INFECTION AND IMMUNITY, Nov. 1980, p. 588-600
0019-9567/80/11-0588/13$02.00/0

Vol. 30, No. 2

# Morphology and Ultrastructure of Oral Strains of *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*

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Selected human oral and nonoral strains of the genera *Actinobacillus* and *Haemophilus* were examined by transmission and scanning electron microscopy. The strains examined were morphologically identical to recognized *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, and *Haemophilus paraphrophilus*. By transmission electron microscopy, the cells were typically gram negative in morphology, with several strains possessing some extracellular ruthenium red-staining polymeric material. Numerous vesicular structures, morphologically identical to lipopolysaccharide vesicles, were seen to originate from and be continuous with the surface of the outer membrane. Large numbers of these vesicles were also found in the external environment. Scanning electron microscopic observations revealed that both actinobacilli and haemophili possessed surface projections and an amorphous surface material which connected and covered adjacent cells.

The past several years have seen the development of a large body of experimental evidence which implicates specific groups of microorganisms in both periodontal health and the pathogenesis of several forms of gingivitis and periodontitis (4, 5, 10, 24, 30-33, 43, 44, 46, 50, 51).

Electron microscopic and histological examinations of periodontal tissue during the course of periodontitis reveal quite clearly the absence of intact microorganisms within host tissue. This absence of intact bacteria from or within periodontal tissues makes it more than likely that for a specific periodontopathogen to be implicated in the development of a lesion or to exert its influence on the host, it must in some way be closely associated with or adhere to host epithelial or mucosal surfaces. This adherence may be mediated by surface exopolymers (polysaccharides) or external appendages (pili and fimbriae) and is especially critical in the initial stages of disease. Some examples of host bacterial interactions critical to the disease process include streptococcal pharyngitis and pneumonia (17, 40, 41), bacterial endocarditis (11), and numerous other gram-negative bacterial-epithelial tissue associations (see reference 9 for pertinent citations).

Members of the genus *Actinobacillus* (14, 22, 38) and *Haemophilus aphrophilus* (21, 42) have been recovered from several pathologies (42, 47, 49), including chronic periodontitis pockets, and are constituents of dental plaques (23). As a first step in our examination of the interactions of these gram-negative bacteria with host tissue, we report here the morphological characterization of representative strains of oral and nonoral strains of *Actinobacillus actinomycetemcomitans, H. aphrophilus*, and *Haemophilus paraphrophilus*.

## MATERIALS AND METHODS

**Organisms.** *A. actinomycetemcomitans* strains were isolated at the Forsyth Dental Center, Boston, Mass., from localized periodontosis or periodontitis patients and from a chest aspirate (American Type Culture Collection, ATCC 29525); *H. aphrophilus* and *H. paraphrophilus* strains were obtained from M. Kilian, Aarhus, Denmark, and from the American Type Culture Collection (strains 13252 and 29242, respectively).

**Growth of organisms.** All strains of *Actinobacillus* and *Haemophilus* used were grown as previously described (3). Briefly, cells were streaked on the surface of Trypticase soy blood agar plates (BBL Microbiology Systems) and incubated under an atmosphere of 10% $H_2$-10% $CO_2$-80% $N_2$ at 37°C in Brewer anaerobic jars. In most instances, plates were incubated for at least 48 h, at which time surface growth was transferred to a sterile liquid medium consisting of the following (per liter of distilled water): 10 g of Trypticase peptone, 50 mg of dithiothreitol, 1 mg of biotin, 5 g of sodium bicarbonate, 5 g of yeast extract, and 2 g of glucose. A 75-ml amount of a stock salt solution and 0.1 ml of a stock vitamin mixture (Socransky, unpublished data) were added per liter of medium. The final pH was 7.2 and required no adjustment. Liquid-grown cultures were incubated statically in air; we noticed no morphological or growth differences

between anaerobically or aerobically incubated cultures of liquid-grown cells.

**Light microscopy.** Both 72-h and 6-day plate- and liquid-grown cultures were examined by phase-contrast microscopy. Plate-grown cells were removed from the agar surface with a sterile loop and suspended in a drop of sterile distilled water on a clean microscope slide. Liquid-grown cells were placed on the microscope slide. Representative microscope fields were photographed.

**Electron microscopy. (i) Transmission electron microscopy.** All cells that were examined in this study by transmission electron microscopy were fixed in 2% (vol/vol) cold (4°C) freshly prepared glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) and postfixed in 1% (vol/vol) cold osmium tetroxide in phosphate buffer for at least 2 h in the cold. In some cases, cells were fixed in osmium tetroxide overnight (approximately 12 h) but no morphological differences were observed between short or long fixation. As a general rule, the petri plate surface of agar-grown cells was flooded with cold glutaraldehyde, and the cells were scraped from the agar surface, decanted into centrifuge tubes, and fixed at 4°C. Liquid cultures were fixed by dilution of 8% (vol/vol) glutaraldehyde to 2% (final concentration) directly into the liquid-grown cells. Cells were fixed at 4°C for 1 h and then centrifuged at low speed to sediment them into an intact pellet. The cell pellets were washed three to four times with cold phosphate buffer, and postfixation was carried out in cold 1% (vol/vol) osmium tetroxide for 1 h at 4°C. In most cases, cell pellets were used; if the cells could not be maintained as pellets, they were immobilized in 2 to 3 drops of warm (45°C) ion agar (in phosphate buffer) before osmification.

Ruthenium red staining and fixation was accomplished by the procedure of Luft (26) and Pate and Ordal (37).

All samples were dehydrated through ethanol and embedded in Epon 812, and ultrathin sections were prepared on a Porter-Blum MT-2 ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate.

All samples were examined in a JEOL 100S transmission electron microscope operating at 80 kV and photographed on Kodak electron image film 4463 photographic emulsion.

**(ii) Scanning electron microscopy. (a) Agar-grown cells.** Cell surface growth was flooded with freshly prepared 2% (vol/vol) glutaraldehyde–phosphate buffer, and the entire cell population was fixed for at least 2 to 4 h at 4°C in situ. The glutaraldehyde was removed by vacuum aspiration, and the surface was washed at least once with cold phosphate buffer. Immediately after washing, 1-$cm^2$ agar-cell cubes were cut out with a clean razor blade, and the cubes were either fixed in 1% (vol/vol) osmium tetroxide for 1 h at 4°C or dehydrated and critical-point dried as described below. Osmium-fixed cells were washed once with buffer and then dehydrated and critical-point dried.

**(b) Liquid-grown cells.** Liquid-grown cells were filtered onto 0.22-μm Nuclepore filters. The filter-impinged cells were fixed in glutaraldehyde for 2 h at 4°C. Small squares of the filters were cut out, dehydrated, and critical-point dried as described previously.

**Dehydration, critical-point drying, and sputter coating.** All samples for scanning electron microscopy were dehydrated through a graded acetone series consisting of 10, 25, 50, 75, 95, and 100% acetone for 5 min, twice for each step. Samples either were stored in 100% acetone in the cold or were immediately dried by liquid $CO_2$ replacement in a Polaron E3000 (Polaron Instruments Inc., Warrington, Pa.) critical-point drying apparatus.

The dried samples were immediately mounted on copper stubs with double-coated tape and vacuum coated in a Polaron sputter-coating unit (E5000) with approximately 10-nm thick Au–Pd deposited from a circular target mounted approximately 3 cm from the sample. The samples were cooled with a continuous flow of cold water through cooling coils mounted in the base of the sample holder.

In some instances, a thin layer of carbon was evaporated onto the sputter-coated surface to stabilize the sample.

All samples for scanning electron microscopy were examined in a JEOL 25S scanning electron microscope operating at either 15 or 25 kV and photographed on Polaroid type 665 positive-negative Land film.

## RESULTS

**General morphology.** When examined by phase-contrast microscopy, 48-h-old plate-grown *A. actinomycetemcomitans* (strains Y4 and N27), *H. aphrophilus* (strain 80), and *H. paraphrophilus* were seen as short, slightly curved rods of approximately 1.5 to 1.7 by 0.45 to 0.55 μm (Fig. 1a, b, d, e, and f). Since *H. aphrophilus* and *H. paraphrophilus* strains were morphologically identical, we will use only *H. aphrophilus* in our morphological description. The haemophili were either curved (Fig. 1d and f) or coccobacillary shaped (Fig. 1e). Liquid-grown cells (Fig. 1b and e) of both genera were similar to plate-grown cells in morphology; however, in liquid media, there was more of a tendency towards cell chaining and the formation of "C"-shaped cells. Incubation of both *Actinobacillus* and *Haemophilus* strains for extended periods of time (i.e., up to 16 days) resulted in a shortening and rounding of the cells (Fig. 1c). There was no evidence of involution forms after a 16-day incubation at 37°C.

**Electron microscopy. (i) Transmission electron microscopy.** Transmission electron microscopic examination of thin sections of *A. actinomycetemcomitans* and *H. aphrophilus* strains (Fig. 2 to 4) revealed several morphological features which were characteristic for solid (agar) surface-grown cells. Both genera were, for the most part, covered with adhering exopolymeric material (Fig. 2a, b, f, and g; 3c; 4a to f). Liquid-grown cells (Fig. 2d) appeared to lack significant surface-associated or adhering exo-

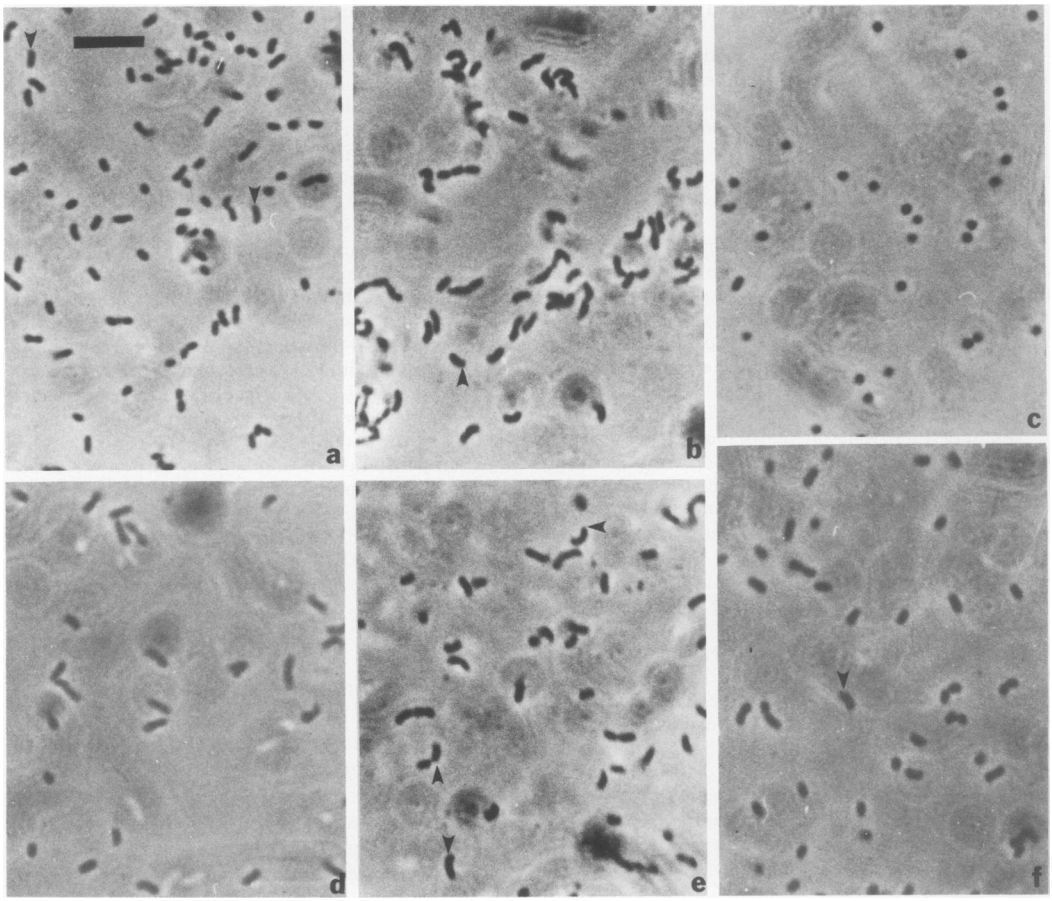


FIG. 1. *Phase-contrast photomicrographs of 72-h plate- and liquid-grown A. actinomycetemcomitans strains Y4 (a and b) and N27 (d and e) and 72-h plate-grown H. aphrophilus strain 80 (f). (c) A. actinomycetemcomitans strain Y4 incubated for 16 days. Both agar-grown and liquid-grown cells tended toward a curved morphology (arrowheads). In liquid, the cells tended to clump and chain. Bar = 5 μm. Unless otherwise indicated, cells shown in all figures are of agar-grown organisms.*

polymeric material. Agar-grown cells appeared to form more outer membrane-associated blebs or vesicles and to release large numbers of these structures into the external milieu (Fig. 2c, f, and g; 3c; 4a to f). With the exception of what appeared to be a qualitatively larger amount of surface-associated exopolymeric material and outer membrane vesicles in agar-grown cells of both genera, liquid-grown cultures were morphologically identical to those grown on an agar surface.

*A. actinomycetemcomitans* strain 511 (Fig. 2a and b) was pleomorphic and displayed branching.

**(a) Outer membrane.** The outer membrane of *A. actinomycetemcomitans* and *H. aphrophilus* (e.g., Fig. 2a, d, and e; 3a; 4b) was morphologically identical to that observed in other gram-negative bacteria and was approximately 9.5 nm thick. In *A. actinomycetemcomitans* strain 511 (Fig. 2a and b), the outer membrane was approximately 15.5 nm thick, whereas in *H. aphrophilus* it was of variable thickness; in strain 77 (Fig. 4a and b), it was approximately 18.5 nm thick, and in American Type Culture Collection strain 29524 (Fig. 2d), it was 6.7 nm thick. Further, the *Haemophilus* outer membrane appeared thin and delicate, as compared with the *Actinobacillus* outer membrane, and also tended to be more irregular. Both genera shed large amounts of outer membrane vesicles into the external environment (Fig. 2a and f; 3c; 4a to f).

Characteristic of both genera was the presence of numerous surface-associated fibrils or loosely fitting microcapsules (Fig. 2b, f, and g; 4a to f).

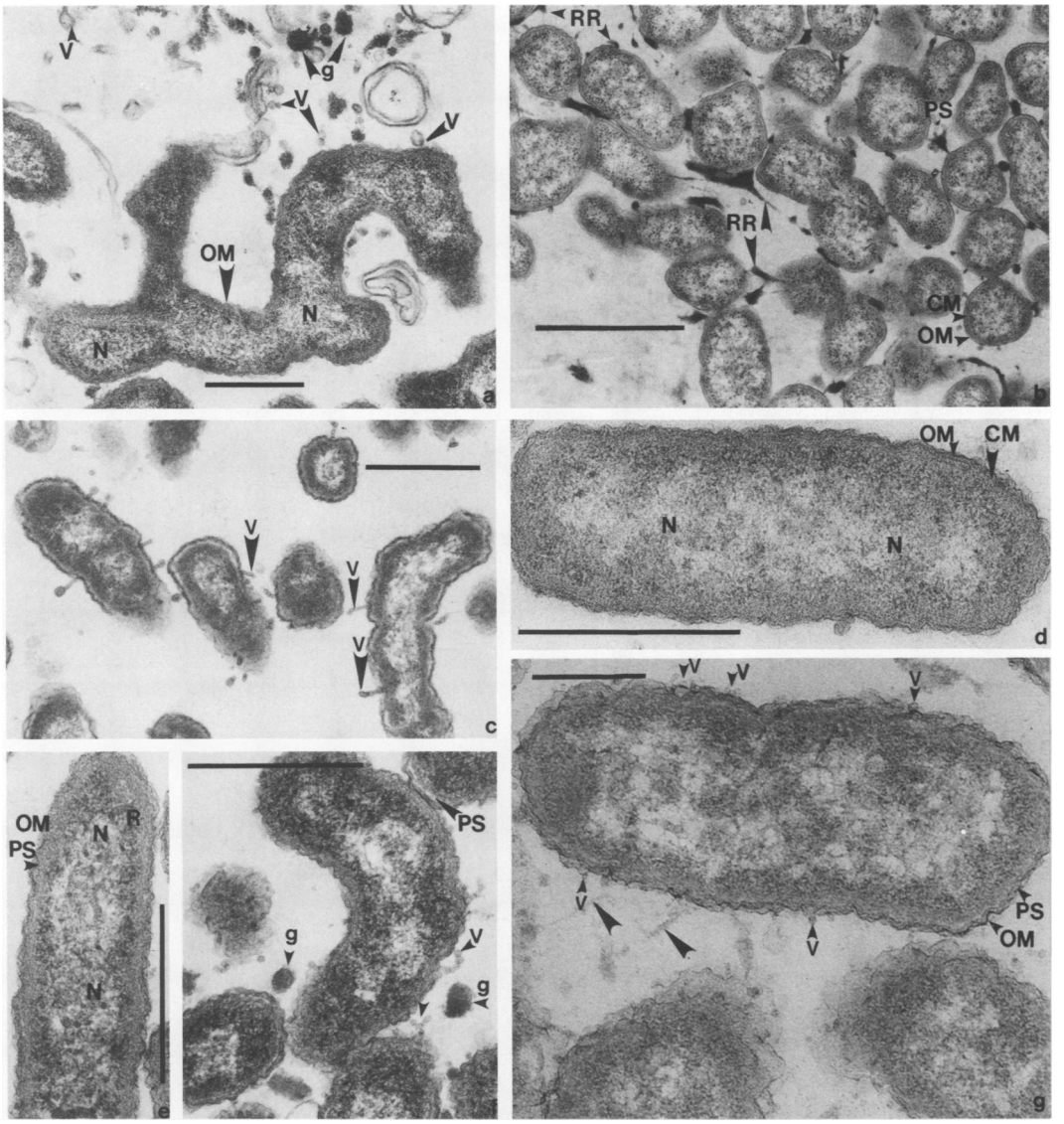


FIG. 2. *Thin sections of A. actinomycetemcomitans strains 511 (a and b), N27 (c and e), ATCC 29524 (d), 2097 (f), and Y4 (g). Strain 511 is pleomorphic to branching in morphology. The outer membrane (OM) encloses a narrow periplasmic space (PS). The inner or cytoplasmic membrane (CM) is, in most instances, difficult to see due to its juxtaposition to the electron-opaque cytoplasm. Numerous electron-opaque granules (g) of various sizes and shapes are apparent. Blebs or vesicle extensions (V) of the outer membrane are apparent. When fixed and stained with ruthenium red (RR), cells of strain 511 (b) and Y4 (g) possessed a ruthenium red-positive outer membrane as well as ruthenium red-positive material in close association with the outer membrane. Thin ruthenium red fibrils (arrowheads) also join adjacent cells. R, Ribosome; N, nucleoid. (d) Liquid grown. Bars = 0.5 μm (a, b, d, e, and f), 1 μm (c), and 0.25 μm (g).*

The fibrils occurred either as thin hairs (Fig. 4a, c, and d) or as electron-opaque regions on the outer membrane surface (Fig. 2f). In several cases, the fibrils emerged from the surface of the outer membrane. Where several cells were juxtaposed, the fibrils appeared to connect them.

Ruthenium red fixation and staining revealed the presence of an electron-opaque layer seen most clearly in *A. actinomycetemcomitans* strains 511 (Fig. 2b) and Y4 (Fig. 2g). The stain appeared to stain the outer membrane of these genera more intensely (Fig. 3a and b) than was

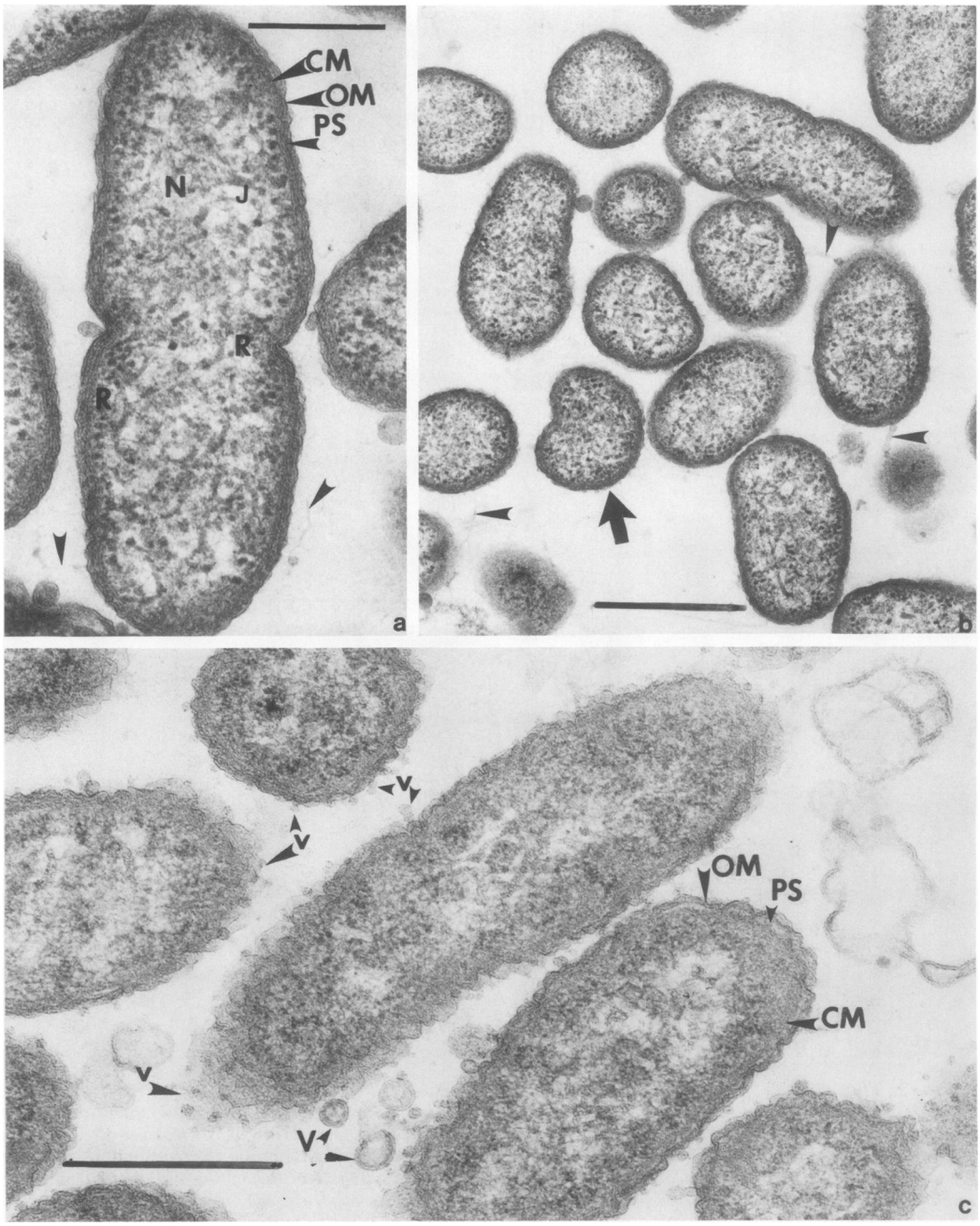


FIG. 3. *Thin sections of A. actinomycetemcomitans strain 2112. (a and b) Ruthenium red-prepared cells; (c) glutaraldehyde-osmium control. A thin fibrillar ruthenium red coating lines the surface of the outer membrane in a and b (arrowheads) which is absent in (c). The periplasmic space is lightly electron opaque. Large (V) and small vesicles (v) line the surface of the outer membrane. The large arrow in (b) indicates numerous small vesicles associated with the surface of the outer membrane. Bars = 0.5 μm (a and c) and 0.25 μm (b).*

observed in other gram-negative bacteria similarly prepared and was not as thick as that found, for example, in *Capnocytophaga* (14) or *Eikenella corrodens* (39).

**(b) Periplasmic space.** The periplasmic space resides between the layers of the outer and cytoplasmic membranes (Fig. 2b; 3b). In *Actinobacillus* and *Haemophilus*, the periplasm

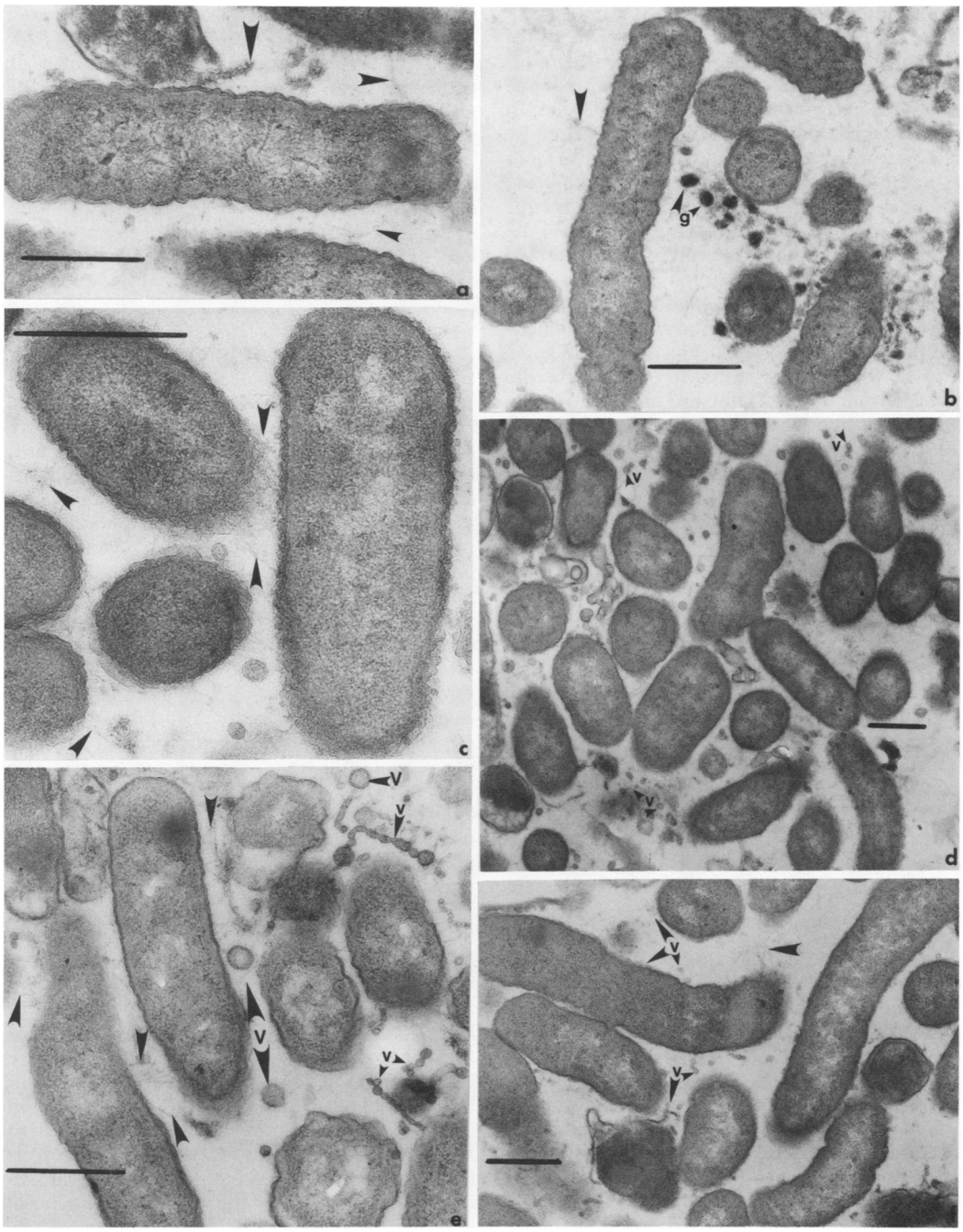


FIG. 4. *Thin sections of H. aphrophilus strains 77 (a and b), 80 (c and d), 81 (e), and 655 (f). Characteristic of these strains is a loosely fitting outer membrane with numerous associated thin fibers (arrowheads). Vesicles (V), both free in the background and outer membrane associated, and granules (g) of various sizes and electron-opacities are apparent. Bar = 0.5 μm.*

was poorly defined. When observed, it represented only a thin and, for the most part, electron-transparent space (Fig. 2c). In *A. actinomycetemcomitans* strains N27 and Y4 (Fig. 2c, e, and g, respectively), it appeared in most cases as a tight-fitting, electron-opaque, or granular region. It could not be distinguished with any degree of clarity from the enclosing outer and

cytoplasmic membranes.

**(c) Cytoplasmic membrane and cytoplasm.** The cytoplasmic membrane is the innermost membrane of the cell and was approximately 5.8 nm wide and of unit construction. In most instances this membrane was obscured by the electron-opaque cytoplasm, the latter consisting of a centrally disposed fibrillar nucleoid surrounded by tightly packed electron-opaque ribosomes. Storage granules and mesosomal membranes were not observed in these studies.

**(d) Extracellular vesicles and particles.** These oral *Actinobacillus* and *Haemophilus* strains possessed large numbers of extracellular membrane-bound vesicles (Fig. 2a, c, and g; 3c; 4a to f), which most probably originated from the outer membrane. They are morphologically identical to lipopolysaccharide-containing vesicles (25). Electron-opaque granules of approximately 80 to 100 nm in diameter were also seen in the background of some strains (Fig. 2a and f; 4d). There was no evidence of their being membrane bound.

**(ii) Scanning electron microscopy.** Scanning electron microscopic observations of both the *Actinobacillus* and *Haemophilus* species (Fig. 5 to 7) revealed the presence of a thick, amorphous layer which not only covered the outer surface of the cells, but appeared to attach adjacent cells (Fig. 5a; 7a and d) by thick fibrous threads or strands (Fig. 5b, d and f; 6a to g; 7a and b). These strands may be an artifact of the preparative procedures and, in fact, may represent a tear or contraction of the outer capsular material away from the cell surface. In *H. aphrophilus* strains 654 and 655 (Fig. 7c to e) the surface matrix was seen to cover many adjacent cells.

Several of the *H. aphrophilus* strains, notably strains 77, 80, 81, and 655 (Fig. 6a and e; 7a to e), possessed numerous small particles on the outer cell surface. *H. aphrophilus* strains 654 and 655 possessed the most densely packed surface particles (Fig. 7c to e), whereas *H. aphrophilus* strain 80 possessed the fewest particles (Fig. 6e to g). *A. actinomycetemcomitans* possessed few surface-associated particles. Careful examination of these particles revealed that they originate from the surface of the outer membrane. When observed at an angle to the cell surface, they were, in fact, fibrils or strands (Fig. 5b; 6a, c, and d; 7a). The distal portion of the fibril terminated in a large knob or bulb (Fig. 5b; 6c; 7a).

Several of the *Actinobacillus* (511, N27, and 2097) and *Haemophilus* strains (80) possessed long, thick strands which traversed long distances, connecting distant cells (Fig. 5e; 6g). From the scanning electron microscopic observations, these strands appeared to be associated with the outermost layer of the cell (Fig. 5e; 6g). Clearly, they are morphologically different (i.e., in length and apparent flexibility) from the short, thick fibrils which connect adjacent cells (Fig. 5c; 6b and d).

The *Actinobacillus* and *Haemophilus* strains displayed a cellular morphology characteristic of segmented bacteria. The extracellular slime or capsular material covered the segmented cells and, in several instances, could be seen to delineate the individual cell segments (Fig. 5b; 6a and d; 7e).

## DISCUSSION

Members of the genera *Actinobacillus* and *Haemophilus* are capnophilic gram-negative rods isolated from both oral and systemic pathologies. In recent years, *A. actinomycetemcomitans* has assumed a role of possible importance in oral diseases such as juvenile periodontitis (46). Not only has *A. actinomycetemcomitans* been isolated in high numbers from periodontal pockets of juveniles and young adults (4), but also strains Y4 and N27 have been shown to cause alveolar bone loss when inoculated as a monocontaminant in gnotobiotic rats. Of interest is the observation that in these gnotobiotic rat infections with strain Y4 there was little apparent leukocytic infiltration of the developing periodontal lesion (16). Strain Y4 has also been shown to possess a "leukotoxin" (2) active against polymorphonuclear leukocytes (48) and possess large amounts of lyso-containing phosphatides, which are known to be active in mammalian tissue destruction.

*Haemophilus* species (see reference 19 for a discussion of *Haemophilus* taxonomy) have been found associated with the mucous membranes of both humans and animals and are frequent inhabitants of the upper respiratory tract (20, 22). They have also been recovered from epithelial mucosal tissues of the respiratory tract; however, there have only been a few isolations of haemophili (i.e., *H. aphrophilus*) from dental plaque material (21, 23). In contrast to *H. aphrophilus*, *A. actinomycetemcomitans* is found more widely disseminated in the human body as a member of the indigenous bacterial flora of the mouth and as a member of the predominant flora in cases of bacterial endocarditis (22, 47, 49).

Our observation that *A. actinomycetemcomitans* and *H. aphrophilus* are covered with surface exopolymers is consistent with the increasing number of observations of these components (i.e., capsules, fibrils, and slime layers) on gram-negative bacteria recovered from disease situa-

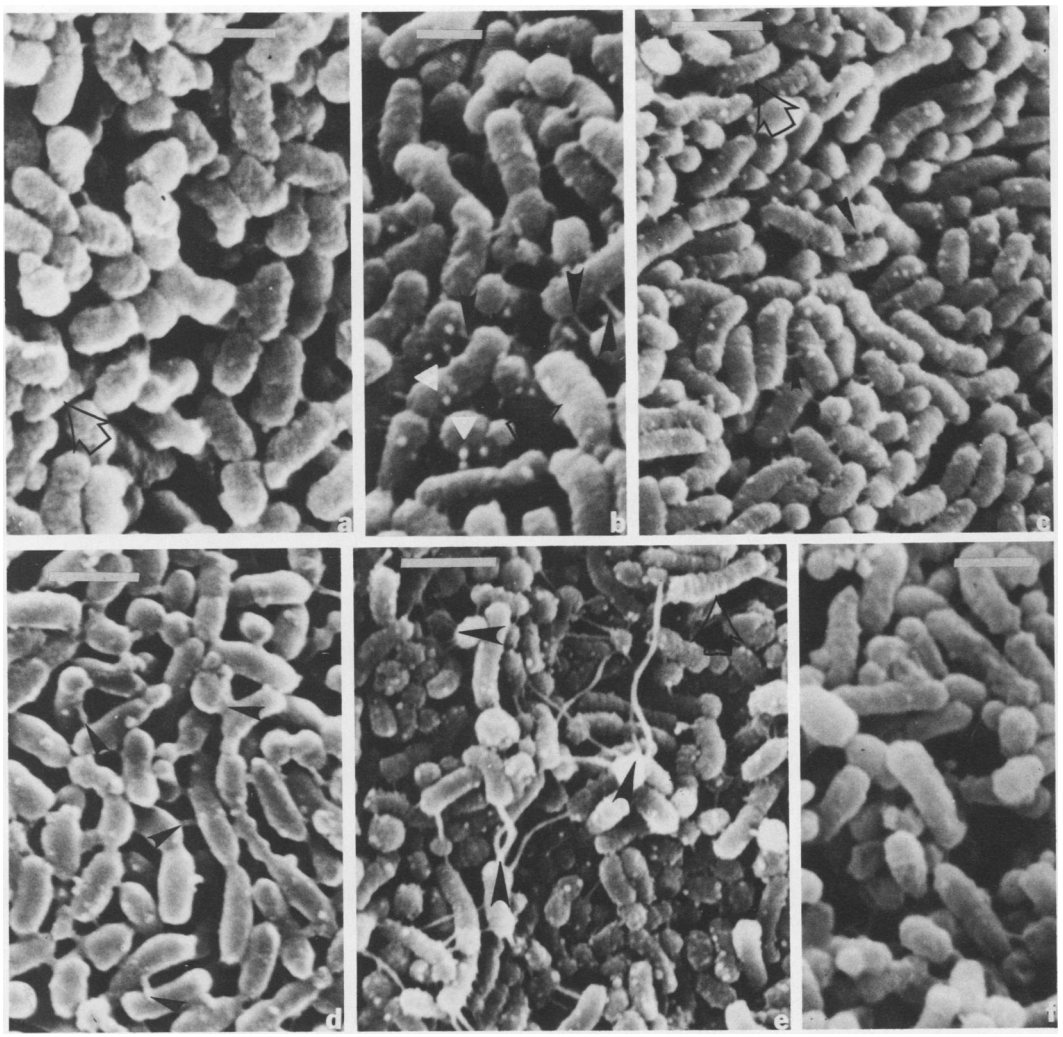

FIG. 5. *Scanning electron photomicrographs of A. actinomycetemcomitans strains Y4 (a and b), N27 (c), 2097 (d), 511 (e), and ATCC 29524 (f). All strains were covered with a thick amorphous material which connects adjacent cells by protruding fibrils (arrowheads). (e) Surface fibrils traverse long distances; however, they still are cell-cell associated. In some cells, multiple fibrils appear to be emerging from one pole of the cell (arrowheads). (b) Several protruding fibrils have on their most distal portion a large terminal knob (white arrowheads). The terminal knob is seen more clearly in Fig. 6c. Note the segmented nature of the cells (large open arrows). Bars = 0.5 μm (a and b) and 1 μm (c to f).*

tions, such as bacterial endocarditis (11), pharyngitis and pneumonia (17, 41), gastroenteritis (13, 29), and, of course, dental caries (8, 9). Other gram-negative bacteria of both numerical significance and disease-producing potential in periodontal lesions have also been observed to possess significant amounts of exopolymeric material morphologically similar to that observed in the studies presented here. *Capnocytophaga sputigena* (strain 4) and *Bacteroides asaccharolyticus* are covered by thick, ruthenium red-positive slime or capsule layers (14, 52). These acidic polyanionic layers (37) not only cover the entire cell surface but connect adjacent cells into a tightly fitting matrix. A similar amorphous surface layer has recently been observed in both oral and nonoral *E. corrodens* species (39). Onderdonk et al. (35) showed that it was the polyanionic polysaccharide polymer on the surface of *Bacteroides fragilis* which was responsible for abscess formation in the pelvic region of the rat. The abscess potentiating ability was related to the capsular polysaccharide, possibly functioning as a "virulence factor" (18). There is a growing body of evidence which implicates both bacterial surface glycoproteins and carbohy-

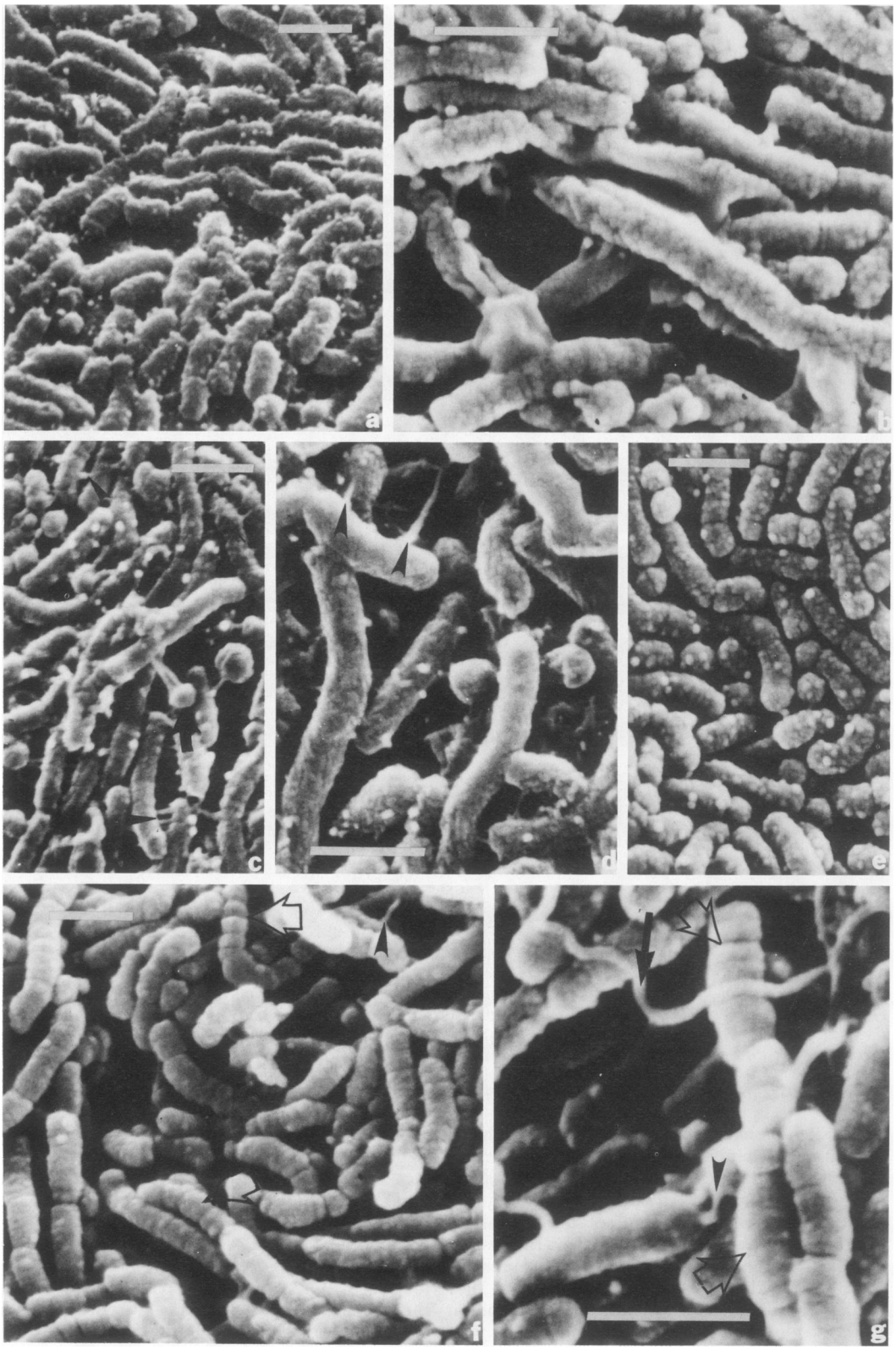

FIG. 6. *Scanning electron photomicrographs of H. aphrophilus strains 77, 78, and 80 (a and b; c and d; e to g; respectively). These strains displayed numerous cell-cell interconnections (arrowheads) as well as a thick, amorphous extracellular material (arrows). (b; e to g) Haemophili are clearly seen to be segmented (large open arrows), with the extracellular material following the individual cell segments. As in the Actinobacillus strains, the surface of the Haemophilus strains was covered with fibrils which emerged from the cell as stalks with a large, knoblike terminus. Bar = 1 μm.*

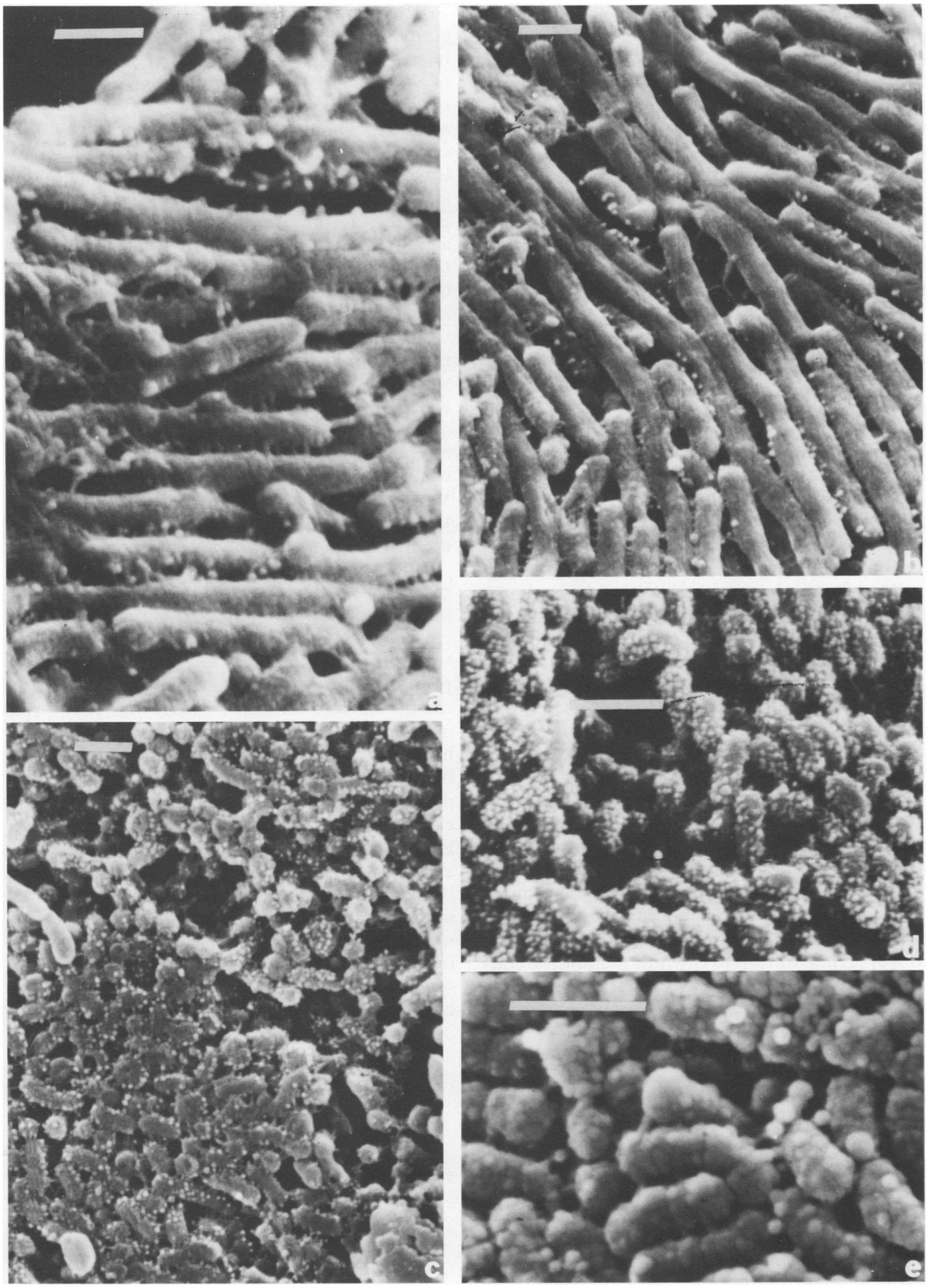

FIG. 7. *Scanning electron photomicrographs of H. aphrophilus strains 81, 654, 655, and 656 (Fig. 7a and b; c; d; e; respectively). In addition to the surface knobs, these strains were covered with thick amorphous material which takes on the appearance of a slime matrix. This matrix not only joined adjacent cells, but appeared to hold the cells together. Bar = 1 μm.*

drates in bacterial-mammalian cell recognition phenomena, especially in specific cell-cell adherence (34, 36).

Microorganisms morphologically similar to the actinobacilli and haemophili have been observed residing on the dorsal surface of the tongues of cattle (28). By transmission and scanning electron microscopy, these bacteria possessed large amounts of ruthenium red-positive exopolymer. Morphologically, it appeared that the polymer in these bacteria was either deposited or synthesized in an asymmetric fashion on the segmented surface.

In all of the studies concerned with cell-associated polysaccharides in bacterial-host tissue interaction, especially as this interaction is manifested in disease, the actual role of these compounds in the disease process is unclear; it is, however, intriguing to invoke their involvement in mixed bacterial infections, such as those described by Macdonald et al. (27), Socransky and Gibbons (45), and Ingham et al. (15). This involvement of surface exopolymers is especially attractive since essentially all of the microorganisms (both gram negative and gram positive) isolated from mixed infections have been shown to be enrobed or covered with extracellular material. *Actinobacillus* and *Haemophilus* appear to be no exception.

In addition to these exopolymers observed on the surface of *Actinobacillus* and *Haemophilus* cells, these bacteria also contained thin cell surface fibrils or strands. In the majority of gram-negative bacteria, these thin fibrils are referred to as pili; however, in both *A. actinomycetemcomitans* and *H. aphrophilus* we could not discern pili by negative staining or any other ultrastructural technique.

The morphology of the outer membrane of the actinobacilli and haemophili was similar to that found in other gram-negative bacteria. However, in addition to the unit membrane configuration, the outer surface of the membrane contained numerous associated blebs or vesicles, which have been identified as lipopolysaccharide. The vesicles in *A. actinomycetemcomitans* and *H. aphrophilus* were not only associated with the outer membrane, but were also released in large numbers into the external environment during growth. DeVoe and Gilchrist (6) have observed that the formation of lipopolysaccharide vesicles in *Neisseria meningitidis* was related to the stage of growth of the neisseria organisms. For example, the vesicles were not observed when *N. meningitidis* was grown to and examined in stationary growth phase. Andersen et al. (1), on the other hand, observed lipopolysaccharide-associated vesicles in stationary-phase *N. meningitidis*. The absence of "extra" or extruded lipopolysaccharide in the studies of DeVoe and Gilchrist (6) may have been associated with the growth conditions (type of N or C source) or the atmosphere under which the cells were cultivated. Andersen et al. (1), for example, showed that only two of the four strains of *N. meningitidis* they examined released any detectable amounts of free lipopolysaccharide and that an increase in cellular growth rate and culture aeration resulted in a significantly increased lipopolysaccharide content, as if the cells were compensating for increased growth by releasing "excess" membrane into the external environment.

Tubular vesicles similar to those observed in the actinobacilli and haemophili were also observed in group B meningococci (7). Not only were these tubular structures observed to be directly linked to or continuous with the outer membrane of *N. meningitidis*, they were also observed in the culture supernatant. Hill and Weiss (12) were able to isolate the tubular structures from cell envelope fractions and chemically identify them as lipopolysaccharide vesicles.

The mechanism of lipopolysaccharide release may be related to the amount synthesized in the outer membrane, which itself may be related to the chemical and physical environment in which the cells are found (e.g., see reference 7). Other properties such as the strength of the outer membrane and osmotic fragility may play a role in lipopolysaccharide liberation and, ultimately, bacterial virulence. Anaerobic gram-negative bacteria in deep periodontal pockets, for example, bathed by host secretions and fed by the carcasses of dead bacteria, may be in a position to elaborate significant amounts of lipopolysaccharide which not only would bathe the pocket, but also could cross epithelial barriers, penetrating host cells and tissues, with resultant disease manifestations. The ability of various strains found resident in a pocket to release lipopolysaccharide and ultimately to interact with the host to cause disease may be a reflection of the outer membrane and may be related to different clinical pictures observed in various periodontal disease and bacterial-host interactions.

## ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant DE 05123 and DE 03488 from the National Institute of Dental Research and National Science Foundation equipment grant PCM 78-05656.

We thank Erika Musante for her expert technical assistance.

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