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ION TRANSPORT IN THE SIMPLEST SINGLE FILE PORE

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Summary

A kinetic scheme is developed to describe single-file transport through pores containing up to two ions which may be of different species. The solution for the fluxes in terms of rate constants for entry, exit, and transfer is derived without specific assumptions about symmetry or the voltage and activity dependence of the constants. For a symmetrical pore the relation between the slope conductance at zero applied potential and ion activity can have two distinct regions in which the conductance increases linearly. Zero current or reversal potentials depend on the absolute values of the activities as well as their ratios. The use of this theory to describe the cation fluxes through the pores formed by gramicidin A will be considered in a subsequent paper. Here the model is discussed for a number of more specific assumptions, most extensively the following combination: (1) while entry to a pore is less likely when the pore is already occupied at the opposite end, this entry is still rapid; (2) exit is much more rapid when the pore is occupied by two ions; and (3) transfer from one end to the other of a singly occupied pore is rapid. With these assumptions and for a range of concentrations over which the fluxes are proportional to ion activities, the model predicts a flux ratio exponent nearly equal to 2, blocking by impermeant ions, rectification due to blocking particles on one side only, relief of block by increase in the permeant ion concentration on the opposite side, and anomalous variations of the conductance and zero current potential with mole ratio when the total concentration of the two permeants is held constant.

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Introduction

This paper describes the simplest plausible model which allows consideration of interaction between ions within a pore and single-filing. Because the model is kept simple, it can only suggest qualitative explanations for the behaviour of the sodium and potassium channels of nerve. It is intended, however, to describe quantitatively the simpler pores formed by gramicidin A. Urry and coworkers [1,2] have proposed that these pores are formed from two gramicidin monomers each coiled in a helix and linked head-to-head via three hydrogen bonds. Their structure satisfies the requirements deduced [3,4] from kinetic data that the portion exposed to the core of the membrane is hydrophobic while the ends and the hole running between them are hydrophilic. The hole is large enough to accept the alkali cations but small enough to stop tetramethylammonium ions. Water permeability measurements [5] have confirmed the earlier inferences [4] that a chain of water molecules extends through the hole.

The single-channel conductances for the alkali cations parallel their aqueous solution mobilities, but no clear evidence has been found that anions or multivalent cations pass through the channel [4,6–8]. The relative exclusion of anions suggests that the electrostatic potential inside the pore is 60 mV or more negative with respect to the aqueous phases [6]. In gramicidin only the >C=O and >N-H groups of the peptide backbone are hydrophilic and thus a series of these must line the pore. A negative potential would arise if the carbonyl oxygens protruded slightly further into the pore than the amino hydrogens. The failure of divalent cations to permeate may be a consequence of a high potential energy barrier which they encounter in the middle of the pore. Parsegian [9] has estimated that the attraction of a monovalent ion towards the water makes the centre less favourable than the ends by an energy several times the average thermal energy of an ion, kT, and that this energy increases with the square of the ionic charge. While divalent cations do not contribute to the conductance, they do interfere with the transport of monovalent cations [8,10,11].

Neither from the structure proposed for gramicidin nor from the flux data is there any reason to postulate a small number of specific binding sites. However, electrostatic calculations [9,12] suggest that ions will rarely be in the middle of the pore. Therefore it seems reasonable to assume that the pore can only exist in a small number of states — e.g. empty (i.e. occupied only by water), one ion on the left, or one each on the left and right. The kinetic model therefore has two sites or regions, each of which can be empty or occupied by an ion. Consequently the pore can be in any of nine states as indicated in Fig. 1. Each transition between states occurs at a rate equal to the product of a rate constant and the probability of the pore being in the initial state. Knock-on reactions [13–16] in which an ion can enter a region already occupied thereby displacing the occupant are assumed not to occur.

Heckmann [17–19] has treated a pore containing at most two molecules of either of two identical but still distinguishable neutral species. With these restrictions there is a straightforward choice of three rate constants which gives rise to relatively simple flux equations. For ions the equations are unavoidably

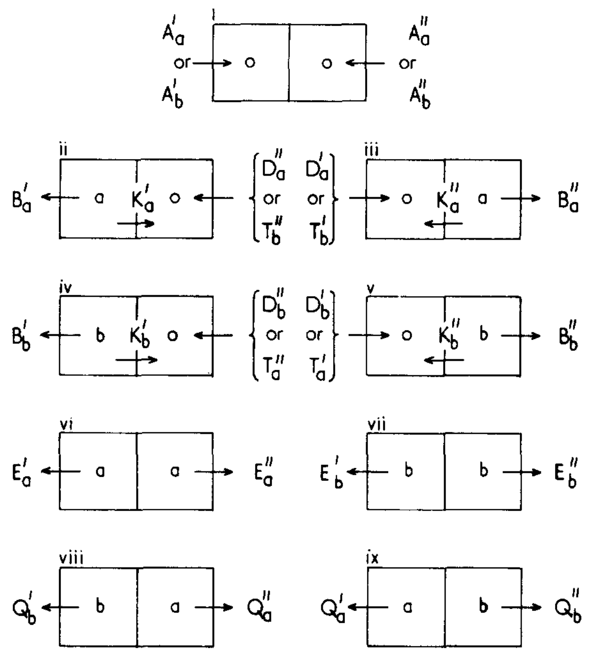

Fig. 1. The nine states of the two ion pore. Each arrow represents a transition which can occur when the pore is initially in that state. Thus for a pore in state ob the ion can leave, rate constant B''_b, or move to the other end, K''_b, or another ion can enter the opposite end, rate constant D'_b if the second ion is of species b, T'_a if it is of species a. After any one of these transitions the pore is in a new state, e.g. if an ion of species a enters a pore in state ob the new state is ab. In the steady-state, the number of transitions occurring per unit time is just the product of the rate constant and the probability the pore was in the original state, e.g. transitions from ba to bo occur at a rate $Q''_a P_{ba}$.

more complex since the potential applied across the membrane introduces an asymmetry in the forward and backward rate constants. In addition, if the model is to deal with ions and pores filled with water, the equations must allow for long range interaction between the ions. This possibility is introduced into the model by using different entrance and exit rate constants if there is another ion in the pore. Since ions of different species may bind at different places and their movements may be limited at different positions, these constants may depend on the species of both ions.

A number of partial solutions to the equations which describe this model have already been given. Zwolinski et al. [20] derived the flux relations for an *n*-site channel which is rarely occupied and Lauger [21] extended the treatment to a pore which is never more than singly occupied. Flux equations for a two site pore and a single species of ion have been derived by Hill and Chen [22] who assumed that the binding constants for the first and second ions are equal and by Levitt [23] who assumed that only the rate constants for transfer depend on the applied potential. In his treatment of biionic potentials [23] Levitt assumes that ions cannot enter pores already occupied by an ion of another species. Markin and Chizmadjev [24] and Chizmadjev and Aityan [25] have derived equations with particular emphasis on the special case, not considered here, in which transfer through the pore is assumed to be slow. Aityan et al. [26] have treated doubly occupied pores in which transfer is so rapid that the rare vacancies which do occur always equilibrate between the two ends *. In this limit they found that the net flux is independent of ion activity (cf. Eqn. 24) while the flux ratio is the square of that expected from independence (cf. Eqn. 42). They also derived an expression for the zero current potential using reasonable but arbitrary assumptions for the potential dependence of the rate constants. Finally Hille [27,28] and Hille and Schwarz [29] have compared predictions from this model with data obtained for the potassium channels of nerve, muscle and eggs. Ref. 29 contains computer-generated examples of many of the effects described here.

In this paper we present the complete solution for the fluxes with two ion species present and arbitrary voltage and activity dependence of the rate constants. A number of specific examples are then considered in order to illustrate interesting behaviour exhibited by this type of pore. Some of the results from Sections II, III.1 and III.2 have received limited circulation in Refs. 8 and 10. The application of this model to the kinetic data for gramicidin has been considered by Hladky and Haydon [4]; Hladky [10,30]; Hladky et al. [31]; Levitt [23]; Urban et al. [7]; and Urban [8]. A thorough numerical analysis of our results will be presented in a subsequent paper.

II. Development of the model

II.1 Notation and the formal flux equations

Rate constants, probabilities, and auxiliary mathematical functions are denoted by capital letters followed by indices. The probability for the pore to be in any particular state is denoted by P_{xy}. The subscripts x and y indicate whether the first (left) and second (right) sites are empty (=o) or occupied by an ion of species a or species b. Permeabilities will be denoted by an italic capital *P* and have only a single index.

Transitions from one state to another are described by the rate constants A_x^i, B_x^i, K_x^i, D_x^i, T_x^i, E_x^i and Q_x^i (see Fig. 1). The subscript refers to the ion species involved; the superscript to whether it is a transition to or from the first site (′) or second site (″). A_x^i (D_x^i or T_x^i) represents ion movement from the outside compartment into the empty (or singly occupied) pore, B_x^i (E_x^i or Q_x^i) the rate at which ions leave the singly (or doubly) occupied pore. D_x^i and E_x^i apply if the second ion in the pore belongs to the same species, T_x^i and Q_x^i if not. K_x^i describes the transfer of the ion x from the site i of the pore to the other site. With two ions in the pore exchange of sites is assumed to be impossible.

In the steady state the current through the pore may be calculated as the net current from one site to the other:

$$I = e[z_a J_a + z_b J_b] \tag{1}$$

$$= e[z_a(K_a' P_{ao} - K_a'' P_{oa}) + z_b(K_b' P_{bo} - K_b'' P_{ob})] \tag{2}$$

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* As Aityan et al. appreciated, the assumptions they required are less stringent than those they stated. In the present notation, their first and third approximations are minor variations of a single approximation with $K_a, K_b >> A_a, A_b > D_a, D_b >> E_a, E_b > B_a, B_b$. In their equation 14, the factor $K_2^A K_2^B$ is missing from the numerator. Their second approximation allows only single occupancy.

where z_a and z_b are the respective valencies and e is the charge on a proton. The solution (Eqns. 3—8) for the fluxes and the current has been derived by standard means (see Appendix). It was found convenient [8] to define indexed auxiliary functions, as these help to display the formal symmetry which exists both with respect to ion species and sites.

$$I = e[z_a(K'_a P_{ao} - K''_a P_{oa}) + z_b(K'_b P_{bo} - K''_b P_{ob})] \tag{3}$$

$$P_{oo} = D/DD \quad P_{ao} = N''_a/DD \quad P_{oa} = N'_a/DD \quad P_{bo} = N''_b/DD \quad P_{ob} = N'_b/DD \tag{4 a—d}$$

$$\begin{aligned} N^i_x = &-(A^j_x G^j_x + A^i_x F^i_x)(F^j_y F^i_y - G^j_y G^i_y) + G^j_x H^j_x(A^j_y F^j_y + A^i_y G^i_y) \\ &+ F^i_x H^i_x(A^j_y G^j_y + A^i_y F^i_y) + A^i_x H^j_x H^j_y F^j_y - A^j_x H^i_x H^j_y G^j_y - A^i_y H^j_y H^j_x H^i_x \end{aligned} \tag{5}$$

$$\begin{aligned} D = &(F'_a F''_a - G'_a G''_a)(F'_b F''_b - G'_b G''_b) - F'_a F'_b H'_a H'_b - F''_a F''_b H''_a H''_b - G'_a G''_b H''_b H'_a \\ &- G''_a G'_b H'_b H''_a + H'_a H'_b H''_a H''_b \end{aligned} \tag{6}$$

$$DD = D + M'_a \times N'_b + M''_a \times N''_b + M'_b \times N'_a + M''_b \times N''_a \tag{7}$$

$$F^i_x = \left(K^i_x + \frac{E^i_x D^j_x}{E^i_x + E^j_x}\right) \qquad M^i_x = \left(1 + \frac{T^i_x}{Q^i_x + Q^j_y} + \frac{D^i_y}{E^i_y + E^j_y}\right)$$

$$G^i_x = \left(\frac{D^i_x E^j_x}{E^i_x + E^j_x} + \frac{T^i_y Q^j_x}{Q^i_y + Q^j_x} + B^j_x + K^j_x\right) \qquad H^i_x = \frac{T^i_x Q^j_y}{Q^j_y + Q^i_x} \tag{8a—d}$$

The remainder of this paper will be devoted to presentation of simpler formulae. which apply in special cases and discussion of their interpretation.

If only one species of ion is present which can enter the pore, the formula for the current (Eqn. 3) takes a much simpler form. Omitting the index referring to the ion species [10],

$$\begin{aligned} I = ze \times &\left[K' \times \left(\frac{A'B''}{A' + A''} + \frac{D'E''}{E' + E''}\right) - K'' \times \left(\frac{A''B'}{A' + A''} + \frac{D''E'}{E' + E''}\right)\right] \\ &\times \left[\left(\frac{A''B'}{A' + A''} + \frac{D''E''}{E' + E''} + K'\right)\left(1 + \frac{B''}{A' + A''} + \frac{D'}{E' + E''}\right)\right. \\ &\left. + \left(\frac{A'B''}{A' + A''} + \frac{D'E''}{E' + E''} + K''\right)\left(1 + \frac{B'}{A' + A''} + \frac{D''}{E' + E''}\right)\right]^{-1}. \end{aligned} \tag{9}$$

In a biionic experiment where there is a single salt on one side of the membrane and a different one with $z_b = z_a$ on the other side, the zero current condition is of comparable simplicity [8,10]:

$$\begin{aligned} &K'_a\left(B''_a + \frac{D'_a E''_a}{E'_a + E''_a}\right) \times \left[\left(\frac{A'_a B''_b}{A'_a + A''_b} + \frac{T'_a Q''_b}{Q'_a + Q''_b}\right)\right. \\ &\quad \left. \times \left(Kb' + B'_b + \frac{D''_b E'_b}{E'_b + E''_b}\right) + K''_b \frac{A'_a B'_b}{A'_a + A''_b}\right] \\ &- K''_b\left(B'_b + \frac{D''_b E'_b}{E'_b + E''_b}\right) \times \left[\left(\frac{A''_b B'_a}{A'_a + A''_b} + \frac{T''_b Q'_a}{Q''_b + Q'_a}\right)\right. \\ &\quad \left. \times \left(K''_a + B''_a + \frac{D'_a E''_a}{E'_a + E''_a}\right) + K'_a \frac{A''_b B''_a}{A''_b + A'_a}\right] = 0. \end{aligned} \tag{10}$$

The potential enters this equation implicitly via the rate constants (see Section II.3).

For $z_a = z_b$, it is customary to define the permeability ratio, P_b/P_a, via

$$V_0 = [kT/(z_a e)] \times \ln \frac{a'_a + a'_b(P_b/P_a)}{a''_a + a''_b(P_b/P_a)} \tag{11}$$

where P_a and P_b are permeability coefficients, k is the Boltzmann constant, T is the absolute temperature, and V_0 is the zero current potential (right minus left). This definition was originally based on the Goldman-Hodgkin-Katz equation [32,33] in which P_a and P_b are true constants each equal at zero applied potential to the flux of the corresponding species divided by its concentration difference. More generally if the fluxes are independent, the permeability ratio may vary with potential, but for a given potential it will be independent of the ion activities and the individual permeabilities will still correspond to the basic definition [34]. In the present model, the fluxes are not independent except at very low activities, the permeability ratios depend on both the potential and the activities, and the individual permeabilities may not bear any relation to the ratios of fluxes to concentration differences. Nevertheless in order to facilitate comparison with data in the literature, the predictions of the model will still be discussed in terms of permeability ratios defined by Eqn. 11. The observed zero current potential can always be calculated from the reported ratio and the activities.

Unidirectional fluxes, $J\rightarrow$ and $J\leftarrow$, for a single species present at the same concentration on both sides may be calculated as the fluxes J_a and J_b of two identical species with

$$a'_a = a''_b = a_a \text{ and } a''_a = a'_b = 0. \tag{12}$$

The flux ratio, $J\rightarrow/J\leftarrow$, then equals the ratio of the terms in Eqn. 10 and similarly for small potentials the ratio of the conductance to the unidirectional fluxes is

$$\begin{aligned} G/J\rightarrow &= -(e/V)(J\rightarrow - J\leftarrow)/J\rightarrow = -(e/V) \\ &\times \Bigg\{(A' + A'')\Bigg[\Bigg[\left(\frac{D'E''}{E' + E''} + B'' + K''\right)\left(\frac{D''E'}{E' + E''} + B' + K'\right) - K'K''\Bigg] \\ &\times \Bigg[K'\left(\frac{D'E''}{E' + E''} + \frac{A'B''}{A' + A''}\right) - K''\left(\frac{D''E'}{E' + E''} + \frac{A''B'}{A' + A''}\right)\Bigg]\Bigg]\Bigg\} \\ &\times \Bigg\{K'\left(\frac{D'E''}{E' + E''} + B''\right)\Bigg[A'\left(\frac{D'E''}{E' + E''} + B''\right)\left(\frac{D''E'}{E' + E''} + B' + K'\right) \\ &+ A'K''B' + A''\left(\frac{D''E'}{E' + E''} + B' + K'\right)\frac{D'E''}{E' + E''}\Bigg]\Bigg\}^{-1} \end{aligned} \tag{13}$$

II.2 Activity dependence of the rate constants

The rate constants will depend on the ion species involved, their respective concentrations on both sides of the pore, and the voltage applied across the membrane. The simplest assumptions are that for a given loading of the pore the values of B^i_x, K^i_x, and E^i_x do not vary with the concentrations of the ions in the aqueous phases and that the rate constants of entry into the pore take the form:

$$A^i_x = AA^i_x a^i_x, \qquad D^i_x = DD^i_x a^i_x, \qquad \text{and} \quad T^i_x = TT^i_x a^i_x, \tag{14}$$

where AA^i_x, DD^i_x, and TT^i_x are independent of the ion concentrations, a^i_x denotes the activity of species x in compartment i, and TT^i_x is independent of activity, but a function of the species 'y' of the other ion in the channel.

II.3 Voltage dependence of the rate constants

The rate constants may vary in any manner with potential subject only to the restrictions imposed by the principle of microscopic reversibility [35,36]. Together with the assumptions in the preceding section this principle requires (see Appendix):

$$AA'_x B''_x K'_x/(AA''_x B'_x K''_x) = \exp(-z_x\phi),\ x = a \text{ or } b\ , \tag{15}$$

$$DD'_x E''_x K'_x/(DD''_x E'_x K''_x) = \exp(-z_x\phi),\ x = a \text{ or } b, \tag{16}$$

$$(A'_b/B'_b)/(T'_b/Q'_b) = (A''_a/B''_a)/(T''_a/Q''_a), \tag{17}$$

$$(A'_a/B'_a)/(T'_a/Q'_a) = (A''_b/B''_b)/(T''_b/Q''_b), \tag{18}$$

and as a consequence of these

$$\left(\frac{TT'_a Q''_a K'_a}{TT''_a Q'_a K''_a}\right) \times \left(\frac{TT'_b Q''_b K'_b}{TT''_b Q'_b K''_b}\right) = \exp(-z_a\phi) \times \exp(-z_b\phi) \tag{19}$$

with $\phi = Ve/(\boldsymbol{k}T)$.

III. Predictions and Interpretation

III.1 The conductance versus activity for a symmetrical pore

For a symmetrical pore and the same solution on the two sides, the conductance at each activity has been derived from Eqns. A1—A10 by expanding each rate constant and state probability in a power series in V. After discarding terms of order higher than the first and using Eqns. 15—19 to specify the potential dependence of the remaining combinations of constants

$$\begin{aligned} G = \frac{e^2 P_{oo}}{\boldsymbol{k}T} \times \Bigg\{ & z_a^2 \times \frac{A_a}{B_a} K_a \Bigg[\left(D_b + B_b + 2K_b + \frac{T_a Q_b}{Q_a + Q_b}\right)(D_a + B_a) \\ & + \frac{T_b Q_a}{Q_a + Q_b}(D_b + B_b + 2K_b[1 + z_b/z_a])\Bigg] \\ & + z_b^2 \times \frac{A_b}{B_b} K_b \Bigg[\left(D_a + B_a + 2K_a + \frac{T_b Q_a}{Q_a + Q_b}\right)(D_b + B_b) \\ & + \frac{T_a Q_b}{Q_a + Q_b}(D_a + B_a + 2K_a[1 + z_a/z_b])\Bigg]\Bigg\} \\ \times \Bigg\{ & \left(D_a + B_a + 2K_a + \frac{T_b Q_a}{Q_a + Q_b}\right)\left(D_b + B_b + 2K_b + \frac{T_a Q_b}{Q_a + Q_b}\right) \\ & - \frac{T_a T_b Q_a Q_b}{(Q_a + Q_b)(Q_a + Q_b)}\Bigg\}^{-1} \end{aligned} \tag{20}$$

where P_{oo} is given by

$$P_{oo} = 1/[1 + 2A_a/B_a + 2A_b/B_b + A_aD_a/(B_aE_a) + A_bD_b/(B_bE_b) + 2A_aT_b/(B_aQ_b)] \tag{21}$$

and rate constants without a superscript represent the value at zero potential. If all entry and exit rates are fast compared to transfer through the pore, then

$$\begin{aligned} P_{ao} &= P_{oa} = (A_a/B_a)P_{oo}, \\ P_{bo} &= P_{ob} = (A_b/B_b)P_{oo}, \end{aligned} \tag{22}$$

and the conductance formula takes on the simpler form,

$$G = [e^2/(kT)]\,[z_a^2P_{ao}K_a + z_b^2P_{bo}K_b]. \tag{23}$$

The elaborate form of Eqn. 20 compared to Eqn. 23 describes the effect on the flux of the changes in the concentrations which were themselves caused by the flux.

If only one species of ion can enter the pore [10],

$$G = [(ze)^2/(kT)](AK/B)P_{oo}/[1 + 2K/(D + B)] \tag{24}$$

and

$$P_{oo} = 1/[1 + 2A/B + AD/(BE)]. \tag{25}$$

III.2 Fluxes and biionic potentials for various activities

III.2a. Low activities. For a single species present at low concentration, Eqn. 9 simplifies to the well known result [20]

$$J = (A'K'B'' - A''K''B')/(K'B'' + K''B' + B'B''). \tag{26}$$

If the rate of transfer, K, is much slower than the rate of exit, B, then the binding of ions at each end remains near equilibrium with the adjacent solution and the current increase reflects the full potential dependence of the product of the binding constant and the rate constant for transfer. If instead transfer is rapid, then the rate of entry, A*a*, becomes limiting. If this process depends only weakly on potential, the current approaches a constant value at high potentials.

The expression for the biionic permeability ratio, Eqns. 10 and 11, also reduces to a much simpler form using Eqn. 15:

$$\frac{P_b}{P_a} = \frac{AA_b''B_b'K_b''}{AA_a''B_a'K_a''} \times \frac{B_a'K_a'' + B_a''K_a' + B_a'B_a''}{B_b'K_b'' + B_b''K_b' + B_b'B_b''} \tag{27}$$

Thus as required by the independence principle [34,37], the fluxes are linearly dependent on activity, the permeability ratio is independent of activity, and the ratio of the currents (Eqn. 26) at the biionic potential is equal to the permeability ratio.

III.2b. Intermediate activities. If ions in the pore repel each other strongly and transfer in single occupied pores is rapid, it is possible to satisfy

$$B \ll A, \quad B \ll D \ll K \text{ and } D \ll E \tag{28}$$

simultaneously. The flux formula (Eqn. 9) then reduces to

$$J = (D'K'E'' - D''K''E')/[(E' + E'') \times (K' + K'')] \tag{29}$$

Thus when the rate of exit from a singly occupied pore is very slow it is possible to have the flux proportional to activity even though the pore is usually occupied. The dependence on activity occurs since ions now leave the pore as a consequence of the arrival of a second ion. The interaction between the ions is not, however, a knock-on process for the entry of a second ion promotes the departure of either of the ions which are then present. If the rate of entry for second ions is only weakly potential dependent, then for high potentials the flux tends towards a constant value.

From Eqns. 10, 11, and 15—18, $B << A, T$ and $B << D << K$, it follows for the biionic permeability ratio that

$$\frac{P_b}{P_a} = \sqrt{\left(\frac{DD''_b K''_b E'_b}{DD''_a K''_a E'_a}\right)\left(\frac{AA''_b B''_a K'_a}{AA''_a B''_b K'_b}\right)\left(\frac{E'_a + E''_a}{E'_b + E''_b}\right)} \tag{30}$$

which is independent of a'_a and a''_b but different from the value at low activities. Thus a linear flux-activity relation and a constant zero current potential can originate from two physical situations: in one there is always an ion in the pore, in the other the pore is hardly ever occupied. However, in contrast to low activities, when the rate constants satisfy (Eqn. 28), the current ratio is usually not equal to the permeability ratio.

It is instructive to derive Eqn. 30 directly since it illustrates why certain combinations of the constants recur frequently in the equations. When species a is present on the left and b only on the right ($a'_b = a''_a = 0$) and B_a and B_b are both very small, an ion of species b can leave the pore into the left hand solution only when another b has entered from the right i.e. it leaves at a rate, $E'_b\,P_{bb}$. Expressed in another way entry of ions of species b into pores already occupied by another b occurs at rate, $D''_b\,P_{bo}$, and each of these entries results in the first b completing its passage across the membrane with probability, $E'_b/(E'_b + E''_b)$. The remaining proportion of entries, $E''_b/(E'_b + E''_b)$, end with the second ion returning to the right. Thus the flux ratio is

$$\frac{J\rightarrow}{J\leftarrow} = \left(\frac{D'_a E''_a}{E'_a + E''_a}\right) P_{oa} \Big/ \left[\left(\frac{D''_b E'_b}{E'_b + E''_b}\right) P_{bo}\right]. \tag{31}$$

Pores containing only species a can be created from pores containing only species b solely by entry of an a forming state ab and departure of the b rather than the a, i.e. at a rate $T'_a\,Q''_b\,P_{ob}/(Q'_a + Q''_b)$. Similarly pores containing only a's are lost at a rate $T''_b\,Q'_a\,P_{ao}/(Q'_a + Q''_b)$. Since the fraction of pores containing only a's is constant in the steady-state,

$$\frac{T''_b Q'_a}{Q'_a + Q''_b} P_{ao} = \frac{T'_a Q''_b}{Q'_a + Q''_b} P_{ob}. \tag{32}$$

Since the rate of transfer is assumed to be rapid,

$$P_{bo}/P_{ob} = K''_b/K'_b$$

and

$$P_{ao}/P_{oa} = K''_a/K'_a. \tag{33}$$

Thus

$$\frac{J\rightarrow}{J\leftarrow}=\frac{D'_aE''_a}{(E''_a+E'_a)}\,\frac{K'_a}{K''_a}\,\frac{T'_aQ''_b}{T''_bQ'_a}\,\frac{K'_b}{K''_b}\,\frac{(E''_b+E'_b)}{D''_bE'_b} \tag{34}$$

while the zero current condition is just $z_aJ_a + z_bJ_b = 0$ or for $z_a = z_b$

$$J\rightarrow/J\leftarrow = 1. \tag{35}$$

The primary potential dependence can be extracted using the conditions of microscopic reversibility (Eqns. 15—18) which leads to Eqn. 30. In this derivation it has been assumed that $B << A$ and $B << D << K$ but the E's and Q's have not been restricted.

III.2c. High activities. At high activities the flux formula is again simple [29]

$$J=\frac{E''K'}{D''}-\frac{E'K''}{D'}. \tag{36}$$

The conductance now depends on the number of vacancies which can be created to allow transfer between the ends. Since $D >> K$, the concentrations of ions and vacancies at each end remain near equilibrium with the adjacent solution and the flux increases steeply with potential.

For the permeability ratio, Eqns. 10, 11, and 15—19 lead to

$$\frac{P_b}{P_a}=\frac{AA''_bK''_bB''_a}{AA''_aK''_aB''_b} \tag{37}$$

and thus the value in constant but different from those in Eqns. 27 and 30. While the permeability ratio is directly proportional to how well the two ions bind $(AA_b/B_b)/(AA_a/B_a)$, the current ratio varies reciprocally with binding strength $(DD_a/E_a)/(DD_b/E_b)$.

III.3 Flux ratios and single-filing

From either the independence principle [37,38] or models allowing only one ion in the pore at a time [21], the ratio of the unidirectional fluxes of a single species across the membrane, J→ and J←, is related to the ion activities on the two sides and the potential by

$$\frac{J\rightarrow}{J\leftarrow}=\frac{a'_a}{a''_b}\,e^{-z\phi}. \tag{38}$$

Single-filing is normally said to occur whenever

$$\frac{J\rightarrow}{J\leftarrow}=\left(\frac{a'_a}{a''_b}\,e^{-z\phi}\right)^n. \tag{39}$$

As Hodgkin and Keynes [13] noted this relation in turn implies for $a'_a = a''_b$ that the conductance and unidirectional flux at $V = 0$ are related by

$$\frac{G}{J\rightarrow}=\frac{nz^2e^2}{kT}. \tag{40}$$

Using Eqns. 13 and 40 the present model predicts for a symmetrical pore [29,31]:

$$n = 1+\frac{2KD}{D^2+D(3B+2K)+2B^2+4KB} \tag{41}$$

As expected at very low concentrations $n \rightarrow 1$ since the ion fluxes are then independent. For intermediate concentrations which satisfy $B << A$ and $B << D << K$, it follows directly from Eqns. 16 and 34 regardless of symmetry that

$$\frac{J\rightarrow}{J\leftarrow} = \left(\frac{a'_a}{a''_b} e^{-z\phi}\right)^2 \tag{42}$$

and thus, as indicated by Eqn. 41, $n = 2$. The square appears since the flux ratio is proportional to $E''_a P_{aa}/(E'_b P_{bb})$. It is noteworthy (cf. Refs. 18, 29) that $n = 2$ can correspond to an average pore occupancy anywhere between one ion ($B << D << E, K$) and two ions ($B < E << D << K$). In the first limit the fluxes are proportional to ion activity while in the second [26] the fluxes are independent of activity. For high concentrations, $D >> K, B, E$, both ends of the pore are almost always occupied, each end remains near equilibrium with the adjacent solution, and an ion which crosses the central barrier will amost always escape into the solution rather than crossing back. The unidirectional fluxes are therefore

$$J\leftarrow = -J_b = K''_b P_{ob} = K''_b (Q'_a/T'_a) P_{ab} = K''_b Q'_a/T'_a$$

and

$$J\rightarrow = K'_a Q''_b/T''_b$$

and the net flux is

$$J = Q''_b K'_a/T''_b - Q'_a K''_b/T'_a. \tag{44}$$

The zero current condition (Eqn. 37) follows directly from Eqn. 44. The flux ratio equation is

$$\frac{J\rightarrow}{J\leftarrow} = \frac{T'_a K'_a Q''_b}{T''_b K''_b Q'_a} = \frac{a'_a}{a''_b} e^{-z\phi} \tag{45}$$

and thus as indicated by Eqn. 41, $n = 1$.

III.4 Blocking by impermeant ions

Ions which enter the pore, but are unable to cross at least one of the barriers can block the pore to movements of other ions. Assume for illustration that the pore is always at least singly occupied but rarely doubly occupied (as in Section III.2b), that the blocker, present only in the right hand solution, cannot cross the central barrier, and that transfer of the permeant ions through the pore is rapid ($a'_b = 0$, $B_a = B_b = K_b = 0$, $K_a >> D_a$, $D_a << E_a$, $T_a << Q_a$, and $T_b << Q_b$) then (for the equation valid at higher activities and/or when the blocker cannot cross the left hand barrier see Appendix),

$$I = \frac{z_a e(D'_a K'_a E''_a - D''_a K''_a E'_a)}{(E'_a + E''_a)[K''_a(1 + T''_b Q'_a/[T'_a Q''_b]) + K'_a]}. \tag{46}$$

Clearly as T''_b increases the current decreases, i.e. there is blocking. The strength of the blocking will depend on the applied potential. When $z_a eV/(kT)$ is large and positive, it will usually be true that $K''_a >> K'_a$, and the reversed inequality will usually hold for $z_a eV/(kT)$ large and negative, therefore the blocking will tend to be greater when the potential is driving the permeant ions away from the side containing the blocker rather than towards it, i.e. the system rectifies.

In qualitative terms Eqn. 46 shows that when transfer through the pore is rapid and $z_a eV/(kT)$ is large and negative, there will almost always be a permeant ion at the right end of the pore, and thus entry of the blocker is prohibited. When $z_a eV/(kT)$ is large and positive, there will rarely be a permeant ion at the right end, and entry of the blocker may proceed unopposed.

Armstrong [16] has suggested that potential dependent block by internal particles might explain the rectification seen in inward rectifying potassium channels [39,40,41]. He also stressed that pore models could explain the relief of block caused by increasing the concentration of K on the outside. This effect and the initially surprising result [16,28,39—42] that an increase in permeant concentration outside (on the left) can increase the outward current follow from Eqn. 46. It should be stressed that the extent of block does not depend simply on the difference between the applied potential and the reversal potential since in Eqn. 46 the extent of block does not depend on a''_a. Hille and Schwarz [29] discuss blocking at length.

The equations which describe rectification in the presence of a blocking particle can also describe physical models in which the blocked states of the pore correspond to different pore conformations. In Eqn. 46, changes to or from the blocked state can occur only while a permeant ion is bound at the left hand end of the pore but not at the right, and the binding constant to the left end is much less when the pore is blocked.

III.5 Anomalous mole fraction effect: conductance

If the ion concentrations are sufficiently low, then as one species is progressively replaced with another of the same valency, the conductance varies linearly between its values for the two species, i.e.

$$G = G_a X_a + G_b(1 - X_a) \tag{47}$$

where X_a is the mole fraction of permeant ions which are of species a. If the ions compete for a single site, the conductances saturate for each species, but mixtures still have intermediate conductances. Neher [43,44] has demonstrated for gramicidin in glyceryl monooleate membranes that complete replacement of NaCl with TlCl increases the conductance of the single channel but replacement of only a small proportion of the NaCl with TlCl substantially reduces it. Since the conductance can still be increased by adding sufficiently more NaCl or TlCl, one can exclude the trivial explanation that following addition of TlCl the pore is predominantly in the doubly occupied state and thus blocked to both species of ions. A similar effect was observed earlier for the inward currents across the membrane of a starfish egg by Hagiwara and Takahashi [45].

The present model predicts a type of selective block to the movement of one ion species caused by the presence of another which may explain these findings. The effect is seen clearly by considering a particular example. Assume that the ions carry the same charge and that both species satisfy the conditions in Section III.2b. The conductance is then ($a'_a = a''_a$, $a'_b = a''_b$, $z_a = z_b$)

$$G = \frac{z_a^2 e^2}{4kT} \times \left[\frac{D_a T_a}{Q_a} + \frac{4T_a T_b}{Q_a + Q_b} + \frac{D_b T_b}{Q_b}\right] \Big/ \left(\frac{T_a}{Q_a} + \frac{T_b}{Q_b}\right) \tag{48}$$

while the conductance for each species alone is

$$G_x = [z_x^2 e^2/(kT)]D_x/4. \tag{49}$$

The total conductance has a minimum if as the species with the greater conductance (b) replaces some of that with the lesser (a) the conductance initially decreases, i.e. if

$$-DD_a + TT_b Q_a[4/(Q_a + Q_b) - DD_a/(TT_a Q_b)] < 0. \tag{50}$$

This condition may be satisfied either for

$$Q_a > Q_b(4TT_a - DD_a)/DD_a \tag{51}$$

in which case b blocks a as apparently occurs for a = K and b = Tl with gramicidin [21] or for

$$Q_b > Q_a(4TT_b - DD_a)/DD_a \tag{52}$$

in which case a blocks b. If the ions produce equal conductances when present alone and the rate of entry does not depend on the species of ion already present ($DD_a = DD_b = TT_a = TT_b$), then the minimum conductance occurs when

$$\frac{a_b}{a_a} = \sqrt{\frac{Q_b}{Q_a}} \tag{53}$$

and the conductance at the minimum is

$$G = [(z_a^2 e^2)/(kT)][DD_a a_0/4][2\sqrt{Q_a Q_b/(Q_a + Q_b)}],\ a_0 = a_a + a_b \tag{54}$$

For $Q_a/Q_b = 14$ or 0.07, the conductance at the minimum is about one half the conductance in pure a or b, and the minimum occurs for a mixture containing 20% of the species with the smaller Q.

The origin of the selective blocking when either Q_a or Q_b is much smaller than the other can be understood in qualitative terms. Let species b have the higher conductance when it is present alone ($DD_b > DD_a$). If species b leaves the pore much less rapidly than species a ($Q_b << Q_a$), then as species b replaces species a, the pore becomes usually occupied by species b ($T_b/Q_b > T_a/Q_a$) while most of the ions entering are still of species a ($D_b < T_a$). In this case, whenever an ion of type a enters, it is much more likely to return back into the solution from which it came than to promote the exit of the blocker ($Q_a >> Q_b$). Thus little net flux is produced by these entries and the conductance is less than when only species a was present. As the replacement continues entries by species b become more frequent. After such an entry either ion in the pore may leave and the conductance increases towards its value when only species b is present. If alternatively species a leaves the pore much less rapidly than b ($Q_a << Q_b$), then entries by species b are unlikely to displace ions of species a from the pore. Thus for small replacements the pore remains occupied by ions of species a ($T_a/Q_a >> T_b/Q_b$), there is little flux of b, and the principal effect is to decrease the activity of a and hence the flux of a and the conductance. Only when most of species a has been removed do the flux of b and the conductance increase towards their final values.

III.6. Permeability ratios from addition experiments

For ions with the same charge, Eqn. 11 can always be used to calculate a permeability ratio from a measured or predicted zero current potential. For low activities this ratio will be the same whether or not each species is restricted to only one side of the membrane. For higher concentrations a simple expression for the zero current potential with two species present on both sides can be obtained if it is assumed that the pore is symmetrical and always at least singly occupied ($B_a = B_b = 0$), only the rate constant for transfer depends on potential, and transfer through the pore is rapid ($D_x^i << K_x^i$). The result is

$$V_o = -\frac{kT}{2ze}\ln\frac{a_a''^2\dfrac{DD_aTT_a}{2Q_a} + 2a_a''a_b''\dfrac{TT_aTT_b}{Q_a+Q_b} + a_b''^2\dfrac{DD_bTT_b}{2Q_b}}{a_a'^2\dfrac{DD_aTT_a}{2Q_a} + 2a_a'a_b'\dfrac{TT_aTT_b}{Q_a+Q_b} + a_b'^2\dfrac{DD_bTT_b}{2Q_b}} \tag{55}$$

which does not have the same form as the defining equation for the permeability ratio (Eqn. 11).

If species a is present only on the left and b only on the right,

$$\frac{P_b}{P_a} = \sqrt{\frac{DD_bTT_bQ_a}{DD_aTT_aQ_b}} \tag{56}$$

which could have been obtained from Eqn. 30. If instead a small addition of b is made to the right side ($a_a' = a_a''$, $a_b' = 0$ and a_b'' is small) then using $\sqrt{1+x} \approx 1 + x/2$,

$$\frac{P_b}{P_a} = \frac{2TT_bQ_a}{DD_a(Q_a+Q_b)} \tag{57}$$

which is clearly not the same as Eqn. 56. Thus if $DD_a = TT_a$, $DD_b = TT_b$, and $Q_a > Q_b$, the values of P_b/P_a determined with a and b on opposite sides, with small additions of a, and with small additions of b will all be different. The ratio for additions of a will be larger than the other two. In qualitative terms this effect is interpreted in the same manner as the anomaly in the dependence of the conductance on mole fraction. When the solutions contain predominantly b, the pore is blocked to a, the net current is determined by the activity gradient of b, and thus the reversal potential is extremely insensitive to small additions of a.

III.7 Anomalous mole fraction effect: zero current potential

Hagiwara et al. [46] have measured zero current potentials (inside minus outside) for the membranes of starfish eggs when the bathing medium is varied by progressive substitution of Tl for K. For all mixtures the zero current potentials varied by 58 mV per decade change of total permeant cation (K + Tl) concentration and there was pronounced inward rectification. When a small proportion of the K was replaced by Tl, the zero current potential became more negative and the conductance for inward currents declined, but for complete replacement the zero current potential was more positive and the conductance larger. As Hille and Schwarz [29] have noted by inspection of numerical examples, the present model can at least partially accomodate these results. In Eqn. 55 let the inside (″) activities and the total external activity ($a_o = a_a + a_b$) remain constant while the proportion of a and b outside is changed. Then for $b = a_b/a_o$

$$V_o - V_{b=0} = \frac{kT}{2ze} \times \ln\left\{\left[\frac{DD_aTT_a}{2Q_a} + 2b\left(\frac{TT_aTT_b}{Q_a + Q_b} - \frac{DD_aTT_a}{2Q_a}\right) + b^2\left(\frac{DD_aTT_a}{2Q_a} + \frac{DD_bTT_b}{2Q_b} - \frac{2TT_aTT_b}{Q_a + Q_b}\right)\right] \times \left[\frac{DD_aTT_a}{2Q_a}\right]^{-1}\right\} \tag{58}$$

The condition that V_o have an extremum for some value of b between 0 and 1 may be obtained by standard means. A minimum with

$$V_{min} - V_{b=0} = \frac{kT}{2ze} \ln\left[\frac{TT_b^2 Q_a[(Q_a + Q_b)^2 DD_b/TT_b - 4Q_aQ_bTT_a/DD_a]/(Q_a + Q_b)}{(DD_aTT_aQ_b + DD_bTT_bQ_a)(Q_a + Q_b) - 4TT_aTT_bQ_aQ_b}\right] \tag{59}$$

exists at

$$b = \frac{TT_aQ_b[DD_a(Q_a + Q_b) - 2TT_bQ_a]}{(DD_aTT_aQ_b + DD_bTT_bQ_a)(Q_a + Q_b) - 4Q_aQ_bTT_aTT_b} \tag{60}$$

provided

$$(DD_aTT_aQ_b + DD_bTT_bQ_a)(Q_a + Q_b) - 4Q_aQ_bTT_aTT_b > 0, \tag{61}$$

$$\frac{TT_b}{DD_a} < \frac{Q_a + Q_b}{2Q_a} \tag{62}$$

and

$$\frac{TT_a}{DD_b} < \frac{Q_a + Q_b}{2Q_b}. \tag{63}$$

If species a leaves mixed occupancy pores faster than species b ($Q_a > Q_b$), these conditions require that ions of species a enter pores occupied by a more rapidly than do ions of species b ($DD_a > TT_b\, 2Q_a/[Q_a + Q_b]$), but that they must not enter pores occupied by b too much more rapidly ($TT_a < DD_b\, [Q_a + Q_b]/[2Q_b]$). The first condition (Eqn. 62) ensures that as b replaces a the zero current potential initially declines, the second (Eqn. 63) ensures that as the replacement is completed the potential rises again, i.e. the two conditions together ensure the existence of a minimum.

Minima may occur in both the conductance and zero current potential relations whenever, for $DD_b > DD_a$, the rate constants satisfy Eqns. 50, 62, and 63. If $DD_b = TT_b$ and $DD_a = TT_a$, as assumed by Hille and Schwarz [29], and $DD_b > DD_a$, then $DD_a < TT_b$ and from Eqn. 62 a minimum in the zero current potential potential is only possible when $Q_b > Q_a$. Thus in agrement with the statements of Hille and Schwarz [29] these assumptions and the data of Hagiwara et al. would require that the species with the lower conductance, K, blocks the species with the higher conductance, Tl (i.e. the reverse of the effect seen with gramicidin). However, if it is not assumed that $DD_b = TT_b$, this conclusion does not necessarily follow since it is then possible to satisfy the inequalities $DD_b > DD_a > TT_b$. Thus if species a enters and leaves occupied pores more rapidly than b,

$$DD_a > TT_b,\ TT_a > DD_b, \text{ and } Q_a > Q_b. \tag{64}$$

Similarly if species a is more effective, while bound, at retarding the entry of other ions, for instance by binding further into the pore,

$$TT_a > DD_a \text{ and } DD_b > TT_b. \tag{65}$$

It is thus plausible that the rate constants for entry could satisfy

$$TT_a > DD_b > DD_a > TT_b, \tag{66}$$

in which case minima can occur in both the conductance mole fraction and the zero current potential mole fraction relations with the ion with the higher conductance, b, blocking the other, a. The minima observed by Hagiwara et al. occur for mixtures containing primarily K as predicted when Tl blocks K rather than the reverse.

IV. Discussion

The present model is the simplest description of ion transport through a pore which allows for electrostatic repulsion between the ions (when they have charge of the same sign), variable occupancy of the pore, and single filling. The model has been considered in detail for three reasons. Firstly, for our experimental system, gramicidin A, the assumptions made in the development of the model are plausible. Secondly any model which was more complicated would have far more constants than could be determined from kinetic data. Finally, it is important to investigate the predictions of simple models in some detail, for it is only when these fail that appeal to more complicated models can be justified.

This model predicts that at very low activities the conductance should be proportional to activity while at very high activities the relation should be inverse. The transition between these behaviours may (cf. Ref. 29) entail a plateau corresponding to one ion present always, a second linear increase as both the rate of entry of second ions and the consequential exit of either the first or second becomes appreciable, a second plateau limited by the rate of exit from doubly occupied pores [26], and finally a bending over as the pore becomes always doubly occupied and hence blocked. At low activities the permeability ratios equal the ratios of conductances determined with each ion species present alone provided these are measured at the same potential. For higher concentrations both types of ratio vary with activity since the currents may be limited by different processes when the pore is usually empty or usually occupied. One important reason for the variation in the permeability ratio is a specific blocking effect whereby ions which bind strongly can block the fluxes of others through the pore. Thus with mixtures of two ion species which separately produce similar conductances it is possible for almost all of the current to be carried by the more strongly bound species to the exclusion of the other (see Sections III.5—III.7). The distribution of the bound species then determines the zero current potential and hence the ratio of its permeability to that of the other species appears high. Similar interaction between the ions can account for minima in the conductances and zero current potentials measured with mixtures of varying mole ratio but constant total concentration.

It would of course be possible to extend this model to allow for more sites and any data could then be fitted more closely. In two respects such changes may be qualitatively significant. The present model predicts an upper limit of 2 for the flux ratio exponent (Eqns. 39, 40) and only a weak potential dependence of blocking by univalent ions. Additional sites within the pore would certainly allow both the flux ratio exponent to be adjusted to a larger value [29,47] and the potential dependence of blocking to be stronger [29], but while the extended theory would not involve any new concepts, it would require many more rate constants. For gramicidin values of the flux ratio exponent somewhat greater than 2 might be explained by a less elaborate extension of the model. Thus, Andersen and Procopio [48] have found that the fluxes at high potentials and low ion concentrations can be limited by diffusion in the aqueous phase. It may be possible to include the effects of this limitation by postulating the existence of superficial sites to represent the diffusional path the ions must follow through the aqueous phase in order to reach the mouth of the pore. Provided ions bound to such superficial sites did not change the energy of ions actually in the pore, these sites could be added to the theory without great complication and the extended theory would allow the flux ratio exponent to be increased. Other limitations of the model and possible extensions to eliminate them are discussed elsewhere [8].

An alternative model which seeks to describe the same types of experimental results has been presented by Sandblom, Eisenman and Neher [49]. In their model there is an inner site and an outer, modulatory site on each side of the membrane. Thus with two permeant species the pore can exist in any of 81 different states. So many states were required to fit their experimental data since it was assumed that the fluxes do not affect the probability of finding the pore in each state. While more than one linear region in the conductance-activity relation follows naturally from this modulation model, concentration dependent permeability ratios are predicted only if the binding constants to the inner sites and the rate constants for transfer between them depend on the species as well as the charge of the ions bound to the outer sites. Single-filing is excluded [47] and the current-voltage relations have the same shape at all ion activities [31,49].

In his theoretical discussion of ion transport via gramicidin, Levitt [12,23] has made the first attempt to go beyond the present kinetic model to predict the values of the rate constants from a physical theory of the transport process. Thus using a simplified model of the dielectric properties of the pore and continuum electrostatic theory, he has predicted the potential energy for one or two ions present at various positions within the pore. From these he concludes that there will be binding sites very near the mouths of the pore and that the ratio of the second binding constant ($DD_a/E_a = TT_a/Q_a$) to the first (AA_a/B_a) should lie between 1/2 and 1/8. However, his physical model may be oversimplified. For instance, if as suggested by the exclusion of anions from the channel, there are oriented dipoles in the wall of the pore, these might serve to draw cations more strongly and further into the pore. Furthermore the separation of ions in doubly occupied pores may be determined not by electrostatic forces but by the number of water molecules trapped between them. If so, one of the ions at least would not be able to sit at its preferred location, and the apparent repulsion between the ions would be strong. Because of these and other uncertainties, we have preferred to develop a formal model with arbitrary constants which can be fitted to experimental data. The results of such fitting for gramicidin, which have been reported briefly [7,31] and will be described in detail in a subsequent paper, can then be compared with the predictions of theories such as Levitt's which are based on more specific assumptions.

Appendix

In the steady state the probability of any particular state should not vary with time, i.e.

$$\frac{dP_{oo}}{dt} = 0 = -(A'_a + A'_b + A''_a + A''_b)P_{oo} + B'_aP_{ao} + B''_aP_{oa} + B'_bP_{bo} + B''_bP_{ob} \qquad \text{(A-1)}$$

$$\frac{dP_{oa}}{dt} = 0 = A''_aP_{oo} + E'_aP_{aa} + Q'_bP_{ba} - (D'_a + T'_b + B''_a + K''_a)P_{oa} + K'_aP_{ao} \qquad \text{(A-2)}$$

$$\frac{dP_{ob}}{dt} = 0 = A''_bP_{oo} + E'_bP_{bb} + Q'_aP_{ab} - (D'_b + T'_a + B''_b + K''_b)P_{ob} + K'_bP_{bo} \qquad \text{(A-3)}$$

$$\frac{dP_{ao}}{dt} = 0 = A'_aP_{oo} + E''_aP_{aa} + Q''_bP_{ab} - (D''_a + T''_b + B'_a + K'_a)P_{ao} + K''_aP_{oa} \qquad \text{(A-4)}$$

$$\frac{dP_{bo}}{dt} = 0 = A'_bP_{oo} + E''_bP_{bb} + Q''_aP_{ba} - (D''_b + T''_a + B'_b + K'_b)P_{bo} + K''_bP_{ob} \qquad \text{(A-5)}$$

$$\frac{dP_{ab}}{dt} = 0 = T'_aP_{ob} + T''_bP_{ao} - (Q'_a + Q''_b)P_{ab} \qquad \text{(A-6)}$$

$$\frac{dP_{ba}}{dt} = 0 = T'_bP_{oa} + T''_aP_{bo} - (Q'_b + Q''_a)P_{ba} \qquad \text{(A-7)}$$

$$\frac{dP_{aa}}{dt} = 0 = D'_aP_{oa} + D''_aP_{ao} - (E'_a + E''_a)P_{aa} \qquad \text{(A-8)}$$

$$\frac{dP_{bb}}{dt} = 0 = D'_bP_{ob} + D''_bP_{bo} - (E'_b + E''_b)P_{bb} \qquad \text{(A-9)}$$

One of these equations is redundant, e.g. Eqn. A-1. The ninth independent equation arises from the assumption that the nine states listed exhaust all the possible states of the pore,

$$1 = P_{oo} + P_{oa} + P_{ao} + P_{ob} + P_{bo} + P_{ab} + P_{ba} + P_{aa} + P_{bb} \qquad \text{(A-10)}$$

These equations have been solved [8] by eliminating P_{ba}, P_{ab}, P_{aa} and P_{bb}, introducing the auxiliary variables (Eqn. 8), and completing the algebraic manipulations. The use of diagrams for generating the solutions [22,25,26,50] was found to be more cumbersome.

Solutions for Eqns. A-1 to A-10 do not automatically satisfy the requirements of microscopic reversibility. This physical principle has been postulated and formulated by Tolman [36] as follows:

'. . . we may expect, on the average, for a system at equilibrium the same frequency of transition from the condition k to v as from v to k. This means that we can regard the steady state of affairs at equilibrium as maintained by a direct balance between the rates of opposing processes; that is, the transitions from k to v do not have to be thought of as balanced with the help of some indirect route such as v to l to k.'

When this principle is applied to the transitions allowed by the two-site model with two species of ions, six identities are obtained which hold at equilibrium. With the assumptions of Section II.2 concerning the activity dependence of the rate constants, these identities can be rewritten, and identities (Eqns. 15—18) obtained which are independent of activity and hold for all conditions, not just at equilibrium.

Blocking

If species b which is present only on the right cannot cross the left hand barrier, then provided the pore is always at least singly occupied

$$J = \left\{\frac{D'_aK'_aE''_a}{E'_a + E''_a} - \frac{D''_aK''_aE'_a}{E'_a + E''_a}\right\} \times \left\{\left(1 + \frac{D''_a}{E'_a + E''_a} + \frac{T''_b(T'_a + Q'_a)}{T'_aQ''_b} + \frac{K''_bT''_bQ'_a}{K'_bT'_aQ''_b}\left[1 + \frac{D''_b}{E''_b} + \frac{T''_a}{Q''_a}\right]\right)\right.$$
$$\left. \times \left(K''_a + \frac{D'_aE''_a}{E'_a + E''_a}\right) + \left(1 + \frac{D'_a}{E'_a + E''_a}\right)\left(K'_a + \frac{D''_aE'_a}{E'_a + E''_a}\right)\right\}^{-1} \qquad \text{(A-11)}$$

If species b cannot cross the central barrier Eqn. A-11 still applies but with $K''_b/K'_b = 0$. If in addition the pore is rarely doubly occupied and $K_a >> D_a$ then Eqn. A-11 reduces to Eqn. 46.

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