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Gliding Motility of *Cytophaga* sp. Strain U67

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Video techniques were used to analyze the motion of the gliding bacterium *Cytophaga* sp. strain U67. Cells moved singly on glass along the long axis at a speed of about 2 μm/s, advancing, retreating, stopping, pivoting about a pole, or flipping over. They did not flex or roll. Cells of different lengths moved at about the same speed. Cells sometimes spun continuously about a pole at a frequency of about 2 Hz, the body moving in a plane parallel to that of the glass or on the surface of a cone having either a large or a small solid angle. Polystyrene latex spheres moved to and fro on the surfaces of cells, also at a speed of about 2 μm/s. They moved in the same fashion whether a cell was in suspension, gliding, or at rest on the glass. Two spheres on the same cell often moved in opposite directions, passing by one another in close proximity. Small and large spheres and aggregates of spheres all moved at about the same speed. An aggregate moved down the side of a cell with a fixed orientation, even when only one sphere was in contact with the cell. Spheres occasionally left one cell and were picked up by another. Cells pretreated with small spheres did not adhere to glass. When the cells were deprived of oxygen, they stopped gliding, and the spheres stopped moving on their surfaces. The spheres became completely immobilized; they no longer moved from cell to cell or exhibited Brownian movement. *Cytophaga* spp. are known to have a typical gram-negative cell envelope: an inner (cytoplasmic) membrane, a thin peptidoglycan layer, and an outer (lipopolysaccharide) membrane. Our data are consistent with a model for gliding in which sites to which glass and polystyrene strongly adsorb move within the fluid outer membrane along tracks fixed to the rigid peptidoglycan framework.

Most motile bacteria swim or glide. Mechanisms for swimming are known, but gliding remains "curious, strange, and at present still unexplained" (54). Common gram-positive and gram-negative organisms swim by rotating filaments that project from the surface of the cell into the surrounding medium (2, 4, 38, 41, 52, 53). Spirochetes, another group of gram-negative organisms, swim by rotating filaments that run between the protoplasmic cylinder and the outer membrane (3, 5, 12). Gliding bacteria, a third major group of gram-negative organisms, have no flagella and do not swim but move in a nonrandom manner when in contact with a solid surface. They creep steadily back and forth along their long axes and sometimes roll, bend, or lash about a fixed pole; no locomotor organelles associated with this motion have been identified (9, 11, 18, 19, 28, 31, 46, 55, 62). This group of organisms (48) includes the phototrophic cyanobacteria (57) and some of the phototrophic green bacteria (45) as well as the chemoheterotrophic filamentous gliding bacteria,

fruiting myxobacteria (40), and cytophagae (13).

We chose to study gliding of a *Cytophaga* sp. because cytophagae are vigorous gliders but are not very complex morphologically. We used video techniques to record the motion of individual cells on glass in the presence of polystyrene latex spheres (42). The evidence strongly supports a model in which adsorption sites within the outer membrane are driven along tracks that run the length of the cell. The data are not consistent with most other models for gliding motility. Rotary movements similar to those exhibited by other bacteria tethered to glass by flagellar filaments (52), polyhooks (52), or hooks (6) inspired a search for incomplete flagellar structures (60, 61), but no such structures were found.

MATERIALS AND METHODS

Strains and culture conditions. *Cytophaga* sp. strain U67 was the gift of J. Henrichsen (31). This strain was isolated by H. Lautrop in 1959 from an ulcer on the jaw of a frog, but it was not the causative agent. It is an oxidase-positive, yellow-pigmented, saccharolytic, gram-negative rod that glides but fails to form microcysts or fruiting bodies (J. Henrichsen, personal communication). *Escherichia coli* strain MS912, an amber

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hag derivative of MS1350, was the gift of M. Simon (51).

Strain U67 was grown overnight at 22°C in Henrichsen medium 62 (0.05% tryptone [Difco Laboratories]–0.05% yeast extract [Difco] adjusted to a final pH of 7.0) and harvested in midexponential phase. The cells were derivatives of a single-colony isolate obtained by streaking on 1% agar (Difco) in medium 62, picking from the edge of the spreading zone after 2 days at 22°C, and restreaking on 1% agar in medium 70 (as medium 62 but with a tryptone concentration of 0.5%), a medium on which spreading is much reduced (31).

Reagents and particles. Unless otherwise noted, water was glass distilled and chemicals were reagent grade. Lysozyme (egg white, salt-free, 2× crystallized) was from Worthington Diagnostics. DNase (bovine pancreas, purified precrystalline), Brij 58, and Triton X-100 were from Sigma Chemical Co. Nonidet P-40 (Shell) was from Particle Data Laboratories.

Polystyrene latex spheres 0.13, 0.26, 0.36, 0.56, 0.80, and 1.30 μm in diameter were from Dow Chemical Co. (10% [vol/vol] suspensions). Carboxylate, hydroxylate, and primary amino derivatives 0.49, 0.52, and 0.52 μm in diameter, respectively, were from Polysciences, Inc. (2.5% [vol/vol] suspensions). Silica spheres 0.4 μm in diameter were made by hydrolyzing 4 ml of tetra-*n*-pentoxy silicate in 50 ml of *n*-propanol-methanol (3:1) and 4 ml of ammonium hydroxide (reagent ACS) as described by Stöber et al. (58). The spheres were washed with water before use or mixed with ca. 100 volumes of medium 62. India ink was taken directly from the bottle.

Light microscopy. Inverse phase-contrast video recordings were made with a Nikon Optiphot microscope equipped with a phase-contrast turret condenser, a CF Plan 40BM objective, a Zeiss Optovar (magnification ×2.0), a CF Photo 10× eyepiece, and a Panasonic WV-1300A vidicon camera. Recordings were made on a Sony VO-2800 video cassette recorder on KCA60 cassettes. The recordings were monitored and played back on a 9-inch (23-cm) Hitachi VM-910U video monitor. Photographs of this monitor were taken with an oscilloscope camera (Tektronix C-30A). Tracings were made from this monitor or from a 19- or 23-inch (48- or 58-cm) Sony monitor. The magnification of the system was calibrated by recording the image of an objective micrometer.

Dark-field video recordings were made with the same microscope with a different objective (CF Plan 40), eyepiece (CFW 15×), and camera (RCA TC-1030H silicon intensifier target). Unless otherwise noted, however, the data were collected with the standard vidicon.

Dark-field photographs were taken with the same microscope with the CF Plan 40 objective, a CF PL 5× eyepiece, and a PFX Microflex with an M-100 Polaroid back. Exposures of 30 s were made on Polaroid type 667 film (3000 ASA) with the microscope lamp (50-W tungsten-halogen) set at maximum intensity.

Samples were taken directly from the cultures, or the cells were pelleted by centrifugation and suspended in fresh growth medium, used as such or mixed with a small sample of spheres or India ink, placed within a ring of grease (Apiezon L) on a glass slide, and covered with a cover slip. Care was taken to include one or more bubbles of air so that cells could be observed at different levels of oxygenation. The slides and cover slips were used out of the box or cleaned in fuming nitric acid and rinsed with water. In one experiment, acid-cleaned glass was coated with Siliclad. All observations were made at room temperature (23 ± 1°C).

The displacement of cells and particles could be measured accurately from single-frame tracings of video images, but their sizes could not, owing to spreading of bright highlights into adjacent areas of the televised scene (blooming). This problem was particularly serious for the largest polystyrene latex spheres and for large aggregates of spheres, which were much brighter than the cells. The spatial resolution was higher when the recordings were viewed in real time, because each frame consisted of only half of the scan lines (2:1 interlace); therefore, the tracings were verified by repeated playback. The temporal resolution was high (recording rate, 60 frames per s), but the single-frame advance on the recorder skipped over every sixth or seventh frame, moving the tape ahead 116 ± 1 frames per 100 actuations. We corrected for this by multiplying the number of actuations by 1.16. The system is now equipped with a digital clock TV display, based on an MM58106 integrated circuit (National Semiconductor). Although many tracings were made of images of cells moving on the undersurface of the cover slip, rotations were scored as clockwise (CW) or counterclockwise (CCW), according to the direction that would be seen if the cell were gliding on the top of the slide, i.e., in the frame of reference of an observer looking down on the cell from within the aqueous medium.

Saturation of sites with spheres. A drop of an exponential-phase culture of *Cytophaga* sp. strain U67 was mixed with a drop of a suspension of 0.13-μm-diameter polystyrene latex spheres (diluted 1:100 in medium 62), and the mixture was allowed to stand at room temperature for 20 min. In a parallel experiment, a drop of the culture was placed within a ring of grease on a slide and allowed to stand for 20 min, and then a drop of the suspension of spheres was added. A slide was prepared of the first mixture, a cover slip was added to the second, and the preparations were compared by phase-contrast microscopy.

Search for flagellar structures. We followed the procedures of Suzuki et al. (60) involving spheroplast formation in lysozyme-EDTA, a low-speed spin (5,000 × *g*, 10 min) to pellet spheroplasts, spheroplast lysis in Brij-58, DNA hydrolysis in DNase-MgCl$_2$, one or two moderate-speed spins (27,000 × *g*, 15 min) to wash and pellet membrane fragments, membrane lysis in Triton X-100–EDTA, a moderate-speed spin to remove any remaining membrane fragments, and a high-speed spin (340,000 × *g*, 30 min) to pellet flagellar structures. The pellet was dispersed and examined in the electron microscope, treated further with Nonidet P-40 (to give fraction BMII), or fractionated on sucrose gradients containing Triton X-100 (by the methods of Suzuki et al. [60] or Bryant et al. [8]). Preparations also were made by the methods of Bryant et al. (8) to purify cyanobacterial phycobilisomes, involving the passage of cells through a French press into a buffer containing Triton X-100, a moderate-speed spin to remove any remaining membrane-wall fragments, and fractionation on a sucrose gradient. All procedures carried out on *Cytophaga* sp. strain U67 were carried out in parallel on *E. coli* strain MS912, a strain with hook-

basal-body complexes (see Fig. 3 of reference 60 or Fig. 3a of reference 61). Samples were negatively stained with 2% uranyl acetate on glow-discharged, carbon-stabilized, collodion-coated grids (8).

RESULTS

Cells of strain U67 adhered strongly to a variety of solids. Cells of strain U67 glided on glass, acid-cleaned glass, and even Siliclad-coated glass, but they did not glide at an air interface, e.g., on bubbles of air or at the bottom of a hanging drop. The only motion evident when cells were in suspension was Brownian movement. The cells readily adsorbed and propelled along their surfaces spheres of silica, polystyrene, and derivatized polystyrene (carboxylate, hydroxylate, and primary amino). The spheres were adsorbed and propelled as effectively by cells in suspension as they were by cells on a solid surface. Adherence to glass was so strong that gliding could be followed for hours either on the undersurface of the cover slip or on the top of the slide. Cells were not easily displaced by flow of the intervening medium, so it was possible to change the medium, as is done with tethered bacteria (7), without resorting to dialysis chambers (20, 21). Most of the experiments described here were done with glass out of the box and with polystyrene latex spheres.

Cells did not produce large amounts of slime. Cultures of strain U67 appeared to be no more viscous than those of *E. coli* of a similar density. Slime trails were not detectable by either phase-contrast or dark-field microscopy. Particles of India ink moved freely in close proximity to the cell surface. Sometimes such a particle stuck to the surface and moved along it, but this was rare, even when the particle density was so high that the preparations were nearly opaque. There was no indication in the motion of these particles of currents in the vicinity of a gliding cell. There was some evidence suggesting the presence of slime that involved the interaction of polystyrene latex spheres with glass after contact with the cell surface. When a large number of 0.13-μm-diameter spheres were added to cells, the cells became completely covered by them and soon stopped gliding. Before they did so, however, they shed some of these spheres, which left trails on the glass extending for a few micrometers behind each cell. Isolated spheres also stuck to the glass, but the polystyrene appeared to be more adherent after having been in contact with cells.

Cells moved singly. Cells of strain U67 glided as soon as they came into contact with the glass, but the motion often was more vigorous after a few minutes. The cells moved independently of one another. If a cell happened to collide with another cell, it might stop or change its direc-

tion, but it would move in front, behind, or alongside of another cell without perturbation. The cells did not follow preestablished paths.

Cells executed a sequence of glides and pivots. Cells moved backward or forward along their long axes in slightly irregular, gently curved paths (glided), then abruptly spun clockwise (CW) or counterclockwise (CCW) about either pole, rotating with the cell body nearly parallel to the surface of the glass through an angle of less than 360 degrees (pivoted). They glided at speeds on the order of 2 μm/s and pivoted at rates of about 0.5 Hz. Instead of pivoting, cells sometimes lifted one end off the glass and set it down somewhere else (flipped), changing their orientation by a few degrees to as many as 180 degrees. The cell body might move on the surface of a cone during this maneuver, but this was difficult to quantify because the events were brief and the cell moved out of focus. A cell might stop or pivot more than once about either pole in the same or in the opposite direction before continuing to glide. Figure 1 shows behavior of this kind for a cell moving on glass for a period of 0.7 min. Figure 2 is a schematic record of the behavior of the same cell for a period of 8.3 min. A summary of these data is given in Table 1.

Gliding cells need not flex or roll. Cells of strain U67 usually glided without changing shape. No undulations, contractions, or oscillations were visible. Occasionally, a long cell might bend, but the bending was clearly passive; for example, the bent trailing end of a long cell might stick to the glass, straighten to some extent, and then snap back into its original shape. A cell with a planar bend could glide lying flat on the glass in either of two possible orientations; it might glide for a minute or more in one orientation and then suddenly roll about its long axis from one orientation to the other. Similar behavior was observed with cells carrying one or more stationary polystyrene latex spheres. Such a cell might glide for several seconds with the particles on its port side and then abruptly roll about its long axis so that the particles appeared to starboard. Later, the cell might roll another 180 degrees in the same or in the opposite direction so that the particles resumed their original positions.

Cells of different lengths glided at about the same speed. Figure 3 shows the speed and length of 24 cells picked from the same preparation. There was a large variation in the sample of both speed and length but no obvious cross-correlation. Since the sample was small and was selected on the basis of the ability of the cells to glide steadily in one direction, it is not possible to conclude from this data that cells of any particular length glide most rapidly.
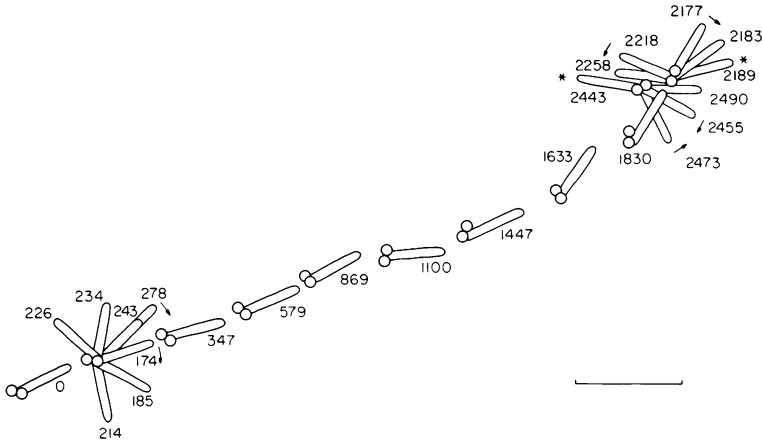
FIG. 1. *Cytophaga* sp. strain U67 gliding on the undersurface of a glass cover slip, drawn as viewed from below. Selected frames of a frame-by-frame analysis spanning 41.5 s (2,490 frames) are shown. The frame number is shown next to each image. Arrows indicate the beginning of pivots; asterisks indicate the beginning of flips. The cell carried on its trailing end two 0.56-μm-diameter polystyrene latex spheres which remained nearly stationary relative to the cell during the period shown. The cell appeared at the edge of the screen at frame 0; glided 8 μm between frames 0 and 174 (2.9 s, 2.8 μm/s); pivoted 334 degrees CW between frames 174 and 243 (1.1 s, 0.8 Hz); glided about 2 μm between frames 243 and 278 (0.6 s, 3.4 μm/s); pivoted 27 degrees CW between frames 278 and 301 (the latter frame not shown; 0.4 s, 0.2 Hz); continued its glide along a path of length about 60 μm between frames 301 and 2177 (31.3 s, 1.9 μ/s); pivoted 45 degrees CW between frames 2177 and 2189 (0.2 s, 0.6 Hz); lifted one end off of the glass and flipped over between frames 2189 and 2218 (0.5 s), changing its orientation by 144 degrees; pivoted 15 degrees CCW between frames 2218 and 2258 (0.7 s, 0.1 Hz); glided about 3.5 μm between frames 2258 and 2443 (3.1 s, 1.1 μm/s); flipped over again between frames 2443 and 2455 (0.2 s), changing its orientation by 162 degrees; pivoted CW 36 degrees between frames 2455 and 2473 (0.3 s, 0.3 Hz); and finally pivoted CCW 64 degrees between frames 2473 and 2490 (0.3 s, 0.6 Hz), at which time the recording ended. These data were collected during the last 0.7 min of the record shown in Fig. 2. Bar, 10 μm.
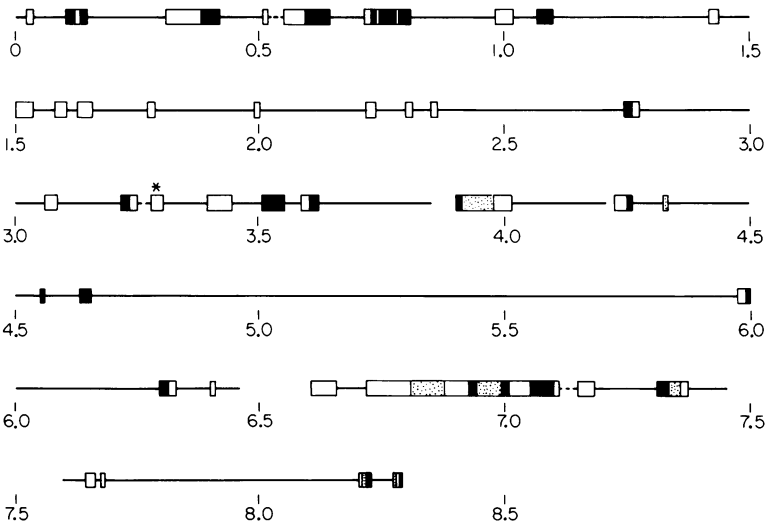

FIG. 2. A schematic record of the behavior of the cell shown in Fig. 1 over a period of 8.3 min. The numbers denote the elapsed time in minutes. The behavior included forward glides (——), backward glides (·····), CW pivots (□), CCW pivots (■), and flips (▣). All pivots and flips but one (*, ending at 3.3 min) were made about the trailing end of the cell. The preparation was moved from time to time so that the cell remained in view; these intervals are shown blank. A statistical summary of the behavior of this cell is given in Table 1.

TABLE 1. Statistics for the data shown in Fig. 2

Event	No. (% total time)	Mean ± SD[a]				
		Interval (s)	Path (μm)	Speed (μm/s)	Displacement (degrees)[b]	Rate (Hz)
Forward glide	38 (79)	10.1 ± 13.9	15.8 ± 18.7	1.7 ± 0.6		
Backward glide	3 (1)	1.7 ± 0.3	3.8 ± 0.9	2.2 ± 0.2		
CW pivot[c]	40 (12)	1.4 ± 1.3			152 ± 124	0.6 ± 0.7
CCW pivot[c]	24 (5)	1.0 ± 0.9			124 ± 110	0.5 ± 0.4
Flip	7 (3)	2.1 ± 1.7			102 ± 59	0.5 ± 0.8

[a] Each individual event was weighted equally, including events bordering gaps in the record.

[b] Net angular displacement (≤180 degrees in the plane of the glass).

[c] CW and CCW in the frame of reference of an observer looking down on the cell from within the aqueous medium.

Cells could glide or pivot on particles fixed to glass or to other cells. Cells glided on fixed polystyrene latex spheres at about the same speed that they glided on glass. The particles could be fixed to glass or other cells, even cells in suspension. Pivots also could occur on such particles. Sometimes a cell would glide on a fixed sphere in a cyclic fashion (Fig. 4). When the cell had gone as far as it could in one direction, it swung through an arc of 180 degrees and moved in the same direction as before, back-end first. In the frame of reference of the cell, the sphere moved down the length of the cell, around the pole, back up the length of the cell, around the other pole, and down the length of the cell once again.

Cells sometimes spun continuously. On rare occasions, cells of strain U67 pivoted or spun continuously in the fashion of tethered *E. coli* (1, 52). The first cell we saw doing this completed 90 CW revolutions around one pole in 39 s (mean rotation rate, 2.3 Hz) and then continued to glide, moving both backward and forward and executing both CW and CCW pivots. Figure 5 shows a more recent example. This cell was bent. The concave side of the bend always led, the convex side always lagged; the cell was rotating, not gyrating (bending in a rotary fashion, as one might move one's arm). Cells spun at a reasonably uniform rate. This is documented for a third cell in Fig. 6, which shows the distribution of 88 successive rotation periods. One cell was observed to complete more than 300 CW revolutions without stopping or changing direction.

When most cells spun, the cell body remained nearly parallel to the surface of the glass, as in a pivot, but with some cells, the cell body lifted off of the glass and moved on the surface of a cone having either a large or a small solid angle. The axis of the cone usually was not perpendicular to

FIG. 4. A cell 4.6 μm long gliding on a polystyrene latex sphere 0.56 μm in diameter fixed to the slide. The figure shows selected frames of a frame-by-frame analysis spanning 4.6 s. The cross is fixed in the frame of reference of the microscope. The numbers are elapsed time in seconds. The cell moved lengthwise at a speed of about 1.5 μm/s until it reached a pole, swung 180 degrees CW in about 0.5 s, and then moved along the sphere again in the original direction. This cycle was repeated more than 75 times. The mean period of the cycle was 4.5 s. Bar, 10 μm.
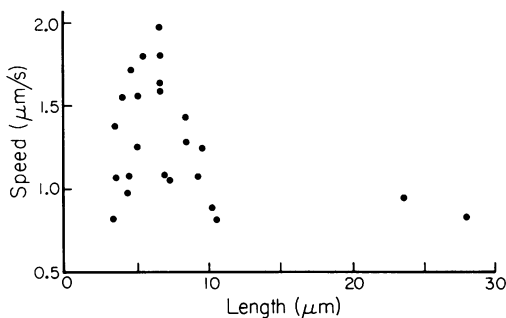

FIG. 3. Gliding speed versus cell length for 24 cells from the same preparation. Cells of a variety of lengths that moved steadily in one direction for at least 10 s were selected for analysis. The sample was not a random sample of the population as a whole.
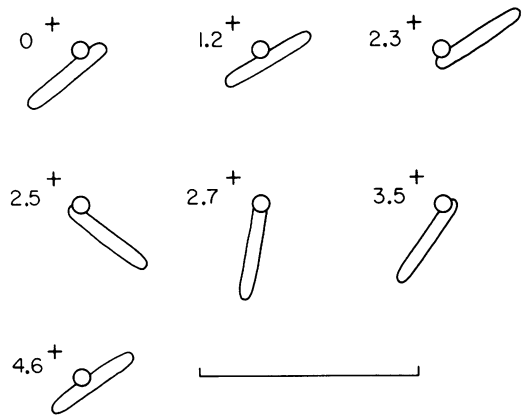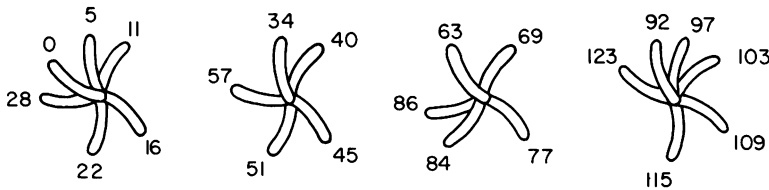
FIG. 5. A bent cell spinning on the top of a slide. Selected frames of a frame-by-frame analysis spanning 2.0 s (123 frames) are shown. The frame number is shown next to each image. Four revolutions are shown, each traced separately. The cell completed 15 CW revolutions in 6.6 s (the last 11 revolutions are not shown; mean rotation rate, 2.3 Hz) and was 6.7 μm long.

the surface of the glass. This situation was difficult to analyze because the cell moved in and out of focus, but in cases involving bent cells that we were able to study closely, the cell appeared to rotate, not gyrate; the concave side always led or lagged, never both.

Spheres remained at rest or moved on the surface of a cell in either direction. A polystyrene latex sphere adsorbed to the surface of a cell could remain stationary or move at a fairly constant speed from one end of the cell to the other. This was true for cells in suspension, cells stuck to glass, or cells gliding on glass. Often, a sphere would move the entire length of a cell, loop around the pole, and then move back again at roughly the same speed, but it might stop or change direction before reaching the end of the cell. Sometimes it crossed over from one side of the cell to the other. If there were several spheres on the same cell, one could be stationary, another could be moving along the cell in

one direction, and a third could be moving along the cell in the same or in the opposite direction. A sphere could move on the surface of the cell in the direction of the glide or in a direction opposite to the glide. Spheres on the port and starboard sides of the same cell sometimes moved in the same direction and sometimes in opposite directions (Fig. 7).

The behavior of 0.13-μm-diameter polystyrene latex spheres adsorbed to the surface of a cell was remarkable. The cell could be covered with spheres, many moving at the same time. A sphere might complete several transits down the length of the cell and around the pole and back again while the cell continued to glide in one direction. The sphere often remained on one side of the cell, e.g., the dorsal side; it did not need to move around the cell's circumference. Indeed, two spheres on the same side of the cell could pass close by one another going in opposite directions, or two spheres moving side by side a short distance apart could meet a third sphere going in the opposite direction, and the third sphere could pass in between!

Spheres moved on the surface of a cell at about the same speed that the cell glided. Spheres tended to remain at rest on the surface of a cell or to move backward or forward at about the speed that the cell glided, approximately 2 μm/s. The adsorption of spheres did not appear to affect the motion of the cell. A sphere that remained stationary relative to the cell moved across the field of view at the same velocity as the cell, a sphere that moved forward relative to the cell tended to move across the field of view at twice this velocity, and a sphere that moved backward relative to the cell tended to remain stationary in the field of view. This observation is documented in Fig. 8, which shows a comparison of the velocities of two polystyrene latex spheres moving on the surface of the same cell over a period of 23 s.

This motion bore little resemblance to Brownian movement; it was not random. A sphere 1.3 μm in diameter could move the length of a cell 10 μm long in about 5 s. The time required for such a sphere to diffuse in one dimension
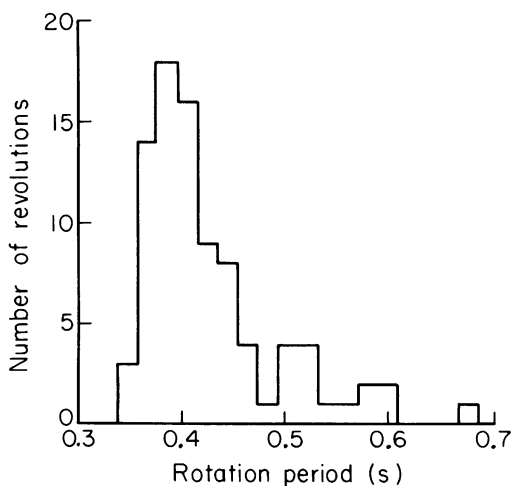

FIG. 6. Distribution of rotation periods for a spinning cell. The cell completed 88 CW revolutions in 37.5 s, with pauses of less than 2 s each after the 22nd and 38th revolutions. The mean period ± standard deviation was 0.43 ± 0.07 s, corresponding to a rotation rate of 2.3 Hz.
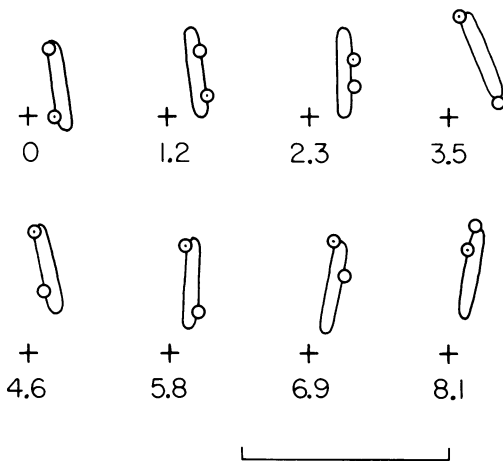
FIG. 7. Two 0.26-μm-diameter polystyrene latex spheres moving on the surface of a cell, 4.4 μm long, gliding on the top of a slide. Selected frames of a frame-by-frame analysis spanning 8.1 s are shown. The cross is fixed in the frame of reference of the microscope. The numbers are the elapsed time in seconds. The first sphere (marked with a dot) was moving downward at time 0. It looped around the lower pole, traveled up the right side of the cell, crossed upward from right to left over the dorsal surface of the cell, and stopped (at 3.5 s); later, it started moving again (at 7.9 s) and moved down the left side of the cell. The second sphere also was moving downward at time 0. It crossed downward from left to right over the dorsal surface of the cell, moved down the right side, passed the first sphere (at 1.9 s), looped around the lower pole, traveled up the left side of the cell, backed up (at 4.6 s), looped around the lower pole once again, traveled up the right side of the cell, and finally looped around the upper pole. In subsequent frames (not shown), both spheres moved down the left side of the cell. Both spheres moved at a speed of about 1.6 μm/s. The cell glided upward between 0 and 1.2 s, stopped between 1.2 and 2.3 s, continued to glide upward between 2.3 and 5.2 s, glided downward between 5.2 and 6.9 s, and finally glided upward again between 6.9 and 8.1 s. In subsequent frames (not shown), it continued to glide upward. The speed of the glide varied from about 1 to 2 μm/s. Note that the times at which the spheres and the cell stopped or changed direction were not the same. This recording was made in dark field with a silicon intensifier vidicon. Bar, 10 μm.

through water a root-mean-square distance of 10 μm is about 150 s, or 30 times as long.

Small and large spheres or aggregates of spheres moved on the surface of a cell at about the same speed. The speed at which a sphere moved over the surface of a cell was independent of the size of the sphere; it was about the same whether the sphere was 0.13, 0.56, or even 1.3 μm in diameter. This was easily seen when spheres of different sizes were adsorbed to the same cell or

when monomers and large aggregates of spheres of the same size were adsorbed to the same cell; small particles and large particles moving in the same direction maintained a fixed separation for considerable periods of time.

Linkages between spheres and a cell tended to be rigid. When an aggregate of two or more spheres moved on the surface of a cell, the angle between the major axis of the aggregate and a line perpendicular to the surface of the cell at the point of contact tended to remain constant. Thus, an aggregate of particles in the shape of a pear with its stem in contact with the surface of a cell could move down one side of the cell, swing around the pole, and then move back again without bending the stem (Fig. 9). This aggregate completed an entire circuit around the cell and returned to its initial position without significantly changing its orientation relative to the surface of the cell (Fig. 10). We never saw aggregates tumble end-over-end or rotate in any systematic way as they moved along the surface of a cell. However, an aggregate might remain at a fixed point on the surface of a cell and rotate about the axis normal to the surface at the point of contact, but this was rare. Dimers and larger aggregates often moved along the surface of a cell making multiple contacts.

Linkages between spheres and a cell could be broken. Spheres 0.36 μm in diameter or larger had a tendency to come off of the surface of a cell, move freely in the medium (undergo Brownian movement), and then return to the same cell or be picked up by another cell. This did not appear to happen with spheres 0.13 μm in diameter, but our experience with this preparation is more limited. A dimer of 0.80-μm-diameter polystyrene latex spheres was followed for a period of 18 min. It behaved in the following way: at 0 min, it was moving on cell 1; at 4 min, it moved to cell 2; at 7 min, it moved to cell 3 and then back and forth between cells 3 and 4; at 9 min, it was carried off by cell 4; at 10 min, it moved to cell 5; at 14 min, it came off of cell 5 and stuck to the glass; at 15 min, it was picked up by cell 6; at 16 min, it came off of cell 6, stuck to the glass, and then moved to cell 7; at 17 min, it moved to cell 8 and then back to cell 7; at 18 min, it came off of cell 7 and stuck to an aggregate of other polystyrene latex spheres.

In the absence of oxygen, all motion ceased. After 1 or 2 h, in regions of a preparation far away from air bubbles, nearly all motion ceased. Cells stopped gliding, and spheres stopped moving on their surfaces. Spheres on the surfaces of cells did not exhibit Brownian movement, nor did they leave one cell and move to another. Most cells remained fixed on the glass, and most spheres remained fixed on the surface of a cell for minutes or hours without perceptible dis-
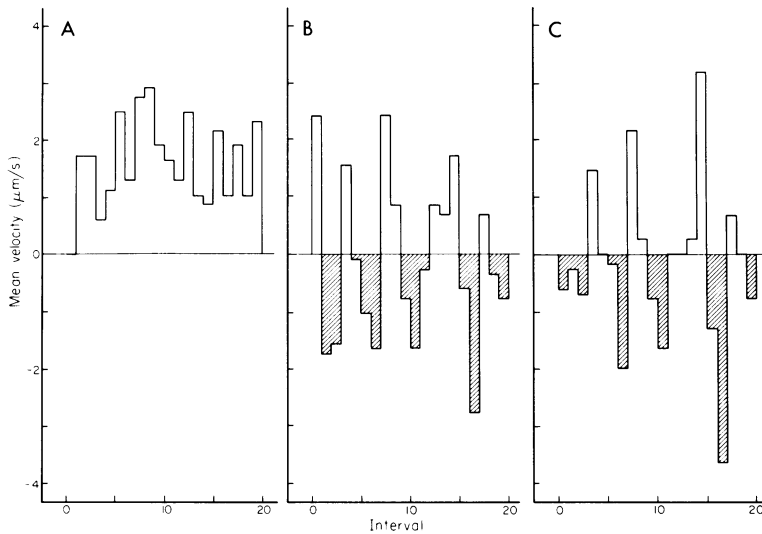
FIG. 8. Mean velocity of a cell in the reference frame of the microscope (A) and mean velocities of two spheres in the reference frame of the cell (B and C) over 20 successive intervals spanning 23 s. The cell was about 5 μm long, and the spheres were 0.56 μm in diameter. The cell was stationary during the first interval but then advanced steadily in one direction along a nearly straight path. The spheres remained at rest relative to the cell or moved forward (toward the leading end) or backward (toward the trailing end; velocity negative, shaded areas) along its entire length. The mean velocity of the cell over the 23-s period (ignoring the first interval) was 1.7 μm/s. For the first sphere, the mean forward velocity was 1.4 μm/s, the mean backward velocity was 1.2 μm/s, and the mean speed (ignoring the fifth interval) was 1.3 μm/s. For the second sphere, the mean forward velocity was 1.3 μm/s, the mean backward velocity was 1.2 μm/s, and the mean speed (ignoring the 5th, 12th, 13th, and 19th intervals) was 1.2 μm/s. These numbers are all of the same order of magnitude. The cell was at rest relative to the glass or a sphere was at rest relative to the cell during the intervals noted.

placement. Some cells or spheres jiggled slightly, but for most, the cessation of movement was absolute. This phenomenon is shown in Fig. 11 for cells coated with 0.13-μm-diameter polystyrene latex spheres. The photographs were made with exposures of 30 s, during which time the images remained completely sharp. A sphere 0.13 μm in diameter, able to diffuse freely in two dimensions in a medium of viscosity equal to that of water, would be expected to diffuse a root-mean-square distance of 20 μm in 30 s. For it to diffuse a root-mean-square distance less than its diameter (0.1 μm) in 30 s, the viscosity of the medium would have to be 4×10^4 times larger than that of water. Evidently, the spheres are adsorbed to the surface of the cell or to sites on the surface of the cell that are linked tightly to its rigid framework. If the cover slip was lifted off such a preparation for a few seconds and then replaced, full activity was restored; cells glided on glass, spheres moved on cells, and large spheres migrated from cell to cell, as before.

Sites on the cells could be saturated. If 0.13-μm-diameter latex spheres were added to cells and the mixture was allowed to stand before a slide was prepared, the cells remained in suspen-

sion. Some appeared to glide on the glass, but closer examination revealed that they glided on spheres or aggregates of spheres stuck to the glass. In contrast, if the cells were added to the slide first and allowed to stand before the spheres were added, most of the cells continued to glide on the glass. In both cases, many spheres moved on the surfaces of the cells; few spheres remained in suspension. The simplest explanation for this dramatic difference is that the spheres and the glass compete for the same sites on the surface of a cell. A site occupied by a sphere no longer interacts with the glass; the cell glides only when this sphere is fixed to the glass. When these preparations were deprived of oxygen, the cells that remained in suspension continued to exhibit Brownian movement, but the spheres on their surfaces stopped moving.

No incomplete flagellar structures were found. No rings, rods, hooks, filaments, or combinations thereof that might be components of an incomplete flagellar structure were detected in pellets or in fractions from sucrose gradients in five separate preparations, under conditions in which hook-basal-body complexes of *E. coli* were readily detected (60, 61). However, the
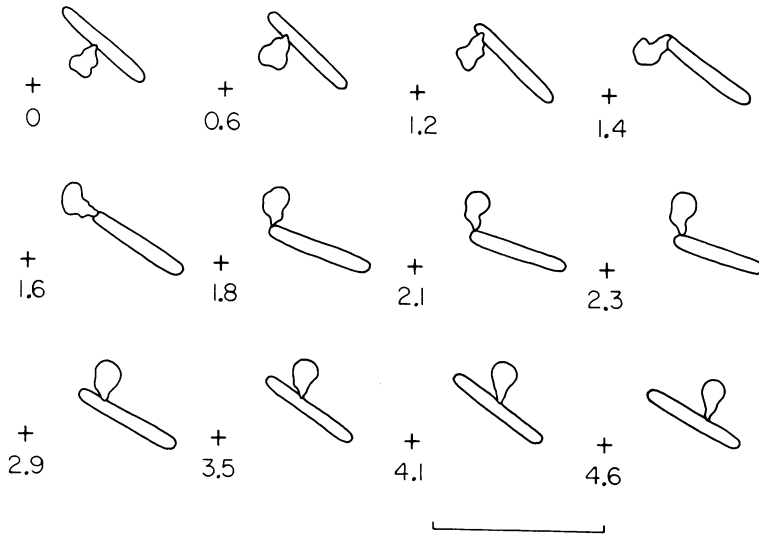
FIG. 9. A large aggregate of 0.13-μm-diameter polystyrene latex spheres moving on a cell in suspension near a glass slide. Selected frames of a frame-by-frame analysis spanning 4.6 s are shown. The numbers are the elapsed times in seconds. The aggregate moved CW around the cell at a speed of about 1.4 μm/s: upward to the left toward the first pole (0 to 1.4 s), around this pole (1.4 to 1.8 s), and downward to the right toward the second pole (1.8 to 4.6 s). In the next 4.9 s (not shown), it pivoted around the second pole (6.2 to 6.7 s) and returned to its initial position (at 9.5 s); the initial and final frames are compared in Fig. 10. The same part of the aggregate remained in contact with the surface of the cell at all times. The angle between the long axis of the aggregate and a line normal to the surface of the cell at the point of contact remained constant. Evidently, the aggregate was strongly adsorbed to a site traveling on a track with hairpin turns at both ends of the cell. The cell body remained nearly parallel to the glass during the period shown (4.6 s) but moved about 1.3 μm lengthwise and 10 degrees CCW. The cell was 6.2 μm long. This recording was made in dark field with a silicon intensifier vidicon. Bar, 10 μm.

search was seriously hampered by the presence in all fractions of large numbers of vesicles of a variety of sizes, ranging from tens to hundreds of nanometers in diameter. These vesicles were not seen in preparations from *E. coli*. They appeared at all levels of the sucrose gradients in bands ranging in color from white to straw yellow to orange, and no means of eliminating them were devised.

One object found in preparations from strain U67 that we thought for a time might be an incomplete flagellar structure is shown in Fig. 12. In some micrographs, as shown in Fig. 12A and B, it appeared as two rings on a rod connected to a nearly straight hook terminated by a distal cap. But in preparations obtained from the French press under conditions in which the hook-basal-body complexes of *E. coli* were not seen, as shown in Fig. 12C, the rings appeared as a base plate with hexagonal symmetry. This object probably is an incomplete virus.

DISCUSSION

These results extend observations made by others. The spreading of *Cytophaga* sp. strain U67 on agar has been described by Henrichsen (31).

This spreading is critically dependent on the wetness of the agar. If the agar is very dry, the cells do not glide; if the agar is slightly wet, the cells glide, but only in rafts and bundles; if the agar is wet, cells also move singly; if the agar is very wet, gliding ceases (31; J. Henrichsen, personal communication). Gliding on agar also is known to depend on the stresses in the substrate (56). We did not encounter such variations with wet mounts.

As this work was under way, Pate and Chang (42) published a description of the motion of cells of *Cytophaga johnsonae* and *Flexibacter columnaris* (also called *Chondrococcus columnaris* and *Cytophaga columnaris*) on glass and the motion of 0.76-μm-diameter latex spheres on these cells while on glass. *Cytophaga johnsonae* and *F. columnaris* appear to glide in a manner similar to that of *Cytophaga* sp. strain U67, except that they spin CW about the long axis when attached to the glass by one pole, not about an axis normal to this (Fig. 5). Pivots and flips were not noted. A description of gliding that is closer to our observations on strain U67 is given by Perry (44) for *Flavobacterium aquatile*: "The movement of a cell on glass was that of
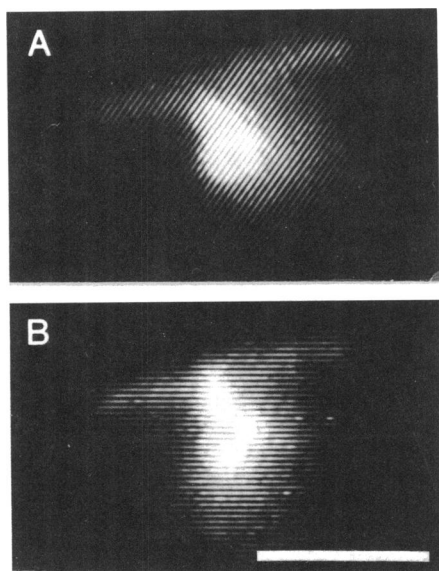
FIG. 10. Photographs at time 0 (A) and 9.5 s (B) of the video images of the aggregate and cell of Fig. 9. The top photograph was printed so that the cell appeared at its final orientation. Note the angle between the two sets of scan lines. The aggregate completed one CW cycle, returning to its initial position and retaining its initial orientation relative to the cell. The cell rotated CCW about 54 degrees, the direction expected given the viscous drag on the aggregate. The images of the aggregate are not quite identical in the two photographs. The cell may have rotated a few degrees about its other axes or moved slightly in or out of focus, or the aggregate may have twisted a few degrees about its long axis. Note that the whole assembly was undergoing Brownian movement. Bar, 5 μm.

gliding to and fro, merging with or interrupted by swinging horizontally, or flipping over or possibly somersaulting. In other tests, on rare occasions, the movement was apparently that of a pendulum describing a conical surface and rotating constantly at *c.* 1 rev./sec." Garnjobst (23) gives a vivid description of this spinning for *Flexibacter columnaris*: ". . . peculiar rotary or waving movements, sometimes combined with flexion, were observed in rods which had suddenly assumed a perpendicular position on glass. The waving was so regular that automatically one began to count. The result of counts made on several different individuals are: 111, 218, 193. The horizontal position was suddenly resumed and sometimes just as suddenly the rod became perpendicular again to repeat the process." The movements in all of these species are much more rapid than those observed in many gliders, for example, in species of *Myxococcus*; the rotary movements are more regular (cf. reference 47).

Cytophaga johnsonae and *Flexibacter columnaris* move polystyrene latex spheres in a manner similar to that of strain U67, except pairs of spheres are said to rotate CCW about the long axis of the cell when localized at one pole and frequently to flip end-over-end when moving down the side of a cell (42); we never saw the latter maneuver. In strain U67, pairs of spheres or aggregates of spheres are not free to rotate or tumble when moving down the side of a cell (Fig. 9 and 10). Pate and Chang describe only the motion of spheres along the port or starboard side of a cell, with transits around the pole or across the body from one side to the other. In strain U67, particles also move on the dorsal surface of the cells, but this is easier to document with spheres smaller than 0.76 μm in diameter. By using spheres as small as 0.13 μm in diameter, we found that particles can move in close lateral proximity, even in opposite directions.

Pate and Chang (42) also note that active movement ceases when cells are deprived of oxygen or treated with certain inhibitors (see below); however, they do not comment on the
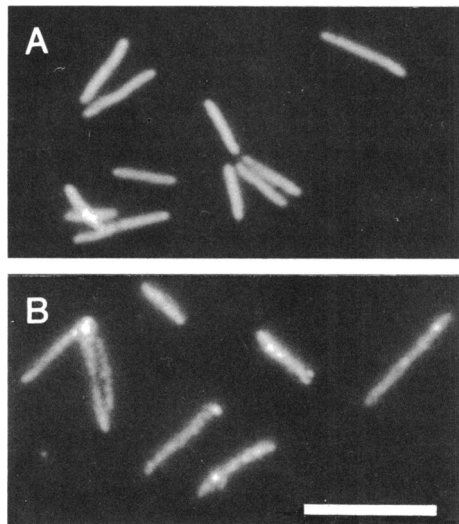

FIG. 11. Dark-field photographs of cells of *Cytophaga* sp. strain U67. (A) Without further treatment; (B) after addition of 0.13-μm-diameter polystyrene latex spheres. Both preparations were made from the same culture. The cells were allowed to glide on glass, the particles were added (B), and then the preparations were depleted of oxygen. Note the distinct spots of light sprinkled widely over the surfaces of the cells (B). The spots are of different brightness because the spheres tend to form monomers, dimers, trimers, and higher aggregates; successive exposures of the same cells were identical. Bar, 10 μm.
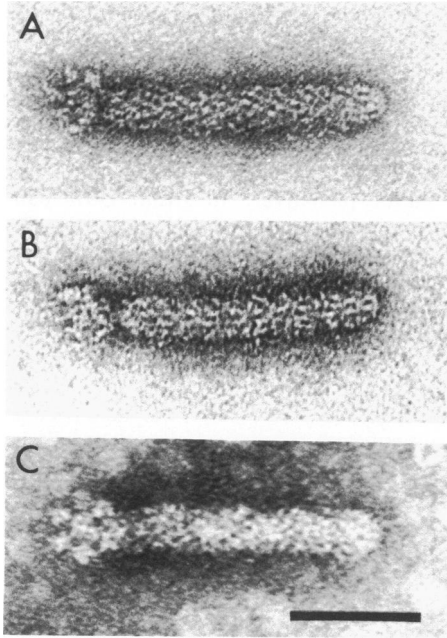
FIG. 12. Three micrographs of an object found in an unsuccessful search for incomplete flagellar structures. (A) From a pellet in Triton X-100; (B) from a pellet in Nonidet P-40; (C) from a sucrose gradient after passage through a French press. The first two images were selected because the base plate, shown more clearly in the third image, appeared to be a pair of rings. Bar, 50 nm.

cessation of Brownian movement that we found so striking (Fig. 11).

There is a long history of the use of small particles (India ink, carmine, indigo, etc.) in studies of gliding motility (see, for example, the reviews of Pringsheim [46], Weibull [62], and Drews and Nultsch [19]), but most of these studies have been made with cells that produce copious amounts of slime or whose trichomes move in slime sheaths, e.g., species of the cyanobacterium *Oscillatoria* (36, 50). In these cases, the particles probably tell us about the movement of slime rather than about the movement of structural components of the cell's outer membrane. One of the most remarkable things about the interaction of polystyrene latex spheres with *Cytophaga* sp. strain U67 (and probably with other species of *Cytophaga* and *Flexibacter*) is that the coupling is rigid, not viscous.

These results support a model in which adsorption sites within the cell's outer membrane move along tracks fixed to the rigid framework of the cell wall. The motion of polystyrene latex spheres on cells of *Cytophaga* sp. strain U67 is not random. The spheres sometimes move

down the length of a cell, around at the pole, and back again, repeatedly. Different spheres on the same cell move in the same or in opposite directions (Fig. 7 and 8). The direction in which a sphere moves is not correlated with the direction of the glide. The spheres are not swept along by bulk flow of slime or by movement of the outer cell membrane as a whole; they move along discrete paths or tracks. The coupling of the particles to the tracks is rigid, not viscous. Small spheres move at about the same speed as large spheres or aggregates of spheres, even though the viscous drag exerted by the external medium varies by factors of 10 or more; the viscous drag on a sphere of radius a moving at velocity v in a medium of viscosity η is $6\pi\eta av$, i.e., linearly proportional to the radius. Aggregates attached at a point on the surface of a cell did not move freely about the point of attachment (Fig. 9 and 10). The whole system becomes immobile when cells are deprived of oxygen (Fig. 11). Evidently, the spheres adsorb strongly to sites within a fluid outer membrane that moves relative to the rigid framework of the cell wall in a tightly coupled manner, step by step or tooth by tooth. The rate of movement is not limited by the external load. The sites remain tightly coupled to the cell wall when the cell is deprived of oxygen. The structures (tracks) along which the sites move might lie within the outer membrane, between the outer membrane and the peptidoglycan layer, or even within the peptidoglycan layer. In any case, they must be anchored to the rigid framework of the cell wall.

We suggest that a cell glides when one or more sites moving in the same direction adsorbs strongly to the glass. Consistent with this model are the observations that a cell glides on a sphere fixed to the glass as readily as it glides on the glass itself (Fig. 4) and that a cell glides at the speed at which spheres move along its surface (Fig. 8).

When a cell glides several body lengths in the same direction (Fig. 1, frames 301 to 2177), sites that have moved to the back of the cell must break their attachments to the glass, and sites that have returned to the front of the cell and turned around at the pole must reform them. Note that sites that move toward the back of the cell are nearly stationary relative to the glass, whether they adsorb or not. Gliding would be facilitated if these sites formed attachments to the glass more readily than those moving relative to the glass at high velocities. It would be advantageous if several sites moving in the same direction adsorbed to the glass; however, if sites moving in opposite directions adsorbed, the cell would have to stop or break some of these attachments. Sites not adsorbed

to the glass would not necessarily affect gliding, because relatively little water or slime between these sites and the glass would reduce their drag to a small value.

Competition between sites moving in opposite directions might explain why spheres 0.36 μm in diameter or larger tend to come off of gliding cells, whereas particles 0.13 μm in diameter remain more firmly attached; the latter spheres might not be large enough to interact with more than one site. It would also explain why all of the spheres remain fixed to cells deprived of oxygen; adsorption to more than one site would prolong attachment when the sites are stationary.

If a site adsorbed to the glass moves along a track through a hairpin turn, the cell must pivot through an angle of 180 degrees or flip over, depending on whether the hairpin is on the ventral side of the cell in a plane parallel to the glass or loops around the pole in a plane normal to the glass. Pivots or flips through other angles are harder to understand, unless tracks make other kinds of turns at the ends of the cell, or a site moving along one track can switch to another at their points of intersection.

Spins, either in a plane parallel to the glass or on conical surfaces having either a large or a small solid angle, are readily understood if some tracks exist as closed circles. If a site moves along a track at a speed of about 2 μm/s, the speed at which a cell glides, and the track is bent into a circle about 0.2 μm in diameter (0.6 μm in circumference), then the site will move around the circle at a frequency of about 3 Hz, the frequency at which a cell spins (Fig. 6). The data at hand are not accurate enough to distinguish rotation about a fixed shaft from motion about a circle as small as this, but note that rotation of a cell on a conical surface is easily explained if circular tracks are laid out on the hemispherical caps at the ends of the cell. If rotation of a cell on a conical surface had to be explained in terms of rotation about a fixed shaft, the shaft would have to flex.

Data obtained with spheres 0.13 μm in diameter indicate that there are many tracks per cell. Some tracks appear to exist as closed loops running the length of the cell in the direction of its long axis. Some have tight hairpin turns at the poles. Others go around the pole from one side of the cell to the other. The tracks probably overlap, particularly at the poles of the cell.

These results are not consistent with most other models for gliding motility. The ability of cells in suspension to adsorb small particles and to propel them at a uniform speed to and fro along their length, with particles in close proximity often moving in opposite directions, argues against most mechanisms previously proposed for gliding motility. These movements cannot be understood in terms of differences in interfacial tension generated at the ends of the cell by excretion of surface-active agents, by the excretion of slime, by inchworm contractions or other undulations of the cell body as a whole, by waves of contraction running from one end of the cell to the other, or by the motion of polar fimbriae. (For discussions of these theories, see the reviews cited in the introduction.)

Mechanisms in which particles are passed from one site to the next along the cell surface, e.g., from crest to crest in a traveling wave of adsorption or from ring to ring in an array of rotating rings (42), seem doubtful but cannot be ruled out. Several connections would have to be maintained at all times between a sphere and a cell to explain why spheres of different sizes move at the same speed, why aggregates of spheres move so rigidly, or why a cell can glide on a sphere in a cyclic fashion or spin continuously. A series of make-then-break connections of such high order would be more likely to occur between components of a molecular machine designed for the purpose, e.g., between interacting protein complexes, one in the outer membrane (the adsorption site), the other fixed to the peptidoglycan layer (an element of the track).

When we began this work, we considered the possibility that gliding bacteria might have incomplete flagella, anlage of the endoflagella of spirochetes, composed of a rotor embedded in the cytoplasmic membrane, a stator just outside the cytoplasmic membrane, a drive shaft that penetrated the peptidoglycan layer, and a proximal hook that ran between the peptidoglycan layer and the outer membrane. If a cell had only a few such structures, they would be very difficult to find in electron micrographs of negatively stained, sectioned, or freeze-etched cells. If the hook were to roll about its axis in viscous contact with the outer membrane, the outer membrane would flow, and this flow, in turn, might cause the cell to glide. If the hook were to adsorb strongly to a component of the outer membrane, it could no longer roll about its axis, but it could still revolve rigidly about the axis of the drive shaft. This would cause the outer membrane to swirl, and this swirl, in turn, might cause the cell to spin. This scheme has the merit that a similar mechanism would underlie motility in flagellated bacteria, spirochetes, and gliding bacteria. Energy transmission from the cytoplasmic membrane to the outer membrane would be explained by direct mechanical linkage. But the data at hand do not support a model in which local regions of the outer membrane undergo viscous flow, nor have we been able to find such incomplete flagellar structures.

Machinery that propels the adsorption sites is

not known. *Cytophaga* spp. have a typical gram-negative cell envelope: an inner (cytoplasmic) membrane, a thin peptidoglycan layer, and an outer (lipopolysaccharide) membrane (13). Strain U67 has neither pili (32) nor flagella (31). When negatively stained with 2% uranyl acetate (G. Guglielmi, unpublished observations), it shows an irregularly undulating surface typical of other gliding bacteria (14, 17, 22, 37). We were not able to find any incomplete flagellar structures by differential centrifugation, but as noted above, this search was severely hampered by large numbers of contaminating vesicles. We have no reason to believe that the object that we did find (Fig. 12) has anything to do with motility.

Some time ago, Pate and Ordal (43) described in *Flexibacter columnaris* arrays of fibrils in the gap between the peptidoglycan layer and the outer membrane that run parallel to each other along the length of the cell and continue in overlapping bands across the poles. Burchard and Brown (10) failed to find these fibrils in freeze-etched preparations of the same cells unless the cells were treated with glutaraldehyde, one of the fixatives used by Pate and Ordal. Without this treatment, Burchard and Brown observed only 10- to 11-nm particles on the inner face of the outer membrane. They did not find such particles in another gliding bacterium, *Myxococcus xanthus*. At about the same time, Glaser and Pate (25) described a mutant of *Flexibacter columnaris*, nonmotile under all conditions tested, that lacked the system of fibrils. More recently, Strohl (59) described in *Cytophaga johnsonae* double-stranded longitudinal fibers, 10 to 12 nm in width, in a fracture plane between the inner and outer membranes, but he considers these fibers to be different from those seen in *Flexibacter columnaris*. Artifacts or not, the fibrils seen in *Flexibacter columnaris* are oriented in precisely the manner required for a system that would generate or direct the motion that we have observed. A similar argument has been used to implicate a system of fibrils in the gliding motility of the cyanobacterium *Oscillatoria* (27–29). It is tempting to speculate that such fibrils (or arrays of 10- to 12-nm particles) are tracks along which adsorption sites in the outer membrane move, but we have no direct evidence for this.

More recently, Pate and Chang (42) described the isolation of ringlike structures from cells of *Cytophaga johnsonae* and *Flexibacter columnaris* that they suggest drive gliding motility. Cell suspensions were homogenized in a buffer containing 1.2 M KI, EDTA, and dithiothreitol. The soluble fraction was clarified by centrifugation at $100,000 \times g$ for 3 h and dialyzed against a buffer containing 0.1 M KCl, ATP, $MgCl_2$, and dithiothreitol. The rings appeared during the dialysis and were pelleted by centrifugation at $100,000 \times g$ for 3 h. The rings also were seen in envelopes of cells treated with a buffer containing $MgCl_2$ and dithiothreitol. Their resemblance to the M- and S-rings of the basal-body complex of flagella from *E. coli* and *Bacillus subtilis* (16), rings thought to serve as the rotor and stator, respectively, of the flagellar rotary motor (1), is at best superficial; the rings from the gliding bacteria are smaller in diameter and much thicker than those from the flagellated bacteria. Whether they exist in the cell envelopes of *Cytophaga johnsonae* and *Flexibacter columnaris* as such or form only after treatment with $MgCl_2$ and dithiothreitol is an open question. We did not see them in our search for incomplete flagellar structures, using methods known to work in *E. coli* (61) and *Salmonella typhimurium* (60).

There is a growing body of evidence that the energy for motility in gliding bacteria, as in flagellated bacteria (see discussion in reference 6) and spirochetes (26), is supplied by a proton-motive force in the form of an electrical potential difference or a pH difference acting across the cytoplasmic membrane. Evidence for this theory has been obtained in *Flexibacter polymorphus* by Ridgway (49), in *Cytophaga johnsonae* and *Flexibacter columnaris* by Pate and Chang (42), in *Flexibacter* FS-1 by Dayrell-Hart and Burchard (15), in *Flexibacter* BH3 by Duxbury et al. (21), and in *Phormidium uncinatum* and *Oscillatoria* spp. by Glagoleva et al. (24; see also 30). If gliding is generated by the motion of adsorption sites in the outer membrane, or, indeed, by the motion of any component of the outer membrane, how is the power required for this motion transmitted from the cytoplasmic membrane through the rigid peptidoglycan layer? In flagellated bacteria and spirochetes, the linkage is mechanical: a drive shaft penetrates the peptidoglycan layer. The existence of fibrils between the peptidoglycan layer and the outer membrane or of adsorption sites or rings in the outer membrane is not enough; some kind of coupling to the cytoplasmic membrane is essential.

The genetics of gliding motility has been studied only in *M. xanthus*. This fruiting myxobacterium has two gene systems controlling movement (33–35). One system, designated A (adventurous), allows cells to glide when they are far apart; it has 21 loci. The second system, designated S (social), allows cells to glide when they are close together; it has at least 10 loci and is correlated with the presence of pili (39). The two systems have only one locus in common. If the machinery for gliding in *Cytophaga* sp. strain U67 is similar to that in *M. xanthus*, then

it must be programed by a gene system similar to the A system; cells of strain U67 glide singly (31; present study), and they have no pili (32).

We hope to learn more about the number, distribution, and nature of adsorption sites in the outer membrane. In summary, our work supports a model for gliding motility in bacteria in which adsorption sites within the outer membrane of the cell move along tracks fixed to the rigid framework of the cell wall. Our observations are inconsistent with most, if not all, alternative models. We would not have obtained these results if the adsorption of polystyrene latex spheres or glass to cells of *Cytophaga* sp. strain U67 were prevented by a thick intervening layer of slime. In this respect, species of *Cytophaga* may be particularly well suited to studies of gliding motility.

A number of intriguing questions remain. How many adsorption sites are there on the surface of a cell? How are they distributed? What is their chemical nature? When we have the answers to these questions, we may be in a position to learn how these sites are propelled.

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