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The Gradient-Sensing Mechanism in Bacterial Chemotaxis

(temporal gradient apparatus/stopped-flow/*S. typhimurium*/motility tracks/memory)

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ABSTRACT A "temporal gradient apparatus" has been developed that allows the motility of bacteria to be studied after they have been subjected to a sudden change from one uniform concentration of attractant to another. A sudden decrease elicits the tumbling response observed with spatial gradients; it was found, however, that a sudden increase also elicits a response, namely supercoordinated swimming. This demonstrates that chemotaxis is achieved by modulation of the incidence of tumbling both above and below its steady-state value. The initial responses gradually revert to the steady-state motility pattern characteristic of a uniform distribution of attractant. The apparent detection of a spatial gradient by the bacteria therefore involves an actual detection of a temporal gradient experienced as a result of movement through space. Potential models for the chemotactic response based on some "memory" mechanism are discussed.

The phenomenon of chemotaxis occurs widely in biological systems. Its presence in bacteria was detected by Pfeffer (1) in 1881, and has been clarified further in recent years, in particular by the recent studies of Adler and his coworkers (2, 3). In many ways bacterial chemotaxis appears analogous to sensory reception in higher species as in (*a*) the specificity of the response to attractants (2, 4), (*b*) the indication that the receptor molecules are located in the outer membrane (3, 5), and (*c*) the sensitivity of the response to ratios of concentrations rather than to differences (1, 6).‡ However, bacterial chemotaxis poses a special problem: how can such a small organism detect the concentration differences necessary to sense a gradient in space?

The "size problem" in relation to gradient sensing can be readily calculated. In an exponential gradient with a decay distance of 20 mm, the difference in concentration of attractant at the two extremes of a 2-μm long bacterium is only 1 part in 10^4. Since bacteria respond strongly in such a gradient, an analytical device capable of discerning 1 part in 10^4 is required if the sensing system simply utilizes spatial separation. A further problem arises in relation to the statistical fluctuations of attractant in the vicinity of the receptors. Assuming hypothetical sampling volumes of 1 μm $\times$ 1 μm $\times$ 0.1 μm near the "head" and "tail" of a bacterium, only 60 molecules of attractant would be present at 1 μM, yet chemotaxis is known to occur at such concentrations. The standard deviation of 60 molecules is $\pm\sqrt{60}$, showing that statistical fluctuations can be much greater than the needed accuracy.

‡ Further evidence for the fact that bacteria sense ratios of concentrations has been provided by Mesibov, R., Ordal, G. W. & Adler, J. (personal communication).

The difficulties of an instantaneous spatial comparison are removed if one postulates a mechanism for comparison of concentrations over a time interval (7). Since there are indications that time-dependent processes may be present in phototactic organisms (8), and are present in higher neural processes (9), it seemed worthwhile to test the chemotactic system for the ability to make temporal comparisons. The difficulty was to devise an experimental method that isolated the time dependence from ambiguities of spatial sensing.

We accomplished this by developing a "temporal gradient apparatus," analogous to the stopped-flow apparatus of chemical kinetics. In this apparatus (Fig. 1), the bacteria initially present in a uniform attractant concentration (C_i) are plunged by a rapid mixing device into a final uniform concentration (C_f). They are then observed by microscopic and photomicrographic techniques. Since the bacteria are observed only after mixing is complete, they will respond as if they are in a uniform environment if they utilize instantaneous spatial sensing, whereas they will respond as if they are in a gradient if they utilize temporal gradient sensing, provided the mixing time is short compared to their time-dependent response.

MATERIALS AND METHODS

Salmonella typhimurium, strain LT2-S2, was taken from a stationary culture in nutrient broth and grown overnight at 30° with agitation in Vogel-Bonner citrate medium (10), with 1% w/v glycerol as an additional carbon source.

The bacteria were observed in dark-field and photographed with a stroboscopic high-pressure xenon-arc lamp. By a suitable choice of flashing rate (typically in the range 3–5 Hz), successive images of a bacterium generate its motility track across the photograph. A similar technique has recently been described for paramecium motility measurements (11). Open-shutter photography had been used in the very early studies of Harris (12).

The temporal gradient apparatus is shown schematically in Fig. 1. Both bottles *A* and *B* were charged with medium (1% glycerol in citrate medium). The bacterial culture was added to bottle *B* to give about 2×10^7 cells per ml, and the bottle was stirred continuously to aerate the culture. Attractant was added to either bottle *A* or *B*, or both, depending on whether a positive, negative, or zero gradient was desired. The two bottles were connected via a peristaltic pump to the inlets of the rapid-mixing device. Use of capillary tubing was minimized; a pump with widely-spaced rollers was used to reduce damage to the bacteria. The mixing tube, 25-mm long (0.9 mm inside diameter), contained two strands of no. 32-gauge wire twisted together as shown in Fig. 1 to provide

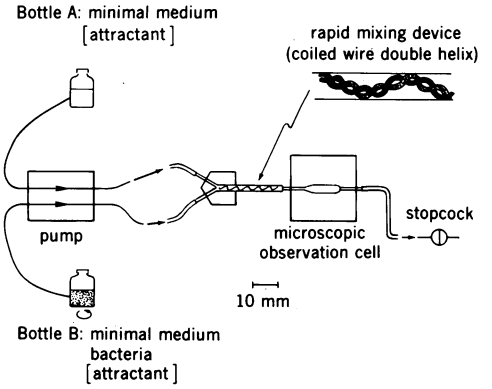

FIG. 1. Schematic illustration of temporal gradient apparatus. Attractant concentrations are: (*i*) Bottle *B*, C_i (≥ 0) (*ii*) bottle *A*, C_i' ($>$, $=$ or $<C_i$) (*iii*) observation cell (as a result of stream mixing) C_f ($>$, $=$, or $<C_i$). Bacteria experience $C_i \rightarrow C_f$, and thus can be subjected to positive, zero, or negative temporal gradients as desired. Gradient is given by $\Delta C/\Delta t$, where $\Delta C = C_f - C_i$ and Δt is mixing time.

effective mixing. Residence time in the mixing tube was about 0.2 sec, and observation commenced about 0.5 sec after flow was stopped. The observation cell consisted of microscope cover slips or slides separated by lucite or Teflon spacers. Flow was stopped by switching off the pump and closing the stopcock. Repeated observations could be made with one filling of bottles *A* and *B*.

RESULTS

Control Experiments (Zero Gradient of Attractant). Three types of control experiment have been done: (*i*) no attractant in either stream, (*ii*) attractant (L-serine) at the same concentration in both streams, and (*iii*) nonattractant in the bacterial stream (e.g., L-histidine at various initial concentrations ranging from 10 nM to 1 mM). In all three cases, motility after stoppage of flow was as follows: bacteria swam in fairly straight lines; slight changes in their direction, achieved by a twitching movement, occurred often; occasionally a bacterium would tumble and then start swimming again in a completely new direction. The overall impression was one of coordinated motility that did not change over a long period of observation, i.e., no relaxation process was observed. This pattern is the same as that of bacteria, in a uniform medium, that have not been subjected to the mixing process. A stroboscopic multiple-exposure photograph of such a control is shown in Fig. 2 (*middle*).

A minority (10–20%) of the population was either totally nonmotile or had severely impaired motility. Such impaired motility is observed in any bacterial population, although the proportion may be somewhat higher in the present case as a result of mechanical damage.

Positive Gradient of Attractant. In a typical experiment, L-serine was present at 1 mM in the nonbacterial stream and was absent from the bacterial stream. With a total flow rate of about 2 ml min^{-1} contributed by the two streams in the ratio 3.2:1 the serine in the bacterial environment rose, in about 200 msec, from zero to 0.76 mM. The motility of the bacteria, when flow was stopped, was much smoother and better-coordinated than normal [Fig. 2 (*upper*)]. Gradually, the slight aberrations in movement characteristic of normal motility were restored, the interval for this relaxation process being as long as 5 min for some concentration changes.

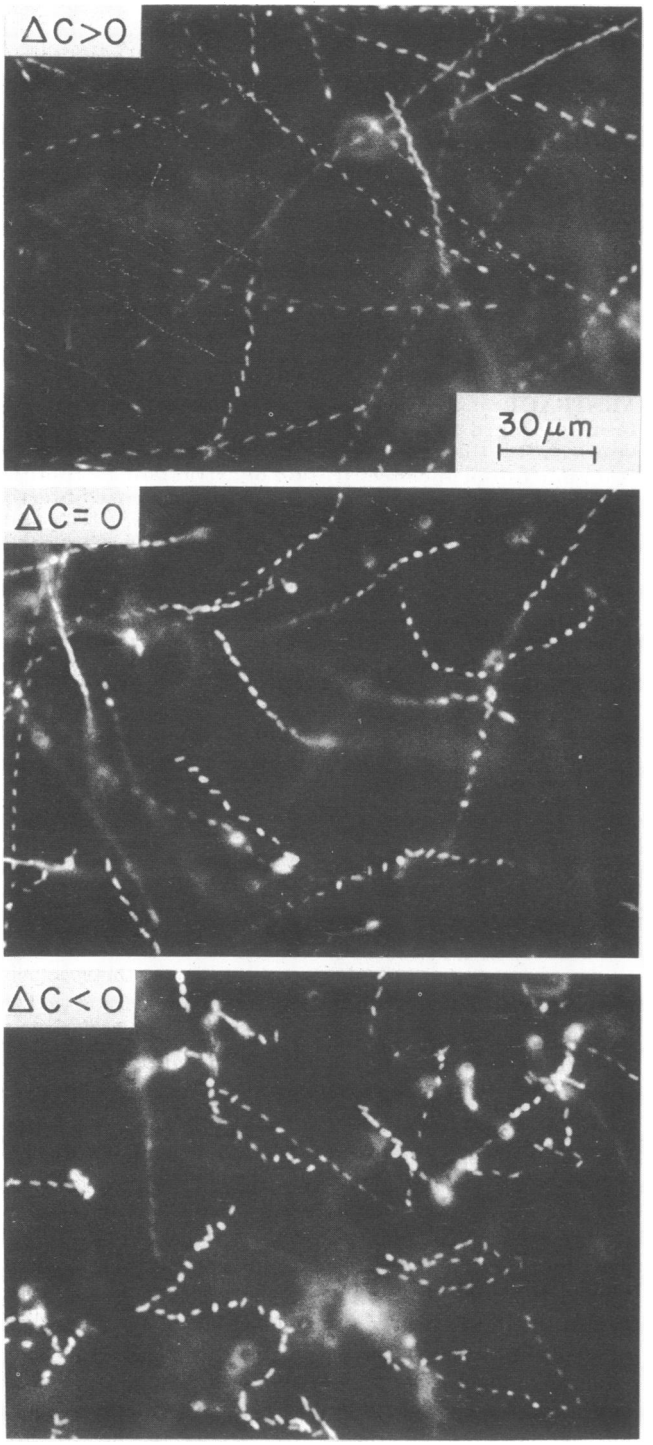

FIG. 2. Motility tracks of *S. typhimurium*, taken in the time interval 2 → 7 sec after subjection of bacteria to a sudden (200 msec) change in attractant (serine) concentration in the temporal gradient apparatus. *Upper:* $C_i = 0$, $C_f = 0.76$ mM. Smooth, linear trajectories. *Middle:* $C_i = C_f = 0$ (control). Some changes in direction; bodies often show "wobble" as they travel. Bright spots indicate tumbling or nonmotile bacteria. *Lower:* $C_i = 1$ mM, $C_f = 0.24$ mM. Poor coordination; frequent tumbles and erratic changes in direction. (Photomicrographs were taken in dark-field with a stroboscopic lamp operating at five pulses sec^{-1}. Instantaneous velocity of bacteria in straight line trajectories is of the order of 30 $\mu m\ sec^{-1}$.)

Negative Gradient of Attractant. With 1 mM serine in the bacterial stream, none in the nonbacterial stream, and the flow conditions described above, the bacteria experienced a sudden fall from 1 to 0.24 mM serine. After flow stopped, they displayed erratic behavior [Fig. 2 (*lower*)] involving frequent tumbling and changes of direction. Coordination seemed almost completely lacking in the initial interval but gradually returned, so that after 12 sec motility was indistinguishable from the control experiments. The recovery of normal motility was fairly synchronous throughout the population.

Velocity at Different Uniform Concentrations of Attractant. To examine the effect of absolute concentration, the velocities of bacteria were measured with uniform spatial distributions (zero gradient) at different absolute concentrations. The results, as shown in Table 1, reveal that the velocities of the bacteria are essentially unchanged by absolute levels of attractant in the range of the experiments reported here. Moreover, there was no relaxation process when the bacteria were observed over long intervals.

Assessment of the Validity of These Observations. The temporal gradient experiments described above have been repeated many times by different observers. For example, two different observers examined 15 cases in which zero, positive, or negative gradients were randomly selected in a blind experiment; both correctly identified all cases. Direct visualization offers the most complete description of the phenomenon, but photomicrographs provide further objective evidence. In addition to serine, aspartate and ribose have been tested and found to elicit responses in the temporal gradient apparatus, in agreement with their known functions as chemotactic attractants (2).

The conclusion that a time-dependent process is important in chemotaxis has been reached independently by Toothman and Dahlquist, using a totally different approach (personal communication).

DISCUSSION

The results of the investigations we describe lead to the conclusion that bacteria detect gradients by the use of a temporal sensing mechanism, i.e., they have some sort of "memory" device that compares present and past environmental concentrations. This conclusion is based on four main observations: (*i*) The mechanical turbulence of the temporal gradient apparatus is not responsible for the observed abnormal motility, since a zero gradient experiment leads to normal motility. (*ii*) The bacteria show normal motility in a uniform distribution of attractant in the range measured, namely 0–1 mM serine.* (*iii*) When the bacteria are suddenly thrust from one uniform concentration to another and are then observed in a uniform spatial gradient, they initially respond abnormally—i.e., a positive temporal gradient leads to supercoordinated motion, a negative temporal gradient leads to lack of coordination and frequent tumbling. (*iv*) The abnormal motions themselves have a time dependence, i.e., they "relax" to normal motility as would be expected if a time comparison were involved. Thus, the apparent sensing of a chemical gradient in space is actually achieved by a translation through space coupled to a sensing of concentration as a function of time.

TABLE 1. *Motility of S. typhimurium at constant attractant levels*

Serine concentration (mM)	Velocity of bacteria in sample (μm sec^{-1})	Overall average velocity (μm sec^{-1})
0	27.4 ± 4.7	
	29.9 ± 6.0	28.8
	29.0 ± 4.6	
0.01	27.6 ± 4.7	
	28.7 ± 3.5	27.2
	25.2 ± 6.0	
1.0	30.2 ± 4.0	
	29.0 ± 2.9	28.8
	27.1 ± 6.0	

Procedure: each sample represents 15 bacterial trajectories taken from a single photograph. The trajectories each contained 10 successive images of a bacterium taken at $^1/_8$-sec intervals. Three samples were examined for each set of conditions.

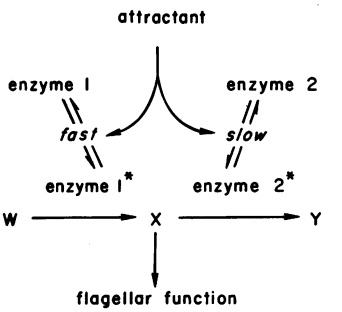

FIG. 3. Schematic illustration of one possible time-dependent mechanism. Attractant alters conformation of enzymes 1 and 2 to catalytically more active forms, enzyme 1 rapidly and enzyme 2 slowly. The compound *X*, which controls flagellar function, therefore tends to increase in positive gradients, decrease in negative gradients, and remain unchanged in zero gradients.

The utilization of a time-dependent process allows the bacterium to overcome the two difficulties mentioned in the *Introduction.* In the first place, by integrating over time it can overcome the problem of statistical fluctuations, even at very low attractant concentrations. Secondly, by comparing concentrations over substantial intervals of time (and, hence, space), it obviates the problem of its own small size. For example, if the bacterium possesses a memory with a decay time of 1 min, and is travelling at 30 μm sec^{-1}, the effective distance over which the concentration comparison is being made is about 2 mm, or roughly 1000 body-lengths. The needed analytical accuracy discussed previously is then reduced from 1 part in 10^4 to 1 part in 10.

How does the bacterium use this time-dependent process to migrate towards higher attractant concentrations? Previous workers have noted the tumbling of the bacteria and suggested some type of biased random-walk mechanism (13, 14). The present results support such a mechanism, and allow us to form a reasonably good picture of the chemotactic process.

* The detailed motions of bacteria have been recorded quantitatively by a tracking device. Also, population diffusion at a sharp boundary of attractant has been studied. Both sets of data provide further quantitative support for the conclusion that absolute uniform concentrations of attractant affect motility slightly, if at all (Dahlquist, Lovely, and Koshland, manuscript in preparation).

Bacteria travelling up an attractant gradient in space sense a positive temporal gradient and tumble less frequently than normal, while bacteria travelling down the gradient sense a negative temporal gradient and tumble more frequently than normal. Each tumble disorients the organism and starts it in a random direction. Since this randomization of direction occurs more often when it is travelling down a gradient, the net effect is that more time is spent travelling up—hence the chemotactic migration. The term "avoidance response" has been used to describe the loss of coordination, but we feel this is misleading since the new direction is taken randomly and is not, therefore, a direct avoidance such as occurs with other organisms [e.g., spirillum phototaxis (8)]. It should also be noted that since the frequency with which tumbles occur is modulated by positive as well as negative gradients, the term phobotaxis is not strictly applicable.

It remains to consider what mechanism for sensing temporal gradients could be developed in such a simple organism. In order to compare values of a parameter at different times, two component responses to that parameter are required, with different relaxation times. This can be expressed roughly by saying that the fast component response reflects the present value of the parameter, while the slow component response reflects the past value. To generate the differential response, the component responses must then act in a subtractive or opposing manner on yet another parameter, whose value determines the ultimate response, in this case loss of flagellar coordination. Fig. 3 offers one possible scheme of this type. The effector (either the attractant itself or a species generated by it—possibly the attractant–chemoreceptor complex) activates both enzymes 1 and 2 by inducing conformational changes, which are rapid in enzyme 1 and slow in enzyme 2. These enzymes catalyze the synthesis and degradation, respectively, of compound X, whose pool size must exceed a critical value for the flagella to function in a coordinated manner. In a positive gradient of attractant, enzyme 1 will be more highly activated than enzyme 2, the pool size of X will rise, and tumbling will diminish. In a negative gradient the pool size of X will be depleted and consequently tumbling will increase. It must be emphasized that this example is only one of a number of possible permutations of this general idea. For example, the enzyme roles could be reversed if high rather than low concentrations of X were responsible for loss of coordination. The response might be achieved by diffusion or transport processes across the cell membrane rather than by conformational changes in enzymes. All such models, however, must involve the elements of a temporal comparison.

The results of these studies imply that bacteria have some type of rudimentary "memory" in the sense that they retain and use information about past events. Moreover, bacteria respond to an external stimulus operating on specific receptors that trigger an appropriate motor response. Analogies can thus be made to neural systems operating in higher organisms. It remains to be seen whether such analogies are purely formal or whether there are mechanistic features in common.

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