STRATEGIES FOR CHEMOTAXIS

M. J. SCHNITZER*, S. M. BLOCK†, H. C. BERG†, E. M. PURCELL*

Departments of Physics, and Cellular and Developmental Biology†, Harvard University, Cambridge MA 02138, and The Rowland Institute for Science†, Cambridge MA 02142, USA*

INTRODUCTION

The problem of migration of cell or animal populations has often been formulated in terms of a differential equation containing terms for both diffusion and drift. Controversy has arisen over the correct form of this equation when the diffusion coefficient varies over a region of space. Here we show, from a model for a one-dimensional random walk, that different microscopic mechanisms for variation in the diffusion coefficient require distinct differential equations. From these derivations we argue, in particular, that if behaviour is determined locally (i.e. does not depend on earlier events) and speed is constant, organisms cannot actively accumulate. They do so only where speeds are low. However, spatial variations in diffusion coefficent will affect the way in which cells approach equilibrium. One has to be precise about the conditions imposed by a given experimental set-up.

Earlier work in which these microscopic mechanisms were not properly distinguished includes that of Patlak (1953), Keller & Segel (1971), and Lapidus (1980, 1981). More recent work in which these distinctions have been recognized, explicitly or implicitly, includes that of Futrelle (1982) and Rivero *et al.* (1989).

To be certain of our ground, we modelled the behaviour by Montecarlo simulation. In the interest of understanding wild-type *Escherichia coli* and mutants defective in adaptation (*cheR cheB* mutants), we included mechanisms in which turning frequency depends on a measurement of concentration made over the recent past, or in a comparison of two such measurements made sequentially. If speed is constant, we find that cells will drift up gradients of attractants only if they are able to make temporal comparisons.

Students of animal behaviour use a different terminology (cf. Diehn *et al.*, 1977). A strategy in which speed is constant but turning frequency depends on the local intensity of a stimulus is called 'klinokinesis without adaptation'. In this case, the equilibrium distribution is uniform. A strategy in which speed is constant but in which turning frequency depends on temporal comparisons of stimulus intensity is called 'klinokinesis with adaptation'. In this case, if turns are suppressed when organisms move up a

gradient, they will accumulate near the top of that gradient. A strategy in which turning frequency is constant but in which speed depends on the local intensity of a stimulus is called 'orthokinesis'. Here, organisms tend to accumulate where speeds are low.

Clearly, the term 'bacterial chemotaxis' is a misnomer. The phrase was coined by Pfeffer at a time when he believed that bacteria could steer directly toward the source of a chemical attractant (Pfeffer, 1884; reviewed by Berg, 1975). Molecular biologists have been more interested in understanding the genetics and biochemistry of chemotaxis (i.e. of chemoklinokinesis with adaptation) than in being scrupulous about nomenclature. So the name has stuck. It is commonly used to refer to any directed movement of bacteria towards, or away from, chemicals, regardless of the underlying mechanism.

ANALYTICAL TREATMENT

We were led to a protocol for deriving diffusion equations by the following thought experiment. Fill a long, capped pipe with an ideal gas at a low pressure, so that the mean-free path of a molecule is comparable to the length of the pipe. Keep the pipe at constant temperature, so that the mean speed of a molecule is everywhere constant. Now, add a number of solid objects to the pipe, more at one end than at the other. Collisions with these obstructions will increase the turning frequency of the gas molecules. Will this lead to an increase in the mean number of molecules per unit volume of free space at one end of the pipe as compared to the other? Any such accumulation would increase the local pressure of the gas. If so, we ought to be able to harness the pressure difference between the two ends of the pipe in order to do external work and, thereby, achieve perpetual motion. So, naïvely, we would not expect molecules to accumulate in regions of enhanced turning frequency.

Consider the following one-dimensional analogue. Let an ensemble of non-interacting particles move parallel to the axis of a long pipe. Distribute a series of semi-permeable barriers along that axis. Half of the surface of each barrier is covered with openings, so that whenever a particle reaches a barrier, it has an equal probability of bouncing back or continuing in the original direction. In either event (by construction) it adopts the speed characteristic of the local region, as defined in Fig. 1. In addition, we assume that the concentration of particles, C, in particles per unit volume, changes slowly enough over space that we can take it to be constant over a region of length δ. In other words, $\delta(\partial C/\partial x) \ll C$. We also assume that, when δ and τ vary over space, they do so slowly enough that we can take each of these quantities to be constant over a region of length δ. In order words, $\delta(\partial\delta/\partial x) \ll \delta$ and $\delta(\partial\tau/\partial x) \ll \tau$. These assumptions are made so that we can neglect higher-order terms in our derivations.

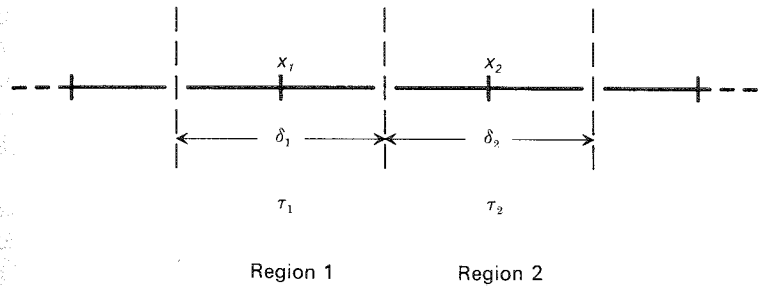


Fig. 1. A series of semi-permeable barriers (shown as vertical dashed lines) distributed along the x-axis, dividing the space into region 1, region 2, etc. The distance between the barriers adjacent to the point x_1 is δ_1 and the travel time between these barriers (in either direction) is τ_1.

Now, returning to Fig. 1, what is the particle flux, J, across the barrier separating region 1 and region 2? Half of the particles in region 1 are moving toward region 2, and half of the particles in region 2 are moving toward region 1, at velocities of δ_1/τ_1 and δ_2/τ_2, respectively. Half of the particles in each group are destined to pass through the barrier and half to be reflected. Thus, the net number of particles per unit area and unit time that cross the barrier from left to right (in the positive x-direction) is:

$$J = -(1/4)[C(x_2)(\delta_2/\tau_2) - C(x_1)(\delta_1/\tau_1)]. \tag{1}$$

The simplest case is one in which the distance between barriers, δ, and the travel time between barriers, τ, are constant. In this case, $\delta_1 = \delta_2 = \delta$, $\tau_1 = \tau_2 = \tau$, and

$$J = -(\delta/4\tau)[C(x_2) - C(x_1)], \tag{2}$$

which can be written

$$J = -(\delta^2/4\tau)[C(x_2) - C(x_1)]/\delta. \tag{3}$$

Since C changes slowly over space, $[C(x_2) - C(x_1)]/\delta \approx \partial C/\partial x$, and we arrive at Fick's First Equation:

$$J = -D(\partial C/\partial x), \tag{4}$$

with the diffusion coefficient $D = \delta^2/4\tau$. [*Note:* in a derivation in which particles at region x_n jump every τ seconds with probability 1/2 to region x_{n-1} and with probability 1/2 to region x_{n+1}, $D = \delta^2/2\tau$ (cf. Berg, 1983). The difference of a factor of 2 arises because in the barrier model, half of the particles in region x_n are destined to remain in that region after an interval τ. But this does not change any of the basic physics.] Note also that if particles had been subjected to an external force, eqns 1–4 would have contained an additional term of the form Cv_{drift}, where v_{drift}

is the drift velocity generated by that force. The equations, as they stand, refer only to the flux due to random motility.

To see what happens at equilibrium, we cap the pipe with reflective lids and wait for the particles to move around until $J=0$. Then by eqn 4, $\partial C/\partial x=0$. This implies that at equilibrium, C is uniform. There is no accumulation.

Let us generalize this derivation to allow for situations in which D changes over space. We can effect this change in four ways. *Case 1*: Vary the barrier interval, δ, and the travel time, τ, but keep the speed, δ/τ, constant. *Case 2*: Keep the barrier interval, δ, constant, but vary the travel time, τ. (The speed is not constant.) *Case 3*: Vary the barrier interval, δ, but keep the travel time, τ, constant. (The speed is not constant.) *Case 4*: Vary the barrier interval, δ, the travel time, τ, and the speed, δ/τ.

Case 1 (δ/τ constant)

We reach eqn 1, as before. Since the ratio δ/τ is constant, eqn 1 factorises to give eqn 2. With the assumption that δ varies slowly over space, eqns 3 and 4 follow, with $D=\delta^2(x)/4\tau(x)$. Note that D varies with position. When $J=0$, $\partial C/\partial x=0$, as before, and the equilibrium distribution is uniform. It does not matter how we arrange the barriers: the equilibrium distribution is uniform, provided that the speed is constant. This is the conclusion that we reached earlier in our thought experiment. But here we have said nothing about ideal gases: the proposition holds equally well for self-propelled objects, such as bacteria.

Case 2 (δ constant)

Eqn 1 now factors to give

$$J=-(\delta/4)[C(x_2)/\tau_2-C(x_1)/\tau_1]. \tag{5}$$

To distinguish contributions to J due to spatial variations in C and τ, we add and subtract (within the brackets) $C(x_2)/\tau_1$ and group terms:

$$J=-(\delta/4)\{C(x_2)[1/\tau_2-1/\tau_1]+[C(x_2)-C(x_1)]/\tau_1\}. \tag{6}$$

This can be written

$$J=-(\delta^2/4)\{C(x_2)(1/\tau_2-1/\tau_1)/\delta+(1/\tau_1)[C(x_2)-C(x_1)]/\delta\}. \tag{7}$$

By our earlier assumptions, $(1/\tau_2-1/\tau_1)/\delta\approx\partial(1/\tau)/\partial x$, and $[C(x_2)-C(x_1)]/\delta\approx\partial C/\partial x$. Since C and t are slowing varying:

$$J=-(\delta^2/4)\{C[\partial(1/\tau)/\partial x]+(1/\tau)(\partial C/\partial x)\}. \tag{8}$$

But this is just

$$J=-D(\partial C/\partial x)-C(\partial D/\partial x)=-\partial(DC)/\partial x, \tag{9}$$

with $D=\delta^2/4\tau(x)$. Now, when $J=0$, DC is constant, and C is inversely proportional to D. The particles accumulate where the travel time is large, i.e. where the speed, δ/τ, is low.

Case 3 (τ constant)

Eqn 1 now factors to give

$$J=-(1/4\tau)[C(x_2)\delta_2-C(x_1)\delta_1]. \tag{10}$$

By adding and substracting (within the brackets) $C(x_2)\delta_1$ and grouping terms we get

$$J=-(1/4\tau)\{C(x_2)(\delta_2-\delta_1)+[C(x_2)-C(x_1)]\delta_1\}. \tag{11}$$

Proceeding as before,

$$J=-(\delta/4\tau)\{C(x_2)(\delta_2-\delta_1)/\delta+\delta_1[C(x_2)-C(x_1)]/\delta\}, \tag{12}$$

$$\approx-(\delta/4\tau)[C(\partial\delta/\partial x)+\delta(\partial C/\partial x)], \tag{13}$$

or, given $D=\delta^2(x)/4t$,

$$J=-D(\partial C/\partial x)-C(\partial D/\partial x)/2. \tag{14}$$

Now, when $J=0$, $D^{1/2}C$ is constant, and C is inversely proportional to $D^{1/2}$.

Case 4 (δ, τ, δ/τ all varying)

By adding and subtracting terms within the brackets of eqn 1 and approximating derivatives, as before, we obtain,

$$J=-(\delta/4)\{C\delta[\partial(1/\tau)/\partial x]+(C/\tau)(\partial\delta/\partial x)+(\delta/\tau)(\partial C/\partial x)\}. \tag{15}$$

This equation cannot be formulated in terms of a diffusion coefficient, but one can show that the equilibrium particle density varies inversely with the speed. In fact, in all four cases, the flux equation can be rewritten as

$$J=-(\delta/4)[v(\partial C/\partial x)+C(\partial v/\partial x)], \tag{16}$$

where v is the speed, δ/τ. If v is constant, the equilibrium particle density is uniform. If v varies, the equilibrium particle density is inversely proportional to v. If, in this situation, one starts with a uniform distribution, then particles will drift until their concentration becomes inversely proportional to v. By eqn 16, the drift velocity is $-(\delta/4)(\partial v/\partial x)$; if v increases with x, the particles drift in the $-x$ direction. For cases 2 or 3 (eqns 9 or 14) this drift velocity is $-\partial D/\partial x$ or $-(\partial D/\partial x)/2$, respectively.

Eqn 4 has been adopted by a number of workers in the field of bacterial chemotaxis. Lapidus (1981) derived eqn 9. Futrelle (1982) derived eqn 14 and noted the different equilibrium distributions expected from eqns 4, 9, and 14.

It is known that the way in which *E. coli* modulates its behaviour is by extending runs (relatively straight segments of its track) when the direction of travel is favourable; changes in swimming speed are small (Berg & Brown, 1972). Therefore, the present analysis implies that *E. coli* cannot do chemotaxis solely on the basis of local cues: it must monitor concentration over a finite interval of time. This also is required by counting statistics: a cell cannot measure the concentration of chemicals in its environment without taking a reasonable period of time. The standard deviation in a count is proportional to the square-root of the count, and the counting rate is limited by diffusion of chemicals in the vicinity of the cell (Berg & Purcell, 1977). On the other hand, tumbles are not instantaneous, so, if a cell tumbles frequently enough, its average speed will fall. Eqn 16 implies that, in the absence of other stimuli, such cells will tend to accumulate where the average speed is small. For wild-type cells, this is a minor effect. It will not be considered further here.

OTHER THEORIES

It is worth noting where we disagree with earlier work. Patlak (1953) presents a generalized formulation of the random walk. In addition to allowing for the possibility of a preferred direction, he assumes that there are distributions of possible values for δ, τ, and v, all of which depend on position and time. This leads to an elaborate diffusion equation. In examining the limit in which there is no preferred direction and no correlation between runs, Patlak arrives at a simpler equation, $\partial C/\partial t = \nabla^2(DC)$, which is equivalent to $J = -\nabla(DC)$, the three-dimensional analogue of our eqn 9. Unfortunately, in making this simplification, he inadvertently holds the mean run length constant (by setting the deviation from the mean run length equal to zero, p. 329). As we have seen (*Case 2*) the equation $J = -\partial(DC)/\partial x$ is not valid in cases in which the mean run length varies while the speed remains constant. Patlak and those who rely heavily on his work (e.g. Okubo, 1980; Doucet & Wilschut, 1987; Turchin, 1989) believe incorrectly that simply altering the frequency of turning as a function of position without changing speed can result in accumulation. One encounters misleading phrases such as 'the well-known phenomenon of trapping . . . by enhancement of the turning frequency' (Alt, 1980) or the 'flypaper effect' (Stock & Stock, 1987).

Keller & Segel (1971) derive an equation in which the flux due to random motility is given by Fick's Equation (with $D = \mu$, which they call the mobility) and the flux due to chemotaxis is given by a drift velocity times

the concentration (with drift velocity equal to the product of a chemotaxis coefficient, χ, and the slope of the gradient of the chemoattractant). In their derivation, the step time varies with the concentration of the attractant but the step length is held constant, so they have, in fact, assumed that the speed changes as a function of position (*Case 2*). As a consequence, the drift velocity is the gradient of the diffusion coefficient, in disguise. Sight is lost of this in later applications, where the mobility and chemotactic coefficients are treated as phenomenological parameters (e.g. Nossal, 1980). Segel (1984) considers it a matter of taste whether or not one regards $-(\partial D/\partial x)$ as an effective drift, 'indeed not brought about by intrinsic directional preferences at point x, but rather due to spatial differences in the vigor of random motion'. Our point is that one must be precise in defining what is meant by the word 'vigor'. The consequences of varying δ or τ, or both, are not the same (*Cases 2–4*), and if δ/τ happens to be constant, the effective drift vanishes.

Lapidus (1981) derives a diffusion equation from a random walk with a constant step length and a stepping probability per unit time that varies spatially. Within the limit that steps in either direction are equally probable, his equation reduces to $\partial C/\partial t = \partial^2(DC)/\partial x^2$, which is equivalent to our eqn 9. Lapidus concludes that 'it is clear that cells aggregate at those places where D is small'. However, if the step length is held constant while the stepping probability per unit time varies spatially, then the cell's speed will also vary spatially. Lapidus's mistake is assuming that this particular flux equation is generally applicable; aggregation will not occur when the speed is constant.

In earlier work, Lapidus (1980) assumes that the diffusion coefficient is a function only of the local concentration of a chemical attractant and also that Fick's Equation (eqn 4) is valid. He finds numerically that cells, initially distributed far from equilibrium, transiently shift towards regions in which the diffusion coefficient is large (i.e. move up the gradient of a substance that suppresses tumbles). However, this 'pseudochemotaxis', as he calls it, fades with time, and the distribution becomes uniform. This analysis is correct. But once again, it is not generally applicable. It applies only when the speed is constant.

The moral of all this is that different assumptions about the microscopic behaviour of particles or cells require distinct diffusion equations. One cannot claim, for example, that $J = -\partial(DC)/\partial x$ and then assert that D varies over space solely because of differences in turning frequency. Nor can one claim that $J = -D(\partial C/\partial x)$, and then assert that D varies because of differences in speed.

Recent workers have been more careful. Rivero *et al.* (1989) derive a flux equation (their eqn 14) in which the dependence on turning frequency and speed are made explicit. In the absence of chemotaxis and at constant speed, their equation reduces to Fick's Equation, as it should.

COMPUTER EXPERIMENTS

Some of the assumptions on which our derivations are based, notably that all parameters change slowly over space, might be restrictive. Are there difficulties that arise from neglected higher-order terms? In the interest of uncovering any such problems and extending the analysis to situations that are difficult to solve analytically, e.g. to bacterial chemotaxis, we developed a series of Montecarlo simulations.

Equilibrium distributions

In the first simulation, a probability per step that a tumble will occur is assigned to every point in a one-dimensional lattice, either with reflecting or periodic boundary conditions. When the cell arrives at a lattice point, a random number is selected, uniformly distributed between 0 and 1. If the value is less than the tumble probability, the next step is taken in the opposite direction; otherwise, it is taken in the same direction. (Tumbles could just as well have been defined so that the cell sets off with equal probability in either direction, rather than reversing, but this would merely double the mean run length without changing any essential feature.) Before the computer writes anything down, the cell is allowed to walk along the lattice for a number of steps which is large compared to that required for an ensemble of such cells to approach the equilibrium distribution. After this initialization, the program is allowed to run for a much larger number of steps, while the computer records the number of visits to each lattice point. We assume that a collection of non-interacting cells will distribute themselves at equilibrium with concentrations proportional to these visitation numbers (i.e. to the fraction of time that the cell spends in a given region of space).

Case 1 (speed constant)

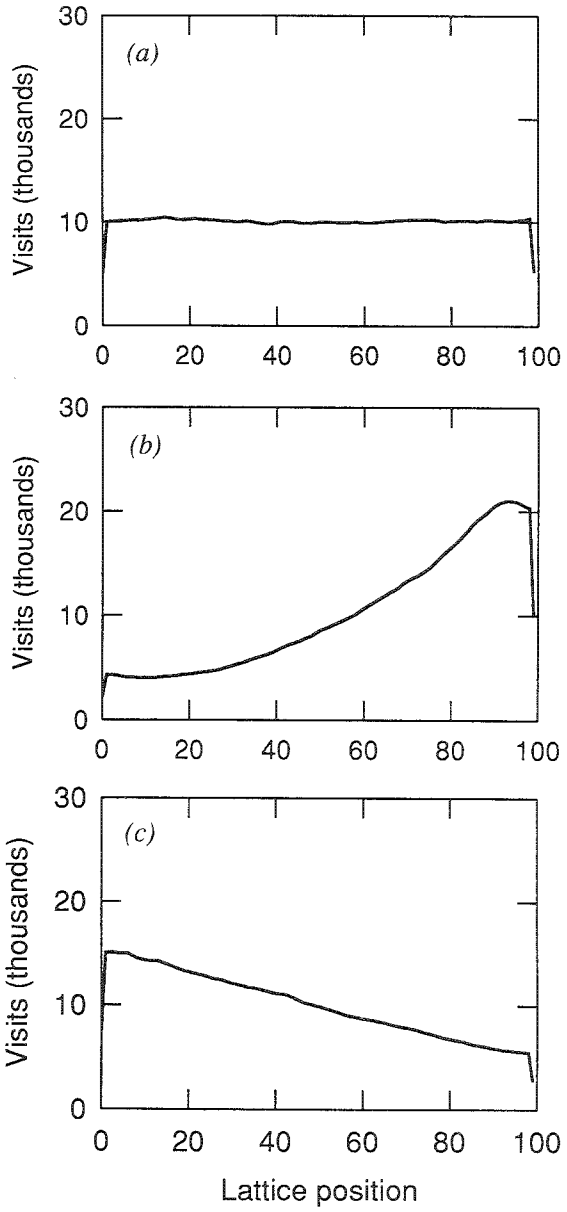
After many trials with lattices of different sizes, with both reflecting and periodic boundary conditions, and with different probabilities of tumbling, we found no evidence that cells moving at constant speed accumulate in regions of higher tumble probability. An example with reflecting boundary conditions is shown in Fig. 2(*a*). In the analytical derivation for this case, the tumble probability is 1/2. Here, it can be much smaller, so that a cell can continue for many lattice points before being reflected. This allows us to probe for changes in behaviour over distances which are short compared to the mean run length. If the tumble probability is approximately constant, runs are distributed exponentially, with a mean length equal to the reciprocal of the tumble probability per unit length, as is the case for *E. coli* (Berg & Brown, 1972).

Cases 2–3 (variable speed)

The essential information provided by the computer simulations just described is that changes in tumble probability have no effect upon the number of visits to each lattice point (the total amount of time per unit length spent in a given region of space). All that the computer does is to select a random number when the cell arrives at a given lattice point and to record the visit. The same process would be carried out in a simulation in which the lattice spacing or the step time varied. For example, if in one region the lattice spacing were doubled and the step time were held constant (i.e. if the speed were doubled), the number of visits to the lattice points would remain the same, but the number of visits per unit length would halve. Thus, the equilibrium particle density would halve. If the lattice spacing were held constant and the step time were halved (again, if the speed were doubled) the time spent in a given region would halve. Thus, the equilibrium particle density would halve. A cell that changes its speed as a function of position will spend a total amount of time in a given region of space that is inversely proportional to the local speed. Thus, we arrive at the distribution predicted by eqn 16 (and by inference eqns 9 and 14).

Measurements extending over time

In these simulations, we follow the strategy used in the first simulation but assign the tumble probability for a given lattice point in a manner that depends upon the cell's past history, in particular, upon the concentrations of an attractant that the cell has recently measured. The weightings are approximations to those determined from measurements of responses of tethered cells to impulsive stimuli (cf. Segall *et al.*, 1986). We use reflecting boundary conditions. To simulate the behaviour of wild-type cells, we assign a weighting factor $1/N$ to the concentrations sensed at the previous N lattice points, and a weighting factor $-1/M$ to the concentrations sensed at the M lattice points before that, and take the sum. This is equivalent to taking the difference between two sequential averages, and approximates to a derivative operator with memory. If the cell has been moving up the gradient, this sum is positive. If so, we reduce the probability of a tumble from a baseline value by an amount (gain $\times$ sum). The gain is picked so that a cell moving steadily up the gradient still has a finite probability of tumbling. If the cell has been moving down the gradient, the sum is negative. In this event, the tumble probability is set to the baseline value. Thus, runs are lengthened as a consequence of favourable measurements but not shortened as a consequence of unfavourable ones (cf. Brown & Berg, 1974). In simulating the behaviour of cells that do not adapt (e.g. *che*R *che*B mutants, cells that cannot methylate or demethylate their membrane transducers; cf. Segall *et al.*, 1986) we assign a weighting factor $1/N$ to the concentrations sensed at the previous N lattice points and take the sum. As before, we reduce the probability of a tumble from the baseline value by an amount (gain $\times$ sum), where the gain has a smaller value



than before. Once again, the gain is chosen so that cells near the top of the gradient have a finite probability of tumbling. Thus, tumbles are suppressed on the basis of measurements made over the previous N lattice points, but temporal comparisons are not made. The results of these simulations are shown in Figs. 2(b) and 2(c). Wild-type cells drift up the gradient, Fig. 2(b), while cells that fail to make temporal comparisons drift down, Fig. 2(c).

Approach to equilibrium

These simulations are done in the same way, except that we start cells at the centre of a lattice and record where they end up after a number of steps, n. The specifications for setting the tumble probabilities are the same as before. Fig. 3 shows a control experiment, in which the tumble probabilities are constant. Figs 4, 5, and 6 refer to the same experiments as Figs 2(a), 2(b), and 2(c), respectively. In Fig. 3 the cells spread symmetrically (the distribution is a Gaussian). In Fig. 4 they spread more rapidly in the direction in which tumbles are suppressed (up the gradient), and then they relax to a uniform distribution. In Fig. 5 the cells drift up the gradient, and in Fig. 6 they drift down.

OTHER COMPUTER EXPERIMENTS

Other simulations have been run in which organisms move at constant speed and modulate their turning frequencies. Some workers reach conclusions that appear similar to ours and others do not (Rohlf & Davenport, 1969; Van Houten & Van Houten 1982; Doucet & Drost, 1985; Doucet & Wilschut, 1987; and Dusenbery, 1989). One possible source of

Fig. 2. Equilibrium distributions for cells moving at constant speed. (a) Tumble probabilities defined locally (pseudochemotaxis). The tumble probability per step varied linearly from 0.3 near the left reflecting boundary (position 0) to 0.03 near the right reflecting boundary (position 99). The tumble probabilities at the boundaries were set equal to 1. At lattice point 50 the tumble probability was 0.135, corresponding to a mean run length of 7.4 steps. One million steps were logged. A similar distribution was obtained from a computation in which the tumble probability was constant (0.135 everywhere; data not shown). A portable pseudorandom number generater was used that is known to be free from sampling artifacts (Press *et al.*, 1988). A different seed was used for each figure shown in this paper. (b) Tumble probabilities defined on the basis of the difference between two sequential averages (real chemotaxis). The number of lattice points used for the two averages was $N = 7$ and $M = 21$. The baseline probabability was 0.135. The gain was chosen so that a cell moving steadily up the gradient (toward the right) had a tumble probability 0.03. See the text. One million steps were logged. (c) Tumble probabilities defined on the basis of a single average (no adaptation). The same gradient was sensed as in the previous computation. The number of lattice points used for the average was $N = 7$. The baseline probability was 0.135. The gain was chosen so that a cell moving near the right reflecting boundary had a tumble probability 0.03. Note that the tumble probability depends on position; for a cell moving near the left boundary, it was 0.111. See the text. One million steps were logged.

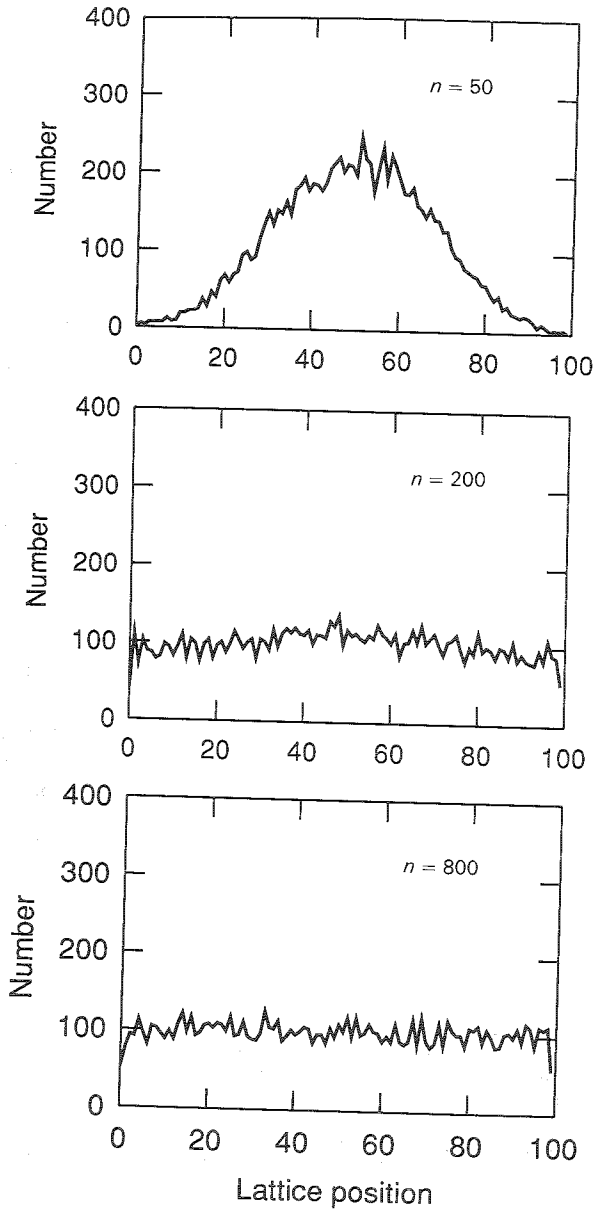


Fig. 3. Approach to equilibrium at constant tumble probability (simple diffusion). Ten thousand cells were released at lattice point 50 and allowed to step for $n = 50$ times (top), 200 times (middle), or 800 times (bottom). The number of cells ending up at each lattice point was recorded. The tumble probability was 0.135 at all points. Note the symmetrical spreading.

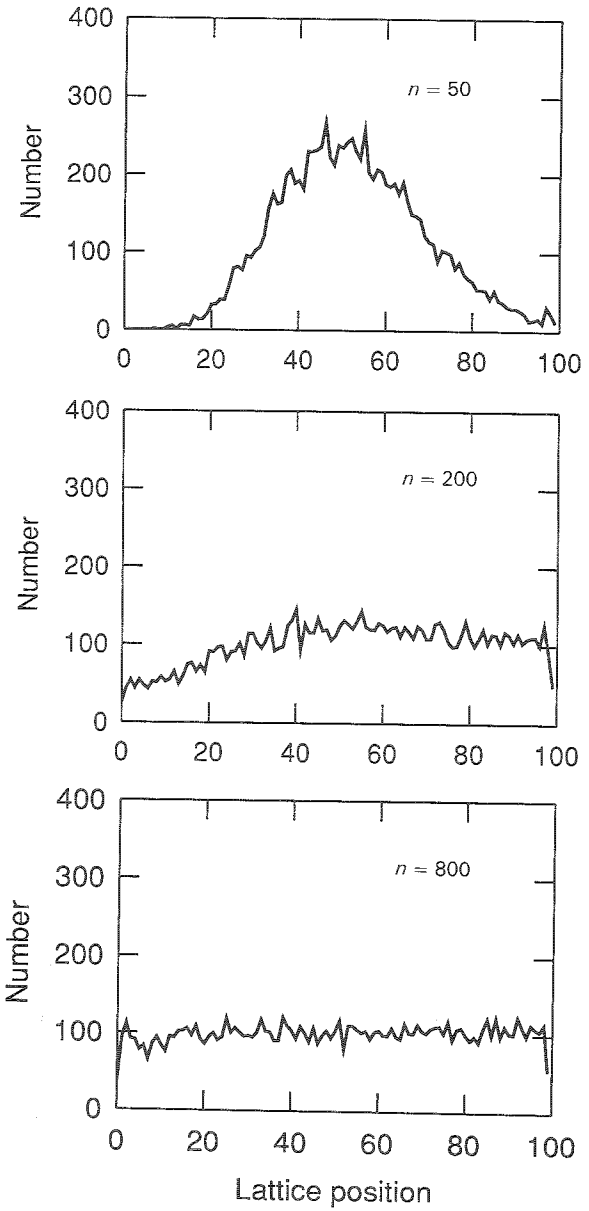


Fig. 4. Approach to equilibrium with tumble probabilities defined locally (pseudochemotaxis). The computation was carried out as in Fig. 3 with the tumble probabilities set as in Fig. 2(*a*). Note the feint to the right (in the direction of lower tumble probability).

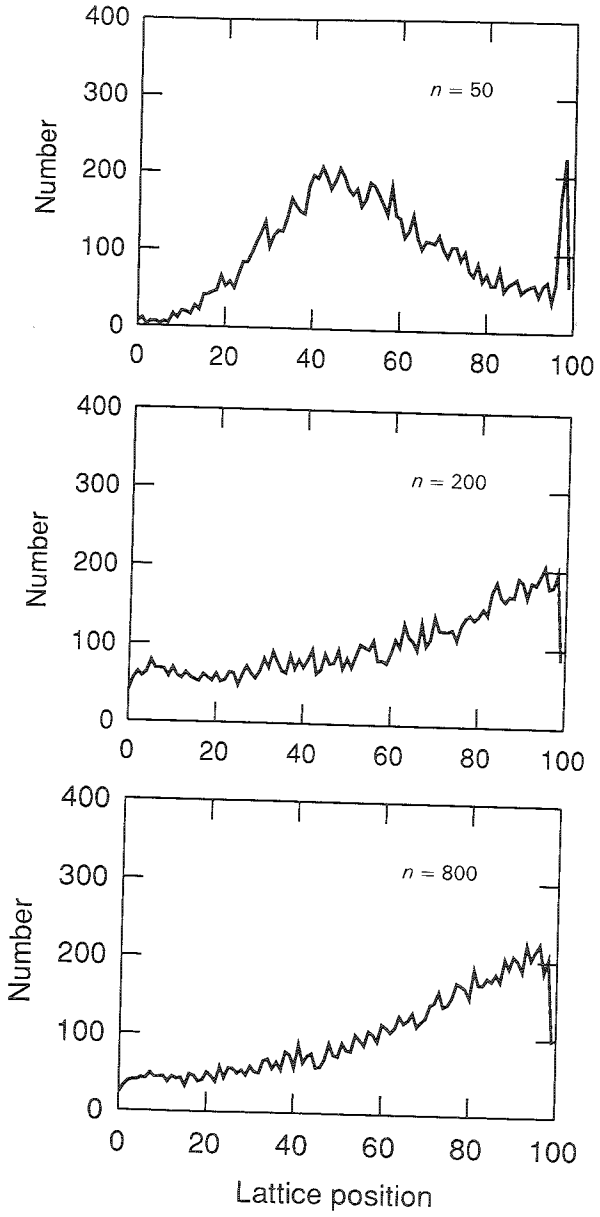


Fig. 5. Approach to equilibrium with tumble probabilities defined on the basis of the difference between two sequential averages (real chemotaxis). The computation was carried out as in Fig. 3 with the tumble probabilities set as in Fig. 2(*b*). Note the progressive shift to the right (up the gradient of an attractant).

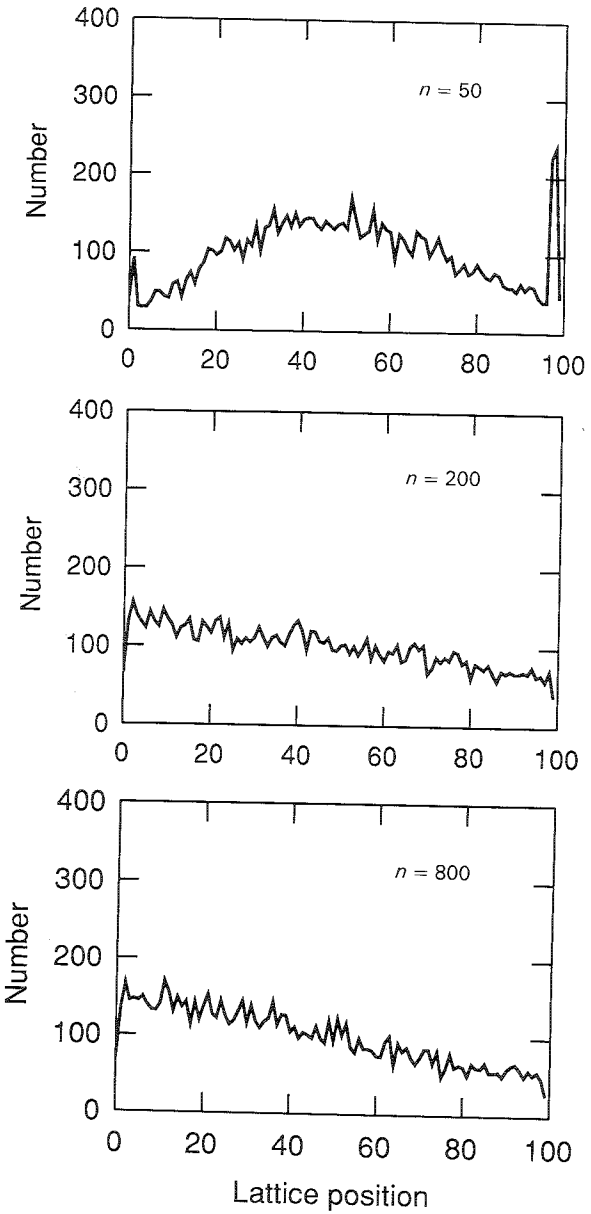


Fig. 6. Approach to equilibrium with tumble probabilities defined on the basis of a single average (no adaptation). The computation was carried out as in Fig. 3 with the tumble probabilities set as in Fig. 2(*c*). Note the progressive shift to the left (down the gradient of an attractant).

disagreement is made particularly clear by Figs 4 and 2(*a*), namely, that, if one follows the displacements of cells for a limited period of time, the conclusions reached might well be different than if one were to determine equilibrium distributions. This is why Lapidus (1980) coined the term 'pseudochemotaxis'. Note that the transient movement in Fig. 4 is up the gradient, i.e. in the direction in which tumbles are suppressed. If one adds sources and sinks and computes fluxes, the differences can appear even more dramatic. For example, if, in the experiment of Fig. 4, the boundaries at positions 0 and 99 are made absorbing, the fraction of cells arriving at position 99 is substantially larger than that arriving at position 0 (by a factor of 2.7). But this merely reflects the fact the the diffusion coefficient is substantially larger over the right half of the figure than over the left. It does not tell us what the equilibrium distribution might be.

DISCUSSION

Patterns of accumulation in bacteria resulting from changes in swimming speed are well known. Vivid descriptions have been given by Metzner (1920; reviewed by Berg, 1975), who watched cells of *Spirillum volutans* as they responded to chemicals placed near the edge of a coverslip. In some cases, the cells became trapped, with their flagellar bundles spinning in a head–head or tail–tail configuration (i.e. working against one another); in other cases the cells shuttled back and forth rhythmically a body length or less, or stopped moving altogether. Clayton (1957) analysed such patterns quantitatively, both in theory and experiment, using the phototactic organism *Rhodospirillum rubrum*. A distinction between effects arising from modulation of turning frequency, as opposed to those arising from modulation of swimming speed, was drawn by Gunn *et al.* (1937) and Fraenkel & Gunn (1940), who assigned to them the terms 'klinokinesis' and 'orthokinesis', respectively. The definition of klinokinesis was based on the work of Ullyott (1936), who studied the photoresponses of a flatworm, *Dendrocoelum lacteum*; however, this work did not stand the test of time (cf. Gunn, 1975). Fraenkel & Gunn argued (correctly) that organisms moving at constant speed could not accumulate (alter the shape of a uniform distribution) by modulating their turning frequencies, unless they could adapt (compare the stimulus in the present with that in the past). This conviction was undermined by the analysis of Patlak (1953), which we have found to be in error. However, as stressed in the previous section, variations in diffusion coefficient will affect the way in which cells approach equilibrium. One of the best ways to determine whether cells actively accumulate is to start with a uniform distribution and then to ask whether that distribution changes with time. This can be done experimentally with the layered-gradient assay (Dahlquist *et al.*, 1972; Weis & Koshland, 1988).

The results of Fig. 2 can be rationalized as follows. Imagine yourself

at an arbitrary lattice point watching cells arriving from the left or the right. In (*a*) the probability that a cell will tumble does not depend on whether it has arrived from the left or the right, so the same fraction of cells of either type will cross the boundary. At equilibrium, when the net flux is zero, an equal number of cells must arrive from the left or the right. Therefore, their distribution must be uniform. In (*b*) the probability of a tumble is smaller when a cell arrives from the left than when it arrives from the right, because cells have been taking derivatives with respect to time, and some have been going up the gradient, while others have been going down. More of the cells that arrive from the left will cross the barrier than those that arrive from the right. Therefore, at equilibrium, there must be fewer cells on the left than on the right. In (*c*), the situation is reversed, because the probability that a cell will tumble is larger if it has arrived from the left, where the concentration of the tumble-suppressing substance is smaller, than if it has arrived from the right. Therefore, at equilibrium, there must be more cells on the left than on the right.

We began this work with the hope of learning whether the weighting function used by *E. coli* for making temporal comparisons (called the impulse response) reflected an optimum design (cf. Block *et al.*, 1982; Segall *et al.*, 1986; Berg, 1988). *En route*, we became aware that there was a great deal of confusion about the success of more rudimentary strategies. These became the main subject of the present paper. In the linear, noiseless gradients of the sort considered in Figs 2(*b*) and 5, similar drift rates are obtained for weighting functions of the same total width, regardless of the relative size of the positive and negative lobes (assuming that the areas of the two lobes are the same, i.e. that the cells adapt). We know from an analysis of how cells count molecules that in the real world the lobes should have a width of the order of 1 second (Berg & Purcell, 1977). The first lobe cannot be much longer than this, because then several tumbles could occur before a decision were reached, and the results would not be relevant. In addition, the baseline tumble probability cannot be much lower than it is, because rotational Brownian movement carries a cell off course by as much as 90° within 10 seconds. In wild-type cells, the widths of the two lobes are about 1 and 3 seconds, respectively. Is the ratio of these values a matter of physics or biochemistry? One way to find out would be to extend the simulations by working in three dimensions, making the gradients lumpy rather than smooth, adding the fluctuations expected in counting molecules, and including the meandering due to rotational Brownian movement. Drift rates computed in this manner for cells using the wild-type impulse response in a smooth, noiseless gradient agree well with drift rates measured experimentally (Berg, 1988). A broader range of parameters remains to be explored.

CONCLUSIONS

We re-examined the problem of migration of motile organisms in spatial gradients of chemical attractants. We showed analytically and by Montecarlo simulation that organisms whose turning frequencies (tumble probabilities) depend solely on the local concentration of an attractant, but whose speeds remain constant, do not accumulate at the top of such a gradient: once uniformly distributed, they remain uniformly distributed. On the other hand, organisms whose swimming speeds depend on the local concentration of an attractant do accumulate in regions where the speeds are low. This analysis resolves a long-standing controversy in the literature that arose because different microscopic mechanisms for generating variations in the diffusion coefficient (a macroscopic parameter) were not properly distinguished. These mechanisms lead to distinct diffusion equations. We extended the Montecarlo simulation to non-local strategies and found that cells that respond (by suppressing tumbles) to concentrations of an attractant sensed over the recent past, but do not make temporal comparisons, drift down rather than up the gradient. Cells that compare concentrations sensed over the recent past with those sensed earlier are able to drift up the gradient. This is the strategy used by *E. coli* for chemotaxis.

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