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STUDIES OF BACTERIAL CHEMOTAXIS IN DEFINED CONCENTRATION GRADIENTS. A Model for Chemotaxis Toward L-Serine

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The details of the chemotactic response of Salmonella typhimurium to gradients of L-serine have been examined in some detail. Two relatively macroscopic techniques have been employed to measure the bacterial response. These include measurements of the average velocity as the bacterial population moves toward attractants, and measurement of the upward-to-downward flux ratio, R, in the stable preformed attractant gradients. The dependence of the average velocity on gradient appears to be hyperbolic in nature, while the flux ratio depends linearly on the gradient. These data suggest a microscopic model for the dependence of bacterial behavior on the serine gradient. The model involves a linear dependence of the mean lifetime of a bacterial trajectory on the gradient for those bacteria moving toward higher attractant concentration. Those moving toward low concentrations of attractant do not change the mean duration of their trajectories, or the speed at which a given bacterium swims through the solution. This model generates the observed dependences of the average velocity and flux ratio on gradient. Interpretation of the experimental data suggests that a gradient which increases serine concentration by a factor of 2 in 10 mm is sufficient to double the average duration of a trajectory for a bacterium moving directly up the gradient. The concentration dependence of the chemotactic response to serine is more complicated. It suggests that more than one receptor of serine may be involved in determining chemotactic behavior to this attractant.

Much interest in recent literature has been devoted to physical measurements of the behavior of motile bacteria (1–4), with the aim of understanding the molecular mechanisms of motility, especially during chemotaxis. An experiment is most interesting if it yields detailed microscopic information. The tracking techniques of Berg (5), the temporal gradient experiments of Macnab and Koshland (6), and the tethered-cell techniques (13) employed by Adler's group (7) provide such microscopic information. Experiments done with less detailed tracking (8) or with macroscopic measurement of concentration of bacteria at different positions in a solution (2) can still yield valuable microscopic information, provided the results of these experiments can be related to a detailed microscopic description of bacterial behavior. The great advantage of these less-detailed approaches is that a statistically significant number of bacteria may be examined. Our purpose here is to relate certain measurable statistical quantities concerning bacterial chemotaxis to the parameters of a model for bacterial motion for Escherichia coli and Salmonella typhimurium. The general model, described below, is not very restrictive, in that it leaves the probability distribution for turning angles and the direction dependence of the mean trajectory duration unspecified. It is merely a logical idealization of the experimental evidence, which simplifies analysis, without, one hopes, suppressing any of the crucial

features of bacterial chemotaxis. We shall apply this general model to the specific case of taxis of S. typhimurium toward L-serine to understand the concentration and gradient dependence of the response to this attractant.

THE GENERAL MODEL (9)

Let us approximate the three-dimensional motion of a bacterium by making the following assumptions:

(a) The path of a bacterium is a sequence of straight-line trajectories joined by instantaneous turns, each trajectory being characterized by a speed, direction, and duration.

(b) All trajectories have the same constant speed.

(c) When a bacterium turns, its choice of a new direction is governed by a probability distribution which is azimuthally symmetric about the initial direction.

(d) The angle between two successive trajectories is governed by a probability distribution which is independent of any other information, such as the direction or duration of the initial trajectory.

(e) The duration of a trajectory is governed by a probability distribution which may depend on the direction of that trajectory, but on no other information, such as the parameters of previous trajectories.

(f) for any particular direction, the probability density function for the duration of a trajectory is a decaying exponential.

Assumptions (a) and (b) are not perfect, and their degree of validity may be judged from the data of Berg and Brown (3). In most of what follows, trajectories and turns correspond respectively to "runs" and "twiddles," as defined quantitatively by Berg and Brown (3). However, as is shown elsewhere (9), rotational diffusion during runs can be accommodated by this model when there is no chemotaxis, by letting a trajectory be much shorter than a run. Also, the nonzero duration of a twiddle can be included in the calculation of the diffusion constant.

The data of Berg and Brown (3) are consistent with assumption (c), and support (d), (e), and (f). They include measurements of a distribution for the angle between successive trajectories, and of a nearly exponential distribution of trajectory durations.

We will not need assumption (f) except for discussing the direction correlation function. This assumption is equivalent to saying that the probabilty of turning in any small time interval depends only on the direction of travel at that time, and has no dependence on previous events. For the isotropic case it means that the number of turns in any time interval is governed by a Poisson distribution. For discussing persistence time and average velocity, we need only to assume that the first moment (mean duration) of the duration probability function exists. For diffusion we must require that the second moment (mean square duration) exist also. We shall not be directly concerned with bacterial diffusion in this communication.

Chemotaxis arises from the dependence of the average duration of a trajectory on direction in assumption (e). The data of Berg and Brown (3) show that this dependence exists, but they are insufficient to describe it in detail.

Statistical measures which depend on large numbers of bacteria are complicated by the fact that all the bacteria are not identical. Even though each bacterium may obey this

model, they will not all have the same speed or other parameters. To describe the behavior of an ensemble of independent bacteria with different properties is straightforward and will not be discussed here, but the problem must be borne in mind when interpreting experiments.

In terms of this model, five different statistical measures, each of which has been studied experimentally, can be described. These are summarized in Table I and are related to the mean duration of a trajectory. In the absence of gradients of attractant or repellent chemicals, this trajectory is to be of duration T_o. When gradients are present, the mean duration becomes a function of the direction the bacterium is swimming and is defined by T(u), where u is the direction cosine with respect to the gradient direction. The dependence of this function on the concentration and gradient of chemotactically active compounds controls the dependence of the observed chemotactic behavior on both the concentration and gradient of the attractants or repellents. We shall be concerned with the determination of this functional dependence by comparing measured values of the quantities given in Table I to a particular model for the dependence of the trajectory duration on concentrations and gradients of attractants.

We shall consider two relatively macroscopic measures of chemotaxis. One of these, the average velocity with which the bacterial population moves past a particular point in a chemical gradient has been discussed before (2). The second measure, the up/down ratio, R, has not been fully described elsewhere.

If freely swimming bacteria are viewed through a microscope with a single crosshair across the field of view, an observer can count the numbers of bacteria which cross the line in both directions. These numbers will depend on the time period of the observations; on the width and depth of the field of view; and on the concentration, speed, and direction distribution of the bacteria. But their ratio will depend only on this direction distribution. If both the crosshair and the direction of view are perpendicular to the direction of a chemical gradient, such an experiment gives the ratio of the rate of flow of bacteria traveling up the gradient to the rate of flow of bacteria traveling down the gradient. We call this the up/down ratio, R. It has proved to be a very practical assay of the strength of chemotactic response.

Another similar assay involves placement of the crosshair parallel to the direction of the attractant gradient. This makes it possible to determine the rate of flow of bacteria perpendicular to the attractant gradient direction. Comparison of the flux ratios then provides interesting semimicroscopic information concerning the symmetry of chemotaxis.

The great advantage of this approach is that a large number of individual bacteria may be observed. The individual bacteria vary quite dramatically in their swimming speed and other parameters of chemotactic response. This variability exists even in a bacterial population derived from a single colony. In order to generate reproducible results, it is necessary to observe a sufficiently large number of bacteria to insure that the population has been satisfactorily sampled. The up/down ratio offers this property. The ratio measurements are reproducible within the counting statistics of the number of bacteria observed. This usually implies reproducibility in the flux ratios of about 3–5%.

One advantage of this approach is that only a small region of the attractant gradient is observed. Therefore, the concentration of attractant does not change significantly over the field of view. Therefore, both the gradient and concentration of attractant can be controlled.

The flux ratios are remarkably constant with the time if exponential attractant gradients are employed and the bacteria are observed away from the extremes of the gradient.

THE SPECIFIC MODEL FOR SERINE RESPONSE

Figure 1 shows the gradient dependence of the average velocity, $\bar{V}$, of the response of S. typhimurium to defined gradients of serine. The data are presented as a plot of average velocity vs gradient. The gradients all begin at 1.0 mM L-serine and decay exponentially in distance such that $(\frac{dc}{c}/\,dx)$ is constant throughout the gradient. The gradients are expressed as this fractional change in concentration per millimeter, or the reciprocal of the distance over which the concentration of serine changes by a factor of 1/e.

As shown in Fig. 1, the average velocity shows saturation in its response to the gradient at a constant concentration 1.0 mM L-serine. This behavior can be seen more clearly in Fig. 2, in which the data of Fig. 1 have been recast as a double reciprocal plot of $1/\bar{V}$ vs 1/gradient. As seen in Fig. 2, this plot is remarkably linear, suggesting that the average velocity has a hyperbolic dependence on the gradient. This may be quantitated by using an equation of the form

$$\bar{V} = V_{max} \frac{\gamma}{\gamma_o + \gamma} \qquad [1]$$

where V_{max} is the rate at which bacteria move up an infinitely steep gradient, γ is the gradient, and γ_o corresponds to the half-saturating gradient. From the data shown in Fig. 2, V_{max} is 7μ/sec and γ_o corresponds to 0.25 mm^{-1} or a gradient which changes by a factor of e in 4.0 mm.

Somewhat more insight can be gained by examination of the dependence of the up/down ratio on the gradient in the same range of gradients as used for the average velocity measurements. Figure 3 shows a plot of the ratio, R, vs the gradient, γ. This is remarkably linear. Therefore the up/down ratio appears to follow the form

$$R = 1 + a\gamma \qquad [2]$$

where a is the slope of the plot shown in Fig. 3 and has a value of approximately 8 mm.

These two measures of the gradient dependence of the serine response can be used to help establish the gradient dependence of the average duration of a trajectory. The data of Berg and Brown (3) show the behavior of bacteria moving down a moderately steep attractant gradient to be essentially that of bacteria moving in the absence of a gradient. Those bacteria moving up the gradient have relatively longer average trajectory lengths. Similar conclusions were reached by Macnab and Koshland (6) using temporal gradient stimulation techniques. This suggests that the response has a "one-sided" element which generates a significantly more sensitive response to positive attractant gradients than negative ones. We shall therefore assume that the lifetime of a trajectory for a bac-

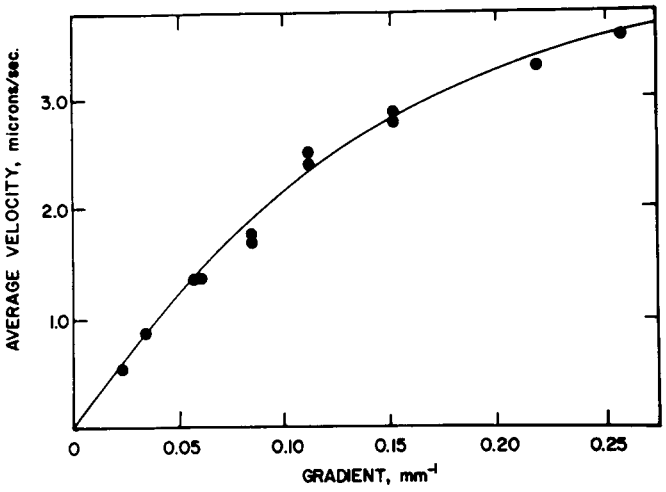

Fig. 1. A plot of the average velocity, $\bar{V}$, the bacterial population moves up an exponential gradient of serine, vs the gradient, γ, at a constant concentration of 1.0×10^{-3} M L-serine. The gradient is expressed as the reciprocal of the decay distance, the distance over which the serine concentration decreases by a factor of 1/e. The bacteria concentration was $1-3 \times 10^{-6}$ cells/ml. Other conditions have been described previously (2).

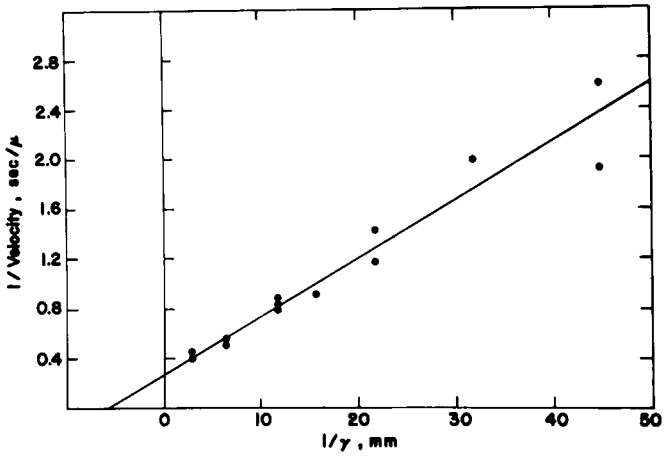

Fig. 2. The data of Fig. 1, replotted as a double reciprocal plot, $1/\bar{V}$ vs $1/\gamma$. The $1/\bar{V}$ axis intercept gives a value $\bar{V}_{max}$ equal to 7μ/sec and the $1/\gamma$ axis intercept gives the half-saturating gradient value of 0.25 mm^{-1}.

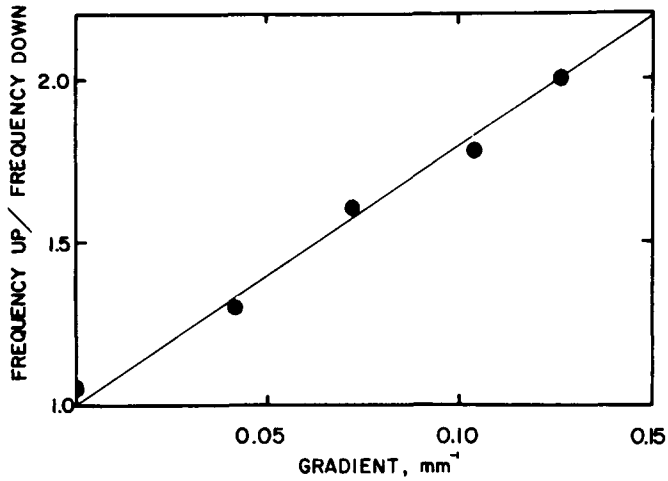

Fig. 3. A plot of the up/down flux ratio, R vs the gradient. The conditions were identical to those in Fig. 1, except that $1-2 \times 10^6$ cells/ml were used to measure the response. The procedure for determination of R is described in the text.

terium moving down the attractant gradient is simply T_o, the same as in the absence of a gradient. In mathematical terms,

$$T(u) = T_o \quad \text{for } u < 0$$

where the u is the cosine of the angle the trajectory makes with the gradient direction.

From Table I, we can now establish the gradient dependence of T(u) for increasing serine gradients by the relationship

$$R = \frac{\int_0^1 uT(u)du}{\int_0^{-1} uT(u)du} = \frac{\int_0^1 uT(u)du}{\frac{1}{2}T_o} \qquad [3]$$

Combining this with equation [2] yields

$$1 + a\gamma = \frac{\int_0^1 uT(u)du}{\frac{1}{2}T_o} \qquad [4]$$

which can be solved directly, provided we make the reasonable assumption that T(u) depends in some undefined way on the effective gradient seen by the bacterium, which is given by $u \cdot \gamma$. The solution is

$$T(u) = T_o(1 + b \cdot \gamma \cdot u)$$

where $b = 3/2\ a$. When the data of Fig. 3 is used, the value of b corresponds to 12 mm.

This provides for us the important microscopic conclusion that the lifetime of a

trajectory varies linearly with the magnitude of the gradient observed by the bacterium.

This conclusion may be further examined by substitution of the gradient dependence of the trajectory lifetime into the expression for the average velocity given in Table I. This gives the following relationship:

$$\bar{V} = \frac{2}{3} V_o \frac{\gamma}{\frac{4}{b} + \gamma} \qquad [5]$$

TABLE I. Summary of Formulae Relating Statistical Measures to Microscopic Behavior Information

τ_c	$=$	$\frac{T_o}{1-\alpha}$		
D	$=$	$\frac{1}{3} v^2 \frac{T_o}{1-\alpha} = \frac{1}{3} v^2 \tau_c$		
τ_+	$=$	$\frac{\pi}{\bar{\psi}} \int_0^1 T(u)du$	$=$	$\frac{\pi}{\bar{\psi}} \frac{1}{\gamma} \int_0^\gamma F(x)dx$
$\bar{V}$	$=$	$v \frac{\int_{-1}^{1} uT(u)du}{\int_{-1}^{1} T(u)du}$	$=$	$v \frac{1}{\gamma} \frac{\int_{-\gamma}^{\gamma} xF(x)dx}{\int_{-\gamma}^{\gamma} F(x)dx}$
R	$=$	$\frac{\int_0^1 uT(u)du}{\int_0^{-1} uT(u)du}$	$=$	$\frac{\int_0^\gamma xF(x)dx}{\int_0^{-\gamma} xF(x)dx}$

Correlation time, τ_c, diffusion constant, D, persistence time, τ_+, average velocity, $\bar{V}$, and up/down ratio, R, are related to the mean isotropic trajectory duration, T_o, the direction dependent mean duration, T(u), the speed, v, the mean turn angle, $\bar{\psi}$, and the mean cosine, α. The dependence of τ_+, $\bar{V}$, and R on the proportional attractant gradient γ is expressed in terms of the function F describing the dependence of mean trajectory duration on the attractant concentration and its time deviation. (The concentration dependence of F is not shown.)

This has the observed hyperbolic dependence of the average velocity on the gradient. The half-saturating gradient, γ_0, is now related to the same parameter, b, as was determined from the up/down ratio measurement. The average velocity measurement gives a value of b equal to 16 mm. This is in good agreement with the value of 12 mm determined above, considering the different sensitivities of the two measures to heterogeneity of response in the bacterial population.

The parameter b represents the real microscopic sensitivity of the bacterium to the gradient it perceives along its trajectory. The value b is 12–16 mm. This means that an effective serine gradient which changes concentration by a factor of e in 12–16 mm along the trajectory will double the lifetime of that trajectory. Comparison of this value to typical bacterial dimensions clearly demonstrates an enormous sensitivity of the decision-making machinery to the gradient. Macnab and Koshland (6) have shown that the bacteria respond to temporal gradients of attractants. So a measure of the length of a trajectory is required before a more quantitative examination of the sensitivity can be made. This will also give us T_0, the mean lifetime of a trajectory in the absence of an attractant or repellent gradient.

Figure 4 shows a plot of the x, y, and z displacements generated by a bacterium swimming in the absence of gradients. The data were obtained with our low-resoltuion tracking device (8). The figure shows that sudden changes in direction occur at apparently random intervals. This behavior may be quantitated by correlating the direction the bacterium is moving at any instant to the direction it is moving at some later time. If large numbers of these direction correlations are determined, and then averaged, the turning statistics of the bacterium may be quantitated. The quantification involves the determination of the cosine of the angle between the bacterium's direction at time, t, and its direction at a later time, $t + \tau$. If τ is much shorter than the lifetime of a trajectory, the cosine will have a value near unity when averaged over many such determinations, since the two directions should be nearly the same. If, on the other hand, τ is much longer than the mean lifetime of a trajectory, the two directions will be essentially randomly oriented with respect to one another. Therefore, the value of the cosine will be averaged over these random directions to zero. The details of the exact functional dependence of this decay to zero as τ increases depend on the statistics of the process by which changes in direction occur. If it is a Poisson process, the average value of the cosine will decay exponentially (12) with τ.

Figure 5 shows a logarithmic plot of the average value of the cosine vs τ. This is remarkably linear and gives a time constant, τ_c, of 10 sec for this process. This implies that the turning times (or trajectory lifetimes) are governed essentially by Poisson statistics. That means that the probability that a bacterium will turn at some instant does not depend on how long ago it last turned.

From Table I, the correlation time, τ_c, can be related to the lifetime of a trajectory in the absence of a gradient, T_0, by

$$\tau_c = \frac{T_0}{1-\alpha} \qquad [6]$$

where α is the mean cosine of the angle between successive trajectories. From the data

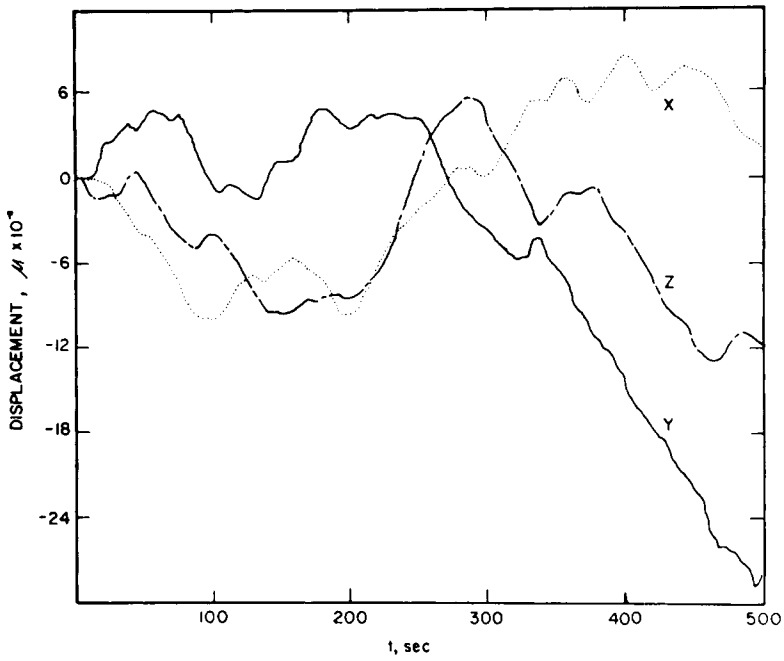

Fig. 4. A plot of the x, y, and z coordinate displacement of an individual S. typhimurium cell, swimming in the absence of attractant gradients.

of Berg and Brown (3), α can be calculated to be approximately 0.33. Therefore T is approximately 7 sec. Since this determination used our S. typhimurium data and Berg and Brown's data (3) from E. coli, it is only approximate.

This allows us to refine our microscopic picture of bacterial chemotaxis to the following. The bacteria maintain a given direction for 7 sec. We find an average swimming speed of the bacterial population to be approximately 15 μ/sec. This suggests a trajectory length of approximately 100 μ. The serine gradient which doubles this trajectory for bacteria swimming up the gradient corresponds to a decay distance of about 12–16 mm. We may now calculate the sensitivity of the sensory mechanism. The bacteria must sense the gradient over about 100 μ since this is the mean length of a trajectory. The concentration changes by about $e^{(-100)/12000} = 0.99$. Thus, a 1% increase in concentration over the length of the trajectory doubles the length of the trajectory. This unusual sensitivity is not easily explained and further demonstrates the very interesting nature of the chemotaxis problem.

Brown and Berg (10) have concluded from their studies of the response of E. coli to controlled temporal gradients of glutamate that the "memory time" of the bacterium must be on the order of the mean duration of a trajectory. Their measurements suggest a similar time scale for gradient detection for this attactant which is sensed by the aspartate receptor.

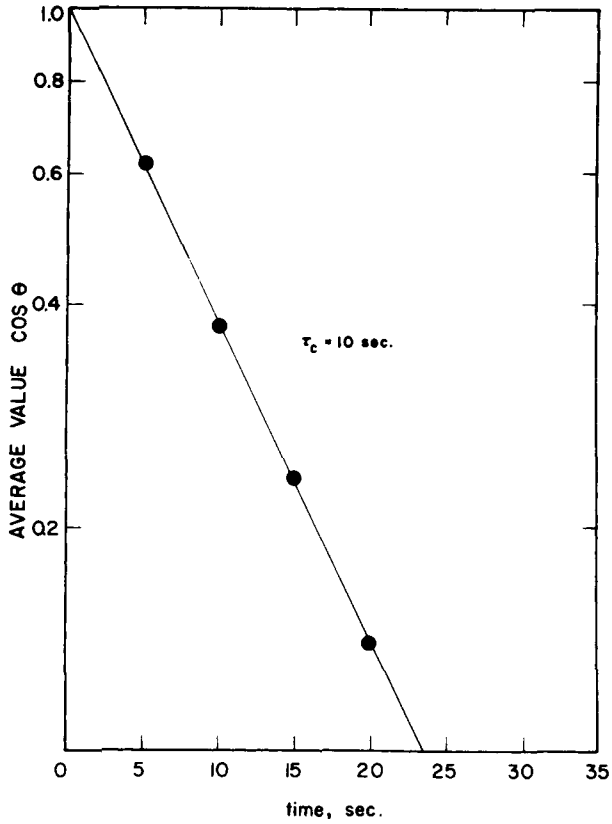

Fig. 5. A first-order plot of the average value of the cosine of the angle between trajectories at times t and t + τ as a function of τ. This data was averaged over a 28 min track of a single bacterium using our low-resolution tracking system (8). The slope of the first-order plot gives the correlation time, τ_c of 10 sec.

We may now employ this model to interpret more exactly the concentration dependence of the chemotactic response to L-serine. Figure 6 shows a plot of the average velocity, $\bar{V}$, of the bacterial population moving up a serine gradient as a function of concentration. At all concentrations the relative gradient $(\frac{dc}{c})/dx$ was held constant at a decay distance of 6.4 mm (2).

This plot does not properly represent the real concentration dependence of the response mechanisms, since both the attractant concentration dependence and the hyperbolic saturation of the average velocity affect the measurement. The data may be recast to eliminate the complications due to the gradient saturation of the observed value of the average velocity. The average velocity appears to be described by equation [1],

$$\bar{V} = V_{max} \frac{\gamma}{\gamma_0 + \gamma}$$

The parameter V_{max} appears to be related only to the swimming speed of the population. This swimming speed is not dependent on attractant concentration if normal growth medium is used for the assay. Therefore, the concentration dependence should be associated with γ_0, the half-saturating gradient. Since γ was held constant, we may easily calculate γ_0 as a function of serine concentration from the data used in Fig. 6 and values of V_{max} equal to 7 μ/sec and γ_0 equal to 0.25 mm^{-1} at 1.0×10^{-3} M L-serine. These data have been summarized in Table II. We have also included the calculated values for the parameter, b, the microscopic sensitivity of the bacterial gradient-sensing machinery to the gradient. As discussed above, the value of b and γ_0 are related simply by $\gamma_0 = 4/b$. As seen in Table II, the value of the half-saturating gradient goes through a minimum at 1.0×10^{-3} serine concentration. This means that the response is most sensitive at this concentration since the half-saturating gradient is smallest in this concentration region. This can be seen more clearly in Fig. 7, where the value of b has been plotted as a function of the logarithm of serine concentration. The solid line is drawn through the observed data points and is not based on any particular model. As shown in the figure, the curve has a distinct maximum at 1.0×10^{-3} M serine, and decays in a fairly symmetric fashion about this maximum. Remembering that the parameter b reflects the microscopic sensitivity of the gradient-sensing machinery of the bacteria, we again conclude that this sensitivity is greatest near millimolar serine concentration.

Two theoretical lines have been included in Fig. 7. These have been labeled "ratio mechanism" and "difference mechanism," respectively. These curves are calculated on the basis of the idea that the information concerning the attractant gradient is transmitted from a receptor protein to the decision-making machinery of the bacterium. We supposed that this information could be transmitted by either of two distinct mechanisms. One such mechanism would involve taking the ratio of the fraction of the receptor bound at some instant in time to the fraction bound later in time (6). This would correspond to determination by the bacterial data-processing machinery of the derivative of the receptor fraction bound. Thus, if K represents the affinity of the receptor for serine, and c is serine concentration,

$$\frac{d \ln [\text{fraction bound}]}{dt} = \frac{d \ln \frac{K \cdot c}{1+K \cdot c}}{dt} = \frac{1}{c} \frac{1}{1+K \cdot c} \frac{dc}{dt}$$

$$= \frac{1}{1+K \cdot c} \frac{\frac{dc}{c}}{dx} \frac{dx}{dt} \qquad [7]$$

where x is distance and dx/dt corresponds to the swimming speed of the bacterium. Since the speed is constant (3) and $(\frac{dc}{c})/dx$ is held constant, the response should vary as $\frac{1}{1+K \cdot c}$. This curve has been plotted and labeled "ratio mechanism." If the data from the receptor were to involve taking the difference in the fraction of receptor bound at any

TABLE II. Concentration Dependence of the Serine Chemotactic Response of S. Typhimurium

Serine concentration, M	$\bar{V}$, μ/sec	γ_0, mm^{-1}	b, mm
1.0×10^{-6}	0.32	3.48	1.15
3.1×10^{-6}	0.72	1.45	2.8
1.0×10^{-5}	1.0	0.95	4.2
3.1×10^{-5}	1.5	0.63	6.3
1.0×10^{-4}	1.8	0.50	8.0
3.1×10^{-4}	2.5	0.28	13.6
1.0×10^{-3}	2.8	0.25	16.0
3.1×10^{-3}	2.4	0.33	12.2
1.0×10^{-2}	1.9	0.43	9.2
1.0×10^{-2}	1.6	0.27	7.3

time minus the fraction bound at some later time, the response should be proportional to the derivative of the fraction bound itself. Thus,

$$\frac{d[\text{fraction bound}]}{dt} = \frac{d\,\frac{K \cdot c}{1+K \cdot c}}{dt} = \frac{K \cdot c}{(1+K \cdot c)^2}\,\frac{1}{c}\,\frac{dc}{dt}$$

$$= \frac{K \cdot c}{(1+K \cdot c)^2}\,\frac{\frac{dc}{c}}{dx}\,\frac{dx}{dt}, \qquad [8]$$

the response should vary as

$$\frac{K \cdot x}{(1 + K \cdot x)^2} \qquad [9]$$

for constant speed and relative gradient. The theoretical curve has been plotted and labeled "difference mechanism." Both curves were scaled using the measured value of b at 16 mm serine.

Both theoretical curves agree well with the measured decrease in b at high concentrations. The difference mechanism provides the observed maximum in this curve while the ratio mechanism does not. As a result, the difference mechanism agrees better with the low concentration data. Neither mechanism gives good agreement at very low concentration, although the difference mechanism is more closely related to the measured value. It seems clear that the difference mechanism is the preferred explanation. The lack of agreement at low concentrations could be explained by proposing a second serine receptor protein with an affinity in the range of 10^5 M^{-1}. The unsuccessful attempts of many workers to isolate serine receptor mutants of E. coli have been used to suggest that serine response may depend on more than one distinct receptor (11). Thus, a multiple mutant lacking all such serine receptors would be exceedingly rare. Of course we cannot interpret

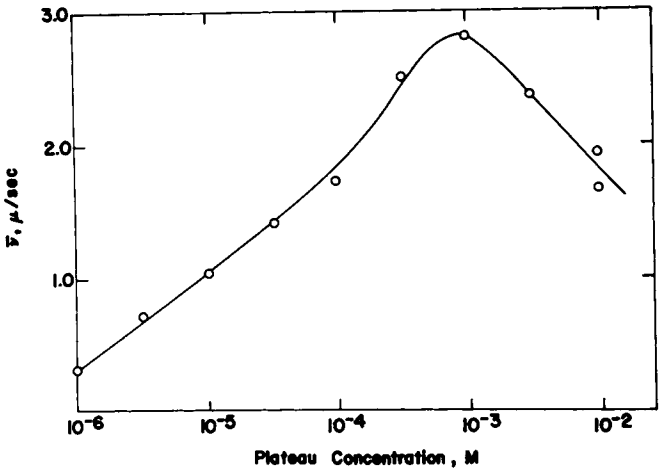

Fig. 6. A plot of the average velocity of the response of the bacterial population vs plateau concentration at constant proportional gradient of decay distance 6.4 mm. These data were collected at 25° C in VBC medium.

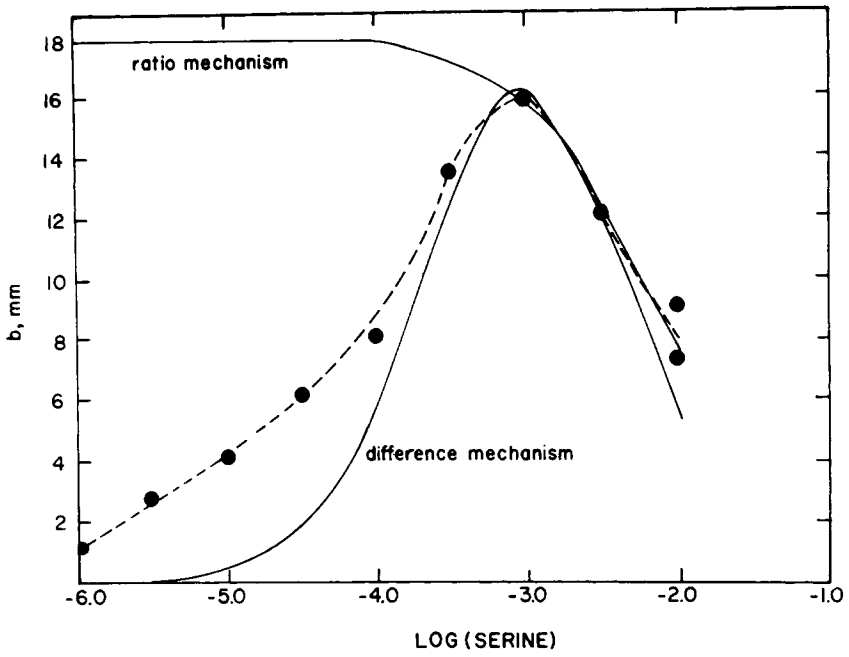

Fig. 7. A plot of the gradient-sensing parameter b vs concentration. The theoretical lines shown in the figure have been calculated for two model mechanisms, the ratio mechanism and difference mechanism. The details of these mechanisms are explained in the text.

such negative information in any rigorous fashion, but it at least suggests that more than one serine receptor may exist.

Brown and Berg (10) have suggested a model involving the time rate of change of chemoreceptor bound to explain the response of E. coli to temporal gradients of glutamic acid which is sensed by the aspartate receptor. This dependence was first suggested by Mesibov et al. (12) using the capillary assay technique. It seems to fit best the more detailed data of Brown and Berg (10). Their detailed tracking in enzyme-produced temporal gradients of glutamate did not extend over a very large concentration range of attractant. Thus, a lack of agreement at low and high attractant concentrations could not be examined in detail.

Both our data and those of Brown and Berg (10) suggest that a strict ratio-taking mechanism can be ruled out for response to serine or aspartate. The difference mechanism is not sufficient to explain our concentration results simply. It requires the presence of a high affinity receptor which does not alter the behavior pattern of the bacteria as strongly as the proposed weak receptor.

Most chemical mechanisms which one might imagine to be important for chemotaxis are ratio taking in nature. For example, one might imagine a membrane depolarization mechanism in which temporal gradients in the chemical potential of the attractant are converted into an electrical potential charge. Such a mechanism involves a determination of the ratio of concentrations in the temporal gradient. Macnab and Koshland (4) tentatively suggested a model in which some transmitter substance χ is formed and degraded in response to the attractant concentration. They proposed a difference in the rate of equilibration with attractant of the forming and degrading enzyme for χ. As a result, the steady concentration of χ, which depends on the ratio of its rate of formation to its rate of degradation, would vary with the temporal gradient of the attractant. This mechanism also involves ratio determinations. The details of the correct biochemical mechanism must await further experimentation. However, if a ratio-taking mechanism is involved, some other steps in the data-processing machinery must be involved which modify the observed concentration dependence of the response.

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