation appear as circular microcolonies in which the cells are arranged collaterally about the colony center. In *Capnocytophaga*, cells emerge or migrate as aggregates or swarms from the edge of a compact microcolony and then, as single cells or aggregates, form new or pioneer microcolonies. Why the single cells and aggregates in the halo zone do not return to the colony area from which they migrated is not at all clear; it may reflect the lack of deposition of a slime track or trail which is characteristic of so many cytophagas and myxobacters.

Not all capnobacterial strains form such complete, cell-filled microcolonies, for colonies of some strains are devoid of cells in the central, core region as is also the case for *Beggiatoa* and some *Vitreoscilla* sp. (14, 34). We have no evidence for the rotation in place noted for the *Beggiatoa* and *Vitreoscilla*. Some *Capnocytophaga* (notably no. 25) seem more prone to form swarms which then loop back and coalesce with the original cell mass so as to form circular colonies, as is also the case with *Cytophaga diffluens* (42) and *Beggiatoa alba* (7).

Although we have adduced no evidence for agar liquefaction or dissolution by *Capnocytophaga*, we have noted a rather unusual, or at least not well recognized trait of gliding bacteria—the ability to grow, or migrate, into the interstices of the agar. A rather limited invasion of agar has been noted for non-agarolytic gliders such as *Oscillatoria princeps* (15), *Beggiatoa*, and a canine isolate of *Simonsiella* which produces grooves in agar surfaces (33).

Gliding bacteria not only display cell-to-cell

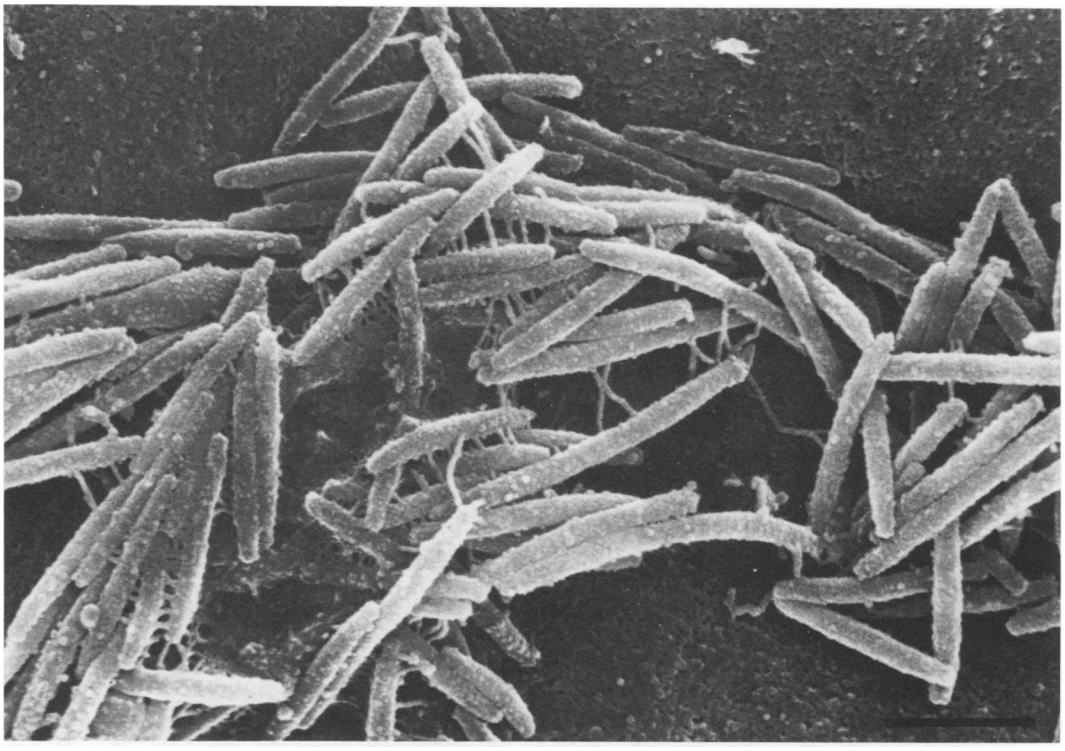

FIG. 8. *SEM of a fresh isolate of a surface translocating capnobacterium grown on the surface of TS-blood agar for 24 h. Numerous filaments and thick extracellular matrix enveloped the cells, as well as connecting adjacent cells. Bar = 2 μm.*

interactions with other members of their clones, but they also presumably attach to, or otherwise interact with, and comigrate with other quite unrelated bacteria. This latter attribute may be of ecological significance in the oral cavity since the capnobacters, which are found in both supra- and subgingival plaques, may be able to move from one location to another by virtue of their ability to glide on a solid surface (i.e, tooth). Two laboratories (E. R. Leadbetter and S. C. Holt, Abstr. Int. Assoc. Dent. Res. 1978, 967, p. 316; L. To, S. Sasaki, and S. S. Socransky, Abstr. Int. Assoc. Dent. Res. 1978, 968, p. 316; S. S. Socransky, S. Sasaki, and L. To, Abstr. Int. Assoc. Dent. Res. 1978, 969, p. 317) have already demonstrated passive translocation or "piggy-back" mechanisms for transport of motile (by flagella) and nonmotile bacteria by gliding bacteria. Surface characteristics of the gliding as well as the nongliding bacteria undoubtedly play a determinant role in the specificity of these organismic interactions.

The interaction of the bacterial and host cell surface in the primary events of a bacterial infection has, in recent years, come under increasing scrutiny. A large body of evidence already indicates that specific microorganisms must first colonize a specific host surface as a prerequisite to infection. This specificity of bacteria-host interaction, commonly referred to as adherence, has been observed in several important and potentially debilitating infections (5, 6, 8, 10, 17, 37, 47). Gibbons (11) and Gibbons and Van Houte (12) have described a variety of potentially pathogenic microorganisms which attach to mucosal surfaces, both during natural and artificially induced infections.

Clearly, the SEM has played a major role in understanding from a morphological aspect the close interaction that exists between bacteria and tissue cells. Muse et al. (28) have shown quite convincingly that *Bordetella pertussis* phase I cells attach only to ciliated epithelial cells, whereas Savage and Blumershine (36) elegantly showed that the murine gastrointestinal tract contained numerous bacterial types which existed in very close association with epithelial surfaces. Numerous spirochete-host interactions have been demonstrated in recent years (18).

There is every reason to believe that in order for a given bacterial species to colonize a particular host tissue it must be capable of adherence,

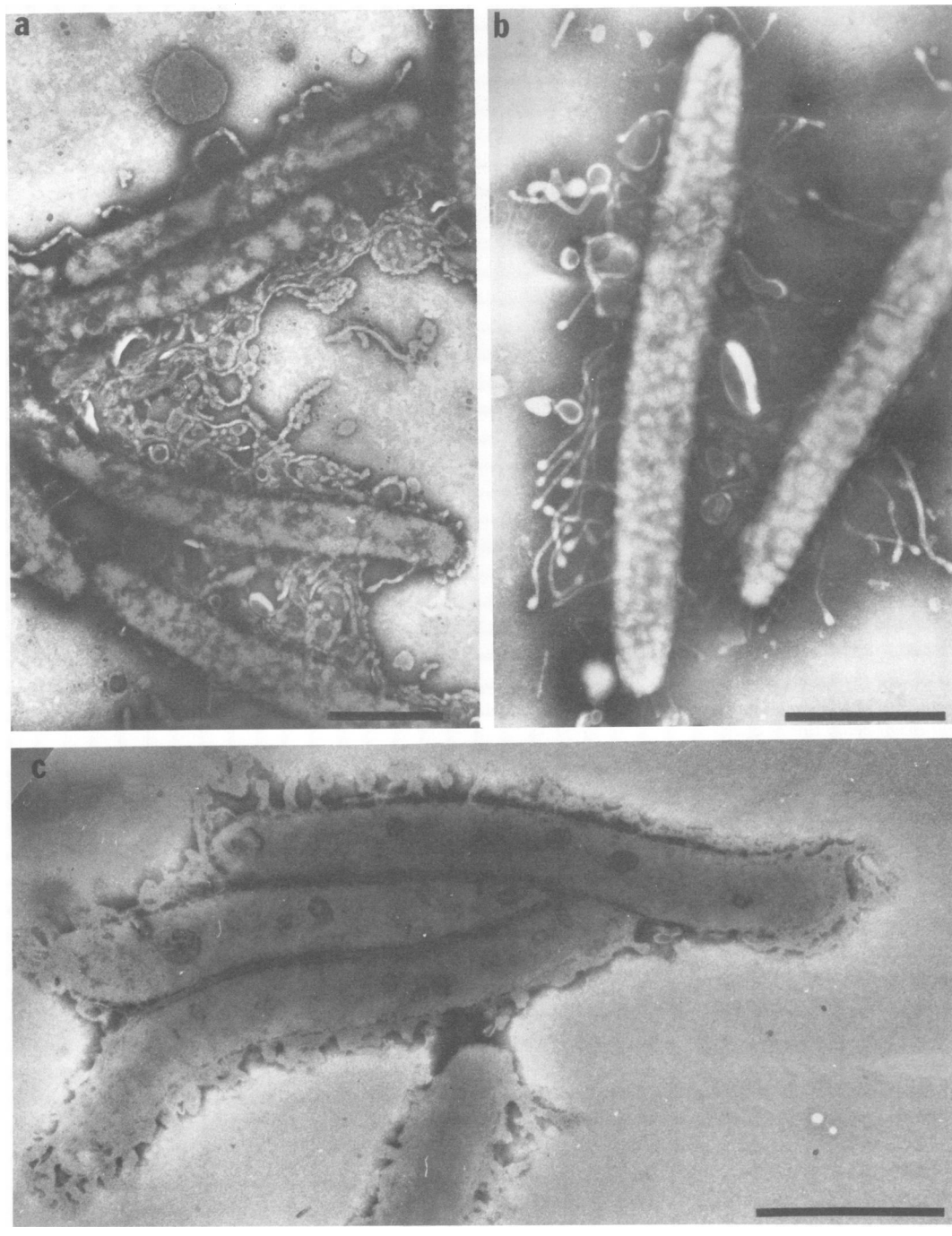


FIG. 9. *Transmission electron micrographs of negatively stained (PTA) cells of C. sputigena (a), C. gingivalis (b), and C. ochracea (c) grown on TS-blood agar for 96 h. Numerous filaments emerged from the surface of the cells. In most instances the filaments appear to be cell associated, with very few of them lying free in the background. Note that C. sputigena which exhibited a rough textured surface by SEM (Fig. 6b) contains the most extracellular material by negative staining. Bar = 1 μm.*

a mechanism probably mediated by surface-associated exopolymeric material. In most instances the surface of *Capnocytophaga* was found to be covered with ruthenium red-positive material, as well as small blebs or vesicles and long thin filaments. The long filaments were morphologically similar to lipopolysaccharide (22, 25). Significantly, *C. sputigena* possesses large amounts of surface-associated material and appears to adhere better than *C. gingivalis* and *C. ochracea* (S. C. Holt and B. Rosan, unpublished data). Interestingly, in an oral *Bacteroides* sp., R. A. Celesk, R. McCabe, and J. London, (Abstr. Int. Assoc. Dent. Res. 1979, 638, p. 252) have observed that these bacteria preferentially adhered to the root surface of extracted teeth in culture vessels. Thin sections of this tooth-bacteria association revealed membranous appendages which emerged from the bacterial cell surface. Woo et al. (48) have also observed numerous long thin filaments emanating from the surface of several *Bacteroides* sp. of oral origin.

Whether the surface material observed in *Capnocytophaga* serves in in vivo environments in an adherent capacity similar to that described by Gibbons (11), Gibbons and Van Houte (12), and Takeuchi et al. (45) for solid and tissue surfaces, or, for that matter, to neighboring microorganisms (44), is unclear.

Taken as a whole, the *Capnocytophaga* possess the numerous traits which could place the genus in a central position in the etiology of periodontal disease. These thin, flexuous bacteria could, by their ability to glide over smooth surfaces (i.e. the tooth surface) in an apical direction through narrow crevices, enter into deep subgingival locations within the developing periodontal pocket. Where exopolymers are produced these bacteria could attach to host tooth or epithelial surfaces or both and ultimately colonize these surfaces. Of considerable importance in the infective process is the ability of *Capnocytophaga* to cotransport nonmotile organisms. This phenomenon of the movement of nonmotile bacteria (i.e. *Bacteroides* sp.) from one location to another may be critical in the microbiological evaluation and understanding of mixed anaerobic infections (20, 35, 40). Ultimately, the pathological effect of the bacterium upon its host could, by the action of hydrolytic or proteolytic enzymes (23, 31), by exopolymeric material, or by an adverse effect on the host defense mechanisms (32, 39) result in host tissue damage.

## ACKNOWLEDGMENTS

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