mice. In addition, Sk has not been reported to be present on kidney tissue. Thus, the antigen recognised by the rat anti-C1300 antiserum is a normal tissue antigen of A mice present on brain and, to a lesser extent, on kidney, but not on liver, lung, muscle, spleen or testes and distinct from the alloantigens previously determined to be present on both C1300 tumour cells and A brain. In subsequent communications, this antigen will be referred to as mouse brain antigen-1 (MBA-1).

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Flagellar rotation and the mechanism of bacterial motility

BACTERIAL flagella are generally composed of three morphologically distinguishable regions: (*a*) the long flagellar filament which accounts for more than 95% of the flagellar protein; (*b*) the hook, which is generally 80–90 nm long and has a characteristic shape, and (*c*) the basal structure which is composed of an intricate set of disks and rods attaching the hook to the cell membrane and cell wall[1–3].

Explanations of how the flagella move[4] include the suggestion that helical waves are propagated along the filament[5,6], or that the filament behaves as a semrigid helical rotor[4,7,8]. Berg and Anderson[9] concluded that the evidence ". . . favours a model in which each filament rotates". The data presented here adds strong support to the existing evidence and provides a way to follow flagellar function that is independent of translational motility.

The methods that have been used to study motility depend on observation of the behaviour of the whole organism. This is a complex result of a series of events and is thus, at times, difficult to analyse and interpret. It would be useful to be able to follow the motion of a single flagellum or to assay for its activity in a way that does not depend on chemataxis or cell motility. To do this, a series of experiments was designed based on the observation that bacteria can be found which appear to be 'tethered' by their flagellum to a particle

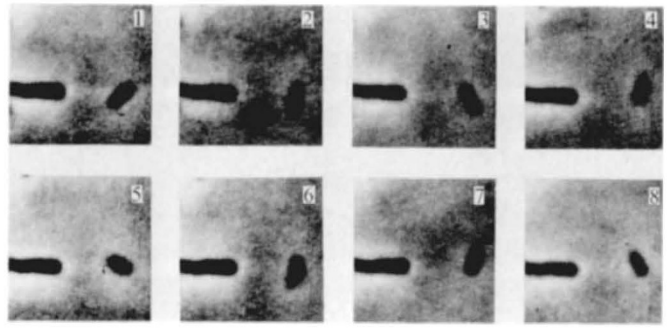

FIG. 1 The rotation of a cell bound to the microscope slide. *E. coli* strain MS1381 was grown on minimal medium with glycerol as the carbon source. Cells were put on a microscope slide and an equal volume of antipolyhook antibody diluted to 1:200 was added. After a 10 m lag, the cells began to spin. Their behaviour was recorded through a Zeiss phase contrast microscope onto video tape and then transferred to film. The pictures presented represent frames taken at intervals of 1/12 of a second. The cell at the left did not move during the sequence and it provides a reference point.

of debris or to the surface of the microscope slide[7]. These bacteria rotate rapidly. The rotation could reflect the motion of the tethered flagellum or it could result because the remaining untethered flagella cause the cell to move. To examine this motion in greater detail, we used 'polyhook' mutants of *E. coli*[10], which carry two mutations, one in the *hag* gene which eliminates the formation of the flagellar filament and another in the *flaE* gene which causes the loss of a function necessary to terminate the assembly of the hook structure. These cells make continuous polyhooks which can be 1–2 μm long and they are nonmotile. However, when dilute anti-polyhook antibody is added to a suspension of cells, they form clumps and begin to rotate rapidly. Several situations have been observed: the bacteria seem to be trapped or attached to the slide; the bacteria are bound to each other in pairs and counter-rotate or one rotates while the other is stationary; large groups of bacteria are cross linked and rotate or move with a jerking type of motion. Ten to twenty per cent of the bacteria in a microscope field can be found to be rotating. Figures 1 and 2 illustrate the first two situations. In Fig. 1, the cell seems to be attached to the surface of the microscope slide. It rotates 360° in a counterclockwise direction around an axis through one end of the cell body. The speed of rotation of an individual cell varies from two to nine revolutions per second. The cells can be observed to modulate their spinning in three ways: (*a*) by continuing to spin counterclockwise; (*b*) by stopping and then restarting in the same direction, or (*c*) by changing direction and spinning clockwise. The cells generally spin

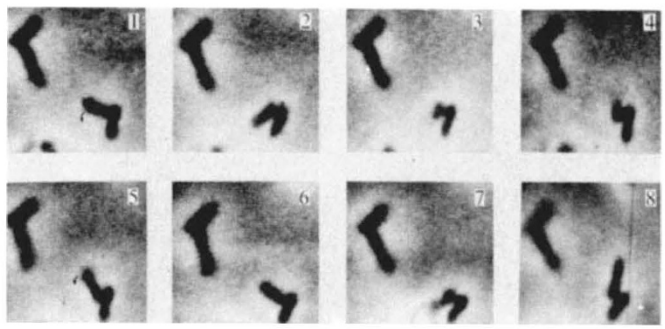

FIG. 2 The rotation of one cell bound to another. The preparation was the same as that described in the legend to Fig. 1. The cells in the upper left corner serve as reference.

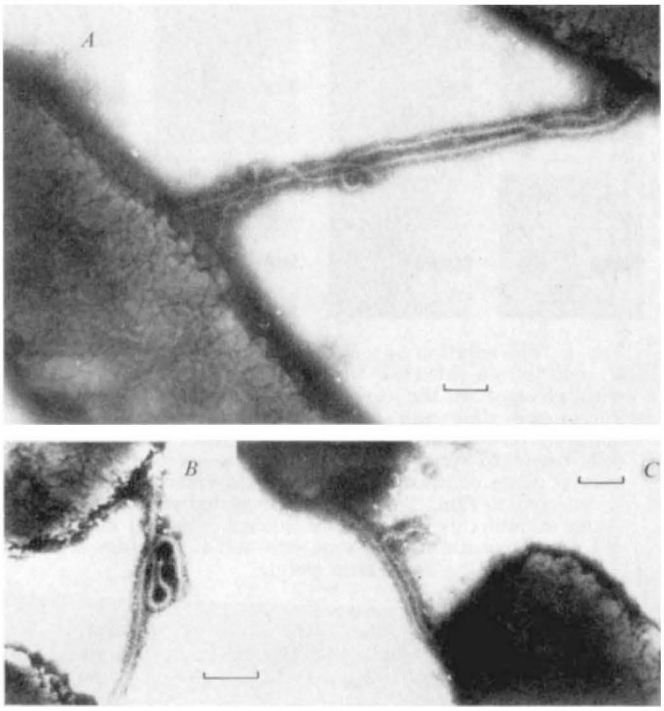

FIG. 3 Electron microscopy of cells bound together via their polyhooks. The cells were prepared as described in Fig. 1. They were placed on copper grids covered with carbon coated formover and stained with 0.5% phosphotungstic acid. The polyhook structure generally appears to be helical with a diameter of about 16 nm (ref. 10). These, however, are straightened and thickened as a result of the antibody molecules bound to them. The line in the photograph represents 0.2 nm. *A*, two cells held together by antibody bound to three polyhooks, *B* and *C*, cells held together by antibody bound to polyhooks derived from each of them.

counterclockwise and occasionally stop or reverse direction for one or two turns and then resume spinning counterclockwise. Individual cells tethered in this way have been observed to spin with intermittent short pauses for more than 30 m. Figure 2 illustrates the motion observed with a pair of tethered bacteria. Again the observations are most consistently interpreted as resulting from the complete rotation of one of the cells about a point of attachment to the other cell. The attachment appears as a narrow gap between the cells (Fig. 2, frame 4 and frame 8) which could correspond to the polyhook-antibody complex.

Thus, it is clear that the cells can rotate. However, it is possible that they rotate around the point of attachment, using the antibody as a swivel rather than rotating around the point of insertion of the basal structure into the cell body. Figure 3 shows electron micrographs of the tethered cells. It is clear that the polyhook structures are coated with antibody molecules, they bind the structures together at many points. The polyhooks have lost some of their characteristic helical appearance and are straightened, suggesting that they have some structural flexibility. The multiple attachment points along the surface of the polyhook appear to stitch them together and make it seem highly unlikely that the cells could rotate around the attachment site.

While these experiments were all done using the polyhook mutant, similar experiments were possible with bacteria that had flagellar filaments. *E. coli* strain W3110 which we used carries a mutation in the *hag* gene that results in the synthesis of straight flagellar filaments lacking the characteristic helical appearance. The cells are nonmotile. Nonetheless, indirect evidence suggests that the flagella are active[11]. To decrease the number of flagella per cell so that they would not tangle and crosslink, the bacteria were grown in minimal medium with glucose as a carbon source[12,13]. When dilute antiflagellar filament antibody was added to these cells they were tethered to the glass and to each other. They began to rotate in the same manner as the polyhook mutants. Thus, even with the straight flagellar filament the cell rotation is observed.

All these observations are concerned with the rotation of the cell, and the rotation of the filament is inferred. To observe the activity of the filaments themselves, we used small latex beads 0.7 μm in diameter (Dow), which were incubated with a 1:1,000 dilution of anti-flagellar antibody and then washed in 0.01 M Tris buffer, *p*H 7.8, and 0.1 M sodium chloride. They were added to suspensions of *E. coli* W3110 that had been grown in minimal medium with glucose. Beads were found attached 1–2 μm from the bacterium and they rotated very rapidly. In the same way, beads could be attached to flagella on wild-type bacteria and these were again observed to rotate rapidly, trailing behind motile bacteria.

All these observations, taken together, can be explained best if the hook is driven in a rotary fashion, probably by a mechanism anchored to the cell body at the base of the flagellum. This results in the rotation of the flagellar filament. Furthermore, the cell has the capacity to vary the speed and direction of rotation as well as the frequency of stopping and restarting. The ability to modulate the rotation of flagella may be the basis for the mechanism of chemotaxis.

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Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*

BERG and Anderson[1] recently argued from existing evidence that bacteria swim by rotation of their helical flagella. Silverman and Simon[2] have now provided a clear demonstration of this. By means of antibodies specific for flagellar components, they tethered cells to microscope slides or to each other and observed rotation of the cell bodies. The cells were able to stop and to rotate in either direction. It seemed possible, as they proposed[2], that cessation or reversal of flagellar rotation might be involved in bacterial chemotaxis. Accordingly, we used wild-type and chemotaxis-defective mutant cells of