Ultrastructures of *Bacteroides gracilis*, *Campylobacter concisus*, *Wolinella recta*, and *Eikenella corrodens*, All from Humans with Periodontal Disease

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Using transmission electron microscopy, we examined the ultrastructures of 13 isolates and 2 reference strains of *Eikenella corrodens*, 6 isolates of *Bacteroides gracilis*, 6 isolates of *Wolinella recta*, 5 isolates of *Campylobacter concisus*, 2 reference strains of *Bacteroides ureolyticus*, 2 reference strains of *Campylobacter fetus*, 2 reference strains of *Campylobacter sputorum*, and 1 reference strain (VPI 9584) that was originally identified as *Vibrio succinogenes*. All *E. corrodens* strains were nonflagellated rods that were ultrastructurally distinct from another group of nonflagellated rod-shaped bacteria (*B. gracilis* and *B. ureolyticus*). The cells of *W. recta* strains were short rods, each having a single polar flagellum and a characteristic outer membrane covered with a distinctive array of hexagonally packed macromolecular subunits. *C. concisus*, *C. fetus*, *C. sputorum*, and strain VPI 9584 formed a heterogeneous group of monoflagellated rod-shaped bacteria. All of the strains which we examined had cell wall structures compatible with the structures of gram-negative microorganisms. The morphological distinctions among species appeared to be helpful in the identification of similar isolates.

Recently, a number of gram-negative, asaccharolytic, rod-shaped bacteria were isolated from human periodontitis lesions. Typically, these organisms formed pitting or corroding colonies on agar, but they also produced colonies of different types. Under dark-field microscopy, these bacteria appeared as either straight or curved rods, some of which were actively motile. Because some of these isolates could not be identified readily, a comprehensive study was undertaken to determine which of the isolates could be fitted into presently known taxa and which required the creation of new taxa.

A number of fresh isolates, including strains of *Bacteroides*, *Bacteroides gracilis*, *Wolinella recta*, and *Campylobacter concisus*, were compared with reference strains of *Eikenella corrodens*, *Vibrio succinogenes*, *Bacteroides ureolyticus*, and several *Campylobacter* species.

In this paper we describe the ultrastructures of the new species and of some reference strains to which they were compared.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. corrodens* which we examined included Forsyth Dental Center strain 384 from a human with gingivitis, strains 373, 374, 470, and 1073 from humans with periodontitis, strains 1078, 1079, and 1080 from humans with periodontosis (juvenile periodontitis), and strains 1006, 1007, 1008, 1010, and 1012 from humans with alveolar

abscesses; we also studied *E. corrodens* reference strains VPI 597 and ATCC 23834. The *B. gracilis* strains which we used were Forsyth Dental Center strains 401, 402, 404, 406, 1083, and 1084 from humans with periodontitis. The strains of *W. recta* included Forsyth Dental Center strain 1086 from a human with gingivitis, strains 267, 285, 371, and 372 from humans with periodontitis, and strain 1219 from a human with periodontosis. The *C. concisus* strains studied were Forsyth Dental Center 483, 484, and 569 from humans with gingivitis and strains 288 and 522 from humans with periodontitis. The additional reference strains included in this study were *B. ureolyticus* strains VPI 7814 and VPI 7815, *Campylobacter sputorum* strains 616 and VPI S17, *Campylobacter fetus* strains VPI H641 and VPI 1176, and strain VPI 9584, which was originally identified as *V. succinogenes*.

Isolation and culture. The initial isolation and passage of the microorganisms studied are described in an accompanying paper (34). For ultrastructural studies the strains were obtained as either fresh or freeze-dried cultures. The strains were grown on commercially available Trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) in an anaerobic atmosphere generated by the GasPak system (BBL). Microbial cells for ultrastructure studies were obtained from 3-day-old cultures after the second or third transfer.

Electron microscopy. Colonies were scraped from an agar surface and immediately fixed in a mixture containing 5% glutaraldehyde and 4% paraformaldehyde buffered to pH 7.3 with sodium cacodylate (12). After postfixation in 2% s-collidine-buffered osmic acid, the cells were washed, dehydrated in graded

ethanol solutions, and embedded in Epon (16). Sections (0.1 μm) were cut, mounted on bare grids, stained with uranyl acetate and lead citrate (23), and examined and photomicrographed with a Philips EM-300 transmission electron microscope.

For selected strains, negatively stained preparations were obtained by applying 1 drop of cellular material suspended in 2% phosphotungstic acid to a carbon-reinforced, Formvar-coated grid. The excess liquid was removed by touching the edge of the grid to a piece of filter paper and allowing the preparation to air dry. Electron micrographs of bacteria were prepared at standard magnifications of $\times 32,800$ and $\times 68,000$. Measurements were made from cells sectioned perpendicular to their surfaces, as determined from the micrographic clarity of the peripheral layers. Average measurements were derived from 10 to 30 cells of each strain.

RESULTS

On the basis of their ultrastructural features, the new isolates could be divided into four morphological groups. The dimensions of the isolates are listed in Table 1.

***E. corrodens*.** The cells of 13 new isolates, the type strain, and a reference strain of *E. corrodens* were short rods with flattened ends but no flagella (Fig. 1a and b). Cellular cross-sections revealed an inner cytoplasmic membrane, an outer membrane, and a distinct intervening electron-dense layer, presumably the peptidoglycan layer (Fig. 1c and d). These features are typical of many gram-negative bacteria. The outer membrane was relatively smooth with flattened undulations. The intervening layer was relatively straight and was 4.5 to 7.5 nm from either membrane (Fig. 1d). The cytoplasm had a relatively electron-dense periphery and a fibrillar, less-electron-dense nucleoid area. Electron-dense inclusions were observed rarely.

***B. gracilis* and *B. ureolyticus*.** The cells of

six isolates of *B. gracilis* had ultrastructures similar to the ultrastructure of *B. ureolyticus* (Fig. 2a and b). However, *B. ureolyticus* cells tended to be larger than *B. gracilis* cells (Table 1). The cells of all of the strains were nonflagellated rods with round or flattened conical ends and had cell wall structures typical of the structure reported for the majority of gram-negative bacteria. The outer membrane tended to have an undulating profile in thin sections (Fig. 2e and f). However, the individual layers of the cell periphery could not be resolved clearly. Negatively stained preparations of *B. gracilis* cells revealed a more or less well-preserved cell wall periphery (Fig. 2c). In contrast, *B. ureolyticus* cells often had a disrupted cell periphery associated with extracellular membranous vesicular structures (Fig. 2d). In general, the middle layer was not detectable except in sections of partially lysed cells. In contrast to *E. corrodens* cells, we observed electron-dense cytoplasmic inclusions (some membrane bound) of relatively uniform size (mean diameter, approximately 40 nm) in all strains of this group.

***W. recta*.** The ultrastructures of the *W. recta* strains differed from the ultrastructures of all other species examined. The cells were short rods (Table 1) with rounded ends and single polar flagella (Fig. 3c and g).

The peripheral layers of each cell consisted of clearly delineated inner and outer membranes which were straight and parallel to one another; each membrane layer had a typical trilaminar structure. The membranes were separated from each other by approximately 20 nm. The intervening space was occupied by moderately electron-dense material but did not contain a distinct peptidoglycan layer (Fig. 3b, c, and f). The outer surface of the outer membrane was covered by an array of bullet-shaped macromolecular subunits with circular bases which were approximately 17 nm in diameter. These were packed in hexagonal arrays consisting of a central subunit surrounded by six subunits, with a center-to-center distance of about 20 nm (Fig. 3d and f). The packing arrangement was best visualized in negatively stained specimens (Fig. 3h). The subunits were attached to the outer surface of the outer membrane by their flat surfaces, with the convex portion facing outward. When cells were disrupted, the subunits could be detected on both sides of the outer membrane (Fig. 3d and e). We observed no subunits on either surface of the inner membrane. Electron-dense cytoplasmic inclusions ranging in diameter from 5 to 45 nm (mean diameter, 30 nm) occurred in all strains (Fig. 3a and b), but with varying frequencies.

TABLE 1. Cellular dimensions^a

Organism	Cell length (μm)		Cell diam (μm)	
	Range	Mean	Range	Mean
<i>E. corrodens</i> ^b	1.00–16.00	2.40	0.35–0.55	0.45
<i>B. gracilis</i> ^b	0.65–4.90	1.85	0.25–0.40	0.35
<i>B. ureolyticus</i> ^b	1.45–4.00	2.70	0.40–0.50	0.45
<i>W. recta</i> ^b	1.40–4.60	2.50	0.30–0.50	0.45
Strain VPI 9584	0.80–3.85	1.95	0.30–0.35	0.30
<i>C. concisus</i> ^b	0.80–5.25	1.80	0.30–0.40	0.35
<i>C. fetus</i> strain VPI 1176	1.10–5.60	2.65	0.30–0.50	0.40
<i>C. fetus</i> strain VPI H641	1.65–5.65	3.05	0.25–0.35	0.30
<i>C. sputorum</i> strain VPI S17	1.65–9.80	2.85	0.25–0.30	0.30
<i>C. sputorum</i> strain 616	1.30–10.35	2.45	0.35–0.40	0.35

^a All dimensions are rounded off to the nearest 0.05 μm .

^b Based on all strains examined in this study.

FIG. 1. *E. corrodens*. (a) General appearance in pure culture. The cells are short rods with square or flattened ends. $\times 20,400$. (b) Negatively stained preparations. Note the absence of flagella. $\times 32,800$. (c) Organism with cell wall typical of gram-negative bacteria. Note the relatively slight undulation in the cell wall contour. N, nucleoid area. $\times 68,000$. (d) Cell wall periphery consists of a plasma or cytoplasmic membrane (arrow 1), an electron-dense peptidoglycan layer (arrow 2), and an outer membrane (arrow 3). $\times 136,800$. (Unless otherwise indicated, the bars in all micrographs represent $0.5\ \mu\text{m}$.)

FIG. 2. *B. gracilis* and *B. ureolyticus*. (a) General appearance of *B. gracilis*. (b) General view of *B. ureolyticus*. Note the size difference between these two species. Some cells of both species have flattened, conical ends (arrows). $\times 24,600$. (c) Negatively stained preparation of *B. gracilis*. (d) Negatively stained preparation of *B. ureolyticus*. Note the relatively disrupted outer membrane of *B. ureolyticus*. $\times 24,600$. (e) *B. gracilis*. (f) *B. ureolyticus*. Note the poorly defined layers of the cell periphery. Also note the cytoplasmic inclusions (arrows 1 and 2), one of which is membrane bound (arrow 2). $\times 64,600$.

***C. concisus*, *C. fetus*, and strain VPI 9584.**

Five strains of *C. concisus*, two reference strains of *C. fetus*, and strain VPI 9584 were ultrastructurally similar. The cells of these strains were either curved short rods or long helical rods with rounded or tapered ends, and they bore single, polar flagella (Fig. 4a and c). However, the cells of both strains of *C. fetus* commonly had a single polar flagellum at both ends of each cell (Fig. 4d).

All of the strains in this group had a typical gram-negative cell wall structure, with the exception that generally a peptidoglycan layer was not detected. In most cells the individual layers of the cell periphery were not resolved clearly because of the irregular contour of the outer layers. In section, the outer membrane often appeared to follow a twisted course, with irregular undulations (Fig. 4e and f).

Several strains in this group also exhibited cytoplasmic condensations near the ends of some cells. These cytoplasmic condensations were in the form of a cap, the external surface of which was bordered by the internal surface of the cytoplasmic membrane. These terminal cytoplasmic condensations were particularly prominent in *C. concisus* strain 483 and *C. fetus* strain VPI 1176 (Fig. 4e and g). Membrane-bound, electron-dense cytoplasmic inclusions similar to those described above for *B. gracilis* and *B. ureolyticus* were also observed in *C. concisus*, *C. fetus*, and strain VPI 9584 (Fig. 4a and b).

***C. sputorum* reference strains.** The morphologies of the two strains of *C. sputorum* differed from the morphologies of the species described above and from each other. The cells of both of these strains were curved or helical rods with single, polar flagella (Fig. 5a through d) and similar dimensions (Table 1). The periphery of each cell exhibited distinct outer and inner membranes, but a peptidoglycan layer was not readily apparent (Fig. 5f). A moderately electron-dense extracellular layer that was approximately 7 to 12 nm thick was located close to the outer surface of the outer membrane (Fig. 5e through g). In *C. sputorum* strain VPI S17, some sections suggested that the extracellular layer consisted of regularly packed subunits (Fig. 5g). However, neither the shape of the subunits nor the pattern in which they were packed could be resolved clearly with the techniques which we used. Although cells of both strains contained electron-dense cytoplasmic inclusions, the strain VPI S17 inclusions were distinctly larger and were visible in negatively stained preparations (Fig. 5c), whereas the *C. sputorum* strain 616 inclusions could be detected only in ultrathin sections.

DISCUSSION

Although within the last 20 years numerous reports concerning ultrastructural variations of the cell periphery of gram-negative bacteria have been published, the peripheral layer generally consists of a trilaminar outer membrane, an inner (cytoplasmic) membrane, and an intervening electron-dense layer (4, 5, 9, 10). It has been shown that the outer membrane is the site of the lipopolysaccharide moiety of the cell wall (2, 3, 7, 18), and the intervening electron-dense layer is considered to represent the peptidoglycan or murein layer of gram-negative bacteria (10).

Among isolates from oral cavities, typical gram-negative cell walls with distinct peptidoglycan layers have been reported for *Veillonella* (2), *Leptotrichia buccalis* (11, 14), *Bacteroides oralis* (7), *Bacteroides fragilis* (36), *Fusobacterium nucleatum* (7), *Selenomonas sputigena* (13), *Bacteroides melaninogenicus*, and *Bacteroides asaccharolyticus* (15, 36). However, among the strains included in this study, only *E. corrodens* had a clearly detectable middle layer.

The method of fixing and staining bacterial specimens may affect the clarity of membranous structures. Sousa and Silva (33) tested a number of gram-negative microorganisms with typical cell walls and reported that uranyl acetate used as a postfixative, as in the Kellenberger technique, may decrease the contrast of the peptidoglycan layer. On the other hand, when uranyl acetate is used as a "stain" for ultrathin sections, it tends to enhance the contrast of the peptidoglycan layer. Since uranyl acetate was used only to stain sections, the frequent absence or lack of definition of the peptidoglycan layer cannot be attributed to the staining procedure, nor can its absence be attributed to a lack of calcium in the fixative, a condition which may also decrease the contrast of this layer (33).

Only *W. recta* cells had a distinctive outer membrane covering consisting of hexagonally packed subunits. Such hexagonally packed macromolecular arrays in bacterial cell walls have been reported in gram-positive cells as well as gram-negative cells. They have also been demonstrated after treatment with heat, detergent, or enzymes in several other microorganisms (8, 16, 17). For additional details on the substructure of bacterial cell walls, see the reviews by Thorne (35) and Glauert and Thornley (10).

Most of the studies dealing with the ultrastructures of bacterial cell walls have relied on shadowed preparations of surface replicas or freeze-etched materials or on negatively stained preparations. Only a few of these studies also dealt simultaneously with ultrathin sections. It

is evident that despite the great similarity in hexagonal packing observed when surfaces alone are studied, there are distinct differences in the cell wall ultrastructure when ultrathin sections are examined. For example, this can be determined by comparing *Lampropedia hyalina* (20, 21), *Ectothiorhodospira mobilis* (22), "*Sprillum serpens*" (19) (not on the Approved Lists of Bacterial Names [28]), and *Clostridium thermohydrosulfuricum* (29-31), which were studied both in section and by using negatively stained or shadowed preparations of whole cells or their replicas. As shown by electron micrographs of thin sections, each of these species has a cell wall ultrastructure which is distinctive and clearly distinguishable from the ultrastructures of the other species and from the ultrastructures of the strains of *W. recta* described above.

It has been shown that the outer membrane plays an important role in determining the packing arrangement of the subunits. Sleytr (29) was able to detach the surface subunits of *Clostridium thermosaccharolyticum*, which has tetragonally packed subunits, and *C. thermohydrosulfuricum*, which has hexagonally packed subunits. Cell wall fragments of each cell type were able to reaggregate subunits from either species to their surfaces in the original packing arrangement observed in the intact cells from which the wall fragments originated. This may explain the occurrence of hexagonally packed subunits on both sides of fragments of the outer membrane of *W. recta* cells (Fig. 3d and e). Presumably, subunits which became detached from the outer surface of the outer membrane were able to reaggregate on the inner surfaces of the outer membrane fragments. In intact cells, no subunits were detected on the inner surface of the outer membrane.

Polar cytoplasmic condensations were observed in close proximity to the inner cytoplasmic membrane in some strains of *C. concisus* and *C. fetus* and in strain VPI 9584. These structures were probably identical to the structures described by Ritchie et al. (25) as thickened inner cytoplasmic membranes in the polar regions of cells of *Campylobacter* species. Smi-

bert (32) also refers to these structures as multilaminar polar membranes and points out that they have been described in a number of other helical bacteria. It is our impression that they are not thickened portions of the inner membrane, since the inner membrane can be observed as a distinct structure that is in contact with the electron-dense zones. Furthermore, although some layering can be observed occasionally within these structures, the structures do not appear to be composed of multiple membranous layers. Therefore, these structures are better described as polar cytoplasmic condensations or polar caps (6).

Cells of *W. recta* and *C. concisus* strains had polar flagella. Rhodes has reviewed the value and reliability of flagella as a taxonomic criterion (24). With the increased resolution provided by electron microscopy, the difficulty and unreliability of flagellar stains for light microscopy are no longer problems. Of greater importance is the effect which altered environmental conditions, including genetic manipulations, may have on the presence or absence of flagella and their distribution at the cell surface. Rhodes (24) indicated that if the environmental conditions are standardized, little change occurs in flagellation patterns and that flagellar characteristics "may well be of great significance for both natural and artificial classification purposes, or for practical diagnosis." It should be noted in this connection that certain cultural conditions may favor or suppress flagellation, including factors such as the consistency of the medium, temperature, pH, degree of aeration, and time of incubation (24, 27).

The cultural conditions may also influence cell length measurements. For example, some of the *Campylobacter* reference strains and strain VPI 9584 tended to be longer in broth cultures (34) than when they were grown on solid media. The longer measurements reported by Tanner et al. (34) for the same microorganisms may have been due in part to cellular divisions being detected more readily by electron microscopy than by light microscopy.

Using a microagglutination technique, Badger

FIG. 3. *W. recta*. (a) General appearance. Note the insertion of the polar flagellum (arrow). $\times 32,800$. (b) Cell periphery consists of a well-defined inner membrane (arrow 1) and an outer membrane (arrow 2). Note the bullet-shaped subunits covering the outer surface of the outer membrane (short arrows) and the lack of a distinct peptidoglycan layer. $\times 68,000$. (c) Insertion of polar flagellum. Note the attachment disk (arrow). $\times 68,000$. (d) Tangential section through hexagonally packed subunits at the cell surface (arrow). Note the bullet-shaped subunits on both sides of a fragment of the outer membrane. $\times 68,000$. (e) Ruptured outer membrane is covered by bullet-shaped subunits on both inner and outer surfaces. $\times 68,000$. (f) Cell periphery. Note the absence of a peptidoglycan layer between the inner membrane (arrow 1) and the outer membrane (arrow 2) and the regularly arranged subunits at the cell surface (arrow 3). $\times 102,000$. (g) Negatively stained cell. Note the single polar flagellum. $\times 13,800$. (h) Negatively stained fragment of outer membrane. Note the size and packing of the surface subunits. $\times 68,000$.

FIG. 4. (a) *C. concisus*. $\times 32,800$. (b) Strain VPI 9584. $\times 32,800$. Note the curved or spiral contour of the cells. (c) *C. concisus* negatively stained cell with a single polar flagellum. $\times 16,400$. (d) *C. fetus* strain 1176 negatively stained cell with single polar flagella at both ends. $\times 16,400$. (e) *C. concisus* cell. Note the cap-shaped cytoplasmic condensation near the upper end of the cell, just beneath the inner cytoplasmic membrane (arrows). $\times 76,500$. (f) *C. concisus*. Note the highly convoluted cell periphery with indistinct peripheral layers. $\times 110,000$. (g) *C. fetus* strain 1176. Note the cap-shaped cytoplasmic condensation near the end of the cell (unmarked arrows) in contact with the inner surface of the inner membrane (arrow 1). Arrow 2, Outer membrane. $\times 102,000$.

FIG. 5. (a) *C. sputorum* strain Loesche 616 negatively stained preparation. Note the unusually long single polar flagellum in this cell. $\times 9,200$. (b) Same cell as in (a). Note the absence of detectable cytoplasmic inclusions. $\times 27,500$. (c) *C. sputorum* strain VPI S17 negatively stained preparation. Note the cytoplasmic inclusions (arrowheads) and the single polar flagellum at each end of the cell. $\times 27,500$. (d) *C. sputorum* strain VPI S17. Note the curved or spiral cells containing relatively large inclusions near the cell ends (arrows). $\times 32,800$. (e) Magnified cell from (d). Note the moderately electron-dense covering over the outer membrane (arrowheads). $\times 68,000$. (f) and (g) Surface layer appears to be composed of individual subunits that are closely packed together (arrows). F, flagellum. $\times 129,000$.

and Tanner (1) have demonstrated that *E. corrodens*, *B. gracilis*, and *W. recta* constitute serologically distinct groups. The results of these workers compare well with the morphological results reported here. Although *B. ureolyticus* reference strains were morphologically similar to the *B. gracilis* strains studied, the serological results and the guanine-plus-cytosine contents of the deoxyribonucleic acids clearly distinguished the two species. Morphologically, strains of *B. ureolyticus* and *B. gracilis* could be separated only on the basis of size (Table 1). Although minor differences were observed, ultrastructural criteria were not sufficiently distinctive to separate *C. concisus*, *Campylobacter fetus* strains VPI 1176, VPI H641, and VPI 9584. Serologically, however, *C. concisus* was identifiable as a distinct group (1).

The clear distinction between the cell wall ultrastructures of *W. recta* strains and strain VPI 9584 raises a question as to whether organisms with such morphologically distinct ultrastructures should be placed in the same genus. Some genera do contain organisms with distinct ultrastructural characteristics. We believe that the guanine-plus-cytosine contents of the deoxyribonucleic acids and the physiological characteristics of the strains of *Wolinella* are so similar that the strains should indeed be included in the same genus. However, we feel that the weighting of ultrastructural characteristics in the definition of species and genera should be considered carefully. The ultrastructural features of *W. recta* cell walls appear to be sufficiently distinct to permit separation of this species from all other isolates from dental plaque reported thus far.

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REPRINT REQUESTS

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LITERATURE CITED

1. Badger, S. J., and A. C. R. Tanner. 1981. Serological studies of *Bacteroides gracilis*, *Campylobacter concisus*, *Wolinella recta*, and *Eikenella corrodens*, all from humans with periodontal disease. *Int. J. Syst. Bacteriol.* **31**:446-451.
2. Bladen, H. A., and S. E. Mergenhagen. 1964. Ultrastructure of *Veillonella* and morphological correlation of an outer membrane with particles associated with endotoxic activity. *J. Bacteriol.* **88**:1482-1492.
3. Braun, V., and K. Hantke. 1974. Biochemistry of bacterial cell envelopes. *Annu. Rev. Biochem.* **43**:89-121.
4. Chapman, G. B. 1959. Electron microscopy of ultrathin sections of bacteria. III. Cell wall, cytoplasmic membrane, and nuclear material. *J. Bacteriol.* **78**:96-104.
5. Clarke, P. H., and M. D. Lilly. 1962. A general structure for cell walls of gram-negative bacteria. *Nature (London)* **195**:516-517.
6. Cohen-Bazire, G., and R. Kunisawa. 1963. The fine structure of *Rhodospirillum rubrum*. *J. Cell Biol.* **16**:401-419.
7. Dahlen, G., H. Nygren, and H.-A. Hansson. 1978. Immunoelectron microscopic localization of lipopolysaccharides in the cell walls of *Bacteroides oralis* and *Fusobacterium nucleatum*. *Infect. Immun.* **19**:265-271.
8. Fischman, D. A., and G. Weinbaum. 1967. Hexagonal pattern in cell walls of *Escherichia coli*. *B. Science* **155**:472-474.
9. Glauert, A. M. 1962. Fine structure of bacteria. *Br. Med. Bull.* **18**:245-250.
10. Glauert, A. M., and M. J. Thornley. 1969. The topography of the bacterial cell wall. *Annu. Rev. Microbiol.* **23**:159-198.
11. Hofstad, T., and K. A. Selvig. 1969. Ultrastructure of *Leptotrichia buccalis*. *J. Gen. Microbiol.* **56**:23-26.
12. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**:137A.
13. Kingsley, V. V., and J. F. M. Hoeniger. 1973. Growth, structure, and classification of *Selenomonas*. *Bacteriol. Rev.* **37**:490-521.
14. Listgarten, M. A., and C.-H. Lai. 1975. Unusual cell wall ultrastructure of *Leptotrichia buccalis*. *J. Bacteriol.* **123**:747-749.
15. Listgarten, M. A., and C.-H. Lai. 1979. Comparative ultrastructure of *Bacteroides melaninogenicus* subspecies. *J. Periodontal Res.* **14**:332-340.
16. Luft, J. H. 1966. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409-414.
17. McCoy, E. C., H. A. Wiltberger, and A. J. Winter. 1976. Major outer membrane protein of *Campylobacter fetus*: physical and immunological characterization. *Infect. Immun.* **13**:1258-1265.
18. Mergenhagen, S. E., H. A. Bladen, and K. C. Hsu. 1966. Electron microscopic localization of endotoxic lipopolysaccharide in gram-negative organisms. *Ann. N. Y. Acad. Sci.* **133**:279-291.
19. Murray, R. G. E. 1963. On the cell wall structure of *Spirillum serpens*. *Can. J. Microbiol.* **9**:381-392.
20. Murray, R. G. E. 1963. Role of superficial structures in the characteristic morphology of *Lampropedia hyalina*. *Can. J. Microbiol.* **9**:593-600.
21. Pangborn, J., and M. P. Starr. 1966. Ultrastructure of *Lampropedia hyalina*. *J. Bacteriol.* **91**:2025-2030.
22. Remsen, C. C., S. W. Watson, J. B. Waterbury, and H. G. Trüper. 1968. Fine structure of *Ectothiorhodospira mobilis* Pelsh. *J. Bacteriol.* **95**:2374-2392.
23. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208-212.
24. Rhodes, M. E. 1965. Flagellation as a criterion for the classification of bacteria. *Bacteriol. Rev.* **29**:442-465.
25. Ritchie, A. E., R. F. Keeler, and J. H. Bryner. 1966. Anatomical features of *Vibrio fetus*: electron microscopic survey. *J. Gen. Microbiol.* **43**:427-438.
26. Rosenbusch, J. P. 1974. Characterization of the major envelope protein from *Escherichia coli*. *J. Biol. Chem.* **249**:8019-8029.
27. Shinoda, S., and K. Okamoto. 1977. Formation and function of *Vibrio parahaemolyticus* lateral flagella. *J. Bacteriol.* **129**:1266-1271.
28. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* **30**:225-420.

29. Sleytr, U. B. 1975. Heterologous reattachment of regular arrays of glycoproteins on bacterial surfaces. *Nature* (London) **257**:400-402.
30. Sleytr, U. B., and A. M. Glauert. 1975. Analysis of regular arrays of subunits on bacterial surfaces; evidence for a dynamic process of assembly. *J. Ultrastruct. Res.* **50**:103-116.
31. Sleytr, U. B., and A. M. Glauert. 1976. Ultrastructure of the cell walls of two closely related clostridia that possess different regular arrays of surface subunits. *J. Bacteriol.* **126**:869-882.
32. Smibert, R. M. 1978. The genus *Campylobacter*. *Annu. Rev. Microbiol.* **32**:673-709.
33. Sousa, J. C. F., and M. T. Silva. 1973. Ultrastructure of the cell wall and cytoplasmic membrane of gram-negative bacteria with different fixation techniques. *J. Bacteriol.* **113**:953-962.
34. Tanner, A. C. R., S. Badger, C.-H. Lai, M. A. Listgarten, R. A. Visconti, and S. S. Socransky. 1981. *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., and description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. *Int. J. Syst. Bacteriol.* **31**:432-445.
35. Thorne, K. J. I. 1977. Regularly arranged protein on the surfaces of gram-negative bacteria. *Biol. Rev.* **52**:219-234.
36. Woo, D. D. L., S. C. Holt, and E. R. Leadbetter. 1979. Ultrastructure of *Bacteroides* species: *Bacteroides asaccharolyticus*, *Bacteroides fragilis*, *Bacteroides melaninogenicus* subspecies *melaninogenicus* and *B. melaninogenicus* subspecies *intermedius*. *J. Infect. Dis.* **139**:534-546.