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CHEMOTAXIS IN BACTERIA

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"I am not entirely happy about my diet of flies and bugs, but it's the way I'm made. A spider has to pick up a living somehow or other, and I happen to be a trapper. I just naturally build a web and trap flies and other insects. My mother was a trapper before me. Her mother was a trapper before her. All our family have been trappers. Way back for thousands and thousands of years we spiders have been laying for flies and bugs."

"It's a miserable inheritance," said Wilbur, gloomily. He was sad because his new friend was so bloodthirsty.

"Yes, it is," agreed Charlotte. "But I can't help it. I don't know how the first spider in the early days of the world happened to think up this fancy idea of spinning a web, but she did, and it was clever of her, too. And since then, all of us spiders have had to work the same trick. It's not a bad pitch, on the whole."

E. B. White, *Charlotte's Web*

Bacterial chemotaxis, the movement toward or away from chemicals, was discovered nearly a century ago by Engelmann (1) and Pfeffer (2, 3). The subject was actively studied for about fifty years, but then there were very few reports until quite

recently. For reviews of the literature up to about 1960, see Berg (4), Weibull (5), and Ziegler (6). This review will restrict itself to the recent work on chemotaxis in *Escherichia coli* and *Salmonella typhimurium*. Some of this work is also covered in Berg's review (4), and a review by Parkinson (7) should be consulted for a more complete treatment of the genetic aspects.

OVERVIEW

Motile bacteria are attracted by certain chemicals and repelled by others; this is positive and negative chemotaxis. Chemotaxis can be dissected by means of the following questions:

1. How do individual bacteria move in a gradient of attractant or repellent?
2. How do bacteria detect the chemicals?
3. How is the sensory information communicated to the flagella?
4. How do bacterial flagella produce motion?
5. How do flagella respond to the sensory information in order to bring about the appropriate change in direction?
6. In the case of multiple or conflicting sensory data, how is the information integrated?

DEMONSTRATION AND MEASUREMENT OF CHEMOTAXIS IN BACTERIA

Work before 1965, although valuable (4–6), was carried out in complex media and was largely of a subjective nature. It was therefore necessary to develop conditions for obtaining motility and chemotaxis in defined media (8–11) and to find objective, quantitative methods for demonstrating chemotaxis.

(*a*) Plate method: For positive chemotaxis, a petri dish containing metabolizable attractant, salts needed for growth, and soft agar (a low enough concentration so that the bacteria can swim) is inoculated in the center with the bacteria. As the bacteria grow, they consume the local supply of attractant, thus creating a gradient, which they follow to form a ring of bacteria surrounding the inoculum (8). For negative chemotaxis, a plug of hard agar containing repellent is planted in a petri dish containing soft agar and bacteria concentrated enough to be visibly turbid; the bacteria soon vacate the area around the plug (12). By searching in the area of the plate traversed by wild-type bacteria, one can isolate mutants in positive or negative chemotaxis (for example, 10, 12–15).

(*b*) Capillary method: In the 1880s Pfeffer observed bacterial chemotaxis by inserting a capillary containing a solution of test chemical into a bacterial suspension and then looking microscopically for accumulation of bacteria at the mouth of and inside the capillary (positive chemotaxis) or movement of bacteria away from the capillary (negative chemotaxis) (2, 3). For positive chemotaxis this procedure has been converted into an objective, quantitative assay by measuring the number of bacteria accumulating inside a capillary containing attractant solution (10, 11). For negative chemotaxis, repellent in the capillary decreases the number of cells that will

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enter (12). Alternatively, repellent is placed with the bacteria but not in the capillary; the number of bacteria fleeing into the capillary for refuge is then measured (12). Unlike in the plate method, where bacteria make the gradient of attractant by metabolizing the chemical, here the experimenter provides the gradient; hence nonmetabolizable chemicals can be studied.

(*c*) Defined gradients: Quantitative analysis of bacterial migration has been achieved by making defined gradients of attractant (16) or repellent (17), and then determining the distribution of bacteria in the gradient by measuring scattering of a laser beam by the bacteria. The method allows the experimenter to vary the shape of the gradient.

(*d*) A change in the bacterium's tumbling frequency in response to a chemical gradient, described next, is also to be regarded as a demonstration and a measurement of chemotaxis.

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THE MOVEMENT OF INDIVIDUAL BACTERIA IN A CHEMICAL GRADIENT

The motion of bacteria can of course be observed microscopically by eye, recorded by microcinematography, or followed as tracks that form on photographic film after time exposure (18, 19). Owing to the very rapid movement of bacteria, however, significant progress was not made until the invention of an automatic tracking microscope, which allowed objective, quantitative, and much faster observations (20). A slower, manual tracking microscope has also been used (21). A combination of these methods has led to the following conclusions.

In the absence of a stimulus (i.e. no attractant or repellent present, or else a constant, uniform concentration—no gradient) a bacterium such as *E. coli* or *S. typhimurium* swims in a smooth, straight line for a number of seconds—a "run," then it thrashes around for a fraction of a second—a "tumble" (or abruptly changes its direction—a "twiddle"); and then it again swims in a straight line, but in a new, randomly chosen direction (22). (A tumble is probably a series of very brief runs and twiddles.)

Compared to this unstimulated state, cells tumble less frequently (i.e. they swim in longer runs) when they encounter increasing concentrations of attractant (22, 23) and they tumble more frequently when the concentration decreases (23). For repellents, the opposite is true: bacteria encountering an increasing concentration tumble more often, while a decreasing concentration suppresses tumbling (17). (See Figure 1.) [Much smaller concentration changes are needed to bring about suppression of tumbling than stimulation of tumbling (22, 24).]

All this applies not only to *spatial* gradients (for example, a higher concentration of chemical to the right than to the left) but also to *temporal* gradients (a higher concentration of chemical now than earlier). The important discovery that bacteria can be stimulated by temporal gradients of chemicals was made by mixing bacteria quickly with increasing or decreasing concentrations of attractant (23) or repellent (17) and then immediately observing the alteration of tumbling frequency. After a short while (depending on the extent and the direction of the concentration change), the tumbling frequency returns to the unstimulated state (17, 23). A different way to

provide temporal gradients is to destroy or synthesize an attractant enzymatically; as the concentration of attractant changes, the tumbling frequency is measured (25). [For a history of the use of temporal stimulation in the study of bacterial behavior, see the introduction to (25).] The fact that bacteria can "remember" that there is a different concentration now than before has led to the proposal that bacteria have a kind of "memory" (23, 24).

The possibility that a bacterium in a spatial gradient compares the concentration at each end of its cell has not been ruled out, but it is not necessary to invoke it now, and in addition, the concentration difference at the two ends would be too small to be effective for instantaneous comparison (23, 24).

These crucial studies (17, 22, 23, 25) point to the regulation of tumbling frequency as a central feature of chemotaxis. The results are summarized in Figure 1.

By varying the tumbling frequency in this manner, the bacteria migrate in a "biased random walk" (24) toward attractants and away from repellents: motion in a favorable direction is prolonged, and motion in an unfavorable direction is terminated.

[Bacteria that have one or more flagella located at the pole ("polar flagellation," as in *Spirillum* or *Pseudomonas*) back up instead of tumbling (26, 27). Even bacteria that have flagella distributed all over ("peritrichous flagellation," as in *E. coli* or *S. typhimurium*) will go back and forth instead of tumbling if the medium is sufficiently viscous (28).]

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THE DETECTION OF CHEMICALS BY BACTERIA: CHEMOSENSORS

What is Detected?

Until 1969 it was not known if bacteria detected the attractants themselves or instead measured some product of metabolism of the attractants, for example ATP. The latter idea was eliminated and the former established by the following results (10). (*a*) Some extensively metabolized chemicals are not attractants. This includes chemicals that are the first products in the metabolism of chemicals that do attract. (*b*) Some essentially nonmetabolizable chemicals attract bacteria: nonmetabolizable analogs of metabolizable attractants attract bacteria, and mutants blocked in the metabolism of an attractant are still attracted to it. (*c*) Chemicals attract bacteria even in the presence of a metabolizable, nonattracting chemical. (*d*) Attractants that are closely related in structure compete with each other but not

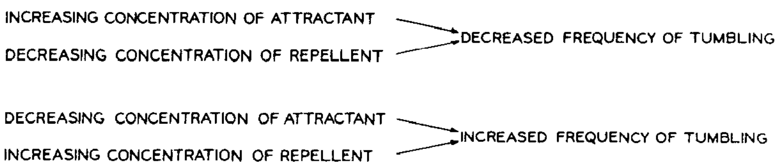

Figure 1 Effect of change of chemical concentration on tumbling frequency.

with structurally unrelated attractants. (*e*) Mutants lacking the detection mechanism but normal in metabolism can be isolated.(*f*) Transport of a chemical into the cells is neither sufficient nor necessary for it to attract.

Thus, bacteria can sense attractants per se: these cells are equipped with sensory devices, "chemoreceptors," that measure changes in concentration of certain chemicals and report the changes to the flagella (10). It is a characteristic feature of this and many other sensory functions that when the stimulus intensity changes, there is a response for a brief period only, i.e. the response is *transient* (17, 23). In contrast, all other responses of bacteria to changes in concentration of a chemical *persist* as long as the new concentration is maintained. For example, when the concentration of lactose is increased (over a certain range), there is a persisting increase in the rate of lactose transport, the rate of lactose metabolism, or the rate of β-galactosidase synthesis. To emphasize this unique feature, the sensory devices for chemotaxis will now be called "chemosensors."

"Chemoreceptor" will now be used for that part of the chemosensor that "receives" the chemicals—a component that recognizes or binds the chemicals detected. A chemosensor must in addition have a component—the "signaller"—that signals to the flagella the change in the fraction of chemoreceptor occupied by the chemical. Further, a chemosensor may contain transport components for the sensed chemical, needed either directly or to place the chemoreceptor in a proper conformation. Bacteria have "receptors" for other chemicals—phage receptors, bacteriocin receptors, enzymes, repressors, etc, but these do not serve a *sensory* function in the manner just defined.

Since metabolism of the attractants is not involved in sensing them (10), the mechanism of positive chemotaxis does not rely upon the attractant's value to the cell. Similarly, negative chemotaxis is not mediated by the harmful effects of a repellent (12): (*a*) repellents are detected at concentrations too low to be harmful; (*b*) not all harmful chemicals are repellents; and (*c*) not all repellents are harmful. Nevertheless, the survival value of chemotaxis must lie in bringing the bacteria into a nutritious environment (the attractants might signal the presence of other undetected nutrients) and away from a noxious one.

The chemosensors serve to alert the bacterium to changes in its environment.

The Number of Different Chemosensors

For both positive and negative chemotaxis the following criteria have been used to divide the chemicals into chemosensor classes. (*a*) For a number of chemosensors, mutants lacking the corresponding taxis, "specifically nonchemotactic mutants," have been isolated (10, 12–15, 29–31). (*b*) Competition experiments: chemical A, present in high enough concentration to saturate its chemoreceptor, will completely block the response to B if the two are detected by the same chemoreceptor but not if they are detected by different chemoreceptors (10, 12, 17, 30–33). (*c*) Many of the chemosensors are inducible, each being separately induced by a chemical it can detect (10, 15).

Table 1 lists chemosensors identified so far for positive chemotaxis in *E. coli*, and Table 2 for negative chemotaxis in *E. coli*. Altogether evidence exists for about

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Table 1 Partial list of chemosensors for positive chemotaxis in *Escherichia coli*

Attractant		Threshold Molarity[a]
	N-acetyl-glucosamine sensor	
N-Acetyl-D-glucosamine		1×10^{-5}
	Fructose sensor	
D-Fructose		1×10^{-5}
	Galactose sensor	
D-Galactose		1×10^{-6}
D-Glucose		1×10^{-6}
D-Fucose		2×10^{-5}
	Glucose sensor	
D-Glucose		3×10^{-6}
	Mannose sensor	
D-Glucose		3×10^{-6}
D-Mannose		3×10^{-6}
	Maltose sensor	
Maltose		3×10^{-6}
	Mannitol sensor	
D-Mannitol		7×10^{-6}
	Ribose sensor	
D-Ribose		7×10^{-6}
	Sorbitol sensor	
D-Sorbitol		1×10^{-5}
	Trehalose sensor	
Trehalose		6×10^{-6}
	Aspartate sensor	
L-Aspartate		6×10^{-8}
L-Glutamate		5×10^{-6}
	Serine sensor	
L-Serine		3×10^{-7}
L-Cysteine		4×10^{-6}
L-Alanine		7×10^{-5}
Glycine		3×10^{-5}

Data from (15, 29, 33); see more complete listing of specificities there. O_2 is also attractive to *E. coli* (8, 34), as are certain inorganic ions (unpublished data).

[a] The threshold values are lower in mutants unable to take up or metabolize a chemical. For example, the threshold for D-galactose is 100 times lower in a mutant unable to take up and metabolize this sugar (15).

20 different chemosensors in *E. coli*, but the evidence for each of them is not equally strong. Oxygen taxis (8, 34) has not yet been studied from the point of view of a chemosensor. *S. typhimurium*, insofar as its repertoire has been investigated, shows some of the same responses as *E. coli* (16, 17, 23, 30, 35).

Nature of the Chemosensors

Protein components of some of the chemosensors have been identified by a combination of biochemical and genetic techniques. Each chemosensor, it is believed,

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Table 2 Partial list of chemosensors for negative chemotaxis in *Escherichia coli*[a]

Repellent	Threshold Molarity
Fatty acid sensor	
Acetate (C2)	3×10^{-4}
Propionate (C3)	2×10^{-4}
n-Butyrate, isobutyrate (C4)	1×10^{-4}
n-Valerate, isovalerate (C5)	1×10^{-4}
n-Caproate (C6)	1×10^{-4}
n-Heptanoate (C7)	6×10^{-3}
n-Caprylate (C8)	3×10^{-2}
Alcohol sensor	
Methanol (C1)	1×10^{-1}
Ethanol (C2)	1×10^{-3}
n-Propanol (C3)	4×10^{-3}
iso-Propanol (C3)	6×10^{-4}
iso-Butanol (C4)	1×10^{-3}
iso-Amylalcohol (C5)	7×10^{-3}
Hydrophobic amino acid sensor	
L-Leucine	$1 \times 1\ ^{-4}$
L-Isoleucine	1.5×10^{-4}
L-Valine	2.5×10^{-4}
L-Tryptophan	1×10^{-3}
L-Phenylalanine	3×10^{-3}
L-Glutamine	3×10^{-3}
L-Histidine	5×10^{-3}
Indole sensor	
Indole	1×10^{-6}
Skatole	1×10^{-6}
Aromatic sensors	
Benzoate	1×10^{-4}
Salicylate	1×10^{-4}
H^+ sensor	
Low pH	pH 6.5
OH^- sensor	
High pH	pH 7.5
Sulfide sensor	
Na_2S	3×10^{-3}
2-Propanethiol	3×10^{-3}
Metallic cation sensor	
$CoSO_4$	2×10^{-4}
$NiSO_4$	2×10^{-5}

[a] Data from (12). See more complete listing of specificities there.

has a protein that recognizes the chemicals detected by that chemosensor—the "chemoreceptor" (or "receptor") or "recognition component" or "binding protein." Wherever this protein has been identified, it has also been shown to function in a transport system for which the attractants of the chemosensor class are substrates. Yet both the transport and chemotaxis systems have other, independent components, and transport is not required for chemotaxis. These relationships are diagramed in Figure 2.

Transport and chemotaxis are thus very closely related; but not all substances that are transported, or for which there are binding proteins, are attractants or repellents (12, 15).

The first binding protein shown to be required (14, 36) for chemoreception was the galactose binding protein (37). This protein is known to function in the β-methylgalactoside transport system (36, 38, 39), one of several by which D-galactose enters the *E. coli* cell (40). This is one of the proteins released from the cell envelope of bacteria—presumably from the periplasmic space, the region between the cytoplasmic membrane and the cell wall—by an osmotic shock procedure (41).

The evidence that the galactose binding protein serves as the recognition component for the galactose sensor is the following: (*a*) Mutants (Type 1 in Figure 2) lacking binding protein activity also lack the corresponding taxis (14), and they are defective in the corresponding transport (38). Following reversion of a point mutation in the structural gene for the binding protein (39), there is recovery of the chemotactic response (M. Goy, unpublished) and of the ability to bind and transport galactose (39). (*b*) For a series of analogs, the ability of the analog to inhibit taxis towards galactose is directly correlated to its strength of binding to the protein (14). (*c*) The threshold concentration and the saturating concentration for chemotaxis toward galactose and its analogs are consistent with the values expected from the dissociation constant of the binding protein (42). (*d*) Osmotically shocked bacteria exhibit a greatly reduced taxis towards galactose, while taxis towards some other attractants is little affected (14). (*e*) Galactose taxis could be restored by mixing the shocked bacteria with concentrated binding protein (14), but this phenomenon requires further investigation and confirmation.

Binding activities for maltose and ribose were revealed by a survey for binding proteins released by osmotic shock, which might function for chemosensors other

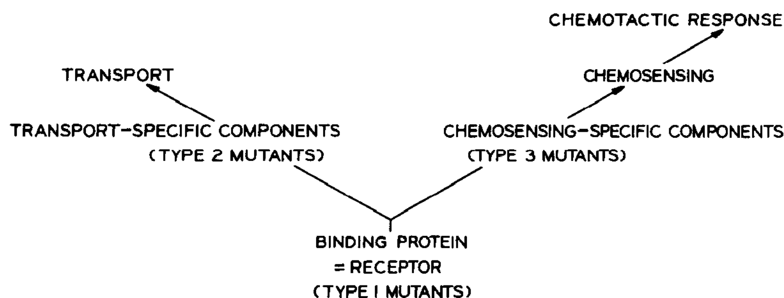

Figure 2 Relation between chemosensing and transport.

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than galactose (14). The binding protein for maltose has now been purified (43), and mutants lacking it fail to carry out maltose taxis (31), as well as being defective in the transport of maltose (31, 43). That for ribose has been purified from *S. typhimurium* and serves as ribose chemoreceptor by criteria *a* to *c* above (30, 35).

Mutants of Type 2 (Figure 2) are defective in transport but not necessarily in chemotaxis, even though the two share a common binding protein. Thus, certain components of the transport system, and the process of transport itself, are not required for chemotaxis (at least for certain chemosensors). This has been studied extensively in the case of galactose, where transport is clearly not required (10, 14, 44). Two genes of Type 2 were found for the β-methylgalactoside transport system for galactose (44). Some of the mutations in these genes abolished transport without affecting chemotaxis; other mutations in these genes affected chemotaxis as well (44). Such chemotactic defects may reflect interactions, direct or indirect, that these components normally have with the chemosensing machinery, or some kind of unusual interaction of the mutated component with the binding protein that would hinder its normal function in chemosensing. Two genes whose products are involved in the transport system for maltose (45) can be mutated without affecting taxis toward that sugar (15, 31). Type 2 gene products are most likely located in the cytoplasmic membrane, since they function in transport.

Mutants of Type 3 are defective in chemosensing but not in transport. Presumably they have defects in gene products—"signallers" (44)—which signal information from the binding protein to the rest of the chemotaxis machinery without having a role in the transport mechanism. Such mutants, defective only for galactose taxis or jointly for galactose and ribose taxis, are known (44). The chemistry and location of Type 3 gene products are as yet undescribed.

A mutant in the binding protein gene (by the criterion of complementation) is known that binds and transports galactose normally but fails to carry out galactose taxis, presumably because this binding protein is altered at a site for interaction with the Type 3 gene product (44). Conversely, some mutations mapping in the gene for the galactose (44) or maltose (31) binding proteins affect transport but not binding or chemotaxis. The binding protein thus appears to have three sites—one for binding the ligand, one for interacting with the next transport components, and one for interacting with the next chemotaxis components.

Whereas the binding proteins mentioned above can be removed from the cell envelope by osmotic shock, other binding proteins exist that are tightly bound to the cytoplasmic membrane. Examples of such are the enzymes II of the phosphotransferase system, a phosphoenolpyruvate-dependent mechanism for the transport of certain sugars (46, 47). A number of sugar sensors utilize enzymes II as recognition components: for example, the glucose and mannose sensors are serviced by glucose enzyme II and mannose enzyme II, respectively (29). In these cases, enzyme I and HPr (a phosphate-carrier protein) of the phosphotransferase system (46) are also required for optimum chemotaxis (29). This could mean that phosphorylation and transport of the sugars are required for chemotaxis in these cases; that enzyme I and HPr must be present for interaction of enzyme II with subsequent chemosensing components; or, as seems most likely, that the enzyme II binds

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sugars more effectively after it has been phosphorylated by phosphoenolpyruvate under the influence of enzyme I and HPr. The products of the phosphotransferase system, the phosphorylated sugars, are not attractants, even when they can be transported by a hexose phosphate transport system (15, 29). This rules out the idea that the phosphotransferase system is required to transport and phosphorylate the sugars so that they will be available to an *internal* chemoreceptor, and indicates instead that interaction of the sugar specifically with the phosphotransferase system somehow leads to chemotaxis (29). Certainly it is not the metabolism of the phosphorylated sugars that brings about chemotaxis: several cases of non-metabolizable phosphorylated sugars are known, yet the corresponding free sugars are attractants (29).

Bacteria detect changes over time in the concentration of attractant or repellent (17, 23, 25), and experiments with whole cells indicate that it is the time rate of change of the binding protein fraction occupied by ligand that the chemotactic machinery appears to detect (25, 42). How this is achieved remains unknown. A conformational change occurs when ligand (galactose) interacts with its purified binding protein (48, 49), and possibly this change is sensed by the next component in the system, but nothing is known about this linkage.

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COMMUNICATION OF SENSORY INFORMATION FROM CHEMOSENSORS TO THE FLAGELLA

Somehow the chemosensors must signal to the flagella that a change in chemical concentration has been encountered. The nature of this system of transmitting information to the flagella is entirely unknown, but several mechanisms have been suggested (10, 23, 50).

(*a*) The membrane potential alters, either increasing or decreasing for attractants, with the opposite effect for repellents. The change propagates along the cell membrane to the base of the flagellum. The cause of the change in membrane potential is a change in the rate of influx or efflux of some ion(s) when the concentration of attractant or repellent is changed.

(*b*) The level of a low molecular weight transmitter changes, increasing or decreasing with attractant or repellent. The transmitter diffuses to the base of the flagella. Calculations (4) indicate that diffusion of a substance of low molecular weight is much too slow to account for the practically synchronous reversal of flagella at the two ends of *Spirillum volutans*, which occurs in response to chemotactic stimuli (26, 27). Thus for this organism, at least, a change in membrane potential appears to be the more likely of the two mechanisms.

Although the binding protein of chemosensors is probably distributed all around the cell because it is shared with transport, possibly only those protein molecules at the base of the flagellum serve for chemoreception. In that case, communication between the chemosensors and flagella could be less elaborate, taking place by means of direct protein-protein interaction.

Several tools are available for exploring the transmission system. One is the study of mutants that may be defective in this system; these are the "generally non-

chemotactic mutants," strains unable (fully or partly) to respond to any attractant or repellent (10, 12, 51). Some of these mutants swim smoothly, never tumbling (51–53), while others—"tumbling mutants"—tumble most of the time (28, 53–55). Genetic studies (56–58) have revealed that the generally nonchemotactic mutants map in four genes (53, 58). One of these gene products must be located in the flagellum, presumably at the base, since some mutations lead to motile, nonchemotactic cells while other mutations in the same gene lead to absence of flagella (59). The location of the other three gene products is unknown. The function of the four gene products is also unknown, but it has been suggested (7, 53) that they play a role in the generation and control of tumbling at the level of a "twiddle generator" (22).

A second tool comes from the discovery that methionine is required for chemotaxis (60), perhaps at the level of the transmission system. Without methionine, chemotactically wild-type bacteria do not carry out chemotaxis (11, 55, 60, 61) or tumble (55, 60, 62). This is not the case for tumbling mutants (55), unless they are first "aged" in the absence of methionine (62), presumably to remove a store of methionine or a product formed from it. There is evidence that methionine functions via S-adenosylmethionine (55, 61–64), but the mechanism of action of methionine in chemotaxis remains to be discovered.

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THE FUNCTIONING OF FLAGELLA TO PRODUCE BACTERIAL MOTION

For reviews of bacterial flagella and how they function, see (5, 65–70).

For many years it was considered that bacterial flagella work either by means of a wave that propagates down the flagellum, as is known to be the case for eucaryotic flagella, or by rotating as rigid or semirigid helices [for a review of the history, see (71)]. Recently it was argued from existing evidence that the latter view is correct (71), and this was firmly established by the following experiment (72). *E. coli* cells with only one flagellum (obtained by growth on D-glucose, a catabolite repressor of flagella synthesis) (9) were tethered to a glass slide by means of antibody to the filaments. (The antibody of course reacts with the filament and just happens to stick to glass.) Now that the filament is no longer free to rotate, the cell instead rotates, usually counterclockwise, sometimes clockwise (72). By using such tethered cells, the dynamics of the flagellar motor were then characterized (73).

Energy for this rotation comes from the intermediate of oxidative phosphorylation (the proton gradient in the Mitchell hypothesis), not from ATP directly (64, 74), unlike the case of eucaryotic flagella or muscle; this is true for both counterclockwise and clockwise rotation (64).

In *S. typhimurium,* light having the action spectrum of flavins brings about tumbling, and this might in some way be caused by interruption of the energy flow from electron transport (75).

It is now possible to isolate "intact" flagella from bacteria, i.e. flagella with the basal structure still attached (Figure 3) (76–78). There is the helical filament, a hook, and a rod. In the case of *E. coli* four rings are mounted on the rod (76), whereas flagella from gram-positive bacteria have only the two inner rings (76, 78). For

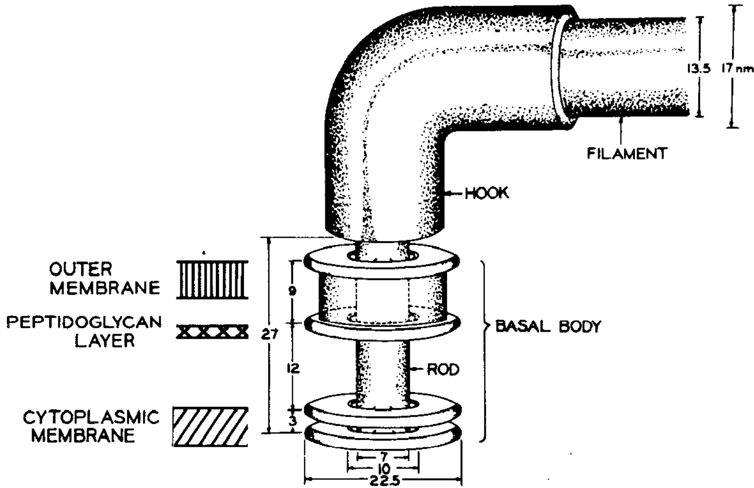

Figure 3 Model of the flagellar base of *E. coli* (76, 77). Dimensions are in nanometers.

E. coli it has been established that the outer ring is attached to the outer membrane and the inner ring to the cytoplasmic membrane (Figure 3) (77). The basal body thus (*a*) anchors the flagellum into the cell envelope; (*b*) provides contact with the cytoplasmic membrane, the place where the energy originates; and (*c*) very likely constitutes the motor (or a part of it) that drives the rotation.

The genetics of synthesis of bacterial flagella is being vigorously pursued in *E. coli* and *S. typhimurium* (28, 79, 80). It is consistent with such a complex structure that at least 20 genes are required for the assembly and function of an *E. coli* flagellum (79) and many of these are homologous to those described in *Salmonella* (28, 80).

THE RESPONSE OF FLAGELLA TO SENSORY INFORMATION

Addition of attractants to *E. coli* cells, tethered to glass by means of antibody to flagella, causes counterclockwise rotation to the cells as viewed from above (52). (Were the flagellum free to rotate, this would correspond to clockwise rotation of the flagellum and swimming toward the observer, as viewed from above. But since a convention of physics demands that the direction of rotation be defined as the object is viewed moving away from the observer, the defined direction of the flagellar rotation is counterclockwise.) On the other hand, addition of repellents causes clockwise rotation of the cells (52). These responses last for a short time, depending on the strength of the stimulus; then the rotation returns to the unstimulated state, mostly counterclockwise (52).

Mutants of *E. coli* that swim smoothly and never tumble always rotate counterclockwise, while mutants that almost always tumble rotate mostly clockwise (52).

From these results and from the prior knowledge that increase of attractant concentration causes smooth swimming (i.e. suppressed tumbling) (22, 23, 25) while

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addition of repellents causes tumbling (17), it was concluded that smooth swimming results from counterclockwise rotation of flagella and tumbling from clockwise rotation (52).

When there are several flagella originating from various places around the cell, as in *E. coli* or *S. typhimurium*, the flagella function together as a bundle propelling the bacterium from behind (75, 81, 82). Apparently the bundle of flagella survives counterclockwise rotation of the individual flagella to bring about smooth swimming (no tumbling), but comes apart as a result of clockwise rotation of individual flagella to produce tumbling. That tumbling occurs concomitantly with the flagellar bundle flying apart has actually been observed by use of such high intensity light that individual flagella could be seen (75).

Presumably less than a second of clockwise rotation can bring about a tumble, and the long periods of clockwise rotation reported (52) result from the use of unnaturally large repellent stimuli. (The corresponding statement can be made for the large attractant stimuli used.) Some kind of a recovery process is required for return to the unstimulated tumbling frequency. The mechanism of recovery is as yet unknown, but it appears that methionine is somehow involved (55, 62).

The information developed so far is summarized in Figure 4.

The reversal frequency of the flagellum of a *Pseudomonad* is also altered by gradients of attractant or repellent, and reversal of flagellar rotation can explain the backing up of polarly flagellated bacteria (27).

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INTEGRATION OF MULTIPLE SENSORY DATA BY BACTERIA

Bacteria are capable of integrating multiple sensory inputs, apparently by algebraically adding the stimuli (17). For example, the response to a decrease in repellent concentration could be overcome by superimposing a decrease in concentration of attractant (17). Whether bacteria will "decide" on attraction or repulsion in a "conflict" situation (a capillary containing both attractant and repellent) depends on the relative effective concentration of the two chemicals, i.e. how far each is present above its threshold concentration (3, 83). The mechanism for summing the opposing signals is unknown.

ROLE OF THE CYTOPLASMIC MEMBRANE

There is increasing evidence that the cytoplasmic membrane plays a crucial role in chemotaxis. (*a*) Some of the binding proteins that serve in chemoreception—the enzymes II of the phosphotransferase system (29)—are firmly bound to the

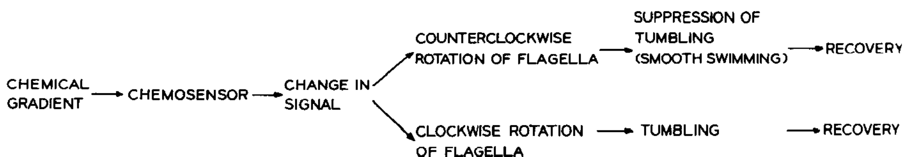

Figure 4 Summary scheme of chemotaxis.

cytoplasmic membrane (46, 47). Binding proteins that can be released by osmotic shock are located in the periplasmic space (41), perhaps loosely in contact with the cytoplasmic membrane. (*b*) The base of the flagellum has a ring embedded in the cytoplasmic membrane (77). (*c*) The energy source for motility comes from oxidative phosphorylation (64, 74), a process that along with electron transport is membrane-associated (84). (*d*) Chemotaxis, but not motility, is unusually highly dependent on temperature, which suggested a requirement for fluidity in the membrane lipids (11). This requirement for a fluid membrane was actually established by measuring the temperature dependence of chemotaxis in an unsaturated fatty acid auxotroph that had various fatty acids incorporated (85). (*e*) A number of reagents (for example, ether or chloroform) that affect membrane properties inhibit chemotaxis at concentrations that do not inhibit motility (26, 86–88).

This involvement of the cytoplasmic membrane in chemotaxis, especially the location there of the chemoreceptors and flagella, makes the membrane potential hypothesis for transmission of information from chemoreceptors to flagella plausible, but of course by no means proves it.

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UNANSWERED QUESTIONS

While the broad outlines of bacterial chemotaxis have perhaps been sketched, the biochemical mechanisms involved remain to be elucidated: How do chemosensors work? By what means do they communicate with the flagella? What is the mechanism that drives the motor for rotating the flagella? What is the mechanism of the gear that shifts the direction of flagellar rotation? How does the cell recover from the stimulus? How are multiple sensory data processed? What are the functions of the cytoplasmic membrane in chemotaxis?

RELATION OF BACTERIAL CHEMOTAXIS TO BEHAVIORAL BIOLOGY AND NEUROBIOLOGY

The inheritance of behavior (see opening quotation) and its underlying biochemical mechanisms are nowhere more amenable to genetic and biochemical investigation than in the bacteria. From the earliest studies of bacterial behavior (2, 3, 89–91) to the present (8, 10, 24, 42, 50, 92, 93) people have hoped that this relatively simple system could tell us something about the mechanisms of behavior of animals and man. Certainly, striking similarities exist between sensory reception in bacteria and in higher organisms (16, 24, 42, 92, 93).

Already in 1889 Alfred Binet wrote in *The Psychic Life of Micro-organisms* (89)

> If the existence of psychological phenomena in lower organisms is denied, it will be necessary to assume that these phenomena can be superadded in the course of evolution, in proportion as an organism grows more perfect and complex. Nothing could be more inconsistent with the teachings of general physiology, which shows us that all vital phenomena are previously present in non-differentiated cells.

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