1969

Chemoreceptors in Bacteria

J. ADLER

For a hundred years it was known that motile bacteria are attracted to a variety of small organic molecules. However, few scientists were interested in bacterial chemotaxis, probably because they were unwilling to believe that these lowly organisms possessed any capability for information processing or could exhibit even simple forms of behavior. Despite evidence to the contrary, it was generally assumed that chemotaxis and metabolism were hopelessly entwined. Bacteria simply congregated where the food was; after all, that was where growth rates were fastest. Julius Adler broke this prejudice.

Undaunted by peer pressure, Adler set out to uncover the molecular basis for bacterial chemotaxis and, in particular, to test rigorously the perceived connection between this phenomenon and metabolism. First he modified a method developed by Pfeffer in the 1880s to permit a quantitative analysis of chemotaxis with *Escherichia coli*, an experimentally tractable organism. Basically this method involves inserting a capillary containing an attractant solution into a suspension of bacteria and then counting the cells that swim into the tube after a defined incubation period. Legend has it that he searched the sewers of Madison, Wis., to find an intelligent strain of *E. coli*. Domesticated strains, which are used to a life of luxury, had become either stupid or paralyzed.

The paper is written in a beautifully clear, Socratic style; questions are posed and answers are provided. With this quantitative assay, Adler presented five lines of evidence demonstrating that bacteria have chemoreceptors for attractants: (i) some metabolites fail to attract, (ii) some attractants cannot be metabolized, (iii) attractants can be detected even when cells are flooded with metabolites, (iv) competition is observed with structurally related attractants, and (v) mutants defective in chemotaxis can still metabolize the molecule in question. Moreover, using attractant competition and mutant analysis, he went on to identify at least five different chemoreceptors. Appropriately enough, the paper ends with a section entitled "Implications for neurobiology and behavioral biology."

Adler's elegantly simple experiments demonstrated that bacteria such as *E. coli* can sense and process environmental information with surprising sophistication. Now many scientists were "attracted" to chemotaxis, and the field grew exponentially. What is remarkable is the diversity of these scientific converts. They include mathematicians and physicists, biochemists and structural biologists, geneticists and molecular biologists, and neurobiologists. Despite the fact that the components of *E. coli's* "brain" have been identified and analyzed in great detail, important questions remain, including the basis for the large range of ligand sensitivity and the mechanisms of signal amplification and adaptation. Because these questions are fundamental to any sensory system, it is likely that bacterial chemotaxis will remain at the forefront of this important research field. Julius Adler spawned an enormously productive enterprise.

THOMAS J. SILHAVY

Chemoreceptors in Bacteria

Studies of chemotaxis reveal systems that detect attractants independently of their metabolism.

Julius Adler

Motile bacteria are attracted to a variety of chemicals—a phenomenon called chemotaxis [for a review, see (*1*)]. Although chemotaxis by bacteria has been recognized since the end of the 19th century, thanks to the pioneering work of Engelmann, Pfeffer, and other biologists, the mechanisms involved are still almost entirely unknown. How do bacteria detect the attractants? How is this sensed information translated into action; that is, how are the flagella directed? This article deals primarily with the first question.

To learn about the detection mechanism that bacteria use in chemotaxis, it is important first to know *what* is being detected. One possibility is that the attractants themselves are detected. In that case, extensive metabolism of the attractants would not be necessary for chemotaxis. There is another possibility: the attractants themselves are not detected but, instead, some metabolite of the attractants is detected (for example, the pyruvate inside the cell); or the energy produced from the attractants, perhaps in the form of adenosine triphosphate, is detected. In these cases, metabolism of the attractants would be necessary for chemotaxis. The idea that bacteria sense the energy produced from the attractants has, in fact, gained wide acceptance for explaining chemotaxis (and also phototaxis) (*2*).

To try to determine which of these possibilities is correct, experiments were carried out with *Escherichia coli* bacteria, which had previously been

demonstrated to exhibit chemotaxis toward various organic nutrients (*3*). The results show that extensive metabolism of the attractants is not required, or sufficient, for chemotaxis. Instead, the attractants themselves are detected.

The systems that bacteria use to detect chemicals without metabolizing them are here called "chemoreceptors." Efforts to identify the chemoreceptors are described.

A Quantitative Method for Studying Chemotaxis

In the 1880's Pfeffer (*4*) demonstrated chemotaxis by exposing a suspension of motile bacteria to a solution of an attractant in a capillary tube and then observing microscopically that the bacteria accumulated first at the mouth of the capillary (Fig. 1) and later inside. A modification of this method, which permits quantitative study of chemotaxis, is here described briefly (*5*).

Wild-type *Escherichia coli* K12, strain W3110, was used, except where otherwise indicated. A capillary tube containing a solution of attractant was pushed into a suspension of bacteria on a slide (*6*). After incubation at 30°C (*7*) for 60 minutes, the capillary was taken out of the bacterial suspension and washed to remove bacteria adhering to the outside. The number of bacteria inside the capillary was then measured by plating the contents of the capillary and counting colonies the next day. The error is ± 15 percent.

A typical result for glucose (*8*) at various concentrations is shown in Fig. 2. From such a dose-response curve—or, better, from a double log plot—one can estimate a threshold concentration for accumulation inside the capillary, in this case about $4 \times 10^{-7} M$. (The threshold is actually lower than this, since the glucose is being used up.) At the highest concentrations, so much attractant diffuses out that the bacteria which have accumulated outside the capillary do not enter in the time allowed. The peak concentration varies with time of incubation, rate of use of the attractant, and other factors (*5*).

Results similar to that shown in Fig. 2 were obtained for other attractants—for example, galactose, ribose, aspartate, and serine (*8*).

Are the attractants themselves detected, or is it something that results

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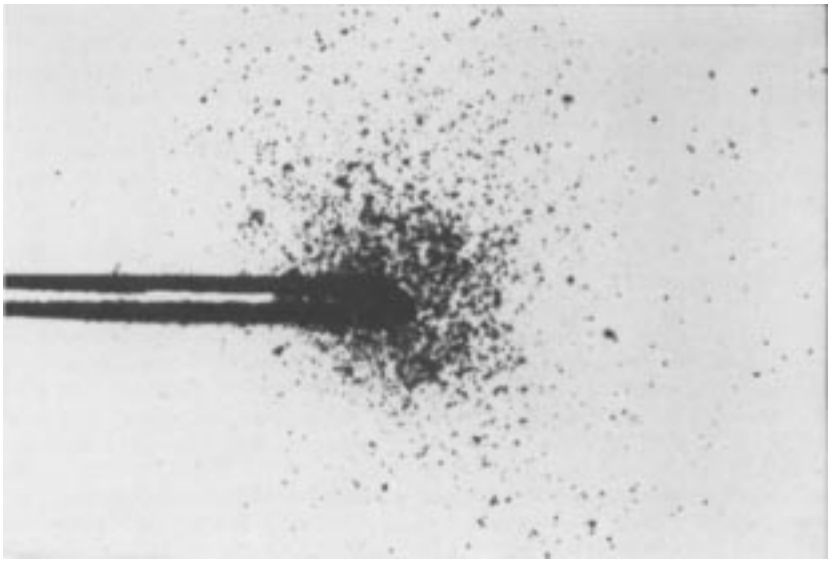


Fig. 1. Photomicrograph showing attraction of *Escherichia coli* bacteria to aspartate. The capillary tube (diameter, ~ 25 microns) contained aspartate at a concentration of $2 \times 10^{-3} M$. [Photomicrograph by Scott W. Ramsey; dark-field photography]

Table 1. The ability of various metabolizable chemicals to attract *Escherichia coli.*

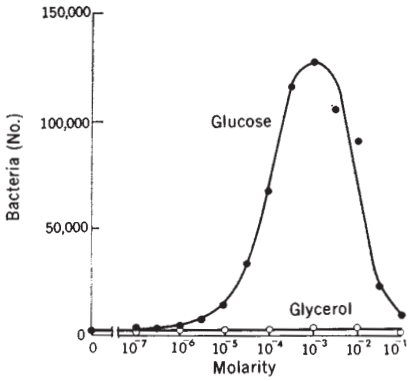
Attractant	Chemotaxis*			Doubling time for growth (hours)
	Threshold molarity	Maximum response†		
		Molarity	No. of bacteria attracted	
Galactose	4×10^{-7}	10^{-3}	125,000	2.6
Galactonate	10^{-1}	10^{-1}	5,000	2.0
Glucose	4×10^{-7}	10^{-3}	187,000	1.2
Gluconate	$> 10^{-1}$	(No response)		1.1
Glucuronate	$> 10^{-1}$	(No response)		1.1
Glycerol	$> 10^{-1}$	(No response)		1.8
α-Ketoglutarate	$> 10^{-1}$	(No response)		2.5
Succinate	10^{-2}	10^{-1}	8,000	2.0
Fumarate	$> 10^{-1}$	(No response)		2.0
Malate	10^{-1}	10^{-1}	5,000	1.7
Pyruvate	$> 10^{-1}$	(No response)		3.0

* The chemotaxis studies were carried out for 1 hour with wild-type (W3110) bacteria grown on each chemical (0.025*M*) as sole source of carbon and energy, in a medium described elsewhere (*45*). † "Maximum response" refers to the number of bacteria attracted into a capillary tube in 1 hour at the peak concentration of attractant. The peak concentration was determined from a dose-response curve for concentrations between $10^{-6} M$ and $10^{-1} M$ (as in Fig. 2) for each chemical. A background value (the value obtained when there is no attractant in the capillary tube) of about 3000 bacteria has been subtracted (see *46*).

Table 2. The ability of L-aspartate and L-serine and of some of their products to attract *Escherichia coli.*

Attractant	Chemotaxis*			Oxygen uptake† (μl/hr per 10^{-9} cells)
	Threshold molarity	Maximum response		
		Molarity	No. of bacteria attracted	
Aspartate	6×10^{-8}	3×10^{-3}	330,000	12
Serine	2×10^{-7}	10^{-3}	194,000	25
Succinate	10^{-2}	10^{-1}	43,000	61
Fumarate	10^{-1}	10^{-1}	3,000	30
Malate	10^{-1}	10^{-1}	3,000	44
Oxalacetate	$> 10^{-1}$	(No response)		5
Pyruvate	$> 10^{-1}$	(No response)		61

* The bacteria were grown on glycerol as sole source of carbon and energy. Otherwise the conditions were as described for Table 1. † Oxygen uptake was measured in chemotaxis medium (see *6*) at 30°C.



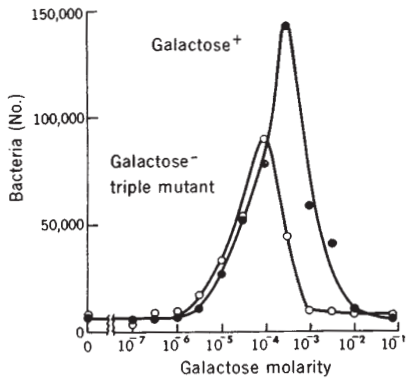


Fig. 2 (left). Graph showing chemotaxis toward glucose (solid circles) but not toward glycerol (open circles). Wild-type (strain W3110) bacteria, grown on glycerol, were used, and the experiment lasted 1 hour. Fig. 3 (right). Graph showing chemotaxis toward galactose by a wild-type strain, W3110 (solid circles), and by a galactose⁻ mutant, W4690 (open circles), which has a mutation in each of the genes for three enzymes needed in the metabolism of galactose. Both strains of bacteria were grown on mannose (see *30*). The experiment lasted 1 hour.

from the metabolism of the attractants —an intermediate, or the energy produced—that is detected? The following five approaches lead to the conclusion that chemotaxis is not a consequence of the metabolism of the attractants but, rather, that the attractants themselves are detected.

Inert Metabolizable Chemicals

1) *Some chemicals that are extensively metabolized fail to attract bacteria.* This result makes it clear that metabolism of a chemical and energy production from it are not sufficient to make a chemical an attractant.

Table 1 shows that among a number of chemicals that are readily metabolized and yield energy, as judged by the ability of *Escherichia coli* to grow on them in the absence of any other carbon and energy source, there are some that fail to attract the bacteria or that attract them very weakly. (The weak response of some of the citric acid cycle compounds could result from their structural resemblance to aspartate, and this resemblance might permit slight detection by an aspartate receptor.) The case of glycerol is shown in more detail in Fig. 2. The inability of glycerol to attract bacteria had already been shown by Pfeffer in 1888 (*1, 4*). The failure of some of the chemicals of Table 1 to attract bacteria was evidently not attributable to an inhibition of chemotaxis, since the presence of each of these chemicals in combination with chemicals that are attractants did not prevent the response of bacteria to the attractants, as documented below for the case of pyruvate and succinate.

Among the chemicals that are extensively metabolized but fail to attract bacteria (or attract them very weakly) are some that are the first products in the metabolism of the attractants aspartate and serine. First products should also attract, if bacteria detect metabolites of the attractants, or energy produced from the attractants.

In *Escherichia coli*, aspartate is known to be converted to fumarate, a reaction catalyzed by aspartase (*9*). Some aspartate may also be converted to oxalacetate by oxidation or transamination. The resulting fumarate and oxalacetate would give rise to succinate, malate, and pyruvate by way of the citric acid cycle. Table 2 shows that cells that are strongly attracted to aspartate are not attracted to any of these intermediates or are attracted to them very weakly; these cells are able to use the intermediates readily, as one finds by measuring the rates of oxidation under the same conditions and by the same cells that are used in the chemotaxis studies. (The inertness of most of these chemicals in chemotaxis is shown in Table 1, but in those chemotaxis studies the bacteria are not strictly comparable to one another since they are first grown on the chemical in question with that chemical as the sole source of carbon and energy before being tested for chemotaxis.)

One of the prominent routes of L-serine metabolism in *Escherichia coli* is conversion to pyruvate by L-serine deaminase (*10*). Table 2 shows that the bacteria are attracted strongly to L-serine but not at all to pyruvate, though they oxidize pyruvate readily.

Pyruvate, oxalacetate, malate, fumarate, and succinate are, of course, also intermediates in the metabolism of glucose, galactose, and ribose, which are good attractants.

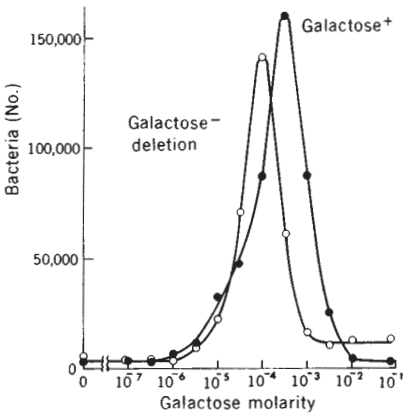


Fig. 4. Graph showing chemotaxis toward galactose by a wild-type strain, W3110 (solid circles), and by galactose⁻ mutant, SU742 (open circles) which has the genes for the three enzymes of galactose metabolism deleted. Both strains of bacteria were grown on mannose (see *30*). The incubation time was 1 hour.

Nonmetabolizable Attractants

2) *Some chemicals that are essentially nonmetabolizable attract bacteria.* It has now been found that mutant bacteria that have lost the ability to metabolize an attractant are still attracted to it, and that bacteria are attracted to largely nonmetabolizable analogs of attractants.

2a) *Mutant bacteria that have lost the ability to metabolize a chemical are attracted to it.* An *Escherichia coli* mutant, W4690, which lacks three enzymatic activities essential for the metabolism of galactose (galactokinase, galactose-1-phosphate uridyltransferase, and uridine diphosphogalactose-4-epimerase) because of a point mutation in each of the genes for these three enzymes (*11*) and another *E. coli* mutant, SU742, in which these three genes are deleted altogether (*12*) are both strongly attracted to galactose, as compared to wild-type bacteria (Figs. 3 and 4). The response peak occurs at a somewhat higher concentration for the wild-type bacteria than for the mutants; this may reflect the fact that the wild-type bacteria consume the galactose and in this way alter the gradient. The galactose used in these experiments had been purified (*13*) to remove any contaminating attractants, such as glucose.

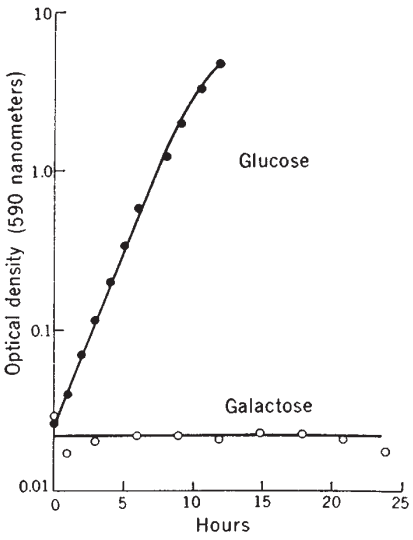


Fig. 5. Graph showing lack of growth on galactose by the galactose⁻ mutant W4690. Bacteria that had been grown on mannose were washed and inoculated into growth medium (*45*) containing galactose (open circles) or glucose (solid circles) at concentration of 0.05*M*, and then shaken at 35°C.

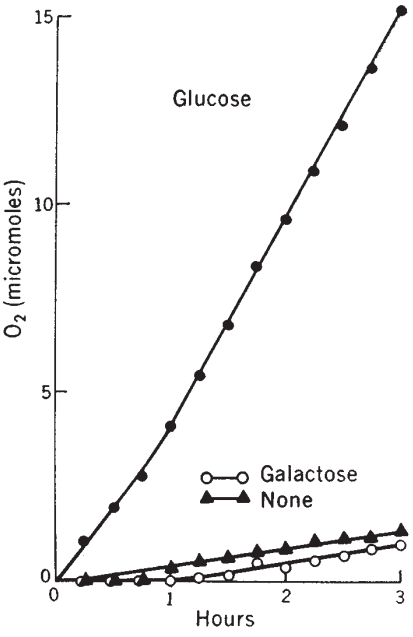


Fig. 6. Graph showing lack of oxidation of galactose by the galactose⁻ mutant W4690 as measured by oxygen uptake. The bacteria (5×10^8 per milliliter) were suspended in a Warburg flask containing 3 milliliters of chemotaxis medium (see *6*) to which galactose (open circles) or glucose (solid circles) was added at a concentration of $1.3 \times 10^{-3}M$, or to which no substrate was added (triangles), and the uptake of oxygen was measured in a Warburg respirometer at 30°C. The results are expressed as micromoles of O_2 taken up per flask. The bacteria had been grown on mannose.

The following evidence shows that mutant W4690 does not metabolize galactose. It failed to grow on galactose, though it grew normally on glucose (Fig. 5), and there was no detectable oxygen uptake on galactose, though there was on glucose (Fig. 6). Measurement of C^{14}-labeled carbon dioxide released from uniformly labeled C^{14}-galactose (*14*) showed that the mutant's production of carbon dioxide is 99.9 percent blocked relative to that of a wild-type strain (Fig. 7). Assays for the three enzymes of galactose metabolism were negative (*11*). Chromatography of an incubation mixture of a heavy suspension of bacteria in a medium containing radioactive galactose (Fig. 8) showed that the galactose was unused, and that slight amounts of only one, or perhaps two, products could be detected. The radioactivity near the origin may be galactose-6-phosphate, known to be produced from galactose and phosphoenolpyruvate (*15, 16*) in mutants that do not metabolize galactose (galactose⁻ mutants) (*17*). That reaction, however, could not be required for chemotaxis, since mutants unable to carry out this reaction showed normal taxis toward galactose.

The deletion mutant SU742 also failed to grow on galactose or to take up any detectable oxygen on galactose, and its production of C^{14}-labeled carbon dioxide from uniformly labeled C^{14}-galactose was 99.5 percent blocked relative to that of a wild-type strain.

An *Escherichia coli* mutant, DF2000, defective in its ability to metabolize glucose because of mutations in the genes for phosphoglucose isomerase and glucose-6-phosphate dehydrogenase (*18*) was attracted to glucose as strongly as its wild-type parent, strain K10, was (Fig. 9). The mutant failed to grow on glucose, and both its oxygen uptake on glucose and its production of C^{14}-labeled carbon dioxide from uniformly labeled C^{14}-glucose (*19*) were 97 percent blocked, relative to the wild-type parent. Being unable to metabolize glucose, the mutant is also unable to metabolize galactose; as expected, the mutant was attracted to galactose as strongly as its parent was.

Analogs That Attract

2b) *Some essentially nonmetabolizable analogs of metabolizable chemicals attract bacteria.* D-Fucose (6-deoxy-D-galactose) is a galactose analog that is not a source of carbon and energy for growth (*20*). Nevertheless the bacteria are attracted to it. In Fig. 10 the responses of bacteria to D-fucose and D-galactose are compared. It may be seen that D-fucose is an effective attractant, though its threshold concentration for chemotaxis is higher than that of D-galactose, as might be expected for an analog. The D-fucose had been purified (*13*) to remove metabolizable impurities such as galactose or glucose. (L-Fucose, while an excellent source of carbon and energy for growth, is inert as an attractant.)

The evidence that D-fucose is essentially nonmetabolizable may be summarized as follows. Buttin has reported (*20*) that D-fucose does not support the growth of *Escherichia coli* (this is demonstrated in Fig. 11), that it is not detectably phosphorylated by galactokinase or by a crude extract of *E. coli*, and that it is not consumed by these bacteria at an appreciable rate. Figure 12 shows that D-fucose, unlike D-galactose, is not detectably oxidized. Chro-

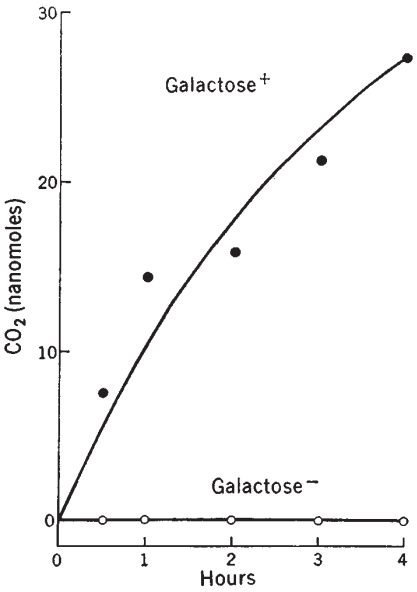


Fig. 7. Graph showing lack of oxidation of galactose by the galactose⁻ mutant W4690 as measured by production of C^{14}-labeled carbon dioxide. Mutant bacteria (open circles) or wild-type bacteria, W3110 (solid circles), each at a concentration of 4×10^8 bacteria per milliliter, were suspended in 1 milliliter of chemotaxis medium containing uniformly labeled C^{14}-galactose (*14*) ($1 \times 10^{-4}M$; 5×10^5 counts per minute per milliliter). Then portions (0.2 milliliter) were incubated at 30°C for various times in flasks equipped with a center well (*47*) for trapping C^{14}-labeled carbon dioxide in ethanolamine and ethylene glycol monomethyl ether (1 : 1) (*48*); a count was made of the trapped carbon dioxide (*48*). The results are expressed as nanomoles of carbon dioxide per flask. The bacteria had been grown on mannose.

Table 3. Effect of added metabolizable chemical on chemotaxis.

Attractant	Number of bacteria attracted			
	No added metabolizable chemical	Pyruvate ($3 \times 10^{-3}M$)	Succinate ($3 \times 10^{-3}M$)	Glucose ($3 \times 10^{-3}M$)
	*Wild-type strain**			
None	3,000	4,000	3,000	3,000
Galactose ($10^{-3}M$)	83,000	69,000	71,000	
Fucose ($10^{-2}M$)	168,000	149,000	152,000	
Glucose ($10^{-3}M$)	138,000	109,000	86,000	
Aspartate ($10^{-3}M$)	537,000	548,000	752,000	503,000
Serine ($10^{-3}M$)	382,000	299,000	418,000	494,000
	Galactose⁻ strain†			
None	10,000	9,000	6,000	
Galactose ($10^{-4}M$)	67,000	128,000	68,000	
	Glucose⁻ strain‡			
None	5,500	3,000	4,000	
Glucose ($10^{-4}M$)	17,000	72,000	87,000	

* The wild-type strain, W3110, was grown on galactose as sole source of carbon and energy. † The galactose⁻ strain, W4690, was grown on mannose (see *30*); the same result was obtained with the galactose⁻ deletion strain, SU742. ‡ The glucose⁻ strain, DF2000, was grown on mannose. All incubations were carried out for 1 hour at 30°C.

matography of an incubation mixture of a heavy suspension of *E. coli* with radioactive D-fucose has revealed that some metabolism does occur (*21*). Five products were formed by wild-type *E. coli*, and three of these were formed by a mutant of *E. coli*, W4690, that lacks the enzymes of galactose metabolism but is attracted to D-fucose as strongly as wild-type bacteria are. In wild-type *E. coli*, D-fucose is converted into products at only 0.5 to 1.0 percent of the rate at which D-galactose metabolism occurs.

Three glucose analogs which failed to support growth and which were oxidized at less than 5 percent of the rate for glucose are also attractants: 2-deoxyglucose, α-methyl glucoside, and L-sorbose. It had been known that these analogs do not undergo extensive conversion (*22*). The thresholds for chemotaxis were $3 \times 10^{-4}M$, $10^{-3}M$, and $3 \times 10^{-4}M$, respectively, and the maximum responses were 125,000 bacteria at $10^{-1}M$, 210,000 at $10^{-1}M$, and 123,000 at $3 \times 12^{-2}M$, respectively. The three analogs were purified before use in order to remove glucose or other contaminants (*13*).

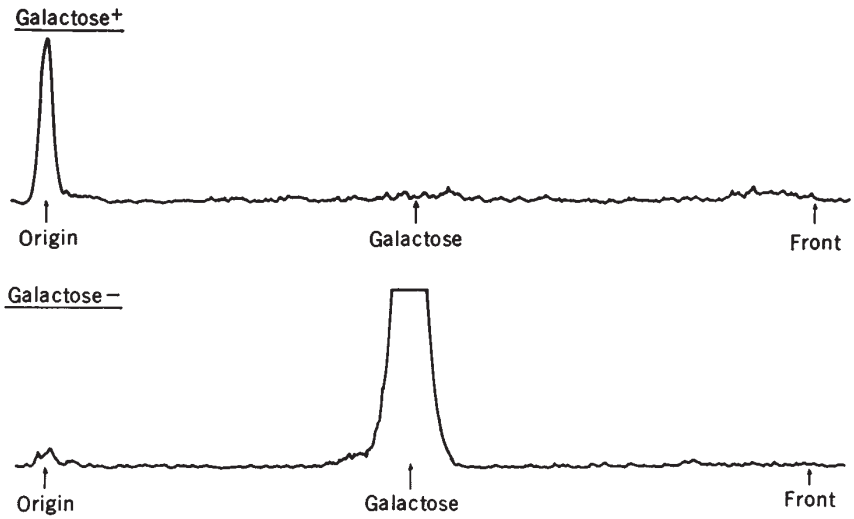


Fig. 8. Chromatographic records showing lack of metabolism of galactose by the galactose⁻ mutant W4690. Mutant bacteria (bottom record) or wild-type bacteria, W3110 (top record), each at a concentration of 2×10^9 bacteria per milliliter in 1 milliliter of chemotaxis medium containing uniformly labeled C^{14}-galactose (*14*) ($2 \times 10^{-4}M$; 6×10^5 counts per minute per milliliter), were incubated at 30°C for 2.5 hours. A 0.05-milliliter sample from each was then chromatographed in an ethylacetate, pyridine, H_2O (8:2:1) system for 12 hours. The top record shows that the wild-type bacteria consumed the galactose; radioactivity at the origin probably represents incorporation into cellular materials. The bacteria had been grown on mannose.

Metabolized Chemicals Do Not Block

3) *Chemicals attract bacteria even in the presence of a metabolizable chemical.* If bacteria detect metabolites of an attractant, or energy produced from it, then the addition of a metabolizable chemical should stop chemotaxis by flooding the cells with metabolites and energy. This was not found to be the case for either metabolizable or nonmetabolizable attractants.

Table 3 shows that the presence of the metabolizable chemicals pyruvate or succinate in the bacterial suspension and in the capillary did not block chemotaxis toward galactose, glucose, aspartate, or serine. Even the chemicals that are not metabolized—fucose, or galactose in the case of the galactose⁻ mutant, or glucose in the case of the glucose⁻ mutant—attracted bacteria perfectly well in the presence of pyruvate or succinate. Nor did glucose block chemotaxis toward aspartate or serine.

[It should be pointed out that no energy source has been added in any of the chemotaxis experiments reported in this article except for those of Table 3. Instead, the bacteria rely on an endogenous energy source (*23*). Stimulation by an added energy source in the case of the glucose⁻ mutant indicates that its endogenous energy source is inadequate, and this conclusion is supported by the observation that the endogenous uptake of oxygen is lower in this strain than it is in its glucose⁺ parent.]

Pyruvate, succinate, and glucose are in fact readily metabolized under these conditions, as shown by oxygen uptake measurements under exactly the conditions of the chemotaxis experiments. They are metabolized even in the presence of the attractants: the oxygen uptake on pyruvate or succinate at a concentration of $3 \times 10^{-3}M$ was not blocked by the addition of fucose ($10^{-2}M$; there was no inhibition), or by the addition of galactose in the case of the galactose⁻ mutant ($10^{-3}M$; there was no inhibition), or by the addition of glucose in the case of the glucose⁻ mutant ($10^{-4}M$; inhibition, 40 percent).

Structurally Related Attractants Compete

4) *Attractants that are closely related in structure compete with each other but not with structurally unre-*

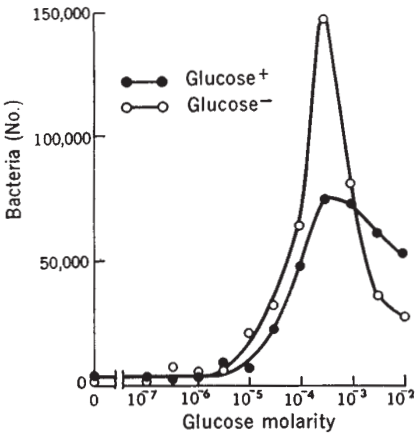


Fig. 9. Graph showing chemotaxis toward glucose by a glucose⁻ mutant, DF2000 (open circles), and its glucose⁺ parent, K10 (solid circles). Both strains of bacteria were grown on mannose. The experiment was carried out for 1 hour in the presence of succinate ($3 \times 10^{-3}M$) as energy source.

lated compounds. This finding supports the conclusion that it is the attractants themselves that are detected, and that there exists a variety of specific receptors.

In these experiments, one attractant at its optimum concentration is put into the capillary tube, and another attractant at a concentration of $0.01M$ is put into both the capillary and the bacterial suspension. If the two attractants use the same chemoreceptor, the response should be inhibited; if they do not, the response should not be affected. (Inhibition could result from other causes too, but failure to obtain inhibition is strong evidence that the attractants use different receptors.) Experiments of this nature were first carried out in the late 19th century (*26*). Some of our results follow (for a more detailed report, see *27* and *28*).

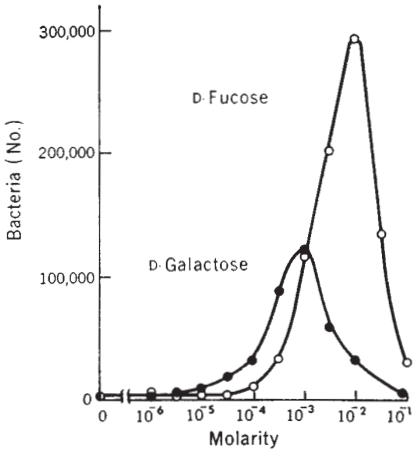


Fig. 10. Graph showing chemotaxis toward D-fucose (open circles) and D-galactose (solid circles). Wild-type bacteria (W3110), grown on D-galactose, were used in this experiment, which lasted 1 hour.

Chemotaxis toward fucose was completely inhibited by the presence of galactose, and in the reciprocal experiment there was nearly complete inhibition. This suggests that fucose and galactose use the same chemoreceptor (the "galactose receptor").

Glucose completely eliminated taxis toward galactose, but in the reciprocal experiment the inhibition was only about 60 to 70 percent, no matter how high the concentration of galactose was. This suggests that the receptor which detects galactose also detects glucose but that, in addition, there is another receptor that detects glucose but not galactose (the "glucose receptor").

Similar experiments show that ribose is detected by yet another receptor (the "ribose receptor"), which fails to detect either galactose or glucose.

Glucose did not block taxis toward serine or aspartate (Table 3), and neither did galactose, fucose, or ribose. In the reciprocal experiments, aspartate failed to inhibit taxis toward any of the sugars. (Serine slightly inhibits chemotaxis toward all other attractants, and this inhibition remains unexplained.) These results show that the receptors which detect the sugars are different from those which detect the amino acids.

Aspartate did not inhibit taxis toward serine, so evidently there are separate receptors for detecting these two amino acids (the "aspartate receptor" and the "serine receptor"). On the other hand, aspartate completely inhibited taxis toward glutamate, and complete inhibition was found in the reciprocal experiment, so it appears that aspartate and glutamate use the same receptor.

The various chemicals that are not attractants or that attract very weakly all failed to inhibit chemotaxis toward the attractants. (See, for example, the case of pyruvate and succinate in Table 3.)

Mutants Lacking Specific Taxes

5) *There are mutants which fail to carry out chemotaxis to certain attractants but are still able to metabolize them.* If there are chemoreceptors in bacteria and if they are specific, there

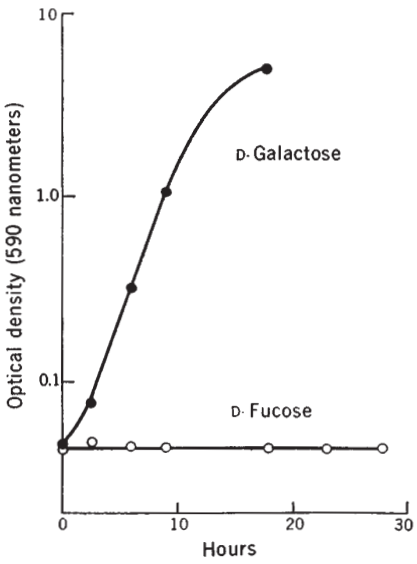


Fig. 11. Graph showing lack of growth on D-fucose by wild-type bacteria, W3110. Bacteria that had been grown on D-galactose were washed and inoculated into growth medium (*45*) containing D-fucose (open circles) or D-galactose (solid circles) at concentration of $0.05M$, and then shaken at 35°C.

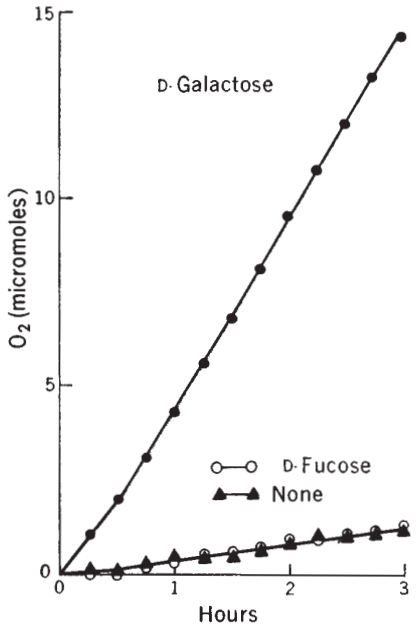


Fig. 12. Graph showing lack of oxidation of D-fucose. Wild-type bacteria (W3110), at a concentration of 8×10^8 bacteria per milliliter, were suspended in a Warburg flask containing 3 milliliters of chemotaxis medium to which D-fucose ($1 \times 10^{-2}M$) (open circles) or D-galactose ($1.5 \times 10^{-3}M$) (solid circles) was added, or to which no substrate was added (triangles), and uptake of oxygen was measured in a Warburg respirometer at 30°C. The results are expressed as micromoles of O_2 taken up per flask. The bacteria had been grown on D-galactose.

should be mutants that are defective in their response to some attractants but not to others, because of a defect in a single receptor. Such mutants of *Escherichia coli* have now been found by Hazelbauer and Mesibov (*24, 25*).

One mutant, defective in the "serine receptor," fails to be attracted to serine, except for a very weak response at the highest concentrations, and shows much-reduced taxis toward alanine, cysteine, and glycine. (These residual responses result from the "aspartate receptor.") The mutant is attracted normally to aspartate and glutamate, and to galactose, glucose, and ribose. It oxidizes and takes up L-serine at the same rate that its parent does.

Another mutant, lacking the "aspartate receptor," shows no chemotaxis toward aspartate and glutamate, and nearly normal taxis toward alanine, cysteine, glycine, serine, galactose, glucose, and ribose. The rate of oxidation and uptake of aspartate is the same for the mutant and its parent.

A third mutant, missing the "galactose receptor," is not attracted to galactose and fucose and is attracted to glucose at a higher-than-normal threshold ($3 \times 10^{-5}M$ instead of $4 \times 10^{-7}M$). (This response to glucose results from the "glucose receptor.") It is attracted normally to ribose, aspartate, and serine. At high concentrations of galactose, growth and oxidation are as fast for the mutant as for a strain, derived from the mutant, that has taxis toward galactose fully restored, but at low concentrations the rates are slower for the mutant. This slowness is caused by a defect in the uptake, rather than in the metabolism, of galactose.

The existence of these mutants argues for specific receptors and provides additional support for the idea that detection of the attractants is independent of their metabolism.

How Many Chemoreceptors?

To determine how many kinds of chemoreceptors there are, three approaches are being used. The first is to ask whether a given attractant is still effective when another attractant is present. The second is to try to isolate mutants defective in individual receptors. A third approach is to study the inducibility of specific taxes (presumably the inducibility of specific receptors) (*27*; see also *29*). For example, taxis toward galactose and fucose is inducible by galactose (*30*).

Table 4. Partial list of chemoreceptors in *Escherichia coli*.* [Data from this article and from *24, 27,* and *28*]

Attractant	Threshold† molarity
Galactose receptor	
D-Galactose	4×10^{-7}
D-Glucose	4×10^{-7}
D-Fucose	3×10^{-6}
Glucose receptor	
D-Glucose	3×10^{-6}
Ribose receptor	
D-Ribose	3×10^{-7}
Aspartate receptor	
L-Aspartate	6×10^{-8}
L-Glutamate	1×10^{-5}
Serine receptor	
L-Serine	2×10^{-7}
L-Cysteine	5×10^{-6}
L-Alanine	5×10^{-5}
Glycine	5×10^{-5}

* A more complete description of the specificity of each receptor is in preparation (*24, 27, 28*). † "Threshold" is the concentration of attractant at which bacterial accumulation in the capillary tube first exceeds background accumulation. It is determined by plotting the log of the number of bacteria accumulated relative to the log of the attractant concentration. The threshold value depends on whether or not the chemical is taken up and used (see discussion of Fig. 13 in text). The chemicals listed here were all taken up and, except for D-fucose, were all utilizable; therefore the actual thresholds are considerably lower than those listed.

The conclusion from results obtained so far (*24, 27, 28*) is that there are at least the five chemoreceptors shown in Table 4. There are probably no additional receptors for amino acids, since no amino acids besides those listed in Table 4 are strongly attractive (*28*). Among the monosaccharides, current work indicates that there is, in addition, a receptor specific for fructose. Among the disaccharides, research in progress indicates a receptor specific for maltose and another for trehalose. The attraction of bacteria to lactose probably results from chemotaxis toward the galactose (and possibly toward the glucose too) produced from the lactose; lactose itself is actually an extremely poor attractant (*27*). Oxygen is known to be an attractant for *Escherichia coli* (*3*), so there could be a receptor for it, but this question has not been investigated so far. A survey of possible other attractants or of repellents has not been completed.

It is conceivable that, besides chemoreceptors, at least some bacteria might have receptors specialized to detect light, gravity, or temperature, since all these stimuli are known to elicit tactic responses in some bacteria (*1*).

What is the nature of the chemoreceptors? One possibility is that they are the first enzymes in the metabolism of the chemicals. This possibility has been excluded in the case of galactose, because mutants that lack galactokinase still respond perfectly well. Another possibility is that the chemoreceptors are the permeases.

Role of Permeases

What role, if any, is played in chemotaxis by permeases and other components essential for transport of substances into the cell? To find out, mutants defective with respect to transport have been investigated from the standpoint of chemotaxis. It has been found in this study that the permeases and other transport-essential components that have been tested are not required for chemotaxis.

Figure 13 shows good attraction to galactose by an *Escherichia coli* mutant, 20S0K− (*20*), that is defective in the uptake of galactose (*20, 31*) to the extent of a 99.5 percent block (Fig. 14), owing to the absence of both galactose permease and methyl galactoside permease (*31*), and that, in addition, is unable to grow on, or to metabolize, galactose, as a result of a mutation in the gene for galactokinase. The threshold concentration for taxis toward galactose appears to be even lower (about $2 \times 10^{-9}M$) for the mutant than for strains that are wild-type with respect to galactose transport (compare Fig. 13 with Figs. 3 and 4); actually the thresholds are probably the same for the mutant and the wild-type bacteria, but the latter consume the galactose and in this way destroy the gradient.

Thus the two permeases most responsible (*31*) for transport of galactose in *Escherichia coli*—the galactose permease and the methyl galactoside permease—are not required for taxis toward galactose. A third permease that is in part responsible for transport of galactose, the thiomethyl galactoside permease II, is destroyed at 37°C (*32*). the temperature used for this experiment, and it was, moreover, not induced under the conditions of growth used. The lactose permease (thiomethyl galactoside permease I), normally capable of transporting galactose, does not transport galactose in this strain (*31*) and also was not induced under these conditions of growth. The small amount of galactose which does enter these mutant bacteria is known to be present

in a phosphorylated form (*31*), so it is probably transported by the phosphorylation system described next. Mutants blocked in this system carry out chemotaxis toward galactose normally.

A two-enzyme system which catalyzes the phosphorylation of glucose and certain other sugars (*15, 16*) is required for the transport of these chemicals (*16, 33*). Enzyme I catalyzes the phosphorylation of a heat-stable protein by phosphoenolpyruvate; enzyme II then catalyzes the transfer of phosphate from the heat-stable protein to the sugar, and there are specific enzyme II's for different sugars. Three mutants of *Escherichia coli* that lack enzyme I activity [X17 and X19 (*34*) and W327 (*35*)] and a mutant [1101 (*36*)] defective in the heat-stable protein all showed normal chemotaxis toward glucose. An *E. coli* mutant (W1895-D1) defective in the enzyme II activity that phosphorylates glucose and α-methyl glucoside (*37*) was attracted normally to these chemicals. Thus neither enzyme I, nor the heat-stable protein, nor enzyme II activity is required for this chemotaxis.

Neither are the permeases and related transport systems sufficient for chemotaxis: many chemicals for which transport mechanisms exist fail to attract bacteria. Examples are the following amino acids for which active transport is known in *Escherichia coli* (*38*): L-glutamine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-tryptophan, L-tyrosine, and L-valine.

The results presented so far show that the chemoreceptors are not the enzymes that catalyze the metabolism of an attractant, nor are they the parts of the permeases or related systems of transport that have been tested. It remains possible that the chemoreceptors are components of the transport machinery which are intact in the transport mutants studied, or they may be new entities whose special function is the detection of chemicals during chemotaxis.

As discussed above, the *Escherichia coli* mutant that is not attracted to galactose (the "galactose taxis mutant") is defective in the uptake of galactose. This suggests that there may indeed be some component of the transport machinery that plays a role in chemoreception. Since the serine and aspartate taxis mutants show normal uptake of serine and aspartate, respectively, chemoreceptors (at least the serine and aspartate receptors) must contain a component that is not involved in transport.

The chemoreceptors appear to be located somewhere on the "outside" of the cell, since mutants which fail to transport the attractants still are attracted to them. However, this can be offered only as a suggestion, in view of the fact that some amount of attractant would be able to enter even these mutant cells.

Further efforts to identify the chemoreceptors, and attempts to isolate them, are in progress.

How Do Chemoreceptors Work?

The mechanism of chemoreception in bacteria is completely unknown. Somehow the gradient of the chemical affects the receptors (*39*), and this in turn causes a change that directs the flagella. This change could be in the cell membrane—a change in conformation of the membrane or a change in membrane potential. [In the protozoa such changes in potential have been observed prior to the reversal of cilia or to emission of light (*40*).] The change might be propagated along the membrane so as to reach all the flagella. In fact, the base of the flagellum is in close association with the cell membrane (*41*). The flagella could then respond by changing their orientation in some way to bring about an avoiding reaction (see *42*).

We have isolated and reported on 40 mutants which fail to carry out

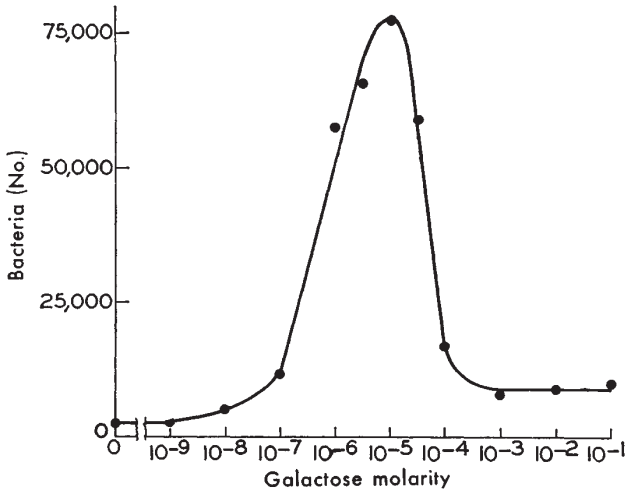


Fig. 13 (left). Graph showing chemotaxis toward galactose by a mutant, 20S0K⁻, defective in the uptake of galactose and also lacking galactokinase. The bacteria were grown on gycerol (*30*) at 37 °C. The experiment was carried out at 37°C and lasted for 1 hour.

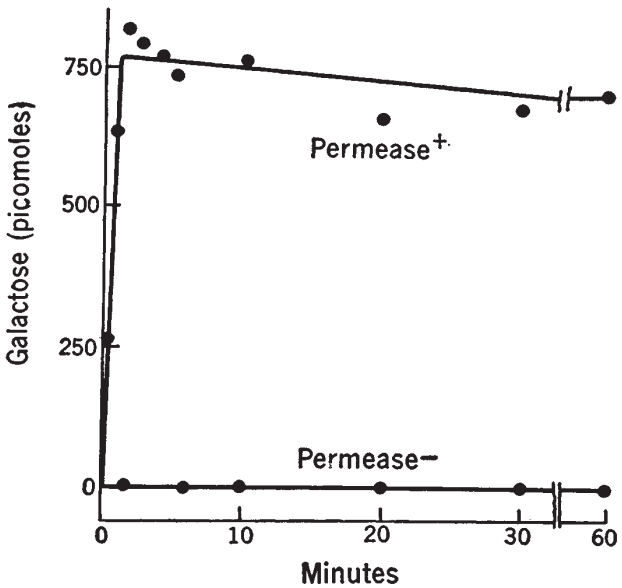


Fig. 14 (right). Graph showing uptake of galactose. About 5×10^8 bacteria were incubated in 1 milliliter of chemotaxis medium containing uniformly labeled C^{14}-galactose (*14*) ($10^{-6}M$; 7×10^4 counts per minute per milliliter), and at various times 0.05-milliliter samples were removed and washed on Millipore filters to measure the uptake of radioactivity. The permease⁺ strain is W3092 C, a mutant lacking galactokinase (*49*); the permease⁻ strain is 20S0K⁻, a mutant lacking galactokinase and defective in the uptake of galactose (*20*). Bacteria of both strains had been grown on glycerol. The results are expressed as picomoles of galactose taken up by bacteria in 1 milliliter of the incubation mixture.

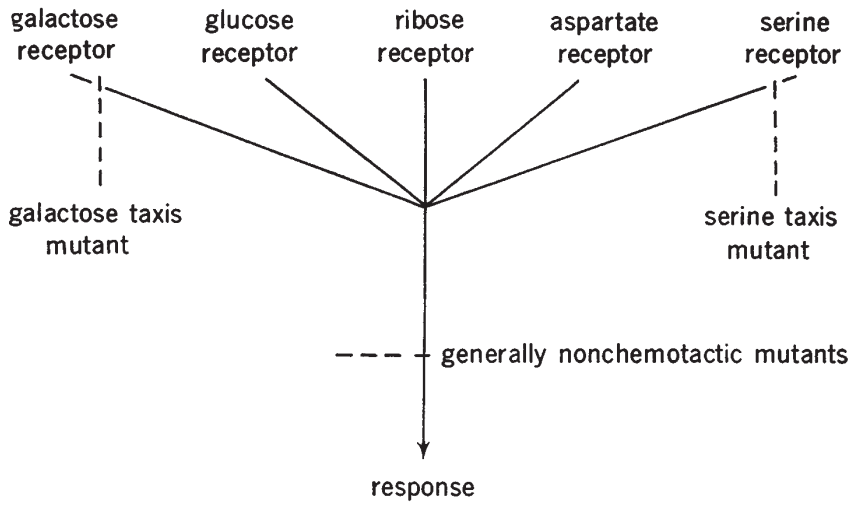


Fig. 15. The scheme for chemotaxis suggested in this article, showing location of defects in the various mutants.

chemotaxis toward any of the attractants—sugars, amino acids, or oxygen—though the bacteria are perfectly motile (*43*). Since it is unlikely that a single mutation would lead to a loss of all the kinds of chemoreceptors, these mutants are probably defective at some stage beyond the receptors, as shown diagrammatically in Fig. 15. The defect could be in a transmitting system through which information from all the receptors is channeled to the flagella, or in the responding mechanism itself. Genetic analyses of the mutants have shown that three genes are involved (*43*). Further studies of these mutants may lead to an understanding of the way in which the chemoreceptors direct the flagella.

Implications for Neurobiology and Behavioral Biology

The study of such stimulus-response systems in bacteria may have relevance for neurobiology and for behavioral biology of higher organisms. Possibly the chemoreceptors of bacteria are related to chemoreceptor sites in animal chemoreceptor cells, and perhaps knowledge of the way in which bacterial receptors function might lead to an increased understanding of the mechanism of smell and taste and other kinds of sensory reception (*44*). If there is an electrical signal that transmits information from the bacterial receptor to the flagellum, it might be similar to changes in membrane potential in higher organisms. The response of the flagellum to this signal may, in some ways, resemble the response of muscle to a nerve impulse.

The availability of behavioral mutants of bacteria—for example, mutants of the types reported here—together with the existence of a great body of knowledge about the genetics and biochemistry of *Escherichia coli*, should make the bacterial system a favorable one for studying simple forms of behavior and perhaps even some primitive kinds of "learning." From such studies might emerge a set of facts and concepts that can be applied to investigations of more complex phenomena in higher organisms.

Summary

Extensive metabolism of chemicals is neither required, nor sufficient, for attraction of bacteria to the chemicals. Instead, the bacteria detect the attractants themselves. The systems that carry out this detection are called "chemoreceptors." There are mutants that fail to be attracted to one particular chemical or to a group of closely related chemicals but still metabolize these chemicals normally. These mutants are regarded as being defective in specific chemoreceptors. Data obtained so far indicate that there are at least five different chemoreceptors in *Escherichia coli*. The chemoreceptors are not the enzymes that catalyze the metabolism of the attractants, nor are they the parts of the permeases and related transport systems that have been tested.

References and Notes

1. C. Weibull, *The Bacteria*, I. C. Gunsalus and R. Y. Stanier, Eds. (Academic Press, New York, 1960), vol. 1, p. 153.
2. J. Links, thesis, University of Leiden (1955) (in Dutch; summary in English); R. K. Clayton, in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1964), vol. 2, p. 51. Another possibility was that bacteria respond to a physical property of the attractant solution—for example, the viscosity or osmotic strength. This is ruled out by the fact that the responses are highly specific: only certain sugars and certain amino acids are attractive. In addition, the attractants are active at low concentrations such as $10^{-6}M$.
3. J. Adler, *Science* **153**, 708 (1966).
4. W. Pfeffer, *Untersuch. Botan. Inst. Tübingen* **1**, 363 (1884); *ibid.* **2**, 582 (1888).
5. A full account of the method will be given in J. Adler, "A quantitative method for studying chemotaxis," in preparation.
6. The capillary tubes are 1-microliter disposable micropipettes obtained from Drummond Scientific Company, Broomall, Pennsylvania. They are 3 centimeters long and have an internal diameter of 0.2 millimeter. One end was sealed in a flame. A solution was drawn into the capillary by passing the tube quickly through a flame several times and then immediately plunging the open end into the liquid. A chamber formed by laying a U-tube (bent from a 5-centimeter length of capillary tube, Kimax No. 34502, obtained from Owens-Illinois, Toledo, Ohio) between a microscope slide and a cover slip was filled with about 0.25 milliliter of a suspension of bacteria. The bacterial suspension (5×10^7 bacteria per milliliter) and the capillary both contained "chemotaxis medium": potassium phosphate buffer ($1 \times 10^{-2}M$) at *p*H 7.0; ethylenediaminetetraacetic acid ($1 \times 10^{-4}M$) to protect against inhibition of motility by heavy metal ions [J. Adler and B. Templeton, *J. Gen. Microbiol.* **46**, 175 (1967)]; and L-methionine ($1 \times 10^{-6}M$), which is an absolute requirement for chemotaxis in those strains unable to synthesize it [J. Adler and M. M. Dahl, *J. Gen. Microbiol.* **46**, 161 (1967)], and stimulates chemotaxis slightly even in wild-type strains. For all experiments reported in this article (chemotaxis experiments and others), the bacteria were grown at 35°C on the carbon and energy source indicated and then washed free of the growth medium, according to procedures described in J. Adler, *J. Bacteriol.* **92**, 121 (1966).
7. The incubations were carried out on a slide warmer obtained from Fischer Scientific Company, Pittsburgh, Pa.
8. All sugars mentioned in this article (including fucose) have the D- configuration, and all amino acids mentioned have the L- configuration.
9. For a recent reference on aspartase, see K. Liess, D. Mecke, H. Holzer, *Biochem. Z.* **346**, 244 (1966).
10. For a recent work on this enzyme, and a list of references, see L. Alföldi, I. Raskó, E. Kerekes, *J. Bacteriol.* **96**, 1512 (1968).
11. Strain W4690, prepared by E. M. Lederberg, carries the mutations *gal* 1, 2, and 22. The content of enzymes for the metabolism of galactose was measured by H. E. Echols.
12. J. A. Shapiro, thesis, University of Cambridge (1967); ——— and S. Adhya, *Genetics*, in press.
13. D-Galactose, already "substantially glucose-free" (from Sigma Chemical Company, St. Louis, Mo.) was separated from any remaining glucose or other impurities by descending chromatography on Whatman No. 40 paper in an ethyl acetate, pyridine, H_2O (120:50:40) system for 12 hours [see I. Smith, *Chromatographic and Electrophoretic Techniques* (Interscience, New York, ed. 2, 1960), vol. 1, p. 248]. To remove any galactose, glucose, or other impurities, chemicals as follows were purified by descending chromatography on Whatman No. 40 paper. D-Fucose (Sigma Chemical Company) and α-methyl glucoside (Pfanstiehl Laboratories, Waukegan, Ill.) were chromatographed in an *n*-butanol, ethanol, H_2O (5:1:4) system for 25 hours [see E. L. Hirst and J. K. N. Jones, *Discussions Faraday Soc.* **7**, 268 (1949)]; 2-deoxyglucose (Calbiochem, Los Angeles) was chromatographed in an isopropanol, H_2O (4:1) system for 27 hours [see I. Smith, *Chromatographic and Electrophoretic Techniques* (Interscience, New York,

ed. 2, 1960), vol. 1, p. 248]; and L-sorbose (Sigma Chemical Company) was chromatographed in an ethyl acetate, ethanol, H_2O (20:3:2) system for 90 hours (B. E. Butterworth, unpublished).
14. To remove an impurity oxidized by galactose⁻ bacteria, uniformly labeled C^{14}-D-galactose (Mallinckrodt Chemical Works, Orlando, Fla.) was purified by descending chromatography on Whatman No. 40 paper in an *n*-butanol, pyridine, H_2O (63:35:48) system for 30 hours [see B. Rotman, A. K. Ganesan, R. Guzman, *J. Mol. Biol.* **36**, 247 (1968)]; it was then rechromatographed in an ethylacetate, pyridine, H_2O (8:2:1) system for 18 hours (B. E. Butterworth, unpublished). D-Fucose-6-^{3}H (Calbiochem) was purified by descending chromatography on Whatman No. 40 paper in an ethyl acetate, pyridine, H_2O (8:2:1) system for 8 hours, to remove a radioactive impurity.
15. W. Kundig, S. Ghosh, S. Roseman, *Proc. Nat. Acad. Sci. U.S.* **52**, 1067 (1964).
16. W. Kundig, F. D. Kundig, B. Anderson, S. Roseman, *J. Biol. Chem.* **241**, 3243 (1966).
17. D. Rogers and S. Yu, *J. Bacteriol.* **84**, 877 (1962); W. Kundig, F. D. Kundig, B. E. Anderson, S. Roseman, *Fed. Proc.* **24**, 658 (1965).
18. D. G. Fraenkel, *J. Bacteriol.* **95**, 1267 (1968).
19. Uniformly labeled C^{14}-D-glucose was obtained from Volk Radiochemical Company, Burbank, Calif.
20. G. Buttin, *J. Mol. Biol.* **7**, 164 (1963).
21. The procedure was the same as that described in the legend to Fig. 8, except that D-Fucose-6-^{3}H (purified as described above) was used ($5 \times 10^{-4}M$, 2×10^5 counts per minute per milliliter) and chromatography was carried out in an *n*-butanol, ethanol, H_2O (5:1:4) system for 24 hours [see E. L. Hirst and J. K. N. Jones, *Discussions Faraday Soc.* **7**, 268 (1949)]. Wild-type (W3110) bacteria were grown on galactose or on mannose, and galactose⁻ bacteria (W4690), on mannose.
22. See, for example, H. Hagihira, T. H. Wilson, E. C. C. Lin, *Biochim. Biophys. Acta* **78**, 505 (1963); D. P. Kessler and H. V. Rickenberg, *ibid.* **90**, 609 (1964).
23. J. Adler and B. Templeton, *J. Gen. Microbiol.* **46**, 175 (1967).
24. G. L. Hazelbauer, R. E. Mesibov, J. Adler, *Proc. Nat. Acad. Sci. U.S.*, in press.
25. The serine taxis mutant was isolated from a strain (AW 405) that is wild-type with respect to chemotaxis, by repeatedly picking the center of the swarm that forms on a semisolid tryptone agar plate, according to the procedure described in J. B. Armstrong, J. Adler, M. M. Dahl, *J. Bacteriol.* **93**, 390 (1967). Several more such mutants were isolated similarly, but the tryptone was replaced by an amino acid mixture that had serine as the chief attractant. Likewise, the aspartate taxis mutant was isolated through use of an amino acid mixture that had aspartate as the chief attractant. The galactose taxis mutation was found in W3109 (gal_0^-), a galactose⁻ strain isolated by E. M. Lederberg [see *Microbiol. Genetics*, W. Hayes and R. C. Clowes, Eds. (Cambridge Univ. Press, Cambridge, England, 1960), p. 115)]. Strains able to metabolize galactose, derived from W3109 either by reversion or by transduction with the bacteriophage lambda dg, retain the galactose defect. The strain that has taxis toward galactose fully restored was isolated from such a strain that had acquired, by reversion, the ability to metabolize galactose.
26. W. Rothert, *Flora* **88**, 371 (1901).
27. J. Adler, G. L. Hazelbauer, M. M. Dahl, "Chemotaxis toward sugars by *Escherichia coli*," in preparation.
28. R. E. Mesibov and J. Adler, "Chemotaxis toward amino acids by *Escherichia coli*," in preparation.
29. R. W. Fleming and F. D. Williams, *Abstr. Amer. Soc. Microbiol.* (1968), p. 29.
30. Taxis toward galactose and fucose is much stronger when the bacteria have been grown on galactose than when they have been grown on glycerol. Growth on mannose, while not as effective as growth on galactose, gives very good taxis toward galactose and fucose. Galactose⁻ bacteria were generally grown on mannose, but in a few cases (strain 20S0K⁻, for example) growth of galactose⁻ bacteria on mannose resulted in very weak taxis toward galactose; in these cases growth on glycerol proved satisfactory.
31. B. Rotman, A. K. Ganesan, R. Guzman, *J. Mol. Biol.* **36**, 247 (1968).
32. L. S. Prestidge and A. B. Pardee, *Biochim. Biophys. Acta* **100**, 591 (1965).
33. R. D. Simoni, M. Levinthal, F. D. Kundig, W. Kundig, B. Anderson, P. E. Hartman, S. Roseman, *Proc. Nat. Acad. Sci. U.S.* **58**, 1963 (1967); H. R. Kaback, *J. Biol. Chem.* **243**, 3711 (1968).
34. Strains X17 and X19 were isolated by C. F. Fox from strain MO, an F⁻ derivative of strain Hfr H. He found (personal communication) that extracts of the two mutants contain no detectable amounts of enzyme I (less than 0.1 percent of the amount in MO). The doubling time of MO, X17, and X19, on glucose, was found to be 1.0, 8.5, and 8.5 hours, respectively. Chemotaxis experiments were carried out with bacteria grown on lactate. In such mutant cells in chemotaxis medium (see *6*) the rate of accumulation of radioactivity from glucose was found to be inhibited by 88 percent relative to the parental strain MO.
35. Strain W327 is a glucose⁻ mutant [M. Doudoroff, W. Z. Hassid, E. W. Putman, A. L. Potter, J. Lederberg, *J. Biol. Chem.* **179**, 921 (1949)] which fails to accumulate α-methyl glucoside [G. N. Cohen and J. Monod, *Bacteriol. Rev.* **21**, 169 (1957)]. Extracts of W327 lack detectable enzyme I activity (E. C. C. Lin and S. Tanaka, unpublished). The doubling time on glucose was found to be 15 hours. Chemotaxis experiments were carried out with bacteria grown on glycerol. In such mutant cells in chemotaxis medium the rate of accumulation of radioactivity from glucose was found to be inhibited by 91 percent relative to a wild-type strain W3110.
36. Strain 1101 has less than 2 percent of the heat-stable protein found in the parental strain (1100), according to C. F. Fox and G. Wilson, *Proc. Nat. Acad. Sci. U.S.* **59**, 988 (1968). The doubling time on glucose was found to be 1.2 hours for the parent and 8.5 hours for the mutant. Chemotaxis experiments were carried out with bacteria grown on lactate. In such mutant cells in chemotaxis medium the rate of accumulation of radioactivity from glucose was found to be inhibited by 78 percent relative to the parental strain.
37. Strain W1895-D1 was isolated by D. P. Kessler as a mutant resistant to α-methyl glucoside; the growth of the parent, W1895, is inhibited by α-methyl glucoside. Cells of the mutant, unlike those of the parent, have been shown by Kessler (personal communication) not to accumulate α-methyl glucoside to any appreciable extent and not to phosphorylate the compound. Extracts of this mutant are defective in the enzyme II system for phosphorylation of glucose and α-methyl glucoside, according to C. F. Fox and G. Wilson [*Proc. Nat. Acad. Sci U.S.* **59**, 988 (1968)]. The doubling time on glucose was found to be 0.8 hour for the parent and 2.8 hours for the mutant. Chemotaxis experiments were carried out with bacteria grown on lactate. Accumulation of α-methyl glucoside by such mutant cells in chemotaxis medium was found to be inhibited by 70 percent relative to the parental strain.
38. For a review of the literature, see J. R. W. Piperno, thesis, University of Michigan (1966).
39. Does a bacterium compare the difference in the concentrations of the attractant at its two ends, or the difference between the concentration in its present location and in the place where it was a short time ago? The latter interpretation appears to be correct for phototaxis: Engelmann discovered that phototactic bacteria, swimming randomly, all back up—that is, carry out an avoiding reaction—when uniform illumination suddenly is dimmed (*1*). No comparable experiment has as yet been carried out for chemotaxis. The following phenomenon must be taken into account in investigating the mechanism of chemoreception. Pfeffer found (see *1*) that bacteria "suspended in 0.01% meat extract were attracted by a 0.05% meat extract solution but not by weaker solutions. When the bacteria were suspended in a 1% extract solution, a 5% solution was required in order to cause a tactic response. Thus the Weber law was found to hold within the concentration range tested, the relative threshold being 500%." Similar results have been obtained by R. E. Mesibov for *Escherichia coli* undergoing chemotaxis toward aspartate and serine.
40. Y. Naitoh, *Science* **154**, 660 (1966); H. Kinosita and A. Murakami, *Physiol. Rev.* **47**, 53 (1967); R. Eckert, *Science* **147**, 1140 (1965); ———, *ibid.*, p. 1142; Y. Naitoh and R. Eckert, *ibid.* **164**, 963 (1969).
41. For a review of the literature, see W. van Iterson, J. F. M. Hoeniger, E. N. van Zanten, *J. Cell Biol.* **31**, 585 (1966).
42. The avoiding reaction (or shock reaction) (see *1*) may be described as follows. A bacterium that happens to swim from a higher into a lower concentration of attractant tumbles for a fraction of a second, or jumps back, and then it goes off in a new direction; in some species that can swim equally well forward or backward, the organism just swims away backward. If the new direction takes the bacterium farther into the region of low concentration of attractant, the avoiding reaction is repeated, but if the bacterium encounters a higher concentration it continues to swim in the new direction. Thus the bacteria avoid low concentrations and accumulate in the region of higher concentration.
43. J. B. Armstrong, J. Adler, M. M. Dahl, *J. Bacteriol.* **93**, 390 (1967); J. B. Armstrong and J. Adler, *ibid.* **97**, 156 (1969); ———, *Genetics* **61**, 61 (1969).
44. In this connection, it is noteworthy that sensory receptor cells of animals generally contain, or are derived from, flagella (or, what is essentially the same thing, cilia); indeed, the suggestion has been made that animal receptors have evolved from single-celled, flagellated organisms [see J. A. Vinnikov, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 293 (1965); R. M. Eakin, *ibid.*, p. 363].
45. J. Adler, *J. Bacteriol.* **92**, 121 (1966).
46. Essentially the same results were obtained when chemotaxis toward each of these chemicals was tested with bacteria grown on galactose (in the case of the sugar derivatives) or glycerol (in the case of the citric acid cycle compounds). In the case of glucose, bacteria grown on galactose were used for the experiment shown, since bacteria grown on glucose have poor motility (see *23*) and respond to glucose poorly, with a high threshold. In every case the bacteria were examined with the microscope for motility, and they were tested for their response to glucose or aspartate to make sure they were capable of carrying out chemotaxis. As in the case of bacteria grown on glucose, bacteria grown on gluconate or glucuronate had poor motility, and their response to glucose was poor.
47. The flasks were obtained from Kontes of Illinois, Franklin Park, Ill.
48. H. Jeffay and J. Alverez, *Anal. Chem.* **33**, 612 (1961).
49. H. C. P. Wu and H. M. Kalckar, *Proc. Nat. Acad. Sci. U.S.* **55**, 622 (1966); H. C. P. Wu, *J. Mol. Biol.* **24**, 213 (1967).
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