CHEMOTAXIS IN BACTERIA

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INTRODUCTION

This review is intended as an introduction to both the early work on sensory transduction in bacteria and problems of current interest. It deals with the ways in which bacteria move and respond (by moving) to changes in their environment. The emphasis is on their response to chemical stimuli (chemotaxis). Bacteria are the simplest free-living organisms amenable to genetic manipulation (1), the organisms of choice for studies of the molecular biology of the gene (2, 3); they are proving of equal value in work on the molecular biology of behavior.

Bacteria swim by rotating thin helical filaments which arise from one or more points on their surface (Figure 1). A filament is joined via a proximal hook to a rod and a set of rings embedded in the cell wall and the cytoplasmic membrane (5).[2] If there are several filaments per cell, they tend to form bundles and move in unison. When viewed by high-speed cinematography, the bundle shows a screw-like motion. It rotates at about 50 rps in one direction while the body of the cell rotates more slowly in the other. Translation occurs at speeds of order 20 cell diam/sec. Depending on the flagellation, the cells either suddenly back up or choose new directions at random. It is the occurrence of these events that is biased by sensory reception.

THE CLASSIC LITERATURE

Leeuwenhoek was intrigued by the movements of bacteria when he first observed them in pepper-water in 1676 (8). Leeuwenhoek, Müller, Ehrenberg, and Cohn all were aware of the swarms of bacteria which collected around bits of food or near

[1] Work supported by grants from the National Science Foundation (GB30337) and the National Eye Institute (EY01263).

[2] The bacterial flagellum (6) is an organelle entirely different from the eukaryotic flagellum (7). In *E. coli* the filament is about 130 Å in diameter. It is made from a single protein with no known enzymatic activity. The eukaryotic flagellum is a much larger appendage that is enclosed by the cytoplasmic membrane and contains complex bending machinery.

the surface of a culture (9), but the first systematic studies of these accumulations did not appear until 1881–1884 (10–13). This was the age of medical microbiology, the period in which Koch published his methods for the study of pathogenic organisms, described the tubercle bacillus and the cholera vibrio, and promulgated his postulates; and Pasteur developed the vaccines for anthrax, swine erysipelas, and rabies. Those interested in sensory phenomena were ahead of their time (14).

Responses to Oxygen

In 1881 Engelmann (10) described the accumulation of *Bacterium termo,* a small, rod-shaped, polarly flagellated "putrefactive" bacterium, in regions of high oxygen tension surrounding cells of higher and lower plants undergoing photosynthesis.[3] By varying the points of illumination and recording the distribution of the bacteria, he was able to prove that oxygen is generated by the chloroplasts [see the drawings of these experiments published in 1894 (16)]. *Spirillum tenue* showed a different kind of behavior: it was attracted to oxygen at low partial pressures but repelled by it at high partial pressures (11). When a drop containing *S. tenue* was covered with a piece of glass, the bacteria accumulated a certain distance from its edges. When the oxygen tension was reduced by exposure of the preparation to hydrogen, the bacteria moved closer to the edges; when it was increased by exposure of the preparation to oxygen, they moved farther away. Observations of this kind were made later on a variety of species by Beijerinck (17), who studied the migration of bands of bacteria in tubes containing oxygen and a nutrient.

Responses to Light and Carbon Dioxide

In 1883 Engelmann (12) published a remarkable paper on *Bacterium photometricum,* a purple sulfur bacterium (1, 18) similar to *Chromatium okenii* (Figure 1). He had found it in the waters of a branch of the Rhine (in Utrecht) and had been captivated by its most peculiar behavior towards light. It swam in the light but eventually stopped in the dark. Unlike *B. termo,* it tended to avoid oxygen. When a uniformly illuminated preparation was suddenly darkened, all the bacteria backed up, stopped for a few moments, and then resumed their normal motion. An identical response occurred when the preparation was suddenly exposed to carbon dioxide. Engelmann called this response a shock reaction ("Schreckbewegung"), because it gave him the impression of fright. Since all the cells backed up, regardless of their orientation, the response depended on the time rate of change of the intensity of the illumination or of the concentration of carbon dioxide, not on the direction of illumination (12) or on spatial differences of intensity or concentration (19). When the initial intensity of illumination or the oxygen tension was high, a larger change in intensity was required to produce the response. The cells failed to back up if the intensity was gradually decreased or if it was suddenly increased, although, in the latter case, they sometimes swam faster. Given these results, Engelmann was able to explain why *B. photometricum* accumulated in a spot of light: the cells

[3] Beijerinck (15) described *B. termo* as a rod-shaped bacterium about 1 μm in diameter and 1.5 μm long with a single polar flagellum. According to Pfeffer (13), it swims forward and backward without changing the orientation of its long axis.

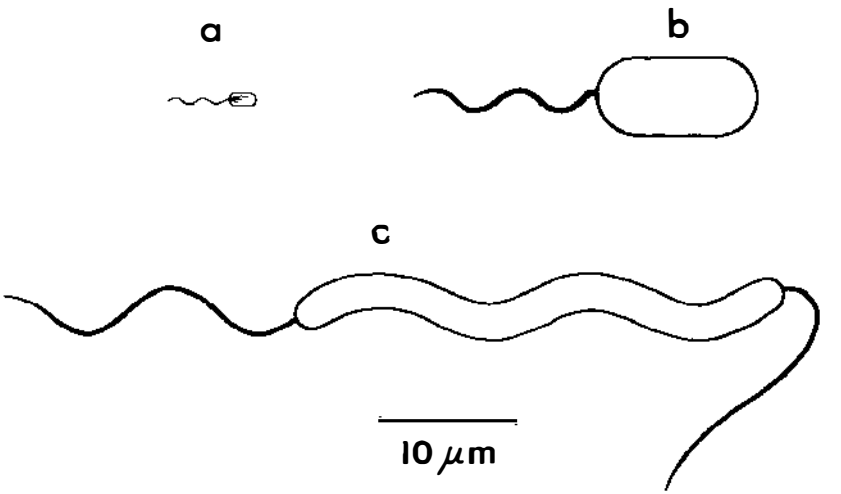

Figure 1 Scale drawings of some of the bacteria whose sensory responses have been studied. (*a*) *Escherichia coli* (or *Salmonella typhimurium*). About six filaments arise at random from the sides of the cell and form a bundle which appears near one pole. The bundle pushes the cell. When it changes its orientation, the cell goes off in a new direction. (*b*) *Chromatium okenii*. About 40 filaments arise at one pole. The bundle either pushes or pulls the cell. When it changes its direction of rotation, the cell backs up. (*c*) *Spirillum volutans*, shown swimming from left to right. The body is helical. About 25 filaments arise at each pole. Those on the left are in the tail configuration; those on the right in the head configuration. When the bundles change their direction of rotation and flip over (tail to head, head to tail), the cell swims in the opposite direction (as if reflected in a mirror normal to its long axis). For a description of the other species mentioned in the text, see Stanier et al (1) and Leifson (4). The flagellation of *E. coli* is peritrichous (*peri*, around; *trichos*, hair), that of *C. okenii* and *S. volutans* lophotrichous (*lophos*, tuft), and that of a cell with a single filament monotrichous.

could swim into the spot but not out of it; they accumulated in the light because they avoided the dark. When he illuminated a culture with a spectrum, the cells accumulated in bands at the wavelengths at which they could respond. This led to the discovery of bacteriochlorophyll *a*, a pigment absorbing in the near infrared (850 nm).[4]

Responses to Other Chemicals

Pfeffer published his first major work on chemotaxis in 1884 (13). He worked with a number of eukaryotic organisms, *B. termo*, and *Spirillum undula*. He inserted into a suspension of bacteria a small capillary tube partially filled with a solution

[4] Pfennig has made an extraordinary movie illustrating these responses in *C. okenii* (20). His films (20, 43, 44) can be rented from the Pennsylvania State University Audio-Visual Services, University Park, Pa. 16802, or purchased from the Institut für den Wissenschaftlichen Film, 34 Göttingen, Nonnenstieg 72, W. Germany.

of attractant. The bacteria responded to the gradient formed by the diffusion of the attractant out of the capillary tube and accumulated near its mouth and later inside.[5] Under optimum conditions, the accumulation began in a few seconds and was well advanced in 1–2 min. The bacteria were attracted to 1% meat extract or to 1% asparagine. However, when *S. undula* was exposed to a mixture of 1% meat extract and 4% potassium nitrate, the attractive effect of the former was surpassed by the repellent effect of the latter, and the bacteria avoided the capillary tube. When the potassium nitrate concentration was reduced to ½%, many more bacteria reached the tube, but the accumulation was smaller than for the meat extract alone. *B. termo* was practically inert to the repellent; it accumulated when exposed to a mixture of 1% meat extract and 20% potassium nitrate. This ruled out the possibility that the repulsion by 4% potassium nitrate was due to diffusion pressure or convective flow. Substances which proved to be attractants at one concentration were repellents at another; for example, *S. undula* was repelled by 4% meat extract or 4% asparagine. Pfeffer compared these results with those obtained with oxygen by Engelmann (11). When an attractant was present in both the capillary tube and the bacterial suspension, the concentration in the capillary tube had to be larger by a fixed factor for accumulation to occur. Finally, attraction occurred even if the solution in the capillary tube would not support growth: both *B. termo* and *S. undula* were attracted to a 1% aqueous solution of asparagine.

Pfeffer expanded on these results in 1888 (23). The best attractants proved to be potassium salts and peptone: *B. termo* responded noticeably to 0.0019% potassium chloride or to 0.001% peptone. All the neutral salts of the alkali and alkaline earth metals were effective to varying degrees; at high concentrations they repelled *S. undula* but not *B. termo*. Asparagine was a good attractant but not as effective as peptone. Mannitol, lactose, and D-glucose were less effective. Glycerol, which was a perfectly good carbon source, neither attracted nor repelled; it was tested at concentrations up to 17%. Even substances that eventually killed the bacteria proved to be effective: the bacteria were attracted to sodium salicylate, morphine hydrochloride, and mixtures of mercuric chloride and potassium chloride. Free acids and alkalis proved to be excellent repellents, as did ethyl alcohol. Pfeffer confirmed his earlier observation that attractants and repellents were antagonistic by adding varying amounts of a repellent (e.g. alcohol) to a fixed amount of attractant (e.g. potassium chloride). For example, a mixture of 0.019% potassium chloride and 2% alcohol repelled *S. undula,* while *B. termo* was attracted to a small degree. The latter did not occur when the alcohol concentration was raised to 10%. Pfeffer also found that a mixture of two attractants worked better than either alone. For example, 0.00095% potassium chloride and 0.0005% peptone failed to attract *B. termo* when tested separately, but they did so when mixed and tested together. Finally, he confirmed his earlier observation that accumulation would occur when an attractant was present in both the capillary tube and the bacterial suspension

[5] Asymptotic solutions for the concentrations of attractants outside capillary tubes have been given by Brokaw (21) and Futrelle & Berg (22). In the latter reference, for $\sqrt{Dt}$ read $\sqrt{(Dt)}$; for γ^2 in the argument of erfc read 2γ.

provided that the concentration in the tube was larger by a fixed factor; when meat extract was present in the bacterial suspension at concentrations of 0.01%, 0.1%, or 1%, the accumulation thresholds for *B. termo* occurred at capillary concentrations of 0.05%, 0.5%, and 5%, respectively.

Evidence for Specific Receptors

Pfeffer (23) was not able to decide if a substance that stimulated the cells needed to be absorbed by them, but he was certain that its effectiveness was based on its specific chemical structure. A given substance affected different species to varying degrees; its effectiveness could not be correlated with molecular weight, size, diffusion rate, osmotic pressure, or other purely physical parameters. Rothert (19) considered it likely that structurally related substances produced qualitatively similar changes in the protoplasm. If so, a response to a substance rarely found in nature, such as a rubidium salt, could be understood in terms of cross specificity in a system developed for a more abundant substance, such as a potassium salt. He also considered it likely that structurally unrelated substances produced qualitatively different changes in the protoplasm. He proved this experimentally by using a competition assay suggested by Pfeffer. If the attractants *A* and *B* produce qualitatively different changes in the protoplasm, the presence of *B* in the capillary and in the suspension (at the same concentration) should not block the response caused by *A*. This would not be the case if the perception of *A* and *B* depended on the same protoplasmic process, for a response would occur then only if the total concentration of attractant *A* and *B* in the capillary were a certain multiple of the concentration of the attractant *B* in the suspension (recall Pfeffer's experiments on accumulation thresholds). Rothert worked with a species of *Clostridium*. To his surprise, it was attracted to ethyl ether (but repelled by it at high concentrations). It also was attracted to meat extract. The response to meat extract (*A*) was not blocked by ethyl ether (*B*) even at concentrations at which the ethyl ether, when used alone, was repellent.

Evidence for a Motor Reflex

How did the bacteria swim into the capillary tube? Pfeffer (13) originally thought that a cell oriented its axis in the direction of the gradient and swam directly toward the capillary mouth; hence, his use of the term chemotaxis. Since, in the absence of the gradient, the cells generally swam backward and forward (changing the direction of locomotion but not the orientation of the body), this meant that reversals were suppressed when they moved up the gradient; indeed, Pfeffer commented on the tendency of both *B. termo* and *S. undula* to go straight to the capillary tube without once backing up. Reversals occurred at the capillary mouth, but only when the bacteria tried to swim out. Other species in his preparations, however, while tending to move up the gradient, occasionally backed up. If the tube contained nutrients, the bacteria generally swam more rapidly when in its vicinity, but this also occurred in isotropic solutions of the nutrients. *S. undula* accumulated in a capillary tube containing 2.5% meat extract without any change in speed, provided that a small amount of meat extract was added to the suspen-

sion. *B. termo* swam more slowly on accumulating in a capillary tube containing 1% meat extract and 25% calcium chloride. Pfeffer concluded that changes in speed were not important.

Pfeffer (24) abandoned the view that a cell swam directly toward the capillary mouth following the work of Rothert (19) and Jennings & Crosby (25). In examining *Bacillus solmsii,* a large, slow-moving, peritrichously flagellated bacterium, Rothert (19) noted that cells swam past the capillary mouth without being deflected, only to back up after they had proceeded some distance beyond. An occasional cell would stop at this point and then continue in the same direction. This led him to study his *Clostridium, S. tenue, S. undula,* and a species of *Chromatium.* Although the responses differed in detail, depending on the kind of flagellation, he never observed active deflections; a bacterium went into the capillary mouth only if it happened, by chance, to be swimming in that direction. Since the paths along which the bacteria moved were only approximately straight and the reversals were rarely precise, a bacterium was likely to find its way into the capillary tube sooner or later. Rothert concluded that the response involved abrupt changes in direction (of a nature characteristic of the flagellation), and that the stimulus always acted repulsively, that is, that the changes in direction occurred when the conditions became unfavorable. Referring to Engelmann's observation that *B. photometricum* backed up when suddenly exposed to carbon dioxide (see above), Rothert concluded that the sensory cue was temporal not spatial, that is, that the bacteria backed up on swimming down the gradient because the concentration decreased in time, not because it differed from one end of the cell to the other.

Jennings & Crosby (25) studied the motion of *Spirillum volutans* in the vicinity of oxygen-generating green algae, near the edges of coverslips (or air bubbles), and in the vicinity of drops of 0.2% sodium chloride (or acids, bases, and other salts). The bacteria swam past the algae without changing their orientation, only to back up at the boundary of the oxygenated zone. They backed up when swimming away from the edges of the coverslip or when approaching them too closely. They also backed up when, by chance, they encountered the edge of the drop of sodium chloride. As a result, they accumulated around the algae and in a band near the edges of the coverslip, while the drop of sodium chloride remained empty. The responses of other bacteria found in decaying vegetable matter were found to be similar. They concluded, in agreement with Rothert, that bacteria respond by a motor reflex triggered by the stimulus.

In 1904 Rothert (26) demonstrated that the motor reflex could be sclectively disabled. He did this by monitoring the response of various bacteria to meat extract (or oxygen) in the presence of increasing amounts of ethyl ether or chloroform. Some bacteria (e.g. his *Clostridium*) remained chemotactic as long as they were able to swim, while others (e.g. *S. tenue*) failed to respond chemotactically but remained motile; they swam past the capillary mouth without ever backing up. Every bacterium stopped swimming if the concentration of the narcotic was high enough. The effect on the tactic response was immediately reversible. It depended on the concentration of the narcotic but not on the duration of exposure. The effect on the motility depended on both.

Motion of the Flagella

With the development of dark-field condensers of high numerical aperture, it became possible to observe the motion of the flagella of even the smallest bacteria. Reichert (27) determined optimum conditions for visualizing moving flagella and described them in great detail. With the exception of old preparations, in which the cells were near a standstill, and of cells now known to have sheathed flagella, for example, *Vibrio metchnikovii* (28), visualization depended on the formation of compact flagellar bundles. With peritrichously flagellated species (e.g. *Salmonella typhosa* and *Proteus vulgaris*) solutions of moderate ionic strength and moderate viscosity were required (e.g. 0.25 to 0.5% sodium phosphate and 1% gelatin); with lophotrichously flagellated species (e.g. *Spirillum volutans* and *Pseudomonas syncyanea*) solutions of moderate ionic strength were sufficient; with the *Vibrio* (the only monotrichously flagellated species examined) even distilled water would do. Reichert determined the number of filaments on a cell by killing it with 1% osmic acid and staining with 5% hematein. If the hematein was used alone, the bundles remained intact. He was able to show, for example, that *S. volutans* formed a bundle in distilled water even though it was not visible on the moving cell. Since a loose bundle will scatter less light than a compact one (through destructive interference), this result is reasonable. Reichert concluded that salts caused the filaments to move closer together by shielding negatively charged groups. He was not able to decide whether substances such as gelatin thickened the filaments, caused them to move closer together, or both. He found all the flagella he examined to be right handed (in the physicist's sense: an object moving away from an observer along such a helix goes clockwise), but this result is suspect (29).

Reichert interpreted his results on the motion of the cells in terms of the theory of propulsion developed for certain flagellates by Bütschli (30). Bütschli considered the forces on a cell with a single helical flagellum. Although his analysis was not exact, he realized that if the flagellum were to rotate as a rigid body about its helical axis, the fluid in which the cell was immersed would resist this motion in such a way as to cause the body of the cell to move along the axis (to be pushed or pulled by the flagellum, depending on the handedness of the helix and its direction of rotation), and to rotate about it (in a sense opposite to that of the flagellum). The same thing would happen if the flagellum were rigidly attached to the body of the cell (as Bütschli believed), if the flagellum were to bend in a helical fashion (to propagate a helical wave). In this case, the flagellum would not rotate relative to the body of the cell, but it would appear to do so. Bütschli proposed that the bending was generated by the sequential contraction and relaxation of elements (lines) running longitudinally along the sides of the flagellum (see the model built by Lowy & Spencer 31). Hydrodynamic treatments of helical wave propulsion have been given by Ludwig (32), Taylor (33), Chwang & Wu (34), Schreiner (35), and Coakley & Holwill (36). Reichert (27) also worried about a net imbalance in the forces perpendicular to the axis of the helix which would cause the body to wobble or gyrate at the frequency of the flagellar motion ("die Trichterbewegung" or funnel movement), but this perturbation is not considered

here. Remarkable as it may seem, we now believe that bacterial flagella actually do rotate (37, 38).

The *Vibrio* swam backward or forward with equal ease, the flagellum pulling or pushing the cell, depending on its direction of rotation, the body rotating more slowly in the opposite direction (27).[6] The *Spirilla* swam as outlined in Figure 1, the body rotating to enhance the translational movement (see below); Reichert thought that the bundle at the head was inert, and that the thrust was generated by the bundle at the tail. *Pseudomonas syncyanea,* a rod-shaped cell with a tuft of flagella at one end, swam with the bundle in front or behind; when in front, it was either extended, as in *Vibrio,* or flipped over, as in *Spirillum.* The bundle of the peritrichously flagellated species (*Salmonella typhosa* and *Proteus vulgaris*) always pushed the cell; it appeared behind, often at an angle to the body axis. It generally was thicker near the base, where there were more filaments, than at the tip. The cell body sometimes turned 180°, sometimes end over end. At other times it stopped, a number of the filaments (or the entire bundle) changed their orientation, and then it moved off in the opposite direction. Sometimes both the filaments and the body changed their orientation.[7] In old preparations of *Proteus,* in which the cells were near a standstill, the bundles came apart and the individual filaments moved the body laterally in different directions, so that it began to shake. When the motion ceased completely, the filaments could be seen extending out from the body in all directions. In general, if a flagellum or a flagellar bundle pushed a cell, its amplitude appeared to be smaller when the cell swam more rapidly or when it moved in a more viscous medium.

Buder (42) described the changes in the motion of the flagella which occurred when *C. okenii* executed a shock reaction. He observed the cells with visible light and stimulated them in the near infrared. The bundle (rotating initially to push the cell) slowed down, stopped, and started up in the opposite direction; it retained its helical shape and remained intact, even when at rest. The body of the cell changed its direction of rotation in synchrony with the flagellar bundle. The cells then swam bundle first but not for very long. If they were stimulated during this period, the normal direction of motion was restored; if they were not, the reversal occurred spontaneously. Buder concluded that this spontaneous change from backward to forward swimming was purely mechanical, that it had nothing to do with the stimulus per se. Given the rigid rotation of the flagellar filaments (37), it probably resulted from their tangling (31). Buder also studied *Thiospirillum jenense,* a purple sulfur bacterium (1, 18) which looks somewhat like *S. volutans* but has only a short flagellar bundle at one pole. When stimulated, this organism simply flipped its bundle over (in the manner of *S. volutans,* Figure 1) and swam in the opposite direction. Pfennig has filmed the flagellar motions of both *C. okenii* (43) and *T. jenense* (44).

Studies of a similar character were made by Metzner (45) on the responses of

[6] See also the description given by Pijper & Nunn (39). Warning: from 1946 on, Pijper (40) believed that bacterial flagella were polysaccharide twirls, not organs of locomotion; his arguments have been discussed by Weibull (29).

[7] See also the description given by Pijper (41).

Spirilla to chemical, photodynamic, and thermal stimuli. In the absence of a stimulus, a cell swam in a manner that depended on its length. If the body helix was as short as one quarter to one half turn, the motion was irregular; if it had three quarters to one turn, the paths were curved; if it had two to three turns (the usual case) they were straight. A cell would occasionally stop or abruptly change its direction without apparent reason. There was no preference for one direction over another until lack of oxygen caused the cells to accumulate near the edges of the coverslip. The handedness of the body was such that its rotation, in reaction to that of the bundle, tended to screw it through the medium. It did so with circumferential slip. In water at room temperature *S. volutans* and *S. undula* swam about 110 μm/sec, the body rotated about 13 rps, and the bundles rotated relative to the body about 40 rps. The pitch of the body helix was 15 μm/turn. To move without slip, a cell would have to swim $13 \times 15 = 195$ μm/sec. Metzner noted that the slip was smaller at higher viscosities.

Metzner gauged the forces generated by the bundles by examining the motion of particles of colloidal silver or fine mastic in the vicinity of cells stuck to bits of detritus. In the main, the tail bundle pushed the fluid backward (as did the bundle of *C. okenii*), while the head bundle moved it circumferentially. Since cells with only one bundle swam in either direction at about the same speed, he concluded that the bundles were equally efficient in either mode: the tail bundle mostly pushed; the head bundle made the body screw itself through the medium (46).

If the cultures were crowded, the bundles often came apart. Sometimes a few filaments remained stationary (extending off to the side) while the rest rotated; sometimes they rotated in separate groups (in the same direction). The filaments originated from separate yet closely spaced points on the surface of the cell. When a bundle flipped over, first the sense of rotation changed, then the basal section began to bend, and finally the distal portion followed. Metzner believed that active bending occurred, in the main, near the base, whereas the distal part of the flagellum, although screwlike, was passively deformed by its motion through the medium. The bundles usually flipped over together (as described in Figure 1), but sometimes only one did. In this case the cell was left with both bundles in the head or the tail configuration. The flagella continued to function, but the cell remained stationary. A head-head to tail-tail transformation could occur without the cell going anywhere. This meant that the thrust generated by the bundles was the same, and that their reversals were tightly coupled.

Metzner was unable to use Pfeffer's capillary method for stimulating the cells, because the dark-field setup required that the preparation be quite thin. If the attractant or the repellent was solid, he placed a small piece of it near the edge of the coverslip; if it was liquid, he placed a drop of it next to a drop of the cell suspension and covered both. With sodium chloride the cells rapidly accumulated in a band some distance away, the boundary closest to the salt being quite sharp, the one farther away diffuse. At the sharp boundary the cells flipped their bundles in synchrony and backed up with great precision; at the diffuse boundary some backed up but others did not. After 10 min three regions could be distinguished: one near the grain of salt, in which the flagella were inert; one farther away, in

which the flagella were active but in the tail-tail configuration; and one in which the flagella were normal. The band, now relatively diffuse, was in the latter region. Potassium chloride, potassium bromide, and potassium iodide gave similar results, but the boundaries of the bands were not as sharp. Metzner assumed that a cell swimming along a steep gradient encountered a large stimulus (a large temporal gradient), and thus responded abruptly; this made the boundary sharp. He also assumed that the more rapidly swimming cells, or those which swam parallel to the gradient (rather than obliquely), responded first, but he did not test this proposition. As the gradient ran down, some organisms stopped for a moment and then backed up. They did this because the head bundle flipped over before the tail one did. Metzner reasoned as follows: if the head flagella perceived the stimulus independently of the tail flagella, reacted, and signaled this fact to the tail flagella, which responded in turn, the deviation from normal behavior could be understood if the salt lengthened the conduction time.

Pepsin gave rise to a single band with boundaries that were absolutely sharp. Lead nitrate proved to be a repellent. Most of the cells backed up at a single boundary. Some, swimming obliquely, crossed this boundary, only to be immobilized. If a particle of nutrient material was placed next to the lead nitrate, all the cells suffered this fate. No response occurred with copper sulfate. The cells which swam close simply stopped swimming. Benzene, xylene, and bromonaphthalene had the same effect, except that a peculiar disorderly movement occurred before the cells died. There was no avoidance response with strong alkalis. The cells slowed down, trembled, and came to a stop, while the bundles split up into single filaments and then disappeared. Most of the cells avoided cocaine hydrochloride. Those which failed to back up started shuttling back and forth rhythmically (at the normal speed, one half to one body length in either direction), then slowed down and wriggled irregularly. The cells failed to avoid chloroform. Those close to the drop stopped, their bundles opposed, while those farther away shuttled back and forth. With ethyl ether and acetone, the cells stopped, their bundles in the head-head mode.

If a photodynamic dye was added, such as eosin (0.01%), cells in the dark field swam normally, while those in the bright focus promptly stopped. If the exposure was short enough ($\frac{1}{4}$ to $\frac{1}{3}$ sec), about one third recovered and swam away; this experiment could be repeated any number of times on the same individual. In the absence of oxygen, the light had no effect. When the light was attenuated by an eosin filter (a 1% solution 0.45 cm thick), cells swimming into the bright focus promptly backed up. They did so as soon as the front end of the cell was intensely illuminated; thus, the stimulus perceived at the front of the cell was relayed to the back. In a preparation in which the oxygen was largely depleted, the ends of the cell were uncoupled: cells already in the focus backed up when the eosin filter was removed; when a cell swam into the focus (unfiltered) the bundle at the head flipped over, but the one at the tail did not; if the focus was moved up to a cell from the rear, the bundle at the tail flipped over but the one at the head did not; the cells remained in the tail-tail or the head-head mode until either end was stimulated again.

Metzner also examined the responses of the *Spirilla* to changes in temperature.

On the basis of the behavior of *S. volutans* in the vicinity of a heated platinum wire, he concluded that an avoidance response occurred as the *Spirilla* were cooled. He proved this by building a chamber which could be uniformly heated or cooled. As it was heated, the cells swam more rapidly but failed to back up; as it was cooled, they shuttled rhythmically back and forth (as they had near a source of cocaine or chloroform). The response was well developed when *S. volutans* and *S. undula* were cultured at 20–22°C. When *S. serpens* was cultured at 11–13°C, however, the response occurred when the chamber was heated, not when it was cooled. Metzner did not determine whether this was a species difference or whether it had to do with the temperature at which the cells were grown.

THE MODERN LITERATURE

On the basis of a review of the early work (and experiments on the alga *Polytoma*) Links (47) proposed that the first (or only) step in the chain of events leading to the chemotactic response is a sudden decrease in the rate of utilization of energy by the motor apparatus. According to this hypothesis, a bacterium swimming out of a spot of meat extract backs up because of a decrease in metabolic rate; a bacterium swimming out of a spot of ethyl ether backs up because the ether inhibits some other energy-requiring process, and thus makes more energy available to the flagella, etc. Manten (48) had found that the action spectrum for phototaxis in *Rhodospirillum rubrum*, a purple nonsulfur bacterium (1, 18), was identical to that for its photosynthesis. He proposed that an avoidance response occurs as a result of a sudden drop in the rate of photosynthesis. Manten's work was extended by Clayton (49–51), who popularized Link's hypothesis. We know now, from work on the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, that bacteria detect, process, and respond to sensory information in a much more sophisticated manner than this hypothesis would imply.

Specific Receptors

In 1969 Adler (52) proved that bacteria have specific chemoreceptors, that is, that they sense attractants per se. He grew *E. coli* on a chemically defined medium, washed and suspended the cells in a medium that supported motility but not growth (53), and then exposed the cells to capillary tubes containing various amounts of an attractant (13, 23, 54). By counting the number of bacteria that swam into the tubes in a given period of time, he was able to establish a quantitative dose-response curve. Using this method he showed that 1. chemicals that are extensively metabolized need not attract (even if they are the first metabolic products of chemicals that do attract); 2. chemicals that are not metabolized (because they are nonmetabolizable per se or because the cells have lost the ability to metabolize them) may attract; 3. the response to an attractant is not generally blocked by the presence of structurally unrelated compounds (even if metabolizable); 4. structurally related compounds compete; 5. mutants exist which lack specific taxes (yet still may metabolize the attractant); and 6. transport of a chemical into the cell is neither necessary nor sufficient for it to attract.

Adler began his work on chemotaxis by studying the migration of bands of

E. coli in tubes containing oxygen and a nutrient (55, 56), a method pioneered by Beijerinck (17) and used by Sherris et al (57) and Baracchini & Sherris (58) in work on aerotaxis. Adler found that the bands formed in response to gradients which appeared as the bacteria consumed oxygen and/or the nutrient, that is, that the migration depended on metabolism as well as chemotaxis. He avoided this complication by perfecting the capillary assay. In the latter, the gradient is generated by diffusion.[8] Effects of metabolism are negligible provided that the number of bacteria is small.

Chemoreceptors for amino acids (59), sugars (60), and a number of repellents (61, 62) are now known.[9] The recognition components for D-galactose, D-ribose, and maltose are shockable binding proteins (64) located in the periplasmic space (65, 66). The role of the galactose binding protein in chemotaxis has been studied by Hazelbauer & Adler (64) and that of the ribose binding protein by Aksamit & Koshland (67, 68). Genetic analysis has shown that the binding protein for galactose interacts with other components that are specific either for taxis or for transport (69). The recognition components for D-glucose, D-fructose, and D-mannitol are sugar-specific enzymes of the phosphotransferase system (60, 70) located in the cytoplasmic membrane (71). Other components in these systems specific for taxis but not for transport remain to be identified.

For L-aspartate (72, 73) and D-galactose (72) the response depends, at least formally, on the time rate of change of the fractional amount of chemoreceptor bound. For L-serine the cells are sensitive over such a wide concentration range (74) that this can be true only if more than one binding site is involved (73). Pfeffer's observations on the interactions between attractants and repellents have been extended by Tsang et al (61) and by Adler & Tso (75). The cells integrate these inputs, but the way in which they do so is not known.

Motion of Individual Cells

My interest in this field was aroused by the possibility of making detailed measurements of the motion of *E. coli* and of determining the precise manner in which it responds to various stimuli. To this end, a microscope was developed which automatically follows the movement of individual cells in three dimensions (76).[10] A bacterium able to respond to attractants or repellents executes a three-dimensional random walk; it moves along as straight a path as rotational diffusion will allow (runs), abruptly changes its direction (twiddles), and then runs again (80).[11] A twiddle is a rare random event; the probability per unit time that a twiddle will

[8] See note 5 above.

[9] Not all would agree: see Seymour & Doetsch (63) and the work cited there.

[10] See this reference for a discussion of other methods of measuring bacterial motility. To these should be added techniques in which time-exposure photographs (77) or videotape recordings (78) are made through an ordinary microscope. A manually operated tracking device has been developed by Lovely et al (79); it can follow cells over large distances, but its time resolution is relatively poor.

[11] This paper has been reprinted with an addendum that includes additional technical and mathematical detail (81). The standard deviations of the run lengths and the twiddle lengths given in the tables seem large. This is a consequence of the fact that the runs and

occur is a constant, and the distribution of run lengths (intervals) is exponential. Except for a fixed bias toward smaller angles, the change in direction from the end of one run to the beginning of the next is random. The flagella remain active during a twiddle; the angular deviation is much larger than can be accounted for on the basis of diffusion. When a bacterium swims up a spatial gradient of an attractant (L-aspartate, L-serine), the probability per unit time that a twiddle will occur is smaller than it is in an isotropic solution; when it swims down the gradient, the probability is the same as it is in an isotropic solution. Other parameters of the random walk remain constant. Thus *E. coli,* under the conditions of our experiment, does not exhibit an avoidance response: its motor reflex is inhibited when the stimulus is favorable. These results were confirmed in experiments in which an attractant (L-glutamate) was generated or destroyed enzymatically (73). As the concentration increased, the bacteria changed directions less frequently; as it decreased, they swam as they did in the absence of the stimulus.

In experiments in which solutions of an attractant at different concentrations were mixed, Macnab & Koshland (82) found that *S. typhimurium* could be made to twiddle more frequently, although the effect was relatively short lived. Twiddles could be suppressed for much longer periods of time. Attractants and repellents had opposite effects (61). The apparent inconsistency between these results and ours might be accounted for by the difference in the size of the stimuli (73); the gradients in the mixing experiments are several hundred times larger than those in the tracking experiments.

All the bacteria described in the early literature, from *B. termo* to *S. volutans,* were noted to change directions spontaneously. Only Pfeffer considered this significant from the point of view of sensory physiology, although he was dissuaded later by the work of Rothert and Jennings and Crosby (see above). It is likely that all bacteria have a "twiddle generator" (80), or its equivalent, which is modulated by the sensory inputs. If so, the behavior of a cell would depend on the rate at which the generator fired in the absence of a stimulus, and on the extent to which the firing could be enhanced or suppressed by sensory reception. Might not *B. termo,* for example, swim more smoothly in a preparation in which the partial pressure of oxygen is gradually increased?

Parkinson (83) has concluded from an analysis of a large number of generally nonchemotactic mutants (84, 85) that the twiddle generator in *E. coli* involves three gene products. *S*-adenosylmethionine may be required for its function (86–88).

Motion of the Flagella

Polarly flagellated bacteria back up. As we have seen from the work of Reichert, Buder, and Metzner, this is a consequence of the change in direction of rotation

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twiddles are distributed in accord with the Poisson interval distribution rather than the normal distribution. The Poisson interval distribution is exponential, and its standard deviation equals the mean. The standard deviation of the mean can be estimated by dividing the standard deviation given in the table by the square root of the number of runs or twiddles (computed by dividing the total tracking time by the sum of the mean run length and the mean twiddle length).

of the flagella (or, as they thought, of the direction of propagation of the flagellar waves). There is now a great deal of evidence that the flagellar filaments rotate relative to the cell body as rigid or semirigid helices (37). The most dramatic proof has been obtained with *E. coli* by tethering a cell with an abnormally long hook or a straight filament to a glass slide with antihook or antifilament antibodies; when this is done, the body of the cell rotates, alternatively clockwise and counterclockwise (38). The cell is nonmotile when free, but it rotates when the hook or the filament is linked to the glass. Larsen et al (89) have shown that mutants that swim smoothly rotate counterclockwise (when tethered to the top of a slide and viewed from above),[12] while mutants that twiddle incessantly rotate clockwise. Stimuli that cause the wild type to swim smoothly (or to twiddle) cause such cells, when tethered, to rotate counterclockwise (or clockwise). Thus, the chemotactic response in *E. coli*, like that in the polarly flagellated bacteria, results from a change in the direction of the rotation of the flagella [see also studies of *Pseudomonas citronellolis* and *S. typhimurium* (90)]. The only difference seems to be that the bundle of a peritrichously flagellated bacterium is able to push the cell but not to pull it; when it tries to pull the cell it either changes its orientation or comes apart (27, 41, 91).[13] The twiddle in *E. coli*, like the shock reaction in *C. okenii* (42), is terminated by a mechanism that does not depend on the stimulus per se; the run length increases in response to the stimulus, but the twiddle length does not (73, 80).

A model for the flagellar motor and a number of its dynamic properties are discussed elsewhere (92). It utilizes as an energy source an intermediate of oxidative phosphorylation rather than ATP (93). It is a remarkable molecular machine.

Coupling of the Receptors and the Flagella

It is evident from Metzner's work on *Spirilla* that some means of long-range intracellular communication is possible. Recall that the flagellar bundles reversed synchronously except when cells were exposed to high concentrations of salt or were partially deprived of oxygen in the presence of a photodynamic dye. One is tempted to think of a bacterium as a one-neuron nervous system and to suppose that the signaling occurs via a change in the membrane potential. This is suggested, for example, by the finding of Krieg et al (94) that flagellar reversals in *S. volutans* are synchronous when filmed at 48 frames/sec. This implies that one end of the cell can signal the other in an interval of order 10^{-2} sec. If the signaling were done by diffusion of a substance of low molecular weight, either through the cytoplasm or along the membrane, the interval would be at least $t = x^2/2D$ sec, where x is the length of the cell and D is the diffusion constant; for $x = 50$ μm and $D = 10^{-5}$ cm^2/sec, $t = 1$ sec. This is too long; an electrical disturbance could be transmitted much more rapidly. That a change in membrane potential may trigger the motor reflex is suggested by the finding of Caraway & Krieg (95) that *S. volutans* can be

[12] If the cell were free, an observer on its surface would find the shaft of the motor rotating in this direction.

[13] A possible exception has been noted by Pijper & Nunn (39): when a drop of sodium bicarbonate was added to a suspension of *S. typhosa* in an acid medium, cells "were seen swimming with their tails in front."

made to back up when an electric field is switched on or off. If a cell is swimming in the direction of the field, this should result in a sizable jump in the trans-membrane potentials at its ends; only such cells were affected. Some work has been done with drugs known to affect excitable membranes (49, 94–97), but the results are hard to interpret. In *E. coli* very little is known about the mode of coupling between the chemoreceptors and the flagella.

SUMMARY

Bacteria swim by rotating their flagella. They alter course by abruptly changing the direction of this rotation. The probability of the occurrence of this event is biased by chemoreception. The bias depends on the way in which the concentration of the attractant or repellent changes with time. Sugars are detected as they bind to specific proteins which also play a role in transport. The way in which the receptors are coupled to the flagella is not known. The coupling may involve changes in membrane potential.[14]

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