

# Surmounting barriers in ionic channels

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## I. INTRODUCTION

Biological cells are defined by the membrane that shields their vital molecules from the environment. The lipid bilayer of the membrane is an effective dielectric shield (Parsegian, 1969; Andersen, 1978; Honig *et al.* 1986), preventing penetration by charged molecules: the lipid presents a large electrostatic energy

barrier because it cannot neutralize the charge of solute molecules nearly as well as water. This energy barrier inhibits the permeation of solutes with local charge, even metabolites. Of course, metabolites do enter cells, and so physiologists have suspected (for a very long time, Hille, 1984, ch. 8) that the membrane shield is pierced by aqueous channels, through which solutes diffuse (with their local charge substantially neutralized) as they cross the membrane. These aqueous pores now have molecular reality (e.g. Noda *et al.* 1984; Miller, 1986). Each is formed by a specialized protein, integral to membranes, perhaps shaped like a thick-walled pipe, called ionic channels. Channels control the movement of many important molecules in and out of cells by the 'gating' mechanism that controls their opening and closing and by the selective properties of their open channel.

The macroscopic flux of solutes across membranes is then determined by the average number of pores open at any time and the flux through each one. When a pore is open, ions move through it by diffusion much as they move in free solution, at least judging from qualitative properties and quantitative estimates of diffusion constant, flux ratio, and so on (Hille, 1984, ch. 8). The classical theory of diffusion was in fact used to describe ion permeation through biological membranes almost as soon as the theory was created, which is less surprising than it seems, given that Fick, the founder of diffusion theory, was a physiologist! (Hille, 1984, pp. 153, 182). Classical theory of membrane permeation assumes that ions move through just one type of permanently open channel. But classical experiments (at their best) measured macroscopic fluxes through whole membranes containing billions of channels of many different types, channels which are open only a fraction of the time, as we now know.

Thanks to the techniques of reconstitution and patch clamping (Sakmann & Neher, 1983, and Miller, 1986, respectively), experiments are finally able to measure the fluxes analysed by the classical theory of the permanently open channel. Using their gigaseal/patch-clamp method, physiologists can measure the current through single open channels, without struggling to separate channel types, channel gating, and open channel current from insufficient macroscopic data. The ambiguity and confusion of earlier macroscopic analyses (with notable exceptions: Fatt & Katz, 1951; Hodgkin *et al.* 1952) are bypassed by experimentation, and unequivocal measurements can be made of ionic movement in a single aqueous pore.

Measurements of open channels have been analysed in two distinct traditions, that of continuum theory, using classical diffusion theory (in the form of the Nernst-Planck equations: Sten-Knudsen, 1978; Levitt, 1982) to describe the concentration of ions within a pore, and that of transition state theory (Läuger, 1973; Frehland, 1982; Hille, 1984), using the theory of gas phase chemical kinetics (i.e. Eyring rate theory) to describe the movement of ions within a pore. The traditions can be fused if discrete occupancy states are defined and the rate constants between those states are interpreted with a continuum diffusion theory (Hladky & Haydon, 1984; Levitt, 1986; Hladky, 1986).

This review presents a stochastic derivation of the diffusion theory of ionic

movement within a pore, using the theory of Brownian motion found in the literature of physical chemistry (e.g. Hynes, 1985; Hänggi, 1986; Weiss, 1986). It uses the well established theory of first-passage times (Weiss, 1966) to derive expressions for the rate constants of such motion. In this manner, we hope to make available to membrane biologists the powerful results of the statistical theory of diffusion and to forge links between statistical physics and membrane biology. For example, the rate constants we derive from diffusion theory are analogous to the traditional expressions of Eyring transition-state theory and so can be used in state diagrams describing permeation through biological channels with complex properties.

This review starts with the collisions between atoms of solvent, solute, and channel protein, occurring in femtoseconds in the idealized case of hard spheres. So many collisions occur in the shortest time interval of biological interest that much can be derived independent of the details of the collision. In the case of many collisions, the distribution of position of an ion around its starting point is Gaussian (Gardiner, 1983, p. 53), a consequence of the central limit theorem of probability theory (Parzen, 1960, ch. 10), if the displacement produced by each collision is uncorrelated and the ion's interaction with the channel protein can be described in a simple way (by what is called a Markov process, Cox & Miller, 1965, ch. 5). Probability theory then yields an equation (called the Fokker-Planck equation: Cox & Miller, 1965, pp. 208-215; Gardiner, 1983, ch. 5; Risken, 1984, ch. 5) that describes the (stochastic) concentration of ions within the pore, depending on the assumed potential profile within the channel and the boundary conditions. The Fokker-Planck equation contains as its only parameters the diffusion constant and gradient of potential energy determined by the applied membrane potential and the structure and dynamics of the protein forming the channel. The entire interaction of ion and channel is captured in these terms. The classical Nernst-Planck equation is recovered by integrating this Fokker-Planck equation, but the independent variable of this Nernst-Planck equation is the probability density function of concentration, not the macroscopic concentration. This stochastic derivation shows the validity of the Nernst-Planck equation even on the microscopic scale of Brownian motion. It is a complete description of the microscopic random walk of ions, describing the stochastic concentration, the thermal chaos resulting from the molecular nature of solutions and the kinetic nature of heat. Its validity is not restricted to distances much larger than atomic, as many have feared. Rather, it is an accurate description to the extent that the interaction of ion and channel protein can be described by a potential function and diffusion 'constant'.

We then turn to the calculation of rates of ion flux over barriers. The Fokker-Planck equation for the stochastic concentration can be solved giving the flux indirectly, but we use an alternative approach, first-passage time theory (following Weiss, 1966), that gives explicit expressions for the rates. This theory describes diffusive (i.e. Brownian) motion by the (distribution of) times an ion takes to reach a given position, for the first time. In the steady-state, we show (following Hardt, 1979, 1981) that the mean value of the first-passage time

distribution is always equal to (the reciprocal of) the rate constant of unidirectional flux, independent of the size of the potential barrier: this rate constant can be determined simply by computing the 'flux over content' of a steady-state system, without considering transients at all and without restriction on the barrier shape.

## 2. STOCHASTIC MODEL OF IONIC MOTION

We seek a simple expression describing the flux of an ion diffusing across an energy barrier in an ionic channel, related to the familiar expressions of transition state (i.e. Eyring rate) theory. The path of each ion can be directly determined by integrating the differential equations of motion for tens of picoseconds, using techniques of molecular dynamics (Berne, 1985), but the equations cannot yet be integrated for the tens of nanoseconds it takes an ion to drift (without diffusion) in a constant electric field of 100 mV across 5 nm.

Eyring rate theory (also called transition-state theory) has often been used in place of extensive numerical analysis. In this theory ions are supposed to move discontinuously, to jump distances of picometres in picoseconds from the bottom of wells of potential energy to the tops of barriers. In that case, simplified state theories of ionic movement can be derived, which have been widely used to describe channels (Hille, 1984, ch. 7).

The chemical literature (e.g. the treatises of Miller, 1976 and Baer, 1985) shows, however, that Eyring theory is appropriate only when reactions involve just a few atomic collisions, as in the gas phase. Eyring theory assumes that atomic or molecular motion over barriers occurs in (essentially) two hops, one from the energy valley to the top of the barrier, the other from the top of the barrier to the next valley. Energy barriers in channels are thought to be 2–20 Å wide and so Eyring theory applied to channels assumes that ions hop comparable distances.

But ionic motion in solutions does not occur by hops of this size. The experimental evidence (also see Fleming *et al.* 1986) is summarized in Tyrrell & Harris, 1984, *Diffusion in Liquids* which concludes (p. 286) that 'There is now rather direct evidence that diffusion in fluids does not occur by individual molecular "jumps" over distances of a molecular diameter.' Simulations of atomic motion (the most powerful theoretical tool in modern physical chemistry) give the same result (Hoare, 1971; Murthy & Singer, 1987, p. 25): 'The free path distribution shown is incompatible with theories which postulate activated jumps of the order of magnitude of the interparticle spacing.'

These statements are perhaps more understandable if they are put into the context of an (oversimplified) analytical theory (see Berry *et al.* 1980, pp. 830–851). It is possible to analyse the properties of a hypothetical 'liquid' made of spherical atoms which collide only one pair at a time (McQuarrie, 1976, chs 16–19). In that hypothetical case the frequency of collisions is high,  $8 \times 10^{14} \text{ s}^{-1}$  for spheres with mass and diffusion constant\* of  $\text{Na}^+$ . The thermal velocity is also very large ( $v_{\text{rms}} \approx 325 \text{ pm/ps}$ ), but there is very little room between spheres. Thus, we

\* The diffusion constant for  $\text{Na}^+$  is taken as  $1.4 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$  (Dani & Levitt, 1981), the value appropriate for the ion and its column of water in a gramicidin molecule.

expect and find that the mean path  $\lambda \approx 0.4$  pm is much smaller than the radius ( $\sim 100$  pm) of a sphere. The thermal velocity  $v_{\text{rms}}$  dominates the local motion of these spheres, exceeding by  $\sim 3000\times$  the purely electrical drift velocity  $\mu \approx 0.1$  pm/ps of a singly charged ion in an electric field of 100 mV across 5 nm. These conclusions follow from fundamental relations [Skinner & Wolynes, 1978, equation (2.19); Frauenfelder & Wolynes, 1985, equation (11); Risken, 1984, p. 2].

$$\mu(x) = -\frac{D}{kT} \frac{\partial U(x)}{\partial x}, \quad g = \frac{\gamma}{m} = \frac{kT}{Dm}; \quad \lambda = D \sqrt{\frac{2m}{kT}}; \quad v_{\text{rms}} = \sqrt{\frac{kT}{m}}, \quad (1)$$

$\gamma$  is the friction coefficient (units in  $\text{g s}^{-1}$ );  $m$  is the ion mass (g);  $\lambda$  is the average distance before the ion reverses direction (cm);  $U(x)$  is an electrical potential function, really the 'potential of mean force' (Berry *et al.* 1980, pp. 842–851). It is the sum of two terms. One term is the potential  $U_{\text{m}}(x)$  at position  $x$  within the pore, resulting from the transmembrane potential. The other term is the interaction potential  $U_{\text{i}}(x)$  between the ion and the channel protein. The interaction potential is assumed independent of  $U_{\text{m}}$ , because we do not consider voltage dependent conformations (i.e. gating) of the channel protein in this paper;  $kT$  is the temperature in units of energy (e.g. ergs, see Feynman *et al.* 1963, pp. 39–10):  $k$  is the Boltzmann constant and  $T$  the absolute temperature;  $D$  is the diffusion constant ( $\text{cm}^2 \text{s}^{-1}$ ). We use  $D$  throughout equation (1) to lessen the chance of confusing absolute and conventional mobilities (which are correctly defined in Bockris & Reddy, 1970, pp. 371, 376).

The picture we have of the motion of these hard spheres is thus exceedingly chaotic, the result of large numbers of collisions. This picture, arising from kinetic theory, fits well with the results of simulations and experimental data cited earlier. The stochastic derivation of the diffusion equation proceeds from that 'axiom', namely the motion of an individual ion is sufficiently chaotic that it is well described by a Gaussian distribution in the smallest distance of physiological interest.

Or to put the argument more formally, if each collision produces a stochastic displacement in position (Skinner & Wolynes, 1978, pp. 2145–2146), the central limit theorem (Parzen, 1960, p. 374) ensures a Gaussian distribution of the running sum of these displacements. The sum of enough uncorrelated displacements is a random variable  $X(t)$  with a Gaussian distribution (Parzen, 1962, pp. 22–24; Ricciardi, 1977, pp. 1–10). Define  $f_{\Delta}(x_0 + \Delta x, t_0 + \Delta t | x_0, t_0)$  ( $\text{cm}^{-1}$ ) as the probability density (i.e. probability per unit length) that an ion (moving with constant drift velocity  $\mu$  because the field  $\partial U/\partial x_0$  is constant) starting at  $x_0, t_0$  is found at  $x_0 + \Delta x$  at time  $t_0 + \Delta t$ . Then, the relevant Gaussian with drift is written

$$f_{\Delta}(x_0 + \Delta x, t_0 + \Delta t | x_0, t_0) = \frac{1}{2\sqrt{[\pi \Delta t D(x_0)]}} \exp\left[-\frac{[\Delta x - \Delta t \mu(x_0)]^2}{4\Delta t D(x_0)}\right], \quad (2)$$

This local Gaussian with drift is discussed in many references, for example, Cox & Miller, 1965, p. 209; Ricciardi, 1977, p. 58; Risken, 1984, p. 73.\*

\* In Cox & Miller,  $\sigma^2 = 2D$ , and their  $t$  equals our  $\Delta t$ . Unfortunately, Ricciardi's expression is written in unconventional form and Risken's equation (4.49) is in error, while his equations (5.3) and (5.4) are correct.

Surprisingly, the Gaussian distribution arises quite independently of the nature of the collisions between atoms; indeed, it arises from models of diffusion which do not satisfy the (Markov) assumptions used here to describe the diffusion and interaction of ion and channel protein. Elementary analysis (Drake, 1967, p. 215) suggests 10 collisions are enough to ensure this Gaussian, even if each collision produces a binomially distributed jump in location. Many more refined analyses give the same result: see the argument of van Kampen, 1983; the (Monte Carlo) simulations of simple collisions of Hoare (1971, p. 209); and the simulations with more elaborate models of collisions (Williams, 1966, p. 48; Skinner & Wolynes, 1979, 1980; Hänggi, 1986, p. 126), some even non-Markovian (Dygas *et al.* 1986; Nitzan, 1987).

Equation (2) is only a local distribution function, valid in a region of constant field, after an ion has had (say) 10 atomic collisions. In the hard sphere model, the ion has then moved a distance of  $\sqrt{2D\Delta t} = \sqrt{(2D \times 10/g)} \simeq 1.9$  pm in a time interval of  $10/g \simeq 13$  fs. The restriction to constant field means that equation (2) is valid in a small time interval  $\Delta t < \tau$  and region  $\Delta x < \xi$ , in which the derivative  $U'(x)$  of the potential (namely, the electric field) is nearly linear, with error

$$\epsilon(\xi) \equiv U(\xi) - U(0) - \xi U'(0) = \frac{1}{2}\xi^2 U''(0), \quad (3)$$

where the right-hand equality comes from a three term Taylor expansion and  $\epsilon$  has units of energy, i.e. of  $kT$ . The potential lies within  $\epsilon(\xi)$  of the linear approximation in a region of width  $2\xi = 2\sqrt{[2\epsilon/U''(0)]}$ . This distance can be written in more familiar form if the potential is the parabola  $U_p(\xi)$  shown in Fig. 1 with width  $2\Delta$ . In that case, the potential is linear in a small region that depends on the acceptable deviation from linearity but is independent of the location because  $U_p$  is a parabola, with constant second derivative. If the acceptable nonlinearity is  $\pm\epsilon/U_0$ , the linear region extends over  $\Delta x = \pm\xi = 2\Delta\sqrt{(\epsilon/U_0)}$ .

The range of validity of equation (2) is then established, given that the narrowest structure relevant to ionic movement in channels is likely to be 500 pm =  $2\Delta$ . The central limit theorem shows that equation (2) is valid after the ion diffuses some 1.9 pm (after some 10 collisions taking 13 fs). The linearity argument shows that equation (2) is valid before the ion diffuses some  $\xi = 50$  pm (before some 10 ps, which is  $\Delta t \simeq \xi^2/[2D]$ );  $\xi = 50$  pm is the edge of the nearly linear region of potential, computed for a nonlinearity of  $|\epsilon/U_0| \simeq 1\%$  with  $\Delta = 250$  pm.

A general validity condition (well known in physical chemistry, see Hänggi, 1986, p. 111) can be similarly derived for non-parabolic potentials. The local Gaussian formulation is useful if the mean displacement after  $n_c$  collisions is within the region  $\xi$  of nearly linear potential, where  $n_c$  is enough to ensure a local Gaussian distribution: the maximum second derivative of potential  $|U''|_{\max}$  must be less than  $(4\epsilon/n_c)(g/D)$ . For the parabolic potential of Fig. 1, this becomes the long known [Chandrasekhar, 1943: eq. (508)] criterion  $[n_c kT]/[2m(g\Delta)^2] < \epsilon/U_0$ , derived under more general conditions by Skinner & Wolynes, 1978: equations (5.1), (5.5), 1980, equations (5.7)–(5.9)

The large frequency  $g$  of collisions fortunately ensures that the local

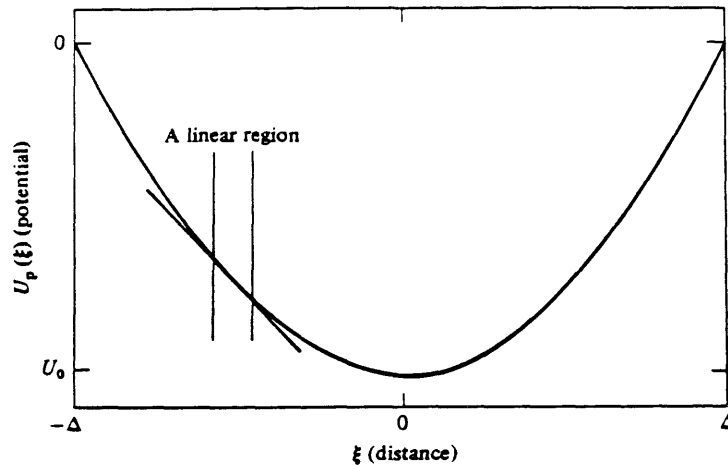


Fig. 1. The figure shows a parabolic potential  $U_p = U_0[1 - (\xi/\Delta)^2]$  of width  $2\Delta$ , depth  $U_0$ , and second derivative  $U'' = 2U_0/\Delta^2$ . The potential is linear in a small region around any point. The size of the region is independent of the location of the point (because  $U_p$  is a parabola, with constant second derivative) but depends on the acceptable nonlinearity. If that is  $\pm \epsilon/U_0$ , the linear region extends over  $x - x_0 = \pm \xi = 2\Delta\sqrt{(\epsilon/U_0)}$ . Although the potential function shown is a well, with  $U_0$  negative, the discussion in the text is unchanged for a barrier, with  $U_0$  positive.

displacement of ions is Gaussian on the time/length scale of channel permeation. Interestingly,  $g$  is also the rate constant of (exponential) equilibration of velocity of an ion in a frictional medium (Eisenberg & Crothers, 1979, p. 701). Evidently, ions reach equilibrium in velocity after 3 or 4 collisions and a Gaussian distribution of displacement after 3–10 collisions (Hoare, 1971, p. 169), maintaining both during the entire permeation process [Cooper *et al.* 1985, equation (6)]. The collisions that damp the acceleration are enough to randomize (i.e. to normalize) the motion of the ion as well.

### 3. DERIVATION OF THE FOKKER-PLANCK EQUATION

We wish to describe the movement of an ion through a channel when the microscopic movement of the ion is Gaussian, described by equation (2), and the effect of channel structure is described by the potential energy  $U(\cdot) = U_m(\cdot) + U_1(\cdot)$ , often identified with the 'potential of mean force' (Berry *et al.* 1980, pp. 842–851), and closely related to the potential of Maxwell's electric displacement  $D$  (Purcell, 1985, p. 380). A differential equation can be derived from equation (2) (and remarkably little else) that describes the probability density of finding an ion in an interval  $\Delta x$  at any time, which we call the stochastic concentration.

The stochastic concentration of ions at point  $(x, t)$  is described by the conditional probability density function  $f(x, t | x_0, t_0)$  when all the ions started their motion at the single point  $(x_0, t_0)$ , that is to say, when the initial distribution of

concentration  $p(x_0, t_0)$  is a delta function  $\delta(x - x_0)$ . We assume that diffusion in channels is adequately described by a (time homogeneous, one dimensional) Markov transition probability function and so the motion from  $(x_0, t_0)$  to  $(x, t)$  can be written as the following sum of motions through all possible intermediate points  $(x_1, t_1)$  (Hille & Phillips, 1957, p. 634, 648; Karlin & Taylor, 1975, p. 286).

$$f(x, t | x_0, t_0) = \int_{\mathcal{R}} f(x_1, t_1 | x_0, t_0) f(x, t | x_1, t_1) dx_1, \quad (4)$$

where  $\mathcal{R}$  and  $\mathcal{A}$  are the physical boundaries of the system.

We introduce another variable  $p(x, t)$  ( $\text{cm}^{-1}$ ) to describe the (stochastic) concentration of ions at  $(x, t)$ , because in general all the ions do not start at the same place. This probability density function is not a conditional probability, rather it is the sum over the motions of ions starting from the initial spatial distribution  $p(x_0, t_0)$ , which need not be confined to just one point (Gardiner, 1983, p. 118).

$$p(x, t) = \int_{\mathcal{R}} p(x_0, t_0) f(x, t | x_0, t_0) dx_0, \quad (5)$$

$f(x, t | x_0, t_0)$  will turn out to be the Green's function Zauderer, 1983, ch. 7) of the Fokker-Planck equation (9) and  $p(x, t)$  its solution, for a source distribution  $p(x_0, t_0)$ .

The concentration is obviously influenced by boundary and initial conditions as well as its equations of motion [i.e. equation (2)]. Thus, a partial differential equation and boundary conditions are needed to specify  $p(\cdot)$  uniquely. Appendix 1 derives such an equation (called the Fokker-Planck equation) for  $p(\cdot)$  using the known [i.e. equation (2)] local transition probability  $f_{\Delta}(\cdot)$  that is itself only valid in a small enough region that  $U(x)$  is linear. The integral (5) is converted to the differential equation by expanding the global transition probability function  $f(\cdot)$  of equation (5) into its (spatial) moments (defined in Appendix 1) and taking the limit of  $X^{(j)}(\cdot)/\Delta t$  as  $\Delta t \equiv t - t_0 \rightarrow 0$ . The moments in that limit coincide with the moments  $X_{\Delta}^{(j)}$  of the local transition probability  $f_{\Delta}(\cdot)$ , which are functions of the diffusion constant and drift velocity of an ion, determined by equation (2). The intermediate steps are presented in Appendix 1 since we could not find a derivation at an appropriate level.

The first infinitesimal moment  $X_{\Delta}^{(1)}(x_0, \Delta t)/\Delta t$ , computed around the source location  $x_0$ , is just the average motion of an ion, namely the drift velocity  $\mu(x)$ .

$$\frac{1}{\Delta t} X_{\Delta}^{(1)}(x_0, \Delta t) = \mu(x) = -\frac{D(x)}{kT} \frac{\partial U(x)}{\partial x}. \quad (6)$$

The second infinitesimal moment  $X_{\Delta}^{(2)}(x_0, \Delta t)/\Delta t$  yields a combination of diffusion constant and drift distance, which in the limit becomes the diffusion constant  $D(x)$ .

$$\frac{1}{\Delta t} X_{\Delta}^{(2)}(x_0, \Delta t) = 2D(x) + [\mu(x)]^2 \Delta t \rightarrow 2D(x). \quad (7)$$



The third (and higher) moments (divided by  $\Delta t$ ) are higher order in  $\Delta t$  and vanish in the limit.

$$\frac{1}{\Delta t} X_{\Delta}^{(3)}(x_0, \Delta t) = 6D(x)\mu(x)\Delta t + [\mu(x)]^3[\Delta t]^2 \rightarrow 0. \quad (8)$$

All higher order moments vanish (Pawula, 1967, Risken, 1984, p. 70), and the expansion (A 1-9) truncates into a second order differential equation, the Fokker-Planck equation describing the density function  $p(x, t)$  for the position of the ion,

$$\frac{\partial p(x, t)}{\partial t} = -\frac{\partial}{\partial x}[\mu(x)p(x, t)] + \frac{\partial^2}{\partial x^2}[D(x)p(x, t)]. \quad (9)$$

The location of  $D(x)$  within the parentheses is a well known feature of this derivation of the Fokker-Planck equation but not of other derivations (see van Kampen, 1981, p. 291 and Gardiner, 1983, pp. 96 and 100 for extensive but difficult discussion).

The initial and boundary conditions for equation (9) depend on the problem of interest. Stochastic analysis requires that  $p(\cdot)$  be a probability density function, positive and normalized (at all times) to unity. Those requirements are not satisfied by all the  $p(\cdot)$  functions that satisfy equation (9) and meaningful boundary conditions: only certain boundary conditions guarantee positive and normalizable (i.e. the existence of time independent integrals over the whole system) solutions (Protter & Weinberger, 1984, ch. 3). In this review, we follow the custom of many others and consider only simplified boundary conditions which guarantee the required properties. Analysis of permeation through real biological channels requires quite different boundary conditions to describe the processes by which ions move up to, enter, and leave the channel and explicit treatment of the interaction of ions (e.g. with a model containing a discrete number of occupancy states).

### 3.1 Relation to Eyring models

A great deal of work has been done trying to extend the diffusion model described here to the case where only a few collisions occur while an ion crosses a barrier (reviewed in Hänggi, 1986; see Northrup & Hynes, 1980; Matkowsky *et al.* 1984; Frauenfelder & Wolynes, 1985; Straub & Berne, 1986; Straub *et al.* 1986; Mel'nikov & Meshkov, 1987). In these treatments, rate constants reminiscent of those of transition state theory arise when the collision rate is small enough or potential barriers large enough. But the physical meaning and temperature dependence of the rate constants differ. In transition state theory, ions sit (at equilibrium) in potential wells except when they hop across barriers. Friction (i.e. collisions in the idealized case) is not involved and friction coefficients do not appear in the rate equations. But in any non-equilibrium theory the pre-exponential factor depends strongly on friction and temperature (Kramer, 1940; discussed in Frauenfelder & Wolynes, 1985, pp. 340-343).

### 3.2 Nernst–Planck equation

The central equation of electrochemistry (Bockris & Reddy, 1970), the Nernst–Planck equation, is a consequence of the Fokker–Planck equation, if we introduce the flux  $\mathcal{J}(x, t)$ , the probability ‘current’ (see the long justification in Ricciardi, 1975, p. 42; Gardiner, 1983, p. 119) through the so-called ‘continuity’ equation

$$\frac{\partial p(x, t)}{\partial t} = -\frac{\partial \mathcal{J}(x)}{\partial x}. \quad (10)$$

We write the drift velocity  $\mu(x)$  in terms of the electrical potential  $V(x) \equiv zqU(x)$ , where  $zq$  is the charge on the ion (cou), using equation (1) and introduce the concentration of ions  $n(x) = p(x)/A(x)$  ( $\text{cm}^{-3}$ ), where  $A(x)$  is the system cross-sectional area ( $\text{cm}^2$ ). One spatial integration of the Fokker–Planck equation then gives the Nernst–Planck equation

$$-\mathcal{J}(x) = D(x) \left[ \frac{zq}{kT} \frac{dV(x)}{dx} \right] n(x) + \frac{d}{dx} [D(x)n(x)]. \quad (11)$$

While this equation appears familiar, it differs in a significant respect from the traditional Nernst–Planck equation of electrochemistry (Bockris & Reddy, 1970, pp. 296, 414–416, 693–698). Here the concentration is treated explicitly as a random variable, and so the equation is valid on an atomic scale. It describes stochastic (spatial) variation in concentration just as well as it does the average concentration, to the extent that our description of the ionic motion in a channel (as a Gaussian Markov process) is accurate. Gardiner, 1983, ch. 8 analyses temporal fluctuations in concentration.

## 4. PASSAGE OVER BARRIERS

The Fokker–Planck equation provides a complete description of the movement of ions driven by a deterministic time independent force  $U'(x)$  from an initial location  $x_0$ , restrained by frictional interactions (i.e., collisions) described by  $D(x)$ . It is a well studied parabolic (e.g. heat or diffusion) partial differential equation (Carrier & Pearson, 1976; Gardiner, 1983; Risken, 1984) that can be solved analytically for some potential functions. When analytical solutions are not available, numerical integration is possible (Lapidus & Pinder, 1982, ch. 1.4). Unfortunately, neither analytical nor numerical solutions are very useful without a unifying paradigm to tie them together to experimental work.

A widely used paradigm analyses ionic movement across barriers with the rate constants for unidirectional tracer fluxes, using the law of mass action to describe dependence on concentration and Eyring transition state theory to describe the dependence on potential. Although transition state theory is certainly an inappropriate general description of a Gaussian Markov process (Kramers, 1940; Chandrasekhar, 1943; Skinner & Wolynes, 1978, 1980; Frauenfelder & Wolynes, 1985; Hänggi, 1986, pp. 107–116) or physical diffusion, for that matter (Tyrrell

& Harris, 1984; Murthy & Singer, 1987), it is a better paradigm for fitting data than nothing (e.g. a table of numbers or a many-term eigenfunction expansion) and is widely used in the experimental literature (reviewed in Hille, 1984), probably for that reason.

Stochastic diffusion theory also can describe unidirectional tracer fluxes with rate constants and the law of mass action (Szabo *et al.* 1980; Schulten *et al.* 1981) – thus maintaining contact with the experimental literature. But it describes ions diffusing in picometer steps over barriers angstroms wide, whereas transition state theory describes ions jumping angstrom or even nanometer steps over barriers just as wide.

The description of stochastic flux is most easily done in the steady-state, when transients in flux have damped away. And fortunately, a transient analysis is not needed. Biological processes depending on channels are so slow compared to the time scale of barrier crossing let alone the time scale of ionic collisions (10  $\mu\text{s}$  vs. say 0.1 ps), that it is natural to assume steady-state current flow through the open channel in a first analysis.\* These (quasi) steady-state currents are observed experimentally and are usually called single channel currents. The question is do such measurements determine the apparent rate constant for barrier crossing? Kramers (1940, p. 293) knew they do and so did many earlier workers in compartmental analysis in biology and chemical engineering (see references in Nauman & Buffham, 1983; Buffham, 1985; Jacquez, 1985, ch. 7). We present the following stochastic analysis (following Hardt, 1979, 1981) to convince, if not prove the point in general.

#### 4.1 Steady-state flux

Consider a pore  $x_0 \leq x \leq \mathcal{A}$  filled with non-radioactive ions (empty circle/spheres in Fig. 2), which has a steady flow  $\mathcal{J}$  into it, through it, and out of it, so it is on average filled with  $\mathcal{N}(\cdot)$  ions, radioactive or non-radioactive. The potential energy of an ion in this pore is  $U(x)$  and so the pore can contain barriers and wells. The left hand side of the pore is idealized as a reflecting boundary: once ions enter the pore they cannot leave to the left. This boundary condition must be made more realistic in later work.

At time  $t_0$ , the ions to the left of the pore (in the region  $x < x_0$ ) are replaced by radioactive tracers ( $x$ 's in Fig. 2). We want to know the average time it takes the  $o$ 's, the original non-radioactive ions, to leave, to flow out of the pore into the absorbing boundary at  $x = \mathcal{A}$ . This time is called the mean time of first-passage.

Now consider the pore with steady flow sometime later, at time  $t$ . The flux out of the pore is made of both radioactive and non-radioactive ions. Those ions that have been in the pore for a time less than  $\tau \equiv t - t_0$  clearly entered after time  $t_0$  and so are radioactive, because only radioactive ions were allowed to enter after  $t_0$ .

\* The time course of channel opening, and the corresponding time course of gating current have not yet been resolved, being clearly faster than 10  $\mu\text{s}$  (Stimers *et al.* 1987). In this review, such transient phenomena are assumed not to be involved in conduction through an (already) open channel.

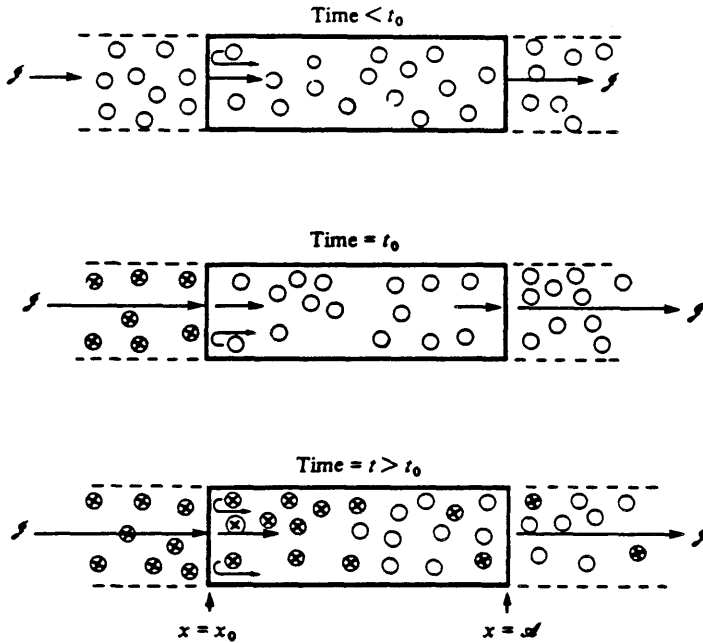


Fig. 2. The steady flux through a one-dimensional channel  $x_0 \leq x < \mathcal{A}$ . At times before  $t_0$ , the ions entering the channel (from the left) are non-radioactive, shown as empty spheres. Ions once in the channel cannot leave from the left (see text). At time  $t = t_0$ , the non-radioactive ions, in the region  $x < x_0$  only, are replaced by radioactive ions (shown as spheres containing  $x$ 's) and from then on (i.e. for  $t > t_0$ ), all the ions entering the channel at  $x = x_0$  are radioactive. After  $t_0$ , the channel contains both radioactive and non-radioactive ions, and so both radioactive and non-radioactive ions leave the channel on the right at the absorbing boundary. The channel (and system) is assumed to be always at steady-state, so the flux is the same at all places and all times. The stochastic derivation in the text [see equation (18)] shows that  $\mathcal{N}/\mathcal{J}$ , the total number of ions in the channel (both radioactive and non-radioactive) divided by the (steady) flux through the channel (carried by both radioactive and non-radioactive ions), equals the mean first-passage time of the ions, as defined by a related transient problem, namely, the ordinary differential equation (32) with a reflecting boundary at  $x = x_0 = \mathcal{R}$  and an absorbing boundary at  $x = \mathcal{A}$ .

Those radioactive ions no longer in the pore are precisely those ions that have been in the pore for less than  $t - t_0$ , i.e. that have taken time  $t - t_0$  or less to pass through the pore and be absorbed at the right hand side, at  $\mathcal{A}$ . The cumulative distribution function  $\phi(t, \mathcal{A} | t_0, x_0)$  (of the times of first-passage) is defined as the fraction of all ions (radioactive and non-radioactive) that have taken time  $t - t_0$  or less to reach  $\mathcal{A}$  and be absorbed. Thus,  $\phi(t, \mathcal{A} | t_0, x_0)$  also describes the fraction of all the ions crossing the boundary (at  $\mathcal{A}$ ) at time  $t$  which are radioactive. Similarly, the ions that are non-radioactive are precisely those that have been in the pore time  $t - t_0$  or longer, and so, the complement of the distribution function, namely  $1 - \phi(t, \mathcal{A} | t_0, x_0)$ , describes the fraction of all the ions crossing the boundary that are non-radioactive.

The formal development follows easily.  $\mathcal{N}(T)$  is the mean number of ions in the pore during time  $T$ , which can be written as

$$\mathcal{N}(T) = \frac{1}{T} \int_0^T d\tau \int_0^{\mathcal{A}} A(x) n(x) dx, \quad (12)$$

where  $\tau \equiv t - t_0$  and the pore extends from  $x = 0$  to  $x = \mathcal{A}$  and has cross-sectional area  $A(x)$  ( $\text{cm}^2$ ) and contains ions of concentration  $n(x)$  ( $\text{cm}^{-3}$ ).  $\mathcal{J}$  is the flux ( $\text{s}^{-1}$ ) through the pore, which is independent of location and time at steady-state. The total number of ions crossing the boundary  $\mathcal{A}$  in time  $dt$  is  $\mathcal{J} dt$ . The number of radioactive ions (all introduced after time  $t_0$ ) crossing the boundary is  $\phi \mathcal{J} dt$ . And

$$\mathcal{J} dt [1 - \phi(\tau)] = \mathcal{J} dt \left( 1 - \int_0^\tau \dot{\phi}(\tau) d\tau \right) \quad (13)$$

is the number of non-radioactive ions that cross the boundary  $\mathcal{A}$ . The time derivative of the cumulative distribution function is written as  $\dot{\phi}$ , and is, of course, just the probability density function of the time of first-passage.

Eventually, all the non-radioactive ions that occupied the pore at time  $t_0$  will be flushed out of the pore (i.e. be absorbed at  $\mathcal{A}$ ), so, remembering that  $\mathcal{J}$  is a constant,

$$\mathcal{N} \equiv \mathcal{N}(\infty) = \mathcal{J} \int_0^\infty [1 - \phi(\tau)] d\tau = \mathcal{J} \int_0^\infty \left( 1 - \int_0^\tau \dot{\phi}(T) dT \right) d\tau, \quad (14)$$

Now, we can do an integration by parts to describe the mean value of a random variable in terms of its cumulative distribution function (Parzen, 1960, p. 211; see also Courant, 1936, p. 245)

$$\int_0^\infty u dv \equiv uv \Big|_0^\infty - \int_0^\infty v du \quad (15)$$

identifying  $dv = d\tau$ , and

$$u = 1 - \phi(\tau) \equiv 1 - \int_0^\tau \dot{\phi}(T) dT \quad (16)$$

Then,  $du = -\dot{\phi}(\tau) d\tau$ ,  $v = \tau$ , and

$$\frac{\mathcal{N}}{\mathcal{J}} = [\tau(1 - \phi(\tau))]_0^\infty + \int_0^\infty \tau \dot{\phi}(\tau) d\tau. \quad (17)$$

The first term vanishes at  $\tau = 0$  and at  $\tau = \infty$  because  $\phi(0) = 0$  and  $\phi(\infty) = 1$ , as with any cumulative probability. The second term is just the definition of the mean value of  $\phi$ , namely the mean first passage time

$$Y(\mathcal{A} | x_0) \equiv \int_0^\infty \tau \dot{\phi}(\tau) d\tau = \frac{\mathcal{N}}{\mathcal{J}}. \quad (18)$$

Thus, the total number of ions in the pore, divided by the flux through the pore is the mean first-passage time  $Y$  for steady flux through the pore.

4.2 *Rate constants and (mean) times of first-passage*

First-passage times can be viewed as a stochastic generalization of rate constants classically used to describe steady unidirectional tracer flux (Sheppard, 1962; Jacquez, 1985; Bass *et al.* 1986): the mean value of the first-passage time is the reciprocal of the rate constant. The rate constant of unidirectional tracer flux describes the movement of tracer from a compartment of one concentration to another compartment of zero tracer concentration, the zero concentration being maintained despite any size flux, by rapid stirring, or rapid dilution by diffusion into a bath of large volume. In the classical theory of tracers the flux of isotope is absorbed into the bath just as the 'flux of probability' is absorbed into the boundary condition in the theory of first-passage. In both theories the unidirectional flux disappears without effect, back-flux being described by a separate unidirectional rate constant. In experiments using this definition of rate constant, the concentration of tracer must be kept negligible at the 'target' (i.e. sink) of the flux. In theories using this definition, the concentration is kept zero by the boundary condition [equation (35)].

Consider the flux of ions across a structure with well defined barriers and wells, something like that shown in Fig. 3. Following tradition, we consider a closed system in which the total number of ions does not change with time, in contrast to the open system (with steady-state flows) shown in Fig. 2 and analysed in equations (12)–(18). 'Concentrations' (more precisely occupancies of the total region)  $N_A(t)$  and  $N_B(t)$  (units: none) are generally defined in two regions, one called A to the left of the maximum (at  $x = 0$  in the figure) and one called B to the right of the maximum. The concentrations are integrals of the probability density  $f(\cdot)$  in each region:

$$N_A(t|x_0) \equiv \int_{-\infty}^0 f(x, t|x_0, t_0) dx; \quad N_B(t|x_0) \equiv \int_0^{\infty} f(x, t|x_0, t_0) dx. \quad (19)$$

The time derivative of the concentration in region A is determined by the unidirectional flux  $\mathcal{J}(t|A \rightarrow B)$  flowing from region A into B and the flux  $\mathcal{J}(t|B \rightarrow A)$  flowing from region B into A:

$$\frac{dN_B(t|x_0)}{dt} = -\frac{dN_A(t|x_0)}{dt} = \mathcal{J}(t|A \rightarrow B) - \mathcal{J}(t|B \rightarrow A). \quad (20)$$

Each flux is described by the following equation, which, in a certain sense, is the definition of the rate constant.

$$\mathcal{J}(t|A \rightarrow B) = k_r(t|x_0) N_A(t|x_0); \quad \mathcal{J}(t|B \rightarrow A) = k_r(t|x_0) N_B(t|x_0), \quad (21)$$

giving

$$\mathcal{J}(t|A \rightarrow B) - \mathcal{J}(t|B \rightarrow A) = k_r(t|x_0) N_A(t|x_0) - k_r(t|x_0) N_B(t|x_0). \quad (22)$$

In traditional simple situations,  $k_r(\cdot)$  and  $k_r(\cdot)$  are (time independent and concentration independent) constants that can be measured experimentally as the (reciprocal) of the time constant of the concentration change.

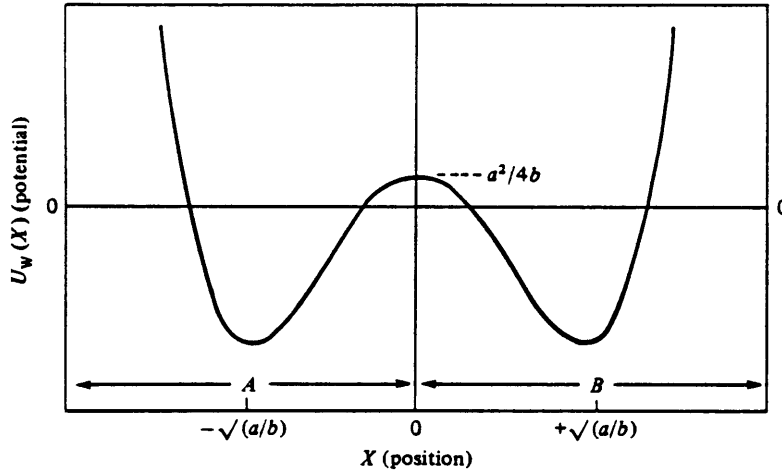


Fig. 3. The symmetrical W-shaped profile of potential energy shown is described precisely by  $U_w(x) = (a^2/4b) - (ax^2/2) + (bx^4/4)$ , and has a minimum of zero at  $x = \pm\sqrt{a/b}$  and a maximum of  $a^2/4b$  at  $x = 0$ . Flux from region A to B is called  $\mathcal{J}(A \rightarrow B)$  and is described by the forward rate constant  $k_f$ ; back flux is defined analogously.

Rate constants have been measured experimentally for many years even when time courses are not exponential, using a definition arising from measurements of so-called 'unidirectional' tracer (isotope) fluxes (e.g. Jacquez, 1985, pp. 100-104), the tracer flux in a particular situation, when isotope is only present in one compartment. If  $\alpha$  is one isotope, and the concentration of  $\alpha$  is negligible when  $x$  is contained in region B, we write  $N_\alpha(t|x) \approx 0$  for  $x \in B$ . In that case, the rate constant for unidirectional flux of isotope  $\alpha$  from  $x_0 \in A$  to  $x \in B$  is defined experimentally by

$$k_f(t|x_0) = \frac{\mathcal{J}(t|\alpha; A \rightarrow B)}{N_\alpha(t|x_0)} \quad \text{for} \quad \left\{ \begin{array}{l} N_\alpha(t|x_0 \in A) \equiv N_A, \\ N_\alpha(t|x_0 \in B) \equiv N_B \approx 0, \end{array} \right\} \quad (23)$$

where we measure the flux  $\mathcal{J}(t|\alpha, A \rightarrow B)$  of the isotope  $\alpha$ , remembering that its concentration is nearly zero in region B, so it does not produce back flux and the second terms (on both sides of) equation (22) are zero. This method allows easy determination of  $k_f$  (or analogously  $k_r$ ) for flux from  $x_0 \in A$  to  $x_0 \in B$  from experimental measurements of  $\mathcal{J}(t|\alpha; A \rightarrow B)$ , when suitable isotopes are available and experimental techniques can keep the isotope concentration  $N_\alpha$  negligible for  $x \in B$ . (Incidentally, this is a preferred experimental method for measuring rate constants because it does not depend on measuring the time course of a concentration, and thus is not sensitive to the well known errors of determining rate constants from the sum of exponentials.)

If we now compare the expression for the flux through a steady-state probabilistic system [equation (18)] with the unidirectional isotope flux computed in this traditional rate constant model [equation (23)], we recover the relationship

of rate constant and mean first-passage time used widely in the literature of physical chemistry (e.g. Hänggi, 1986, p. 116; Weiss, 1986, p. 12):

$$Y_r(x_0) \equiv Y(x \in B | x_0 \in A) = \frac{1}{k_r(x_0)}; \quad Y_r(x_0) \equiv Y(x \in A | x_0 \in B) = \frac{1}{k_r(x_0)}. \quad (24)$$

The appropriate extension of this definition of rate constant to the non-steady state is not known to us (but see the treatment of Szabo *et al.* 1980; Schulten *et al.* 1981; Cox & Miller, 1965, pp. 252–253). Fortunately, the steady-state suffices for problems of channel permeation.

Although the rate equations (20)–(23) seem similar to those of traditional membrane theory (Hille & Schwarz, 1978), their meaning is quite different. In particular, the rate constants of a diffusion theory depend on the starting position  $x_0$ : ions diffusing through solutions take time to cross wells and barriers and so the rate constants depend on how far the ion moves; they depend on the starting position  $x_0$  (see Hladky & Haydon, 1984, p. 347).

## 5. FIRST-PASSAGE TIME

Fortunately, a complete theory is available to describe the time needed for a stochastic process to (first) reach a certain value (Feller, 1950; Parzen, 1962, pp. 226–247; Cox & Miller, 1965; Ricciardi, 1977, ch. 3; and as a problem in combinatorics, Andersen, 1953, 1974). The theory of first-passage times describes the time  $t^*$  an ion released at  $x_0$  takes to reach  $\mathcal{A}$  for the first time. The ion diffuses in a potential field, according to a local probability density [like equation (2)]; the ion is confined to a definite region of space (so the first-passage time is not infinite or otherwise ill defined); but the diffusion process is unrestricted in other respects; it is as general as the Fokker–Planck equation (9) itself. The phrase ‘first-passage’ means an ion is counted only the first time it reaches  $\mathcal{A}$ ; if the ion moves past  $\mathcal{A}$ , reverses direction and returns to  $\mathcal{A}$  a second time, it is not counted. Obviously something at  $\mathcal{A}$  must select from all the trajectories of ions just those representing the first-passage to  $\mathcal{A}$ . A boundary at  $\mathcal{A}$  that absorbs an ion (and thus removes it from the system the first time the ion reaches  $\mathcal{A}$ ) performs this selection. Thus, the diffusion of an ion from  $x_0$  to an absorbing boundary at  $\mathcal{A}$  defines the first-passage time from  $x_0$  to  $\mathcal{A}$ . Without such an absorbing boundary the passage time is not so well defined and is perhaps a less useful construct. An ion found at  $\mathcal{A}$  could have been there many times before.

In this review, first-passage times are described by the random variable, indicated by the asterisk,  $t^*(\mathcal{A} | x_0, \mathcal{R})$  (units in seconds) for an ion starting at time zero, at location  $x_0$ , moving to  $\mathcal{A}$ . The ions are confined to the system (in our discussions) by a physical reflecting boundary at  $\mathcal{R}$ , a boundary which the ions cannot cross.  $t^*(\mathcal{A} | x_0, \mathcal{R})$  is most intuitively described by its cumulative probability distribution function  $\phi(t, \mathcal{A}, x_0) \equiv \phi(t, \mathcal{A} | x_0, \mathcal{R})$ , giving the probability that an ion starting at  $x_0$  takes time  $t$  (or less) to reach  $\mathcal{A}$  for the first time, i.e. to be absorbed at position  $\mathcal{A}$ . The time  $t$  is, of course, a function of  $x_0$ ,  $\mathcal{A}$  and (perhaps) other parameters of the system. When an ion reaches a position



$x_0$  for the first time, that position is a maximum in the excursion of the ion (Lindenberg & West, 1986) and so, interestingly, the statistical theory of extremes (Blake & Lindsey, 1973), also called the theory of reliability (Gumbel, 1958), is closely related to the probability theory of first-passage times.

Probability functions describing the first-passage time can be derived from the probability density functions already used to describe the stochastic concentration of ions, using a conservation law. Consider an ion starting at  $(x_0, t_0)$  and moving to an absorbing boundary at  $\mathcal{A}$ . If  $\mathcal{R}$  is the other boundary of the system, then the number of ions between  $\mathcal{R}$  and  $\mathcal{A}$  is simply the integral of the probability density function between  $\mathcal{R}$  and  $\mathcal{A}$ , i.e.  $\int f(x, t | x_0, t_0) dx$ . The ions in the system are either in this interval or they have reached the boundary  $\mathcal{A}$ , been absorbed, and left the system. The cumulative probability function  $\phi(\text{time} < t | x_0, t_0)$  defined above specifies the probability that an ion has reached the boundary  $\mathcal{A}$  by time  $t - t_0$  (or before). The conservation law is (Cox & Miller, 1965, p. 230) then

$$1 = \phi(t | x_0, t_0) + \int_{\mathcal{R}}^{\mathcal{A}} f(x, t | x_0, t_0) dx. \quad (25)$$

An integral expression for the first-passage time is derived by substituting the consistency equation (4) into equation (25), using the dummy variables  $x_1, t_1 (\mathcal{R} < x_1 < \mathcal{A}; t_0 < t_1 < t)$  to describe the interior intermediate location required by equation (4). Interchanging orders of integration gives

$$1 - \phi(t | x_0, t_0) = \int_{\mathcal{R}}^{\mathcal{A}} f(x_1, t_1 | x_0, t_0) \left\{ \int_{\mathcal{R}}^{\mathcal{A}} f(x, t | x_1, t_1) dx \right\} dx_1, \quad (26)$$

which is just

$$\left. \begin{aligned} 1 - \phi(t | x_0, t_0) &= \int_{\mathcal{R}}^{\mathcal{A}} f(x_1, t_1 | x_0, t_0) \{1 - \phi(t | x_1, t_1)\} dx_1, \\ &= \int_{\mathcal{R}}^{\mathcal{A}} f(x_1, t_1 | x_0, t_0) dx_1 - \int_{\mathcal{R}}^{\mathcal{A}} \phi(t | x_1, t_1) f(x_1, t_1 | x_0, t_0) dx_1. \end{aligned} \right\} \quad (27)$$

The first integral on the right hand side can be rewritten using equation (25),

$$\phi(t | x_0, t_0) = \phi(t_1 | x_0, t_0) + \int_{\mathcal{R}}^{\mathcal{A}} \phi(t | x_1, t_1) f(x_1, t_1 | x_0, t_0) dx_1 \quad (28)$$

If  $t_1$  is close to  $t_0$ , then

$$\phi(t_1 | x_0, t_0) \simeq 0, \quad \text{and} \quad f(x_1, t_1 | x_0, t_0) \rightarrow f_{\Delta}(x_1, t_1 | x_0, t_0),$$

the local Gaussian, equation (2), provided  $x_0$  is not too close to the boundary  $x = \mathcal{A}$ . We then have a local consistency relation for  $\phi(\cdot)$ , originally presented in Weiss, 1966, p. 11 (see also Mandl, 1968, p. 129; Karlin & Taylor, 1981, p. 217).

$$\phi(t | x_0, t_0) = \int_{\mathcal{R}}^{\mathcal{A}} \phi(t | x_1, t_1) f_{\Delta}(x_1, t_1 | x_0, t_0) dx_1 \quad (x_1 \rightarrow x_0; \quad x_0 < \mathcal{A}). \quad (29)$$

The derivation proceeds by expanding  $\phi(t|x_1, t_1)$  in equation (29) about  $x_0, t_1$  and factoring the  $x_1$  independent part out of the integrals.

$$\begin{aligned} \phi(t|x_0, t_0) &= \phi(t|x_0, t_1) \int_{\mathcal{A}} f(x_1, t_1|x_0, t_0) dx_1 \\ &+ \frac{\partial\phi(t|x_1=x_0, t_1)}{\partial x_1} \int_{\mathcal{A}} (x_1-x_0) f(x_1, t_1|x_0, t_0) dx_1 \\ &+ \frac{1}{2} \frac{\partial^2\phi(t|x_1=x_0, t_1)}{\partial x_1^2} \int_{\mathcal{A}} (x_1-x_0)^2 f(x_1, t_1|x_0, t_0) dx_1 + \dots \end{aligned} \tag{30}$$

The first integral is identically 1 from equation (28) above. We pass to the differential equation by subtracting  $\phi(t|x_0, t_1)$  from both sides of the equation, dividing by  $t_1-t_0$ , and letting  $t_1 \rightarrow t_0$ . We use the infinitesimal moments  $(1/\Delta t)X_{\Delta}^{(i)}$  of the Gaussian diffusion process  $X(t)$  derived and discussed earlier (equations (6)–(8)) to derive

$$\frac{\partial\phi(t, x_0)}{\partial t} = X_{\Delta}^{(1)}(x_0) \frac{\partial\phi(t, x_0)}{\partial x_0} + \frac{1}{2} X_{\Delta}^{(2)} \frac{\partial^2\phi(t, x_0)}{\partial x_0^2}, \tag{31}$$

$$\frac{\partial\phi(t, x_0)}{\partial t} = \mu(x_0) \frac{\partial\phi(t, x_0)}{\partial x_0} + D(x_0) \frac{\partial^2\phi(t, x_0)}{\partial x_0^2}. \tag{32}$$

Equation (32) is of the same form as the ‘backwards’ Fokker–Planck equation (e.g. Gardiner, 1983, p. 128) which can lead the unwary to forget how different it is from the (forward) Fokker–Planck equation presented here (9). The physical and probabilistic meaning of the dependent variables are quite different: equation (32), with an absorbing boundary condition, describes the cumulative probability function (dimensionless) for the first-passage *time* while equation (9) describes the probability density [with respect to *distance*, (cm<sup>-1</sup>)] of the ion concentration. Mathematically, the equations are adjoints in form (Siegert, 1951, p. 621; Coddington & Levinson, 1955, ch. 12) but physically they describe different variables with different meanings and different units, related by equation (27).

### 5.1 *Boundary conditions*

The first-passage time is completely specified by its cumulative distribution function  $\phi(\cdot)$ , which in turn is specified by equation (32) and the boundary conditions required to ensure that the solution of the differential equation is a cumulative probability distribution [always positive, satisfying the conservation relation, equation (25)]. The initial condition is that an ion takes no time to move from  $x_0$  to  $x_0$  or equivalently no ion has been absorbed at time  $t = 0$ :

$$\phi(0, x_0) = 0, \quad \mathcal{R} \leq x_0 \leq \mathcal{A} \text{ is the initial condition.} \tag{33}$$

The conservation relation for  $\phi(\cdot)$  implies that ions must be confined to the system by a ‘reflecting’ boundary condition, a physical boundary condition involving the spatial derivative  $\partial\phi(t, x_0)/\partial x_0$ . If this derivative is zero at  $x_0 = \mathcal{R}$ , the cumulative

probability is at a maximum there (remembering that  $\phi$  is a cumulative probability function and so is monotonic), so the time to absorption is maximal. That time, the first-passage time, is maximal if the boundary does not allow ions to leave the system, if the ions are reflected from the boundary. Thus, one type of reflecting boundary condition is (Goel & Richter-Dyn, 1974, p. 58)

$$\frac{\partial \phi(t, x_0)}{\partial x_0} = 0 \Big|_{x_0 = \mathcal{R}} \text{ is a reflecting boundary condition at } \mathcal{R}. \quad (34)$$

Cox & Miller, 1965 (p. 223) give a more general form of the reflecting boundary condition.

The other boundary condition defines the absorbing barrier at  $x_0 = \mathcal{A}$ :

$$\phi(t, x_0) \Big|_{x_0 = \mathcal{A}} = 1 \quad (t \neq 0). \quad (35)$$

Because the cumulative probability is unity at  $\mathcal{A}$ , an ion starting there, at any time  $t \neq 0$ , is certain to reach  $\mathcal{A}$  immediately, and be absorbed and removed from the system. In the classical theory of tracer fluxes, the absorbing boundary condition is an inhomogeneous Dirichlet condition describing the infinite dilution (or infinitely rapid mixing) of the tracer when it reaches  $x_0 = \mathcal{A}$ .

### 5.2 Moments of first-passage time

Ion conduction through an open channel reaches steady-state long before we can measure it, so ion conduction requires only a steady-state analysis of the first-passage time, which is equivalent [as we have shown, equation (18)] to the mean first-passage time, the first moment  $T^{(1)} \equiv Y$ .

$$Y \equiv T^{(1)}(x_0) \equiv \int_0^\infty t \dot{\phi}(t, x_0) dt. \quad (36)$$

Appendix 2 presents most of the steps involved in determining the mean first-passage time  $Y$  from equation (32), yielding the result

$$Y(\mathcal{A} | x_0, \mathcal{R}) \equiv T^{(1)}(x_0) = \int_{x_0}^{\mathcal{A}} \frac{1}{Q(\chi)} \left[ \int_{\mathcal{A}}^{\chi} \frac{Q(\chi)}{D(\chi)} d\chi \right] dx; \quad (\mathcal{R} \leq x_0 \leq \mathcal{A}), \quad (37)$$

which in general includes three integrals, given the definition of the 'integrating factor' (see Appendix 2)  $Q(\cdot)$ :

$$Q(\chi) \equiv \exp \left[ \int_{\mathcal{A}}^{\chi} \mu(x)/D(x) dx \right]. \quad (38)$$

But

$$\mu(x) \equiv -\frac{D(x)}{kT} \frac{\partial U(x)}{\partial x},$$

so the integrating factor simplifies

$$Q(\chi) = \exp \left\{ - \int_{\mathcal{R}}^{\chi} \frac{1}{kT} \frac{\partial U(x)}{\partial x} dx \right\} = e^{-[U(\chi) - U(\mathcal{R})]/kT}, \quad (39)$$

to an expression with simple physical meaning, namely the (exponential of the) difference in potential energy between the boundary  $\mathcal{R}$  and location  $\chi$ .

If we define our zero of potential by  $U(\mathcal{R}) \equiv 0$ , and let  $D(\cdot)$  be independent of position,

$$Y(\mathcal{A} | x_0, \mathcal{R}) \equiv \frac{1}{k_t(x_0)} = \frac{1}{D} \int_{x_0}^{\mathcal{A}} e^{U(x)/kT} \left( \int_{\mathcal{R}}^x e^{-U(\chi)/kT} d\chi \right) dx. \quad (40)$$

Equation (40) is perhaps the key result of the diffusion theory presented here. It gives the rate constant  $k(x_0 \rightarrow \mathcal{A})$  for an ion moving from position  $x_0$  to  $\mathcal{A}$ , through a potential profile of shape  $U(\chi)$  extending from  $\mathcal{R}$  to  $\mathcal{A}$ , and is directly comparable to the rate constants of Eyring transition state theory. It differs from the Eyring expression because it depends on the entire shape of the potential profile, as one would expect from a theory in which ions diffuse, in which they toddle back and forth across a barrier, instead of hopping to the top in one step.

For some potential functions the integrals reduce to quite simple expressions. In particular, if the potential well (at  $x_1$ ) and barrier (at  $x_2$ ) are both large and locally parabolic, the rate constant for crossing depends asymptotically on the height of the barrier  $\Delta U \equiv U(x_1) - U(x_2)$ , the second (spatial) derivative of the potentials at  $x_1$  and  $x_2$ , namely  $U''(x_1)$  and  $U''(x_2)$ , and no other feature of  $U(x)$  [Kramers, 1940; Schulten *et al.* 1981, equation (1.4); Hänggi, 1986, pp. 112–116].

$$K_k \approx \frac{D}{2\pi kT} \sqrt{|U''(x_1)| |U''(x_2)|} e^{-\Delta U/kT}. \quad (41)$$

This expression is similar in form to, but distinct from the rate constant  $K_{\text{ts}}$  of transition state theory [e.g. Hill, 1960, pp. 194–200, particularly equations (11)–(16); Frauenfelder & Wolynes, 1985, equation (7)] for (ballistic) barrier crossing from an equilibrium distribution in a well

$$K_{\text{ts}} \approx \frac{1}{2\pi} \sqrt{|U''(x_1)/m|} e^{-\Delta U/kT}. \quad (42)$$

The similarity of form in these expressions should not hide the drastically different physical models they describe. The transition state model assumes in essence a gas phase with ballistic crossing of potential barriers, with no significant collisions in the process (see, for example, Frauenfelder & Wolynes, 1985; Hänggi, 1986, pp. 106–109). The diffusion model assumes a liquid phase dominated by a large number of collisions in any time or region of interest, including barrier crossing. Diffusion theory is dominated by frictional, not ballistic processes and is in some sense the opposite pole from transition state theory. Thus, the rate constants of diffusion theory involve the parameters which

determine diffusive motion, the diffusion constant, and the path length for diffusion, determined in the general diffusion model by the entire shape of the barrier (determined by the maximum curvature of the barrier in the Kramers special case), whereas the rate constant of transition state theory involves the parameters which determine ballistic motion, the mass of the ion (not its diffusion constants), and the height of the barrier. The rate constants [equations (41) and (42)] both depend on the shape of the potential well, because ions in both models start in the same equilibrium distribution determined by the well shape.

In the simplified case of equation (41), one may apply diffusion theory directly to state diagrams constructed to fit experimental data from channels, which is a considerable advantage, given the large experimental literature (reviewed in Hille, 1984) and powerful theoretical techniques available from theoretical chemistry (Hill, 1977, 1985) and electrical circuit theory (Sandblom *et al.* 1982). The topology of the models (and inferences that depend only on the topology) are unchanged. One simply can replace  $K_{\text{ts}}$  of state models with  $K_{\text{k}}$ , taking care to remember the different functional dependence of the parameters. The diffusion expression depends on parameters describing Brownian motion over barriers, namely  $D$  and the second derivative of potential  $U''(x_2)$ . These parameters have no effect on  $K_{\text{ts}}$ , and so one may expect more complex behaviour from an ion diffusing over a barrier than from an ion jumping it. Indeed, when barriers are smaller, and the  $K_{\text{k}}$  approximation no longer holds, one can expect still more complex behaviour, with the rate constant reflecting the fine structure of the potential profiles, with finite contributions coming even when  $U''(\cdot)$  is identically zero [see Appendix 7.3.3, Constant field (p. 359)]. Appendix 7.3 gives explicit expressions for the mean first-passage time for a variety of potential functions and starting locations of ions.

## 6. PROBLEMS AND POTENTIALS

This review is only a step in the development of a diffusion model of channel permeation. It tries to acquaint the community of membrane biophysicists with the relevant results of statistical physics, particularly the literature of stochastic diffusion theory. But the only problem confronted here is that of ionic movement across a potential barrier. A theory of ion permeation must describe ionic movement into a channel and it must deal with ionic interactions determining the occupancy of a channel (Jakobsson & Chiu, 1987). These issues will be the subject of future work and publications.

The most serious approximations in our treatment of barrier crossing concern the description of the interaction of ion and channel wall by  $D(x)$  and  $U(x)$ , which are supposed to be functions only of position. Simulations of atomic motions, using nonequilibrium molecular dynamics (Bitsanis *et al.* 1987), show that the  $D(x)$  function is a valid description of frictional phenomena and viscosity in liquids under a surprisingly wide range of conditions. On the other hand, the  $U(x)$  function cannot be so robust. A single  $U(x)$  function, independent of all parameters except location, cannot be expected to describe all channels: for example, one can clearly imagine types of channels in which ionic interactions depended on other

variables, e.g. the velocity of ionic motion. A single  $U(x)$  function should suffice to describe the interaction of ion and channel in many cases of biological interest; for example, a single function would suffice if the conformational changes of the protein occur on time scales distinct from that of ion diffusion and the average (rms) velocity of ions permeating the channel does not vary too much under conditions of experimental and biological importance (i.e. if thermal velocity is much greater than drift velocity).

If a single  $U(x)$  function is enough to describe a channel's interaction with ions, the role of a diffusion theory of permeation expands. The Fokker-Planck equation can then be viewed as a transform, a functional operator, that converts the structural model  $U(x)$  into predictions of the behaviour of channels, in particular, into predictions of current-voltage curves at different concentrations, or current-concentration curves at different membrane potentials. The  $U(x)$  function can then be determined, and the Fokker-Planck equation tested, by fitting its predictions to experimental data describing open channel current under a variety of conditions or by solving the appropriate inverse problem (Attwell & Jack, 1978) using the powerful new techniques of the general theory of inverse problems (Gladwell, 1986) which allowed complete well determined solutions of other problems, at least as difficult (Barcilon, 1986).

Simple modifications of the  $U(x)$  function might also account for selectivity between ions. Selectivity in a diffusion model arises from the (doubly) integrated difference of the exponential of two  $U(x)$  functions, one for each type of ion, and so a highly selective channel could be one with a long region in which  $\Delta U(x)$  was rather small. If  $\Delta U(x)$  for different ions could be rationalized, let alone predicted from independently measured physical properties of the ions, one might develop some confidence in the utility, if not reality, of diffusion theory. Interestingly, a diffusion theory may easily be able to account for the seemingly paradoxically large conductance of channels with very high selectivity, without invoking ion-ion interactions. The double integral [equation (40)] defining the rate constant probably can be made small enough to ensure a large open channel conductance while the corresponding double integral of the difference of two exponentials (responsible for the permeability ratio and conductance difference between two ion types) is also kept large enough to give substantial selectivity.

The path ahead is clear in direction, if not outcome. Before diffusion theory can be applied with confidence to problems of selectivity or to the (inverse) problem of determining the potential profile within a channel, it must be tested experimentally. The theory must be extended to predict the current-voltage relations of many types of channels, measured under many conditions. If it is flexible enough to account for most experimental results, but rigid enough to do so using only a plausible class of  $U(x)$  functions, the theory will have made a significant contribution to our understanding and issues of selectivity and channel structure will be worth pursuing.

Only experiments, suitably analysed, will tell, and then only for one channel at a time.

7. APPENDICES

7.1 Appendix 1. Derivation of the Fokker-Planck equation

To begin, we introduce the characteristic function  $\otimes(\omega, t | x_0, t_0)$  (Parzen, 1960, pp. 215-225, 395-413; Stratonovich, 1963, pp. 8-10) defined by the pair of spatial Fourier transforms, with  $i \equiv \sqrt{-1}$ :

$$\otimes_t(\omega, t | x_0, t_0) = \int_{-\infty}^{\infty} f(x, t | x_0, t_0) e^{i\omega(x-x_0)} dx, \tag{A 1.1}$$

$$f(x, t | x_0, t_0) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \otimes_t(\omega, t | x_0, t_0) e^{-i\omega(x-x_0)} d\omega. \tag{A 1.2}$$

The analysis (following Stratanovitch, 1963, p. 57) uses an expansion of these transforms into the moments of the underlying random variable, a technique widely used to derive partial differential equations of probability functions. When the probability functions depend on more than one independent variable, moments can be taken with respect to each independent variable. Moments (with respect to each independent variable) are often computed after subtraction of some special value of the variable; the dependence on the special value is substantial and must be kept in mind. Finally, moments are sometimes studied in the limit of small increments in variables, like the infinitesimal moments appearing later in the appendix. Each type of moment has its own identity and meaning and must be kept distinct. Confusion is best avoided by treating the word 'moment' as a generic term, and using a specific formula as the definition of the moment of interest.

In this Appendix we start with spatial moments of the random variable  $X^*$  computed from  $f(\cdot)$  around the initial position  $x_0$ .

$$X^{(j)}(x_0, t_0, t) \equiv \int_{-\infty}^{\infty} (x-x_0)^j f(x, t | x_0, t_0) dx. \tag{A 1.3}$$

These moments are global functions in the sense that they depend on the entire domain of space and time; in particular, they depend on the boundary conditions for  $f(x, t | x_0, t_0)$  and so cannot be written explicitly until the corresponding Fokker-Planck equation (with boundary conditions) is solved.

A Taylor expansion of the exponential in equation (A 1.1) gives the standard expansion for the characteristic function

$$\otimes_t(\omega, t | x_0, t_0) = 1 + i\omega X^{(1)}(x_0, t_0, t) + (i\omega)^2 (\frac{1}{2}!) X^{(2)}(x_0, t_0, t) + \dots, \tag{A 1.4}$$

giving the moment expansion for the global transition probability

$$f(x, t | x_0, t_0) = \frac{1}{2\pi} \int_{-\infty}^{\infty} [1 + i\omega X^{(1)} + (i\omega)^2 X^{(2)}/2! + \dots] e^{-i\omega(x-x_0)} d\omega. \tag{A 1.5}$$

We use the Dirac delta function and its derivatives (Papoulis, 1962, pp. 36–43, 269–282; Zauderer, 1983, pp. 365–382) defined implicitly

$$\left. \begin{aligned} g(x) &= \int_{-\infty}^{\infty} g(x_0) \delta(x-x_0) dx_0, \\ \frac{\partial^k g(x)}{\partial x^k} &= (-1)^k \int_{-\infty}^{\infty} \frac{\partial^k \delta(x-x_0)}{\partial x^k} g(x_0) dx_0, \end{aligned} \right\} \quad (\text{A } 1.6)$$

or defined explicitly here [in view of equations (A 1.1) and (A 1.2)] as

$$\left. \begin{aligned} \delta(x-x_0) &= \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\omega(x-x_0)} d\omega, \\ \frac{\partial^k \delta(x-x_0)}{\partial x^k} &= \frac{1}{2\pi} \int_{-\infty}^{\infty} (-i\omega)^k e^{-i\omega(x-x_0)} d\omega. \end{aligned} \right\} \quad (\text{A } 1.7)$$

Moments are introduced into the fundamental equation (5) by substituting from equation (A 1.5). The terms  $(-i\omega)^k$  can be rewritten using the explicit definition of the delta function (A 1.7). Then, remembering that the moments are not functions of  $\omega$ , we write

$$\begin{aligned} p(x, t) &= \int_{-\infty}^{\infty} p(x_0, t_0) \delta(x-x_0) dx_0 - \int_{-\infty}^{\infty} p(x_0, t_0) X^{(1)}(x_0, t_0, t) \\ &\times \frac{\partial}{\partial x} [\delta(x-x_0)] dx_0 + \frac{1}{2!} \int_{-\infty}^{\infty} p(x_0, t_0) X^{(2)}(x_0, t_0, t) \frac{\partial^2}{\partial x^2} [\delta(x-x_0)] dx_0 - \dots \quad (\text{A } 1.8) \end{aligned}$$

Since  $p(\cdot)$  and  $X^{(k)}(\cdot)$  in the integrand are not functions of the final position  $x$ , we can interchange the order of integration (with respect to  $x_0$ ) and differentiation (with respect to  $x$ ). We then introduce and divide by  $\Delta t \equiv t - t_0$  (in anticipation of passage to the limit) and use the implicit definition of the delta function (A 1.6) to derive

$$\frac{p(x, t + \Delta t) - p(x, t)}{\Delta t} = -\frac{\partial}{\partial x} \left[ p(x, t) \frac{X^{(1)}(x, t, \Delta t)}{\Delta t} \right] + \frac{1}{2!} \frac{\partial^2}{\partial x^2} \left[ p(x, t) \frac{X^{(2)}(x, t, \Delta t)}{\Delta t} \right] - \dots \quad (\text{A } 1.9)$$

This Kramers–Moyal expansion (Gardiner, 1983, pp. 246–249; Risken, 1984, pp. 67–81) is an infinite sum of indefinite convergence and a partial differential equation of undefined order and validity (van Kampen, 1981), which has nonetheless a central role in stochastic physics.

The expansion (A 1.9) is usually used in the restricted case  $\Delta t \rightarrow 0$ ,  $x \rightarrow x_0$  in which the global transition probability  $f(x, t | x_0, t_0)$  and its moments  $X^{(j)}(x_0, t_0, t)$  become the local transition probability  $f_{\Delta}(x, t_0 + \Delta t | x_0, t_0)$  and its ‘infinitesimal’ moments, here called  $X_{\Delta}^{(j)}(x_0, \Delta t)$ . These moments depend only on the microscopic mechanism of motion from point to point (e.g. local electro-diffusion in our case, as described by equation (2). They do not depend on the boundary conditions. The infinitesimal moments are determined (Papoulis, 1984, p. 115)



from the derivatives (with respect to  $\omega$ ) of the characteristic function  $\otimes_{\Delta t}$  of equation (2), the Gaussian with drift

$$\otimes_{\Delta t}(\omega, \Delta t) = \exp [D\Delta t(j\omega)^2 + (j\omega) \mu\Delta t] \tag{A 1.10}$$

as described in the text.

**7.2. Appendix 2. Moments of the first-passage time : differential equation and solutions**

**7.2.1 Moments**

When studying the first-passage time, we use moments with respect to time  $T^{(j)}$  (units in  $s^j$ ) of the first-passage time probability density namely,

$$T^{(j)}(x_0) \equiv \int_0^\infty t^j \dot{\phi}(t, x_0) dt, \tag{A 2.1}$$

where we remember that  $\phi(t, x_0)$  is a cumulative probability function : thus its time derivative  $\dot{\phi}(t, x_0)$  is the probability density function ( $s^{-1}$ ) of the first-passage time. A differential equation for the moments is derived, following many earlier authors, by differentiating equation (32) with respect to  $t$ ; multiplying by  $t^j$  and integrating; and interchanging the order of integration (with respect to  $t$ ) and differentiation (with respect to  $x_0$ )

$$\int_0^\infty t^j \frac{\partial \dot{\phi}(t, x_0)}{\partial t} dt = \mu(x_0) \frac{\partial}{\partial x_0} \left[ \int_0^\infty t^j \dot{\phi}(t, x_0) dt \right] + D(x_0) \frac{\partial^2}{\partial x_0^2} \left[ \int_0^\infty t^j \dot{\phi}(t, x_0) dt \right]. \tag{A 2.2}$$

The integral on the right of equation (A 2.2) is obviously the  $j$ th moment as defined by equation (A 2.1). The left hand side can be expressed as a moment after integrating by parts:

$$\int_0^\infty t^j \frac{\partial \dot{\phi}(t, x_0)}{\partial t} dt = -t^j \dot{\phi}(0, x_0) + t^j \dot{\phi}(\infty, x_0) - \int_0^\infty j t^{j-1} \dot{\phi}(t, x_0) dt. \tag{A 2.3}$$

The first term on the right hand side is zero because  $\dot{\phi}(t, x_0)$  is the probability density function of  $t^*(x_0)$  and so goes to zero  $t \rightarrow 0$ . The second term is zero because  $\dot{\phi}(t, x_0) \rightarrow 0$  as  $t \rightarrow \infty$ . (Note that  $t^j \dot{\phi}(t, x_0)$  remains finite as  $t \rightarrow \infty$ , because it is the integrand of the  $j$ th moment  $T^{(j)}$ , which is finite for all reasonable distribution functions.) The third term is just  $-jT^{(j-1)}$ , so we have the recurrence relation

$$D(x_0) \frac{\partial^2 T^{(j)}}{\partial x_0^2} + \mu(x_0) \frac{\partial T^{(j)}}{\partial x_0} = -jT^{(j-1)}. \tag{A 2.4}$$

Equation (A 2.4) is a set of (recursive) ordinary differential equations for the moments, the partial derivative sign  $\partial$  being used only to emphasize that all physical parameters are constant during the differentiation. Because  $T^{(j)}$  appears only as a derivative in equation (A 2.4), a simple change in variable yields a first-order inhomogeneous linear ordinary differential equation. The equations can be

solved starting with  $T^{(1)}$ .  $T^{(1)} \equiv 1$  Because the integral (A 2.1) for  $j = 0$  is just the normalization condition for the density function  $\phi$ . Higher order equations can be solved one at a time, i.e., recursively and not simultaneously, because  $T^{(j-1)}$  is a known function when the equation for  $T^{(j)}$  is examined. For example,

$$D(x_0) \frac{\partial^2 T^{(2)}}{\partial x_0^2} + \mu(x_0) \frac{\partial T^{(2)}}{\partial x_0} = -1. \tag{A 2.5}$$

The explicit solution for equation (A 2.5) requires two boundary conditions for the moments, because it is a second order differential equation. The boundary conditions for the moments are derived from the boundary conditions for the probability density function  $\phi$  at a reflecting or absorbing boundary, equations (34) and (35) (see Goel & Richter-Dyn, 1974, p. 58; Weiss, 1966).

$$\left. \begin{aligned} \text{Reflecting boundary, at } \mathcal{R}: 0 &= \left. \frac{\partial T^{(j)}(\mathcal{R}, x_0)}{\partial x_0} \right|_{x_0=\mathcal{R}}, \\ \text{Absorbing boundary, at } \mathcal{A}: 0 &= T^{(j)}(\mathcal{A}, \mathcal{A}). \end{aligned} \right\} \tag{A 2.6}$$

**7.2.2 Explicit representation of mean first-passage time**

The expression for the mean first-passage time (and thus the rate constant of barrier crossing) involves three different integrals of deceptively similar appearance, integrals central to diffusion models of membrane permeation (e.g. Levitt, 1986).

The first integral arises in an integrating factor defined in text equation (38), which assumed a simple form in a channel with a diffusion constant and/or electric field independent of position (see text). The moments of the first-passage time with  $\partial T^{(j)}/\partial x_0 = 0$  at  $x_0 = \mathcal{R}$  are the solution of a first order linear differential equation (see the unusual and excellent presentation in Apostol, 1967, pp. 308-313)

$$\frac{\partial T^{(j)}}{\partial x_0} = -\frac{1}{Q(x_0)} \int_{\mathcal{R}}^{x_0} \frac{Q(\chi)}{D(\chi)} j T^{(j-1)} d\chi. \tag{A 2.7}$$

Then, the solution of  $T^{(j)}(x_0)$  of equation (A 2.4) with the absorbing condition  $T^{(j)} = 0$  at the position  $x_0 = \mathcal{A}$  is

$$T^{(j)}(x_0) = j \int_{x_0}^{\mathcal{A}} \frac{1}{Q(\chi)} \left[ \int_{\mathcal{R}}^{\chi} \frac{Q(\chi)}{D(\chi)} T^{(j-1)} d\chi \right] d\chi \quad (\mathcal{R} \leq x_0 \leq \mathcal{A}). \tag{A 2.8}$$

The mean first-passage time for an ion starting at  $x_0$ , absorbed at  $\mathcal{A}$ , with a reflecting boundary at  $\mathcal{R}$  is then, remembering that  $T^{(1)} \equiv 1$ ,

$$Y(\mathcal{A} | x_0, \mathcal{R}) \equiv T^{(1)}(x_0) = \int_{x_0}^{\mathcal{A}} \frac{1}{Q(\chi)} \left[ \int_{\mathcal{R}}^{\chi} \frac{Q(\chi)}{D(\chi)} d\chi \right] dx \quad (\mathcal{R} \leq x_0 \leq \mathcal{A}). \tag{A 2.9}$$

which in general includes three integrals, given the definition of  $Q(\cdot)$ , equation (38). Equation (A 2.9) can be significantly simplified, see text.

7.3 Appendix 3. Examples of first-passage times

7.3.1 Equilibrium initial distribution

If ions are initially spread out in space, the mean time of first-passage depends on the initial distribution. Consider, for example, ions starting from a (normalized) equilibrium Boltzmann distribution  $p_{\text{eq}}(x_0)$  between  $\mathcal{R}$  and  $\mathcal{A}$  with probability density

$$p_{\text{eq}}(x_0) = \frac{e^{-\beta U(x_0)}}{\int_{\mathcal{R}} e^{-\beta U(x_0)} dx_0} \quad \text{at } t = 0. \tag{A 3.1}$$

The mean first-passage time is determined by integrating over the source location  $x_0$  in equation (38) with source function (A 3.1), because equation (38) is the Green's function (Coddington & Levinson, 1955, p. 192) for the differential equation (A 2.5),

$$Y(\mathcal{A} | p_{\text{eq}}(x_0)) = \int_{\mathcal{R}} p_{\text{eq}}(x_0) Y(\mathcal{A} | x_0, \mathcal{R}) dx_0, \tag{A 3.2}$$

$$= \frac{\int_{\mathcal{R}} e^{-\beta U(x_0)} dx_0 \int_{x_0}^{\mathcal{A}} e^{\beta U(x)} dx \int_{\mathcal{R}} e^{-\beta U(x)} d\chi}{D \int_{\mathcal{R}} e^{-\beta U(x_0)} dx_0}. \tag{A 3.3}$$

The notation used here in the numerator means the following: first integrate with respect to  $\chi$ , giving a function of  $x$  (and  $\mathcal{R}$ ) as a result. Then, integrate with respect to  $x$ , giving a function of  $x_0$  (and  $\mathcal{R}$  and  $\mathcal{A}$ ) as a result. Finally, integrate with respect to  $x_0$ , giving a function of  $\mathcal{R}$  and  $\mathcal{A}$  as the result, the value of the entire numerator. Following this plan, we write

$$\text{numerator} = \int_{\mathcal{R}} \left[ e^{-\beta U(x_0)} \int_{x_0}^{\mathcal{A}} e^{\beta U(x)} \mathcal{J}(x) dx \right] dx_0, \tag{A 3.4}$$

where

$$\mathcal{J}(x) \equiv \int_{\mathcal{R}} e^{-\beta U(\chi)} d\chi. \tag{A 3.5}$$

The double integral in the numerator can be simplified to a single integral as described in Courant, 1936, p. 245, using a technique of considerable power, needed quite often in the application of diffusion theory (and so presented in detail here). The double integral is a surface integral, with area element  $dS \equiv dx dx_0$ , computed over a triangular region TRI (see Fig. (A 3.1)) extending along the horizontal  $x$  axis from  $x = \mathcal{R}$  to  $x = \mathcal{A}$  and vertically along the  $x_0$  axis from  $x_0 = \mathcal{R}$  to  $x_0 = \mathcal{A}$ , with vertices at the set of points  $\{x_0, x\} = \{\mathcal{R}, \mathcal{R}\}, \{\mathcal{R}, \mathcal{A}\}, \{\mathcal{A}, \mathcal{A}\}$ :

$$\text{numerator} = \iint_{\text{TRI}} e^{-\beta U(x_0)} e^{\beta U(x)} \mathcal{J}(x) dS. \tag{A 3.6}$$

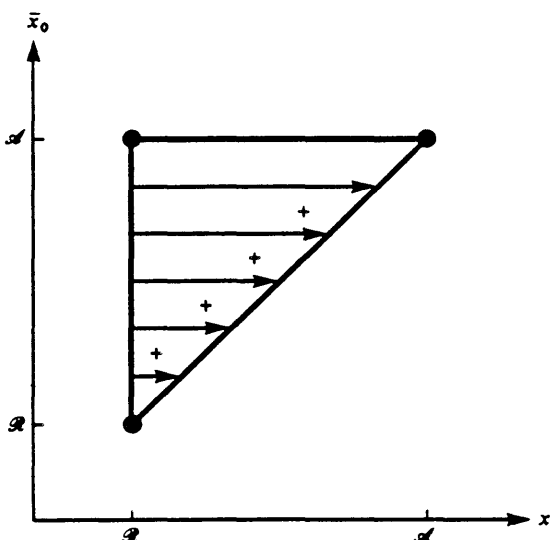


Fig. A 3.1. The integration method for equation A 3.6: the surface integral is over the triangular domain shown. The integral can be performed by two iterated one-dimensional integrations. The first integration is horizontal and is represented by the horizontal arrow. The second integration sums the results of the first: it is the vertical sum of the horizontal arrows and is symbolized by the + signs in the figure. Further details of the integration can be found in the text.

This area integral can be evaluated by repeated one dimensional integrations in which the horizontal integration (with  $x$  ranging from  $x_0$  to  $A$ ) is done first, followed by the vertical integration (with  $x_0$  ranging from  $R$  to  $A$ ), as in equation (A 3.3) and (A 3.4); or the surface integral can be evaluated by repeated integrations in which the vertical integration (with  $x_0$  ranging from  $R$  to  $x_0$ ) is done first, followed by the horizontal integration (with  $x$  ranging from  $R$  to  $A$ ):

$$\text{numerator} = \int_A^A \left[ e^{\beta U(x)} \mathcal{J}(x) \left\{ \int_A^x e^{-\beta U(x_0)} dx_0 \right\} \right] dx. \tag{A 3.7}$$

Now, the integral over  $x_0$  is simply  $\mathcal{J}(x)$  because the name of the dummy variable of integration is immaterial, so we can write equation (A 3.3) as

$$Y(A | p_{\text{eq}}(x_0)) = \frac{\frac{1}{D} \int_A^A e^{\beta U(x)} \left[ \int_A^x e^{-\beta U(\chi)} d\chi \right]^2 dx}{\int_A^A e^{-\beta U(\chi_0)} d\chi_0}. \tag{A 3.8}$$

Equation (A 3.8) is much easier to compute than equation (A 3.3) because it replaces a double integration with a single integration and multiplication!

### 7.3.2 'Pure' diffusion

We consider the diffusion of a solute which does not interact with the channel, that is to say, the case  $U = \text{constant}$ . In that case, an 'ion' starting from  $x_0$  first reaches  $\mathcal{A}$  at a mean time of

$$Y(x_0, \mathcal{A}) = \int_{x_0}^{\mathcal{A}} \left[ \int_{\mathcal{A}}^x dx \right] = \frac{1}{2D} [\mathcal{A}^2 - x_0^2] - \frac{\mathcal{R}}{D} [\mathcal{A} - x_0] = \frac{(\mathcal{A} - x_0)(\mathcal{A} + x_0 - 2\mathcal{R})}{2D}. \quad (\text{A } 3.9)$$

If the absorbing boundary is at  $\mathcal{A} = 0$ , but the reflecting boundary is anywhere and the ion starts anywhere between the boundaries, we recover Berg's result [1983, eq. (3.13), p. 44]  $Y = (2\mathcal{R}x_0 - x_0^2)/(2D)$ . If the absorbing boundary is anywhere, but the ion starts its motion at the reflecting boundary at  $\mathcal{R} = x_0 = c$ , we have the familiar formula  $Y = \mathcal{A}^2/2D$ .

Uncharged particles starting from their equilibrium distribution  $p_{\text{eq}}(x_0) = 1/(\mathcal{A} - \mathcal{R})$  have as their mean first-passage time

$$Y(\mathcal{A} | p_{\text{eq}}(x_0)) = \frac{1}{D(\mathcal{A} - \mathcal{R})} \int_{\mathcal{A}}^{\mathcal{A}} \left[ \int_{\mathcal{A}}^x d\chi \right]^2 dx = \frac{1}{3D} [\mathcal{A} - \mathcal{R}]^2. \quad (\text{A } 3.10)$$

### 7.3.3 Constant field

The potential in a uniform electric field is defined here by

$$\beta U(x) = mx + b \equiv \beta U_{\text{off}} + \beta \left[ \frac{x - \mathcal{R}}{\mathcal{A} - \mathcal{R}} \right] \Delta U, \quad (\text{A } 3.11)$$

where  $\beta \equiv 1/kT$ ,  $m$  and  $b$  are defined in the above expression,  $U_{\text{off}}$  is any offset potential, and  $\Delta U$  is the transmembrane potential, i.e. the potential at one side of the region minus that at the other.

If the ions start at one location  $x_0$ , we have

$$Y = \frac{1}{D} \int_{x_0}^{\mathcal{A}} e^{mx+b} \left[ \int_{x_0}^x e^{-m\chi-b} d\chi \right] dx, \quad (\text{A } 3.12)$$

which becomes

$$Y = \frac{1}{D} \left[ \frac{(\mathcal{A} - \mathcal{R})^2}{\beta \Delta U} \right] \left[ e^{\beta \Delta U} - e^{\beta \Delta U (x_0 - \mathcal{R}) / (\mathcal{A} - \mathcal{R})} - \beta \Delta U \frac{\mathcal{A} - x_0}{\mathcal{A} - \mathcal{R}} \right]. \quad (\text{A } 3.13)$$

Note that the offset potential has no effect on the calculated first-passage time, which is gratifying because we know, from fundamental properties of the electric field, it must not influence the motion of the ion.

If the ions in the constant field start from an equilibrium distribution (in space)  $p_{\text{eq}}$ ,

$$p_{\text{eq}}(x_0) = \frac{e^{-mx_0-b}}{\int_{\mathcal{A}} e^{-mx-b} dx}, \quad (\text{A } 3.14)$$

we eventually derive

$$Y(\mathcal{A} | p_{\text{eq}}(x_0)) = \frac{1}{Dm(e^{-m\mathcal{A}} - e^{-m\mathcal{A}})} \int_{\mathcal{A}} \left[ e^{m(x-2\mathcal{A})} - 2e^{-m\mathcal{A}} + e^{-mx} \right] dx, \quad (\text{A } 3.15)$$

which gives from equation (A 3.11)

$$Y(\mathcal{A} | p_{\text{eq}}(x_0)) = \frac{(\mathcal{A} - \mathcal{R})^2 [e^{\beta\Delta U(\mathcal{A}-2\mathcal{R})/(\mathcal{A}-\mathcal{R})} - e^{-\beta\Delta U\mathcal{A}/(\mathcal{A}-\mathcal{R})} - 2\beta\Delta U e^{-\beta\Delta U\mathcal{R}/(\mathcal{A}-\mathcal{R})}]}{D(\beta\Delta U)^2 [e^{-\beta\Delta U\mathcal{R}/(\mathcal{A}-\mathcal{R})} - e^{-\beta\Delta U\mathcal{A}/(\mathcal{A}-\mathcal{R})}]} \quad (\text{A } 3.16)$$

If the reflecting boundary is at  $\mathcal{R} = 0$ ,

$$Y(\mathcal{A} | p_{\text{eq}}(x_0)) = \left. \begin{aligned} & \frac{\mathcal{A}^2}{(\Delta U)^2} \frac{e^{\beta\Delta U} - e^{-\beta\Delta U} - 2\beta\Delta U}{1 - e^{-\beta\Delta U}}, \\ & = \frac{2\mathcal{A}^2 \sinh(\beta\Delta U) - \beta\Delta U}{D(\beta\Delta U)^2 [1 - e^{-\beta\Delta U}]} \end{aligned} \right\} \quad (\text{A } 3.17)$$

## 8. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of Eric Jakobsson to this work. He introduced us to the stochastic approach to ion permeation and to each other.

It is a pleasure to thank Stephen Hladky for his most thoughtful and perceptive comments, which helped us correct and refine the paper. The paper was also enriched by the suggestions of Olaf Andersen, Fred Cohen, Lane Niles, Eduardo Rios, and the referees: we are grateful for their help.

Support was generously provided by the NIH (a research grant from the National Institute of General Medical Sciences and a training grant from the National Heart, Lung, and Blood Institute) and the Muscular Dystrophy Association.

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