PHYSICS OF CHEMORECEPTION

HOWARD C. BERG AND EDWARD M. PURCELL, *Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309 and the Department of Physics, Harvard University, Cambridge, Massachusetts 02138 U.S.A.*

ABSTRACT Statistical fluctuations limit the precision with which a microorganism can, in a given time T, determine the concentration of a chemoattractant in the surrounding medium. The best a cell can do is to monitor continually the state of occupation of receptors distributed over its surface. For nearly optimum performance only a small fraction of the surface need be specifically adsorbing. The probability that a molecule that has collided with the cell will find a receptor is $Ns/(Ns + \pi a)$, if N receptors, each with a binding site of radius s, are evenly distributed over a cell of radius a. There is ample room for many independent systems of specific receptors. The adsorption rate for molecules of moderate size cannot be significantly enhanced by motion of the cell or by stirring of the medium by the cell. The least fractional error attainable in the determination of a concentration $\bar{c}$ is approximately $(T\bar{c}aD)^{-1/2}$, where D is the diffusion constant of the attractant. The number of specific receptors needed to attain such precision is about a/s. Data on bacteriophage adsorption, bacterial chemotaxis, and chemotaxis in a cellular slime mold are evaluated. The chemotactic sensitivity of *Escherichia coli* approaches that of the cell of optimum design.

INTRODUCTION

In the world of a cell as small as a bacterium, transport of molecules is effected by diffusion, rather than bulk flow; movement is resisted by viscosity, not inertia; the energy of thermal fluctuation, kT, is large enough to perturb the cell's motion. In these circumstances, what are the physical limitations on the cell's ability to sense and respond to changes in its environment? What, for example, is the smallest change in concentration of a chemical attractant that a bacterium could be expected to measure reliably in a given time? We begin our analysis by reviewing some relevant features of diffusive transport and low Reynolds number mechanics. This will lead to certain conclusions about selective acquisition of material by a cell and how this acquisition may be influenced by the cell's movement. We then develop a theory of the signal-to-noise relation for measurement of concentration by a cell with specific receptors, discuss its implications for chemotactic behavior, and compare theory with experiment.

DIFFUSIVE INTAKE

Consider a spherical cell of radius a immersed in an unbounded medium. The medium contains in low concentration some molecules of species X with diffusion

constant D. The local concentration of X will be denoted by c and expressed in molecules per cubic centimeter (1 M = $6 \times 10^{20} \text{cm}^{-3}$). The spatial and temporal variation of c is governed by the diffusion equation

$$D\nabla^2 c = \partial c/\partial t. \tag{1}$$

Suppose the cell is a perfect sink for the molecules X, sequestering or otherwise immobilizing every X molecule that reaches its surface. Then in the steady state the current J of molecules to the cell, in molecules per second, is given by

$$J = 4\pi a D c_\infty, \tag{2}$$

where c_∞ is the concentration far from the cell, assumed to be maintained at a steady value.[1]

For our purposes it will be useful and instructive to relate this formula to an electrical analogue. Comparing the time-independent diffusion equation $\nabla^2 c = 0$ with Laplace's equation for the electrostatic potential ϕ in charge-free space, $\nabla^2 \phi = 0$, we observe that the diffusive current density $\mathbf{F} = -D \text{ grad } c$ is the analogue of the electric field vector $\mathbf{E} = -\text{grad}\,\phi$. The total diffusive current J entering a closed surface S is given by $J = \int_S \mathbf{F} \cdot \text{ds}$, whereas the total electric charge Q on a surface is given by $Q = 1/4\pi \int_S \mathbf{E} \cdot \text{ds}$. Because the cell is a perfect sink, c must be zero at its surface, which therefore corresponds to a surface at constant potential. We see that Eq. 2 is equivalent to the statement that the static charge Q on a spherical conductor in vacuum is $\phi_\infty a$, if ϕ_∞ is the difference in potential between the conductor and points far away. And in general, the steady-state diffusive current to a totally absorbing body of any shape and size can be written as

$$J = 4\pi C D c_\infty, \tag{3}$$

where C is the electrical capacitance (in cgs units of centimeters) of an isolated conductor of that size and shape.

Solutions are available for the capacitances of a variety of conductors. As an example, the capacitance of an isolated thin conducting disk of radius b, $2b/\pi$ in cgs units (1), provides us with the diffusive current to both sides of a disk-like sink: $J = 8bDc_\infty$. The same result provides us with the current through a circular aperture of radius b in a thin membrane which separates regions of concentration c_1 and c_2: $J_{1,2} = 4bD(c_1 - c_2)$.

Another simple result, perhaps not quite so familiar, is easily obtained by way of the electrical analogy. Consider again the completely absorbing sphere of radius a in an unbounded medium. Let a molecule X be released at a point in the medium a distance r from the center of the sphere. What is the probability P_c that the molecule will eventually be captured by the sphere? The exact answer, which we shall make use of

[1] If a steady state has been established, the right-hand side of Eq. 1 is zero. Then a solution with spherical symmetry and with $c = 0$ at $r = a$ is $c = c_\infty(1 - a/r)$. The density of inward current, $D\text{d}c/\text{d}r$, is c_∞/r^2, giving for the total inward current the result in Eq. 2.

later, is

$$P_c = a/r. \tag{4}$$

It can be found by considering the capacitance of concentric spherical shells.[2] Notice that the result has nothing to do with the solid angle subtended by the absorber as seen from the point of release; with increasing distance, P_c goes down not as $1/r^2$, but only as $1/r$.

The electrical analogy does not extend to time-dependent diffusion. The relaxation of a charge distribution within a homogeneous poor conductor, which might be thought to resemble superficially the relaxation by diffusion of a spatially varying concentration, is governed by a first-order equation and is characterized by a size-independent time constant. In contrast, the characteristic time for a change brought about by diffusion in a region of size a is a^2/D.

Absorption by Specific Receptors

Let us apply some of this to a cell that carries on its surface specific receptors for species X. Each receptor has a binding site that we shall idealize as a circular patch of radius s. Suppose N receptors are distributed more or less uniformly over the surface of the cell. The cell's radius is again a; the fraction of its surface occupied by binding sites is $Ns^2/4a^2$. Any X molecule that touches a binding site is immediately (or within a time short compared to the interval between arrivals) captured and transported through the cell wall, clearing the site for its next catch. The surface of the cell between these absorbent patches is impermeable to and does not bind X molecules. Under these rules, what is the total current of X molecules assimilated by the cell, in a medium of X concentration c_∞?

For $N = 1$, and if $s \ll a$, the current is the same as that to one side of the disk-like sink already mentioned, and is given by

$$J_1 = 4Dsc_\infty. \tag{5}$$

With only a few widely separated receptors the total current J will be almost N times as great. But as N increases, the receptors will begin to interfere with one another, the presence of one sink depressing the concentration in the vicinity of a neighboring sink, and vice versa. If N is so large that the surface is entirely covered by absorbent patches, the whole cell becomes a spherical sink, and the current, which is the largest current a cell of that size, however equipped, could collect by diffusion, is given by Eq. 2 as

$$J_{max} = 4\pi aDc_\infty. \tag{6}$$

[2]To derive this, consider the diffusive current from a continuous source distributed uniformly over a shell of radius r. The probability sought is the ratio of the inward current flowing to the sphere of radius a to the total current, inward plus outward. The electrical equivalent is a spherical capacitor with the inner conductor at zero potential, the same as the potential at infinity. The ratio of the inward diffusive current to the total current is the same as the ratio of the charge on the inner surface of the outer conductor to the total charge on that conductor, namely a/r.

We can find the current for any number N of receptors by solving the following analogous problem in electrostatics: Over the surface of an insulating sphere of radius a are evenly distributed N conducting disks of radius s, connected together by infinitesimal wires so as to form a single conductor. The insulating sphere is itself impermeable to electric field, which is to say that its dielectric constant is zero, requiring the electric field just outside the surface of the dielectric to have vanishing normal component. What is the capacitance of this object? The answer, derived in Appendix A, is

$$C = Nsa/(Ns + \pi a). \tag{7}$$

The only assumption made in the derivation of this equation is that the distance between neighboring disks is large compared with the size of a disk, or equivalently, that the fraction of the sphere's surface covered by disks is small. This condition will be satisfied in all our applications of Eq. 7. Translating Eq. 7 into a formula for the cell's intake of X molecules by diffusion, we find

$$J = 4\pi Dc_\infty Nsa/(Ns + \pi a) = J_{max}Ns/(Ns + \pi a). \tag{8}$$

For large N the intake approaches that of the completely absorbing cell, as it ought to. But it can become *almost* that large before more than a small fraction of the cell's surface is occupied by absorbent patches. A reasonably generous allotment of area for one patch might be a few hundred square angstroms. Let us take the patch radius s equal to 10 Å and the cell radius a equal to 1 μm. According to Eq. 8, the intake is half of J_{max} for $N = \pi a/s = 3{,}100$. The receptor patches then occupy somewhat less than 1/1,000 of the cell's surface. The distance between neighboring patches is about 60 times the patch radius. It is important that the receptor patches be well dispersed. If they were combined into a single absorbent patch of the same total area, the current would be severely reduced, from $J_{max}/2$ down to $J_{max}/(3{,}100)^{1/2}$. If the same number of receptors were distributed randomly over the surface of the cell, rather than uniformly, as assumed in the derivation of J/J_{max}, the current would be only slightly smaller than $J_{max}/2$. A numerical calculation comparing uniformly distributed with randomly distributed receptors showed that the difference in current, for the same number of receptors N, does not exceed a few percent if N is larger than 50.

Qualitatively the outcome reflects the fact that a diffusing molecule that has bumped against the surface of the cell is by that very circumstance destined to wander around in that vicinity for a time, most likely hitting the cell many times before it wanders away for good. Insight is gained by developing this idea quantitatively. Fig. 1 shows the path of a diffusing molecule that has touched the cell's surface at a sequence of points A, B, ... F, none of which happened to lie in a receptor patch. This hypothetical path is unrealistic in one respect: the total number of distinct encounters with the surface in a finite interval after one encounter is really very large—in the limit of continuous diffusion, infinite. But clearly, that does not give the molecule an infinite number of independent tries at hitting an absorbent patch. Two contacts such as C and D, close together compared to the dimension s of a patch, must count as only one try. The effective number of independent tries must be something like the number of

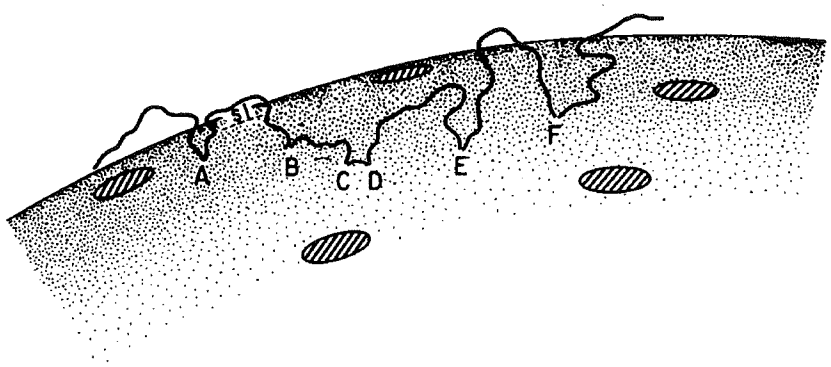

FIGURE 1 The path of a diffusing molecule that has touched the surface of a cell of radius a at a sequence of points $A, B, \ldots F$. The receptor patches, shown shaded, are of radius s. A and B constitute independent tries at hitting a patch, but C and D do not. Note between A and B the excursion of distance s perpendicular to the surface of the sphere.

path segments whose ends on the cell surface are separated by a distance greater than s. Such a path segment is likely to include an excursion of similar magnitude perpendicular to the surface. Let us therefore assume, for a rough calculation, that extension out to a distance s from the surface will serve as the necessary and sufficient condition for the ends of a segment to be at least a distance s apart.

How many such excursions are to be expected after a molecule has once touched the cell? The probability P_s that a molecule now located a distance s from the surface of a sphere of radius a will hit the surface of the sphere at least once before escaping to infinity is precisely equivalent to the "capture probability" P_c given by Eq. 4, which we now rewrite as

$$P_s = a/(a + s). \tag{9}$$

The probability that a molecule now at $r = a + s$ will execute exactly n excursions to the surface, separated by reappearances at $r = a + s$ and followed by diffusion to infinity, is $P_s^n(1 - P_s)$. It follows that the average number of such excursions is

$$\bar{n} = \sum_{n=0}^{\infty} n P_s^n(1 - P_s) = P_s/(1 - P_s) = a/s. \tag{10}$$

The probability of not hitting a receptor patch in a single random encounter is $\beta = 1 - (Ns^2/4a^2)$. If the contacts we have just enumerated can be taken as independent tries, the probability that a molecule starting at $r = a + s$ survives all subsequent contacts until it escapes to infinity is

$$P_{esc} = \sum_{n=0}^{\infty} \beta^n P_s^n(1 - P_s) = (1 - P_s)/(1 - \beta P_s). \tag{11}$$

Eq. 11 reduces to

$$P_{esc} = 4a/(4a + Ns). \tag{12}$$

Since $1 - P_{esc}$ is the fraction of all arriving molecules that ultimately are captured, we have for the resulting current

$$J/J_{max} = Ns/(4a + Ns), \tag{13}$$

to be compared with our exact formula, Eq. 8. The close numerical agreement is fortuitous. But this does show clearly how the remarkable effectiveness of dispersed receptors arises from the multiplicity of encounters of a single diffusing molecule with the surface of the cell. The number $\bar{n}$ would be about 1,000 in our earlier example.

Some general conclusions can now be drawn. The number of receptors a cell can usefully employ is not much larger than the ratio of cell diameter to patch diameter; more receptors than that fail to increase the intake much. Receptor patches of adequate number cover only a small fraction of the surface of the cell. Hundreds of such receptor systems can be accommodated, each capable of collecting its particular molecular species almost as effectively as if the entire surface of the cell were dedicated to that single task. Other constraints aside, the best arrangement of receptors of a given type is maximum dispersal, with different receptor systems thoroughly intermingled.

Two-Stage Capture

Adam and Delbrück (2) considered a two-stage capture process involving adsorption followed by diffusion of the adsorbed molecule over the surface of the cell. Suppose that an X molecule that touches the cell at any point becomes attached, but so weakly that it can migrate by two-dimensional diffusion until it either desorbs or encounters an X-receptor. This will increase the rate at which molecules are captured by receptors by an amount that depends on the coefficient of surface diffusion, D', and the mean time of residence on the surface before desorption, $\bar{t}_r$. Of course, a molecule just desorbed has a very good chance of diffusing back to the surface and being readsorbed, so the total time available to a particular molecule for random exploration of the cell surface will be many times $\bar{t}_r$. But that time and $\bar{t}_r$ itself do not need to be involved explicitly in the result, as we shall see.

Following Adam and Delbrück, we consider first the mean time $\bar{t}_c$ between adsorption on the cell's surface and capture, by a receptor, of a molecule that never desorbs. This time $\bar{t}_c$ is to be averaged over all possible starting positions, that is, over the whole surface of the cell. An approximate formula for $\bar{t}_c$, agreeing closely with the one given by Adam and Delbrück, is

$$\bar{t}_c = (1.1\,a^2/ND') \ln (1.2\,a^2/Ns^2). \tag{14}$$

As before, N is the number of X receptors on the cell, a is the radius of the cell, and s is the radius of the binding site. The receptors are assumed to be uniformly distributed; the binding sites take up only a small fraction of the cell's surface ($Ns^2 \ll 4a^2$). Eq. 14 has been adapted from an exact formula that can be derived for the case of an absorber in the center of a disk with an impermeable perimeter, as explained in Appendix B. The result was checked for a square lattice of absorbers in an independent computation by a relaxation method, and also by a Monte Carlo

calculation. The latter confirmed that the distribution of times-to-capture is exponential. That simplifies our problem, for it implies that, averaged over all positions, the probability for capture within an interval dt is $dt/\bar{t}_c$, independent of the starting time; the mean rate at which X molecules are being captured by receptors at any time depends only on the number of X molecules on the cell's surface at that time. Denote that number by m and its time average by $\overline{m}$. Let J' be the average current absorbed by the cell by way of the two-stage process. Then

$$J' = \overline{m}/\bar{t}_c. \tag{15}$$

As long as J' is small compared with $J_{max} = 4\pi aDc_\infty$, the number $\overline{m}$ will be close to its equilibrium value for the given concentration of X molecules in the medium. In that case we expect $\overline{m}$ to be given approximately by

$$\overline{m} \simeq 4\pi a^2 dc_\infty \exp(E_A/kT), \tag{16}$$

where E_A is the energy of adsorption and d is a distance of molecular size. The factor $4\pi a^2 d$ is roughly the volume accessible to a molecule adsorbed but still free to move over the surface. When Eqs. 14–16 are combined, we find

$$J' \simeq 4\pi ND'dc_\infty \exp(E_A/kT)/\ln(a^2/Ns^2). \tag{17}$$

Let us compare this with the current that would be collected without the aid of surface diffusion (Eq. 8), which for small J/J_{max} is

$$J = 4NDsc_\infty. \tag{18}$$

The two-step process will be dominant if J' exceeds J, that is, if

$$(\pi d/s)(D'/D)\exp(E_A/kT) > \ln(a^2/Ns^2). \tag{19}$$

In order of magnitude, the logarithm will by typically around 10, the factor $\pi d/s$ roughly unity. Suppose D'/D is as large as 0.1. Then the two-stage process will be important if E_A is greater than $kT\ln(100)$, about 3 kcal/mol.

There may well be a number of systems that rely on the two-stage process. On the other hand, as we have shown, it is not necessary to invoke a two-stage process to explain efficient collection by a cell with many receptors. Nor is the advantage of a two-stage process—when Eq. 19 is satisfied and such an advantage exists—to be attributed simply to a reduction from a three-dimensional to a two-dimensional diffusion process, as Adam and Delbrück implied. They noted that the logarithmic factor in Eq. 14, which they called the "tracking factor," is a measure of the difficulty of "finding the target" in a two-dimensional random walk, and that the corresponding factor for three-dimensional diffusion is much larger, being of order (space size/target size), rather than the logarithm of that ratio. But target finding in three dimensions, that is, finding the cell itself, is required as a first step in both the two-stage and the one-stage processes. Any X molecule that arrives at the surface of the cell has already overcome the difficulty of that three-dimensional search and is now assured

a period of diffusion in close proximity to the surface, which it will probe at a large number of points. Indeed, one could regard this motion close to the cell wall as quasi-two-dimensional diffusion—not quite as effective, to be sure, as an equally rapid diffusion on the surface itself, but by no means as poor a substitute as the contrast of the two- and three-dimensional tracking factors might suggest.

These results have been derived for receptors with binding sites that are perfect sinks. It is not obvious how they apply in chemotaxis, where we are concerned with the time-average occupation of receptors that bind molecules of attractant temporarily, subsequently releasing them to the medium. It will turn out, however, that the formula for the current absorbed by receptors with binding sites that are perfect sinks is precisely what we shall need to analyze the fluctuations in the occupation of receptors in the more general case.

EFFECT ON INTAKE OF ACTIVE MOVEMENT

It is natural to ask whether a cell cannot, by some movement of its own, increase its intake of X molecules. Before addressing that question, we remind the reader that in the environment of the microorganism the mechanics of the medium is dominated by viscosity. The ratio of inertial forces to viscous forces is expressed in the Reynolds number:

$$R = L\nu\rho/\eta = L\nu/\nu, \tag{20}$$

where L and ν are a length and a velocity typical of the motion under consideration, η is the viscosity of the fluid, and ρ is its density. The kinematic viscosity ν is defined as η/ρ. The smallest kinematic viscosity we need to consider is that of water, about 10^{-2} cm^2/s, and the largest velocities we shall encounter are well below 10^{-2} cm/s. Then, even if L is as large as 10^{-3} cm, we have $R \lesssim 10^{-3}$. In most cases the Reynolds number will be smaller by one or two orders of magnitude. Inertia is utterly negligible in all the processes we shall consider. To emphasize that, we may remark that if a bacterium the size of *E. coli,* swimming in water at top speed, about 30 μm/s, were suddenly to stop moving its flagella, it would coast less than 10^{-9} cm!

Stirring

Consider first what might be called local stirring. Let the organism be equipped with suitable active appendages with which to manipulate the fluid in its vicinity. Can it thereby significantly increase the rate at which molecules reach its receptors? This has been suggested as a possible major function of flagella (3, 4). Consider the following rather loose dimensional argument: Transport by stirring is characterized by some velocity V_s, the speed of the appendage, and by a length L, its distance of travel, which determine a characteristic time $t_s = L/V_s$. On the other hand, movement of molecules over a distance L by diffusion alone is characterized by a time $t_D = L^2/D$. Roughly speaking, stirring will be more effective than diffusion only if $t_s < t_D$, which is to say, only if

$$V_s > D/L. \tag{21}$$

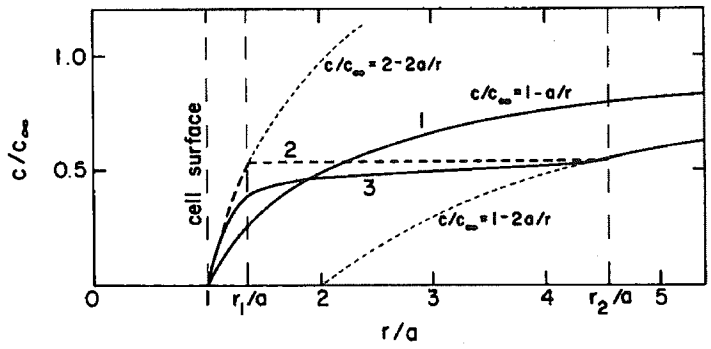

FIGURE 2 Relative concentration in the vicinity of a spherical absorber, for three cases: (1) No stirring; the current absorbed is $J_0 = 4\pi a D c_\infty$. (2) Volume between r_1 and r_2 stirred infinitely rapidly, fluid stationary elsewhere; current absorbed is $2J_0$. (3) Finite stirring speed; region inside r_1 still dominated by diffusion; current absorbed is $2J_0$.

For local stirring the distance L cannot be much larger than the size of the organism itself. With $L = 1$ μm and $D = 10^{-5}$ cm^2/s, Eq. 21 calls for speeds of order 10^3 μm/s. That is faster than any motions bacterial cells exhibit. However, for larger organisms lower speeds suffice. In fact, the feasibility of effective local stirring depends on size even more strongly than Eq. 21 might suggest. That becomes clear when we reckon the cost in energy dissipated in viscous friction, energy which the organism itself is obliged to supply if it is doing the stirring.

Consider a spherical cell of radius a that is a perfect sink for molecules X. Without stirring the cell collects a current of X molecules given by Eq. 2: $J_0 = 4\pi a D c_\infty$. The concentration in the neighborhood of the cell is given by $c = c_\infty(1 - a/r)$, shown as curve 1 in Fig. 2. Now let us introduce local stirring with the aim of doubling the cell's steady intake. The object is to transfer fluid from a distant region of relatively high concentration to a place much closer to the cell, thereby increasing the concentration gradient near the absorbing surface. Of course, the depleted parcels of fluid must be carried back again—some more or less complicated pattern of circulation must be maintained by means we need not specify.

An idealized limiting case of this process could be described as follows. Let the stirred volume extend from a sphere of radius $r_1 > a$ out to a sphere of radius r_2, and let the stirring be so vigorous as to keep the concentration uniform at all times throughout this region. Elsewhere, that is, both for $a < r < r_1$ and $r > r_2$, the fluid is stationary and transport of X molecules is effected by diffusion only. If the current is to be twice J_0, r_1 and r_2 are related as follows:

$$r_1 = 2ar_2/(r_2 + 2a). \tag{22}$$

The dependence of relative concentration on distance is shown by curve 2 in Fig. 2.

This case demanded unlimited rapidity of stirring, and hence unlimited expenditure of energy. We should expect that in a practical case the concentration $c(r)$, which now must be understood as a mean concentration at any given r, will behave more like

curve 3 in Fig. 2, rising somewhat with increasing r through the stirred volume. The boundary at $r = r_1$, though no longer sharp, still locates the essential transition from diffusion-dominated transport to convective transport. We can express this by the condition that at $r = r_1$ half the current density be due to diffusion and half to fluid motion, that is, by the condition $D\,dc/dr = cV_r/4$. Here V_r is the maximum radial velocity of flow at r_1. The factor $\frac{1}{4}$ derives from the assumption of a sinusoidal pattern of radial velocities over the spherical surface, together with an assumption that fluid parcels having the greatest outward velocity are fully depleted. Since the convective current at r_1, $4\pi r_1^2\, c(r_1)\, V_r/4$, is equal to half of the total current, $4\pi\, aDc_\infty$, and $c(r_1)$ is approximately $2c_\infty(1 - a/r_1)$, we get

$$V_r = 2aD/r_1(r_1 - a). \tag{23}$$

This is a specific example of the general relation expressed by Eq. 21.

Whatever the pattern of circulation, the region from $r = a$ out to at least $r = r_1 + (r_1 - a)$ must contain velocity gradients and, in particular, rates of shearing deformation, as large as $V_r/(r_1 - a)$. The square of the rate of shearing deformation determines the local rate of energy dissipation in a viscous fluid (ref. 5, p. 54). Thus, Eq. 23 implies a lower bound on the rate at which energy must be expended in stirring. The mean rate of energy dissipation per unit volume of fluid is approximately

$$\tfrac{1}{2}\eta[V_r/(r_1 - a)]^2 = 2\eta a^2D^2/r_1^2(r_1 - a)^4. \tag{24}$$

The volume involved is approximately $8\pi r_1^2(r_1 - a)$, so the total stirring power expended in this region is $16\pi\eta a^2D^2/(r_1 - a)^3$. We neglect the dissipation in the larger portion of the stirred volume that extends from $2r_1 - a$ out to r_2, since the velocity gradients there can be much smaller. Using Eq. 22 to express the result in terms of r_2, the outer limit of the stirred volume, we find as a lower bound on the stirring power, S, required to double the cell's intake

$$S = \frac{16\pi\eta D^2}{a}\left(\frac{r_2 + 2a}{r_2 - 2a}\right)^3. \tag{25}$$

The energy cost per unit volume of cell is

$$\frac{S}{(4\pi/3)a^3} \gtrsim \frac{12\eta D^2}{a^4}\left(\frac{r_2 + 2a}{r_2 - 2a}\right)^3. \tag{26}$$

For example, if $r_2 = 6a$, which would call for a rather extensive stirring apparatus, the specific power demand would be at least $100\,\eta D^2/a^4$. For $\eta = 10^{-2}$ P, $D = 10^{-5}\text{cm}^2/\text{s}$, and $a = 1\ \mu\text{m}$, this amounts to 0.1 W/cm^3, more than 10^4 times the specific power demand required to propel the sphere at a speed $v_0 = 30\ \mu\text{m/s}$.[3]

For an organism as small as a bacterium in a medium like water, the cost of increas-

[3]The force required to propel a sphere at speed v_0 is $6\pi\eta a v_0$ (Stokes' law). The power dissipated is $6\pi\eta a v_0^2$. Division by the volume of the sphere gives the specific power demand: $9\eta v_0^2/2a^2$.

ing the intake by local stirring would appear to be prohibitive. The prospect is somewhat more favorable if the viscosity of the medium is high. Since D varies as $1/\eta$, the product ηD^2 also varies as $1/\eta$. Stirring may also be more useful if the molecule in question is so large that its diffusion constant is small. Note, however, that for any molecule whose configuration is such that it can be enclosed in a sphere of radius R, the product ηD^2 cannot be smaller than $(kT/6\pi R)^2/\eta$.

The most striking aspect of Eq. 26 is the strong dependence of the specific energy demand on cell radius. If the cell in our previous example had had a radius of 10 μm, the stirring power required would have been reduced to 10^{-5} W/cm^3. Local stirring for the purpose of increasing intake changes from a hopelessly futile to a possibly useful activity somewhere in the range of cell size between a few microns and a few tens of microns. We emphasize that this conclusion does not depend on the details of the stirring mechanism. It should be noted, as well, that the largest possible gain in intake, even if all other constraints could be ignored, would be by the factor r_2/a, because the current is limited ultimately to what can diffuse into the stirred region.

Stirring of the fluid on a larger scale by some external agent can in principle increase the current absorbed by the cell, but to be effective, it must convey fresh solution into the region of low concentration around the cell faster than diffusion into the absorber depletes it, that is, in a time short compared to a^2/D. What is required is a continuous shearing deformation with a transverse velocity gradient greater than D/a^2. It is shown in another article[4] that if a suspension of spheres of radius a is stirred vigorously enough to double the rate at which diffusing material is absorbed, the mechanical power expended in agitating the fluid must be approximately 500 $\eta D^2/a^4$ ergs/cm^3 per s. For the same values of η, D, and a as we assumed in our previous example, the external stirring power required is 0.5 W/cm^3 of fluid stirred. Here again we find strong dependence on the size of the absorbing particles.

Swimming

Can a cell in a medium of uniform concentration increase its material intake by swimming? One is tempted to suppose that a moving cell might scoop up the X molecules that lie in its path (3) or move suddenly to a region in which the local concentration is c_∞ (4). This is not the case. The molecules in front of the cell are carried out of its way along with the fluid it must push aside to move. The cell carries with it a layer of liquid that is practically stationary in its frame of reference. Every molecule that reaches the surface of the cell must cross this layer by diffusion. The controlling relations are essentially the same as those involved in stirring, of which swimming could be viewed as a special case. But here the question can be formulated and answered more precisely.

Let a spherical cell of radius a be propelled at constant velocity v_0 through a fluid containing at concentration c_∞ molecules for which the cell is a perfect sink. By adopt-

[4]Purcell, E. M. 1978. The effect of fluid motions on the absorption of molecules by suspended particles. *J. Fluid Mech.* In press.

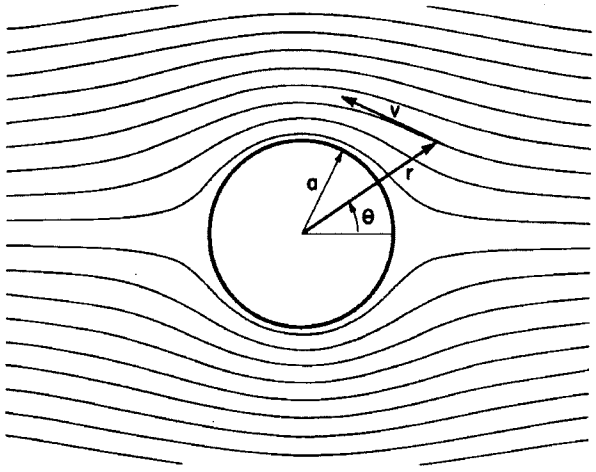

FIGURE 3 A sphere moving at constant velocity through a viscous fluid. Flow lines show the direction of flow in a frame of reference fixed to the sphere. In that frame the components of fluid velocity are given by Eq. 27.

ing the frame of reference of the cell and polar coordinates, as shown in Fig. 3, the flow around the sphere is the Stokes' velocity field described (ref. 5, p. 65) by

$$v_r = -v_0 \cos\theta (1 - 3a/2r + a^3/2r^3),$$
$$v_\theta = v_0 \sin\theta (1 - 3a/4r - a^3/4r^3). \tag{27}$$

To find the current to the cell, the equation to be solved is

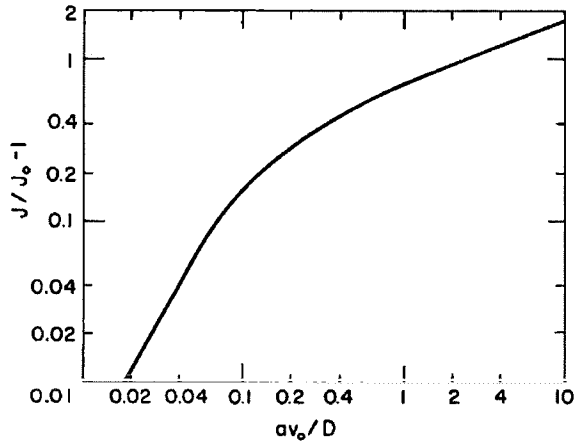

FIGURE 4 The increase in current to a spherical absorber resulting from motion at speed v_0. J and J_0 are the currents collected by moving and stationary spheres, respectively. The curve is a log-log plot of the fractional increase, $J/J_0 - 1$, as a function of the dimensionless velocity parameter av_0/D, where a is the radius of the sphere and D the diffusion constant of the molecules absorbed. The fractional increase $J/J_0 - 1$ is proportional to v_0^2 for $v_0 \ll D/a$ and to $v_0^{1/3}$ for $v_0 \gg D/a$.

$$D\nabla^2 c - \mathbf{v} \cdot \text{grad}\, c = 0, \tag{28}$$

with the components of $\mathbf{v}$ given by Eq. 27. An approximate solution has been obtained by a relaxation method. Thanks to the axial symmetry, the three-dimensional problem can be reduced to diffusion with drift on an appropriately modified two-dimensional grid. The result for $J/J_0 - 1$, the fractional increase in the current collected by the moving sphere compared with that collected by a stationary sphere in the same medium, is plotted in Fig. 4 against the dimensionless velocity parameter av_0/D. For $av_0/D \ll 1$, the increase in current, J–J_0, is proportional to $(av_0/D)^2$. That was to be expected. The increase cannot depend on the direction of motion and can hardly have a singularity at $v_0 = 0$; therefore, it must depend on even powers of v_0 in the neighborhood of $v_0 = 0$. There is, to be sure, an increase in current to the forward half of the sphere that is linear in v_0; our solution shows a fractional increase of approximately $1.5\, av_0/D$. There is a corresponding linear decrease in current to the rear half of the sphere, leaving the total current with an initial quadratic rise. But our major concern is with values of av_0/D large enough to make an appreciable difference in J–J_0. Here the current increases much more slowly with v_0. One can show that in the high-velocity limit J/J_0 is proportional to $(av_0/D)^{1/3}$. For values of av_0/D as large as 10, our numerical solution exhibits that behavior.[5]

For a bacterium in pursuit of a typical nutrient, $a = 1\ \mu$m, $v_0 = 30\ \mu$m/s, $D = 10^{-5}$ cm^2/s, and $av_0/D = 0.03$. The increase in intake is only 2.5% (Fig. 4). The speed required to double the intake is enormous, about 3 mm/s. The situation is somewhat more favorable if the diffusion constant D is very small. For example, if $a = 1\ \mu$m and $D = 5 \times 10^{-8}$ cm^2/s, the intake is doubled at a speed of only $15\,\mu$m/s.

At the speed $v_0 = 2.5D/a$, the speed required to double the intake, the specific power demand is $28\eta D^2/a^4$; see footnote 3. This is to be compared with the result from our earlier example of stirring, $100\ \eta D^2/a^4$. The mechanical efficiency of flagellar propulsion is at best a few percent (8), so the actual power requirement lies well above our estimated lower bound for stirring in general.

We conclude that in a uniform medium motility cannot significantly increase the cell's acquisition of material. At least that is true for a cell as small as a bacterium and for molecules of moderate size. In a nonuniform medium, on the other hand, a motile organism can seek out, as in chemotaxis, more favorable regions. The search must involve the detection of changes in concentration, a comparison of concentrations at different places or different times.

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[5]Approximate analytical treatments of this problem have been published, for example, by Friedlander (6) and Acrivos and Taylor (7). Their results are expressed in terms of the Nusselt number, in our notation $2J/J_0$, and the Peclet number, in our notation $2av_0/D$. While agreeing that for large values of v_0 J/J_0 is proportional to $v_0^{1/3}$, the papers cited predict a linear dependence of J–J_0 on v_0 for small values of v_0. We do not understand how that dependence can arise.

MEASUREMENT OF CONCENTRATION

The Perfect Instrument

We want to see how well a microorganism can determine the concentration of molecules X in its vicinity. We shall assume that the organism derives its information from the state of occupation of specific receptors, and we shall study presently the capability of such a measuring procedure. But it will be instructive to consider first a hypothetical instrument, a device that can instantly count all the X molecules in some fixed volume V of medium. The expected count is $V\bar{c}$, where $\bar{c}$ is the "true" concentration of X molecules, the mean over a very long time. At the dilutions with which we are concerned there is no doubt that the X molecules diffuse independently. If the concentration is inferred from the result of a single count, a fractional error of magnitude $\Delta c/\bar{c} \approx (V\bar{c})^{-1/2}$ is probable. However, given a sufficient length of time to make the determination, we could by repeating the count reduce the uncertainty—provided that we wait long enough between counts to insure that the next population counted is statistically independent of the previous one. The waiting time required is approximately the time it takes a molecule to diffuse out of the sample volume, roughly $V^{2/3}/D$. That is, we can make about $TD/V^{2/3}$ independent counts in the alloted time T, which will reduce the rms fractional error in the determination of $\bar{c}$ to something like

$$\Delta c/\bar{c} \approx (TD/V^{2/3})^{-1/2}(V\bar{c})^{-1/2} = (TD\bar{c})^{-1/2}V^{-1/6}. \tag{29}$$

If the sample space is a spherical volume of radius a, this gives

$$\Delta c/\bar{c} \approx (1.61\, TD\bar{c}a)^{-1/2}. \tag{30}$$

If $a = 1\ \mu$m and $D = 10^{-5}$ cm^2/s, a concentration of 10^{-6} M ($\bar{c} = 6 \times 10^{14}$ cm^{-3}) could be measured with 1% uncertainty in something like 0.01 s.

Numerical factors of order unity would appear in such relations if they were formulated precisely. A "perfect" instrument whose performance can be described precisely is a counter that registers at every instant the exact number m of X molecules that are at that moment inside a spherical region of radius a. The function $m(t)$, the instrument's output, contains all the information about the ambient concentration $\bar{c}$ that can be obtained without looking outside that sphere. If we are given the output for time T, starting at t_1, the best estimate of $\bar{c}$ is $\bar{c} = (3/4\pi a^3)m_T$, where m_T is the average of $m(t)$ over the time of observation:

$$m_T = \frac{1}{T}\int_{t_1}^{t_1+T} m(t)\,dt. \tag{31}$$

The question now becomes, if we repeat this operation many times, starting at widely separated times t_k, what is the expected fluctuation in the values of m_T? We need to compute the mean square deviation of m_T, $\langle m_T^2\rangle - \langle m_T\rangle^2$. The brackets indi-

cate an average over a large number of independent runs, each of duration T. The average of the m_T's, $<m_T>$, is of course just $(4\pi/3)a^3\bar{c}$.

A useful tool is the autocorrelation function of $m(t)$, defined by

$$G(\tau) = <m(t)m(t + \tau)>, \tag{32}$$

in which the average indicated by the brackets is over an unlimited time. $G(\tau)$ is an even function of τ: $G(\tau) = G(-\tau)$. From the definition of m_T it follows that

$$m_T^2 = \frac{1}{T^2}\int_{t_1}^{t_1+T} dt' \int_{t_1}^{t_1+T} m(t)m(t')\,dt, \tag{33}$$

from which by introducing the autocorrelation function $G(\tau)$ we obtain

$$<m_T^2> = \frac{1}{T^2}\int_0^T dt' \int_0^T G(t' - t)\,dt, \tag{34}$$

reducing our problem to the determination of $G(\tau)$.

To find $G(\tau)$, consider a large number N of X molecules confined to a spherical volume of radius $R \gg a$ within which lies our spherical sample volume of radius a. Let $w_j(t)$ be the function which is 1 if molecule j is inside the smaller sphere at time t and 0 if it is not. Then the correlation function of $m(t)$ can be written as follows:

$$<m(t)m(t + \tau)> = \left\langle \sum_{j=1}^{N} w_j(t)w_j(t + \tau) \right\rangle + \left\langle \sum_{j\neq 1}^{N} \sum_{i=1}^{N} w_j(t)w_i(t + \tau) \right\rangle \tag{35}$$

Clearly $<w_j> = a^3/R^3$, and since w_j and w_i are independent, the average of the double sum, in which there are $N(N - 1)$ terms, is $N(N - 1)a^6/R^6$, or $(Na^3/R^3)^2$ for large N. In the single sum the average of one of the N terms is $(a^3/R^3)u(\tau)$, where $u(\tau)$ is the probability that if a certain molecule is found inside the sample volume at time t, it will be found inside it at the later time $t + \tau$, this probability having been averaged over a uniform distribution of initial positions throughout the spherical volume $r < a$. We now have

$$<m(t)m(t + \tau)> = (Na^3/R^3)u(\tau) + (Na^3/R^3)^2. \tag{36}$$

But Na^3/R^3 is just $<m>$, so the correlation function is

$$G(\tau) = <m>u(\tau) + <m>^2. \tag{37}$$

Actually we shall not need $u(\tau)$ itself but only the characteristic time τ_0 defined by

$$\tau_0 = \int_0^\infty u(\tau)\,d\tau, \tag{38}$$

which is easier to calculate. The value of τ_0, derived in Appendix C, is $\frac{2}{5}a^2/D$.

It is an appropriate measure of the time for the contents of the sample volume to be renewed by diffusion in and out.

Returning to Eq. 34, let us consider observation times T much longer than τ_0. In that case, remembering that $G(-\tau) = G(\tau)$, the integral becomes

$$<m_T^2> = (1/T^2) \int_0^T dt'(T<m>^2 + 2\tau_0 <m>)$$

$$= <m_T>^2 + (2\tau_0/T)<m_T>. \quad (39)$$

This gives us an exact formula for the mean square fluctuation in m_T:

$$<\Delta m_T^2> = <m_T^2> - <m_T>^2 = (2\tau_0/T)<m_T> = (4a^2/5DT)\bar{m}, \quad (40)$$

from which we obtain the rms fractional error in concentration $\bar{c}$ in one such measurement:

$$\Delta c_{rms}/\bar{c} = (5\pi TD\bar{c}a/3)^{-1/2}. \quad (41)$$

The rms error is smaller by a factor 0.55 than the estimate of Eq. 30.

This result for the perfect instrument will provide us with a convenient standard of comparison. For instance, any procedure capable of determining the concentration with an rms error of 1% in an observation time T may be said to be equivalent to a perfect instrument sampling a spherical volume of radius about $2{,}000/TD\bar{c}$.

A Single Receptor

We assume that a receptor has a binding site capable of binding one, but only one, X molecule. We shall describe the history of this site by a function $p(t)$ that has the value 1 when the site is occupied and 0 when it is empty. The time average occupation $\bar{p}$ is determined by a single dissociation constant K, the concentration in moles per liter for which $\bar{p} = 0.5$. Let us denote by $c_{1/2}$ the same concentration in molecules per cubic centimeter. In equilibrium at concentration c the expected average occupancy is

$$\bar{p} = c/(c + c_{1/2}). \quad (42)$$

After a molecule has become attached to a binding site there is a constant probability, per unit time, that it will be released. Let the probability of detachment in an interval dt be dt/τ_b. Then τ_b is the average time a molecule stays bound to a site. As before, we shall describe the binding site as a circular patch of radius s. If the patch were a sink, the current to it would be $4Dsc$, as in Eq. 5. Suppose that a molecule that arrives at a vacant binding site sticks with probability α. If it doesn't stick on its first contact, it may very soon bump into the site again—and again. If these encounters occur within a time interval short compared to τ_b, their result is equivalent merely to a larger value of α. As we have no independent definition of the patch radius s, we may as well absorb the effective α into s, writing for the probability that a vacant patch becomes occupied during dt simply $4Dsc\ dt$. Since $\bar{p}$ is

the probability that a receptor patch is occupied and $1 - \bar{p}$ the probability that it is empty, in the steady state the following relation must hold:

$$\bar{p}/\tau_b = 4(1 - \bar{p})\, Dsc. \tag{43}$$

In particular, since $\bar{p} = \frac{1}{2}$ for $c = c_{1/2}$,

$$\tau_b = (4Dsc_{1/2})^{-1}. \tag{44}$$

Thus for $K = 10^{-6}$M, $D = 10^{-5}$cm^2/s, and $s = 10$ Å, we would have $\tau_b = 4 \times 10^{-4}$ s.

If the only information about the ambient concentration c is the function $p(t)$ for one receptor recorded for a time T, the best use that can be made of it is to form the average,

$$p_T = (1/T) \int_{t_1}^{t_1+T} p(t)\, \mathrm{d}t, \tag{45}$$

take that as an estimate of $\bar{p}$, and use Eq. 42 to derive c:

$$c/c_{1/2} = p_T/(1 - p_T). \tag{46}$$

To compute the uncertainty in such a determination of c we proceed exactly as we did above with the measurement of m_T. All we need is the correlation function for $p(t)$, for which we shall use the same symbol, $G(\tau)$:

$$G(\tau) = \langle p(t)p(t + \tau) \rangle. \tag{47}$$

Consider data from a large number n of pairs of observations, one at t, the other at $t + \tau$, with random values of t but always the same value of τ. Segregate those pairs in which the first observation found $p = 1$ and ignore the others. If n is very large, there will be about $n\bar{p}$ such pairs. Of these, according to the definition of $G(\tau)$, the number with $p(t + \tau)$ also equal to 1 will be $nG(\tau)$. These "1,1" pairs are the only ones for which $p(t)p(t + \tau) \neq 0$. Now consider the result of shifting the time of the second observation from $t + \tau$ to $t + \tau + \mathrm{d}\tau$. Some of the $nG(\tau)$ 1,1 pairs will become 1,0 pairs; $nG\, \mathrm{d}\tau/\tau_b$ of them will do so. Some of the 1,0 pairs, of which there were $n\bar{p} - nG$, will become 1,1 pairs; the number doing so will be $n(\bar{p} - G)[\bar{p}/(1 - \bar{p})]\mathrm{d}\tau/\tau_b$. We should now have $nG(\tau + \mathrm{d}\tau)$ 1,1 pairs, which requires that

$$\mathrm{d}G = -G\, \mathrm{d}\tau/\tau_b + (\bar{p} - G)[\bar{p}/(1 - \bar{p})]\, \mathrm{d}\tau/\tau_b. \tag{48}$$

Integrating and requiring that $G(0) = \bar{p}$, we obtain

$$G(\tau) = \bar{p}^2 + \bar{p}(1 - \bar{p}) \exp[-|\tau|/(1 - \bar{p})\tau_b]. \tag{49}$$

We now use Eq. 34 to calculate $\langle p_T^2 \rangle$, assuming $T \gg \tau_b$, with the result

$$\langle p_T^2 \rangle - \langle p_T \rangle^2 = (2/T)\bar{p}(1 - \bar{p})^2 \tau_b. \tag{50}$$

For the rms error in $\bar{c}$ inferred from such a measurement we get

$$\Delta c_{\text{rms}}/\bar{c} = (2\tau_b/T\bar{p})^{1/2}. \tag{51}$$

This result can be expressed in a surprisingly simple and illuminating form. Using Eqs. 42 and 44, we can transform Eq. 51 into

$$\Delta c_{\text{rms}}/\bar{c} = (\nu/2)^{-1/2}, \tag{52}$$

where

$$\nu = 4Ds\bar{c}(1 - \bar{p})T. \tag{53}$$

The current $4\,Ds\bar{c}$ is the rate at which molecules would be captured by the receptor patch if it were a perfect sink. It is therefore the rate at which molecules arrive at the receptor if we only count "new" molecules, those that have not been there before. Since the probability that the patch is already occupied when any molecule arrives is $\bar{p}$, the rate at which new molecules are captured by the patch is $4\,Ds\bar{c}(1 - \bar{p})$. Hence the number ν is just the total number of new molecules that have occupied the receptor patch during the observation period T. We see that the fractional error in the determination of $\bar{c}$ depends on this number ν and nothing else! Evidently, once a particular molecule has occupied a receptor patch, subsequent visits by the same molecule contribute no information whatever about the concentration in the medium. Indeed, that ought to be true if the molecules are diffusing independently. Such diffusion is a Markov process, which is to say that the probability of a future configuration is determined completely by the present configuration, regardless of the past. It follows that we can draw from the molecule's future behavior no inference about the past that is not already implied by its present position. This observation is the key to the generalization of our result to include cells with many receptors.

It is interesting to compare the performance of one receptor, as a "$\bar{c}$-measuring" device, with the perfect instrument described earlier. Using Eq. 41, we find that the single receptor is equivalent to a perfect instrument with a spherical sampling volume of radius $(6/5\pi)(1 - \bar{p})s$, a radius approximating that of the single receptor patch. With $\bar{p} = \frac{1}{2}$, $s = 10$ Å, and $D = 10^{-5}\,\text{cm}^2/\text{s}$, a concentration of 10^{-6} M could be measured with 1% uncertainty in about 17 s.

A System of Many Receptors

As we turn toward our ultimate goal, assessing the performance of a cell with many receptors, a formidable complication looms ahead. The "signal" from which $\bar{c}$ is to be determined is now the total instantaneous occupation of the N receptors on the cell, that is, the function

$$P(t) = \sum_{j=1}^{N} p_j(t). \tag{54}$$

Now the histories of the occupation of two receptors on the same cell, especially of two receptors relatively close to one another, are not statistically independent. A

molecule just released at receptor j is necessarily favorably situated to wander into receptor k. Put another way, we should expect the two fluctuations, $p_j(t) - \bar{p}$ and $p_k(t) - \bar{p}$, to exhibit some positive cross-correlation. The effect would depend on the distance between the receptors and would be extremely difficult to handle rigorously. Fortunately, this threatening complication vanishes when we realize that, as we have just learned in the case of the single receptor, we need be concerned only with the capture of new molecules. Here "new" molecules are those that have not previously occupied *any* receptor on the cell. For once a particular molecule has occupied a receptor anywhere on the cell, its subsequent history is statistically determined and can convey no further information about the ambient concentration. The current of new molecules to a receptor patch is equal to the current that would reach that patch if all the receptor patches on the cell were perfect absorbers. The capture rate for the receptor patch is this current times $(1 - \bar{p})$. Therefore, the rate at which new molecules are captured by the receptor system is just $J(1 - \bar{p})$, where J is given by Eq. 8 and $\bar{p}$ is given, as before, by Eq. 42. This does not mean that the cell is obliged to identify the new molecules and avoid counting the others. What we are asserting is that the statistical error in the cell's inferred value of $\bar{c}$, given the receptor occupation history $P(t)$, will be the same as if new molecules only had been recorded.

The capture of a new molecule by one receptor and the capture of a new molecule (necessarily a different molecule) by another receptor are statistically independent events. With respect to such events, the history of N receptors observed for time T is statistically indistinguishable from that of a single receptor observed for time NT. It follows that the probable error in a value of $\bar{c}$ inferred from this information will be the same as Eq. 52 predicts for a single receptor if $\nu = TJ(1 - \bar{p})$. Thus we arrive rather suddenly at our final result:

$$\Delta c_{\text{rms}}/\bar{c} = [\tfrac{1}{2}TJ(1 - \bar{p})]^{-1/2} = [2\pi TD\bar{c}Nsa(1 - \bar{p})/(Ns + \pi a)]^{-1/2}. \quad (55)$$

If the number of receptors, N, is such as to make $J/J_{\text{max}} = \frac{1}{2}$, namely $N = \pi a/s$, this becomes

$$\Delta c_{\text{rms}}/\bar{c} = [\pi TDca(1 - \bar{p})]^{-1/2}. \quad (56)$$

On comparing this with Eq. 41, we find that the equivalent sample volume for the perfect instrument would be a sphere of radius $\frac{3}{5}(1 - \bar{p})a$.

APPLICATIONS

Bacteriophage Adsorption

Eq. 8 provides a solution to a classic problem in bacteriophage adsorption (see ref. 9). Why is the initial rate of adsorption of phage to a bacterium so close to the diffusion-limited rate for a perfectly adsorbing cell, given that the receptor binding sites cover only a small fraction of the surface? As we have seen, the answer lies in the large number of independent tries that each diffusing particle has at hitting a binding site

once it has bumped into the surface of the cell. This is a statistical property of diffusion per se; it is true regardless of the structure of the diffusing particle. Schwartz (9) found a hyperbolic dependence of the initial rate of adsorption of bacteriophage λ on the number of λ-receptors in samples of *E. coli* grown under different cultural conditions (his Fig. 2*a*). A least-squares fit (10) of Eq. 8 to this data gives $J/c_\infty = (2.14 \pm 0.10) \times 10^{-10}$ cm^3/s and $\pi a/s = 483 \pm 79$. With $a = 8 \times 10^{-5}$ cm, we find $s = 52$ Å. Recall that s is an effective radius; it depends on the size of the binding site and of the phage and on the probability that a phage, having arrived at a binding site, is adsorbed. Adsorption occurs at the half-maximum rate with fewer than 500 receptors per cell, when only 0.5% of the surface is specifically adsorbing. The value for J/c_∞ determined by the data is larger than the value computed from Eq. 2, 5.03×10^{-11} cm^3/s, by a factor of 4. The diffusion constant for bacteriophage λ is quite small, 5×10^{-8} cm^2/s; in this case, the specific energy demand for stirring (Eq. 26) is not prohibitive, and the bacterium could double the adsorption rate by swimming (Fig. 3, $av_0/D \approx 3$). But we do not know whether Schwartz's bacteria were motile. A systematic error in the measurement of the number of phage or bacteria could explain the discrepancy.

Bacterial Chemotaxis

Studies of chemotaxis are most advanced for the enteric bacteria *Escherichia coli* and *Salmonella typhimurium* (for reviews, see refs. 11–14). These cells execute a three-dimensional random walk (15). They swim steadily along smooth trajectories (run), move briefly in a highly erratic manner (tumble or twiddle), and then run in new directions. They sense concentrations of attractants or repellents as a function of time (16, 17) and bias their random walk accordingly. Runs that carry a cell to higher concentrations of an attractant or to lower concentrations of a repellent are extended. The available evidence is consistent with a model in which a bacterium measures the difference in the fraction of receptors bound in successive intervals of time (17–19), i.e., in which the response is proportional to $d\bar{p}/dt$. The random walk can be biased most effectively if the measurements are made in a time interval short compared to the mean run length (ref. 17, Fig. 3); information gathered during a run is of little value once the cell has chosen a new direction at random. The time available for gradient determination and chemotactic response could not, in any case, exceed the time, τ_{rot}, which characterizes the Brownian rotation of the cell. There is no way, even in principle, for a bacterium to preserve an orientation reference frame for a time much longer than τ_{rot}—unless, of course, it could use some external clue such as the direction of illumination. In the case of *E. coli*, τ_{rot} is typically a few seconds, somewhat longer than the length of a run (15), so the run length remains the controlling limit on gradient measurement and response time.

Let the period of time devoted to each measurement of $\bar{p}$ be T. The difference between two successive measurements will be significant if that difference is larger than the standard deviation in the difference, i.e., if

$$(T/\bar{c})\partial\bar{c}/\partial t > \sqrt{2}\Delta c_{\text{rms}}/\bar{c}. \tag{57}$$

This inequality places a condition on T that can be found by substitution of Eq. 55:

$$T > \left[\pi a D\left(\frac{Ns}{Ns + \pi a}\right)\left(\frac{\bar{c}c_{1/2}}{\bar{c} + c_{1/2}}\right)\left(\frac{1}{\bar{c}}\frac{\partial\bar{c}}{\partial t}\right)^2\right]^{-1/3}. \tag{58}$$

If the temporal gradient is generated by the movement of the cell at velocity v through a spatial gradient $\partial\bar{c}/\partial x$, $(1/\bar{c})\partial\bar{c}/\partial t = (v/\bar{c})\partial\bar{c}/\partial x$. The time required to complete the temporal comparison is $2T$.

Working with enzymatically generated temporal gradients of the attractant L-glutamate, Brown and Berg (ref. 17, Fig. 1) found that the mean run length doubled (increased from 0.67 to 1.34 s) for $d\bar{p}/dt = 1.05 \times 10^{-3}$ s^{-1}, i.e., for $\bar{c} = 1.61$ mM, $(1/\bar{c})\partial\bar{c}/\partial t = 4.35 \times 10^{-3}$ s^{-1}, and $c_{1/2} = 2.3$ mM. If $a = 0.8$ μm, $D = 9 \times 10^{-6}$ cm^2/s, and $Ns/(Ns + \pi a) = 0.5$, we find $2T > 0.087$ s. The time required to detect a temporal gradient of $\frac{1}{10}$ the magnitude would be $10^{2/3} = 4.64$ times longer, or about 0.4 s.

Working with defined spatial gradients of the attractant L-serine, Dahlquist et al. (18) found that a trajectory of length 10 μm was doubled by a gradient of decay length about 1.4 cm. With $c_{1/2} = 1.0$ mM, $(1/\bar{c})\partial\bar{c}/\partial x = 0.7$ cm^{-1}, $v = 15$ μm/s, $a = 0.8$ μm, $D = 10^{-5}$ cm^2/s, and $Ns/(Ns + \pi a) = 0.5$, we find $2T \geq 0.27$ s. A gradient $\frac{1}{10}$ as steep could be detected in about 1.2 s.

Using the capillary assay and attractants detected by the aspartate and galactose chemoreceptors, Mesibov et al. (19) found threshold responses for DL-α-methylaspartate and D-galactose at concentrations (the initial concentration in the capillary) $\bar{c}_0 \simeq 4 \times 10^{-7}$ M and 2.5×10^{-8} M, respectively, with $c_{1/2} \simeq 1.3 \times 10^{-4}$ M and 6×10^{-7} M, respectively. These experiments are difficult to analyze, because the response is complex and the gradient near the mouth of the capillary is hard to define. Using Adler's interpolation (ref. 20, Fig. 5), we estimate $\bar{c} = 10^{-2}\bar{c}_0$ and $(1/\bar{c})\partial\bar{c}/\partial x = 80$ cm^{-1}. As before, we assume $Ns/(Ns + \pi a) = 0.5$. With $a = 0.8$ μm, $D = 10^{-5}$ cm^2/s, and $v = 15$ μm/s, we find for α-methylaspartate $2T > 0.6$ s, for galactose $2T > 1.4$ s.

Taken together, these results imply that *E. coli* and *S. typhimurium* are able to make temporal comparisons of concentrations in time intervals of about 1 s. If times much longer than this were required, cells in spatial gradients could not effectively bias their random walks; for *E. coli*, even 5 s would be prohibitive (17). Thus, the design of the chemotaxis machinery appears to be nearly optimum. This implies, for example, that the receptors are dispersed widely over the surface of the cell, rather than concentrated at the base of each flagellum, and that essentially every capture of a molecule by a receptor contributes to the signal controlling the direction of rotation of the flagella.

It is of interest to ask whether, in principle, a bacterium could navigate by comparing the concentration at the front to that at the back, that is, by a strictly spatial mechanism. In this case we require

$$(a/\bar{c})\partial\bar{c}/\partial x > \sqrt{2}\,\Delta c_{\text{rms}}/\bar{c}, \tag{59}$$

where Δc_{rms} is the standard deviation in the measurement made by half of the cell. A condition on T is obtained by substituting Eq. 55 and using for J the current to one half of the cell:

$$T > \left[\frac{1}{2}\,\pi a^3 D\left(\frac{Ns}{Ns + \pi a}\right)\left(\frac{\bar{c}c_{1/2}}{\bar{c} + c_{1/2}}\right)\left(\frac{1}{\bar{c}}\,\frac{\partial\bar{c}}{\partial x}\right)^2\right]^{-1}. \tag{60}$$

For the experiment of Dahlquist et al. (18), we find $T > 1.7$ s. The comparison probably could be made in less time, since the length of the cell is roughly twice its diameter. Thus, on the basis of this analysis a mechanism involving spatial comparisons remains feasible.

But there is a much more serious problem: motion of the cell will generate an apparent spatial gradient. As noted in our discussion of swimming, the flux to a perfectly absorbing sphere of radius a moving at velocity v_0 in a solution of uniform concentration is greater in the front than in the back by a factor of about $1 + 3av_0/D$. As shown in Appendix D, the flux to a stationary perfectly absorbing sphere of radius a in a spatial gradient of decay length L is greater in the front than in the back by a factor $1 + a/L$. Thus, the moving sphere finds itself in an apparent spatial gradient of decay length $D/3v$. In the experiment of Dahlquist et al. (18), this decay length is of order 2×10^{-3} cm: the apparent gradient is 600 times steeper than the real gradient! The problem remains severe but is less dramatic when we realize that the cell takes up only a few percent of the molecules that reach its surface (ref. 4, Table 3); molecules not absorbed by the front half of the cell could still be counted by a receptor system operating independently at the back.

Slime Mold Chemotaxis

The spatial mechanism is much more effective if the cell is large. Mato et al. (21), working with the cellular slime mold *Dictyostelium discoideum*, observed a threshold response for cyclic AMP (with cells 0.6 cm away from a point source of the attractant) when $\bar{c} = 4.3 \times 10^{-9}$ M, $\partial\bar{c}/\partial x = 3.6 \times 10^{-8}$ M/cm, and $c_{1/2} \simeq 10^{-8}$ M. Assuming $a = 5\ \mu$m, $D = 5 \times 10^{-6}$ cm^2/s, and $Ns/(Ns + \pi a) = 0.5$, we find, using Eq. 60, $T > 16$ s. This interval is short compared to the duration of the wave of cyclic AMP diffusing past the cells. If the cells absorb (or destroy) an appreciable fraction of the cyclic AMP, then an apparent gradient of decay length 0.8 cm would be generated were they to crawl through a solution of uniform concentration at about 0.2 μm/s, their usual speed during a chemotactic response. This is comparable to the decay length of the threshold gradient in the experiment of Mato et al. (1.2 cm).

How long would it take the slime mold to sense the spatial gradient by making temporal comparisons? If the crawl velocity is 0.2 μm/s, the spatial gradient of Mato et al. could be detected in about 17 s (Eq. 58). A pseudopod of one-fourth the radius moving twice as fast would require a similar time. This analysis does not allow us to

rule out a temporal mechanism. But note that whatever the mechanism, the measurement must be made over an appreciable period of time.

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APPENDIX A

The Capacitance of N *Conducting Disks of Radius* s *on an Insulating Sphere of Radius* a *and Dielectric Constant Zero*

We first treat the N disks as a system of independent conductors with different charges q_j and potentials ϕ_j, $j = 1, 2, \ldots N$. The charges and potentials are connected by linear relations involving the so-called potential coefficients, h_{jk}:

$$\phi_j = \sum_k h_{jk} q_k. \tag{A1}$$

Put zero charge on all but the kth disk. Then $\phi_k = h_{kk} q_k$. The presence of the $N - 1$ uncharged disks can affect ϕ_k only through dipole or higher moments induced in them by the field of the lone charge q_k. That is a second-order effect which can be neglected if, as we shall now assume, the disk radius s is small compared to the distance between neighboring disks, approximately $a(4\pi/N)^{1/2}$. If the other disks were not there, the capacitance of a single disk on the insulating sphere would be just half that of an isolated conducting disk, or s/π. So we must have, to first order in the ratio $Ns^2/4\pi a^2$, $h_{kk} = \pi/s$. As the disks are all equivalent, this holds for all $k = 1, \ldots N$. Now the potential of one of the uncharged disks is

$$\phi_j = h_{jk} q_k. \tag{A2}$$

Consider the sum

$$[1/(N - 1)] \sum_{j \neq k} \phi_j = [q_k/(N - 1)] \sum_{j \neq k} h_{jk}. \tag{A3}$$

If N is large, this is essentially the average over the spherical surface of the potential due to the single charge q_k. If the sphere were empty space with dielectric constant unity instead of zero, that average would be simply q_k/a, because the average of a harmonic function over a sphere is equal to its value at the center of the sphere. In our case, however, the electric field outside the sphere is not that of a single charge q_k, but rather that plus the field of a set of "image charges" within the sphere, the combined field being such as to satisfy the boundary condition of zero normal component at the spherical surface. So we should add to q_k/a the contribution of the image-charge distribution to the average potential. But that contribution is zero, since the net charge of the image distribution is zero. (If a sphere contains no net charge, the average of the potential over the surface is that due to external charges alone.) Hence we are left with

$$[q_k/(N - 1)] \sum_{j \neq k} h_{jk} = q_k/a. \tag{A4}$$

We have tacitly assumed that N is large, so we can write

$$\sum_{j \neq k} h_{jk} = N/a. \tag{A5}$$

Referring back to Eq. A1, if we now put the same charge q on every disk so that the total charge is Nq, the common potential ϕ will be

$$\phi = \phi_k = h_{kk} q + \sum_{j \neq k} q h_{jk} = \pi q/s + Nq/a, \tag{A6}$$

from which we obtain the capacitance of the combination:

$$C = Nq/\phi = Nsa/(Ns + \pi a). \tag{A7}$$

APPENDIX B

The Mean Time to Capture in Two-Dimensional Diffusion

In a space of two dimensions a particle is released at time $t = 0$ at the point x, y. The particle then diffuses at a rate determined by a two-dimensional diffusion constant D' until it eventually blunders into the boundary of an absorber, where, at time t, it is captured. Let this be repeated very many times, starting always at the same point x, y. Let W denote the mean of all the observed times-to-capture for this starting point. To find the equation satisfied by the function $W(x, y)$, picture the diffusion as a random walk on a square lattice, with step length δ and step time Δt. Consider a particle now at the lattice point x, y, from which the mean time to capture is $W(x, y)$. One step-time later this particle will be with equal probability at one of the four lattice points $x \pm \delta$, $y \pm \delta$. It must therefore be true that

$$W(x,y) = \Delta t + \tfrac{1}{4}[W(x + \delta, y) + W(x - \delta, y) + W(x, y + \delta) + W(x, y - \delta)]. \tag{B1}$$

If we now shrink the step-length and step-time so as to approach the continuous diffusion limit with $D' = \delta^2/4\Delta t$, Eq. B1 becomes

$$D'\nabla^2 W + 1 = 0. \tag{B2}$$

In electrical terms, this is just Poisson's equation for a region of uniform charge density, with W the potential and $1/4\pi D'$ the charge density. As boundary conditions we require $W = 0$ on all absorbing boundaries. On a line of symmetry the normal component of grad W must vanish. The same condition holds at an impermeable, nonabsorbing boundary.

A number of cases are now almost trivially soluble, including the case of diffusion in an annular region treated by Adam and Delbrück (2): a circular absorber of radius s (a in their notation) centered within an impermeable boundary of radius b. All we need is the electrical potential $\phi(r)$ between concentric cylinders, the space being filled with uniform space charge opposite in sign and equal in total amount to the charge on the inner cylinder (thus insuring zero gradient at $r = b$). In this way we find

$$W(r) = (2b^2 \ln r - 2b^2 \ln s - r^2 + s^2)/4D'. \tag{B3}$$

We seek the mean of W over all starting points in the annular space, the quantity Adam and Delbrück call $\tau^{(2)}$ and we call $\bar{t}_c$. In this case

$$\bar{t}_c = \frac{1}{\pi(b^2 - s^2)} \int_s^b 2\pi r W(r) \mathrm{d}r = \frac{b^4}{2D'(b^2 - s^2)} \ln \frac{b}{s} - \frac{3b^2 - s^2}{8D'}. \tag{B4}$$

For our application we might have defined t_c as the average of W over the whole region $r < b$, including the absorber, but the distinction is unimportant if $b \gg s$, which is the case of interest. In this limit Eq. B4 reduces to

$$\bar{t}_c = (b^2/2D')[\ln(b/s) - \tfrac{3}{4}]. \tag{B5}$$

Adam and Delbrück's approximate result for this case, obtained by rather laborious means, is in our notation

$$\bar{t}_c \simeq (0.5b^2/D')[\ln(b/s) - 0.5], \tag{B6}$$

in excellent agreement with Eq. B5 as far as the logarithmic term.

Our actual problem was concerned with an unbounded array of evenly spaced absorbers. Let these be circular patches of radius s on a square lattice of spacing $d \gg s$. We need only solve Eq. B2 in the unit cell, a square of edge b with the absorber at its center. W is zero at the absorber and its normal derivative vanishes on the unit cell boundary. An approximation that ought to be adequate for $b \gg s$ is obtained simply by taking over Eq. B5 for a circular boundary and setting $b^2 = (4/\pi)d^2$. Another approach is to subdivide the unit cell with a grid and solve Eq. B2 numerically. Such a treatment has been carried out by a relaxation method for meshes as fine as $b/40$ and for absorbers consisting of a square set of mesh points ranging in size from a single point to 7^2 points. Both of these approaches gave results adequately represented by Eq. 14, as did a Monte Carlo study of random walks on a square grid with a central sink. The Monte Carlo results included information about the distribution of times-to-capture, in the form of the first four moments of the distribution of all path lengths to capture with all starting points equally weighted. The ratios of the observed moments corresponded closely to those for an exponential distribution, i.e., a distribution in which the probability of a time-to-capture greater than t_c is proportional to $\exp(-t_c/\bar{t}_c)$.

The derivation of Eq. B2 generalizes easily to spaces of higher or lower dimensionality. Eq. B2 remains unchanged, it being understood that in ν dimensions ∇^2 is the ν-dimensional Laplacian and D' the ν-dimensional diffusion constant. For a spherical absorber of radius a in a spherical vessel of radius b we find

$$W(r) = (2b^3/a - 2b^3/r + a^2 - r^2)/6D, \tag{B7}$$

which leads to the mean time to capture

$$\bar{t}_c = \frac{b^6}{3Da(b^3 - a^3)}\left(1 - \frac{9}{5}\frac{a}{b} + \frac{a^3}{b^3} - \frac{1}{5}\frac{a^6}{b^6}\right). \tag{B8}$$

This reduces to $b^3/3aD$ in the limit $b \gg a$. Thus, the three-dimensional "tracking factor" of Adam and Delbrück is $b/3a$.

APPENDIX C

Calculation of τ_0 Defined by Eq. 38.

Let a sphere of radius a contain at time $t = 0$ unit amount of a uniformly distributed diffusing substance. The function $u(\tau)$ is defined as the fraction of the original material that remains inside the sphere at $t = \tau$. If a concentrated source of unit amount were released at any point P at time zero, the concentration in the neighborhood of some other point Q at a later time would be

$$f(\rho, \tau) = (4\pi D\tau)^{-3/2}\exp(-\rho^2/4D\tau) \tag{C1}$$

where ρ is the distance between P and Q. We can use this to express $u(\tau)$ as follows:

$$u(\tau) = \int dv \int (3/4\pi a^3) f(|r - r'|, \tau)\, dv', \tag{C2}$$

both integrals to be extended throughout the volume of the sphere.

We want to calculate τ_0 defined by

$$\tau_0 = \int_0^\infty u(\tau)\,d\tau. \tag{C3}$$

Substitute from Eqs. C1 and C2 into Eq. C3 and carry out the integration over τ first:

$$\int_0^\infty f(\rho,\tau)\,d\tau = (1/4D\rho\pi^{3/2}) \int_0^\infty (e^{-x}\,dx/\sqrt{x}) = 1/4\pi D\rho. \tag{C4}$$

Then

$$\tau_0 = (1/4\pi D) \int dv \int (3/4\pi a^3)(dv'/|r - r'|). \tag{C5}$$

The integral over v' can be recognized as the potential at r within a uniform spherical charge distribution of total charge unity, which is $3/2a - r^2/2a^3$. The integration over v now yields

$$\tau_0 = \tfrac{2}{5}a^2/D. \tag{C6}$$

APPENDIX D

Diffusive Current to Two Halves of a Stationary, Perfectly Absorbing Sphere in a Uniform Gradient

Let a sphere of radius a be immersed in a uniform gradient $\partial c/\partial z = c_0/L$, with $L \gg a$. The solution to the equation $\nabla^2 c = 0$, with boundary conditions $c = 0$ at $r = a$ and $\partial c/\partial z \to c_0/L$ at $r \to \infty$, is

$$c = c_0(1 - a/r) + (c_0a^3/L)(z/r^3 - z/a^3). \tag{D1}$$

The current density at the surface of the sphere is

$$D\left.\frac{\partial c}{\partial r}\right|_{r=a} = Dc_0(1/a + \cos\theta/L), \tag{D2}$$

where θ is the angle measured from the $+z$-axis (spherical coordinates). The current to the forward half of the sphere, J_+, is obtained by integrating Eq. D2 over the surface from $\theta = 0$ to $\theta = \pi/2$; the current to the rear half of the sphere, J_-, is obtained by integrating Eq. D2 over the surface from $\theta = \pi/2$ to $\theta = \pi$. We find

$$J_\pm = 2\pi aDc_0(1 \pm a/2L), \tag{D3}$$

and

$$J_+/J_- = 1 + a/L. \tag{D4}$$