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Temporal Stimulation of Chemotaxis in *Escherichia coli*

(bacterial taxis/temporal gradients/alanine aminotransferase/three-dimensional tracking)

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ABSTRACT **We used the tracking microscope to study the chemotactic responses of *E. coli* to temporal gradients of L-glutamate generated in isotropic solutions by the action of the enzyme alanine aminotransferase. Positive gradients suppress directional changes which occur spontaneously in the absence of a stimulus. Negative gradients have little effect. The data can be fit with a model in which the suppression is proportional to the time rate of change of the fractional amount of chemoreceptor bound. The model accounts for the behavior of individual cells and populations of cells in spatial gradients. A computer simulation of the motion in spatial gradients indicates that if the bacteria have a "memory," its decay time cannot be much longer than a few seconds. The relationship between the responses observed in these experiments and in experiments in which solutions of an attractant at different concentrations are mixed is discussed.**

Tactic responses in bacteria occur when the intensity of a spatially uniform stimulus changes with time. This was shown for phototaxis and for chemotaxis by Engelmann in 1883 (1) and for thermotaxis by Metzner in 1920 (2). Engelmann found that when a uniformly illuminated preparation of his *Bacterium photometricum* was suddenly darkened, every bacterium backed up, stopped, and then resumed its normal motion. An identical response was observed when the preparation was suddenly exposed to CO_2. Metzner examined the motion of *Spirilla* in a thin chamber which could be uniformly heated or cooled. Some species responded by shuttling back and forth only when the temperature was lowered; others did so only when it was raised.

The possibility that tactic responses might also be due to spatial variations in the intensity of the stimulus over the length of a cell was discussed by Rothert in 1901 (3), but he decided in favor of the temporal mechanism on the basis of Engelmann's results. He predicted that tactic responses should occur when solutions containing a chemotactic agent at different concentrations are mixed, but he supposed that mechanical currents and local differences in concentration present during the mixing would invalidate the results. In recent studies with *Salmonella*, Macnab and Koshland (4) showed that transient changes in the motion of the bacteria could be observed after the mixing was complete. When the concentration of an attractant was suddenly increased, the bacteria changed direction less frequently; when it was decreased, they changed direction more frequently. Repellents produced the opposite effects (5).

In our earlier work on chemotaxis in *Escherichia coli* (6) we followed the motion of individual cells in spatial gradients of serine and aspartate. When the bacteria moved up these gradients they changed direction less frequently; when they moved down, their motion was largely unperturbed. However, we were not able to determine the functional dependence of the response on the stimulus; the analysis was complicated by the fact that the stimulus depended on the motion of the cells.

In the experiments described here, the concentration of an attractant is changed enzymatically. The medium is homogeneous and isotropic; therefore, the stimulus is independent of the motion of the cells. We use alanine aminotransferase to generate or to destroy L-glutamate via the reaction:

$$\text{L-alanine} + \text{2-oxoglutarate} \rightleftharpoons \text{pyruvate} + \text{L-glutamate}. \quad [1]$$

Glutamate is sensed by the aspartate receptor and alanine by the serine receptor, so the experiments are done with the serine taxis mutant AW518 (7). Neither 2-oxoglutarate nor pyruvate is chemotactically active (7).

We observe a response when glutamate is generated but not when it is destroyed. The data in positive gradients are best explained with a model, suggested by the work of Mesibov, Ordal, and Adler (8), in which the suppression of directional changes is proportional to the time rate of change of the fractional amount of chemoreceptor bound.

MATERIALS AND METHODS

Reagents. All solutions were prepared from reagent-grade chemicals and glass-distilled water. L-[*U*-^{14}C]alanine and L-[*U*-^{14}C]glutamic acid (Amersham Searle) were diluted with the corresponding nonradioactive amino acids (Calbiochem A grade). Solutions of L-glutamic acid and 2-oxoglutaric acid (Calbiochem A grade) were neutralized with sodium hydroxide. Alanine aminotransferase (EC 2.6.1.2; Sigma G-9880) from pig heart was obtained in suspension in 1.8 M ammonium sulfate and dialyzed 10 hr at 4° against 10^{-2} M potassium phosphate (pH 7.0), 10^{-3} M ethylenediaminetetraacetate (EDTA) and 10^{-3} M 2-mercaptoethanol and for an additional 10 hr against 10^{-2} M potassium phosphate (pH 7.0), 10^{-4} M EDTA. The dialyzed material was divided into 0.2-ml aliquots and stored under nitrogen at −20°. When stored in this way the enzyme was stable for at least 6 months. On days when kinetic or tracking experiments were to be run, an aliquot was thawed and diluted 3- to 10-fold with 2×10^{-5} M pyridoxal phosphate (Calbiochem A grade) in tracking medium, namely, 10^{-2} M potassium phosphate (incorrectly identified in ref. 6 as sodium phosphate), 10^{-4} M EDTA, and 0.18% hydroxypropyl methylcellulose (Dow Methocel 90 HG). This solution was stored at 4° and warmed

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to 32° shortly before use. Its activity was checked with a modified Sigma-Frankel assay (9); the incubation time was shortened to 10 min at 32°, and the absorbance was read at 490 nm.

Enzyme Kinetics. The forward and reverse reactions were studied under the conditions used in the tracking experiments by following the deamination of [^{14}C]alanine and [^{14}C]glutamate, respectively. In a typical measurement of the forward reaction, a 0.25-ml sample of the enzyme solution (see above) was added to 4.75 ml of tracking medium containing 0.1–10 mM L-[^{14}C]alanine and 1–20 mM sodium 2-oxoglutarate at 32°. The conversion of alanine to pyruvate was monitored by withdrawing 0.5-ml aliquots and stopping the reaction by mixing with 0.1 ml of 0.1 M acetic acid. The alanine and the pyruvate in each mixture were separated on a column of DEAE-cellulose (Bio-Rad Cellex-D) by elution with 1.3×10^{-2} M sodium acetate (pH 4.7), and the total amount of [^{14}C]alanine in the eluent was measured with a scintillation counter. Measurements of the reverse reaction were made in a similar fashion; the initial solution contained 1–4 mM sodium L-[^{14}C]glutamate and 1–20 mM sodium pyruvate (Sigma Type II); 0.1 M citric acid was used to stop the reaction; the columns were eluted with 10^{-2} M sodium citrate (pH 3.9); the [^{14}C]glutamate was counted.

Capillary Assays. Chemotactic accumulations were measured by the method of Adler (10) in which one counts the number of bacteria that swim from a suspension into a capillary tube filled with an attractant. Sensitivity assays were done by adding attractant to the suspension, as well, at a concentration 0.316 times the initial concentration in the tube. Sensitivity curves (8) were obtained by repeating these assays at a number of different concentrations. Taxis-inhibition experiments (7) were done by adding a fixed concentration of a second substance both to the tube and to the suspension. All assays were run in duplicate in tracking medium at 32°; the bacteria were suspended at an optical density of 0.01 (590 nm).

Tracking Chamber. The chamber was made from a tantalum cylinder (2.5 mm inside diameter, 2-mm high, with an inlet pipe on one side near the bottom and an outlet pipe on the opposite side near the top) and two glass windows. The inlet and outlet pipes were connected to a vial containing the tracking mixture and to a valve on a vacuum line, respectively. The chamber and the vial were maintained at 32.0°. When the valve was opened the tracking mixture was drawn through the chamber at the rate of 0.05 ml/sec. Bacteria tracked after subjection to this flow were indistinguishable from bacteria drawn into the chamber at a rate of only 0.002 ml/sec. If the vial was replaced by another containing a different solution and the valve was opened for 10 sec, the displacement of the first solution by the second was at least 99% complete, and no spatial inhomogeneities could be detected in the region of the chamber in which the bacteria were tracked (experiments done with tracking medium and tracking medium containing 0.2% methylene blue by the optical absorption methods of ref. 11).

Tracking Procedures. Cultures of *E. coli* were grown and washed as described before (6) and suspended at an optical density of 0.01 (590 nm; about 10^7 bacteria per ml) in tracking medium containing either L-alanine and 2-oxoglutarate or pyruvate and L-glutamate. This mixture was divided into aliquots in Pyrex vials, which were incubated at 32° for at least 20 min before tracking. Up to 0.2 ml of the enzyme solution was added to a vial (final volume of the mixture 1.0 ml), the vial was rocked gently for 5 sec, the vacuum valve was opened for 10 sec, and the tracking was begun. Controls were handled identically, except that the enzyme was omitted. Positive and negative concentration jumps were studied by a similar procedure. For positive jumps, the solution added just before tracking contained L-glutamate. For negative jumps, the vial contained tracking medium and the solution added contained bacteria and L-glutamate. Controls were done by omitting the glutamate. As many as 200 bacteria from 30 different vials were tracked on a given day, each for 6–30 sec. The data acquisition system is described elsewhere (12, 13).

Data Analysis. The analysis which distinguishes runs (intervals during which changes in direction are gradual) from twiddles (intervals during which changes in direction are abrupt) is described in detail elsewhere (13). In the enzyme experiments we looked for a dependence of the mean run length (τ, defined in ref. 13) on several different functions (f) of the glutamate concentration (C) and of its time rate of change (dC/dt). The values of C and dC/dt for a given bacterium were computed from the kinetic equations (see *Results*); the corresponding values of the f's were computed from the mean values of these quantities.

For each function f a plot was made of the data points ($\ln\tau$, f) obtained from all the bacteria studied on a given day. We plotted $\ln\tau$ (rather than τ) as a function of f because the frequency distribution of $\ln\tau$ (for cells from the same culture under identical environmental conditions) is approximately normal (13). If the environmental conditions change (e.g., if serine is added, ref. 6, Fig. 5) the mean of this distribution changes, but its standard deviation remains approximately constant. Therefore, in analyzing the plot we assumed that the values of $\ln\tau$ were distributed normally about their mean ($\langle\ln\tau\rangle$) for any given value of f, but that $\langle\ln\tau\rangle$ varied with f. Equations (models) describing possible dependences of $\langle\ln\tau\rangle$ on f were then fit to the data by the method of Least Squares (ref. 14, p. 78), i.e., the parameters in the equations were adjusted to minimize the experimental standard deviations (σ) of the values of $\ln\tau$ about $\langle\ln\tau\rangle$.

Five different f's were tested (see *Results*). The equations used to fit the data were

$$\langle\ln\tau\rangle = \ln\tau_0 + \alpha f^\beta \quad [2]$$

and

$$\langle\ln\tau\rangle = \ln\tau_0 + \ln(1 + \alpha f^\beta), \quad [3]$$

where β is a constant and $\ln\tau_0$ and α are parameters determined by the Least Squares' fit. The best model was assumed to be the one for which σ was the smallest. The Likelihood Ratio test (ref. 14, pp. 234–237, 257–260) was used to estimate the probability (p) that a model with a larger σ might describe the results of future experiments equally well; we considered such a model to be significantly worse than the best model if $p < 0.01$.

RESULTS

Reaction Kinetics. The concentration of glutamate was given in the forward reaction by

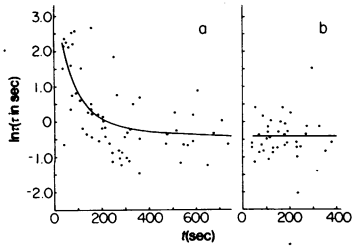

FIG. 1. (*a*) The logarithms of the mean run lengths of *E. coli* (AW518) in positive temporal gradients as a function of the time after the addition of the enzyme. Each point was derived from the analysis of one bacterium. The bacteria were obtained from the same culture. Glutamate was generated in accord with Eq. 4 with $C_e = 3.1$ mM and $T = 213$ sec. The *solid curve* is the function $\langle \ln\tau \rangle = \ln\tau_0 + \alpha(dP_b/dt)$, with $\ln\tau_0 = -0.4$ and $\alpha = 660$ sec; see the *text*. (*b*) Data from control experiments in which the enzyme was omitted.

$$C_{(t)} = C_e[1 - \exp(-t/T)], \quad [4]$$

and in the reverse reaction by

$$C_{(t)} = C_e[1 + 5\exp(-t/T)], \quad [5]$$

where t is the time measured from the time of addition of the enzyme, C_e is the concentration of glutamate measured at equilibrium, and T is a decay time. Values of T accurate to within 5% were determined from linear fits to plots of $\ln|C_e - C_{(t)}|$ versus t. The kinetics were the same when the reactions were run in suspensions of *E. coli*. Strain AW518 at an optical density of 0.01 (590 nm) changed the concentrations of the various substrates by at most 10^{-4} M/hr.

TABLE 1. *Relative goodness of fit of various functions (f) of C and dC/dt to values of lnτ obtained in positive gradients of glutamate*

f	σ*	p†
0	0.99	$\ll 10^{-5}$
C	0.99	$\ll 10^{-5}$
dC/dt	0.83	$<10^{-3}$
$(dC/dt)/C$	0.84	$<10^{-4}$
dP_b/dt‡	0.80	1

The fits were made to data obtained in two experiments in which glutamate was generated in accord with Eq. 4 and in the corresponding controls in which the enzyme was omitted. The bacteria (*E. coli* strain AW518) were taken from the same culture. In the first experiment: initial concentration of alanine = 4 mM, initial concentration of 2-oxoglutarate = 4 mM, $C_e = 1.8$ mM, $T = 184$ sec, number of bacteria tracked in the gradient = 69; number of bacteria tracked in the control = 26. In the second experiment (data shown in Fig. 1): initial concentration of alanine = 4 mM, initial concentration of 2-oxoglutarate = 20 mM, $C_e = 3.1$ mM, $T = 213$ sec, number of bacteria tracked in the gradient = 62; number of bacteria tracked in the control = 40. The two controls were indistinguishable. The fits given are for Eq. 7, i.e., Eq. 2 with $\beta = 1$; no other fits of Eq. 2 or Eq. 3 with $0.25 \leq \beta \leq 3.0$ were significantly better, although some were equally good, e.g., Eq. 3 with $\beta = 1$. Eqs. 2 and 3 are equivalent when $\alpha f^{\beta} \ll 1$.

* The standard deviation of the values of $\ln\tau$ about $\langle \ln\tau \rangle$.

† The probability that the model in which $\langle \ln\tau \rangle$ depends on f will describe the results of future experiments as well as one in which $\langle \ln\tau \rangle$ depends on dP_b/dT.

‡ The time rate of change of the fractional amount of chemoreceptor bound, Eq. 6, with $K_D = 2$ mM.

Response to Positive Gradients. The bacteria changed direction less frequently in positive gradients. Results of a typical experiment with *E. coli* AW518 (7) are shown in Fig. 1. Runs were long when the concentration of glutamate changed rapidly (Fig. 1*a*; $t < T$) but were equal in length to runs in the control (Fig. 1*b*) as the concentration approached its equilibrium value ($t \gg T$). No changes in the lengths of runs were observed if the enzyme was omitted (Fig. 1*b*), if the enzyme was added but alanine and/or 2-oxoglutarate were omitted, or if the experiment was run with the aspartate taxis mutant AW539 (7). Controls also were run with capillary assays: AW518 was not attracted to 2-oxoglutarate or to pyruvate; neither compound inhibited its attraction to glutamate.

In order to determine the functional dependence of the mean value of $\ln\tau$ on C and dC/dt we fit various models to the data as described in *Materials and Methods*. Our major findings are summarized in Table 1. If glutamate and its receptor form a complex characterized by a dissociation constant K_D (8), and the formation of this complex does not appreciably change C, then the time rate of change of the fractional amount of receptor (protein) bound is

$$dP_b/dt = [K_D/(K_D + C)^2]dC/dt \quad [6]$$

The best fits were obtained with equations which assumed a dependence of $\langle \ln\tau \rangle$ on dP_b/dt; the simplest was

$$\langle \ln\tau \rangle = \ln\tau_0 + \alpha(dP_b/dt), \quad [7]$$

where $\ln\tau_0$ and α are constants. K_D and its standard deviation were estimated from this fit to be 2 ± 1.5 mM; if $K_D = 2$ mM, the means and standard deviations of the other constants were $\ln\tau_0 = -0.4 \pm 0.1$ and $\alpha = 660 \pm 70$ sec. Two other experiments with AW518 involving fewer cells gave values for α of 420 ± 140 sec and 1300 ± 900 sec.

The positive gradients did not affect the mean speeds of the bacteria, but they did reduce the fluctuations in the speeds during runs, the mean angular speed while running (6), the lengths of twiddles, and the changes in direction from run to run. All of these results are consistent with a model in

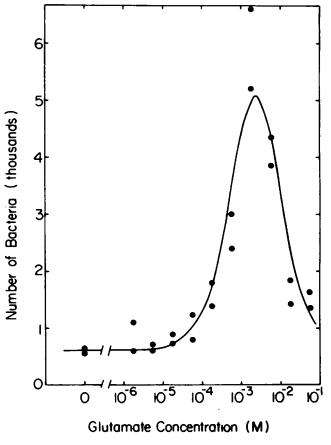

FIG. 2. The results of sensitivity assays for taxis toward glutamate (●) and the theoretical curve $K_DC/(K_D + C)^2$ fit by eye (*solid line*; $K_D = 2.3$ mM). The initial concentration of attractant in the capillary was 3.16 times that in the bacterial suspension; the geometric mean of the two concentrations is plotted on the abscissa. The number of bacteria (*E. coli* strain AW528) accumulating in the capillary after 1 hr at 32° is plotted on the ordinate.

which positive gradients increase the stability of the flagellar bundle (6).

Measurement of K_D with the Capillary Assay. The value of K_D estimated from the sensitivity curve is consistent with the value obtained from the tracking data. If the bacteria sense dP_b/dt, then the response in the sensitivity assay should be proportional to $K_DC/(K_D + C)^2$ (see *Discussion*); the theory and the experiment are compared in Fig. 2, with K_D = 2.3 mM.

Tests of a "Memory" Model. If the bacteria smooth dP_b/dt with some time constant T_m, i.e., if they have a "memory with a decay time" as suggested by Macnab and Koshland (4), then the equation:

$$\langle \ln\tau \rangle = \ln\tau_0 + \alpha \overline{dP_b/dt}, \quad [8]$$

where

$$\overline{dP_b/dt} = \frac{1}{T_m}\int_{-\infty}^{t} (dP_b/dt') \exp[(t' - t)/T_m]\, dt', \quad [9]$$

might fit the data better than Eq. 7. We tested this possibility for the data of Table 1 with 1 sec $\leq T_m \leq$ 150 sec and 0.5 mM $\leq K_D \leq$ 8.0 mM: none of the fits were significantly better than those of Eq. 7; fits with $T_m \geq$ 100 sec were significantly worse. Note that Eqs. 7 and 8 are identical when $T_m = 0$.

Evidence that T_m cannot be much larger than the mean run length was obtained from a computer simulation of *E. coli* swimming in a spatial gradient, Fig. 3. The drift velocities of the bacteria up the gradient were small for $T_m \gg \tau$, but were in the range 2-4 μm/sec for $T_m < \tau$. The velocity we estimated earlier from the tracking data was 0.9 μm/sec (6).

Response to Negative Gradients. Negative gradients had little effect. In two experiments in which glutamate was destroyed (Eq. 5, C_e = 0.67 mM, T = 74 sec; number of bacteria tracked in gradients = 69 and 41; number of bacteria tracked in controls = 35 and 23), we observed no significant dependence of $\langle \ln\tau \rangle$ on dP_b/dt, i.e., the values of α estimated from fits of Eq. 7 were not significantly different from zero (means and standard deviations 80 ± 70 sec and 0 ± 110 sec if K_D = 2 mM). However, the absence of a response could have been due, in part, to the concomitant generation of alanine. Therefore, we did two additional experiments in which the rate of generation of alanine was small (C_e = 0.167 mM, T = 55 and 61 sec; number of bacteria tracked in gradients = 41 and 41; number of bacteria tracked in controls = 25 and 24); the means and standard deviations of the α's were 410 ± 130 sec and −90 ± 140 sec. We also determined the sensitivity curve for alanine. From the shape of this curve ($K_D \simeq$ 10 mM and a peak response less than half as large as that for glutamate, results consistent with the concentration–response curves for AW518 shown in ref. 7) we estimate that the tracking results could have been obtained even if the bacteria responded to negative gradients of glutamate with an α as large as 200 sec. Therefore, these experiments only allow us to conclude that the response, if it exists, is comparatively weak.

Response to Concentration Jumps. No significant change in $\langle \ln\tau \rangle$ was observed when the enzyme was omitted and the bacteria were subjected to jumps from 0 to 0.13 mM glutamate or from 5.0 to 0.5 mM glutamate, increments larger than any which could occur in the experiments with the enzyme. Jumps from 0 to 0.5 mM glutamate, however, produced an initial increase in $\langle \ln\tau \rangle$ and a subsequent gradual decay to the control value (as noted for serine in ref. 4). Larger jumps resulted in altered behavior for longer times, e.g., about 300 sec after a jump from 0 to 10 mM glutamate. The values of $\langle \ln\tau \rangle$ measured at t = 30 sec were never greater than about 1.7.

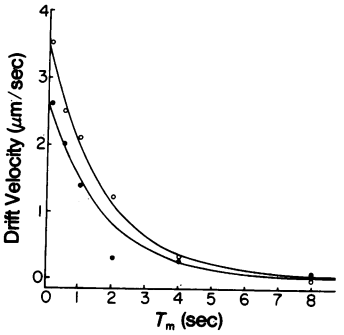

FIG. 3. The results of a computer simulation of the motion of *E. coli* in a spatial gradient of aspartate. The rate of drift up the gradient is shown as a function of the memory decay time (T_m) for two models: in the first (○), the bacteria responded both to negative and to positive values of $\overline{dP_b/dt}$ (α independent of the sign, Eq. 8); in the second (●), they responded only to positive values of $\overline{dP_b/dt}$ (α = 0 for negative values). Each data point is the mean drift velocity of 30 bacteria; the standard deviations were of order 0.3 μm/sec. The *solid lines* are exponential fits made by eye (decay time 1.7 sec). A bacterium started from rest and swam for a time δt = 0.08 sec at a constant speed in a direction chosen at random. Eq. 9 was used to compute $\overline{dP_b/dt}$. The probability that a twiddle would occur, p_t, was determined from Eq. 8: $p_t = \delta t \exp(-\ln\tau_0 - \alpha \overline{dP_b/dt})$. The computer then generated a random number between 0 and 1. If this number was less than p_t, a twiddle occurred, and the bacterium stopped; if it was greater than p_t, the bacterium continued in the same direction for another interval δt, and the test for a twiddle was repeated. The length of the twiddle was picked from the appropriate Poisson interval distribution (ref. 6; the mean interval was assumed to be independent of $\overline{dP_b/dt}$). After the twiddle, the bacterium swam in a new direction. The angle between the new and the old direction was picked from the distribution shown in Fig. 3, ref. 6. The process was continued until the bacterium completed 30 runs, and its drift velocity up the gradient was computed. The gradient was linear; the mean concentration (8.4 μM) and the steepness (20 μM/mm) were the same as in the aspartate tracking experiment (Table 2, ref. 6). We assumed $K_D = 10^{-4}$ M (since the K_D for α-methylaspartate is 1.3 × 10^{-4} M, ref. 8, and the two amino acids give similar responses in the capillary assay, ref. 7), and α = 700 sec (as for glutamate, which binds to the same receptor). The speed (12.3 μm/sec), the mean run length in the absence of a gradient (0.83 sec), and the mean twiddle length (0.19 sec) were the same as in the aspartate control (Table 2, ref. 6).

DISCUSSION

The swimming pattern of *E. coli* resembles a three-dimensional random walk: a bacterium moves along a relatively straight path (runs), abruptly changes direction (twiddles), and then runs again. The twiddles occur at random (6): the distribution of run lengths is exponential and completely specified by the mean run length (τ), i.e., by the probability per unit time that a twiddle will occur ($1/\tau$). Temporal gradients of L-glutamate change the mean run length. The response is asymmetric: positive gradients increase the lengths of runs, but negative gradients have relatively little effect. The mag-

nitude of the response depends (at least formally) on the time rate of change of the fractional amount of chemoreceptor bound (dP_b/dt).

The responses of individual bacteria in spatial gradients (6) can be explained fully by this mechanism. When a bacterium swims up a spatial gradient, the fractional amount of chemoreceptor bound increases, and the runs are long; when it swims down the gradient, the fractional amount of chemoreceptor bound decreases, and the runs are about the same length as in isotropic solutions. The change which occurs when the bacterium swims up the gradient is roughly that predicted by Eq. **7**.

The response depends both on C and on dC/dt. When $C \ll K_D$, dP_b/dt is proportional to dC/dt; when $C \gg K_D$, it is proportional to $(dC/dt)/C^2$; when $C \simeq K_D$ it is proportional to $(dC/dt)/C$; see Eq. **6**. If more than one receptor or more than one binding site is involved, the response may depend on $(dC/dt)/C$ over a wider range of concentrations, but this dependence will break down when C is smaller than the smallest K_D or larger than the largest K_D.

The dependence on C can account for the limited validity of the Weber-Fechner law noted by Dahlquist, Lovely, and Koshland (15). When a bacterium swims in a spatial gradient, dC/dt is proportional to dC/dx (the steepness of the gradient), and the rate of drift up the gradient depends on C and dC/dx in the same way that $\langle \ln \tau \rangle$ depends on C and dC/dt. (This is true if the speeds of the bacteria are independent of C, if the drift velocity is small compared to these speeds, and if the changes in direction from run to run do not depend on the direction of the gradient, conditions which are met in practice, refs. 4, 6, and 15.) It follows from Eqs. **6** and **7** that the rate of drift up a gradient for which $(dC/dx)/C$ is constant is not independent of C, as implied by the Weber-Fechner law (15), but varies as $K_D C/(K_D + C)^2$. The drift velocities of *Salmonella* up exponential gradients of serine varied with C in roughly this way (15).

The temporal mechanism can explain the shapes of the sensitivity curves of Mesibov, Ordal and Adler (8). Since the ratio of the initial concentration of attractant in the capillary tube to that in the suspension is fixed, $(dC/dr)/C$ (at a given distance r from the mouth of the capillary and at a given time after its insertion) is the same from one assay to another (11). If the number of bacteria swimming into the capillary in a given time is proportional to the drift velocity, it follows from the arguments given above that the sensitivity curve should vary as $K_D C/(K_D + C)^2$. This dependence has been noted by Ordal and Adler (ref. 8, appendix).

The mechanism by which the bacteria sense the time rate of change of the fractional amount of chemoreceptor bound is not known. Comparisons must be made at successive intervals in time (4), but there is nothing in our data to suggest that these intervals need be very long. If the memory time were much longer than the mean run length, the rate at which the bacteria could drift up a spatial gradient would be small (Fig. 3). On the other hand, if it were very short, the temporal gradients sensed as a result of random fluctuations in the local concentration of attractant would be large. Since the response to positive and negative gradients is asymmetric, the run lengths would then depend on the mean concentration, even in isotropic solutions. Such a dependence has been observed only for substances sensed by the serine receptor (6).

There are inconsistencies between our results and the results obtained in mixing experiments (4) which may be accounted for on the basis of the difference in the size of the stimuli. The gradients in the enzyme experiments are several hundred times smaller than those in the mixing experiments. We do not observe a sizeable response in negative gradients; in the mixing experiments, the bacteria change direction more frequently, but the response is short-lived (12 sec, ref. 4). We find a dramatic increase in run length in positive gradients; this is also true in the mixing experiments, but the effect persists for a very long time (up to 300 sec, ref. 4). This time is too long to be a memory decay time (Fig. 3). Our finding that the magnitude of the response to a small positive jump may be as great initially as that to a large positive jump, but that the effect persists for a shorter time, suggests that large stimuli may saturate the sensing mechanism. The decay time for a small stimulus, for example, might depend on the rate at which an intermediate diffuses away from its site of action, whereas the recovery time for a large stimulus might depend on the rate at which a transport system pumps the intermediate out of the cell. The fact that the model which fits our tracking data also explains the results of sensitivity assays (in which the temporal gradient repeatedly changes sign, so that the average time rate of change in concentration is small) implies that saturation effects are not important in the enzyme experiments.

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